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## Integrated Control of Pome Fruit Diseases

Denis J. Butt (ed.)

Proceedings of the 3rd Workshop held  
1993 at Lofthus, Norway



Agricultural University of Norway – Advisory Service, Ås, Norway

## NORWEGIAN JOURNAL OF AGRICULTURAL SCIENCES

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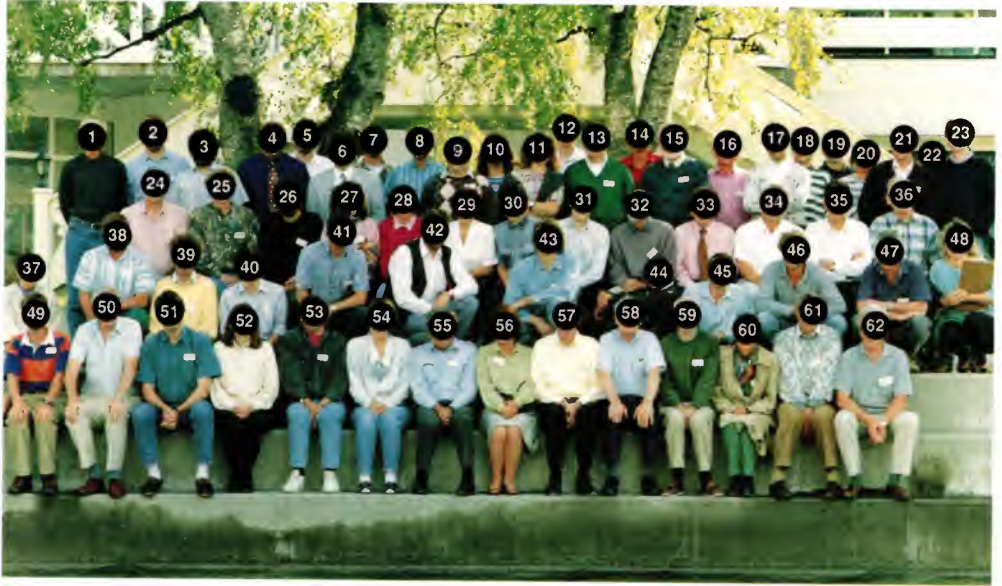
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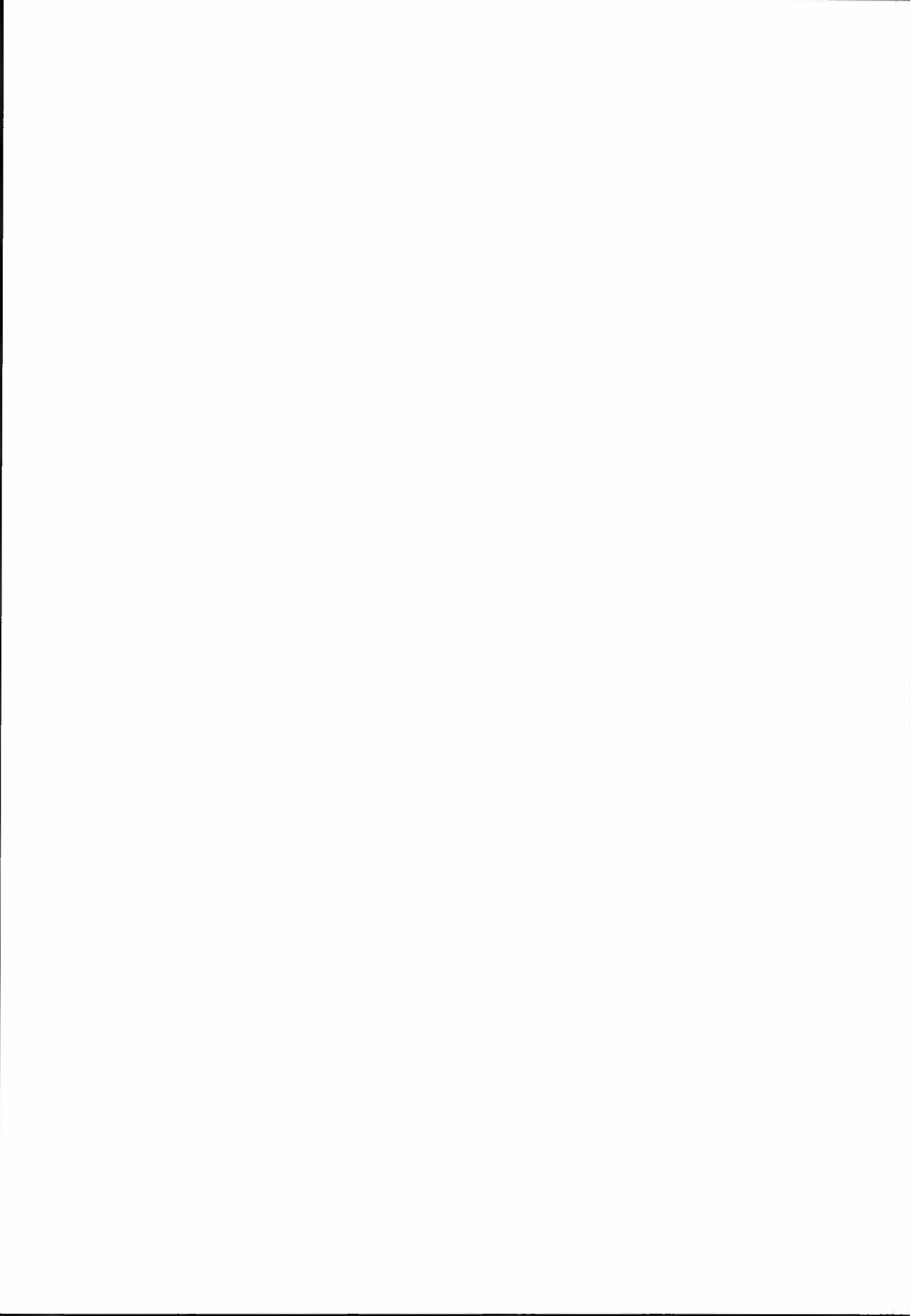
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# Introduction

DENIS J. BUTT

Horticultural Research International, East Malling, West Malling, Kent, United Kingdom

This publication contains most of the contributions presented as papers and posters at the 3rd Workshop on Integrated Control of Pome Fruit Diseases. The meeting, organized by the subgroup "Orchard Diseases" of the IOBC/WPRS<sup>1</sup> Working Group "Integrated Plant Protection in Orchards", was held in June 1993 at the Ullensvang Hotel, Lofthus, Norway. Previous workshops held by the subgroup were at Lana, South Tyrol, Italy in 1987 and at Brissago, Ticino, Switzerland in 1988(1). The subgroup also met at Godollo, Hungary in 1990(2) at the international symposium on integrated plant protection in orchards, organised jointly by the West – and East Palaeartic Regional Sections of IOBC.

The following objectives of the subgroup "Orchard Diseases" were agreed at Lana.

## OBJECTIVES OF THE SUBGROUP

1. To reduce the usage of fungicides before and after harvest
2. To search for biological control agents
3. To promote the breeding and introduction of varieties with durable resistance against multiple diseases
4. To make chemical and non-chemical control methods compatible
5. To avoid harmful side-effects of fungicides on the environment, crop and beneficial organisms used in IPM programmes
6. To develop and implement disease forecasting and infection warning systems
7. To develop and use pathogen and disease assessment methods
8. To determine disease damage thresholds and adopt action thresholds
9. To facilitate the exchange and dissemination of information, collaborative studies and standardisation of methods.

The 3rd Workshop was attended by 63 plant pathologists, advisers and other specialists from 16 countries, including guests from the USA, Canada, South Africa, Poland and Japan. As before, the agenda was limited to diseases of apple and pear. The programme contained 4 keynote lectures, 3 major reviews, offered papers and posters; the posters were each introduced as a mini-paper. Session topics covered integrated fruit production, fungicides, computer-aided disease management, molecular biology, apple scab, storage rots, cankers, and fireblight. The final session included an open forum for "questions and answers".

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<sup>1</sup>International Organisation for Biological and Integrated Control of Noxious Animals and Plants (West Palaeartic Regional Section)

## 12 Introduction

During the week the participants visited Ullensvang Research Station and a nearby commercial fruit farm owned by Lars M. Opedal.

Following precedents set at the previous two workshops, this meeting was held in a region of outstanding natural beauty; the magnificent scenery was enjoyed by all on the final day during the "Norway in a nutshell" guided tour.

The 4th Workshop will be held in England in 1996: Dr. C. Verheyden was elected to succeed D.J. Butt as scientific secretary of the subgroup.

We are pleased to acknowledge financial support from the IOBC(WPRS). Also, to the local organisations as listed above for their generous contribution to the scientific and social success of the meeting. Thanks are also due to those who ran the excellent programme for accompanying persons. Finally, our appreciation to C. Verheyden, H.A.Th. van der Scheer and C. Gessler for much editorial help and for serving as members of the 3rd workshop management team.

Kåre Hesjedal	Local organiser
Lars Sekse	Local organiser
Denis Butt	Scientific secretary
Erich Dickler	Convenor, IOBC Working Group "Integrated Plant Protection in Orchards"

- 1) Integrated Control of Pome Fruit Diseases. Vol. 2. ed. Gessler, Butt & Kollar. Proceedings of Workshop held October 30–November 4, 1988, Brissago, Switzerland, **IOBC/WPRS Bulletin** 1989/XII/6.
- 2) Proceedings of the International Symposium on Integrated Plant Protection in Orchards (ISIPPO), held in Godollo, Hungary July 31–August 5, 1990. Ed. K. Balazs, **Acta Phytopathologica et Entomologica Hungaria**, 27, 1992, numbers 1–4.

# Integrated Fruit Production - current status in Europe

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## UNDERLYING PRESSURES FOR INTEGRATED FRUIT PRODUCTION

Whilst pome-fruit yields fluctuate considerably from year to year, depending on weather conditions at blossom, in most years there is over- production of apples and pears in Europe. This results in an intensely competitive market so that individual growers, cooperatives and production regions are seeking new marketing concepts to improve their competitive edge, or at least are anxious not to lose out to competitors. At the same time the public in most countries have become conscious of environmental and food safety issues. These issues constantly receive media attention.

Most cultivars of apples and pears grown in Europe are susceptible to a range of damaging pests and diseases. In the absence of effective control measures considerable losses of yield and quality would result. To overcome these problems the industry relies heavily on the use of chemicals. Typically, pome fruit crops receive about 20 foliar spray applications per season, multiple active ingredients being applied in many instances. In many countries a significant proportion of harvested fruit is treated with fungicides and/or an anti-oxidant to prevent storage diseases and disorders. This chemical-dependent strategy has been very effective and relatively inexpensive for many years, and is the simplest to operate by growers. On well managed farms control levels approaching 100% are often achieved with the use of modern pesticides.

However, this reliance on chemicals poses apple and pear growers with a difficult dilemma. Throughout Europe the public have a strongly unfavourable attitude towards chemicals. Whilst overall they are beneficial to all parties, they are generally believed (by the uninformed) to be toxic, damaging to human health and to the environment. In a few instances this may be the case, especially when they are not used correctly, but undesirable aspects are generally exaggerated. Agrochemicals are often linked to the loss of countryside and wildlife habitats which have, in fact, resulted from agricultural practices in general and changes in land use.

The top fruit industry would like to improve its image in the market and cease using chemicals, but this is impractical. Organic production of apples and pears has not been widely successful because losses in yield and quality necessitate much higher prices which

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the market will not stand. The basis of organic standards, that natural substances are inherently safe and synthetic ones harmful, is also unsound.

The solution is to reduce reliance on chemicals to the minimum consistent with efficient, profitable production and to enhance and publicise the positive aspects of fruit growing. Integrated Fruit Production (IFP) provides a rationale for achieving this aim and is a guiding philosophy for both the marketing and production sides of the industry. The pressures outlined have been the driving force underlying the adoption of IFP, which has spread rapidly throughout Europe.

#### REQUIREMENTS FOR IFP STANDARDS AND THEIR DEVELOPMENT

The historical development of IFP from its origins in orchard integrated pest management is reported by Dickler & Schafermayer (1991). Switzerland and the South Tyrol region of Italy led with the first regional guidelines, followed by many other regions throughout Europe. Although the interpretation of the IFP philosophy was broadly similar in different regions, there were marked regional differences. The meaning of the term IFP was thus subject to several regional interpretations, leaving the concept open to attack by non-IFP fruit producers, organic producers and pressure groups. It was, therefore, necessary to agree a definition of IFP and to develop general guiding principles and minimum standards, and guidelines on how to meet them. Regional guidelines also require harmonisation to save the IFP concept from falling into disrepute. This task was undertaken by a joint group of the International Organisation for Biological Control (IOBC) and International Society for Horticultural Science (ISHS), under the chairmanship of Professor Dickler, Dossenheim. Each fruit-producing country in Europe was represented by IOBC/ISHS scientists at an intensive series of meetings in 1990 and 1991. As a result, the first edition of the European Guidelines and Standards for Integrated Production of Pome Fruits was published in four languages (Dickler & Schäfermayer, 1991). This great achievement was made possible by the realisation that scientific principles must come first in IFP, and that local or regional difficulties could not be allowed to undermine the ideal of IFP.

#### IFP - A SIGNIFICANT STEP FORWARD

It is crucial to the reputation of IFP that it represents a significant step forward towards environmentally safer, efficient and profitable fruit production. The minimum standards must not simply enshrine good agricultural practice. IFP must remain distinct from conventional production systems. Routine chemical inputs, which are a feature of conventional production, must be replaced to the greatest possible extent by non-chemical ones and IFP must embody the use of scientific methodology and high standards of crop management. A further aspect, which in future must play an increasingly important part in IFP, is the inclusion of positive steps for wildlife and habitat conservation and management.

These requirements pose a dilemma for IFP. On one hand, the standards have to be achievable by a significant body of growers in most regions. On the other hand, high standards must be maintained to give IFP significant credibility. If standards are too high



IFP is unlikely to be practised by more than a small minority of growers and the scheme will have little impact. The answer is to maintain a careful balance.

#### **IFP IN THE MARKET PLACE**

The way in which IFP currently operates in the market, varies considerably between countries and regions. The main purpose has been for cooperatives to seek market advantages with supermarket or export customers. Price premiums, often considerable, have been obtained in some instances, but these have usually not been sustained. In seasons when over-supply has occurred, IFP fruit may have maintained a more secure market. When under-supplied, the market has not generally favoured IFP and growers have been disappointed. In the UK, food safety legislation places a duty of 'due diligence' in matters of food safety on retailers and producers, including in the sphere of chemical use. This has been a significant driving force behind progress towards IFP. Although most IFP regions use a label to identify fruit produced under IFP, it is unlikely that this is having a significant impact upon consumers.

#### **FITTING IFP IN THE INTEGRATED PRODUCTION FRAMEWORK OF IOBC**

Whilst progress with pome fruits has been rapid, and has led the way forward, there is now strong and growing pressure for Integrated Production (IP) to be extended to other crops and, indeed, to whole farm enterprises. The IOBC has recognised here a niche and has set up a commission which has recently published a definition and the general principles for IP, together with a scheme to endorse local IP organisations. The demand for these activities from other sectors has evidently been growing. The scheme is ambitious in its scope and will require considerable administrative input. It is clearly important that IFP fits into the general IOBC scheme and its requirements.

#### **IFP DEVELOPMENT**

Growers' cooperatives adopt IFP for marketing purposes. They can only partake in a scheme if it is supported by the bulk of their members. Otherwise, fruit not meeting the standards of the cooperative would be disadvantaged, and the market place divided. The first edition of the European IFP Guidelines agreed by IOBC scientists was met with dismay by fruit growers in some countries, because local difficulties made the standards unachievable. Some technical aspects of the content of the first edition provoked particular criticism. Foremost of these was the unequivocal prohibition of post-harvest chemical treatments in IFP, whilst pre-harvest sprays were still permitted. Arguments for prohibition, though vigorously fielded, were emotive and political rather than strictly scientific. A value judgement that post-harvest treatments were unacceptable to consumers was taken, overriding the logical and scientific arguments against pre-harvest spraying. Several other aspects caused difficulties in individual regions or countries, including the prohibiting of

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chemical soil sterilisation and use of synthetic plant growth regulators.

The first edition of the guidelines was never regarded as final in every aspect, though the importance of continuity is universally recognised. A meeting of the IOBC/ISHS joint group was therefore held in Bologna, Italy in May 1993, and the contentious issues re-debated. A delegation of three from each country, led by an IOBC scientist, attended. Each delegation was charged with representing the views and interests of growers from their country. Each issue was debated and proposals for changes voted upon, each country having one vote. As agreed at a previous meeting, a 70% majority was necessary before changes to the first edition could be made.

Contrary to expectations it was agreed to permit post-harvest fungicide treatments for storage rot control in IFP, but only under strict conditions. These conditions included rot risk assessment, treatment according to need and adjustments of dose rate so that residues are no higher than for pre-harvest sprays. As far as I am aware, no country is fully ready to meet these stringent requirements and much future research is needed. Although several minor adjustments and some improvements to the first edition were made, no other major changes were agreed. A second edition of the guidelines will be submitted to the IOBC Commission for approval before publication.

### ENDORSEMENT

An endorsement procedure by which conforming of regional guidelines and standards with the European ones is certified by an independent scientific body is important. Regional guidelines and standards are a local interpretation of the European IFP guidelines and standards. The IOBC/ISHS joint group for IFP guidelines is now able to undertake this endorsement as an interim measure until general IOBC procedures are implemented. These latter procedures will cover the whole organisation of IFP in each region. The procedure of the joint group is simple and only covers the regional guidelines themselves. The regional IFP organisations submit their guidelines to the chairman for consideration for endorsement. The chairman appoints two separate referees from other countries who examine the guidelines in relation to the European guidelines and provide a recommendation to the chairman as to whether or not they should be endorsed. If the chairman receives no objections from the referees he is empowered to recommend the regional guidelines to the IOBC for endorsement. This endorsement procedure is now in operation and, if used seriously, will have a profound effect on IFP.

### SCIENTISTS OR GROWERS?

It is important for the integrity of IFP that it is scientifically sound and logical and that it is not based on emotive decisions. Inevitably, pressure will come from growers to adjust the guidelines to make them as easy as possible. Each country has different problems due to climate, varieties grown, methods of culture, pest and disease, flora and fauna, etc. Caution must be exercised in accepting a solution which is merely the lowest common denominator, easy for every grower in every country. It may be a mistake to let growers'

groups and representatives have too much say in the IFP guidelines. At the same time, the standards must not be set too high or unfairly, so that one country could use them as a means of preventing fruit imports. These factors can only be kept in balance by maintaining ruthless scientific impartiality.

## THE WAY AHEAD AND CHALLENGES FOR THE FUTURE

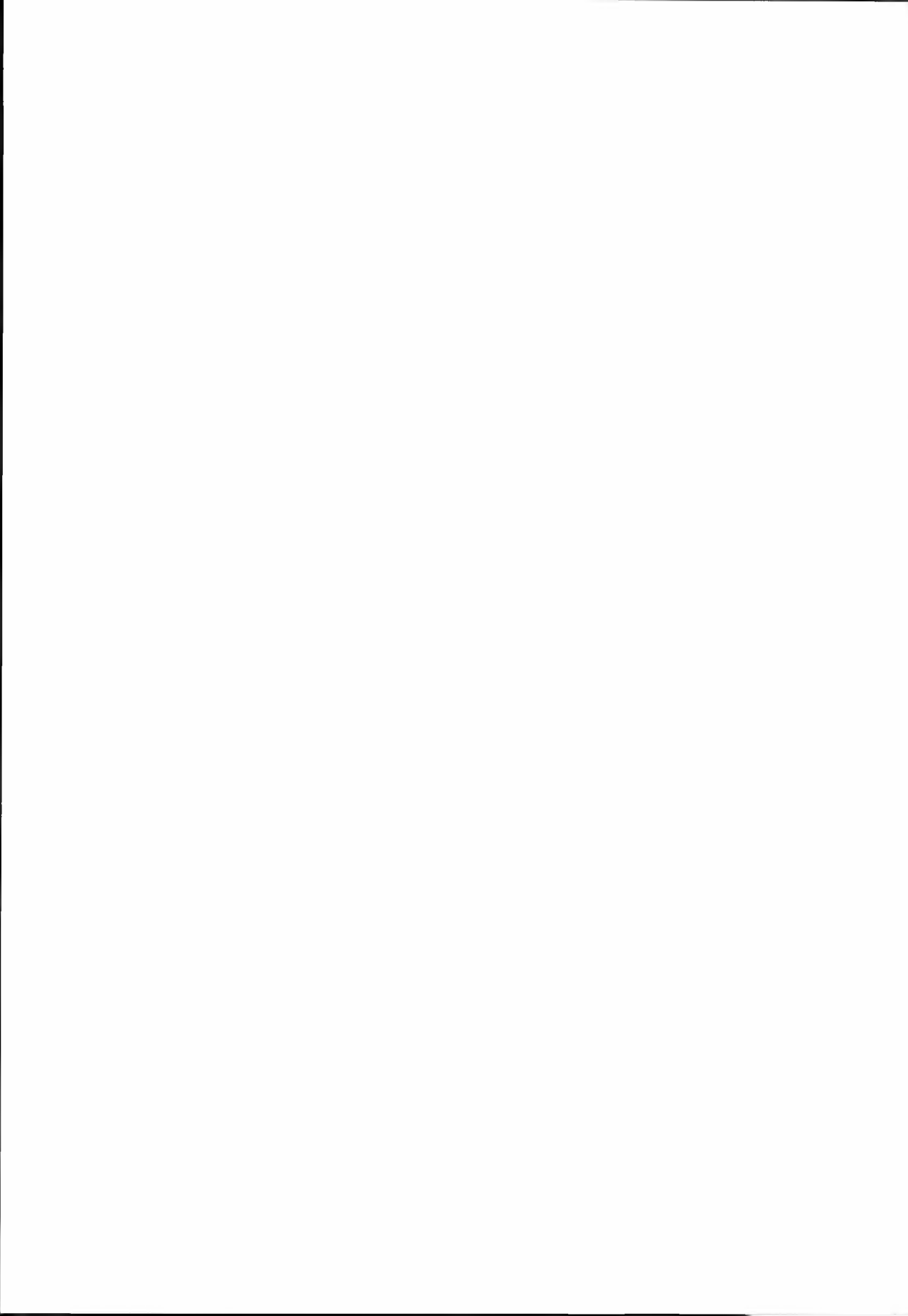
Further progress with IFP is only likely to come with technological progress. Considerable progress with the development of integrated pest management and alternative non-chemical methods for control of arthropod pests has been made. Commercial adoption of these methods remains far from complete. However, the bulk of spray applications in orchards are of fungicides for scab and mildew. In addition, a significant proportion of fruit in some countries is treated with fungicides to prevent post-harvest rotting. The high level of susceptibility of most commercial apple and pear cultivars to scab is regrettable. Breeding for resistance offers the prospect of almost complete elimination of the need for fungicides for scab and mildew control, though other diseases may become important e.g. sooty blotch. It seems it will be many years before resistant varieties with the full range of necessary agronomic characters including yield, internal and external quality and storability are available. When they are, the logical step will be for IFP guidelines to permit only such resistant varieties. In the meantime, plant pathologists must devise ways of reducing fungicide inputs. Forecasting models require careful practical evaluation, though it is probable that only modest reductions in fungicide use will be possible by these means. The whole subject of post-harvest rots and their control is one which requires much greater attention. Biological control has been successful with arthropod pests, but has always seemed difficult with diseases and there are few examples of success.

## CONCLUSION

IFP has expanded rapidly with overall beneficial effects. The linking of higher standards in fruit growing to the market place is an important achievement. Though IFP fruit seldom attracts a premium price, it is often favoured relative to conventionally produced crops: if this does not occur growers will lose interest and IFP will decline. There is always a danger that media or pressure groups will react adversely to IFP, pointing out that the degree of chemical use is only marginally reduced compared to conventional production. We must therefore work to achieve greater benefits and find ways of measuring them. To realise the aims of IFP we must strive in research to find alternatives to chemicals for the control of pests and diseases.

## REFERENCES

Dickler, E. & S. Schäfermeyer 1991. General principles, guidelines and standards for integrated production of pome fruits in Europe. IOBC Bulletin 1991/XIV/3. 67 pp.



# Integrated Fruit Production (IFP) in Norway

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Key words: Apple, fruit, IFP, integrated production, orchards, pear.

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## NATIONAL STRATEGY FOR FOOD PRODUCTION

In Norway, Integrated Fruit Production is part of a national strategy, The Norwegian Food Competitive Strategy launched in 1991 by The Ministry of Agriculture.

The reason for initiating this new strategy is to strengthen the competitive ability of Norwegian agriculture in a more open market. Since 1934, when The Norwegian Parliament passed a law which regulates the import of agricultural products, Norwegian agriculture has operated in a protected market. With possible EEC membership in the near future and the possibility of an agreement on world trade under GATT, Norwegian agriculture and horticulture will face a completely new market situation in the future.

The main objectives of the strategy are:

1. Strengthen the competitive ability of Norwegian food on the domestic market by stimulating use of national competitive advantages.
2. Achieve international market access for products based on Norwegian competitive advantages.

This national strategy comprises all branches of agriculture such as animal husbandry, grain production, vegetables, fruit and small fruit production. For each branch, a steering group has been appointed. These steering groups organize and bring about production systems aimed at product quality control and environmentally sound production methods. In some cases research programmes are initiated in order to obtain a scientific basis for new production systems.

## STRATEGY FOR IMPLEMENTING IFP TO ALL GROWERS

The Norwegian Food Competitive Strategy aims at implementing integrated production as the future production system for top fruit, small fruit, and vegetables.

As a first step the concept of Documented Production will be introduced to the growers on a large scale. In a documented production system the grower has to document

the orchard use of pesticides, herbicides and fertilizers. However, he does not have to justify orchard practices and there are no restrictions on use of agrochemicals. In an Integrated fruit production system there are definite restrictions on the use of agrochemicals as well as other orchard practices.

## INTEGRATED FRUIT PRODUCTION

IFP is organized by a national board on which the two growers' organisations (Norges Bondelag, Norsk Bonde- og Småbrukarlag), the growers' cooperative Gartnerhallen, the Norwegian Fruit and Vegetable Wholesalers Organisation and the Norwegian Agricultural Advisory Service are represented. The growers' organisations form the majority of the board.

At local level IFP is organized by regional steering groups to which growers, local advisers and researchers have been appointed. Regional steering groups have been established in all fruit-producing regions of Norway.

In each region, the regional steering group has appointed control groups of three to six members. According to decisions made by the National Board there should be a minimum of two advisers and one grower in each control group.

The national IFP guidelines are in accordance with the international IOBC guidelines. Growers who want to join the scheme have to sign a declaration in which they state knowledge of the guidelines, willingness to comply with the guidelines and acceptance of the recommendations in the guidelines.

Inspection of orchards has to be carried out during the growing season and before the end of August. At harvest, the field notebook will be inspected to see if there are any discrepancies between observations in the orchard and the records. A properly filled-out notebook and up-to-date records are mandatory. In case of doubt, soil or fruit samples will be taken in order to check if there has been any violation of the guidelines. The samples taken will be analysed by The Norwegian State Pesticide Laboratory. Also, storage and handling practices in the fruit stores will be inspected.

Certification is based on a scoring system where violations of the rules give scores from minus 1 to minus 5. A score of minus 5 leads to exclusion. Inadequate field records or use of plant protection chemicals not allowed give a score of minus 5.

1992 was the first year of organized IFP in Norway. 111 growers participated in the scheme representing about 200 hectares or about 8% of the acreage. However, a larger percentage of the growers is practicing integrated production methods. Only apples and pears are produced following IFP standards; work on IP-guidelines for stonefruit is in progress.

The Norwegian Agricultural Advisory Service, in cooperation with local advisers, assists growers throughout the season. Courses in IFP have been held in all major growing districts. So far, approximately 500 growers have attended the courses.

Over an initiation period of two years the requirements of the production system will be developed and more clearly defined and from 1995 IFP is expected to be effective as a full-scale production system.

## ORGANISATION AND CONTROL OF INTEGRATED FRUIT PRODUCTION

- \* A cooperative of growers or an individual grower who wishes to participate in the IFP scheme must sign a statement which states
  - a) their/his/her knowledge of the IFP guidelines and willingness to comply with the guidelines
  - b) inspection of the orchard will be allowed and samples of fruit, soil or plants can be taken
  - c) decisions made by the inspection team will be accepted and in cases of disagreement a final decision taken by the National Board will be accepted
- \* Normally the entire orchard area of the farm should be included in IFP. In a period of transition no longer than 3 years a grower can participate with certain cultivars only or part of the farm.
- \* Inspection will be carried out by inspectors appointed by the Regional Board. The inspection shall be neutral, representative and reliable. A minimum of 20% of the growers will be inspected each year. On each farm a minimum of one orchard has to be inspected thoroughly to see if the orchard practices are in accordance with the guidelines. In cases of doubt, samples of fruit, leaves, orchard floor vegetation and soil will be taken for analyses of residues of agrochemicals.
- \* The field notebooks will be inspected at harvest. Insufficient records or violations of the guidelines lead to exclusion from the IFP scheme.
- \* The inspectors can approve or disapprove a grower or an orchard, depending on whether the requirements listed in the inspection form have been fulfilled. Disagreements are settled by The National Board. When a grower/orchard is excluded from the IFP scheme his or her wholesaler or warehouse will be informed.
- \* Fruit produced according to the IFP guidelines will be marketed under a special label.
- \* IP-fruit should be marked properly and stored according to storage conditions outlined in the guidelines. In order to secure proper storage and handling procedures, warehouse inspection will be carried out every year. Misuse of the label will lead to suspension of the warehouse for the remainder of the storage season.
- \* Samples for residue analyses will be taken at random and in accordance with procedures worked out by The Norwegian State Pesticide Laboratory.

## I.F.P. ORGANISATION IN NORWAY

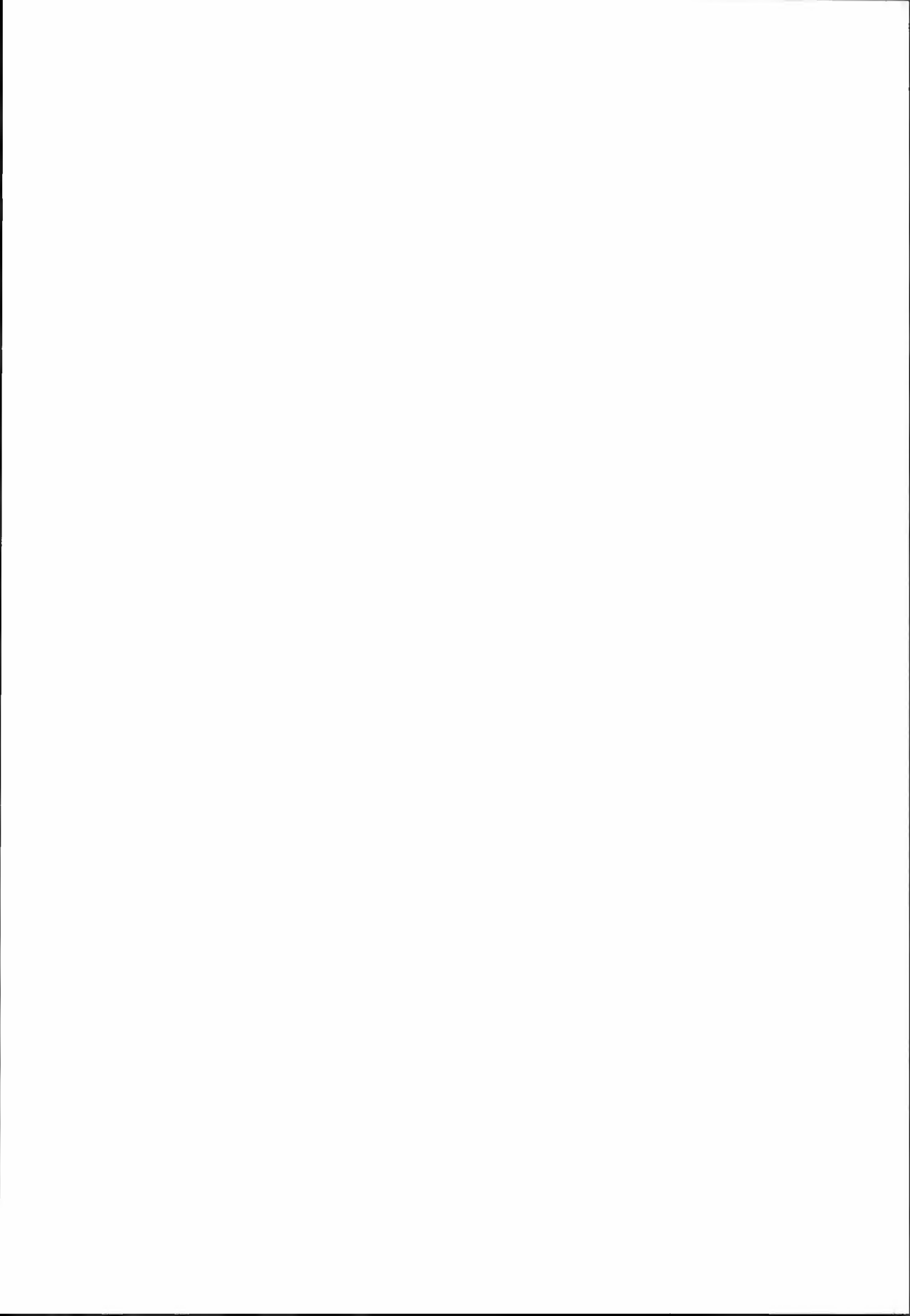
<p><b>NATIONAL LEVEL</b></p> <p><b>NATIONAL BOARD</b></p> <p><b>REPRESENTATION:</b></p>		
<p><b>GROWERS ORGANISATIONS:</b> 4 MEMBERS</p> <p><b>REGIONAL STEERINGGR:</b> 2 MEMBERS</p>	<p><b>WHOLESALERS ORGANISATION</b></p> <p><b>GROWERS COOP:</b> 2 MEMBER</p>	<p><b>ADVISORY SERVICE</b> 1 MEMBER</p>
<p><b>RESPOSIBILITIES: ORGANISE I.F.P. ON A NATIONAL LEVEL</b></p> <p>APPROVE GROWERS</p> <p>APPROVE INSPECTORS APPOINTED BY THE REGIONAL BOARD</p> <p>MAKE FINAL DECISION IN EXCLUSION CASES</p> <p>MAKE INFORMATION ABOUT I.F.P. AVAILABLE TO THE GROWERS</p>		
<p><b>REGIONAL LEVEL</b></p> <p><b>REGIONAL BOARDS</b> (MINIMUM 3 MEMBERS)</p>		
<p><b>MØRE NORDFJORD</b></p>	<p><b>SOGN</b></p>	<p><b>HARDANGER</b></p>
<p><b>ROGALAND</b></p>	<p><b>EAST NORWAY</b></p>	
<p><b>RESPOSIBILITIES:</b></p> <p>ORGANISE I.F.P. AT THE REGIONAL LEVEL</p> <p>APPOINT INSPECTORS</p> <p>ORGANISE INSPECTION ROUTINES</p> <p>DISTRIBUTE WRITTEN INFORMATION TO THE GROWERS</p> <p>COLLECT FIELD NOTEBOOKS</p>		



LITERATURE

Dickler, E. and S. Schäfermeyer, 1991. General Principles, guidelines and standards for integrated production of pome fruits in Europe. IOBC/WPRS Bulletin XIV/3, 67 pp. 1991.

Hesjedal, K. and T. Edland, 1991. Retningslinjer for integrert produksjon av kjernefrukt. Norwegian Agricultural Advisory Service, 10 pp. 1991.



# Evaluation of disease control in apple cv. Cox according to IFP Guidelines in comparison with current commercial practice

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Disease control in 1991 and 1992 using U.K. Integrated Fruit Production (IFP) Guidelines was compared with conventional control methods in a replicated trial. In the conventional plots, fungicide sprays for scab and mildew were applied every two weeks, usually at reduced rates. Fruit from the conventional plots was treated after harvest with a fungicide drench for control of storage rots compared with a programme of pre-harvest fungicide sprays used in IFP plots. Levels of scab and powdery mildew recorded were very low and similar in IFP and conventional plots. Fungicide use to control scab was greater in IFP compared to conventional, although monitoring levels of powdery mildew on IFP plots and matching fungicide rate to mildew level did achieve a reduction in mildew fungicide use in both seasons. Adequate control of storage rots was achieved in fruit from IFP plots using a programme of pre-harvest sprays compared to a single post-harvest fungicide treatment in the conventional system. The progress of IFP in the U.K. is discussed together with strategies for further reducing fungicide use.

Key words: Apple scab, apple powdery mildew, disease assessment, fruit rots, fungicides, integrated fruit production, orchard diseases.

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In the U.K., apple orchards generally receive routine sprays of fungicides to reduce pest and disease problems and to achieve economic yields of first-quality fruit demanded by the market. This orchard protection system has been successful and is the simplest and most convenient to manage. More recently, however, the increase in public awareness of chemicals and concern over possible side effects on health and the environment has made such routine fungicide control less acceptable. Cox is the main dessert apple cultivar grown in the U.K. and is susceptible to most major apple diseases, especially apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and storage rots (e.g. *Monilinia fructigena*, *Botrytis cinerea*, *Nectria galligena*). In seasons favourable for disease, up to 20 sprays of fungicide can be applied. Organic production of such disease-susceptible cultivars

is not economically viable, is inefficient and results in high prices due to large (often >50%) losses. Breeding programmes or genetic manipulation to produce resistant cultivars offer a long-term solution, but for the immediate future the economic production of quality fruit in the U.K. will still rely on the use of chemicals. Consumer concerns, however, cannot be ignored. Therefore, production systems that minimise chemical use must be developed. Integrated Fruit Production (IFP), which combines genetic, chemical, agronomic, biological and natural methods to minimise the use of agrochemicals offers the best approach to reducing chemical use and attendant environmental and safety problems.

Guidelines for IFP for apples and pears in the U.K. were first published in 1990 (Cross et al. 1991). In IFP, chemical use is only permitted according to a clearly identified need. Such decisions for orchard diseases, therefore, must be based on regular risk assessment using information from orchard monitoring to determine disease incidence and/or weather conditions. At present in the U.K. few growers make use of scab warning systems to regulate fungicide use for scab control. Because of the importance of scab control pre-blossom and immediately after bloom, and the difficulties of early scab recognition in the orchard, current U.K. IFP guidelines (and also those of other countries) permit the use of routine sprays during the early part of the season. Fungicide choice in IFP is also regulated by the need to preserve predatory mite populations (*Typhlodromus pyri*). The use of those fungicides (e.g. mancozeb, carbendazim) likely to be harmful to these mites must be avoided or limited; fungicide products differing in mode of action must also be used in a programme to prevent the selection of fungicide-insensitive components of the *V. inaequalis* population. Current U.K. guidelines do not permit the use of post-harvest fungicides to control storage rots.

Although U.K. guidelines for IFP have been published, the entire production system, including integrated orchard protection, has not been demonstrated in commercial practice. The purpose of the trial to be described was therefore to test the practical feasibility of IFP in the U.K. Only aspects of the study relating to disease control will be reported here.

## MATERIALS AND METHODS

In 1991 a trial site was established in a commercial apple orchard, cv. Cox on MM106 rootstock, in Kent in South East England. At the site, control of orchard diseases according to IFP guidelines was compared with current conventional practice in large plots (5 rows of 70-72 trees), each treatment being replicated three times and fully randomised (randomised complete block design). Untreated plots were included to indicate disease incidence and were not part of the experiment design. All fungicide spray treatments were applied by farm staff using a tractor-trailed axial fan orchard air-blast sprayer (mist blower), at 100-150 litres/ha.

Decisions on fungicide treatment (choice of product and rate) were based on the results of regular plot assessments. The centre row in each plot was assessed for disease (scab and mildew) every two weeks from green cluster to harvest, according to the assessment schedule in Table 1. Secondary mildew was assessed on extension growth according to the method of Butt & Barlow (1979).

Blossom wilt (*Monilinia laxa* f.sp. *mali*) and canker (*Nectria galligena*) were assessed after blossom. In July and September the orchards were assessed for rot risk to determine need for pre-harvest sprays for control of storage rots. At harvest, 1000 fruit were picked per plot and assessed for fruit scab and other diseases. Fruit from the centre row in each plot was picked into bins and either drenched (conventional) with a fungicide pre-storage or left undrenched (IFP). The fruit was stored commercially in a controlled atmosphere store at 3.5 - 4°C and 2% oxygen until the following March. After storage, 1000 fruit from each plot were assessed for rots.

Table 1. Disease assessment times and sampling rate

Time	Sample rate/plot	Disease
Green cluster/pink bud	10 blossoms/branch;/4 branches/tree; 25 trees (whole tree)	Primary blossom mildew Apple scab
Petal fall	25 trees (whole tree) Top 5 leaves/shoot; 2 shoots/tree; 25 trees	Apple scab, canker Secondary mildew
Petal fall + 2 weeks	25 trees (whole tree)	Apple scab, canker Blossom wilt
	Top 5 leaves/shoot; 2 shoots/tree; 25 trees	Secondary mildew Apple scab
Every 10-14 days from petal fall - harvest	25 trees (whole tree)	Apple scab, canker
	Top 5 leaves/shoot; 2 shoots/tree; 25 trees	Apple scab/secondary mildew
July to September/ Pre-harvest	Whole plot (rot risk)	Fruit quality (russet, cracking) Crop load Phytophthora rot Brown rot Monilinia rot
Post harvest	100 leaves/plot	Late scab

After harvest in late September, 100 leaves were sampled at random from the centre row in each plot and assessed for late scab infection.

## RESULTS

Assessments of disease incidence, to determine need for treatment, were made every two weeks and took approximately 30 minutes to complete on 25 trees. Fungicide sprays to conventional plots were applied routinely every 14 days, although usually not at the manufacturers full recommended rate. Sprays to IFP plots were applied at similar timings, but usually at reduced rate. At the end of the season, total fungicide applied was greater on the IFP plots than on the conventional plots (Tables 2 & 3).

Table 2. Fungicide product, active ingredient, number of applications and per cent of recommended full dose used to control apple scab and powdery mildew in conventional and IFP treatments in 1991

Fungicide Product	Active Ingredient	Conventional		IFP	
		No. applications	% of full dose	No. applications	% of full dose
Dithianon	dithianon	6	53	2	68
DelanCol	dithianon	0	0	5	50
Topas	penconazole	6	75	2	75
Pallitop	nitrothal isopropyl	6	35	5	37
Nimrod	bupirimate	0	0	2	75
Total spray treatments		13		11	

Table 3. Fungicide product, active ingredient, number of applications and per cent of recommended full dose used to control apple scab and powdery mildew in conventional and IFP treatments in 1992

Fungicide Product	Active Ingredient	1991		1992	
		No. applications	% of full dose	No. applications	% of full dose
Dithianon	dithianon	2	59	7	45
Topas D	penconazole + dithianon	5	55	3	55
Topas	penconazole	0	0	1	43
Systhane	myclobutanil	0	0	1	100
Pallitop	nitrothal isopropyl	3	33	1	25
Nimrod	bupirimate	0	0	2	36
Total spray treatments		10		10	

**Powdery mildew in 1991 and 1992**

Levels of primary mildew were very low and similar in all plots in 1991 (Table 4). In 1992, primary mildew had increased in the untreated area although it was still at a low level and remained similar and at very low levels in IFP and conventional plots. Secondary mildew levels were generally low throughout the season in both years in both treatments. In the IFP plots fungicide rate was matched to secondary mildew levels assessed according to the method of Butt & Barlow (1979). This enabled fungicide to be applied at reduced dose for most of the season in both 1991 and 1992. By monitoring mildew in this way total fungicide applied for mildew control was less on the IFP plots compared to conventional plots. The level of secondary mildew on the untreated plot increased steadily through the season in 1991, reaching a maximum of 80 per cent. Levels in 1992 were much lower, reaching a maximum of 25 per cent in mid June.

Table 4. Mildew-infected blossoms (primary mildew) and mildew-infected leaves (secondary mildew) and assessment dates 1991 and 1992

	Treatment	% Primary Mildew	% Secondary Mildew							
1991		17/4	30/5	10/6	25/6	8/7	22/7	5/8	19/8	2/9
	IFP	0.5	0	2.5	6.5	2.8	1.2	6.3	3.2	5.0
	Conventional	0.4	0	1.3	3.5	1.7	1.3	6.0	4.0	8.3
	Untreated	0.25	7.0	38.0	38.0	26.0	4.0	16.0	21.0	80.0
1992		28/4	19/5	2/6	15/6	30/6	14/7	28/7		
	IFP	0.3	<1	0	1.9	2.4	0.5	0.7		
	Conventional	0.2	<1	0.7	0.3	2.1	1.7	0.7		
	Untreated	1.7	1.5	13.3	25.3	2.0	4.0	10.7		

**Apple scab 1991 and 1992**

In 1991, during the early part of the season no scab was recorded (Table 5) apart from one scabbed fruit in the untreated, despite the favourable weather recorded in June (Table 6). Fungicide sprays for scab were therefore discontinued, at the end of June in IFP plots and one month earlier in May in conventional plots. During August, scab was noted on extension growth in both conventional and IFP plots, although levels in the latter were lower. No scabbed fruit were recorded at harvest. In the untreated plot the number of scab-infected shoots increased steadily from August, although levels of scab on the fruit at harvest were negligible. At the end of the season the total amount of fungicide applied for scab control was greater on the IFP plots than conventional plots (Table 2). The additional fungicide applied to IFP plots were justified by favourable weather in June and early July and did result in a lower scab incidence on IFP plots. However, scab levels were very low and not commercially significant on conventional plots. In 1992, weather was more

30 Evaluation of disease control in apple cv. Cox

favourable for scab (Table 6) and fungicide sprays were continued until early July. A higher incidence of scab was recorded in the untreated plots, with 10% of shoots infected in early June and 15% fruit infection at harvest. No scab-infected shoots were recorded in either the IFP or conventional plots and levels of scabbed fruit at harvest were very low. At the end of the season the total amount of fungicide applied for scab control was again greater on the IFP plots than conventional plots.

Table 5. Assessment dates and levels of apple scab recorded 1991 and 1992

	Treatment	% Infected Shoots				% Infected Fruit	% Infected Leaves
		25/6	5/8	19/8	2/9		
1991						25/9 (harvest)	7/11
	IFP	0	0	2.0	0	0	4.5
	conventional	0	1.7	6.8	0.7	0	8.7
	untreated	0	0	30.0	43.0	0.07	22.8

	Treatment	% Infected Trees		% Infected Shoots	% Infected Fruit	% Infected Leaves
		13/5	15/6			
1992				2/6	8/9	10/10
	IFP	0	0	0	0.03	2.2
	conventional	0	0	0	0.1	0.7
	untreated	33.3	66.7	10.0	15.5	93.0

Table 6. Apple scab infection periods (Smith Periods) recorded at Manston, Kent, between bud burst (April) and harvest (September) in 1991 and 1992

Month	Number of Smith Periods	
	1991	1992
April	3	2
May	1	2
June	13	3
July	8	8
August	2	8
September	7	5



### Fruit rots 1991 and 1992

In 1991, the pre-harvest orchard assessment indicated a possible risk of brown rot (*Monilinia fructigena*) and Phytophthora rot (*Phytophthora syringae*). A programme of two sprays of Captan and one of Mildothane (thiophanate methyl) was therefore applied to IFP plots in July, August and September for control of storage rots. In 1992, high rainfall was recorded in July and August and therefore a high risk of Phytophthora rot. Three sprays of captan were therefore applied pre-harvest. In both years fruit from the conventional plots was drenched post harvest in Ridomil MBC (metalaxyl + carbendazim). In addition, in 1992 conventional plots also received 2 sprays of Captan pre-harvest. Losses due to rotting were low (<2 per cent) in both the IFP and conventional in both years (Table 7). In 1991, fewer rots were recorded in the IFP fruit, principally due to a reduction in the level of Botrytis rot.

Table 7. Per cent losses due to fungal rots after storage in 1991 and 1992

Fungal rot	1991		1992	
	IFP	conventional	IFP	conventional
<i>Botrytis cinerea</i>	0.60	1.23	0.80	0.27
<i>Monilinia fructigena</i>	0.10	0.03	0.07	0.43
<i>Nectria galligena</i>	0.23	0.97	0.16	0.24
<i>Phytophthora syringae</i>	0.00	0.10	0.00	0.00
<i>Penicillium</i> spp	0.30	0.60	0.27	0.50
Other	0.23	0.26	0.13	0.10
Total loss	1.46	3.19	1.43	1.54

### DISCUSSION

Disease control according to IFP Guidelines was successful and similar to that achieved by conventional methods for both orchard diseases and storage rots. Levels of scab and mildew in the orchard were very low and this obviously contributed to the successful result. The definition of conventional treatment needs consideration. Within the U.K. there is considerable variation in current commercial practice in orchards, but in general very few growers apply fungicides at the full dose given on the product label. At the orchard trial site, the grower's usual practice was already to reduce fungicide dose rate as a means of saving costs. Consequently, the differences in fungicide rates used were very small, despite the reduced dose rates used on the IFP plots. By monitoring powdery mildew levels it was possible to achieve a lower fungicide use at the end of the season. With apple scab, however, where the attendant risks of fruit damage are much greater, dose-rate reductions in IFP were more difficult to achieve based solely on available weather data and orchard scab assessments. The grower, with his greater experience of the orchard, was with confidence able to make further reductions in fungicide rate on the conventional plots. A Metos<sup>R</sup> weather station has now been installed in the orchard and in the final year of the

study will be used, with the VENTEM™ scab warning system (Xu et al. 1993), as an aid to decisions on fungicide use in the IFP plots.

Reducing the amount of fungicide applied to IFP plots achieves the aim of IFP to reduce chemical use and obviously results in lower costs. However, balanced against this is the increase in management time resulting from the required disease assessments in order to make treatment decisions. In the U.K., where fruit farms vary in size from 2 to 300 hectares, management time required for orchard assessment can be considerable and may be one of the negative features of IFP perceived by growers (Lovelidge 1993). If fungicide use is to be reduced without jeopardising fruit quality, decisions must be based on accurate assessments of disease risk. Methods of achieving this with minimum management input need to be investigated.

The results presented here represent only two years of a study at one site with low disease incidence. Reducing fungicide use may be more difficult at sites with a high disease incidence and could result in a gradual build up of disease. Long-term trials are needed to evaluate the full implications of the system.

#### REFERENCES

- Butt, D.J. & G.P. Barlow 1979. The management of powdery mildew: a disease assessment method for growers. *Proceedings 1979 British Crop Protection Conference - Pest & Disease*, pp. 77-86.
- Cross, J.V., A.M. Berrie & M.J. Marks 1991. *Integrated production of pome fruits. ADAS Guidelines and Standards for Apples & Pears.*
- Lovelidge, B. 1993. Getting to grips with IFP. *The Grower* April 29, 9.
- Xu, X.M. & D.J. Butt 1993. PC-based disease warning systems for use by apple growers. *EPPO Bulletin* 23: 595-600.

# The use of fungicides in Norwegian integrated apple production compared to conventionally grown apples

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Summer 1992 records of fungicide application in 77 integrated grown and 31 conventionally grown apple orchards were collected from fruit growers in western part of Norway. The object was to document the use of fungicides in Norwegian apple production and to confirm possible differences in protection strategy and fungicide application between the two production methods. The records included the date of spraying, kind of fungicide, fungicide dosage and spray volume used per hectare. The collected data showed that none of the growers used a scheduled application programme. There was no pronounced difference in the number of applications between integrated (6.1 times) and conventional production (6.4 times). On average for all cultivars, 3.9 "normal dosages" of fungicides were applied in integrated production and 4.7 "normal dosages" in conventional production. Growers who participated in the integrated production programme were more aware of the cultivars disease resistance when they choose protection strategy.

Key words: Apple, conventional production, fungicide application, integrated production

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There are no routine registrations of pesticide application in Norway, and the last investigation of pesticide use in Norwegian fruit production was accomplished in 1988 (Edland, 1989). Increasing competition in the market from environmental-friendly and integrated grown fruit from other countries made it necessary to put a figure on to-day's use of pesticide in Norway. Therefore, Ullensvang Research Station started to collect information about the pesticide use in Norwegian apple production in the summer of 1992. In addition to document the total amount of pesticide application, we were interested in confirming possible differences in protection strategy and pesticide use between integrated and conventionally production methods. The investigation covers the application of all kinds of pesticides, but this paper will concentrate on the fungicide use.

## MATERIALS AND METHODS

The integrated fruit production programme in Norway requires that the participants record their use of fungicides during the season. For each application the records include date of spraying, kind of fungicide, fungicide dosage and spray volume per hectare. In 1992 thirty-three fruit growers from the Hardanger region in the western part of Norway participated in the integrated fruit production programme. At the end of the season they were asked by letter to contribute their records to this investigation. Twenty-four of them (72.7 %) gave a positive answer, and their data gave information about fungicide application in 77 integrated grown apple orchards. From the same region eight fruit growers have contributed corresponding information from 31 conventionally grown apple orchards.

Fungicide concentration in the spray liquid varies a lot and the number of spray applications during the season is no good measure of the total amount of fungicide applied in the orchards. For that reason the records are converted to "normal dosage" which is the maximum permitted dosage per spraying in Norway. Table 1 gives a view of the most common fungicides used in Norwegian apple production, the "normal dosage" and the amount of active ingredient per "normal dosage".

Table 1. "Normal dosage" of ordinary applied fungicides in Norwegian apple production. "Normal dosage" is the maximum permitted dosage per application in Norway

TRADE NAME IN NORWAY	COMMON NAME OF CHEMICAL	"NORMAL DOSAGE"	ACTIVE INGREDIENT PER "NORMAL DOSAGE"
Benlate	benomyl	1.2 kg·ha <sup>-1</sup>	0.60 kg·ha <sup>-1</sup>
Baycor	bitertanol	1.6 kg·ha <sup>-1</sup>	0.40 kg·ha <sup>-1</sup>
Melprex	dodine	1.8 kg·ha <sup>-1</sup>	1.17 kg·ha <sup>-1</sup>
Rovral	iprodione	3.0 kg·ha <sup>-1</sup>	1.50 kg·ha <sup>-1</sup>
Kopperkalk	copper oxychloride	5.0 kg·ha <sup>-1</sup>	4.20 kg·ha <sup>-1</sup>
Dithane	mancozeb	4.0 kg·ha <sup>-1</sup>	3.00 kg·ha <sup>-1</sup>
Svovel, Thiovit	sulphur	7.5 kg·ha <sup>-1</sup>	6.00 kg·ha <sup>-1</sup>
Svovelskalk	lime-sulphur	40.0 l·ha <sup>-1</sup>	7.60 l·ha <sup>-1</sup>
Topsin	thiophanate-methyl	1.4 kg·ha <sup>-1</sup>	0.98 kg·ha <sup>-1</sup>
Euparen	tolyfluanid	3.0 kg·ha <sup>-1</sup>	1.50 kg·ha <sup>-1</sup>
Bayleton S	triadimefon	1.0 kg·ha <sup>-1</sup>	0.05 kg·ha <sup>-1</sup>
Saprol	triforine	3.0 l·ha <sup>-1</sup>	0.57 l·ha <sup>-1</sup>
Ronilan	vinclozolin	2.0 l·ha <sup>-1</sup>	1.00 l·ha <sup>-1</sup>

The selection of growers have not been casual nor can observations of fungicide application in one orchard be regarded as independent of observations from another orchard. Despite these facts a T-test has been accomplished to find if the two groups of growers differ according to protection strategy or fungicide application. In the T-test the variance in the two groups of growers was assumed to be unequal. Significant differences between the two groups must be interpreted carefully.

## RESULTS

### Application of sulphur, tolylfluanid and dodine

In 1992 scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) were the most common diseases in the apple orchards in western Norway, and sulphur, tolylfluanid and dodine were the most frequently applied fungicides. As a rule the fungicide concentration in the spray fluid was far below the maximum permitted dosage per hectare. This gave an average "normal dosage" of fungicides both in integrated and conventional production.

Sulphur was applied in 90% of the integrated grown orchards compared to 97% of the conventionally grown orchards. In integrated production the treated orchards on average were applied 2.4 times with sulphur and the average "normal dosage" was 0.7 (4.2 kg of active ingredients per hectare). In conventional production the average number of spray rounds was 2.6, and the average "normal dosage" 1.0 (6.0 kg active ingredient per hectare). Neither one of the integrated nor one of the conventionally grown orchards was treated more than five times with sulphur during the season.

Tolyfluanid was used in 86% of the integrated grown orchards compared to 97% of the conventionally grown orchards, and for both production methods the average number of spray rounds during the season was 2.1. The "normal dosage" applied in the treated orchards was on average 1.0 and 0.9 respectively for integrated and conventional production. This corresponds to approximately 1.5 kg active ingredient per hectare.

Fewer apple orchards, 71% of the integrated and 87% of the conventionally grown, were treated with dodine. The number of spray rounds was on average 1.8 and 2.0 and the average "normal dosage" was 1.0 and 1.1 respectively for integrated and conventional production (approximately 1.2 kg active ingredient per hectare).

### Fungicide application in orchards of the main apple cultivars

The material indicates that the growers take into account the cultivars' disease resistance when they adopt protection strategy. This holds both for integrated and conventional production. The low scab resistant cultivar 'Gravenstein' has approximately got twice as many "normal dosages" compared to the highly scab resistant cultivar 'Aroma' (Table 2). The number of fungicide applications has also been higher in 'Gravenstein' orchards than in orchards with other cultivars.

In integrated production the average "normal dosage" of fungicide application in orchards of the cultivars 'Prins' and 'Summerred' has been slightly higher compared to conventional production, but the difference is not significant at 5% level. For the cultivars 'Aroma' and 'Gravenstein' the situation is opposite, but no significant difference was found.

In orchards of the cultivars 'Aroma' and 'Prins' the number of fungicide applications has been lower in integrated than in conventional production. For both the cultivars the T-test indicates that the differences between integrated and conventional production are significant (5% level). There are no significant differences between the production methods according to number of fungicide applications in orchards of 'Summerred' and 'Gravenstein'.

On average for all the cultivars the T-test gave no significant difference between the two groups of growers according to the "normal dosage" applied. The average number of

### 36 Fungicides in Norwegian apple production

spray rounds was lower in integrated production compared to conventional production, and this difference was significant at 5% level.

Table 2. Application of fungicides in apple orchards. Recorded by growers from the Hardanger region in Norway in the summer 1992

APPLE CULTIVAR	NUMBER OF ORCHARDS		AVERAGE "NORMAL DOSAGE" PER ORCHARD		NUMBER OF APPLICATIONS PER ORCHARDS	
	IP <sup>1)</sup>	CP <sup>2)</sup>	IP <sup>1)</sup>	CP <sup>2)</sup>	IP <sup>1)</sup>	CP <sup>2)</sup>
'AROMA'	18	5	2.6	3.3	4.4	6.0
'PRINS'	13	7	3.7	3.5	4.5	5.3
'SUMMERRED'	11	6	4.9	4.7	7.6	7.2
'GRAVENSTEIN'	21	9	4.9	6.8	7.7	7.7
ALL CULTIVARS	77	31	3.9	4.7	6.1	6.4

<sup>1)</sup> IP : integrated production <sup>2)</sup> CP : conventional production

### DISCUSSION AND CONCLUSION

None of the growers who participated in the investigation, used a scheduled application programme for fungicides. Comparisons between the collected data and messages from the warning system in the district, indicate that both in integrated and conventional production fungicides were applied when needed. The results showed that growers following an integrated production programme were more aware of the cultivars' disease resistance compared to growers with conventional production.

On average for all the cultivars both the "normal dosage" of fungicide applied in the orchards and the number of applications were lower in integrated production than in conventional production. This indicates that the fungicide application can be reduced by an integrated production programme. The same result was found in an investigation in Germany of pesticides costs in integrated and conventional apple production. (Kaub & Beicht 1988)

The fungicides application found in this investigation was on average 4.1 "normal dosages" compared to 4.5 "normal dosages" found in 1988 for 6 growers in the same region (Edland 1989). This can indicate a reduction in fungicide application in Norwegian fruit production, but no significant answer can be given.

Fungicides application according to requirements will necessarily differ from year to year. In 1992 the weather in May and June in western Norway was dry and hot. Powdery mildew had good conditions. The disease attacks in the apple orchards were more seriously than ordinary and this influenced the fungicide application. The registration of fungicide application will be continued in 1993, and with results from two seasons we can draw more certain conclusion about fungicide application in Norwegian apple production.

REFERENCES

Edland, T. 1989. Kvantifisering av kjemikaliebruken nytta mot soppsjukdommer og skadedyr i frukthagar. (*Quantification of agrochemicals applied against fungus diseases and orchards pests. Plant Protection Conference 1989.*) Informasjonsmøte i plantevern 1989. Aktuelt fra Statens fagtjeneste for landbruket. nr. 3, 1989: 297-305.

Kaub, H. & W. Beicht 1988. Integrierter Pflanzenschutz im Apfelanbau. Obstbau - Weinbau 25 (10): 282.





# Combining stable disease resistance with high fruit quality and good yielding capacity in apple

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Disease-resistant apple varieties constitute an important component of advanced integrated fruit production systems. However, they will only be commercially successful if fruit quality and yielding capacity are similar or superior to well-known commercial varieties. Progeny comparisons and disease assessments in orchards not treated with fungicides help to choose appropriate parental varieties and to develop efficient breeding concepts. The main concerns in current breeding programmes for disease resistance are durability of the resistance, fruit quality and yield capacity. Backcrossing tends to erode the resistance especially in combination with highly susceptible parents. Our experiments have demonstrated that susceptible parents with a high level of polygenic scab resistance increase the percentage of resistant seedlings in the progeny. However, we do not know if they strengthen the resistance. To achieve the requirements in respect of fruit quality both parents, the resistant and the susceptible, should carry the highest possible level of quality parameters such as firmness, juiciness and flavour. Precocious parents with the highest and most regular crops tend to give progenies with the best yielding capacity.

Key words: Apple breeding, disease resistance, fruit quality, yield

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Breeding for disease and pest resistance is one approach to reducing the usage of chemicals in fruit growing. Today, many disease-resistant apple cultivars from breeding programmes all over the world are available. However, in respect to scab resistance, most of them carry the Vf-resistance derived from *Malus floribunda* 821 (Kellerhals 1989). An increasing number of observations question the durability of the Vf resistance (Fischer et al. 1983; Krüger 1988; Krüger 1989; Krüger 1991; van der Scheer 1989; Silbereisen 1989).

The nature of the Vf resistance is still not elucidated. Lamb and Hamilton (1969) and Rousselle et al. (1974) postulated the presence of minor genes which can modify the level of scab resistance conferred to the Vf resistance. Cumulative minor genes could be contributed by both the susceptible and resistant parents.

It can be argued that disease resistance always follows a gene-for-gene system. An alternative view is that there is another type of resistance that shows no gene-for-gene inter-

action and is independent of variation in the pathogen. Vanderplank (1963) proposed that there are two distinct classes of resistance, vertical which is effective only against certain races and therefore interacts in a gene-for-gene system, and horizontal which is equally effective against all races and outside a gene-for-gene system. Resistance that is not vulnerable to a gene-for-gene interaction is interesting for the plant breeder because it might be more durable.

In order to exploit durable resistance in breeding it would be beneficial to understand the genetic basis of this resistance. Modern molecular techniques offer the possibility of genetic linkage of important characters to easily assessable markers.

For the success of new apple varieties not only resistance but also fruit quality and the yielding capacity are important features. Yield is the ultimate polygenic trait and involves most genes. Although assessment of the yielding capacity of new selections requires much time it is possible to evaluate this character in the very first years of cropping.

What is quality? Eating quality is difficult to define, being a combination of characters. These include:

- balanced sugar/acid ratio
- crisp texture
- juiciness
- medium and regular fruit size
- resistance to physiological disorders
- good skin finish without russet
- color
- good storage and shelf life.

Fruit criteria which are of importance to the consumer comprise properties related to sensory, nutritional and technological quality as well as wholesomeness and appearance (Höhn 1990). In a Swiss survey, consumers indicated that the ideal apple should be juicy, crisp and flavourful. In our progeny analysis we have focused on the criteria firmness, juiciness and flavour.

## MATERIALS AND METHODS

Apple progenies incorporating disease resistance were screened, at the 4-leaf stage on average, in the glasshouse for scab resistance. The seeds were sown in plastic seed trays with 104 pressblocks of medium, each containing one apple seed. The young seedlings were inoculated in the glasshouse with a suspension of  $4.5 \times 10^6$  conidia  $\text{ml}^{-1}$  of *Venturia inaequalis* by using a hand sprayer. The inoculum consisted of *Venturia* strain 71, originally collected from Boscoop-trees without fungicide treatment, and then multiplied on apple seedlings of different origin in the glasshouse. The conidial suspension was sprayed onto seedlings in sufficient quantity to form small droplets without dripping. The seedlings were incubated under plastic at 20°C and high r.h. for 60 hours. Afterwards they were kept at the same temperature but 70-80% r.h.. The recording of the symptoms was made 13 days after the inoculation, using the following reaction classes (adapted from Chevalier et al. 1991):

- 0 = No visible reaction
- 1 = Pin-point symptom. Depressions of 100-500  $\mu\text{m}$  where the epidermal cells have collapsed. No subcuticular stroma.
- 2 = Wide but shallow depressions. Limited stroma formation. No sporulation.
- 3a = Epidermal cells collapsed over large areas. Close to the centre the abundant mycelial stromata can produce conidiophores with a limited number of conidia.
- 3b = Lesions are a network of mycelial strands. Aborted conidiophores are mixed with normal conidiophores. Sporulating chlorosis and sporulating necrosis occur.
- 4 = Numerous conidiophores are often grouped in clusters and sporulate abundantly. The mycelial stroma forms a dense subcuticular network.

Seedlings assigned to reaction class 4 were considered susceptible and therefore discarded; the other seedlings were labelled with their reaction class and planted to the field. In the summer of the following year the seedlings were assessed for mildew susceptibility, juvenility, growth habit and possible escapes of the glasshouse screening for scab resistance. Selected seedlings were budded on the dwarfing rootstock M27. The 1-year-old trees were planted at a distance of 4 x 0.6 m in an orchard where no fungicides are applied and grown as spindle trees with a minimum of pruning and fruit thinning. The number of trees per cross varied from 18 to 52. The tree and fruit characters were observed regularly on these trees. Most scores for the fruit and tree characters follow a 1 to 9 scale, with 1 as the poorest and 9 as the best score. The whole crop was harvested and a sample put in cold store at 4°C and 92% r.h. Eating quality was assessed according to the picking time once between October and December. Promising selections were kept till February/March and assessed a second time. This second assessment is not considered in the results below.

The incidence of scab and mildew on potential parental varieties was assessed by counting the number of leaves attacked by the respective disease in relation to the total number of leaves per tree. Especially for mildew, the assessment was repeated several times during the season because of differences due to primary and secondary infection.

## RESULTS

### Disease resistance

The glasshouse screening for scab resistance gives indications about the erosion or dilution of the resistance during successive backcrossing. The progeny of Golden Delicious crossed with *Malus floribunda* 821, Florina and Nova Easygro are compared (Fig. 1). Florina carries the Vf resistance derived from *Malus floribunda* 821. Florina is a variety which was selected after 5 backcross generations. Nova Easygro carries the Vr resistance derived from a Russian seedling (*M. pumila* R 127 40-7A). It is seen that the combination Golden x *Malus floribunda* 821 gave a much higher proportion of resistant seedlings than Golden x Florina. Golden x Nova Easygro has an intermediate position showing that the Vr resistance is still quite efficient. It is also demonstrated that the choice of the susceptible parent in combination with the Vf-resistant cv. Florina has an impact on the resistance in the progeny (Fig. 2): varieties which are known for a low susceptibility to scab, such as Discovery, give a higher proportion of resistant progeny than very susceptible cultivars such as Golden Delicious.

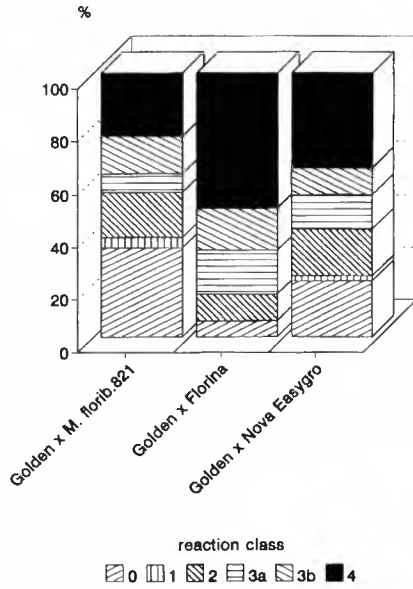


Fig. 1. Erosion of the Vf-resistance compared to Vr resistance from Nova Easygro (Scab screening 1993, Wädenswil)

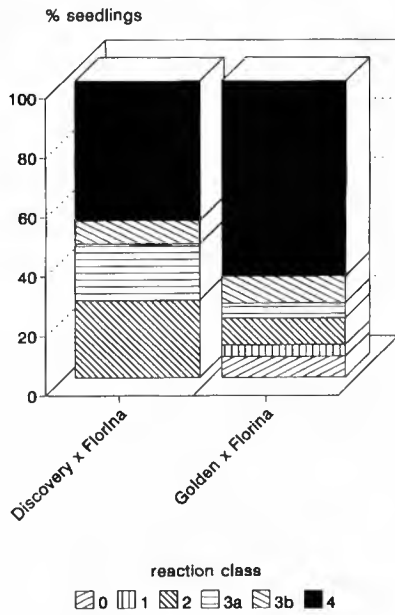


Fig. 2. Effect of the susceptible parent on the scab resistance of the progeny (Glasshouse screening 1992, Angers, France)

The early selection of progenies in our programme for breeding disease resistant varieties consists of two steps: glasshouse screening for scab resistance and the preselection for mildew resistance, juvenility and growth characters in the nursery. Both steps reduce the original seedling population to about 10% on average (Fig. 3). The susceptibility to scab and mildew are the main reasons for a low percentage of preselected seedlings. Parents carrying scab and mildew resistance, such as A 849-7, A 849-8, A 849-6 and A-849-5, induced a higher percentage of preselected seedlings than most other combinations. Green-sleeves x Liberty and Elstar x Jonafree gave a poor output due to a high incidence of scab and mildew in the progeny. It is therefore essential to screen parental varieties for their susceptibility to scab and mildew (Fig. 4, 5). Striking differences between the varieties can be found. The french variety 'Delbard Jubile', a cross between Golden Delicious and the danish variety Lundbytrop showed, for example, a very low scab and mildew susceptibility. The varietal differences may vary to some extent from region to region.

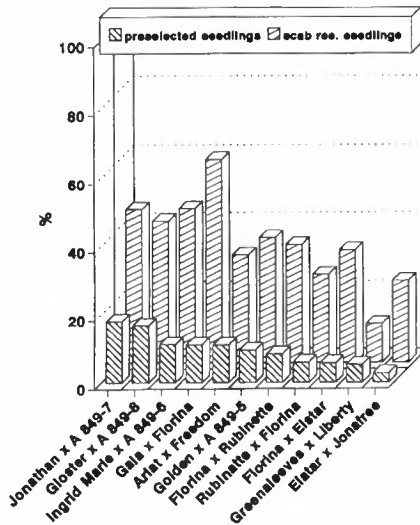


Fig. 3. Early selection of disease-resistant apple progenies 1986 (crosses from Wädenswil and HRI East Malling, England)

### Precocity and yielding capacity

Besides disease and pest resistance the breeder's attention is focused on cropping performance and fruit quality. One aspect which becomes increasingly important in modern high-density orchards is precocity (Table 1). The preselected progenies are assessed for their precocity as trees budded on the dwarfing rootstock M27. In the first year the percentage of fruiting trees is generally very low. In the second year it increases up to 50% in precocious combinations such as Greensleeves x Liberty and RubINETTE x Florina. It is surprising that the reciprocal cross Florina x RubINETTE was the least precocious. However, all the progenies shown in Fig. 5 are acceptably precocious with 70-100% of the trees fruiting in the third leaf. More striking differences show up when considering the yielding capacity (Table 1): Golden Delicious, A 849-5, Elstar and Jonafree seem to be satisfactory parents. Florina and Freedom seem to be less satisfactory.

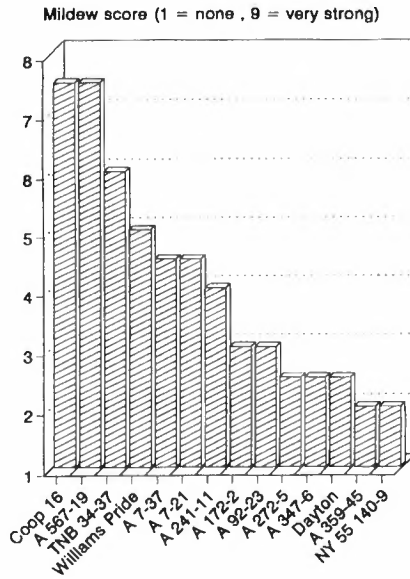


Fig. 4. Mildew susceptibility of Vf resistant varieties and selections (Wädenswil, 1989-1992)

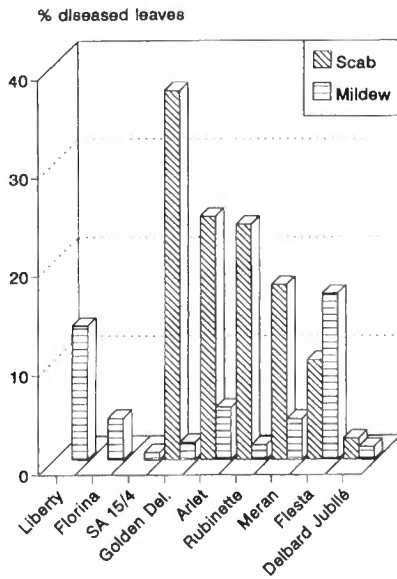


Fig. 5. Incidence of scab and mildew in an orchard without fungicide application at Grabs, Switzerland (1989-1992)

Table 1. Progeny analysis for precocity, yielding capacity and fruit quality

Progeny	Precocity		Yield (g/tree in the 3rd year)	Firmness (1 = soft, 9 = firm)	Juiciness (1 = dry, 9 = juicy)	Flavor (1 = bad, 9 = good)
	% fruiting 2nd year	% fruiting 3rd year				
Arlet x Freedom	21	83	1246	6.2	5.6	5.8
Greensleeves x Liberty	50	86	2245	6.0	5.2	5.1
Gala x Florina	19	80	1713	6.7	5.7	5.4
Elstar x Jonafree	11	79	2528	8.0	6.3	6.4
RubINETTE x Florina	44	92	1947	6.4	5.4	5.3
Florina x RubINETTE	14	72	1632	6.2	5.4	5.2
Florina x Elstar	20	85	1900	6.5	5.4	5.1
Golden x A 849-5	38	100	2872	6.7	5.4	5.2
Gloster x A 849-8	20	87	2018	6.8	5.3	5.5
Ingrid Marie x A 849-6	13	91	1639	5.3	5.2	5.5
Jonathan x A 849-7	18	78	1900	7.6	5.6	5.4

### Fruit quality

Internal fruit quality is a very complex notion but it can be identified in terms of firmness, juiciness and flavour. The progenies were analysed for these characters with sensory scores from 1 to 9. With fruit firmness the differences between the progenies are not very striking (Table 1). Good parents for fruit firmness are Gala and Jonafree. The combinations with Greensleeves and Ingrid Marie gave the lowest scores. Table 1 also shows that fruit firmness and juiciness are often interrelated. Good parents for fruit firmness are also good parents for juiciness. The same association is true for bad parents.

Parents with a good flavour, such as Elstar, the A 849 series, Ingrid Marie and Arlet, had a positive effect on flavour. The influence of Florina and Liberty on flavour was less evident, even in combination with highly flavoured varieties such as RubINETTE and Elstar.

## DISCUSSION

### Disease resistance

To achieve durable disease resistance one must consider the host-pathogen interaction. Johnson (1978) argued that the strongest test for durable resistance occurs when a cultivar is widely grown in an environment favourable to the disease. In Switzerland and especially at Wädenswil, the environment for the development of scab and mildew is in most years favourable. We are therefore able to test the resistance of parents and progenies under these favourable conditions. This allows efficient preselection. However, this analysis should be accompanied by the investigation of the host-pathogen interactions and their genetic basis. Does the erosion of scab resistance through repeated backcrossing, which is shown in these results, only mean that the efficiency of our breeding work is poor, or is there really lost genetic strength? To answer this question it would probably be necessary to carry out test crosses with the same tester and/or elucidate the genetic structure of resistance by using modern molecular techniques. These techniques would also permit more precise investigations into the variation and population genetics of pathogens and the epidemiology of

disease. Collaborative work with the Federal Institute of Technology (ETH) at Zürich is being done in these areas. Pathogen variation is also an important problem for plant breeders. How quickly will changes occur leading to failure of resistance? Work with perennial crops such as fruit trees requires an accurate evaluation of potential risks. The Vf resistance is supposed to be of complex nature (Chevalier et al. 1992; Gessler 1989) and coded by a group of more or less closely linked genes. There is a continuous distribution of reaction types in the progeny. The question arises whether it can be feasible to reinforce the Vf resistance with the partial resistance found in several old and modern varieties, as suggested by Chevalier et al. 1992. Additionally, it is necessary to develop adequate strategies to establish orchard ecosystems favorable for the durability of resistance. These strategies could include an increase of genetic diversity in the host-pathogen interaction through using mixtures of varieties, and other approaches.

### **Yielding capacity**

Precocity, productivity and regularity of yields are the main yield components. In actual breeding programmes first priority is no longer given to these characters. But it would be a mistake to neglect them while breeding for disease resistance and quality. Alston and Bates (1979) found that the yield of young apple seedlings grown on the rootstock M27 was correlated with their subsequent performance as orchard trees. Moreover, they suggest preselection for high yield in the first two years after sowing, by considering plant habit.

### **Fruit quality**

Quality is a complex of many different components and in this paper focusses on firmness, juiciness and flavour. There are many indications that fruit firmness will be a crucial requirement in the future for the grower, the market and the consumer. Successful new varieties such as Gala, Braeburn and Fuji all have very good texture, keeping properties and shelf life. For disease resistant cultivars these characteristics are also extremely relevant. Fruit flavor, one of the most important criteria in the selection of apple seedlings, is a complex combination of acids, sugars, tannins and aromatic substances (Brown 1975).

Redalen (1988) noted that a method of sensory evaluation, using a team of trained judges, gives flavor scores resulting in a reliable evaluation of the eating quality. This method was used for the first evaluation of selections. In later steps precise measurements of firmness, sugar, acidity, etc. are added. A relatively close correlation between firmness and juiciness was found. This is comprehensible because most apples when softening become mealy, which also means not juicy. However, there are also examples where apples are very firm but tough and not juicy, as well as apples which are soft but also juicy. In the cross combinations Jonathan x A 849-7 and Golden Delicious x A 849-5 there was much fruit with internal breakdown. This may have been aggravated by inadequate storage conditions. However, a predisposition to this disorder seems to be present.

### **SUMMARY**

Progeny analysis has shown that Vf scab resistance is gradually eroded through back-crossing. However, apple varieties with differing levels of polygenic scab resistance



combined with the Vf-resistant cultivar Florina (as the tester) have an impact on the resistance of the progenies. Vf resistance could be reinforced by the partial resistance found in several old and modern varieties. Preselection and monitoring of diseases on parental varieties in an environment favourable for the respective diseases, are valuable. To increase the efficiency of the breeding programmes it is essential to screen the parents and the progenies for yield components such as precocity, productivity and regularity of yields. Internal fruit quality is also influenced by the parents. Good parents for firmness are Gala and Jonafree. Good parents for firmness are also good parents for juiciness. Parents with a good flavour, such as Elstar, Ingrid Marie and Arlet, have a positive effect on flavour in the progenies. The effect of Florina and Liberty on flavour was less acceptable, even in combination with highly flavoured varieties such as Elstar and RubINETTE.

## REFERENCES

- Alston, F.H. & J.W. Bates 1979. Selection for yield in apple progenies. Proceedings Eucarpia Fruit Section Symposium, Tree Fruit Breeding, Angers, 1979: 15-27.
- Brown, A.G. 1975. Advances in Fruit Breeding. Apples. J. Janick and J.N. Moore, (Eds.), Purdue University Press, West Lafayette, Indiana.
- Chevalier, M., Y. Lespinasse & S. Renaudin 1991. A microscopic study of the different classes of symptoms coded by Vf gene in apple for resistance to scab (*Venturia inaequalis*). Plant Pathol. 40: 249-256.
- Chevalier, M., L. Parisi & Y. Lespinasse 1992. Sensibilité et résistance à la tavelure. Les interactions hôte-agent pathogène. L'Arboriculture Fruitière 451: 17-20.
- Fischer, C., V.F. Bukartschuk, A.A. Bondarenko & E.S. Artamonova. Erste Ergebnisse zur Stabilität der Schorfresistenz beim Apfel unter verschiedenen ökologischen Bedingungen in der UdSSR und DDR. Arch. Gartenbau 31: 263-264.
- Gessler, C. 1989. Genetics of the interaction *Venturia inaequalis*-*Malus*: the conflict between theory and reality. OILB Working Group "Integrated Control of Pome Fruit Diseases" Vol. II, WPRS Bulletin XII/6: 168-190.
- Höhn, E. 1990. Quality criteris of apples. Acta Horticulturae 285: 111-118.
- Johnson, R. 1988. Durable resistance to yellow (stripe) rust in wheat and its implications in plant breeding. In: N.W. Simmonds & S. Rajaram (Eds.) "Breeding Strategies for Resistance to the Rust of Wheat". CIMMYT, Mexico D.F.: 63-75.
- Johnson, R. 1992. Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63: 3-22.

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Kellerhals, M. 1989. Breeding of pome fruits with stable resistance to diseases. 1. Development of disease resistant pome fruit varieties. OILB Working Group "Integrated Control of Pome Fruit Diseases" Vol. II, WPRS Bulletin XII/6: 116-129.

Krüger, J. 1988. Beständigkeit der Schorffresistenz aus *Malus floribunda* 821 auf dem Versuchsfeld der Bundesforschungsanstalt für gartenbauliche Pflanzenzüchtung in Ahrensburg. Erwerbsobstbau 30: 52.

Krüger, J. 1989. Scab resistance of apple cultivars, selections and progenies with the Vf gene. OILB Working Group "Integrated Control of Pome Fruit Diseases" Vol. II, WPRS Bulletin XII/6: 161-167.

Krüger, J. 1991. Schorfbefall von Nachkommen aus Kreuzungen mit der schorffresistenten Apfelsorte 'Prima'. J. Plant Dis. Protect. 98: 73-76.

Lamb, R.C. & J.M. Hamilton 1969. Environmental and genetic factors influencing the expression of resistance to scab (*Venturia inaequalis* Ckt. Wint.) in apple progenies. J. Amer. Soc. hortic. Sci. 94: 554-557.

Redalen, G. 1988. Quality assessment of apple cultivars and selections. Acta horticulturae 224: 441-447.

Rouselle, G.L., E.B. Williams & L.F. Hough 1974. Modification of the level of resistance to apple scab from the Vf gene. XIX Intern. Hort. Congr. Warszawa: 19-26.

Silbereisen, R. 1989. Experiences at Bavendorf (FRG) with scab-resistant apple cultivars. OILB Working Group "Integrated Control of Pome Fruit Diseases" Vol. II, WPRS Bulletin XII/6: 148-160.

Vanderplank, J.E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York.

Van der Scheer, H.A.Th. 1989. Susceptibility of apple cultivars and selections to scab and powdery mildew in the Netherlands. OILB Working Group "Integrated Control of Pome Fruit Diseases" Vol. II, WPRS Bulletin XII/6: 205-211.

# Apple scab (*Venturia inaequalis*) - patterns of ascospore release in Norway

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Volumetric sporetraps have been stationed in five orchards in different apple growing regions of Norway during the primary inoculum seasons of 1989-92. Weather data were provided by electronic scab warning devices and weather stations in the orchards. The mean number of cumulative degree days for 95% spore release was 735. Periods of warm and dry weather at two locations in 1992 extended the primary inoculum season. Spore release was suppressed during darkness. At one location in 1990 and another in 1992, 14.8 and 26.9%, respectively, of the season's total ascospore release occurred during periods of dew. Data on global radiation and light intensity at 720 nm wavelength are given.

Key words: Apple scab, dew, diurnal pattern, light, spore trapping, *Venturia inaequalis*.

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Apple scab is caused by the fungal pathogen *Venturia inaequalis* (Cooke) Winter. Ascospores are formed within pseudothecia in infected apple leaves overwintering on the ground. In spring and early summer ascospores are released during periods of rain, and are the major cause of primary infections on apple tissue.

To develop a reliable warning system for apple scab, it is necessary to understand how climatic conditions affect ascospore release. Much information is already available on the epidemiology of *V. inaequalis* throughout the world. Our objective in this study was to investigate the effect of climatic conditions in Norway on the pattern of ascospore release by the apple scab fungus. A preliminary report is given below.

## MATERIALS AND METHODS

### Spore trapping

Burkard 7-day recording volumetric spore traps (Burkard Manufacturing Co Ltd., UK) were installed in five orchards in different apple growing regions of Norway during the primary inoculum seasons of 1989-92. The investigations are continued in three of the

locations in 1993.

Two to three m<sup>2</sup> of the orchard floor under the traps were covered with apple leaves heavily infected with *V. inaequalis*. The leaves were collected in the fall and kept in place during winter under a plastic screen at the trap site, exposed to rain and snow. The number of ascospores of *V. inaequalis* recorded was corrected for the proportion of the tape examined and the volume of air sampled.

#### **Weather data**

Weather data were obtained from the electronic scab warning device (KMS-P, Anton Paar KG, Austria) or the weather station (Instrumentjenesten A/S, Norway) in the orchards. The data provided hourly records of precipitation, temperature, relative humidity, and leaf wetness. Degree days were accumulated from the beginning of the green tip phenological stage, with a base temperature of 0°C. To calculate the number of infection periods at different temperatures, additional weather data were supplied from KMS-P devices in four other fruit growing areas in Norway. Predicted infection periods were based on "Mills' table" for light infection (Mills & Laplante 1951).

#### **Light measurements**

Global radiation was measured with an "Eppley" pyranometer (Eppley Laboratory Inc., Rhode Island, USA) at a meteorological station situated at Ås in south east Norway. The measurements are given in watts per m<sup>2</sup> (W/m<sup>2</sup>). Spectral distribution was measured in Ås by means of an "LI-1800" portable spectroradiometer (LI-COR inc., Nebraska, USA). The light intensity was recorded as microwatts per cm<sup>2</sup> (μW/cm<sup>2</sup>). A narrow wavelength band of 710-730 nm has the most stimulating effect on spore release (Brook, 1969b). Below, we only discuss the light intensity at 720 nanometer (nm). Data for sunrise and sunset were provided by the Institute of Theoretical Astrophysics at the University of Oslo. The times (hours) given below are in standard time.

## **RESULTS**

#### **Seasonal pattern**

The total number of ascospores per m<sup>3</sup> of air trapped at each location and year varied greatly. The cumulative hourly observations were between 2 970 and 40 633, with a mean of 15 184. The first spore release always occurred between the green tip and tight cluster stages. At one location in 1992, the major peaks in spore release occurred prior to and at the tight cluster stage. At the other locations and years the maximum in spore release occurred between the tight cluster stage and within one or two weeks after petal fall. The mean numbers of cumulative degree days for 50 and 95% spore release from these observations were 374 and 735.

In 1992, 95% spore release was reached at 1305 and 1092 degree days at two locations in eastern Norway. The primary inoculum season of 1992 in eastern Norway was characterized by two long periods in May and June of dry, warm weather, with no rainfall.

### **Diurnal pattern**

On seven occasions when rain started during night and leaves remained wet for more than 24 hours, 12.3, 4.2, and 2.2% of the spores were trapped between 18:00 and 07:00 hours, 20:00 and 07:00 hours, and 22:00 and 07:00 hours, respectively. All these episodes occurred in April and early May, in the early part of the primary inoculum season. On an average, the spore release had the most significant increase between 09:00 and 12:00 hours, it peaked between 12:00 and 13:00 hours, and decreased rapidly between 20:00 and 21:00 hours.

### **Light conditions**

On April 1, sunrise in the fruit growing regions in Norway is at 05:55 hours  $\pm$  10 minutes. Similarly, on May 1, sunrise is at 04:30 hours  $\pm$  15 minutes, and on June 1, sunrise is at 03:20 hours  $\pm$  20 minutes.

We measured the spectral distribution at the meteorological station at Ås May 7 and 8 1993. May 7 had continuous rainfall and complete cloud cover during the night and morning. May 8 was bright, with no cloud cover during the night and morning. At 720 nm, the spectroradiometer detected the first light May 7 at 04:00 hours, May 8 at 03:30 hours. On May 7, the light intensity varied between 0.63 and 3.8  $\mu\text{W}/\text{cm}^2$  from 05:00 to 12:00 hours. On May 8, the light intensity at 720 nm increased rapidly after sunrise, and peaked 12:00 hours with 76.8  $\mu\text{W}/\text{cm}^2$ . The global radiation varied between 6 and 56  $\text{W}/\text{m}^2$  from 05:00 to 12:00 hours on May 7. On May 8, the global radiation peaked 12:00 hours with 743  $\text{W}/\text{m}^2$ .

### **Temperature**

Of a total of 186 infection periods recorded by the electronic scab warmer in nine different locations in 1989-92, 5, 10, 56, and 115 periods had an average temperature of 2-4°C, 4-6°C, 6-10°C, and above 10°C, respectively. There were no leaf wetness periods long enough to get infection at an average temperature below 2°C. We frequently observed spore release at these temperatures, however, at very low rates.

### **Dew**

We have observed 17 days with dew resulting in spore release. 14.8 and 26.9% of the total number of spores were trapped during dewfall in 1990 at one location and 1992 at another location, respectively. Most of the spores were trapped between 01:00 and 06:00 hours. High spore release during dewfall occurred during the expected peak of the primary inoculum season, after one week or more with warm, sunny weather and no rain.

## **DISCUSSION**

The seasonal patterns of spore release in our investigations are in accordance with other studies on *V. inaequalis* throughout the world. Depending on frequency of rainfall, the first ascospores are released between bud break and the tight cluster stage, and the peak in release is usually found from tight cluster to just after petal fall (Childs 1917, Frey & Keitt 1925, Wiesman 1935, Weber 1934-35, Hirst & Stedman 1962, Szkolnik 1969, Brook 1976,

Gadoury & MacHardy 1982).

One location in 1992 had the major peaks in spore release just prior to and at tight cluster. This area is in a coastal region, and had a relatively mild and rainy winter in 1992. According to James & Sutton (1982) such climatic conditions will stimulate the spore maturation in the spring. This might explain the early peak in spore release.

Periods of dry weather can delay spore maturation in *V. inaequalis* (Keitt & Jones 1926, Wilson 1928, James & Sutton 1982, Schwabe et al. 1989). Prolonged periods with dry, relatively warm weather is common during May and June in Norway, especially in the eastern parts of the country. In 1992, such conditions seemed to extend the primary inoculum season.

Many authors have described suppressed spore release during darkness in *V. inaequalis* (Frey & Keitt 1925, Keitt & Jones 1926, Baumeister 1954, Hirst et al. 1955, Hirst & Stedman 1962, Brook 1966, 1969a, 1969b, Pinto de Torre et al. 1984, MacHardy & Gadoury 1986). However, a diurnal pattern of ascospore release has never been reported in the Nordic countries. The apple growing regions of Norway, Sweden, Finland and Denmark are on the northern limit of commercial fruit production. Apples are grown commercially in Norway from approximately 58 to 62° northern latitude. Because of extended daylight periods, the light conditions during spring and summer are different from those further south. In spite of the short periods of darkness, there is a clear suppression of spore release during night. The period of night time suppression is, however, shorter than described in New Hampshire, USA, by MacHardy & Gadoury (1986). They found that 5% or less of the spores are released between 18:00 and 07:00 hours if rain starts during night and continues throughout the following day.

Palm (1988) indicates that few ascospores of the apple scab fungus are released at light levels below 2 000 lux. In natural daylight 2 000 lux equals approximately 8 W/m<sup>2</sup> global radiation (L. Mortensen, pers. comm.). On a rainy day in early May in Norway, a global radiation of 8 W/m<sup>2</sup> was reached between 05:00 and 05:30 hours. Gadoury et al. (1993) in New York, USA, recently found the light level we recorded at 05:00 hours on a rainy day sufficient to stimulate spore release in the laboratory. This light level is reached 4-5 hours before high spore numbers are recorded in the morning.

On rainy days, there were only small differences between night and day time temperatures. Therefore, lower temperatures during night can hardly be an additional explanation of the diurnal pattern in spore release.

The rate of spore release is reduced at low temperatures, but the effect is not significant until the temperature approach freezing (Hirst & Stedman 1962, Gadoury et al. 1993). We observed spore release at low rates at 0-2°C, but we never had periods of leaf wetness long enough to get infection according to "Mills' table".

High spore release during dewfall has previously never been reported. Brook (1969a) suggests that few ascospores are released during dew because both low temperatures and darkness will suppress spore release when dew forms. Moore (1958) found many ascospores in dew droplets from apple leaves. Because of the thick water film developing during dew, he suggests that the spores are more likely to be submerged in the water than to become airborne.

The higher light intensities during clear mornings with dew compared to rainy mornings can not explain the observed spore release, since considerable spore releases

occurred several hours before any detectable light was measured. The minimum temperature in the night during dew in our observations varied between 5.7 and 11.3°C. Those temperatures are not low enough to suppress spore release completely, since we have observed high spore release in this temperature range during rain.

High spore release during dew was only observed after several days with warm, sunny weather in May. High accumulation of mature spores in the pseudothecia during these periods may interfere with the ordinary diurnal periodicity of spore release.

## LITERATURE

Baumeister, G. 1954. Weitere Untersuchungen zur Biologie des Apfelschorfes. Mitteilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft (Berlin-Dahlem) 80:98-102.

Brook, P.J. 1966. The ascospore production season of *Venturia inaequalis* (Cke.). Wint., the apple black spot fungus. New Zealand Journal of Agricultural Research 9: 1064-1069.

Brook, P.J. 1969a. Effects of light, temperature, and moisture on release of ascospores by *Venturia inaequalis* (Cke.) Wint. New Zealand Journal of Agricultural Research 12: 214-227.

Brook, P.J. 1969b. Stimulation of ascospore release in *Venturia inaequalis* by far red light. Nature 222: 390-392.

Brook, P.J. 1976. Seasonal pattern of maturation of *Venturia inaequalis* ascospores in New Zealand. New Zealand Journal of Agricultural Research 19:103-109.

Childs, L. 1917. New facts regarding the period of ascospore discharge of the apple scab fungus. Oregon Agricultural College Experiment Station Bulletin No. 143, 11 pp.

Frey, C.N. & G.W. Keitt 1925. Studies of spore dissemination of *Venturia inaequalis* (Cke.) Wint. in relation to seasonal development of apple scab. Journal of Agricultural Research 15: 529-540.

Gadoury, D.M. & W.E. MacHardy 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. Phytopathology 72: 901-904.

Gadoury, D.M., R.C. Seem & A. Stensvand 1994. Ascospore release in *Venturia inaequalis*. 3rd Workshop on Integrated Control of Pome Fruit Diseases. 31 May - 4 June 1993, Lofthus, Norway. Norwegian Journal of Agricultural Science, Supplement No. 17: 205-219.

Hirst, J.M., I.F. Storey, W.C. Ward & H.J. Wilcox 1955. The origin of apple scab epidemics in the Wisbech area in 1953 and 1954. Plant Pathology 4: 91-96.

- Hirst, J.M. & O.J. Stedman 1962. The epidemiology of apple scab (*Venturia inaequalis* (Cke.) Wint.) II Observations on the liberation of ascospores. *Annals of Applied Biology* 50: 525-550.
- James, J.R. & T.B. Sutton 1982. Environmental factors influencing pseudothecial development and ascospore maturation of *Venturia inaequalis*. *Phytopathology* 72: 1073-1080.
- Keitt, G.W. & L.K. Jones 1926. Studies of the epidemiology and control of apple scab. Wisconsin Agricultural Experiment Station Research Bulletin No. 73, 104 pp.
- MacHardy, W.E. & D.M. Gadoury 1986. Patterns of ascospore discharge by *Venturia inaequalis*. *Phytopathology* 76: 985-990.
- Mills, W.D. & A.A. Laplante 1951. Diseases and insects in the orchard. Cornell Extension Bulletin 711: 21-27.
- Moore, M.H. 1958. The release of ascospores of apple scab by dew. *Plant Pathology* 7: 4-5.
- Palm, G. 1988. Untersuchungen über den Einfluß der Belichtungsstärke für den Askosporenausstoß des Schorfpilzes (*Venturia inaequalis*, *Venturia pirina*). *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 245: 420.
- Pinto de Torre, A., I.I. Carreño & W. Moller 1984. Control químico de *Venturia* en manzanos. Aplicaciones a calendario fijo o cuando el tiempo favorece la infección. Niveles de inóculo primario. *Agricultura Técnica (Chile)* 44: 123-130.
- Schwabe, W.F.S., A.L. Jones & E. van Blerk 1989. Relation of degree-day accumulations to maturation of ascospores of *Venturia inaequalis* in South Africa. *Phytophylactica* 21: 13-16.
- Szkolnik, M. 1969. Maturation and discharge of ascospores of *Venturia inaequalis*. *Plant Disease Reporter* 53:534-537.
- Weber, A. 1934-35. Undersøgelser over æbleskurvens (*Venturia inaequalis*) overvintring. *Tidsskrift for Planteavl* 40: 754-758.
- Wiesman, R. 1935. Untersuchungen über die Bedeutung der Ascosporen (Wintersporen) und der Konidien an den schorfigen Trieben für die entstehung der Primärinfektionen des Apfelschorfpilzes *Fusicladium dendriticum*. Separatabdruck aus dem Landwirtschaftlichen Jahrbuch der Schweiz 1935, 28 pp.
- Wilson, E.E. 1928. Studies of the development of the ascigerous stage of *Venturia inaequalis*. *Phytopathology* 18: 375-420.



# Development and evaluation of a simulation model for ascospore infections of *Venturia inaequalis*

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The main reason to develop a simulation model of *Venturia inaequalis* is to get a better view of the relative importance of infection periods during spring. On basis of a literature study and discussions with experts, a dynamic simulation program was written that describes the primary epidemiology of *Venturia inaequalis*. The program in its present form was evaluated in three ways:

1. The simulated ascospore discharge was compared with the spore flight recorded in spore traps for locations in Italy, Switzerland, Germany and the Netherlands.
2. The simulated development of lesions was compared to the development of lesions in unsprayed orchard blocks in 1992 and 1993 in the Netherlands.
3. The simulated number of spores entering the host plant during an infection period was used as a Relative Infection Measure (RIM) to guide spraying in replicated plot trials in The Netherlands in 1992.

The program simulated most of the discharge periods relatively well. Some of the differences between simulated and observed ascospore discharge cannot yet be explained. The differences might be due to artifacts introduced by the recording technique. Simulated development of lesions matched quite well the occurrence of scabbed leaves in unsprayed orchard blocks. Treating only 3 infection periods with high RIM values had 96% control of primary infections compared to 98% control when 9 curative sprays followed every Mills Period.

Key words: Apple, apple scab, epidemiology, forecasting, orchard diseases, prediction,

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In The Netherlands from 6 to over 15 Mills Periods occur during the primary infection season. In practice, most primary scab symptoms seem to originate from one to three infection periods. Several authors (Cesari, Soenen 1957, 1960, Waldner: this workshop) report that not all Mills periods lead to visible lesions. Even severe Mills Periods do not always lead to scab symptoms. On the other hand, under certain conditions scab symptoms may occur without fulfilled Mills criteria. This has led to several revisions of the Mills' table. To allow for some extreme situations, these adaptations can lead to even more infection periods during the primary scab season. This cannot be overcome because Mills'

scab infection table is a static model that only describes a very limited part of the epidemiology of *Venturia inaequalis*.

The absolute importance of an infection period is determined by the number of spores that enter the hostplant during a leafwetness period. Excluding the influence of fungicides and the susceptibility of the host, the risk coming from an infection period can be described as:

**Ascospore potential \* discharged fraction \* penetrated fraction**

As the ascospore potential is different for every orchard, it is not possible to incorporate this first factor in a regional warning system. Every measure of infection risk is relative to the ascospore potential of the individual orchard.

While the discharged fraction of the spore potential during rain periods in spring can vary by more than a factor of 100, the dissemination of ascospores can easily overrule Mills' criteria as the key factor determining the scab risk associated with a wet period. It is not possible to have a spore trap in every orchard, we try to develop a simulation model to include ascospore discharge.

**GENERAL STRUCTURE OF THE MODEL**

The model was constructed as a dynamic simulation model using the methods of De Wit and Goudriaan (1974) and Rabbinge (1989). The program was written in Turbo Prolog, using fractional boxcar trains to simulate dispersion. The program structure was obtained by studying literature and from discussions with specialists. Several assumptions were made to fill gaps in knowledge. The flow chart of the program is given in figure 1.

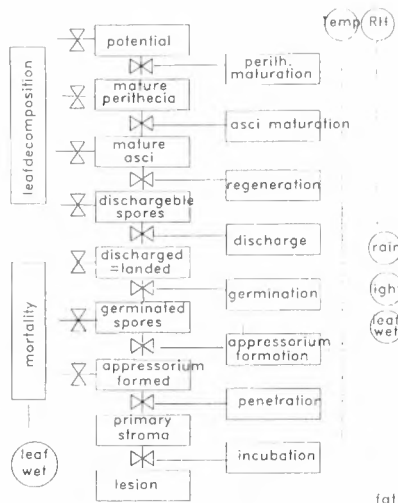


Fig. 1. Flow diagram of the apple scab simulation model for ascospore production and infection

### **Perithecial development**

Temperature and moisture are the main driving factors for the development of perithecia, asci and spores. Several authors have published temperature relations for perithecial development. Gadoury (1982) and MacHardy (1985) published temperature relations describing the cumulative ascospore discharge starting from the moment first mature spores are found in perithecia, for situations in which moisture is not a limiting factor. Their model describes quite well the cumulative catch of ascospore in the field in regions where there is regular rainfall during spring. Prolonged dry periods retard perithecial development and stretch the ascospore flight over the temperature sums given by Gadoury and MacHardy (Schwabe 1989). Cumulative numbers of ascospores, however, are the result of two processes: the process of the perithecia reaching the point of first mature asci, and the maturation of the rest of the spores in the perithecium. To be more accurate in the short term the total process of perithecial development must at least be divided into two stages. The need for this can be seen in several publications that show parallel curves for cumulative number of mature asci or perithecia and cumulative number of empty asci or perithecia.

### **Perithecia**

The model uses first mature spores as a biofix. Temperature and RH are the main driving variables for the development of perithecia in the model. No development is simulated when the temperature is below zero°C, or relative humidity drops below 40%. At temperatures over 19°C development rate is constant.

### **Ascospores**

In the final stage of maturation of the spores in the perithecia moisture seems to be an important factor. Experiments show that leaves under dry circumstances strongly suppresses spore maturation; on the other hand under wet conditions spores can mature very rapidly. In the model, binding spore maturation to leaf wetness and high RH greatly improved the performance compared with temperature as the only driving factor. 'RH > 85% or leafwet' gave the most spores.

### **Dischargeable fraction**

Observations published by different authors make clear that during a rain period not all mature ascospore are released. The dischargeable fraction is therefore an input that holds the relation between total number of mature spores and dischargeable spores. This variable is normally set to 70% of the mature spores.

### **Regeneration**

In the pattern of ascospore discharge it is often seen that after a strong discharge there is a short period in which relatively few spores are discharged. The reason for this is not clear. Stephan (1985) gives some quantitative information on this. This 'regeneration' is implemented as the time it takes to balance the relation between total mature spores and dischargeable spores after a discharge period. Following the data provided by Stephan, in the model regeneration is only driven by time, not by temperature or moisture.

Playing around with this variable did not have an important effect on the results.

### **Discharge**

Discharge rate is driven by temperature, light, a rain threshold and leaf wetness.

For temperature, the highest discharge rates published by Hirst (1962) gave the only reasonable outcome of the model. In the dark, discharge rate is set to 5% of the rate that would occur during daytime under the same conditions.

### **Infection process**

In Mills' table and its revisions, most emphasis is laid on the time it takes the very first spore to become independent from wetness. For a simulation program it's much more important to know average time that spores need to become independent from leaf wetness, and the relative dispersion for this process. From the data published by Schwabe (1979) it can be learned that the average time for a spore to become independent from leaf wetness is about twice the time the first spore needs. Also the data published by Turner (1986) and Boric (1985) indicate at least a relative dispersion of 0.25 for the infection process. The new model uses half the development rate given by the function published by MacHardy (1989) as LAB-a, with a relative dispersion of 0.25 to let the first spores penetrate the leaf according to this curve. The infection process is subdivided into three subprocesses; germination, germ tube growth and appressorium formation.

### **Incubation**

From the data published by Mills for the first visible lesions a latent period of about 170 day-degrees (base 0°C) can be calculated. Other controlled experiments generally confirm Mills' data, but in the field the latent period can be much longer, influenced by the age of the leaves at the time they were infected and the relative humidity (Keitt 1926, Tomerlin 1983). Soenen (1957) published accurate observations on incubation time in the field of infections in 1956. Using the 1956 weather data from the region he worked in the heat sums between infection and first visible lesions have been calculated. An average incubation time of 270 degree days (base 0°C) is used in the new model.

### **Mortality**

Leaf decomposition has an important effect on the relative number of ascospores discharged in the second half of the ascospore flight period. For technical reasons, leaf decomposition is simulated as a linear process. The number of days from Biofix to full leaf decomposition is an input variable. In most cases this variable is set to 100 days.

In the present model development of the host plant is not yet incorporated. For that reason all spores that have been discharged are assumed to land on susceptible host tissue.

When the leaf dries before the 'landed spores' have formed their penetration peg they die after a certain period. When the leaf is dry the only driving factor for the mortality process time. We found no data that support the vision that mortality rate is depending on temperature or air humidity. Schwabe found a higher mortality rate under high temperature and humidity but these values are too extreme to have a noticeable influence on the model under West European conditions.

Most publications lead to the conclusion that ungerminated spores can survive dry periods much longer than the eight hours normally used in Mills-calculations (Boric 1985; Schwabe 1979, 1980; Moore 1963; Becker 1990; Roosje 1959). The new model allows an

average survival of 48 hour for ungerminated spores, and 12 hour during germination and appressorium formation. Relative dispersion for this process is 0.25.

## VALIDATION AND EVALUATION

The performance of the model was tested in three ways:

1. Simulated ascospore discharge was compared to ascospore flight as recorded with spore traps in 1992 in St Michele (Trento, Italy) and Zurich in 1990, 1991 and 1992 at Lake Constance region, and 1992 and 1993 in The Netherlands. In all cases spore flight was monitored in orchards using object glasses placed 5 mm above overwintered apple leaves. In case of the data from Lake Constance and St Michele, for practical reasons ascospore were counted only after rain periods that resulted in an infection period. In Zurich, 1992 data observations were made every day. In the Dutch data of 1992 observations began some time after the start of ascospore flight. On 6 April the leaves in the trap were roughly wetted to free all mature spores and make the situation in the trap comparable to the orchard situation. As the Biofix date was not known, the model was initiated several days before the first spores were found in the trap.
2. Simulated development of lesions was compared to the development of lesions in untreated orchard blocks in The Netherlands in 1992 and 1993. At regular intervals 100 fruit clusters were searched for scab lesions. The number of scabbed leaves in the cluster and on the fruitspur was recorded.
3. The simulated number of spores that infected the host was used as a relative infection measure (RIM) to decide scab management in replicated small plot trials and trials on a practical scale in Germany, France, Belgium and The Netherlands. The preliminary results from one of these trials is presented here. In 1992 in an orchard block of the apple var. Elstar a trial was conducted in which trees were sprayed curatively after every Mills Period, or only after moderate or high RIM values. This trial had 5 replications. The fungicide pyrifenoxy was used. Before the trial started the grower had applied a spray with a pyrifenoxy-captan mixture on 16 March. Counts of primary infections were made in the first week of June.

## RESULTS AND DISCUSSION

### **Ascospore discharge**

#### *St. Michele 1992 (fig.2)*

25 March was used as the date to initiate the model. As the spores in the trap were not counted every day the plotted discharge for the spore trap is often the sum of the spores discharged during several days of rain. The first spores were found in the trap on 6 April after several days of rain. The model overestimated the discharge twice, once on the 1 May after 0.4 mm of rain in the evening, and once on 4-5 May when rain fell during the night and the leaves stayed wet until 09.00 h.

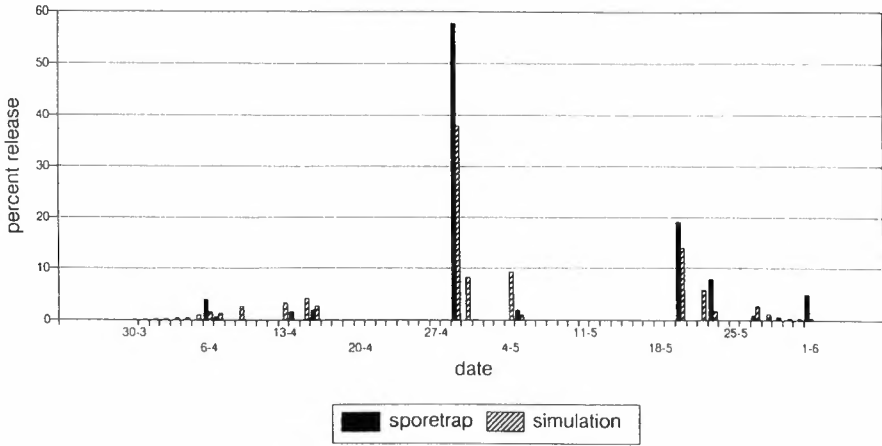


Fig. 2. Ascospore discharge St. Michele 1992

*ETH Zurich 1992 (fig.3)*

As the trap was inspected daily an accurate comparison is possible. During the second discharge period (13-19 April) the model produced spores every day, whereas in the trap an accumulation of spores occurred which were set free in the last two days of this period. Temperature in this period was quite low, partly below freezing point. In this temperature range relations describing the processes in the model are inaccurate. On the 22 April, 0.6 mm of rain fell early in the morning (05.00-05.30) and leaves stayed wet until 09.00. This amount triggered discharge in the model, but hardly any spores in the trap were recorded. At the end of the ascospore flight period, the model produced spores over a longer period than the trap.

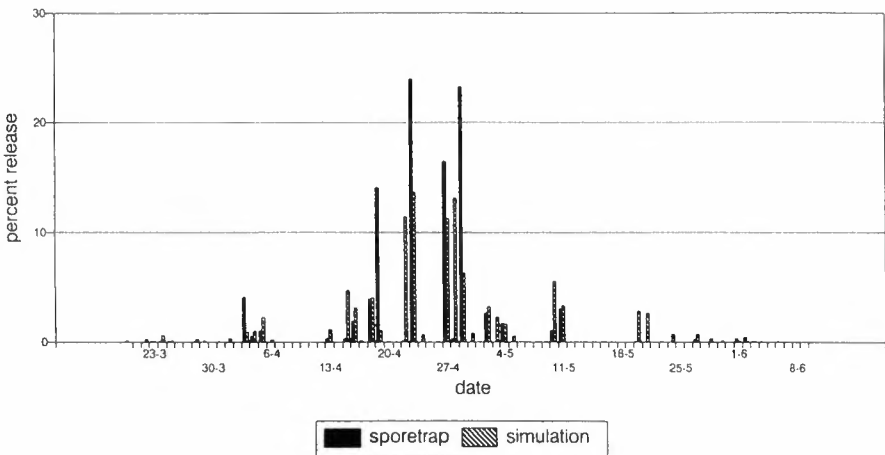


Fig. 3. Ascospore discharge ETH Zurich 1992

Lake Constance 1990 (fig. 4)

The general pattern of the discharge over time fits well. However, the peak discharge on 28 March was strongly underestimated by the model. Changing different variables in the model did not improve the results and made the model less accurate in some cases. In the second half of ascospore flight the model produced more spores than the trap.

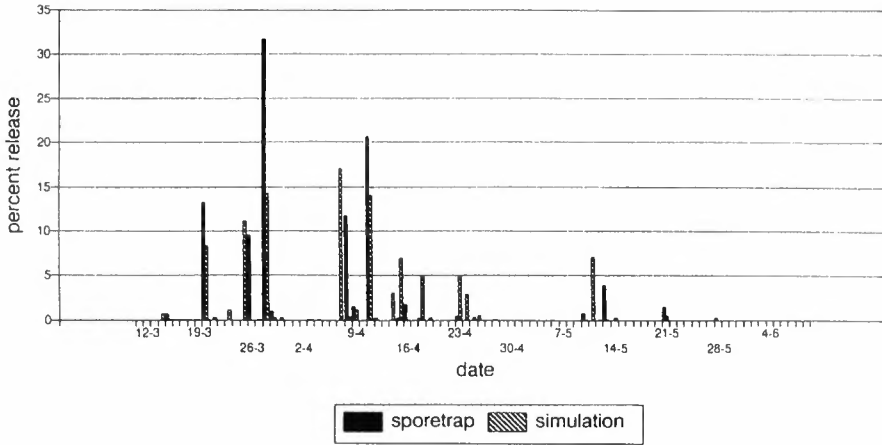


Fig. 4. Ascospore discharge Lake Constance 1990

Lake Constance 1991 (fig. 5)

In the middle of the ascospore flight period a long wet period occurred during which the sporetrap was only checked twice so that only a general comparison could be made.

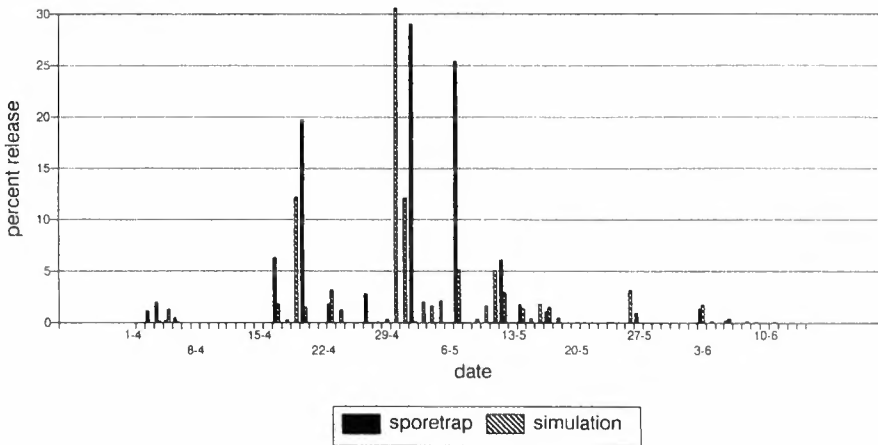


Fig. 5. Ascospore discharge Lake Constance 1991

*Lake Constance 1992 (fig. 6)*

The model fits well to the data. Only the discharge on the 13 April was strongly underestimated by the model. As in the Swiss data, this discharge was recorded during low temperatures.

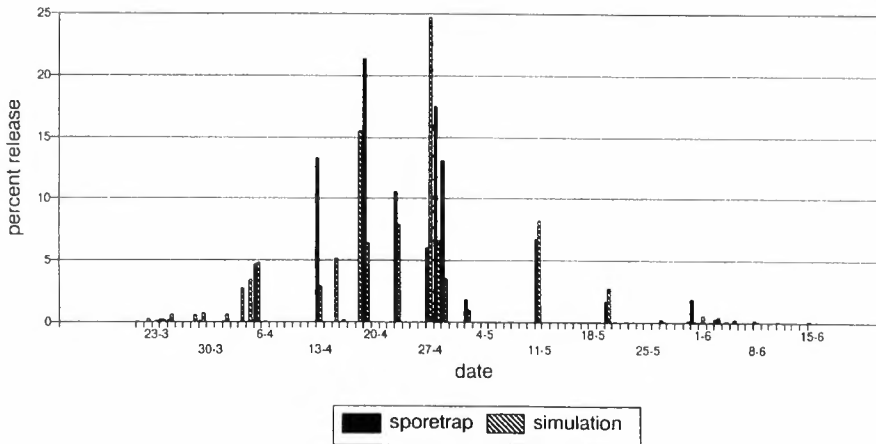


Fig. 6. Ascospore discharge Lake Constance 1992

**Lesion Development**

*Netherlands (Asch) 1992 (fig. 7)*

The observations were started on 6 April by artificially wetting the leaves in the spore trap; in the graph (Fig. 7) the situation in the spore trap was set equal to the simulated discharge on 6 April.

After a dry period the model generated a lot of spores in the first daytime rain period. In the spore trap this peak was recorded two days later. This discrepancy could be caused by an artifact: the trap was initially placed under a tree and it might be possible that the trap was not wetted enough during this first rain period.

The appearance of lesions on cluster leaves and later on the first leaves of fruit spurs fits well to the simulated development of lesions from primary infections. In the field, however, there was some evidence that the lesions on the fruit spurs were at least partly caused by secondary infections.



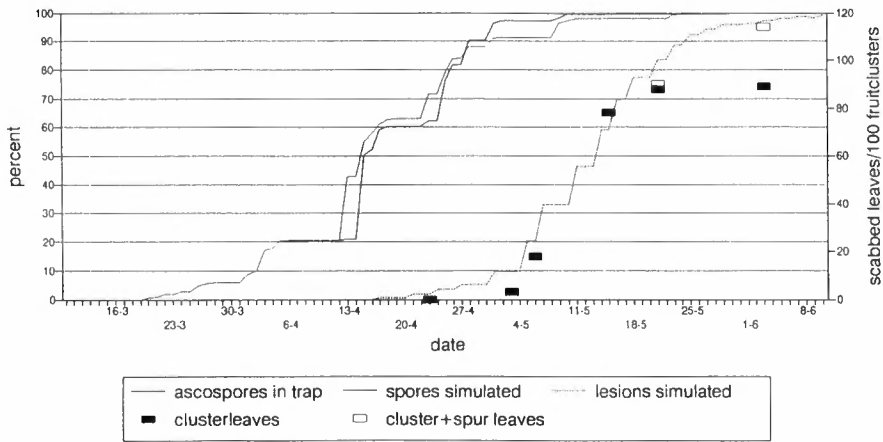


Fig. 7. Primary infection period Netherlands (Asch) 1992

*Netherlands (Eck en Wiel, Asch) 1992 (fig. 8)*

In 1993 there is a poor fit between the data from the sporetrap and the simulated discharge. In the first week of April the trap produced many more spores than the model. The simulation of lesions, however, fits much better to the data, which raises the question whether the problem lays in the model or the trap.

A few drawbacks from the spore trapping technique used:

- the spores are obtained from a limited number of leaves and the variation from leaf to leaf is considerable.
- during drizzle rain it takes a long time for the leaves under the glass trap surfaces to become wet enough to discharge.
- the temperature under the glass may be higher than in the field on sunny days.

Therefore under certain circumstances the number of spores in the trap may not represent ascospore discharge in the field.

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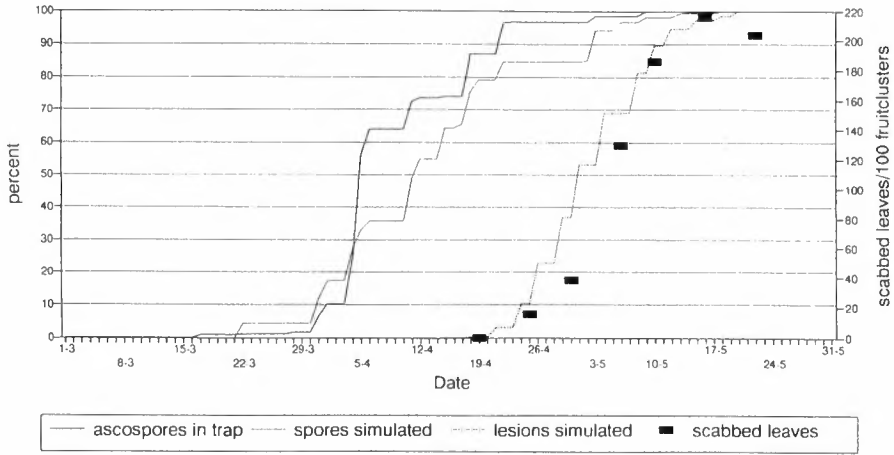


Fig. 8. Primary infection period Netherlands (Eck & Wiel-Asch) 1993

**Disease management**

*Treating according to the Relative Infection Measure (RIM) (figure 9)*

In 1992 14 Mills Periods were recorded. They are shown at the top of figure 9.

In the histogram the number of spores that entered the host plant, as calculated by the model is plotted. Figures are the fraction of the total spore potential in the orchard, which was set to 10,000. As these values are the number of discharged spores and the chance they had to penetrate the host, they are interpreted as a measure of the relative importance of infection periods.

In a small plot trial with 5 replications, the scab development on untreated plots was compared to plots with 9 sprays following each Mills period during the ascospore flight period, plots with 5 sprays in which only the moderate and large peaks in RIM values were treated, and plots with only 3 sprays applied after high peaks in RIM values.

At the end of the primary scab season the untreated blocks had on average 104 leaves with scab on 100 clusters. The 9 sprays gave 98% control and 5 or 3 sprays gave 96% control. During the same period the grower applied 12 sprays to the rest of the orchard. He also achieved 98% control.

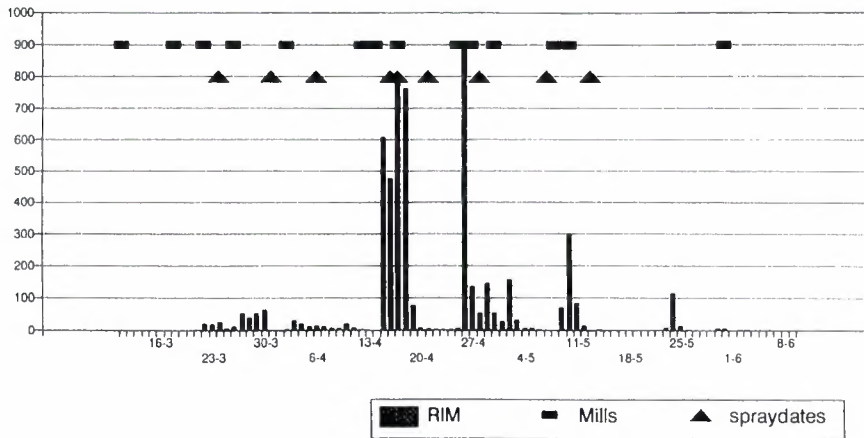


Fig. 9. Relative Infection Measure (RIM) Netherlands (Asch) 1992

### GENERAL CONCLUSION

1. Computer simulations make it possible to apply detailed pieces of knowledge in scab management.
2. The performance of the present model is encouraging.

In some situations there are differences between trap catches and simulation. These differences could not be corrected by changing values of the variables in the model. A further stepwise improvement of the model and evaluation by comparing the outcome of the model with the ascospore flight as monitored with other types of spore trap seems necessary.

### REFERENCES

Becker, C.M. 1990. Overwintering of the anamorph of *Venturia inaequalis* (Spilocaea Pomi) in apple buds and the viability of conidia as affected by discontinuous wetting. Cornell University: 1-86.

Boric, B. 1985. Influence of temperature on germability of spores of *Venturia inaequalis* (Cooke) Winter, and their viability as affected by age. *Zastita bilja* 36: 295-302.

Cesari, A. & R. Fiaccadori. Reliability of forecast parameters in ascospore infections of *Venturia inaequalis* (Ckr) Wint.

Gadoury, D.M. & W.E. MacHardy 1982. Effects of temperature on the development of pseudothecia of *Venturia inaequalis*. *Plant Disease* 66: 464-468.

Gadoury D.M. & W.E. MacHardy 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. *Phytopathology* 72: 901-904.

Hirst, J.M. & O.J. Stedman 1962. The epidemiology of apple scab (*Venturia inaequalis* (Cke.) Wint.). II. Observations on the liberation of ascospores. *The annals of applied Biology* 50: 525-550.

MacHardy, W.E. & D.M. Gadoury 1985. Forecasting the seasonal maturation of ascospores of *Venturia inaequalis*. *Phytopathology* 75: 381-385.

MacHardy, W.E. & D.M. Gadoury 1986. Patterns of ascospore discharge by *Venturia inaequalis*. *Phytopathology* 76: 985-990.

MacHardy, W.E. & D.M. gadoury 1989. A revision of Mills' criteria for predicting apple scab infection periods. *Phytopathology* 79: 304-310.

Moore, M.H. 1963. Glasshouse experioments on apple scab. 1. Foliage infection in relation to wet and dry periods. *Annals of applied Biology* 54: 423-435.

Rabbinge, R. et al. 1989. Simulation and systems management in crop protection. Pudoc, Wageningen: 420 s.

Roosje, G.S. 1959. Het schurftonderzoek in Nederland van 1955 t/m 1958. II. Laboratoriumonderzoek van infecties door ascosporen en conidien. *Mededelingen Dir. Tuinbouw* 22: 441-447

Schwabe, W.F.S. 1979. Wetting and temperature requirements for apple leaf infection by *Venturia inaequalis* in South Africa. *Phytophylactica* 12: 69-80.

Schwabe, W.F.S. 1980. Apple scab infection as influenced by 'dew' following 'rain' wetting periods. *Phytophylactica* 12: 229-230.

Schwabe, W.F.S. et al. 1988. Relation of degree-day accumulations to maturation of ascospores of *Venturia inaequalis* in South Africa. *Phytophylactica* 21: 13-16.

Soenen A. et al. 1957. Het appelschurft. *Landbouwtijdschrift* 10 (6).

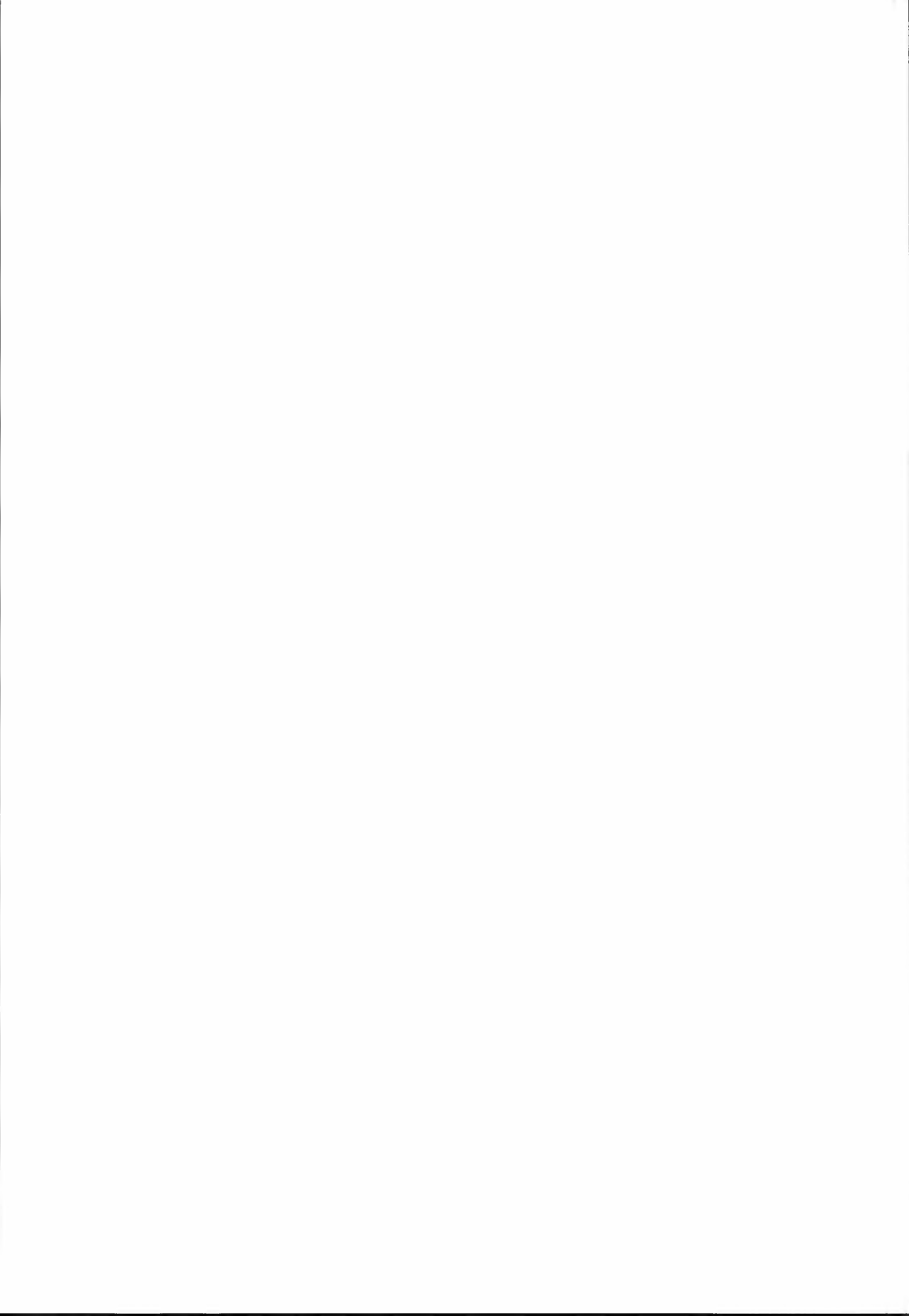
Soenen, A & E. Beauvuin 1960. Het schurft op appel. *Landbouwtijdschrift* 13 (8).

Stephan, S. 1985. Epidemiologische Untersuchungen zum Ascosporenpotential und Ascosporenenflug des Apfelschorfes (*Venturia inaequalis* (Cke.) Wint.). *Arch. Phytopathol. Pflanzenschutz, Berlin* 1987, 23 (1): 43-54.

Tomerlin, J.R. & A.L. Jones 1983. Effect of temperature and relative humidity on the latent period of *Venturia inaequalis* in apple leaves. *Phytopathology* 73: 51-54.

Turner, M.L. et al. 1986. Germination and appressorium Formation by *Venturia inaequalis* during infection of apple seedling leaves. *Plant Disease* 70: 658-661.

Wit, de C.T. & J. Goudriaan 1974. Simulation of ecological processes. Pudoc, Wageningen: 159 s.



# Potential ascospore dose measurements of *Venturia inaequalis* applied to the management of primary scab in commercial apple orchards

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Scheer, H. A. Th. van der 1994. Potential ascospore dose measurements of *Venturia inaequalis* applied to the management of primary scab in commercial apple orchards. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 69-73. ISSN 0802-1600.

In 1990, 1991, and 1992 the primary inoculum of *Venturia inaequalis* - expressed as potential ascospore dose (PAD) - was evaluated as an aid to control primary scab in commercial apple orchards in The Netherlands. In all but one of the commercial trial orchards PAD was low. In 22 out of the 26 orchards with a low PAD the first early-season fungicide spray could be delayed for at least 3 to 4 weeks without causing extra crop loss. However, in 4 orchards with a low PAD no delay could be tolerated, probably because of entrant air-borne inoculum. This problem seriously limits the use of PAD estimations as an aid in a scab management programme.

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In The Netherlands on average 3 to 4 pre-blossom sprays are applied for control of scab mostly post-infection. Post blossom (until the end of July) scab is controlled together with powdery mildew, mostly with a mixture of fungicides, in a calendar spraying scheme. With this type of control measure, up to about 1990, it was common to achieve almost 100% control of fruit scab. However, scab escaped control measures in quite a number of orchards in the last two years because of weather circumstances favourable to the disease.

Until now, application of fungicides has not been based upon the amount of inoculum present, because it has been assumed that the amount of primary inoculum present in every orchard is more than sufficient to cause an unacceptable amount of fruit scab, if the recommended fungicide schedule is not strictly followed. However, this assumption is incorrect, as was demonstrated recently by MacHardy (1990) in a three year study undertaken in four commercial orchards in New Hampshire, USA. MacHardy used the primary inoculum that overwinters in the fallen leaves - expressed as the Potential Ascospore Dose (PAD) - to determine when the action threshold for applying a fungicide to prevent infection has been exceeded.

It is likely that action thresholds will vary from one region to another, due to differences in orchard characteristics and weather patterns. In the autumn of 1989, therefore, we started to evaluate the PAD as an aid to control of primary scab in commercial apple orchards in The Netherlands.

## MATERIALS AND METHODS

In the spring of 1990, 1991, and 1992, the density of overwintering populations of *Venturia inaequalis*, or potential ascospore dose, at 5, 12, and 10 locations respectively, was determined as described by Gadoury & MacHardy (1986). The PAD is the number of ascospores per square meter of orchard floor for the season. The research block in each orchard consisted of sub-plots that received one to four fewer early-season fungicide sprays. The study was conducted using cultivars Elstar, Golden Delicious, and Jonagold on 2, 6 and 21 instances respectively.

The procedure for determining the PAD in each orchard is described hereafter. Disease incidence and severity were recorded just before leaf fall. The numbers of infected leaves and lesions per scabbed leaf were recorded from 10 terminal shoots of 60 trees at each site. At the same time, the leaves of 30 terminal shoots selected at random from trees at each site were counted and the total leaf area of each shoot was determined with an area measurement system (Delta-T device LTD, Burwell, Cambridge, England). In each instance the leaf litter density (LLD) was assessed at bud break, using the point-intercept method mentioned by Gadoury and MacHardy. At four trees randomly selected from each orchard, four transects were run on diagonals across the adjacent rows. A measuring tape was stretched along each transect, and beginning 0-30 cm from the end of the tape, and at 30 cm intervals thereafter, the presence of leaves under the tape was noted. LLD was computed as the proportion of the points under which leaves were found. Furthermore data on mean production of pseudothecia, asci and ascospores per infected leaf as determined by Gadoury and MacHardy were used in PAD calculations.

The scab development in the season was determined direct after blossom time by counting the number of leaf whorls with scab lesions from 10 leaf whorls on each of 20 trees per sub-plot, and at picking time by counting the number of leaves on terminal shoots with scab lesions from 10 terminal shoots on each of 20 trees per sub-plot and the number of infected fruits from 20 fruits on each of 25 trees per sub-plot.

The biofix for determining the length of the period of delay of the first fungicide spray is the last event for the season of either bud burst or first mature ascospore. In 1990, 1991, and 1992 the biofix is 24 March (bud burst), 11 March (first mature ascospore), and 9 March (first mature ascospore) respectively.

Leaf wetness was monitored in the pre-blossom part of the season with a DeWit leaf wetness recorder and the data used for determining unprotected Mills periods (Mills 1944; Mills & Laplante 1951).



## RESULTS

Part of the results have been presented at the 2nd Symposium on Integrated Fruit Production held in August 1992 in Veldhoven in The Netherlands (van der Scheer 1993). A popular presentation of the results for Dutch fruit growers have recently been published (van der Scheer & Wondergem 1993).

### PAD components

Elstar trees produced less leaves per shoot than did Golden Delicious or Jonagold, and the leaf area of Jonagold was significantly larger than that of Elstar or Golden Delicious (table 1).

Table 1. The value and standard deviation (SD) of some PAD parameters assessed in apple orchards on cultivars Elstar (El), Golden Delicious (GD), and Jonagold (Jon) in the period 1989-1992

Parameter	El (n=2)		GD (n=6)		Jon (n=21)	
	Value	SD	Value	SD	Value	SD
Leaf area (cm <sup>2</sup> )	30.1	4.9	31.4	1.0	38.9	0.8
Leaves/shoot (number)	16.7	2.0	19.1	2.1	19.0	0.7
Leaf area/600 shoots (m <sup>2</sup> )	30.6	8.4	36.0	4.3	44.5	2.2
Leaf litter density (%)	47	3.4	36	4.8	41	3.2

Disease incidence at leaf fall was low in the commercial orchards under trial. In only 10 out of 26 instances infection of the leaves was recorded, resulting in a PAD of up to 82. In 9 of these instances less than 0.1% of the leaves on terminal shoots were infected, and in only 1 instance the percentage infected leaves amounted to 0.33%.

No consistent relationships were found between leaf litter density at bud burst, cultivar, and year. At bud burst, on average 40% of the orchard floor was covered by leaf litter. Significantly more leaf litter was found on the weed-free strips under the trees than on the grass alleys.

### Scab development in the season

In the one orchard with a higher PAD the first early-season spray was applied 11 days after the time that the first ripe ascospores were observed at Wilhelminadorp in 1991, thus leaving the trees unprotected during 3 infection periods. This delay resulted in too many diseased fruits (2.2%) and infected leaves on terminal shoots (table 2). In most of the other orchards under trial with low PAD measurements a delay of the first spray of four to seven weeks could be tolerated without causing extra crop loss.

However, in three orchards (one in 1990 and two in 1991) with a PAD of zero a delay of the first spray for some days resulted in too many scabbed fruits. For the one orchard in 1990 the cause of this incidence remains unknown. But, for the other two orchards in 1991 an inoculum source was detected outside the orchards concerned, at a

distance of approximately 350 metres. Air-borne inoculum from these sources could be the cause of the scab infection. At the same time no scab infection was observed in an orchard under trial at a distance of circa 800 meters westerly from the infection source near one of the two orchards in 1991. In 1992 a delay of four weeks in an orchard with a PAD of 81 resulted also in too many scabbed fruits and again an inoculum source was detected nearby.

## DISCUSSION

In general, commercial orchards have a low incidence of apple scab. In that case ascospores are the overwintering inoculum (van der Scheer and Grabowski, 1991). They are the only ones on the spot and focal distributed. In this situation spore traps are not sufficiently efficient for direct determination of the density of overwintering populations of *Venturia inaequalis*. The PAD model overcomes this obstacle.

According to MacHardy (1990), the fungicide program to control scab in an orchard with less than 600 PAD can be delayed until pink bud stage without risk of increased scab compared to a schedule initiated prior to that stage. The result in the only one orchard with a higher PAD value (being 387) does not fit this conclusion. It may be that under the same circumstances in Dutch orchards a lower PAD threshold has to be used, unless an error was the cause of the scab injury (such as application of too low a rate of fungicide). The result with the other cases (PAD lower than 83) indicates that a delay of minimally three to four weeks is possible. Some growers already routinely delay the first fungicide spray in their orchard for two or three weeks without crop loss by scab as is reflected by the delay in the growers schedule and the resulting disease incidence in table 2.

Table 2. Potential ascospore dose (PAD), delay of the first spray and the resulting amount of scab incidence on apple

Year	Orchards (number)	Delayed first spray				Grower's schedule			PAD
		Delay (day)	Unprotected infection periods (number)	Scab (%)		Delay (day)	Scab(%) Leaves	Fruit	
1990	4	30	-	-	0	6	-	0	6
	1	-	-	-	-	5	-	2.8	0
1991	6	52	6.7	0.1	0	9	0.0	0	0
	3	30	5.0	0.1	0.1	5	0.2	0.1	0
	2	-	-	-	-	8	4.6	1.1	0
	1	-	-	-	-	11	16.4	2.2	387
1992	6	45	4.7	1.2	1.9	9	1.0	1.7	15
	3	31	2.7	2.0	3.8	5	1.8	3.7	51
	1	-	-	-	-	29	1.0	7.6	81

However, in three instances with a zero PAD, a delay of the first spray for some days resulted in too many scabbed fruits. Furthermore a delay of four weeks in an orchard with a PAD of only 81 resulted in far too many scabbed fruits. These events are probably due to entrant air-borne inoculum, although Burchill (1966) reported a rapid fall-out of ascospores liberated from a point inoculation source. They seriously limit the use of PAD estimations as aids in a scab management programme.

In orchards with a history of canker, caused by *Nectria galligena*, chemical control is necessary in the period of bud-scale drop to protect the scars from infection by this pathogen. This may interfere with the strategy to delay the first scab spray, since that spray controls canker too if it contains an appropriate fungicide.

#### ACKNOWLEDGMENTS

The author thanks Mr. H.J. Wondergem for his assistance.

#### REFERENCES

- Burchill, R.T. 1966. Air-dispersal of fungal spores with particular references to apple scab (*Venturia inaequalis* (Cooke) Winter). In: Colston Papers XVIII. Butterworth Scientific Publications, London, pp. 137-140.
- Gadoury, D.M. & W.E. MacHardy 1986. Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. *Phytopathology* 76: 112-118.
- MacHardy, W.E. 1990. New, non-fungicidal techniques to aid in the management of apple scab. New England Fruit Meetings 1990. Proceedings 96th annual meeting Massachusetts Fruit Growers' Association: 75-78.
- Mills, W.D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab. *Cornell Extension Bulletin* No. 630, 4pp.
- Mills, W.D. & A.A. Laplante 1951. Diseases and insects in the orchard. In: *Cornell Extension Bulletin* No. 711, pp. 20-27.
- Scheer, H.A.Th. van der 1993. Forecasting ascospore dose of *Venturia inaequalis* as aid to control of primary scab in commercial apple orchards. *Acta Horticulturae* No. 347: 127-131.
- Scheer, H.A.Th. van der & M. Grabowski 1991. Ascosporen meestal dader van schurftinfecties in het voorjaar. *Fruittelt* 81(16): 28-29.
- Scheer, H.A.Th. van der & H.J. Wondergem 1993. Minder spuiten voor de bloei is riskant. *Fruittelt* 83(8): 24-25.



# A "PAD" action threshold: the key to integrating practices for managing apple scab

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Thresholds are fundamental components of an IPM program, and damage threshold, economic damage threshold, action threshold, warning threshold, and perception threshold have been defined recently by a committee of the American Phytopathological Society (Nutter et al. 1993). These thresholds have received little attention to date in the development of apple scab management programs, and the reasons for this have been discussed (MacHardy 1994). One main reason is that they all require an assessment of the pathogen population, and there was no procedure to estimate the ascospore inoculum of *Venturia inaequalis* (Cke.) Wint., the causal agent of apple scab disease, until the recent development of a procedure to forecast the potential ascospore dose (PAD), i.e., the number of ascospores per m<sup>2</sup> orchard floor (Gadoury & MacHardy 1986a). Also, it was commonly accepted that all infection periods during the primary (ascospore) scab season must be protected with fungicide, regardless of how little ascospore inoculum is present, so apparently an action threshold to protect the trees with fungicide was exceeded at bud break.

## THE DEVELOPMENT OF A "PAD" ACTION THRESHOLD

For most of this century, before the development of modern fungicides and air blast sprayers and the planting of cultivars on semi-dwarfing and dwarfing rootstocks, it may have been true that all orchards require fungicide treatment for all infection periods, but that is not true now. Several studies conducted in the past ten years in the northeastern US (Gadoury & MacHardy 1984, 1985, 1986a, 1986b; Gadoury et al. 1989, and MacHardy et al. 1993) have demonstrated that the onset of a fungicide program for control of apple scab in orchards with a low PAD can be delayed until after one or more Mills infection periods (Mills 1944; Mills & LaPlante 1951) had occurred without increasing the incidence of scabbed fruit at harvest. In one continuing study begun seven years ago and including 7 commercial orchards in the states of New Hampshire, Massachusetts, and Maine, plots in 23 orchards with a PAD forecasted at < 600 that were left unprotected with fungicide for three infection periods or until the pink fruit bud stage had an incidence of scabbed fruit

at harvest that was similar to that of plots treated with fungicide for all infection periods (Table 1). In six of these orchards, it was shown that trees could be left unprotected with fungicide for the first four to nine infection periods without exceeding 1% scabbed fruit at harvest, which is an acceptable level of control in the northeastern US.

Table 1. The number of unprotected scab infection periods<sup>1</sup> and the fruit bud development stage, in orchards with a predicted PAD < 600, at which the first fungicide was applied without an increase in scabbed fruit at harvest compared with the scabbed fruit on trees treated with fungicide according to the grower's program, designed to begin soon after bud break and protect the trees from infection by *Venturia inaequalis* during all infection periods. Developed from data by MacHardy et al. (1993) and from unpublished data (W. MacHardy). Reprinted by permission

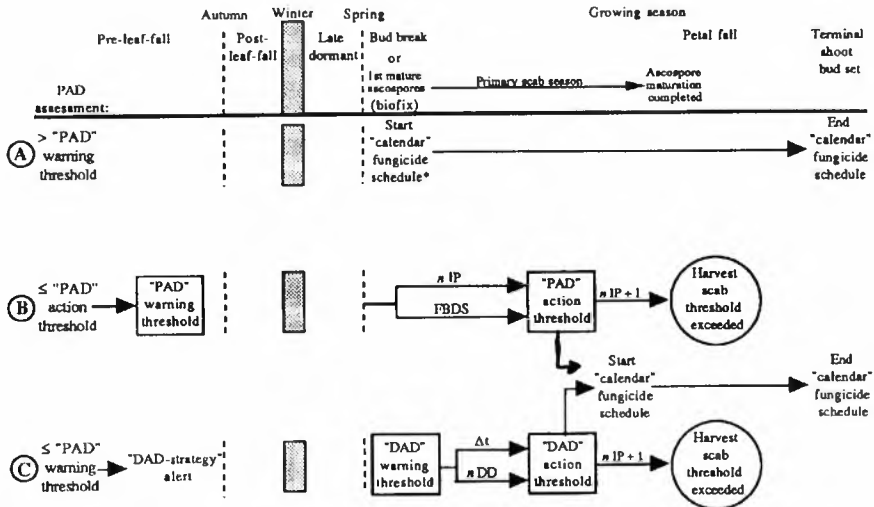
Unprotected infection periods	Number of orchards					
	Tree growth stage at which fungicide applied <sup>2</sup>					
	TC	P	B	PF	FS	1cmF
1		1	3	1		
2	2	2	1	1		1
3		1	2	3	1	
4			1			
5					1	
6		1				
7		1				
8						1
9						1

<sup>1</sup> Infection period determined to criteria reported by Mills (1944, 1951)

<sup>2</sup> TC = tight cluster; P = pink; B = bloom; PF = petal fall; FS = fruit set; 1cmF = 1 cm diameter fruit

In two other orchards (Table 1), fungicide intervention was needed at tight cluster, after only two unprotected infection periods. The incidence of scabbed fruit was higher than expected in plots that received their first fungicide treatment after three or more infection periods had occurred, but plots sprayed according to the grower's regular schedule also had more scab than expected. Inquiry into the spray procedure revealed that errors in calculating fungicide dose and in sprayer calibration were responsible. The experience in these two orchards demonstrates that applying the correct amount of fungicide is critical to the success of the delayed-spray tactic.

The following "PAD" action threshold for initiating a fungicide program at the pink fruit bud stage would have resulted in control of fruit scab comparable to the grower's schedule: if PAD < 600 and  $\leq 3$  infection periods, then delay first spray until the pink fruit bud stage. With this action threshold, the first fungicide spray could be delayed until pink or until after 3 infection periods if they occurred before pink. This definition of action threshold and the definitions for other thresholds being developed for the apple scab management program in New Hampshire are quite different from the definitions published by the American Phytopathological Society committee. The reasons for redefining these terms for apple scab disease are explained elsewhere (MacHardy 1994). Threshold definitions, relationships between the different thresholds, relationships between PAD levels and thresholds, the different means of identifying when the thresholds are met, and relationships between the thresholds and fungicide programs are diagrammed and explained in Figs. 1-5.



\*It is assumed that the harvest scab threshold will be exceeded if the trees are left unprotected for any infection periods.

**Terms, concepts, actions, and symbols:**

**PAD assessment:** ascospores/m<sup>2</sup> sq orchard floor, determined by the method of Gadoury and MacHardy (1986).

**"PAD" warning threshold:** a PAD at which ascospores can infect unprotected trees for at least the first infection period without exceeding the action threshold. The grower is alerted to keep count of infection periods and observe fruit bud development stages and to be prepared to act when the action threshold is reached.

**"PAD" action threshold:** the number of infection periods or stage of fruit bud development at which action must be taken to prevent exceeding the harvest scab threshold.

**Harvest scab threshold:** the lowest cumulative dose of discharged ascospores for which  $\geq 1.0\%$  scabbed fruit at harvest is projected if control action is not taken.

**"DAD-strategy" alert:** an assessment of PAD at the "PAD" warning threshold level. The grower is alerted that the "DAD" threshold strategy can be used next spring. Calculations to identify when the "DAD" action threshold is reached (i.e.,  $\Delta t$  or  $n DD$ ) should be performed before the "DAD" warning threshold is reached.

**"DAD" warning threshold:** the pathogen population density (first matured ascospores) or fruit bud development stage (bud break) at which the grower is warned to prepare to act on the "DAD" action threshold. The grower is alerted to begin cumulating degree days or counting the days to reach  $\Delta t$ .

**"DAD" action threshold:** The cumulative dose of discharged ascospores (DAD) at which action must be taken to prevent the discharged ascospore dose from exceeding the harvest scab threshold.

$n IP + 1$ , once the "DAD" or "PAD" action threshold is reached, the harvest scab threshold will be exceeded at the next infection period ( $n IP + 1$ ) if control action is not taken.

**Different means to identify when an action threshold is reached:**

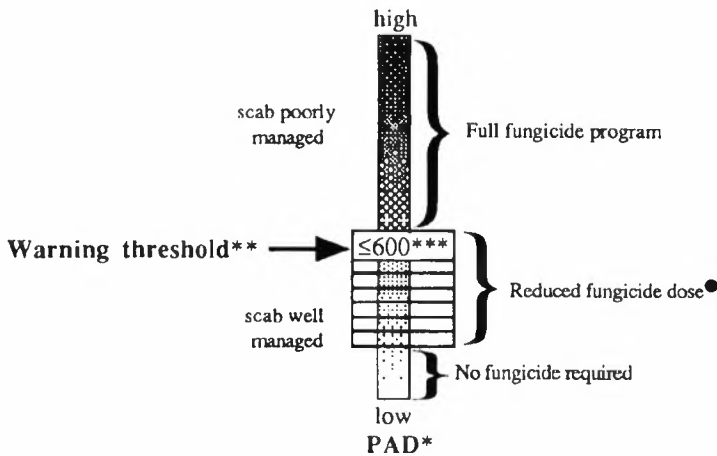
$\Delta t$ , the number of days ( $\Delta t$ ) from a biofix (bud break or the day matured ascospores are first observed or trapped) to when the onset of an apple scab epidemic is reached and control is needed.

$n DD$ , the degree-days ( $n DD$ ) cumulated from the biofix to when the cumulated discharged ascospore dose reaches a threshold level at which control is needed. See text for explanation of how  $n DD$  is determined.

$n IP$ , the number ( $n$ ) of infection periods (IP) that can occur before control is needed, determined by the PAD level assessed in the autumn.

**FBDS**, the most advanced fruit bud development stage (FBDS) at which trees can be left unprotected with fungicide, determined by the PAD level assessed in the autumn.

Fig.1. Three schemes to use an action threshold based on an assessment of potential ascospore dose to determine the time to initiate fungicide treatments to control apple scab caused by *Venturia inaequalis*: A, when the forecasted ascospore inoculum (PAD) is greater than a PAD warning threshold, B, when the forecasted ascospore inoculum is less than a PAD action threshold and the "PAD" action threshold is identified by the number of infection periods or a fruit bud development stage, and C, when the forecasted ascospore inoculum is less than a "PAD" warning threshold and the action threshold ("DAD" action threshold) is identified by the number of days or the cumulated degree-days from a biofix. From MacHardy (1994). Reprinted by permission



\* PAD: ascospores per square meter orchard floor.

\*\* Warning threshold: maximum ascosporic inoculum that will allow a reduction in fungicide dose without risk to the crop.

\*\*\* A warning threshold based on a PAD set at  $\leq 600$ . Empty boxes represent lower levels of PAD (or ascospore dose) at which increasingly less fungicide dose is needed.

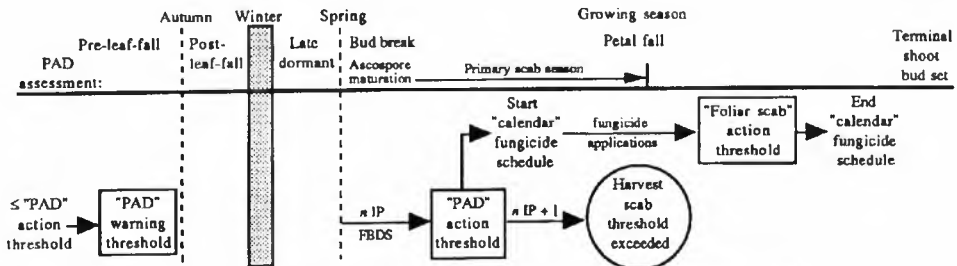
● **Tactics**

- Delay first scab fungicide spray
- Reduce the fungicide rate
- Extend interval between sprays

Figure 2. A warning threshold, based on an assessment of potential ascospore dose (PAD), that alerts a grower to prepare to act on an action threshold that identifies when the first fungicide treatment is needed to control apple scab in an orchard in which the primary inoculum is comprised solely of ascospores of *Venturia inaequalis*. The action threshold is reached when the maximum ascospore dose that can infect unprotected apple tissue without exceeding a harvest scab threshold has been discharged. The action threshold can be identified by a specified number of unprotected infection periods, a stage of fruit bud development, the number of days to the onset of a scab epidemic, or the cumulative degree-days from a biofix in a predictive model of ascospore maturity. The lower the ascosporic level below the warning threshold, the greater the allowable reduction in fungicide dose applied according to the tactics listed. Reprinted by permission

The "PAD" action threshold is reliable apparently because there was not enough inoculum in these orchards prior to the fungicide treatments to cause infections that would eventually result in an unacceptable buildup of scabbed fruit. Weather conditions were highly favorable for scab, i.e., trees were left unprotected for as many as one moderate and four severe Mills' infection periods without excessive buildup of scab, so it would appear that the "PAD" action threshold should hold under future weather conditions, in orchards in which the primary inoculum consists only of ascospores, provided errors by the grower don't place the orchard at risk. Examples of errors include an incorrect assessment of scab in the autumn (the main input for computing PAD) and errors in fungicide application.





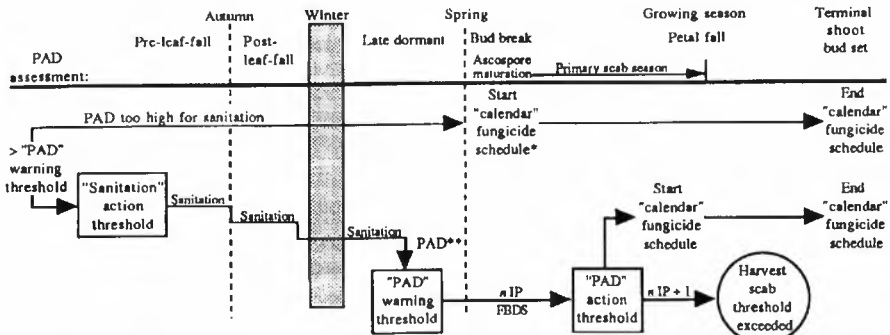
\*It is assumed that the harvest scab threshold will be exceeded if the trees are left unprotected for any infection periods.

**Terms, concepts, actions, and symbols:**

"Foliar scab" action threshold: the maximum percentage of extension shoots with foliar scab during June, July, and August, respectively, that allows the trees to be unprotected with fungicide for the remainder of the growing season without scabbed fruit exceeding the harvest scab threshold.

See Figure 1 for explanation of PAD assessment, "PAD" warning threshold, "PAD" action threshold, harvest scab threshold,  $n$  IP, FBDS, and  $n$  IP + 1.

Figure 3. The relationship between a "PAD" warning threshold, "PAD" action threshold, "foliar scab" action threshold, and harvest scab threshold and the scheduling of fungicides to control apple scab, based on the work of van der Scheer (1980, 1987). Reprinted by permission.



\*It is assumed that the harvest scab threshold will be exceeded if the trees are left unprotected for any infection periods.

\*\*Sanitation reduced the PAD to  $\leq$  "PAD" action threshold.

**Terms, concepts, actions, and symbols:**

"Sanitation" action threshold: a PAD above the "PAD" action threshold at which action (sanitation) will reduce the PAD to at least the "PAD" action threshold and therefore prevent the ascospore dose from causing lesions that will exceed the harvest scab threshold. Sanitation practices (e.g., applying a chemical or biological agent to the leaves; mulching the leaf litter) can be performed in the autumn before or after leaf fall and in the spring before bud break.

A "sanitation" action threshold is considered only when the PAD is greater than the "PAD" action threshold, but at a level at which sanitation will allow the grower to schedule the first fungicide application according to a "PAD" action threshold. Otherwise, no sanitation will be performed and a "calendar" fungicide schedule will begin soon after bud break.

See Figure 1 for explanation of PAD assessment, "PAD" warning threshold, "PAD" action threshold, harvest scab threshold,  $n$  IP, FBDS, and  $n$  IP + 1.

Figure 4. A scheme to use a "sanitation" action threshold to integrate sanitation practices and fungicide applications in an apple scab management program. Reprinted by permission.

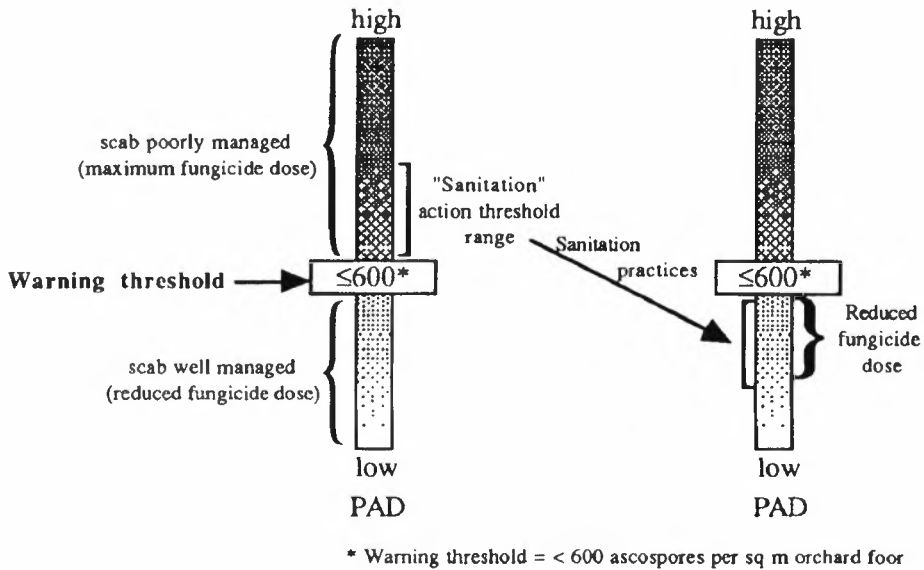


Figure 5. A strategy to utilize sanitation practices to reduce the fungicide dose in the management of apple scab caused by *Venturia inaequalis*. Sanitation practices are employed in an orchard with a potential ascospore dose (PAD) greater than the "PAD" warning threshold for the purpose of lowering the ascospore dose to below the warning threshold. The "sanitation" action threshold is the range of PAD that extends from the "PAD" warning threshold to the maximum PAD at which sanitation can reduce the ascospore inoculum to the "PAD" warning threshold or less. Reprinted by permission.

Delaying the first fungicide application until the pink fruit bud stage is economically beneficial to growers in the northeastern US, because pesticide use prior to pink is limited to 1 or 2 oil sprays for controlling mites and scale (Gadoury et al. 1989). In most seasons, 1-4 early-season fungicide applications recommended in a conventional (calendar) protectant schedule would be eliminated, a reduction in fungicide dose of about 20-40%, and this savings in fungicide and associated spray costs make this strategy appealing to growers. A "foliar scab" action threshold designed to identify the time to end a fungicide schedule for controlling scab (Fig. 3), based on the work of van der Scheer (1980, 1987), is well-suited for the program being developed in New Hampshire (MacHardy 1994).

#### THE DEVELOPMENT OF A "SANITATION" ACTION THRESHOLD

The delayed-spray strategy presented in Fig. 1 can be used only in orchards in which the PAD is less than the "PAD" action threshold. However, it is possible to include orchards with a PAD greater than the "PAD" action threshold, if sanitation practices are employed in the autumn and/or before bud break to reduce the PAD to below the "PAD" action

threshold (Figs. 4 and 5). A sanitation practice is defined as any practice aimed at reducing the primary inoculum, and two sanitation practices, shredding the leaf litter with a flail mower and applying urea to the leaf litter, were evaluated for their potential to reduce the PAD and the number of primary scab lesions. Autumn leaf-shredding reduced the leaf litter density, a main component for calculating PAD, an average 55% in treatment plots at 7 sites and reduced the severity of primary foliar scab an average 62% at 5 of the 7 sites (Sutton 1993; Sutton & MacHardy 1992). The ascospore dose was reduced 55% at the two sites where airborne ascospores were trapped. Spring leaf-shredding and spring urea treatment reduced the ascospore dose 89 and 74%, respectively, and each treatment reduced primary foliar scab 80%. In small plot studies in 1990 and 1992, pre-leaf-fall urea, spring urea, autumn leaf-shredding, spring leaf-shredding, and a combined autumn and spring leaf shredding treatment reduced ascospore productivity 97, 82, 50, 65, and 83%, respectively. The results suggest that employing these sanitation practices in orchards with a PAD as high as 3,000 would lower the PAD below the "PAD" action threshold of 600. From 65-95% of the leaf litter was shredded in the orchard study, with the amount of leaf litter shredded dependent mainly on tree spacing and canopy extension into the row alley. Improvements in flail mowers that will allow more of the leaf litter under the tree canopy to be shredded will improve the efficiency of the leaf-shredding technique and produce more consistent results.

## CONCLUSIONS

It has been shown over several years that commercial orchards in the northeastern US planted with the highly scab-susceptible cultivar McIntosh could be left unprotected with fungicide for several infection periods during the primary scab season when the PAD was <600, even when the weather was highly favorable for infection to occur, without placing the orchard at greater risk. Delaying the onset of the traditional fungicide schedule to the time when the pseudothecial population matures to a level that requires management is practical in low ascospore inoculum orchards, because several early-season fungicide applications are eliminated. At the start, growers participating in these studies were skeptical that this approach would work, but they are now enthusiastic supporters. They are aware that improvements in controlling scab (e.g., better fungicides and improved application equipment) during the approximately 40 years since the standard protectant fungicide programs were developed have resulted in orchards that commonly have an ascospore inoculum level at which an action threshold is applicable. In addition to demonstrating that an action threshold can be coupled to strategies and tactics that use fungicides more efficiently, the threshold approach also makes clear how sanitation practices can be integrated to complement fungicide dose. Using thresholds to make scab management decisions should be applicable to other regions, but because of differences in cultivar, planting density, tree height and shape, overwintering conditions, weather patterns, and economics, similar studies must be conducted in each region to determine their feasibility and to establish the thresholds.

LITERATURE

Gadoury, D.M. & W.E. MacHardy 1984. Integration of fungicide and insecticide applications in low inoculum orchards: 1983. *Fungic. Nematic. Tests* 39: 10.

Gadoury, D.M. & W.E. MacHardy 1985. Epidemiological analysis and the integration of fungicide and insecticide applications in low inoculum orchards: 1984. *Fungic. Nematic. Tests* 40: 6-7.

Gadoury, D.M. & W.E. MacHardy 1986a. Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. *Phytopathology* 76: 112-118.

Gadoury, D.M. & W.E. MacHardy 1986b. Integration of fungicide and insecticide applications in apple orchards. (Abstr.) *Phytopathology* 76: 652.

Gadoury, D.M., W.E. MacHardy & D.A. Rosenberger 1989. Integration of pesticide application schedules for disease and insect control in apple orchards of the northeastern United States. *Plant Dis.* 73: 98-105.

MacHardy, W.E. 1994. *Apple Scab: Biology, Epidemiology, and Management*. APS Press, St. Paul, MN. (in press).

MacHardy, W.E., D.M. Gadoury & D.A. Rosenberger 1993. Delaying the onset of fungicide programs for control of apple scab in orchards of low potential ascospore dose of *Venturia inaequalis*. *Plant Dis* 77: 372-375.

Mills, W.D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab. *Cornell Ext. Bull.* 630. 4 pp.

Mills, W.D. & A.A. LaPlante 1951. Diseases and insects in the orchard. *Cornell Ext. Bull.* 711. 100 pp.

Nutter, F.W. Jr., P.S. Teng & M.H. Royer 1993. Terms and concepts for yield, crop loss, and disease thresholds. *Plant Dis.* 77: 211-215.

Sutton, D.K. 1992. A potential role for orchard sanitation in the management of apple scab (*Venturia inaequalis* (Cke.) Wint.). M.S. thesis. University of New Hampshire, Durham, NH. 91 pages.

Sutton, D.K. & W.E. MacHardy 1993. The reduction of ascosporic inoculum of *Venturia inaequalis* by orchard sanitation. (Abstr.) *Phytopathology* 83:247.

van der Scheer, H.A.Th. 1980. Threshold of economic injury for apple powdery mildew and scab. Pages 49-52 in: *Integrated Control of Insect Pests in the Netherlands*. A.K. Minks and P. Guys, eds. Pudoc, Wageningen.

van der Scheer, H.A.Th. 1987. Supervised control of scab and powdery mildew on apple. *Obstbau Weinbau* 24: 249-251.

# Specific virulence of isolates of *Venturia inaequalis* on "susceptible" apple cultivars

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H. Sierotzki, M. Eggenschwiler, J. McDermott & C. Gessler 1994. Specific virulence of isolates of *Venturia inaequalis* on "susceptible" apple cultivars. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 83-93. ISSN 0802-1600.

Twenty-one single spore isolates, seven from each of the cultivars Boskoop, Spartan and James Grieve, were separated from primary lesions caused by ascospore infections in an orchard. The seven isolates from a particular cultivar were tested as a mixture on all cultivars. The host of origin of the isolates was the most important factor in determining the lesion type. Sporulating lesions developed on the host from which the isolates were derived. On the other hosts, symptoms were consistently either: no symptoms, slight chloroses, or, in particular combinations, necroses with slight sporulation. The 21 isolates were identified and distinguished by RAPD-markers. A mixture of conidia from all 21 isolates (equal concentrations for each isolate) was applied to the three cultivars and maintained for three asexual cycles on each cultivar. Fifty single conidial isolates were then separated from the lesions on each cultivar and the RAPD markers used to characterise them. The isolates originating from a cultivar other than the one on which the asexual cycles were passed, disappeared. These results indicate that cultivars recognised as being generally susceptible may impose strong selection on naturally occurring isolates of *Venturia inaequalis*.

Key words: Cross infection, *Malus x domestica*, RAPD-PCR., selection, *Venturia inaequalis* (Cke.) Wint.

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Early in this century, investigators had already recognised variation in severity of infection of apple scab by *Venturia inaequalis* (Cke.) Wint. on a particular apple cultivar, depending on the location (Aderhold 1899, Wiesmann 1931, Schmidt 1935, 1936a, 1936b, 1937, and Rudloff 1934). Wiesmann (1931) differentiated primary and secondary hosts for isolates taken from one cultivar. This system was extended to many cultivars used at this time.

Further studies (mainly in USA), which included crossing different isolates of *V. inaequalis*, led to the conclusion that pathogenicity is controlled by 19 different genes (Boone 1957, 1971).

Today five physiological races are distinguished by a set of differential cultivars, although all of the commonly used cultivars without a known specific resistance (Vf, Vr, Va, Vb, Vbj) should be susceptible to race 1 (Boone 1971, Williams 1968).

Most breeders nevertheless regard *V. inaequalis* as homogeneous for virulence or aggressiveness (Kellerhals 1991).

It is well recognised that resistant cultivars impose strong selection on naturally occurring populations of plant pathogens and virulent pathogens will usually appear, sooner or later (Wolfe et al. 1992, Leonard 1987), nullifying the efforts of breeders.

Based on the results of Wiesmann (1931) and on several field observations, Gessler (1989) postulated that the population of *V. inaequalis* consists of many different races. In this work we tried to confirm the presence of virulence specific to particular cultivars in natural *Venturia* populations, and to present the first data on the selection exerted on the *V. inaequalis* population by cultivars considered to be susceptible by using the RAPD-PCR method (Williams 1990) to identify isolates.

## MATERIAL AND METHODS

Twenty-one monospore isolates were made from primary lesions from Boskoop, James Grieve and Spartan. The trees were planted in an untreated orchard, alternating the three cultivars within the rows.

### Method of isolation

Spores of a primary lesion (assumed to be caused by ascospores) were washed off and diluted to a concentration of  $15-20 \times 10^4$  conidia/ml. The monospore cultures were made with a ten times diluted inoculum: One- $\mu$ l-drops were placed on water agar (1.2 % agar), spread and incubated for 24 h at 20 °C.

Under an inverted microscope (Olympus IMT-2), lone conidia were marked and transferred to a new agar plate (1.2 % agar, 1.5 % malt extract and 25  $\mu$ g/ml terramycin) and incubated at 20 °C. The resulting cultures were named Boskoop 1-7, James Grieve 1-7 and Spartan 1-7.

### Cross infection

Cross infections were made with inoculum collected in July from each of the cultivars Boskoop, James Grieve and Spartan ( $15-20 \times 10^4$  conidia/ml); each inoculum source was inoculated on to each of the three cultivars (one year old on rootstock M26). The incubation conditions were 19 °C and 100% relative humidity for at least 24 h. The macroscopic symptoms were classified after the 17th day of incubation (Table 1).

Table 1: Classification system of the macroscopic symptoms of infection of *Malus x domestica* with *Venturia inaequalis*

Score	Symptoms
0=	no visible symptom
1=	small, chlorotic flecks
2=	small, chlorotic or weak sporulating flecks
3=	well sporulating but weak chlorotic lesions
4=	obvious and strong sporulating lesions

### **Mixed population trial**

Spores of the 21 collected lesions were mixed in equal amounts (final concentration  $15\text{-}20 \times 10^4$  conidia/ml). The three cultivars were inoculated with this inoculum and, after lesions appeared, the conidia were washed off and each cultivar was inoculated with its "own" inoculum. After three asexual cycles, 50 monospore isolations were made from the inoculum from each cultivar (see above).

### **DNA extraction**

For DNA extraction the fungus was grown in liquid culture (PDA, 2.4 %) for about 3 weeks at 20 °C. The mycelium was washed three times with ice-cold sterile water, frozen at -80 °C for 1 h and lyophilised for 2 days.

The extraction method was a shortened protocol of the total-DNA mini-preparation of Zolan and Pukkila (1986) with the following modifications: the aqueous phase was extracted two times with chloroform:isoamylalcohol (24 : 1, v : v). The pellet was washed with 70 %-ethanol for 30 min, after isopropanol precipitation and centrifugation (15 min, 12000 x g); the ethanol was then poured off and the pellet allowed to dry. The pellet was resuspended in 100 µl TE-buffer (0.09 M Trisphosphat, 0.002 M EDTA, pH 7.4); the RNA digestion was not performed.

### **Amplification conditions**

Amplification reaction volumes were 25 µl containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM MgCl<sub>2</sub> (Stähelin, Basel), 100 µM each of dATP, dCTP, dGTP and TTP (Boehringer), 0.3 µM Primer, 5 ng of genomic DNA and 1 U SuperTaq DNA Polymerase (Stähelin, Basel). Amplification was performed in a Perkin Elmer Cetus Gene Amp PCR System 9600 programmed as follows: 2 cycles of 30 sec at 94°C, 30 sec at 36°C, 120 sec at 72°C; 20 cycles of 20 sec at 94°C, 15 sec at 36°C, 15 sec at 45°C, 90 sec at 72°C; 19 cycles of 20 sec at 94°C (increased 1 sec/cycle), 15 sec at 36°C, 15 sec at 45°C, 120 sec at 72°C (increased 3 sec/cycle), followed by 10 min at 72°C.

Amplification products were electrophoresed in 1.5 % agarose (Biorad) gels with 1 x TPE (0.09 M Tris-phosphate, 0.002 M EDTA) and stained with ethidium bromide. The following primers were used: Primer E 15 (ACGCACAACC), E 7 (AGATGCAGCC), U 19 (GTCAGTGCGG) and U 10 (ACCTCGGCAC) from Operon Technologies Inc. USA.

## **RESULTS AND DISCUSSION**

The ability of the scab pathogen to infect the apple host and the type of symptoms resulting were dependent on the origin of the inoculum and of the host cultivar. Strongly sporulating lesions were observed only on the same host cultivar as that from which the inoculum originated. This confirms what Wiesmann (1931), Schmidt (1935, 1936a, 1936b, 1937) and Rudloff (1934) found for several cultivars that are now little known. The three cultivars that we used, Boskoop, James Grieve and Spartan, are known to be susceptible but populations of the scab pathogen collected from them were, until now, not known to carry virulences specific to these cultivars. On a non-appropriate host, a sub-population can cause minor symptoms in particular host-pathogen combinations (Table 2).

Table 2. Symptoms<sup>1)</sup> caused by *V. inaequalis* spores collected in July from scabbed leaves of the three varieties Boskoop, James Grieve und Spartan

Origin of inoculum	Inoculated host cultivar		
	Boskoop	James Grieve	Spartan
Boskoop	4	1	2
James Grieve	1	3	2
Spartan	0	2	4

<sup>1)</sup> Symptoms: 4, obvious and strong sporulating lesions; 3, well sporulating but weak chlorotic lesions; 2, small, chlorotic or weak sporulating flecks; 1, small, chlorotic flecks; 0, no visible symptoms. Three trees with 4 inoculation spots were classified. No deviations were observed

Arbitrary decamer primers can be used to generate amplified segments of genomic DNA that can differentiate *V. inaequalis* isolates. By pre-screening 60 10-mer primers we found four primers that gave enough information to differentiate 17 of the 21 collected isolates. The arbitrarily primed DNA profile of the 21 *V. inaequalis* isolates with primers E 15, E 7, U 19 and U 10 led to a system of 18 consistent polymorphic bands to identify all 21 isolates except Sp 1, 4, 6, 7 (Figures 1,2,3,4,5). These isolates were indistinguishable even with six other primers (U 1, U 3, U 16, J 12, J 20). It is likely that these four isolates were already secondary infections or that they originated from overwintered conidia. By selecting only strongly (and therefore consistently) amplified DNA segments as informational bands, variation in minor bands resulting from different amplifications can be excluded (Figures 1,2,3,4).

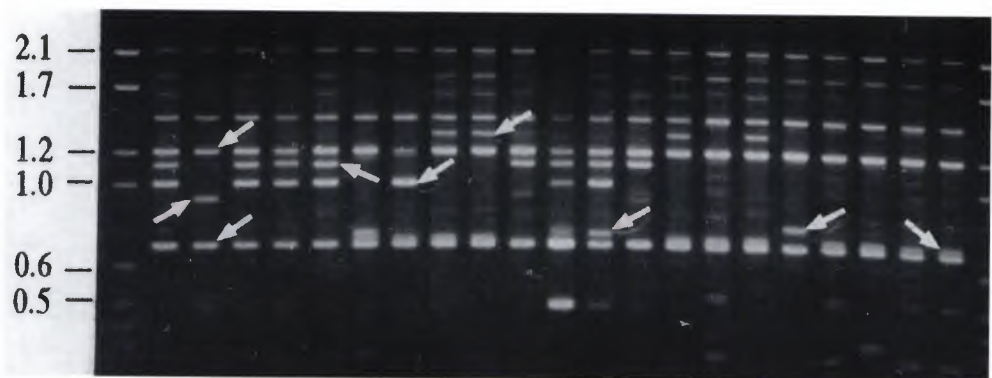


Figure 1. Banding pattern of all 21 isolates in a simultaneous PCR using primer E 15. The arrows indicate the consistent polymorphic DNA segments used for differentiation. The DNA concentration was 5 ng in 15  $\mu$ l reaction volume. The size of the bands of the marker on the right side is given in kbp.



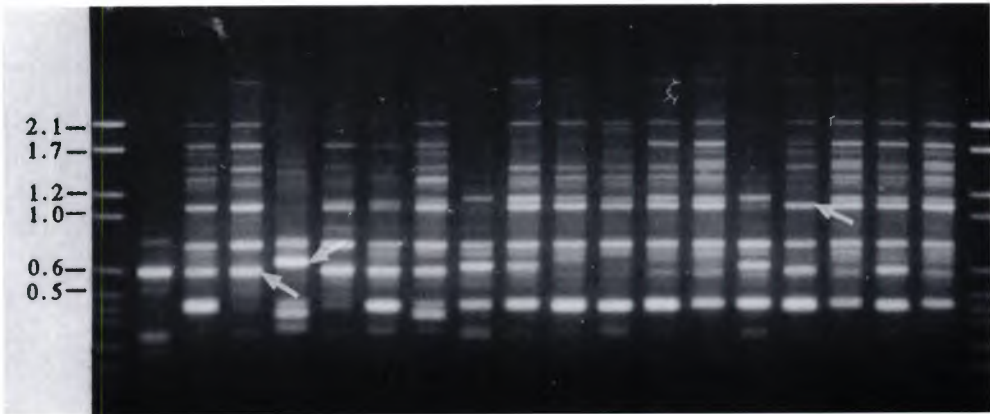


Figure 2. Banding pattern of the isolates Boskoop 1,2,3,4,5,6,7, James Grieve 2,3,4,5,6 and Spartan 1,2,3,4,5,6 in a simultaneous PCR using primer U19. The arrows indicate the consistent polymorphic DNA segments used for differentiation. The DNA concentration was 5 ng in 15  $\mu$ l reaction volume. The size of the bands of the marker on the right side is given in kbp.

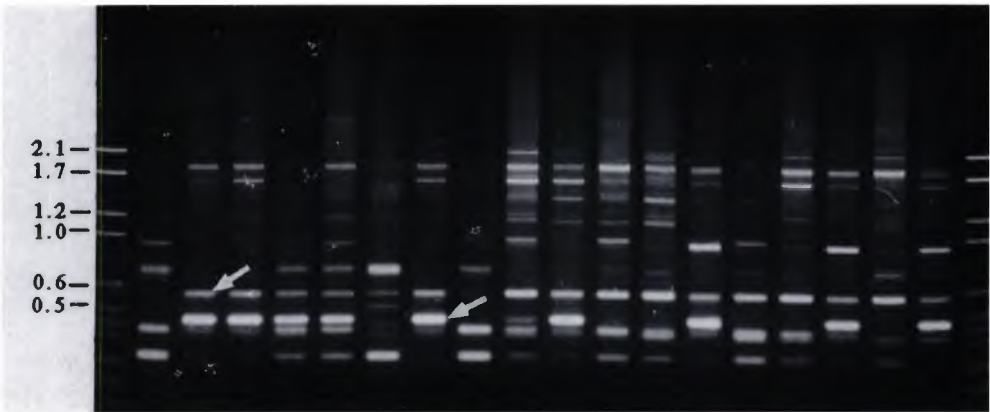


Figure 3. Banding pattern of the isolates Boskoop 1,2,3,4,5,6,7, James Grieve 2,3,4,5,6 and Spartan 1,2,3,4,5,6 in a simultaneous PCR using primer E 7. The arrows indicate the consistent polymorphic DNA segments used for differentiation. The DNA concentration was 5 ng in 15  $\mu$ l reaction volume. The size of the bands of the marker on the right side is given in kbp.

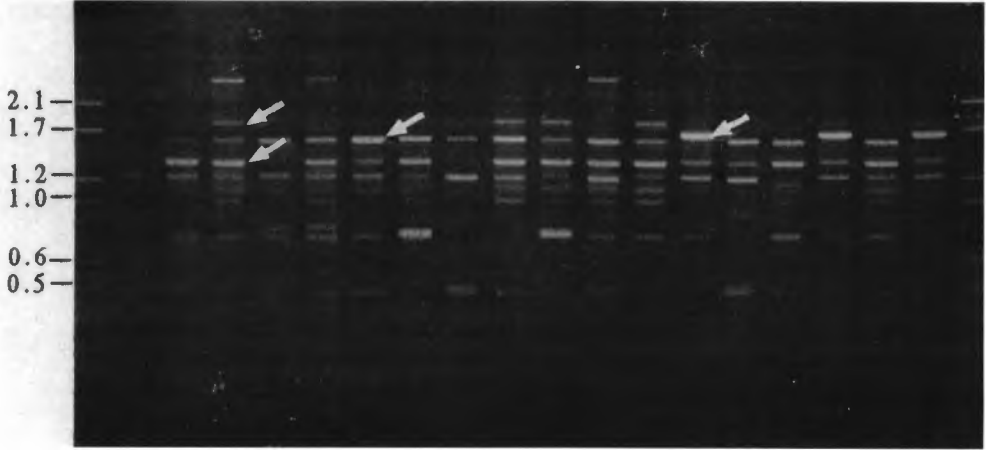


Figure 4. Banding pattern of the isolates Boskoop 1,2,3,4,5,6,7, James Grieve 2,3,4,5,6 and Spartan 1,2,3,4,5,6 in a simultaneous PCR using primer U10. The arrows indicate the consistent polymorphic DNA segments used for differentiation. The DNA concentration was 5 ng in 15  $\mu$ l reaction volume. The size of the bands of the marker on the right side is given in kbp.

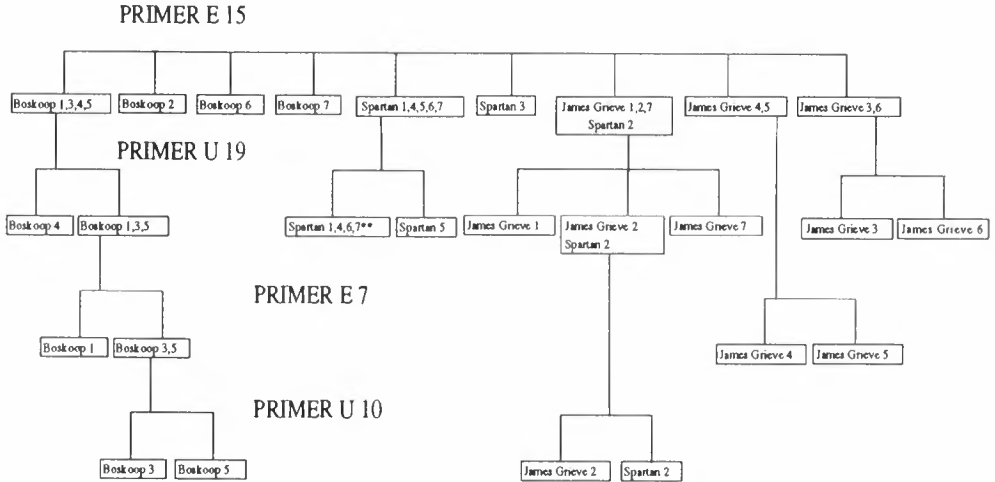


Figure 5. Identification of the 21 isolates collected on three different apple varieties (Boskoop, James Grieve and Spartan) with RAPD-PCR with the primers E 15, U 19, E 7 and U 10 (Operon Technologies, USA). The isolates in each rectangle were not different with the particular primer.

\*\* These isolates were not even distinguishable with the primers U 1, U 3, U 5, U 16, J 12 and J 20.

By applying a mixed inoculum of conidia originating from the three cultivars, followed by three cycles on a particular cultivar, it was possible to detect a shift in the composition of the three populations of *V. inaequalis* (Figure 9). The re-isolations gained after three

asexual cycles were all related to one of the isolates. Some monospore cultures were contaminated so that from Boskoop and James Grieve, 46, and from Spartan, 32, isolates were evaluated. The shift was dependent on the host cultivar. On Boskoop, isolates Boskoop 2 and 3, both originally from Boskoop, dominated. Non-Boskoop isolates disappeared completely. Similarly on the other two cultivars only the isolates specific to a particular cultivar survived although some of these also disappeared. The starting frequency of all isolates was 4.76 %; the four indistinguishable isolates from Spartan (1,4,6 and 7) had a frequency of 19.05 %. After the three asexual propagation cycles, eight isolates disappeared completely (Boskoop 1 and 6; James Grieve 1, 2, 4 and 7; Spartan 2 and 3). Five further isolates appeared at a low frequency (Boskoop 4, 5 and 7; James Grieve 3; Spartan 5). The five remaining isolates showed an increase in frequency (Boskoop 2 and 3; James Grieve 5 and 6; the group of Spartan 1, 4, 6 and 7) (Figures 6,7,8).

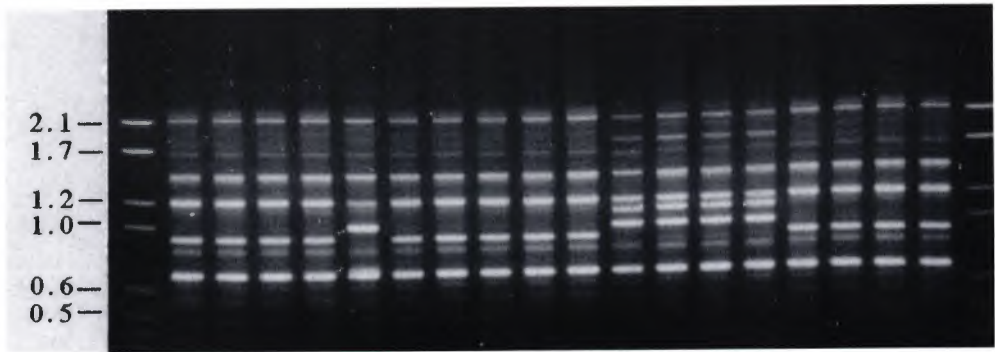


Figure 6. Amplified DNA segments of the first 18 monospore isolates, isolated after three propagation cycles on Boskoop starting from a pool inoculum. The amplification was made with primer E 15 in the same PCR run. The size of the bands of the marker on the right side is given in kbp.

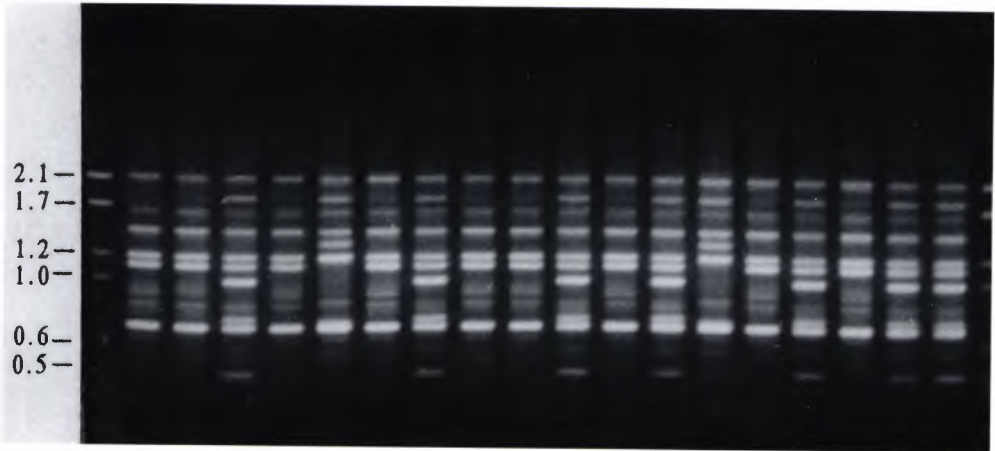


Figure 7. Amplified DNA segments of the first 18 monospore isolates, isolated after three propagation cycles on James Grieve starting from a pool inoculum. The amplification was made with primer E 15 in the same PCR run. The size of the bands of the marker on the right side is given in kbp.

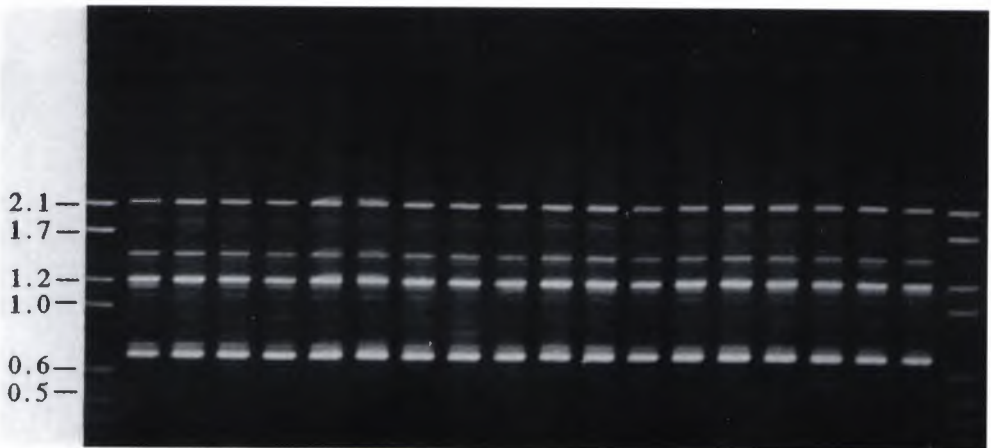


Figure 8. Amplified DNA segments of the first 18 monospore isolates, isolated after three propagation cycles on Spartan starting from a pool inoculum. The amplification was made with primer E 15 in the same PCR run. The size of the bands of the marker on the right side is given in kbp.

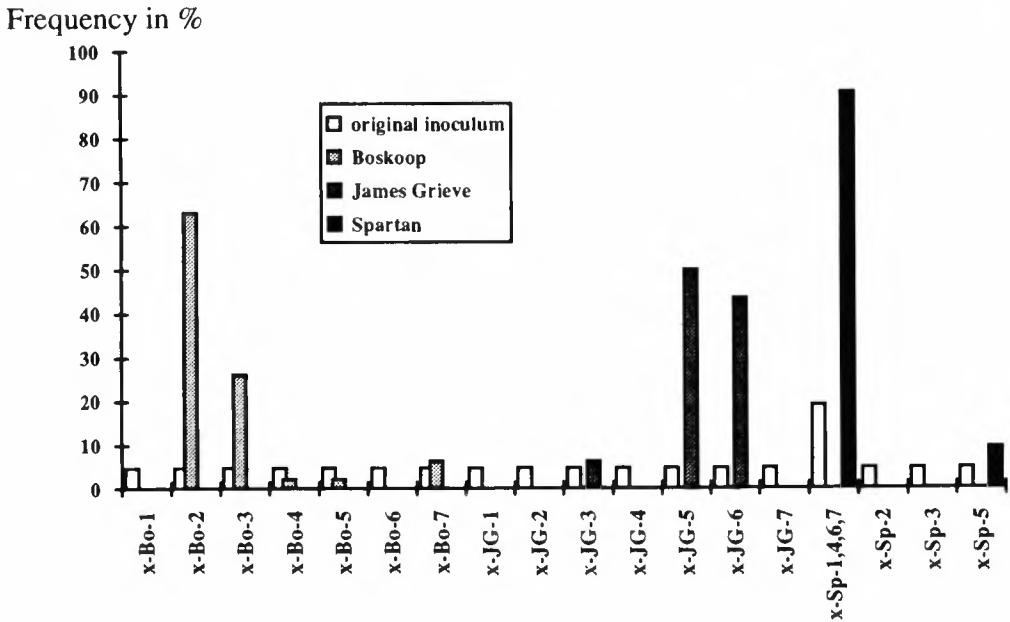


Figure 9. Frequency of single spore isolates after three asexual cycles on Boskoop, James Grieve and Spartan starting from a mixed inoculum of equal amounts of conidia from 21 isolates (7 from each variety). The isolates were identified with RAPD-PCR. The isolates Spartan 1, 4, 6, 7 showed no differences.  
 x-Bo 1-7: Isolates originating from Boskoop (Frequency out of 46)  
 x-JG 1-7: Isolates originating from James Grieve (Frequency out of 46)  
 x-Sp 1-7: Isolates originating from Spartan (Frequency out of 32)

The observation that only the isolates originating from a particular cultivar survived on that cultivar leads to the conclusion that each cultivar imposes strong selection so that only the isolates with the appropriate virulence are able to survive. The second observation, that even cultivar specific isolates disappeared, could be explained by the assumption that within a particular pathotype there are different degrees of fitness (aggressiveness) among the isolates. More research is needed to verify this hypothesis.

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REFERENCES

Aderhold, R. 1899. Auf welche Weise können wir dem immer weiteren Umsichgreifen des Fusicladiums in unseren Apfelkulturen begegnen, und welche Sorten haben sich bisher dem Pilze gegenüber am widerstandsfähigsten gezeigt? Pomologische Monatshefte, XLV: 266-272.

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- Boone, D.M. & G.W. Keitt 1957. *Venturia inaequalis* (Cke.) Wint. XII. Genes controlling pathogenicity of wild-type lines. *Phytopathology* 47: 403-409.
- Boone, D.M. 1971. Genetics of *Venturia inaequalis*. *Ann. Rev. Phytopathology* 9: 297-318.
- Gessler, C. 1989. Genetics of the interaction *Venturia inaequalis*-*Malus*: the conflict between theory and reality. p. 168-190. In: C. Gessler, D. Butt and B. Koller (eds.), 1989. Integrated control of pome fruit diseases. Vol II. *WPRS-Bulletin XII/6*: 346 pp.
- Kellerhals, M. 1991. Apfelzüchtung in Wädenswil. *Erwerbsobstbau* 33: 219-224.
- Leonard, K.J. 1987. The host population as selective factor. p. 163-179. In: M.S. Wolfe & C.E. Caten (eds), *Population of Plant Pathogens: their Dynamics and Genetics*. Blackwell Scientific Publications, Oxford.
- Rudloff, C.F. 1934. *Venturia inaequalis* (Cooke) Aderhold. III. Zur Formenmannigfaltigkeit des Pilzes. *Gartenbauwiss.* 9: 105-119.
- Schmidt, M. 1935. *Venturia inaequalis* (Cooke) Aderhold. IV. Weitere Beiträge zur Rassenfrage beim Erreger des Apfelschorfes. *Gartenbauwiss.* 9: 364-389.
- Schmidt, M. 1936a. *Venturia inaequalis* (Cooke) Aderhold. V. Weitere Untersuchungen über die auf verschiedenen Bäumen lebenden Populationen des Apfelschorfpilzes. *Gartenbauwiss.* 10: 422-427.
- Schmidt, M. 1936b. *Venturia inaequalis* (Cooke) Aderhold. VI. Zur Frage nach dem Vorkommen physiologisch spezialisierter Rassen beim Erreger des Apfelschorfes. Erste Mitteilung. *Gartenbauwiss.* 10: 478-499.
- Schmidt, M. 1936. *Venturia inaequalis* (Cooke) Aderhold. VIII. Weitere Untersuchungen zur Züchtung schorf widerstandsfähiger Apfelsorten. *Der Züchter* 10: 280-291.
- Schmidt, M. 1937. *Venturia inaequalis* (Cooke) Aderhold. VII. Zur Morphologie und Physiologie der Widerstandsfähigkeit gegen den Erreger des Apfelschorfes. *Gartenbauwiss.* 11: 221-230.
- Wiesmann, R. 1931. Untersuchungen über Apfel- und Birnenschorfpilz *Fusicladium dendriticum* [Wallr.] Fuckel und *Fusicladium pirinum* [Lib.] Fuckel sowie die Schorfanfälligkeit einzelner Apfel- und Birnensorten. *Landwirtschaftliches Jahrbuch der Schweiz* 1931: 109-156.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski & S.V. Tingey 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* 18(22): 6531-6535.

Williams, E.B. & A.G. Brown 1968. A new physiologic race of *Venturia inaequalis*, incitant of apple scab. *Plant Disease Repr.* 52: 799-801.

Wolfe, M.S., U. Brändle, B. Koller, E. Limpert, J.M. McDermont, K. Müller & D. Schaffner 1992. Barly mildow in Europe: population biology and host resistance. *Euphytica* 63: 125-139.

Zolan, M.E. & P.J. Pukkila 1986. Inheritance of DNA methylation in *Coprinus cinereus*. *Mol. Cell. Biol.* 6: 195-200.





# A new race of *Venturia inaequalis* overcomes apple resistance due to the Vf gene

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The gene Vf from *Malus floribunda* 821 for resistance to scab (*Venturia inaequalis*), has been used successfully for 50 years in apple breeding programmes. Since 1984, scab symptoms have been observed in the field at Ahrensburg, Germany, on seedlings of apple cv. Prima that had been selected as resistant in the greenhouse. In 1988, small scab lesions were found on some Vf selections in the same orchard. The inoculum from Ahrensburg was compared with the inoculum currently used at Angers, France, for selecting apple seedlings for resistance to *V. inaequalis*. All Vf-gene cvs. or selections tested were susceptible to the Ahrensburg inoculum, whereas *M. floribunda* 821 itself and the ornamental crabapple Evereste were resistant. The progeny from a cross between a resistant (Vf) and a susceptible cv. segregated into the 5 expected classes to Angers inoculum, but were completely susceptible to Ahrensburg inoculum. These results indicate the urgency of diversifying the sources of resistance to *V. inaequalis* in new breeding strategies. The distinction should be made between the resistance of *M. floribunda* 821, that is resistant to Ahrensburg inoculum, and that of the named cvs. and selections that are susceptible. The new race of *V. inaequalis* is named race 6.

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Resistance to *V. inaequalis*, coded by the Vf gene originating from *Malus floribunda* clone 821, has been used in breeding for the last 50 years. Beginning with the resistant hybrids selected in the United States, breeders in several countries released 30 scab-resistant cultivars (cvs) between 1970 and 1989 (Lespinasse 1990). However, those cvs have not been grown on a large scale.

Vf resistance has been considered durable, because Vf cvs have been free of scab for over 50 years in the various countries where they have been grown.

Prima susceptibility to *V. inaequalis* was first reported from Moldavia in 1981 (Fischer et al. 1983). However, this report was not confirmed and, in contrast, a later article reported continuing resistance of Prima in Moldavia (Indenko et al. 1986).

In 1988 at Ahrensburg, Germany, symptoms of scab were observed in the orchard

on cultivars Prima, Coop 7, 9 and 10 and on some seedlings previously selected as resistant in the greenhouse. The first progeny (Prima x A 143/24) was more susceptible than that of Prima x Klon 40. With the latter progeny, 20% of the plants remained scab-free even during years with a high scab pressure (1985-1987). Nevertheless, one tree (no. 53) from this cross showed scab symptoms annually from 1984 and was severely attacked in 1984, 86 and 87. All the progeny of Prima x A 143/24 were infected in 1985 and 1987, whereas all the trees were scab free in July, 1982. Tree no. 22 from this progeny showed scab symptoms every year from 1983 (Krüger 1991; Parisi et al. 1993).

These orchard observations challenged the idea of stability of resistance coded by the Vf gene.

Under a cooperative research program between France (INRA-Angers) and Germany (Institut für Zierpflanzenzüchtung), studies on the variability of *V. inaequalis* populations were undertaken in both countries. One of the priorities of this program was to study the pathogenicity of Ahrensburg inoculum under controlled conditions and to determine whether *V. inaequalis* isolates from Ahrensburg could overcome the resistance coded by the Vf gene.

## MATERIALS AND METHODS

### Laboratory and growth-chamber experiments

#### Plants

Grafted potted trees and young apple seedlings were used. Nineteen clones were grafted onto seedling rootstocks and grown in pots in the greenhouse (Table 1). The mean number of leaves per shoot was 9-11 when the plants were inoculated.

Table 1. Characteristics of 19 apple clones inoculated with *Venturia inaequalis*

Clone	Name or code number	Characteristic
X 972	Golden Delicious	susceptible to scab
X 4238	Topred	susceptible to scab
X 2596	Prima	Vf resistant
X 2775	Florina	Vf resistant
X 3183	Baujade	Vf resistant
X 2851	Priscilla	Vf resistant
X 2811	Liberty	Vf resistant
X 4171	NY 61345-2	Vf resistant
X 3191	Idared x Prima	Vf resistant
X 6246	1082-201 x Prima	Vf resistant
X 6247	Britemac x Prima	Vf resistant
X 6517	Coop 28	Vf resistant
X 6518	<i>Malus floribunda</i> 821	Progenitor of all the Vf selections
X 2369	<i>Malus x "Perpetu"</i> Evereste	Ornamental crab apple, scab resistant
X 2225	9-AR2T196 (Vm/vm)	Differential host for race 5 (h5)
X 2250	TSR34T132	Differential host for race 2 (h2)
X 4811	Dolgo	Differential host for race 2 (h2)
X 2249	TSR33T239	Differential host for race 4 (h4)
X 2253	Geneva	Differential host for race 3 (h3)

Apple seedlings originated from 3 crosses:

- 1 - a cross between two susceptible cvs., Golden Delicious and Granny Smith.
- 2 - a cross between cv. Chevalier Jaune (scab susceptible) and cv. Baujade (Vf-resistant).
- 3 - a cross between cv. Melrose (scab susceptible) and cv. Florina (Vf-resistant).

The methods used to obtain the seedlings were described previously (Olivier & Lespinasse 1980). The progeny of each cross was randomly divided into 2 or more batches, and each subpopulation tested with one type of inoculum or one single strain; the seedlings had 2-3 leaves fully expanded when inoculated.

#### *Inocula*

In July 1988, *V. inaequalis*-infected leaves were collected from seedlings of cv. Prima, in an orchard at the Ahrensburg research station. Ten monoconidial isolates were obtained either from tree no. 22 from the cross between Prima and A 143/24 (Jonathan x *Malus zumi* o.p.), or from tree no. 53 from the cross between Prima and Klon 40 (a seedling from open-pollinated cultivar Goldparmäne) (Table 6). These isolates were grown on malt agar medium (1% Cristomalt, Difal, Villefranche-sur-Saône, France). Eight isolates were selected for high sporulation. A conidial suspension was obtained from each isolate by Keitt and Palmiter's technique (1938). A conidial suspension of each isolate was applied to young apple seedlings from the cross Golden Delicious x Granny Smith. Leaves with abundant sporulation were rinsed in distilled water to obtain a conidial suspension of each isolate. The inoculum "Ahrensburg" was a mixture of the 8 isolates no. 298, 300, 302 to 307 in equal proportions (Table 6). The inoculum "Angers" was composed of a mixture of aggressive isolates from different french orchards. This inoculum is currently used at INRA-Angers to select scab-resistant apple seedlings and stored on frozen apple leaves. The inocula were each adjusted to a concentration of  $2-3 \times 10^5$  conidia/ml. Each monoconidial strain was also tested separately and compared with the reference strain no. 104 (Table 6).

#### *Inoculation*

The inoculation method and the incubation conditions are those used currently at INRA-Angers (Parisi et al. 1993).

#### *Symptom assessment*

Symptom assessment on potted trees was performed 14 to 17 days after inoculation; all leaves were assessed on each shoot and severity was evaluated using a grading scale derived from Croxall et al. (1952) (Table 2). The results were recorded simply on the basis of the presence or absence of symptoms and then expressed as the percentage of scabbed leaves. Disease severity for each clone was expressed by the median obtained from the scores of all leaves. For each clone, 2 to 6 trees were evaluated (representing 4-22 shoots and 58-280 leaves/clone).

Table 2. Grading scale used for the evaluation of severity of infection by *Venturia inaequalis*

Score	Percentage of the leaf area with sporulating symptoms (las)
0	no symptoms
1	> 0
2	> 1
3	> 5
4	> 10
5	> 25
6	> 50
7	> 75

Ten days after inoculation, the young seedlings were classified according to the grading system of Hough et al. (1953): - class 1: pin-point pits with no sporulation; class 2: irregular chlorotic or necrotic lesions with no sporulation; class 3: few restricted sporulating lesions; class M: intermediate between class 2 and class 3; class 4: susceptible reaction, extensive and abundantly sporulating lesions.

The susceptible plants of class 4 were eliminated and a second inoculation was performed to confirm the first one. The results were expressed as the percentage of plants in each class.

### Orchard observations

Symptoms of scab on seedlings from the cross Prima x A143/24 were evaluated twice a year at Ahrensburg (Germany) in 1991 and 1992. The resistance of several V-resistant cvs. and selections was also evaluated in 1992. Incidence and severity were recorded and plants were graded as: healthy, slightly infected, moderately infected, or severely infected.

## RESULTS

The goal of the first experiment was to study the pathogenicity of the inoculum collected in the Ahrensburg orchard. The results showed that this inoculum was able to induce symptoms on all the cvs. and Vf-resistant selections tested (Table 3). However, disease incidence and severity varied among the cvs. and selections. The cv. Golden Delicious was the most susceptible, but did not differ significantly from Florina and X 3191, which both contain the Vf gene. Prima expressed intermediate susceptibility to Ahrensburg inoculum, whereas the two selections X 6246 and X 6247 were least susceptible on the basis of both incidence and severity.

Table 3. Pathogenicity of Ahrensburg *Venturia inaequalis* inoculum to Vf apple clones

Cv. or clone	Presence of symptoms	Incidence		Severity
		Percentage of scabbed leaves <sup>†</sup>		Score
Golden Delicious	+	41.2	a	3
Florina	+	31.3	ab	3
X 3191	+	31.3	ab	3
Prima	+	20.7	b	2
X 6247	+	7.1	c	2
X 6246	+	6.9	c	1

y: Percentages followed by the same letter do not differ significantly by t-test at P = 0.05

In the second experiment, involving more cvs. and Vf selections, including the original source of the Vf gene *M. floribunda* 821 and also the differential hosts for races 2, 3, 4 and 5 (Table 4), inoculated cvs. and selections were susceptible to the Ahrensburg inoculum but they were resistant to the Angers inoculum. In contrast, *M. floribunda* 821 and the ornamental crabapple Evereste were resistant to both inocula. No symptom was ever observed on the differential hosts, whatever the inoculum. Disease incidence and severity on a susceptible host were consistently higher with Angers inoculum than with the Ahrensburg inoculum.

Table 4. Comparison of the pathogenicity of *Venturia inaequalis* inocula Angers and Ahrensburg to apple clones

Cv. or clone	Presence of symptoms of susceptibility	
	Angers inoculum	Ahrensburg inoculum
Golden Delicious	+	+
Topred	+	+
Baujade	-	+
Coop 28	-	+
Florina	-	+
Liberty	-	+
Priscilla	-	+
X 4171	-	+
Evereste	-	-
<i>Malus floribunda</i> 821	-	-
X 2225 (h5)	-	-
X 2250 (h2)	-	-
X 4811 (h2)	-	-
X 2249 (h4)	-	-
X 2253 (h3)	-	-

The difference in pathogenicity of the two inocula was confirmed by a study of the reaction of two progenies to inoculation (Table 5). All progeny from Golden Delicious x Granny

Smith were susceptible to both inocula. The progeny from Chevalier Jaune x Baujade (susceptible x Vf resistant) segregated into the 5 different classes with the Angers inoculum, but were all susceptible to the Ahrensburg inoculum.

Table 5. Effect of the source of *Venturia inaequalis* inoculum on the percentage of plants in the different classes of symptoms in two apple progenies

Inoculum	Percentage of plants in each class and total (T) population											
	Angers						Ahrensburg					
	1	2	M	3	4	T	1	2	M	3	4	T
Chevalier Jaune x Baujade	1	2.9	33.2	7.8	55.1	488	0	0	0	0	100	435
Golden Delicious x Granny Smith	0	0	0	0	100	144	0	0	0	0	100	141

The study of the pathogenicity of each single strain which composed the Ahrensburg inoculum (Table 6) pointed out two different kinds of strains. The strains from tree no. 53 of the progeny Prima x Klon 40 were not significantly different from strain 104 when inoculated on the progeny of a cross between Florina x Melrose (Table 7). Strain 104 is a standard from the INRA-Angers collection, avirulent on the Vf gene, which gave on this cross a segregation close to 1:1 in the two resistant and susceptible groups (Table 7). In contrast, all the strains from the tree no. 22 of the progeny Prima x A143/24 gave a different segregation, with a large majority of the plants in the susceptible classes (Table 7).

Table 6. Strains of *Venturia inaequalis* tested for their pathogenicity on two apple progenies

Strain	Origin	Host variety or hybrid	Characteristics
104	Saint Lézin, France, 1978	Golden Delicious	Race 1 reference strain <sup>c</sup>
298	Ahrensburg, Germany, 1988	81/19-53 <sup>a</sup>	Race 1
299	Ahrensburg, 1988	81/19-53	Race 1
300	Ahrensburg, 1988	81/19-53	Not tested
301	Ahrensburg, 1988	81/19-53	Race 1
302	Ahrensburg, 1988	81/11-22 <sup>b</sup>	Race 6
303	Ahrensburg, 1988	81/11-22	Race 6
304	Ahrensburg, 1988	81/11-22	Race 6
305	Ahrensburg, 1988	81/11-22	Race 6
306	Ahrensburg, 1988	81/11-22	Race 6
307	Ahrensburg, 1988	81/11-22	Race 6

a: Tree n° 53 of the progeny Prima x Klon 40

b: Tree n° 22 of the progeny Prima x A143/24

c: Reference strain from INRA-Angers collection, avirulent on Vf hybrids and on differential hosts of the four known *V. inaequalis* races

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Table 7. Percentage of plants in the different classes of symptoms in two apple progenies inoculated with three *Venturia inaequalis* monoconidial strains. R = classes 1, 2, M and S = classes 3 and 4 of the Hough et al. (1953) grading scale

Strain	Percentage of plants in each group of classes and total population								
	104			301			302		
Cross	R	S	T	R	S	T	R	S	T
Florina x Melrose	52.4	47.6	63	48.5	51.5	130	1.5	98.5	69
Golden Delicious x Granny Smith	0	100	31	0	100	640	0	100	61

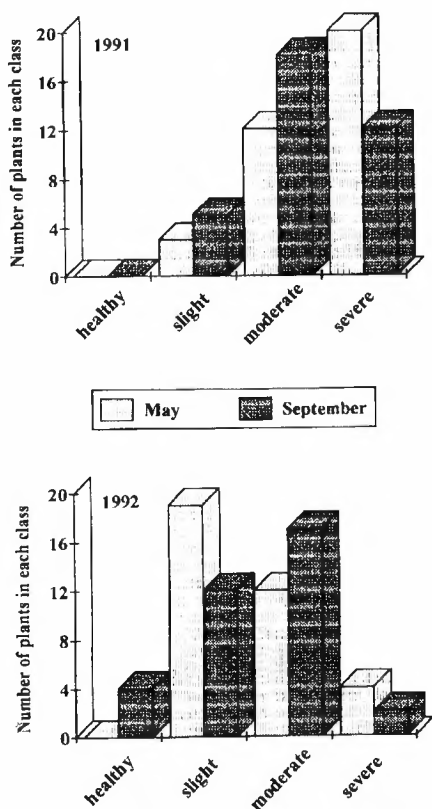


Figure 1. Severity of scab in the progeny Prima x A143/24 in May and September 1991 and 1992

The orchard observations made in Ahrenburg in 1991 and 1992 showed that the progeny Prima x A143/24, which had been selected as resistant to scab in the greenhouse, was

entirely susceptible in 1991 and in May 1992 (Fig. 1). In May 1991, the majority of the progeny was severely attacked, while in May 1992 the majority was slightly attacked. In 1991, of the 22 Vf-resistant selections or cvs. planted in the Ahrensburg orchard, seven only were free of scab; the 15 others had symptoms of the disease, even if only slight on most of them (Table 8).

Table 8. Scab infection at Ahrensburg in 1991 on different Vf clones or cultivars

SEVERITY OF SCAB INFECTION			
Healthy	Slight	Moderate	Severe
Coop 12	Coop 2 (Prima) <sup>a</sup>	Coop 2 (Prima) <sup>b</sup>	A 163/42
Coop 13	Coop 6	HAR 13 T 57	Coop 8
Liberty	Coop 7	5002	Coop 9
Ny 66-300-42	Coop 10		
Ny 62-306-10	Florina		
Priam	Freedom		
TSR 15 T 3	HAR 20 T 106		
	P 22 R 17-66		
	Priscilla		
	Sir Prize		

a - Young tree (orchard planted in 1989)

b - Tree different and older than a

The orchard observations made in Ahrensburg in 1991 and 1992 showed that the progeny Prima x A143/24, which had been selected as resistant to scab in the greenhouse, was entirely susceptible in 1991 and in May 1992 (Fig. 1). In May 1991, the majority of the progeny was severely attacked, while in May 1992 the majority was slightly attacked. In 1991, from the 22 Vf resistant selections or cvs. planted in the Ahrensburg orchard, 7 only were free of scab; the 15 others showed symptoms of the disease, even if slight for most of them (Table 8).

## DISCUSSION

These results clearly demonstrate that the Vf resistance present in recently released cvs. and selections is overcome by *V. inaequalis*. However, the resistance carried by *M. floribunda* 821 and that of Evereste has remained effective.

The Ahrensburg inoculum, which induces symptoms on all the Vf-resistant selections tested, has a specific pathogenicity compared to the Angers inoculum; the latter does not induce symptoms on any of the same hybrids. The differential virulence of the Ahrensburg inoculum is confirmed by the study of the progeny of Chevalier Jaune x Baujade.

It is significant that *M. floribunda* 821, the progenitor of all the current Vf selections, still appears to be resistant. This might be related to the long breeding process from *M.*



*floribunda* 821 to the release of new cvs., and could indicate that *M. floribunda* 821 resistance was rapidly eroded or partly lost.

The resistance of the ornamental crab apple Evereste to the Ahrensburg inoculum must also be stressed. Nevertheless, it is not known if the resistance of Evereste is due solely to the Vf gene; Evereste is a seedling from an open pollination of a Vf hybrid (Decourtye, 1977).

Although the results obtained in the growth chamber show clearly the susceptibility of all the recently released Vf selections and cvs., until 1990 most of the Vf selections present in the Ahrensburg orchard remained scab free (Parisi et al. 1993). The main reason of this change in behaviour could be the inoculum, if different in the two situations. Therefore, it seems important to know exactly the composition of the Ahrensburg inoculum used in the growth chamber experiments. Even though one of the 8 strains was not tested, the results show that all the strains from the tree no. 53 were not virulent on the Vf gene, whereas all the strains from the tree no. 22 overcame this resistance. Our hypothesis is that the tree no. 53 is not a Vf-resistant hybrid, while the tree no. 22 has the Vf gene. So, the Ahrensburg inoculum was composed of 2 strains avirulent on the Vf gene and 6 virulent strains.

This inoculum is probably different from Ahrensburg's orchard *V. inaequalis* population, and so explains the differences between the growth chamber and orchard observations. But if the fitness of the virulent strains is good, their frequency could increase and induce field symptoms in the Vf hybrids that were susceptible in the growth chamber.

This hypothesis seems confirmed by the 1991 and 1992 observations in the Ahrensburg orchard, which point out the field susceptibility of the whole progeny of the cross Prima x A143/24 and of 15 Vf selections or hybrids.

We propose that the new race of *V. inaequalis*, that overcomes the Vf gene, be named race 6; its differential virulence distinguishes it from races 2, 3, 4 and 5 previously identified (Shay & Williams 1956; Williams & Brown 1968). The results show that this new race spreads, even if slowly, in the orchard.

The results emphasize an urgency for defining new breeding strategies. Currently, out of 34 scab-resistant cvs., 30 possess the Vf gene, and 2 the Vm gene which is overcome by *V. inaequalis* race 5. It is very important to diversify the sources of resistance and to combine independent genes, or polygenic and monogenic resistance, in the same cv. Further studies of the variability of *V. inaequalis* pathogenicity in different countries and on the stability of the other sources of resistance seem necessary in order to select durable scab resistance.

## REFERENCES

- Croxall, H.E., D.C. Gwynne & J.E.E. Jenkins 1952. The rapid assessment of apple scab on leaves. *Plant Pathol.*, 1: 39-41.
- Decourtye, L. 1977. "Evereste", un nouveau pommier d'ornement. *L'Horticulture Française* 85: 9-10.

Fischer, C., V. Bukartschuk, A. Bondarenko & E. Artamonova 1983. Erste Ergebnisse zur Stabilität der Schorfresistenz beim Apfel unter verschiedenen ökologischen Bedingungen in der UdSSR und DDR-vorläufige Mitteilung. Archiv Gartenbau 31: 236-264.

Hough, L.F., J.R. Shay & D.F. Dayton 1953. Apple scab resistance from *Malus floribunda* Sieb. Proc. Am. Soc. Hort. Sci. 62: 805-820.

Indenko, I., A.R. Rasulov & K.H.B. Gutov 1986. Highly scab resistant apple varieties Prima and Priscilla. Sadovod. Vinograd. Moldavii, 11: 36-36 (summary).

Keitt, G.W. & D.H. Palmiter 1938. Heterothallism and variability in *Venturia inaequalis*. Am. J. Bot. 25: 338-345.

Krüger, J. 1991. Schorfbefall von Nachkommen aus Kreuzungen mit der schorfresistenten Apfel Sorte "Prima". Z. Pflanzenkrankheiten Pflanzenschutz 98: 73-76.

Lespinasse, Y. 1990. La pomme: l'amélioration génétique. Arboric. Fruit. 434: 17-32.

Olivier, J.M. & Y. Lespinasse 1980. Etude de l'efficacité de fongicides antitavelures après contamination de pommiers en serre. 1 - Méthode d'étude sur jeunes semis. Phytiat. Phytopharm. 29: 13-22.

Parisi, L., Y. Lespinasse, J. Guillaumes & J. Krüger 1993. A New Race of *Venturia inaequalis* Virulent to Apples with Resistance due to the Vf Gene. Phytopathology 83: 533-537.

Shay, J.R. & E.B. Williams 1956. Identification of three physiologic races of *Venturia inaequalis*. Phytopathology 46: 190-193.

Williams, E.B. & A.G. Brown 1968. A new physiologic race of *Venturia inaequalis*, incitant of apple scab. Plant Dis. Rept. 52: 799-801.

# Cultivar mixtures in apple orchards as a mean to control apple scab?

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Early and recent studies showed that populations of *Venturia inaequalis* are composed of races with different virulences against different cultivars. This information could be used to develop strategies for the control of apple scab by planting orchards with cultivar mixtures. To study the possible advantages of such a strategy, we developed a simple simulation which allows different hypotheses to be tested: starting from a known virulence distribution in an ascospore population, the program generates, for a given orchard, the disease pattern resulting from primary infection and subsequent asexual multiplication, as well as the changes in virulence distribution in the conidial population. The main parameters affecting the theoretical spread of the disease can be changed: virulence distribution of the ascospore population, size and spacing of trees, composition of the cultivar mixture, gradient of conidia dispersal. Different strategies have been tested by varying the parameters. The results are presented and discussed, as well as the implications for practical purposes.

Key words: Cultivar mixture, planting strategy, simulation, *Venturia inaequalis*.

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Recently, Sierotzki et al. (this volume) confirmed the presence of virulence specific to particular cultivars in natural *Venturia inaequalis* populations. Moreover, experiments revealed that after as few as three asexual generations on a given cultivar, only races with virulence for this cultivar remain.

This situation is similar to cereals, where populations of the powdery mildew pathogen are composed of races virulent only to some cultivars. This differential virulence-resistance system has been used successfully for the control of powdery mildew by planting cultivar mixtures (Wolfe 1992).

Using the same strategy in apple orchards could lead to a reduction of the disease pressure and thus to a lower pesticide input. However, field experiments with perennial cultures are slow and costs of new plantings are high. It is therefore important to obtain

preliminary theoretical information on the potential effectiveness of such a strategy. Computer simulation is widely used to test the impact of new strategies quickly at low cost and to see the influence of single parameters on complex systems. Some simulators have been developed for cereal mixtures (Kampmeijer & Zadoks 1977, Mundt & Leonard 1986) but no attempt has been made with perennial crops. Moreover, in the mixtures tested, all resistant plants had absolute resistance against some pathogen races, whereas the resistance of apple cultivars towards races of *V. inaequalis* seems to be only partial. To account for these differences, we developed a mechanistic simulation model of epidemics of *V. inaequalis* in an orchard with mixed cultivars.

## MATERIAL AND METHODS

The model was programmed in Pascal and compiled with Turbo Pascal compiler V.7.0 (Borland International Inc, Scotts Valley, Ca) on an IBM-compatible personal computer. All parameters of the program can be changed, allowing different hypotheses and strategies to be tested. To generate the simulations presented, the parameters were set, as far as possible, to values representative for a real orchard.

Starting from an ascospore population with a known virulence distribution against the cultivars in a hypothetical orchard, it generates the development of disease for this orchard. The mechanism for the spread of disease is reduced to its simplest form: spores falling on a tree produce lesions which, depending on the virulence pattern of the spores and the cultivar they land on, can be of 4 types: no, light, medium and normal sporulation. Quantitative data on relative spore production for the different interaction types were estimated from experimental data (Sierotzki et al., this volume). The primary inoculum (ascospores) consists of subpopulations of distinct pathotypes, each virulent on its main host where it produces 10 potential infectious conidia of the same pathotype. On the other, secondary, hosts the production of conidia is limited to 0 (pathotype Boskoop on Spartan and James Grieve, and pathotype Spartan on Boskoop), or 1 (pathotype Spartan on Boskoop and on James Grieve, and pathotype James Grieve on Boskoop and Spartan). No pathotype is able to reproduce on Florina, but all are fully compatible on the susceptible cultivar. The relative size of the subpopulations of the initial inoculum corresponds to the frequency of the cultivars. The interaction type is considered only for the reproduction rate. Therefore each spore landing on a tree causes a lesion regardless of the resulting interaction.

Since multiplication following primary infection is asexual, the virulence pattern of the spores remains identical, for the whole epidemic, with that of the ascospore from which they were descended.

The spatial development of the disease is displayed graphically (Fig. 1) and the disease curve is displayed after a user-selected number of generations, together with the resulting virulence distribution of the population.

Trees are represented through ellipses defined by their centre and axes, and belong to a specific cultivar. They are disposed regularly according to the distance within and between rows and to the distribution pattern of the different cultivars. The values used to generate the results presented were: tree size: 1.60 m x 1.20 m; tree spacing: 2.0 m within rows and 3.0 m between rows; orchard size: 50 m x 70 m resulting in 595 trees (Fig. 1).

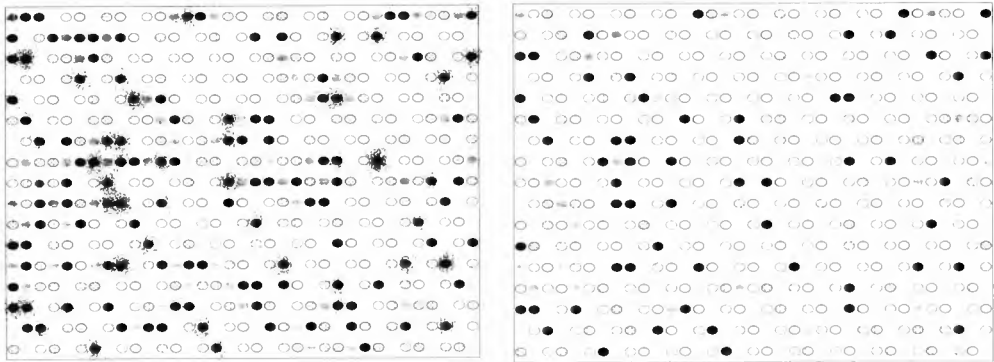


Fig. 1. Hard copies of the graphical display of the spatial development of apple scab in an orchard planted with the three varieties Boskoop, James Grieve and Spartan mixed within rows. Filled ellipses are trees infected with a compatible pathotype; dots represent spores. Left: state after primary infection (1000 ascospores). Right: state four asexual reproduction cycles later.

Gregory (1945) proposed an hyperbole to describe the concentration of spores downwind and Shrum (1975) used a gamma distribution, however, we chose the method adopted by Kempmeijer and Zadoks (1977), and considered the spore source to be momentaneous so that, after Pasquill (1962), the distribution downwind is also normal. We consider the wind being equally distributed in all directions, so that conidia dispersal follows a Gaussian distribution along the  $x$  and  $y$  coordinate, both with the same variance (std. dev: 0.90 m, resulting in a dispersal of max. ca. 4.0 m). Ascospores are dispersed randomly over the orchard.

## RESULTS AND DISCUSSION

Simulations of scab epidemics show clear differences in the course of the epidemic and the final number of total lesions due to the cultivar composition in the model orchard. As expected, the greatest lesion number is generated in an orchard containing a single variety susceptible to all pathotypes (Fig. 2 and 3, curves "1 var."). When the orchard consists of two cultivars with differential resistance, e. g. each cultivar is susceptible to 50 % of the medium-sized initial inoculum (1000 infectious ascospores) and partially resistant to the non-corresponding pathotype, the final lesion number (LN) is smaller. In orchard systems with the two cultivars planted alternatively in homogeneous rows, the reduction is of 34 % (Fig. 2). Alternating the cultivars within the rows reduces the LN by 67 %. Three cultivars planted in alternate rows reduces the LN by 65 %, within-row mixtures by 79 %. This shows that ephemeral resistances, massively overcome by a part of the scab population

present, can reduce scab epidemics considerably if planted correctly, in comparison to an orchard with a single cultivar or planted in blocks of a size where the initial inoculum comes overwhelmingly from the same cultivar.

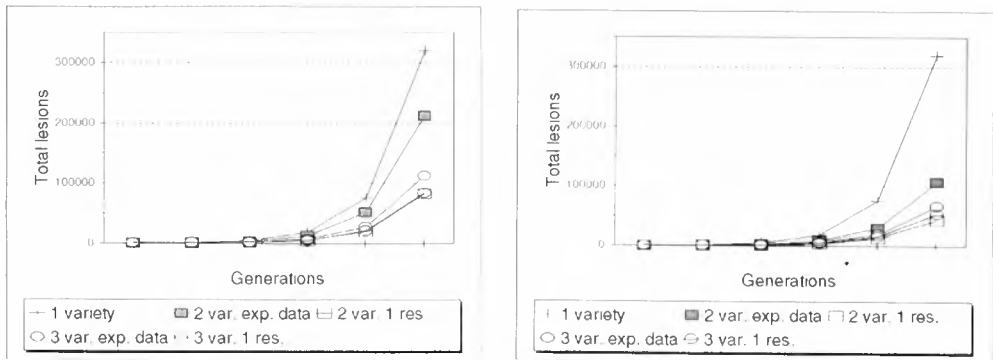


Fig. 2. Comparison of five simulated apple scab epidemics in different orchard planting systems. Initial inoculum was 1000 randomly distributed infectious ascospores. Left: cultivars are mixed row by row. Right: cultivars are mixed regularly within the rows. The different systems were: i) one single susceptible variety, inoculum 100% of a compatible pathotype; ii) mixture of Boskoop and James Grieve, inoculum 50% of each pathotype; iii) mixture of Boskoop and Florina, inoculum 50% of Boskoop pathotype, 50% non-infectious; iv) mixture of Boskoop, James Grieve and Spartan, inoculum one third of each pathotype; v) mixture of James Grieve, Spartan and Florina, inoculum accordingly.

Substituting one of the cultivars by a completely resistant cultivar reduces the lesion number level by 74 % and 74% in the between-row system (Fig. 2, "3 var. 1 res." and "2 var. 1 res."), and respectively by 83 and 86 % in the within-row system. The effect is clearly greater than expected from the proportion of resistant plants present. On the other hand, the gain over the systems with three ephemeral resistances is relatively small. The major fault of the simulations presented lies in the unconfirmed assumption that the conidia are dispersed over only a small surface following quantitatively a Gaussian curve with 95 % of the spores deposited in a radius of less than three meters around the center of the tree. Further research is needed to confirm this assumption or to offer better solutions to this problem. However, the simulations show that appropriate mixtures of cultivars, supposed to be susceptible but shown to carry ephemeral resistances (Sierotzki et al., this volume), can contribute substantially to reducing scab epidemics.

For Burdon (1978), the most important mechanisms leading to the reduction of disease in mixtures compared to pure stands are: i) the reduction of susceptible tissues, which leads to a reduction of inoculum, ii) increase in the average distance that inoculum has to travel to produce infection and iii) the presence of resistant plants acting as a barrier to the spread of inoculum. He observed that although the two first mechanisms may play an important role in reducing disease spread, the third aspect has been considered most in the literature. In our simulations, although only the first two factors can influence the spread of disease

due to the dispersal process, the resulting disease was always lower for a mixture than for a pure stand. This confirms the statement of Burdon, and suggests that under field conditions, disease reduction should be even more important since the barrier effect of resistant cultivars would be superimposed on the other two effects. Chin & Wolfe (1984) observed similar effects and pointed out an additional mechanism, induced resistance. Unfortunately, at the moment, no information is available on the presence of such a mechanism in the interaction between *V. inaequalis* and apple. However, a disadvantage of mixed tree planting compared to cereals has been outlined by Heybroek (1982): the crown of a single tree provides a large volume of leaves with the same genotype so that self-infection can lead to a considerable increase in inoculum. This effect is clearly shown by the localised spore clouds on the graphic display of the disease spread (Fig. 1).

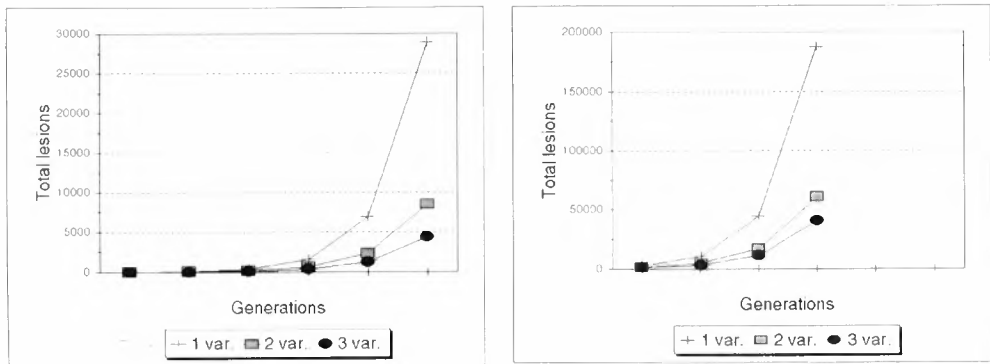


Fig. 3. Effect of initial inoculum on simulated apple scab epidemics in three different cultivar compositions. Spatial arrangement was as in fig. 2 right side, cultivar compositions were as in fig. 2 i), ii) and iv). Initial inoculum was 100 (left) and 10000 (right) randomly distributed infectious ascospores.

In all mixtures tested, the presence of a cultivar resistant to all races decreased the disease further, as expected, but the proportional reduction was greater than the proportion of the resistant cultivar in the orchard. This again confirms the fact that reduction of disease is not only due to dilution effects but to different mechanisms which can ideally superimpose on each other.

Two major, closely related, factors affecting disease spread in a mixed stand are the distance between two susceptible hosts and the distance that infectious spores can travel. Following the work of Frey and Keitt (1925) and Keitt and Jones (1926), it has long been accepted that conidia of *V. inaequalis* are dispersed only by rain, and that they thus travel only short distances. However, Hirst and Stedman (1961) and later Sutton and Jones (1976) were able to catch conidia in the daytime during dry weather. Although no quantitative measurement was made of how far conidia could travel and infect, this indicates that some spores could travel much further than the 4 meters possible with the dispersal function that we used. To take this into account, superposition of the normal distribution with another, wider distribution could be introduced.

For a perennial culture, it is important that the beneficial effects of the mixture last for a long time. However, there is no agreement on the evolution of the virulence pattern of the pathogen population in mixtures. Barrett (1978) divides the arguments in two categories: i) the pathogen population will "stabilise" and consist predominantly of genotypes with simple virulence and disease will be reduced, ii) there will be a rapid selection for pathogen genotypes capable of attacking all the components, thus eliminating disease control. Barrett (1978, 1980) studied the evolution of pathogen populations in multilines and variety mixtures with simulation techniques, but was unable to favour either of the two categories, and concluded that "super-races" would not necessarily evolve. In a review of the use of multilines cultivars and variety mixtures, Wolfe (1985) could not report any case of selection of complex races within a mixture. He reported later (Wolfe, 1993) a shift towards a high frequency of combined virulence for two barley mildew resistance genes in a large area of barley mixtures after some years of use, but the races in question came from outside the mixture area (Schaffner et al. 1992).

The results presented suggest that cultivar mixtures could be a valuable means to reduce apple scab in orchards. Concerning the choice of mixture components, Wolfe (1985) notes that the best control would be achieved by interspersing as many different mixture components as possible. However, although the constraints in apple orchards are different from those in cereal crops, we agree that the number of components should be kept to the minimum consistent with the reasonable restriction of disease progress. The problems mostly cited in a survey we made of the willingness of apple growers to use cultivar mixtures (unpublished) were: harvest, control of other diseases and pests, and chemical thinning. Unfortunately, these problems were considered to be even more important in the most effective spatial arrangement (mixture within rows). This is in agreement with the observation of Wolfe and Gessler (1992) that in orchard crops, because of quality considerations, introduction of mixed cropping is regarded as impracticable. Interestingly, however, a minority of the surveyed growers did not see any insurmountable problems in growing cultivar mixtures, giving some hope for the implementation of such a strategy. Cultivar mixtures will certainly not replace chemical control of apple scab, but could contribute significantly to the requirements of IP which postulate the use of all practical means to reduce fungicide use.

#### ACKNOWLEDGEMENTS

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#### LITERATURE

Barrett J.A. 1978. A model of epidemic development in variety mixtures. in *Plant disease epidemiology* (Scott P. R. & A. Bainbridge Eds.), Blackwell Scientific Publications, Oxford. 329 pp.



- Barrett J.A. 1980. Pathogen evolution in multilines and variety mixtures. *Z. Pflkrkh. Pflschutz.* 87: 383-396
- Burdon J.J. 1978. Mechanisms of disease control in heterogeneous plant populations - an ecologist's view. in *Plant disease epidemiology* (Scott P.R. & A. Bainbridge Eds.), Blackwell Scientific Publications, Oxford. 329 pp.
- Chin K.M. & M.S. Wolfe 1984. The spread of *Erysiphe graminis* f. sp. *hordei* in mixtures of barley varieties. *Plant Pathology* 33: 89-100.
- Frey C.N. & G.W. Keitt 1925. Studies of spore dissemination of *Venturia inaequalis* (Cke.) Wint. in relation to seasonal development of apple scab. *J. Agr. Res.* 30: 529-540.
- Gregory P.H. 1945. The dispersion of air-borne spores. *Trans. Brit. mycol. Soc.* 28: 26-72.
- Heybroek H.M. 1982. Monoculture versus mixture: interactions between susceptible and resistant trees in a mixed stand. In *Resistance to diseases and pests in forest trees: Proc. 3rd Int. Workshop on the genetics of host-parasite interactions in forestry.* H.M. Heybroek, B.R. Stephan, K. von Weissenberg Eds., pp. 342-360. Wageningen: Ctr. Agric. Publ. Doc. 503 pp.
- Hirst J.M. & O.J. Stedman 1961. The epidemiology of apple scab (*Venturia inaequalis* (Cke.) Wint.). I Frequency of airborne spores in orchards. *Ann. appl. Biol.* 49: 290-305.
- Kampmeijer P. & J.C. Zadoks 1977. EPIMUL, a simulator of foci and epidemics in mixtures of resistant and susceptible plants, mosaics and multilines. Wageningen: Pudoc.
- Keitt G.W. & L.K. Jones 1926. Studies of the epidemiology and control of apple scab. *Wisc. Agr. Exp. Sta. Res. Bull.* 73. 104 pp.
- Mundt C.C., K.J. Leonard, W.M. Thal & J.H. Fulton 1986. Computerized simulation of crown rust epidemics in mixtures of immune and susceptible oat plants with different genotype unit areas and spatial distribution of initial disease. *Phytopathology* 76: 590-598.
- Pasquill F. 1962. Atmospheric diffusion; the dispersion of windborne material from industrial and other sources. Van Nostrand, London. 297 pp.
- Schaffner D., B. Koller, K. Müller & M.S. Wolfe 1992. Response of populations of *Erysiphe graminis* f. sp. *hordei* to large-scale use of variety mixtures. *Vortr. Pflanzenzüchtg.* 24: 317-319.
- Shrum R. 1975. Simulation of Wheat stripe rust (*Puccinia striiformis* West) using EPIDEMIC, a flexible plant disease simulator. Penn. State Univ., Agric. Exp. Sta., Progress Report 347.

Sutton, T.B., A.L. Jones & L.A. Nelson 1976. Factors affecting dispersal of conidia of the apple scab fungus. *Phytopathology* 66: 1313-1317.

Wolfe M.S. 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual review of Phytopathology* 23: 251-273.

Wolfe M.S. 1992. Barley diseases: Maintaining the value of our varieties. Proceedings of the Sixth International Barley Genetics Symposium July 22-27, 1991. Helsingborg, Sweden. L. Munck Ed.

Wolfe M.S. 1993. Can the strategic use of disease resistant hosts protect their inherent durability? In *Durability of Disease Resistance*. Th. Jacobs & J. E. Parlevliet Eds. Kluwer academic publisher. 375 pp.

Wolfe M.S. & C. Gessler 1992. The use of resistance genes in breeding: epidemiological considerations. In *Genes Involved in Plant Defence*. T. Boller & F. Meins Eds. Springer-Verlag. 364 pp.

# Isozyme variation and inheritance of *Venturia* species causing scab on pears

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Polyacrylamide gel electrophoresis was used to study the isozyme variation and its inheritance in *Venturia nashicola* and *V. pirina*. Interspecific diversity for esterase and peroxidase banding was found among the strains assayed. Intraspecific isozyme diversity was also present amongst strains isolated from different host species or cultivars, and from many geographical origins. Isozyme electrophoresis should have potential for assisting in race identification of two *Venturia* species. Inheritance of esterase and peroxidase production was demonstrated and the loci *Est* and *Pox* identified in *V. nashicola* strains.

Key words: Esterase, genetics, isozyme, pear, peroxidase, scab, *Venturia nashicola*, *Venturia pirina*.

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*Venturia nashicola*, the scab fungus of Japanese pear (*Pyrus pyrifolia* var. *culta*) and Chinese white pear (*P. breischneideri*), is a distinct species from *V. pirina* which causes scab on European pear, *P. communis* var. *sativa* (Tanaka & Yamamoto 1964; Ishii unpublished). Both species differ in their morphology, crossing ability and pathogenicity to pears.

It is important to assess whether parasitic specialization occurs in fungal populations when disease resistance is considered in breeding programmes. Shabi et al. (1973) reported that five races of *V. pirina* could be isolated from cultivated and wild pears in Israel. For *V. nashicola*, no parasitic specialization was observed in strains collected from commercial pear cultivars (Ishii et al. 1992). More recently, however, it was found that *V. nashicola* strains obtained from the wild pear "mamenashi" (*P. dimorphophylla*) were pathogenic on this host but not on Japanese pear, Chinese white pear, or European pear cultivars (Ishii et al. unpublished). It seemed that races probably exist also in field populations of *V. nashicola*. However, in both *V. nashicola* and *V. pirina*, inoculation tests required for the identification of fungal races are difficult to perform due to poor sporulation in culture, and the long incubation period needed. Therefore, it should be useful if isozyme or molecular markers were developed to allow more efficient testing of races.

Here, we report the results of screening for enzyme activities in fungi using a semi-quantitative micromethod system. Next, the inter- and intraspecific variation of esterase and peroxidase banding was analyzed with polyacrylamide gel electrophoresis (PAGE). Furthermore, inheritance of the enzyme production and its pattern were examined.

## MATERIALS AND METHODS

### Screening for enzyme activities

#### *Activities in mycelia*

Fourteen strains of *V. nashicola* isolated from Japanese pear, Chinese white pear, and Asian-type wild pears were used. Six strains of *V. pirina* obtained from European pear were also included. These strains were collected from various pear species or cultivars grown in field widely separated from each other. Each monoconidial strain was cultured on potato-dextrose agar (PDA) plates at 20°C for 45 days. Mycelial discs, 4 mm in diameter, were cut from the margins of the colonies and placed into microcups of API ZYM kit (API SYSTEM S.A., France). Mycelial discs were covered with sterile distilled water, and the incubation trays kept at 37°C in the dark for 4 hr. Detection and recording of enzyme activities were carried out according to the procedures described in the instructions of the kit.

#### *Activities in spore germ-tube fluid*

Conidial suspensions (ca.  $5 \times 10^5$  conidia/ml distilled water) of *V. nashicola* and *V. pirina* were prepared from scab lesions collected from pear trees which had never been treated with fungicides. Suspensions were placed on the upper surface of detached pear leaves previously sterilized with 70% ethyl alcohol. Pear cultivars employed were as follows: Japanese pear Kosui (susceptible to *V. nashicola*), Suishu (resistant), and European pear Flemish Beauty (susceptible to *V. pirina*). After incubation at 20°C in a moist chamber for 3 days, the suspensions were collected from pear leaves and passed through a membrane filter (MILLEX-GS, 0.22  $\mu\text{m}$ , MILLIPORE, U.S.A.). The spore germtube fluid (ca. 50  $\mu\text{l}$ ) was pipetted into microcups and enzyme activities examined as mentioned above.

### PAGE of enzymes

#### *Fungal strains and preparation of protein extracts*

Twenty-one strains of *V. nashicola* isolated from Japan and People Republic of China were used. Ten *V. pirina* strains derived from Japan, Israel, Uruguay and Republic of South Africa were also examined for isozyme patterns. These strains were collected from different pear species or cultivars. Each strain was first cultured on PDA plates at 20°C for 45 days. Mycelial discs from the edge of fungal cultures were transferred to liquid medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% glucose (w/v). After incubation at 20°C for one month, mycelia were collected from the cultures and washed with sterile distilled water then homogenized aseptically. The mycelial fragments thus obtained were further used to inoculate the liquid medium described above and incubated at 20°C for 7 days on a shaking incubator.

Fresh mycelia, harvested from cultures in the liquid medium, were washed with distilled water and put into a precooled pestle. Protein extracts were then prepared by grinding mycelia with sea sand and mortar in liquid nitrogen. One millilitre extraction buffer (Tris 5 mM, Triton X-100 2% v/v, sucrose 15%, pH 7.0; Clark et al. 1989) was added to approximately 1 g of the mycelia. The mixture was kept at 4°C for 1 hr then transferred to an Eppendorf tube and centrifuged for 10 min at 10,000  $\times g$  at 4°C. The supernatant obtained was kept in an Eppendorf tube at -80°C until use.

#### *Analysis of esterase*

The analysis was carried out on vertical polyacrylamide gels (80 mm x 90 mm) containing 0.16% SDS (w/v) following the methods of Laemmli (1970). The main gel composition was 5% (w/v) acrylamide (29.2:0.8 acrylamide to *N,N*-methylene bisacrylamide) in Tris-HCl buffer (0.375 M Tris, pH 8.8). The stacking gel composition was 4.5% (w/v) acrylamide (29.2:0.8 acrylamide to *N,N*-methylene bisacrylamide) in Tris-HCl buffer (0.125 M Tris, pH 6.8). Each track was loaded with 10-30  $\mu$ l of protein sample and run at 150 V at 4°C for 2 hr. The running buffer was composed of 8.3 mM Tris and 30 mM barbital, pH 7.45. Staining methods were based on Loxdale et al (1983), and contained 2.5 ml 0.5 M Tris-HCl pH 7.1; 25 mg fast blue RR salt; 0.75 ml 1%  $\alpha$ -naphthyl or  $\beta$ -naphthyl acetate in 50% (v/v) acetone-water, in a final volume of 25 ml.

#### *Analysis of peroxidase*

Native polyacrylamide gels (main gel 7.5%, pH 8.9; stacking gel 3.75%, pH 6.7) were prepared and samples run in Tris-glycine buffer (5 mM Tris and 38 mM glycine, pH 8.3) at 175 V at 4°C for 2 hr. The gels were treated with the mixture of 0.2 M sodium acetate with 0.25% *o*-dianisidine and then stained with 0.003% H<sub>2</sub>O<sub>2</sub> (Jang et al. 1990).

#### **Progeny tests for isozyme patterns**

Stock cultures of progeny from crosses described previously (Ishii & van Raak 1988) of *V. nashicola* were used for these tests. Sensitivity of these progeny to the benzimidazole fungicide, carbendazim, was determined based on mycelial growth on fungicide-amended PDA (Ishii et al. 1992).

## RESULTS

### **Screening for enzyme activities**

#### *Activities in mycelia*

The use of API ZYM kit allowed the systematic and rapid study of 19 enzymatic reactions, and results are shown in Table 1. Alkalinephosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, phosphoamidase,  $\beta$ -galactosidase, and  $\alpha$ -glucosidase were detected in all strains tested.  $\beta$ -Glucosidase and  $\alpha$ -galactosidase were found in most strains, but the other enzyme activities were detected in less than half of the strains, or in none at all. There were no clear relationships between activities and either pear species or cultivars from which the fungal strains were isolated.

#### *Activities in spore germ-tube fluid*

An active acid phosphatase was found in all treatments, irrespective of the fungal species from which the germ-tube fluid was taken, or the pear cultivar involved (Data not shown). Faint activities of esterase lipase, valine arylamidase, and phosphoamidase were found in most treatments, whereas alkaline phosphatase, esterase,  $\beta$ -glucosidase, and  $\alpha$ -fucosidase were only detected occasionally.

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Table 1. Screening for enzyme activities in *Venturia nashicola* and *V. pirina* strains using API ZYM kit

Fungal strain	Microcup No. <sup>1)</sup>																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>V. nashicola</i>																				
from Japanese pear																				
JS-2	0 <sup>2)</sup>	3	0	0	0	3	2	0	0	0	3	2	1	2	0	1	1	1	0	0
JS-115	0	2	0	0	0	2	2	0	0	0	2	2	0	1	0	1	1	0	0	0
JS-127	0	3	0	0	0	3	2	0	0	0	3	2	0	1	0	1	1	1	0	0
JS-132	0	4	0	0	0	2	2	0	0	0	4	2	0	2	0	2	1	1	0	0
JS-134	0	4	0	0	0	3	3	0	0	0	4	2	0	1	0	1	1	2	0	0
JS-165	0	4	0	0	0	2	3	0	0	0	4	2	1	4	0	1	1	1	0	0
from Chinese white pear																				
CS-11	0	3	0	0	0	3	1	0	0	0	3	2	1	1	0	1	1	0	0	0
CS-21	0	3	0	0	0	3	3	0	0	0	3	2	1	3	0	2	2	2	1	0
from Asian-type wild pear																				
Mamenashi 12-1	0	5	0	0	0	3	3	0	0	0	4	2	1	2	0	2	1	0	0	0
Hokushimamenashi-1	0	4	0	0	0	3	3	0	0	0	4	1	1	2	0	1	1	0	0	0
<i>P. serotina</i> -1	0	4	0	0	0	3	3	0	0	0	5	1	1	2	0	1	1	0	0	0
Manshumamenashi-1	0	4	0	0	0	3	3	0	0	0	4	1	1	2	0	1	1	0	0	0
Iwateyamanashi-1	0	4	0	0	0	5	3	0	0	0	5	1	2	3	0	1	2	0	1	0
Manshuyaseinashi-1	0	4	0	0	0	3	2	0	0	0	3	2	1	2	0	2	0	2	0	0
<i>V. pirina</i>																				
from European pear																				
Akita FB-1	0	4	0	0	0	4	3	0	0	0	3	2	2	2	0	3	2	0	1	0
Akita SK-1	0	3	0	0	0	3	3	0	0	0	3	2	1	1	0	2	1	0	1	0
BP2-1	0	3	0	0	0	3	3	0	0	0	3	2	0	1	0	3	2	0	0	0
PT-1	0	3	0	0	0	2	3	0	0	0	3	2	1	1	0	3	1	1	0	0
ATCC 38996	0	4	0	0	0	4	3	0	0	0	2	2	1	1	0	1	2	0	0	0
IFO 8777	0	3	0	0	0	4	3	0	0	0	3	2	1	1	0	1	1	1	0	0

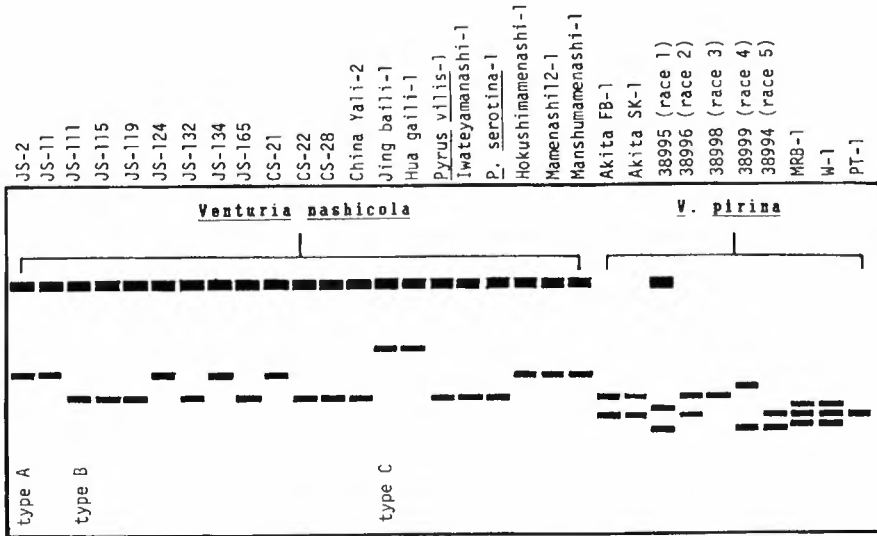
<sup>1)</sup> Enzyme assayed for 1, control; 2, alkaline phosphatase; 3, esterase (C 4); 4, esterase lipase (C 8); 5, lipase (C 14); 6, leucine arylamidase; 7, valine arylamidase; 8, cystine arylamidase; 9, trypsin; 10, chymotrypsin; 11, acid phosphatase; 12, phosphoamidase; 13,  $\alpha$ -galactosidase; 14,  $\beta$ -galactosidase; 15,  $\beta$ -glucuronidase; 16,  $\alpha$ -glucosidase; 17,  $\beta$ -glucosidase; 18, N-acetyl- $\beta$ -glucosaminidase; 19  $\alpha$ -mannosidase; 20,  $\alpha$ -fucosidase

<sup>2)</sup> A value ranging from 0-5 could be assigned corresponding to the colours developed as per the colour chart enclosed in the kit. Zero corresponds to a negative reaction; 5 to a reaction of maximum intensity. Value 1 through 4 are intermediate reactions depending on the level of intensity

**PAGE of enzymes**

*Esterase*

$\alpha$ - and  $\beta$ -esterase activities were detected in mycelial extracts of all strains tested and their patterns on a gel could be clearly distinguished between *V. nashicola* and *V. pirina* strains (Fig. 1). For *V. nashicola*, the pattern of most strains divided into either type A or type B. Two (Jing baili-1 and Hua gaili-1) out of three strains isolated from China revealed a different pattern (type C). In *V. pirina*, on the other hand, variation of esterase patterns was more striking. Five races from Israel each showed a different pattern (Fig. 1). Whereas, two strains isolated in Japan (Akita FB-1 and Akita SK-1) were identical with 38996 (race 2). Two strains collected from Uruguay (MRB-1 and W-1) and one from South Africa (PT-1) were also distinct from the other *V. pirina* strains.

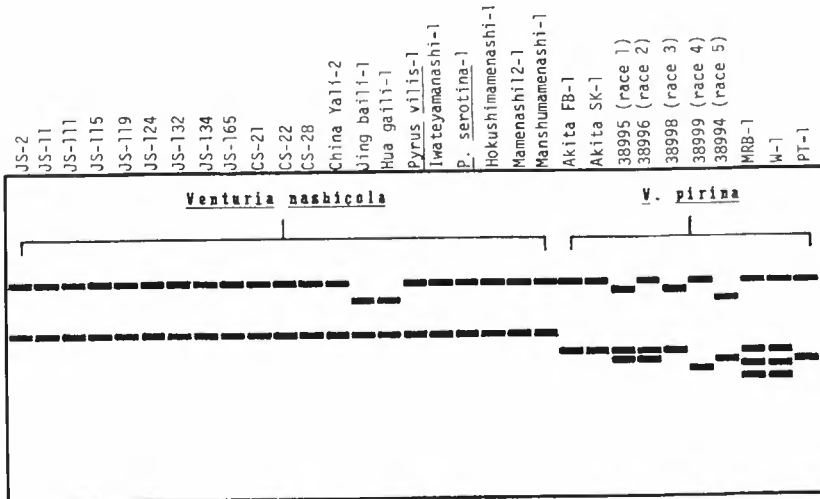


$\alpha$ -esterase patterns of *Venturia nashicola* and *V. pirina* (SDS-PAGE).

Fig. 1.  $\alpha$ -esterase patterns of *Venturia nashicola* and *V. pirina* (SDS-PAGE)

*Peroxidase*

This enzyme was detected in all strains tested (Fig. 2). In *V. nashicola*, no differences were noticed in their patterns except for two Chinese strains (Jing baili-1 and Hua gaili-1). However, *V. pirina* strains were again very variable and differed from those of *V. nashicola* strains. As for esterase in *V. pirina*, intraspecific variation in the peroxidase patterns was observed in individual races and strains isolated from different countries. Two Japanese strains (Akita FB-1 and Akita SK-1) were slightly different from others.



Peroxidase patterns of *Venturia nashicola* and *V. pirina* (Native PAGE).

Fig. 2. Peroxidase patterns of *Venturia nashicola* and *V. pirina* (Native PAGE)

**Progeny tests for isozyme patterns**

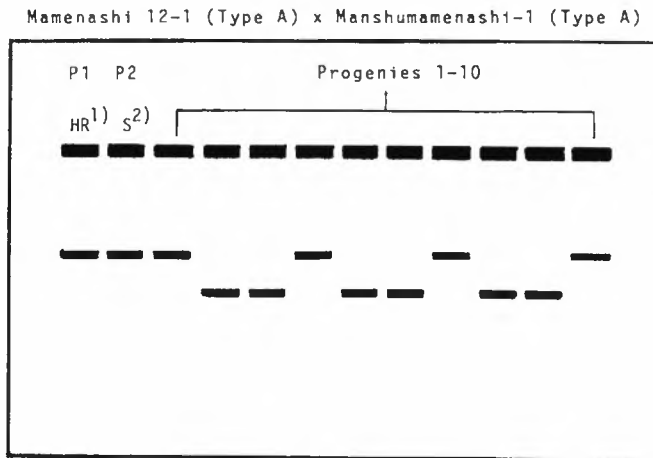
*Esterase*

Results of these tests are given in Table 2. Progeny strains possessed esterase activities and inheritance of esterase production in *V. nashicola* thus demonstrated experimentally. In a cross between a type A strain with type B one, the enzyme patterns segregated in the ratio of 1 type A:1 type B. However, when 2 type A strains were crossed, segregation of 4 type A and 6 type B progenies occurred (Fig. 3). Moreover, non-parental type A progenies appeared from two other crosses between two type B parents (Fig. 4). There was no apparent correlation between the sensitivity of progenies to carbendazim and their esterase patterns.

Table 2. Segregation of  $\alpha$ -esterase patterns in the cross of *Venturia nashicola* strains

Cross	Type of parents	Segregation (A:B)
JS-135 x JS-115	A x B	29:24 <sup>1)</sup>
Mamenashi 12-1 x Manshumamenashi-1	A x A	4:6
CS-1 x <i>P. Serotina</i> -1	B x B	5:5
JS-115 x Iwateyamanashi-1	B x B	2:7
JS-115 x <i>P. vilis</i> -1	B x B	0:9

1) Number of progenies



Segregation of  $\alpha$ -esterase patterns in the cross of *Venturia nashicola* strains.

- 1) HR: Highly resistant to carbendazim.
- 2) S: Sensitive to carbendazim.

Fig. 3. Segregation of  $\alpha$ -esterase patterns in the cross of *Venturia nashicola* strains. <sup>1)</sup>HR: Highly resistant to carbendazim. <sup>2)</sup>S: Sensitive to carbendazim



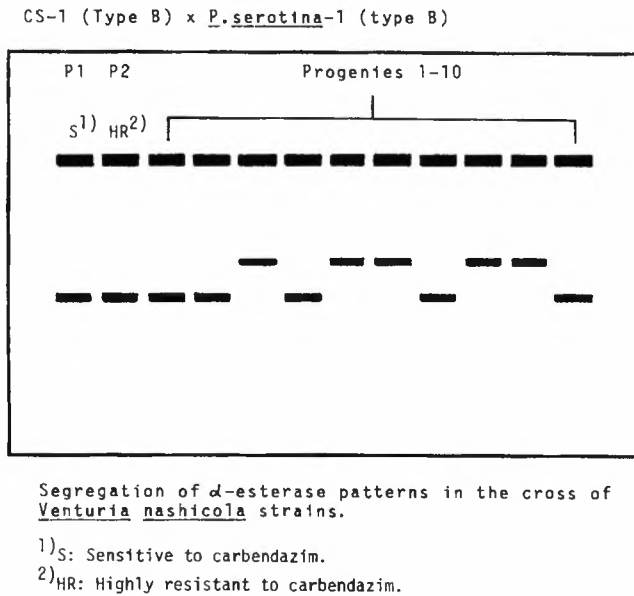


Fig. 4. Segregation of  $\alpha$ -esterase patterns in the cross of *Venturia nashicola* strains. <sup>1</sup>S: Sensitive to carbendazim.  
<sup>2</sup>HR: Highly resistant to carbendazim

### Peroxidase

In 2 crosses so far tested, peroxidase was detected in all progenies and the native PAGE patterns were the same as those of parental strains (Data not shown).

### DISCUSSION

It is known that pear species first originated in the western parts of China but then separated following movement either to the East or West. Consequently, Asian pears such as Japanese pear and Chinese white pear are now distinct from the European pear. Besides cultivated pear species, there are many wild pear species. These changes have been matched by the evolution of the cause of pear scab, and two *Venturia* species have been identified so far. *V. nashicola* causes scab of Japanese pear and Chinese white pear but not of European pear. On the contrary, *V. pirina* is pathogenic to European pear but not to Asian pears. *V. nashicola* is a species distinct from *V. pirina*. Furthermore, the relationship between evolution of *Pyrus* species and acquisition of pathogenicity in *Venturia* species might be explained by the concept of coevolution.

In this study, screening for enzyme activities was carried out in order to get basic information on the enzyme production of both these *Venturia* species. Activity of several

enzymes such as alkaline phosphatase, leucine arylamidase, and acid phosphatase was detected in all strains used irrespective of their source.

Isozyme polymorphisms detected by polyacrylamide and starch gel electrophoresis have been extensively employed in classification of organisms at various taxonomic levels (Clark et al. 1989; Julian & Lucas 1990; Hellmann & Christ 1991). For *V. inaequalis* the cause of apple scab, it was reported that five isolates representing the five races (race 1 to race 5) differed in their esterase patterns (Roig et al. 1990). Umemoto (1982) compared electrophoretic patterns of mycelial soluble proteins, polyphenoloxidase and peroxidase isozymes among strains of *V. nashicola*, *V. pirina*, and *V. inaequalis*. The author mentioned that *V. nashicola* strains seemed to be distinguishable from those of *V. pirina* based on the difference of polyphenoloxidase isozyme patterns. However, peroxidase was not observed in all strains of *V. nashicola*, *V. pirina*, or of *V. inaequalis* which were tested. A similar failure to detect peroxidase activity by PAGE was described by Roig et al. (1990).

In our experiments, the SDS-PAGE esterase patterns could be easily distinguished between *V. nashicola* and *V. pirina* strains. Other results showed a difference of esterase patterns between strains of *V. nashicola* from cultivated pears and a strain isolated from the wild pear "mamenashi" (Data not shown). Moreover, five races of *V. pirina* differed in the esterase patterns. Differences between the fungal species were also observed in peroxidase patterns and each race of *V. pirina* exhibited a distinct peroxidase pattern. These findings indicated the potential of isozyme analysis for assisting in race identification of *Venturia* species. Very recently, a new race of *V. inaequalis* that overcomes the Vf gene (encoding scab resistance) has been found and named race 6 (Parisi et al. 1993). It will be interesting to test this new race for isozyme patterns.

Inheritance of these isozyme variants was examined using stock cultures of progeny strains of *V. nashicola*. The data suggest that more than one gene probably two genes are encoding esterase. We temporarily designated the gene(s) *Est*. Inheritance of peroxidase production was also demonstrated and the locus *Pox* identified. In this fungus, several loci have been identified so far by classical genetic studies. Ishii et al. (1984, 1992) identified the locus *Ben1* which confers benzimidazole resistance. However, no correlation in progeny strains between benzimidazole resistance and type of esterase patterns was recognized. These loci will be used as genetic markers in future.

#### ACKNOWLEDGEMENTS

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## SUMMARY

*Venturia nashicola*, the scab fungus of Japanese pear and Chinese white pear, is a species distinct from *V. pirina* which causes scab of European pear. It might be useful if isozyme or molecular markers are developed for assessing the parasitic specialization in fungal populations.

Screening for fungal enzyme activities was performed using the API ZYM kit. Activities of alkaline phosphatase, leucine arylamidase, acid phosphatase, and some other enzymes were found in extracts of all strains irrespective of their source.

Polyacrylamide gel electrophoresis patterns of esterase and peroxidase differed in strains of *V. nashicola* and *V. pirina*. Furthermore, intraspecific isozyme diversity was also present amongst strains obtained from different host species, or cultivars, and from many geographical origins. Five races of *V. pirina* each showed characteristic patterns for esterase and peroxidase. Thus, the usefulness of isozyme analysis for race identification was suggested.

Esterase and peroxidase production was inherited and the loci *Est* and *Pox* identified in *V. nashicola* strains. None of these loci was same as the locus *Ben1* which encodes benzimidazole resistance.

## REFERENCES

- Clark, J., J. Butters, K.J. Brent & D.W. Hollomon 1989. Isozyme uniformity in *Erysiphe graminis* f. sp. *hordei*. *Mycological Research* 92: 404-409.
- Hellmann, R. & B.J. Christ 1991. Isozyme variation of physiologic races of *Ustilago hordei*. *Phytopathology* 81: 1536-1540.
- Ishii, H., H. Udagawa, S. Nishimoto, T. Tsuda & H. Nakashima 1992. Scab resistance in pear species and cultivars. *Acta Phytopathologica et Entomologica Hungarica* 27: 293-298.
- Ishii, H. & M. van Raak 1988. Inheritance of increased sensitivity to N-phenylcarbamates in benzimidazole-resistant *Venturia nashicola*. *Phytopathology* 78: 695-698.
- Ishii, H., M. van Raak, I. Inoue & A. Tomikawa 1992. Limitations in the exploitation of N-phenylcarbamates and N-phenylformamidoximes to control benzimidazole-resistant *Venturia nashicola* on Japanese pear. *Plant Pathology* 41: 543-553.
- Ishi, H., H. Yanase & J. Dekker 1984. Resistance of *Venturia nashicola* to benzimidazole fungicides. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 49/2a: 163-172.
- Jang, J.T., K. Tanabe, K. Banno, F. Tamura & K. Kawamoto 1990. *Journal of the Japanese Society for Horticultural Science (Supplement 2)*: 136-137.

Julian, A.M. & J.A. Lucas 1990. Isozyme polymorphism in pathotypes of *Pseudocercospora herpotrichoides* and related species from cereals. *Plant Pathology* 39: 178-190.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature* 227: 680-685.

Loxdale, H.D., P. Castañera & C.P. Brookes 1983. Electrophoretic study of enzymes from cereal aphid populations. I. Electrophoretic techniques and staining systems for characterising isoenzymes from six species of cereal aphids (Hemiptera: Aphididae). *Bulletin of Entomological Research* 73: 645-657.

Parisi, L., Y. Lespinasse, J. Guillaumes & J. Krüger 1993. A new race of *Venturia inaequalis* virulent to apples with resistance due to the Vf gene. *Phytopathology* 83: 533-537.

Roig, E., P. Neumann & J.-P. Simon 1990. Growth and isoenzyme comparison of five isolates of *Venturia inaequalis*. *Phytoprotection* 71: 65-71.

Shabi, E., J. Rotem & G. Loebenstein 1973. Physiological races of *Venturia pirina*. *Phytopathology* 63: 41-43.

Tanaka, S. & S. Yamamoto 1964. Studies on pear scab. II. Taxonomy of the causal fungus of Japanese pear scab. *Annals of the Phytopathological Society of Japan* 29: 128-136.

Umemoto, S. 1982. Comparison between mycelial soluble proteins of both fungi of Japanese pear scab and pear scab which are sensitive or tolerant to benomyl by disc electrophoretic method. *Bulletin of The Chiba-Ken Agricultural Experiment Station* 23: 31-39.

# An option for an apple scab warning system apart from the IPM-Guidelines

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Key words: Apple scab, forecasts, fungicides, orchard disease, *Venturia inaequalis*.

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If an Austrian fruitgrower is asked why he sprays against scab, he often cannot tell the reason why. Some farmers spray because they follow a calendar schedule, other farmers spray because their scab warning instrument indicates a Mills Periode. So most farmers in Austria spray 12-15 times a year against scab.

It is easier to encourage a farmer to spray than to convince him not to spray. Scab management is often a matter of emotion and not a matter of clear facts.

Despite the great number of sprays the scab problem in Austria has increased rapidly since the middle of the 1980's. IPM introduction did not reduce the scab attacks or the number of sprays because the guidelines have no strategy for solving this problem. Since IPM was introduced no farmer has saved fungicides against scab. So another option for a scab warning system apart from the IPM-guidelines is needed. Since 1990 a new system created by P. Triloff has been tested.

## CIRCUMSTANCES AND REASONS FOR THE PROBLEMS IN THE PAST

- a) Introducing very early pruning of the trees in the 1980's.  
Advisers encourage the farmers to prune their trees first in June, in July and again in August and also to prune intensely in winter. The result is a long season of vegetative growth and shoot tips infected by conidia. These shoot infections (weed scab) produce a very high number of conidia which cannot be controlled.
- b) Scab infections on the trees coming from the nurseries.  
Bad scab management in the nursery lead to shoot infections with a high number of conidia.
- c) Wrong choice of fungicides.  
Preventive fungicides e.g. dithianon have been used for curative treatments, after rain rather than before rain. Curative fungicides have been used protectively.

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- d) The mere use of electronic scab warning instruments by farmers does not of itself reduce the number of sprays. The instrument says nothing about the tree or the fungus, only the physical factors. In practice, a severe Mills Period may not lead to scab lesions whereas a light Mills Period is. A scab warning system is needed with components which influence the scab infections. These are:
- \* ascospore release
  - \* leaf growth

#### DIFFERENTIATION OF ORCHARDS

There are 2 kinds of orchards:

- a) non-vigorous orchards with few problems
- b) vigorous orchards with permanent problems

a) non-vigorous orchards

no shoot scab.  
no sepal scab.  
no green shoots tips in autumn.  
no scab problem in the past.  
low inoculum (ascospores only).  
reduction of spore potential is possible  
ascospores.  
first spray 3-4 weeks after bud break.  
number of sprays low.  
sprays needed until end of ascospore release.  
low scab attack.

b) vigorous orchards

shoot scab.  
sepal scab.  
green shoot tips in autumn.  
permanent scab problems in past.  
very high inoculum (ascospores + conidia).  
reduction of spore potential impossible.  
conidia are in the tree.  
first spray at bud break.  
number of sprays very high.  
sprays needed until end of shoot growth.  
high scab attack.  
scab problem the following year.

Only non-vigorous trees with only ascospore inoculum are suited for the "less chemical" strategy.

#### SANITARY TREATMENTS TO REDUCE ASCOSPORE POTENTIAL

Sanitary treatments reduce the period of ascospore release and the number of ascospores released.

- a) Spray urea (50kg/ha) in autumn
- b) Spray Cyanamide (10-15 l/ha) in spring 2 weeks before ascospore release begins (fig. 1)
- c) copper, endosulfan and benzimidazol
- d) Shred leaves 1-2 weeks before ascospore release begins if earthworms have not decomposed leaves.

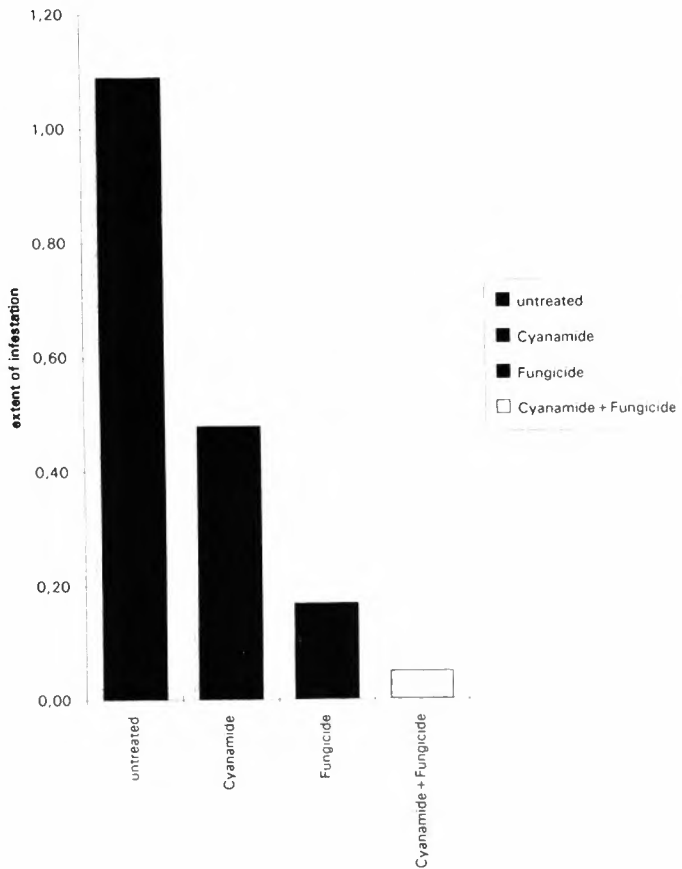


Fig. 1. Reduction of ascospore potential

### THE SYSTEM

- Control ascospore release after every rainy period
- Calculate the infection conditions using a Metos<sup>R</sup> weather station
- Measure leaf growth in rainy periods
- Transfer the scab report to the farmer

### THE RESULTS

Figures 2-5 show ascospore release and Mills Periods, from 1990 to 1993.

Tables 1-4 show the sprays in a conventional system and in a spore trap system from 1990 to 1993.

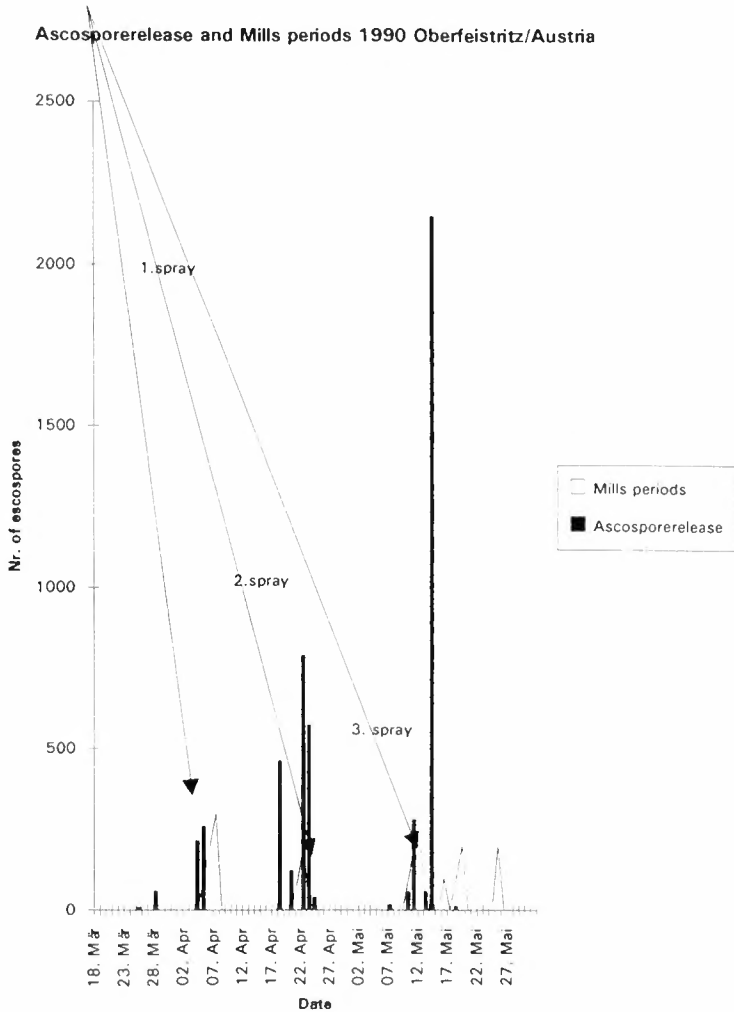


Fig. 2. Ascospore release and Mills Periods 1990 Oberfeistritz(Austria)

- a) In the fruit-growing region, there were only 3-5 infections by ascospores. In 1992 sprays were applied more than 5 times in the ascospore season because of early infection. Probably this infection came from using a curative fungicide at low temperature.
- b) Ascospore release starts 3-4 weeks after bud break and ends in the middle of May. Some farmers say that ten years ago they applied their first spray against scab at the green bud stage or pink flower stage of the trees. Farmers are surprised that this late beginning of sprays had worked.



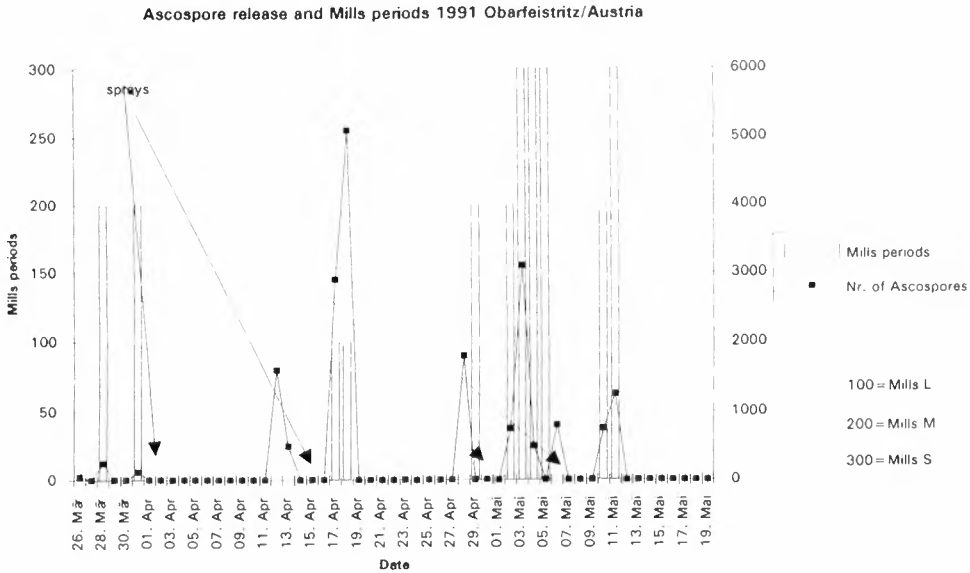


Fig. 3. Ascospore release and Mills Periods 1991 Oberfelstritz/Austria

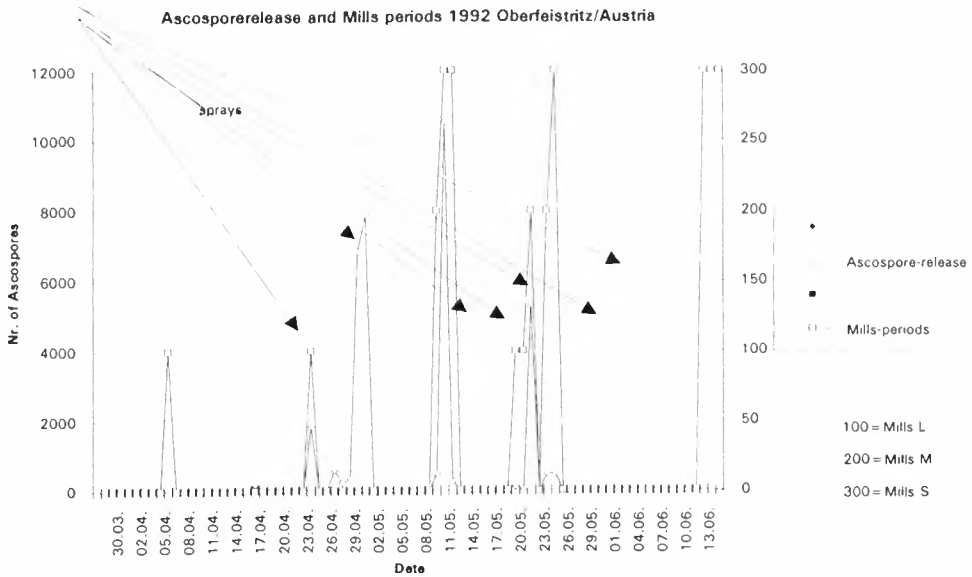


Fig. 4. Ascospore release and Mills Periods 1992 Oberfelstritz/Austria

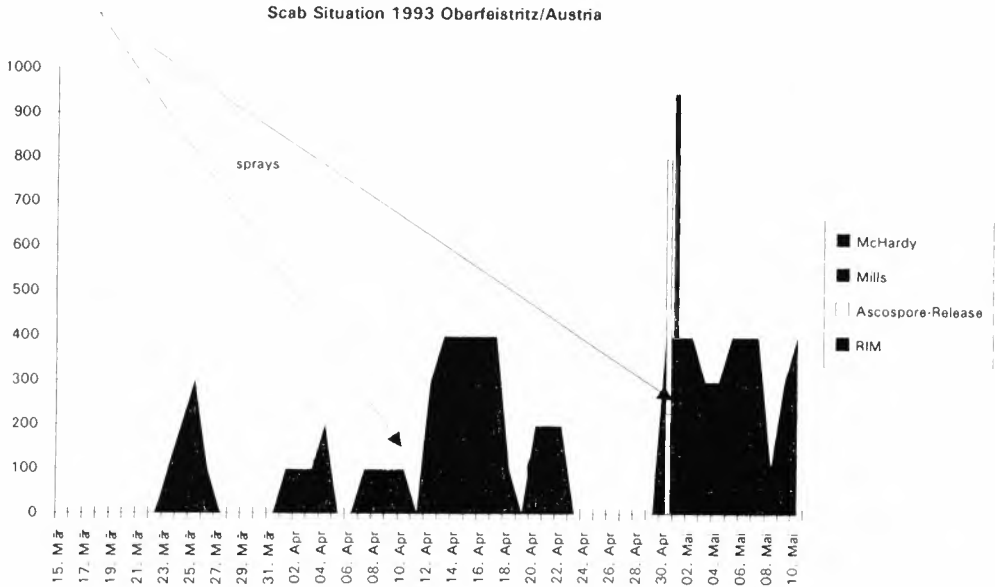


Fig. 5. Scab situation 1993 Oberfelstritz/Austria

Table 1. Sprays against Scab 1990

Conventional system	Spore trap system
28.03. Dodine	28.03. ---
09.04. Dithianon + Myclobutanil	09.04. Myclobutanil
21.04. Dodine	21.04. ---
24.04. ---	24.04. Penconazol
11.05. Dithianon + Penconazol	11.05. Dithianon + Penconazol
19.05. Dithianon + Penconazol	19.05. ---
<b>5 Sprays</b>	<b>3 Sprays</b>

- c) Not every Mills Period results in lesions.  
Susceptible leaves were marked at the time of infection periods. Only infection periods with a high numbers of ascospores result in lesions.
- d) After rainfall in the night only a few ascospores were caught at sunrise.
- e) It is possible to reduce the number of sprays without increasing the scab attack.  
In each year final scab attack at harvest was below 5% scabbed shoots, except in 1992, when there were 10% scabbed shoots at harvest. Fruit scab was below 1%.
- f) The farmers can save more than 50% of the costs of fungicides.  
Tables 5-8 show the economics of both systems and both dosages - the normal and the reduced.

Table 2. Sprays against Scab 1991

Conventional system	Spore trap system
02.04. Myclobutanil	02.04. Myclobutanil
15.04. Dodine	15.04. Dodine
30.04. Dithianon + Penconazol	30.04. Dithianon + Penconazol
07.05. Dithianon + Myclobutanil	07.05. Dithianon + Myclobutanil
02.06. Dithianon + Penconazol	02.06. ---
15.06. Dithianon	15.06. ---
23.06. Dodine	23.06. ---
29.06. Dithianon	29.06. ---
12.07. Dithianon	12.07. ---
23.07. Dithianon	23.07. ---
01.08. Dodine	01.08. ---
02.09. Dichlofluanid	02.09. Dichlofluanid
15.09. Dichlofluanid	15.09. Dichlofluanid
<b>13 Sprays</b>	<b>5 Sprays</b>

Table 3. Sprays against Scab 1992

Conventional system	Spore trap system
21.03. Dodine	21.03. ---
30.03. Dithianon + Penconazol	30.03. ---
07.04. Dodine	07.04. ---
24.04. Dodine	24.04. Dodine
02.05. Dithianon + Myclobutanil	02.05. Dithianon + Myclobutanil
12.05. Dithianon + Penconazol	12.05. Dithianon + Penconazol
18.05. Dithianon	18.05. Dithianon
23.05. Dithianon	23.05. Dithianon
30.05. Dithianon	30.05. Dithianon
04.06. Dithianon	04.06. Dithianon
15.06. Dithianon + Myclobutanil	15.06. Dithianon + Myclobutanil
22.06. Dodine	22.06. ---
02.07. Dithianon	02.07. ---
10.09. Dichlofluanid	10.09. Dichlofluanid
20.09. Captan	20.09. Captan
<b>15 Sprays</b>	<b>10 Sprays</b>

Table 4. Sprays against Scab 1993

Conventional system	Spore trap system
22.03. Copper	22.03. ---
02.04. Dichlofluanid	02.04. ---
10.04. Dithianon	10.04. Dithianon
24.04. Myclobutanil	24.04. ---
01.05. Dodine	01.05. Dodine
06.05. Dodine	06.05. Penconazol + Captan
19.05. Pyrifenox	19.05. ---
28.05. Pyrifenox + Dithianon	28.05. ---

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Table 5. Cost savings of fungicides against Scab 1990

Conventional system Normal Dose	Conventional system Reduced Dose	Spore trap system Normal Dose	Spore trap system Reduced Dose
2x Dodine 2x Novit 1,4l/ha S 607,-/ha	2x Dodine 2x Novit 0,7l/ha S 303,-/ha	---	---
3x Dithianon 3x Delan 0,5l/ha S 918,-/ha	3x Dithianon 3x Delan 0,25l/ha S 459,-/ha	1x Dithianon 1x Delan 0,5l/ha S 306/ha	1x Dithianon 1x Delan 0,25l/ha S 153,-/ha
1x Myclobutanil 1x Prothane 0,3l/ha S 300,-/ha	1x Myclobutanil 1x Prothane 0,15l/ha S 150,-/ha	1x Myclobutanil 1x Prothane 0,3l/ha S 300,-/ha	1x Myclobutanil 1x Prothane 0,15l/ha S 150,-/ha
2x Penconazol 2x Topas 0,25l/ha S 400,-/ha	2x Penconazol 2x Topas 0,125l/ha S 200,-/ha	2x Penconazol 2x Topas 0,25l/ha S 400,-/ha	2x Penconazol 2x Topas 0,125l/ha S 200,-/ha
S 2225,-/ha	S 1112,-/ha	S 1006,-/ha	S503,-/ha
100%	50%	45%	23%

Table 6. Cost savings of fungicides against Scab 1991

Conventional system Normal Dose	Conventional system Reduced Dose	Spore trap system Normal Dose	Spore trap system Reduced Dose
3x Dodine 3x Novit 1,4l/ha S 911,-/ha	3x Dodine 3x Novit 0,7l/ha S 455,-/ha	1x Dodine 1x Novit 1,4l/ha S 304,-/ha	1x Dodine 1x Novit 0,7l/ha S 152,-/ha
7x Dithianon 7x Delan 0,5l/ha S 2142,-/ha	7x Dithianon 7x Delan 0,25l/ha S 1071,-/ha	2x Dithianon 2x Delan 0,5l/ha S 612,-/ha	2x Dithianon 2x Delan 0,25l/ha S 306,-/ha
2x Penconazol 2x Topas 0,25l/ha S 400,-/ha	2x Penconazol 2x Topas 0,125l/ha S 200,-/ha	1x Penconazol 1x Topas 0,25l/ha S 200,-/ha	1x Penconazol 1x Topas 0,125l/ha S 100,-/ha
1x Myclobutanil 1x Prothane 0,3l/ha S 300,-/ha	1x Myclobutanil 1x Prothane 0,15l/ha S 150,-/ha	1x Myclobutanil 1x Prothane 0,3l/ha S 300,-/ha	1x Myclobutanil 1x Prothane 0,15l/ha S 150,-/ha
2x Dichlofluanid 2x Euparen 2kg/ha S 600,-/ha	2x Dichlofluanid 2x Euparen 1kg/ha S 300,-/ha	2x Dichlofluanid 2x Euparen 2kg/ha S 600,-/ha	2x Dichlofluanid 2x Euparen 1kg/ha S 300,-/ha
S 4353,-/ha	S 2177,-/ha	S 2016,-/ha	S 1008,-/ha
100%	50%	46%	23%

Table 7. Cost savings of fungicides against scab 1992

Conventional system Normal dose	Conventional system Reduced dose	Spore trap system Normal dose	Spore trap system Reduced dose
4x Dodine 4x Novit 1,4l/ha S 1215,-/ha	4x Dodine 4x Novit 0,7l/ha S 608,-/ha	1x Dodine 1x Novit 1,4l/ha S 304,-/ha	1x Dodine 1x Novit 0,7l/ha S 152,-/ha
9x Dithianon 9x Delan 0,5l/ha S 2754,-/ha	9x Dithianon 9x Delan 0,25l/ha S 1377,-/ha	7x Dithianon 7x Delan 0,5l/ha S 2152,-/ha	7x Dithianon 7x Delan 0,25l/ha S 1076,-/ha
2x Myclobutanil 2x Prothane 0,3l/ha S 600,-/ha	2x Myclobutanil 2x Prothane 0,15l/ha S 300,-/ha	2x Myclobutanil 1x Prothane 0,3l/ha S 600,-/ha	2x Myclobutanil 2x Prothane 0,15l/ha S 300,-/ha
2x Penconazol 2x Topas 0,25l/ha S 400,-/ha	2x Penconazol 2x Topas 0,25l/ha S 200,-/ha	1x Penconazol 1x Topas 0,25l/ha S 200,-/ha	1x Penconazol 1x Topas 0,125l/ha S 100,-/ha
1x Dichlofluanid 1x Euparen 2kg/ha S 300,-/ha	1x Dichlofluanid 1x Euparen 1kg/ha S 150,-/ha	1x Dichlofluanid 1x Euparen 2kg/ha S 300,-/ha	1x Dichlofluanid 1x Euparen 1kg/ha S 150,-/ha
1x Captan 1x Captan fl 3l/ha S 468,-/ha	1x Captan 1x Captan fl 1,5l/ha S 243,-/ha	1x Captan 1x Captan fl 3l/ha S 468,-/ha	1x Captan 1x Captan fl 1,5l/ha S 234,-/ha
S 5737,-/ha	S 2869,-/ha	S 4024,-/ha	S 2012,-/ha
100%	50%	70%	35%

## DISADVANTAGES

- a) Problems with the transmission of the report to the farmer.  
Most farmers have no fax facility. It is not practical to use postal services (transmission time is too long) or the telephone (the farmer is not in the house).
- b) Some farmers cannot spray within the curative action time of the fungicide.  
In Styria there are orchards with a gradient of nearly 100%. After heavy rainfall such orchards are impossible to treat rapidly.
- c) 2 spraying schemes.  
Farmers have variable farms - one part is suited for the ascospore strategy, one not. Fruitgrowers always tend to spray their whole farm with the same schedule.
- d) Reliance on curative fungicides.  
Can the strategy be based on curative fungicides? Possibly "yes", if there are only 3-5 infection periods and if protective sprays are also used (Marc Trapman)
- e) The amount of time spent by the advisers.  
The amount of time to count the spores is too high.

132 *Another option for a scab warning system*

Table 8. Cost savings of fungicides against scab 1993 (costed 30 May)

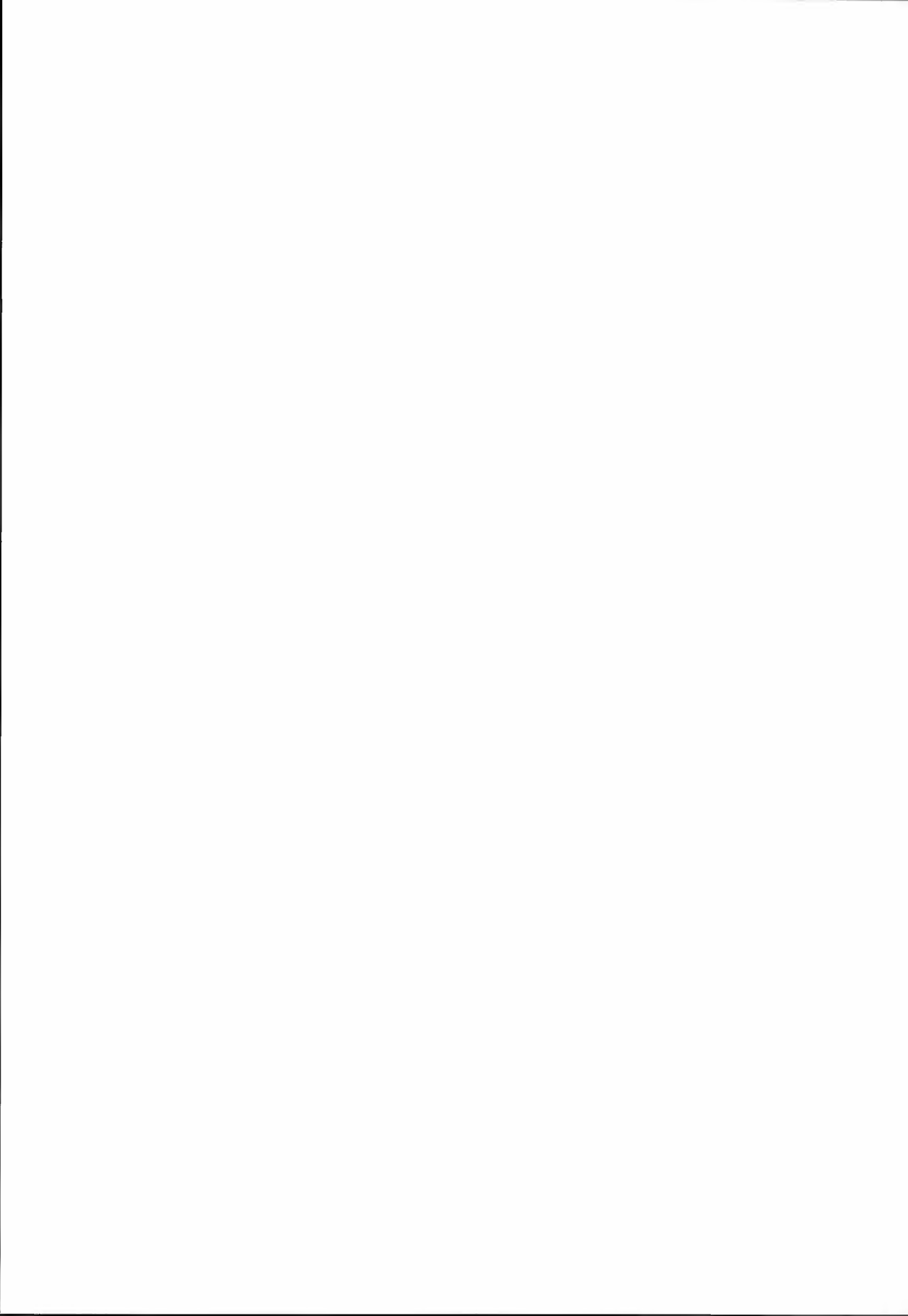
Conventional strategy Normal dose	Conventional strategy Reduced dose	Spore trap system Normal dose	Spore trap system Reduced dose
1x Copper 1x Kupfer fl 3l/ha S 279,-/ha	1x Copper 1x Kupfer fl 1,5l/ha S 140,-/ha	---	---
1x Dichlofluanid 1x Euparen 2kg/ha S 300,-/ha	1x Dichlofluanid 1x Euparen 1kg/ha S 150,-/ha	---	---
2x Dithianon 2x Delan 0,5l/ha S 612,-/ha	2x Dithianon 2x Delan 0,25l/ha S 306,-/ha	1x Dithianon 1x Delan 0,5l/ha S 306,-/ha	1x Dithianon 1x Delan 0,25l/ha S 153,-/ha
2x Dodine 2x Novit 1,4l/ha S 608,-/ha	2x Dodine 2x Novit 0,7l/ha S 304,-/ha	1x Dodine 1x Novit 1,4l/ha S 304,-/ha	1x Dodine 1x Novit 0,7l/ha S 152,-/ha
1x Myclobutanil 1x Prothane 0,3l/ha S 300,-/ha	1x Myclobutanil 1x Prothane 0,15l/ha S 150,-/ha	---	---
---	---	1x Penconazol 1x Topas 0,25l/ha S 200,-/ha	1x Penconazol 1x Topas 0,125l/ha S 100,-/ha
2x Pyrifenox 2x Dorado 0,25l/ha S 564,-/ha	2x Pyrifenox 2x Dorado 0,125l/ha S 282,-/ha	---	---
---	---	1x Captan 1x Captan fl 3l/ha S 468,-/ha	1x Captan 1x Captan fl 1,5l/ha S 234,-/ha
S 2663,-/ha	S 1332,-/ha	S 1278,-/ha	S 639,-/ha
100%	50%	48%	24%

### SAVING FUNGICIDES

To reduce the number of sprays is not the only way to save chemicals. The dosage of fungicides can be reduced by using low-volume sprayers, which were tested in both years. In the fruit-growing region 50% of the recommended dosage is advised for all orchards - vigorous and non-vigorous. Tables 5-8 show the cost-saving of fungicides against scab.

## FUTURE PROSPECTS

- a) A new spore trap needing less time to operate is being developed.
- b) A system to forecast ascospore release (Marc Trapman) for use where orchards are steep.
- c) A good data-transfer system by fax should be developed.





# Apple scab control by curative application of ergosterol biosynthesis inhibiting fungicides

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Schwabe, W.F.S. & J.V. Hadlow 1994. Apple scab control by curative application of ergosterol biosynthesis inhibiting fungicides. *Norwegian Journal of Agricultural Sciences*. Supplement No. 17: 135-136. ISSN 0802-1600.

The requirements for fruit scab infection on apple during all stages of development and the curative action of ergosterol biosynthesis inhibitors (EBI's) against scab have previously been determined in greenhouse trials. To confirm the findings under field conditions scab trials were conducted in an orchard at the Elgin Experiment Farm, Grabouw, on Starking and Granny Smith apple trees during the 1985/86 and 1986/87 growing seasons. While the first season was not very favourable for scab development, the second was very favourable.

In the 1985/86 season treatments were as follows:

- O. An unsprayed control
- A. A scheduled protective mancozeb programme
- B. A curative penconazol/captan programme applied as soon as possible after recording a fruit infection period
- C. As in B, but applied > 120 h after the onset of a fruit infection period
- D. A curative bitertanol plus mancozeb programme applied as in B
- E. As in D, but applied > 120 h after the onset of a fruit infection period.

To ensure a high inoculum pressure, spraying commenced after the full bloom stage, although ascospore discharge had already started before the green tip stage.

For the protective programme, nine sprays were applied, while only three curative sprays were required for the remaining treatments.

Fruit and leaf scab control was more than 90% in treatments B, C and D on both cultivars. The protective programme (A) and the bitertanol plus mancozeb programme applied > 120 h after the onset of a fruit infection period (E) were not so effective.

In the 1986/87 scab trial treatments were as follows:

- O. An unsprayed control
- A. A scheduled protective mancozeb programme commencing at green tip
- B. As in A, but commencing at full bloom stage
- C. A curative flusilazol programme applied > 120 h after the onset of a fruit infection period
- D. Flusilazol plus mancozeb applied as in C
- E. As in D, but applied as soon as possible after the onset of a fruit infection period

*136 Apple scab control by curative application*

Treatments C, D and E commenced after full bloom, as did treatment B. For the long and short protective programmes (A and B) eleven and eight applications were required respectively, while eight applications were required for the curative programmes.

Fruit scab control was highly effective throughout (>95% control). Programme B (mancozeb programme starting at full bloom) gave unsatisfactory leaf scab control, while all other treatments were highly effective.

It is concluded that

1. Index values for light fruit infection determined under controlled greenhouse conditions are applicable in the field
2. EBI fungicides are highly effective for curative fruit and leaf scab control
3. Curative scab control programmes are more cost effective than protective programmes during "dry" seasons

Because of a lack of alternative highly effective curative fungicides and the risk of selecting EBI-tolerant scab strains the exclusive use of EBI fungicides is not recommended season after season. EBI's have to be applied together with an effective protectant.

# The dispersal of ascospores of *Venturia inaequalis*

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MacHardy, W.E. 1994. The dispersal of ascospores of *Venturia inaequalis*. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 137-148. ISSN 0802-1600.

The apple scab management program being developed in New Hampshire is "orchard oriented." That is, decisions for using sanitation practices and scheduling fungicides in the spring are based on an estimate of the amount of ascosporic inoculum (i.e., the potential ascospore dose, or PAD) in each orchard (MacHardy 1994). Ascospores are the sole constituent of the primary inoculum in New Hampshire, and the program assumes that scab lesions that develop in an orchard during early spring result from ascospores produced within that orchard. When this program is presented to growers, questions pertaining to ascospore dispersal invariably enter into the discussion. How far are ascospores dispersed? Aren't there situations when ascospores produced in one orchard disperse to an adjacent orchard and cause scab? What about ascospores produced in an abandoned or poorly managed orchard nearby? Shouldn't that orchard be considered, and, if so, how far away should it be before ascospores produced in it can be ignored in the orchard using the PAD-based management program? What the questioners really want to know is whether or not the proportion of discharged ascospores arriving from an outside source will require a management practice, e.g., a fungicide application, to prevent their causing an unacceptable buildup of scab. In this review of ascospore dispersal of *Venturia inaequalis*, three lines of evidence are considered that address this concern: ascospore dispersal gradients calculated from (i) ascospores caught in mechanical traps or deposited on glass slides placed at known distances from a point source of ascospores, (ii) the amount of scab that developed in a section of an orchard with a negligible ascosporic inoculum compared with the amount of scab that developed in a section of the orchard with a high ascosporic inoculum, and (iii) the amount of scab that developed in an orchard with a negligible ascosporic inoculum compared with the amount of scab that developed in a high inoculum "source" orchard a known distance away.

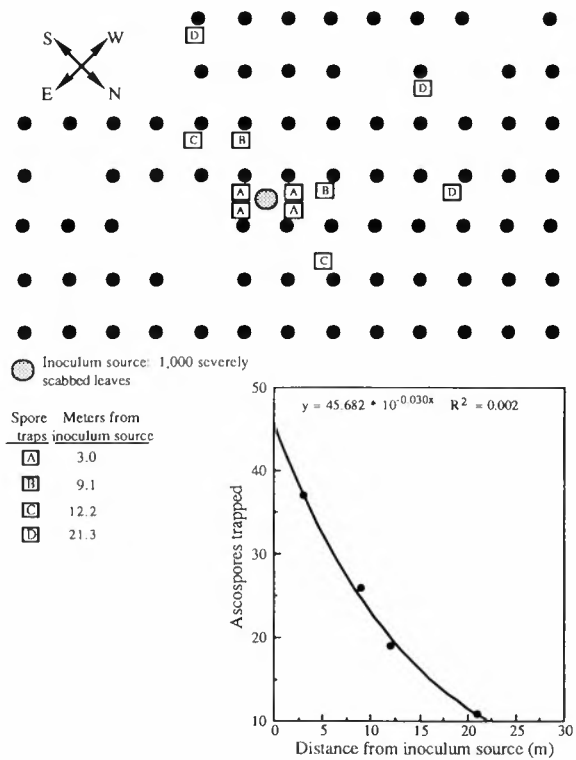
## ASCOSPORE DISPERSAL WITHIN AN ORCHARD

### Spore trapping studies

Nearly all ascospores are ejected 5 mm or less above a scabbed leaf (Aylor & Anagnostakis 1991). Some ascospores are trapped by ground vegetation. Others are swept into the orchard air by air currents or they fall to the ground due to gravitational pull. The dispersal

of ascospores of *V. inaequalis* within an orchard has received little attention, but it was the subject of one detailed study (Kaplan 1986) in which spore traps were placed 3 to 21.3 m from a point source of inoculum (1000 severely scabbed leaves) placed in an orchard (Fig. 1). The airborne ascospore density (AAD), i.e., ascospores per m<sup>3</sup> air at 0.9 m above the orchard floor, decreased exponentially over those distances from the source. In a related study (Kaplan 1986), a steep ascospore dispersal gradient near a source was calculated from spores trapped 0.5 m directly above the source and within the canopies of four apple trees 5.4 m from the edge of the source during four rainy periods (Fig. 2). For the four rainy periods, the AAD was reduced 99.4, 99.7, 99.4, and 99.6%, respectively, at 5-6 m from the source. The mean ratio of ascospores trapped at the source to ascospores trapped in the four tree canopies for the four rainy periods was 223:1, and the mean ratio of ascospore area dose (which considers wind velocity) was 334:1.

Fig. 1. The dispersal gradient of ascospores of *Venturia inaequalis* discharged from a point source of scabbed leaves in a MacIntosh orchard. Developed from data reported by Kaplan (1986). Reprinted, by permission, from MacHardy (1994)



### Studies using apple trees as a "biological trap"

Scabbed apple leaves placed in the centers of a Cox's Orange Pippin plot and a Worcester Pearmain plot did not cause a detectable amount of scab 15 m beyond the point source of inoculum in either of two years the study was conducted (Burchill 1966). Similar results were obtained in field trials by the same researcher (Burchill & Hutton 1965): the ascospores dispersed from a block with a high ascosporic inoculum caused negligible scab in a block nearby that had been treated the previous autumn with a chemical to eradicate

the sexual stage of *V. inaequalis*. From these results, the investigator concluded that the scab that develops in an orchard is likely to be determined almost entirely by the inoculum produced in that orchard.

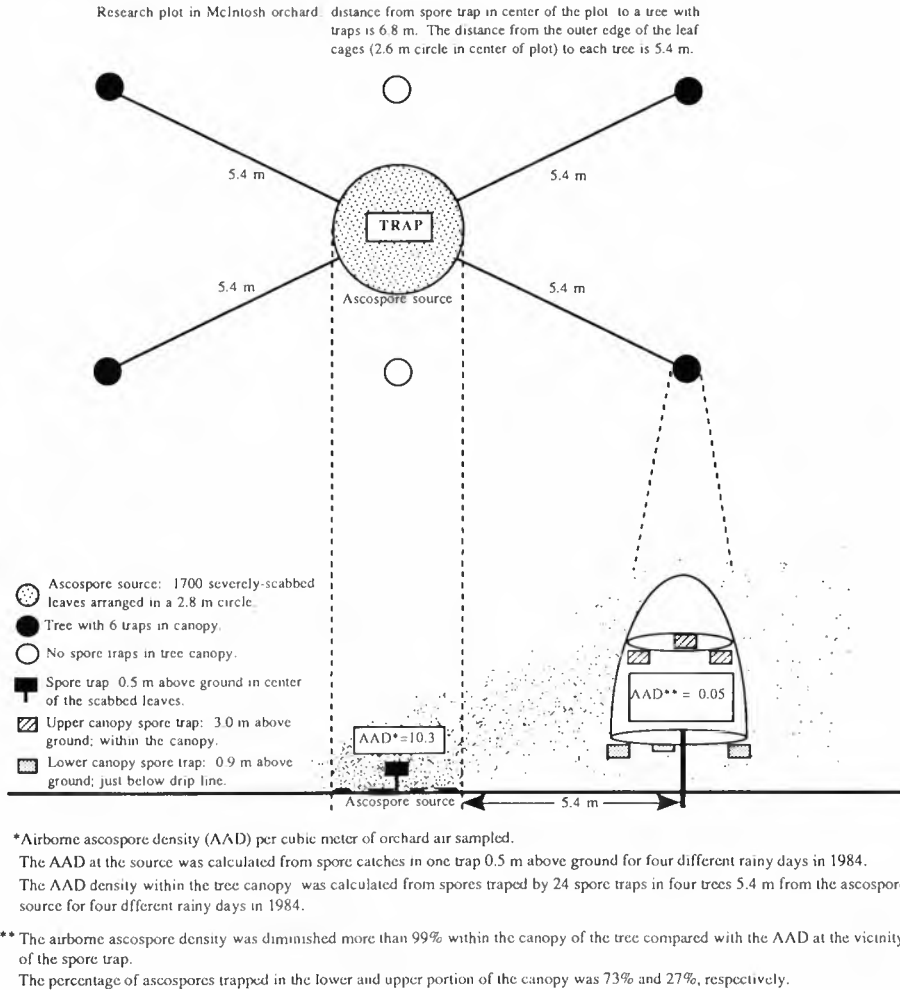
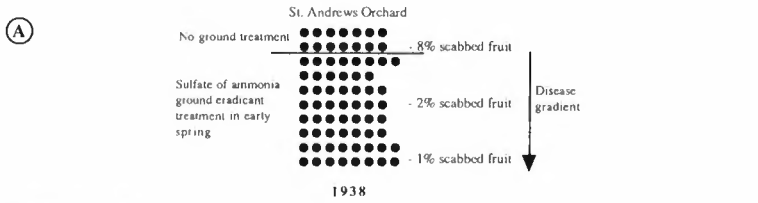


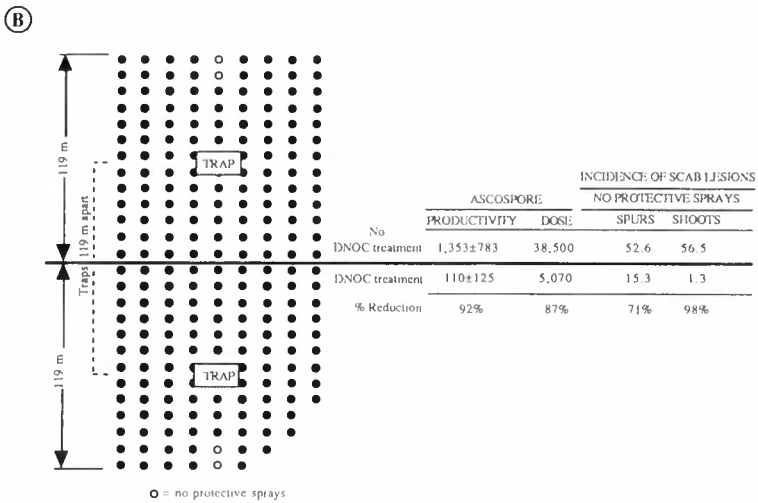
Fig. 2. A comparison of the airborne ascospore density (AAD) of *Venturia inaequalis* ascospores 0.5 m above a point source of severely scabbed apple leaves and the AAD within the canopy of four apple trees 5.4 m from the source. Developed from data reported by Kaplan (1986). Reprinted, by permission, from MacHardy (1994)

Further evidence that the ascospore dispersal gradient is steep within in an orchard is provided in two studies in which an eradicant chemical was applied to one section of a heavily-scabbed orchard. In a study by Palmiter (Palmiter 1946), the incidence of scabbed fruit in the untreated row bordering the treated section was 8% compared with 2% and 1% scabbed fruit in treated rows 4 and 8, respectively, away from the untreated area (Fig. 3A). Palmiter concluded from this pattern that when one section of an orchard is treated with an

eradicator chemical, the full effect of the treatment in reducing scab can be seen in four to eight rows from the untreated area, with the distance dependent primarily on wind direction and the quantity of inoculum in the untreated section. In a study by Hirst & Stedman (1962), ascospore productivity and ascospore dose were reduced 92% and 87%, respectively, and the incidence of scab lesions on unprotected spur and extension shoot leaves was reduced 71% and 98%, respectively, in the section of a severely-scabbed orchard that had been treated with dinitro-ortho-cresol (DNOC) to eradicate the overwintering stage of *V. inaequalis* (Fig. 3B). The similar reduction in ascospores and lesions in the treated section suggests that the number of ascospores that were dispersed the approximately 59 m from the untreated section to the treated section produced an inconsequential number of scab lesions. The authors concluded that the ascospore dose in an orchard is determined by ascospores produced within the orchard.



**IMPORTANT POINT:**  
 Ascospore inoculum from the nontreated portion was largely responsible for the 8% scabbed fruit on trees in the row bordering the nontreated portion, but the low incidence of scab 4 and 8 rows inside the treated portion identifies a steep gradient of ascospore dispersal from the nontreated portion.



**IMPORTANT POINTS:**  
 The similarity between the reduction in ascospore productivity (92%) and the reduction in ascospores trapped (87%) in the DNOC-treated plot when compared with the nontreated plot suggest that the dispersal of ascospores from the nontreated plot contributed insignificantly to the spore catch in the trap in the treated plot 59 m from the boundary between the plots.  
 Correspondingly high reductions of lesion incidence on spur and extension shoot leaves in the treated plot provide additional support for the contention that most ascospores discharged in the nontreated plot remained in the nontreated plot.

Fig. 3. Evidence for limited dispersal of ascospores of *Venturia inaequalis* within an orchard. A. Adapted from data reported by Palmiter (1946). B. Adapted from data reported by Hirst & Stedman (1962b). Reprinted, by permission, from MacHardy (1994)

Disease gradients have been considered indicators of spore dispersal in orchards other than apple and with pathogens other than *V. inaequalis*, and of particular relevance to this review is a two-year study that investigated the incidence of blossom infection caused by *Sclerotinia laxa* (Alderh. & Ruhl) in apricot orchards (Wilson & Baker 1946). Each orchard (3 orchards in 1939; 4 orchards in 1940) was treated with an eradicator chemical, monocalcium arsenite, except for a small untreated section that served as the inoculum (i.e., conidia) source. In each orchard, there was a clearly defined exponential decrease in disease incidence windward to the inoculum source (Fig. 4A and B). The incidence of blossom blight decreased an average 84% between 7 m and 27 m from the inoculum source in 1939 and an average 59% in 1940. The less steep disease gradient in 1940 was directly related to increased wind speed. Presumably, the disease gradient was less steep at the greater wind speeds because the conidia traveled further before settling out due to gravitational pull.

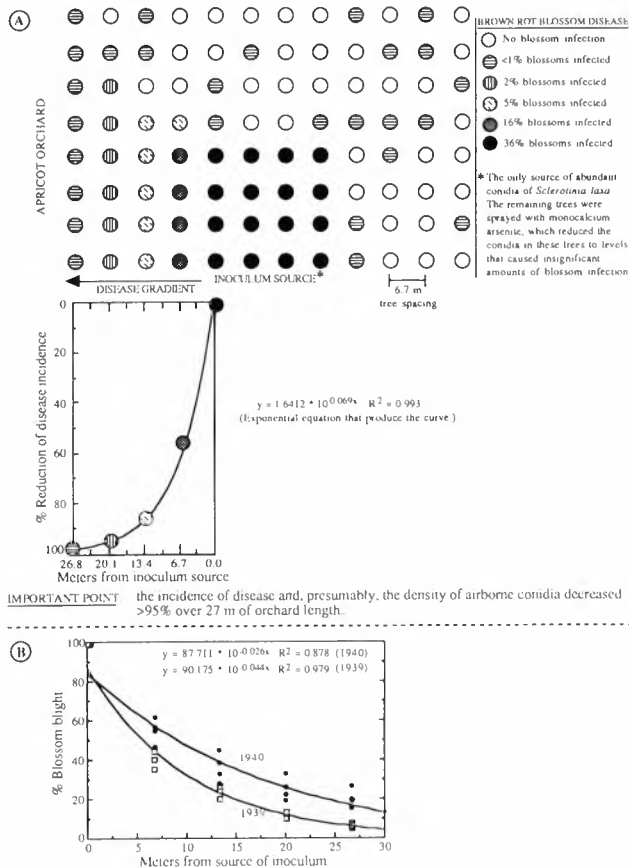


Fig. 4. Gradients of blossom blight, caused by *Sclerotinia laxa*, in apricot orchards. The disease gradients were windward of a delimited source of *S. laxa* conidia that were established by treating all but the source area with an eradicator chemical. A. Disease gradient and pattern of blossom blight in one orchard. B. Gradients of blossom blight for three orchards in 1939 and four orchards in 1940. Curves are mean disease gradients for all orchards assessed in 1939 and in 1940. Figures and equations were developed from data and figures reported by Wilson & Baker (1946). Reprinted, by permission, from MacHardy (1994)

## ASCOSPORE DISPERSAL FROM ONE ORCHARD TO ANOTHER ORCHARD

The potential of chemical ground treatments to eradicate the overwintering stage of *V. inaequalis* and reduce disease was investigated beginning in the late 1920's (Keitt and Wilson 1927, 1928, 1929) in Wisconsin and continuing to the mid-1940's in Wisconsin and New York (Keitt & Palmiter 1937a, 1937b; Keitt & Moore 1943; Keitt et al. 1940, 1941a, 1941b; Palmiter 1940, 1946, Palmiter & Keitt 1936). The studies were conducted in severely-scabbed orchards. In some instances, entire orchards were treated with an eradicant chemical, and this necessitated the selection of another orchard to serve as an untreated check. Orchards were selected that were close enough for comparisons of eradicant treatment effects and fungicide spray schedules to be meaningful. The eradicant treatments usually reduced the ascosporic inoculum 95-99%, and in nearly every instance there was a close correlation between the reduction in ascosporic inoculum and the reduction in the incidence of scab lesions in the treated orchard, when compared with ascospore production and disease development in the untreated orchard. Thus, the dispersal of ascospores from the untreated (source) orchard to the treated orchard did not cause a noticeable increase in the incidence of scab lesions.

It is important to note that wind direction was not reported in these studies. It is obvious from Fig. 4 that an orchard upwind would not be expected to receive much inoculum, so in any of these studies, it is possible that a low amount of scab developed in an untreated orchard because the orchard was upwind of the inoculum source. However, it is unlikely that all storm fronts during the primary (ascospore) season each year would have driven ascospores in the untreated orchard away from the treated orchard. In two instances, significant dispersal of ascospores between the source and recipient orchards did occur. These two instances along with other selected examples from these studies are discussed below.

### **Orchards separated by $\leq 200$ m**

In 1936, an unexpectedly high amount of scab developed in a Wealthy orchard that had been treated with an effective eradicant (Keitt & Palmiter, 1937b). The treated orchard was near an untreated Wealthy orchard, so the scab lesions in each quadrant were assessed separately and analyzed with respect to distance from the untreated orchard. Neither orchard had received a fungicide treatment during the growing season. The average number of scab lesions per extension shoot was approximately 55 lesions in the untreated orchard and 12, 8, 5, and 2 lesions per shoot in the four quadrants of the treated orchard, with near and far borders 91 and 213 m, respectively, from the untreated orchard (Fig. 5). The gradient of scab lesions per extension shoot is highly correlated with distance from the inoculum source, and at approximately 200 m from the inoculum source, there were 95% fewer lesions per extension shoot. The pseudothecial density had been reduced 98% in the treated orchard, so it is reasonable to assume that the lesion gradient shown in Fig. 5 identifies the dispersal gradient of ascospores from the untreated orchard over the primary (ascospore) scab season.



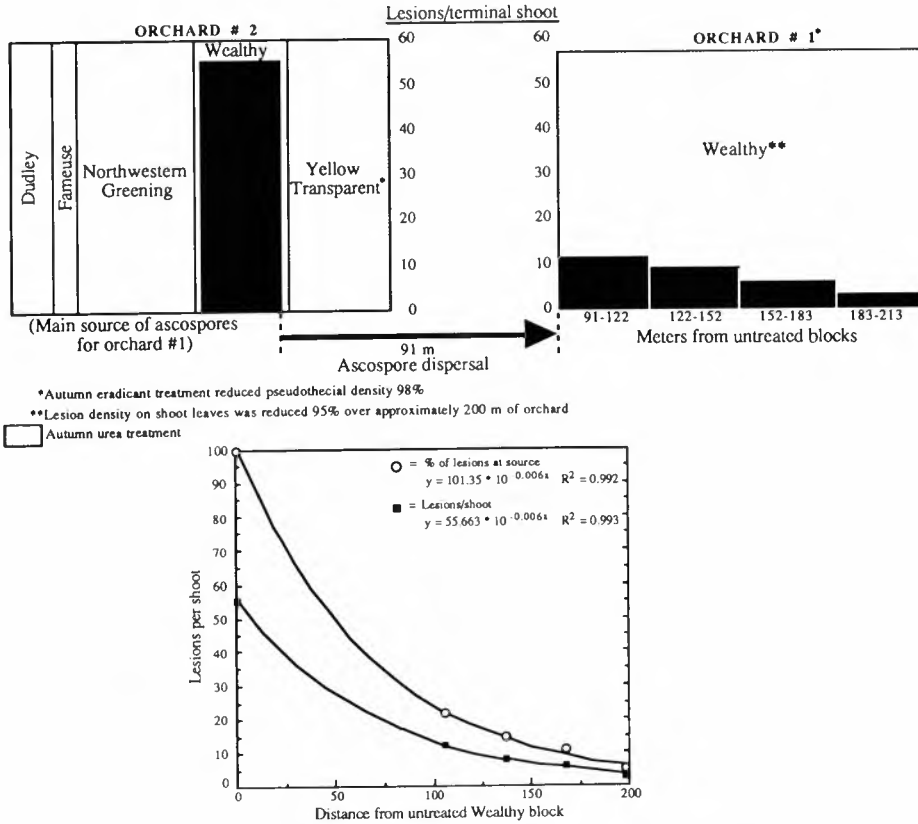
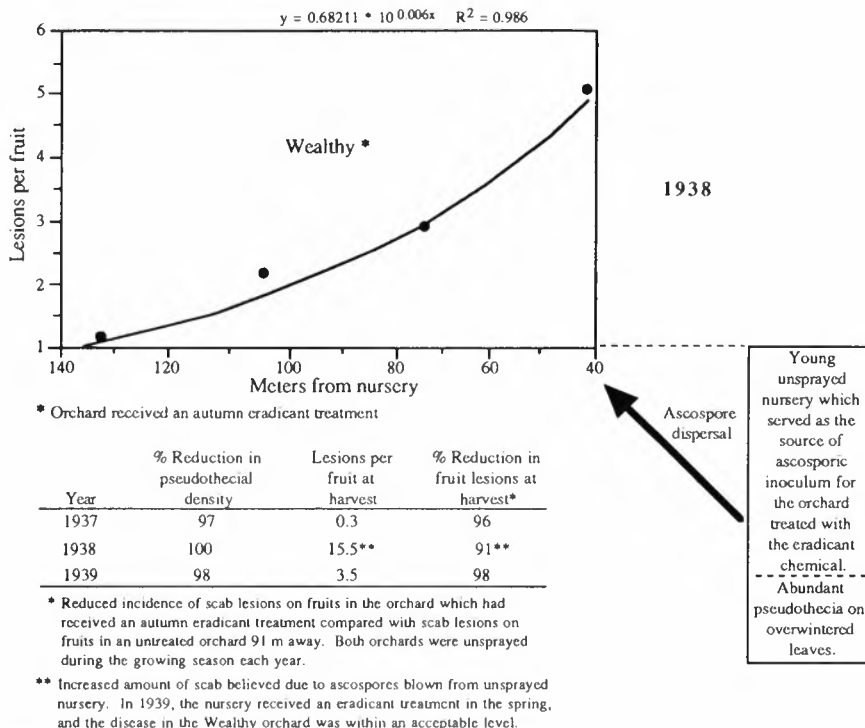


Fig. 5. The severity of scab on terminal shoot leaves (empty circles) and percent reduction in lesion density (filled circles) on shoots in an unsprayed Wealthy apple orchard treated with an eradicant chemical the previous autumn. The orchard was 91 m from the closest boundary of an untreated and unsprayed Wealthy orchard that was the source of the ascosporic inoculum responsible for the disease gradient in the treated Wealthy orchard. Developed from data reported by Keitt & Palmiter (1937b). Reprinted, by permission, from MacHardy (1994)

In 1938, the number of scab lesions per fruit in the quadrants of the treated Wealthy block was 5.1, 2.9, 2.2, and 1.3 lesions; thus, the severity of scab on the fruit was reduced 75% over the approximately 100 m length of the Wealthy orchard. The disease gradient sloped away from a young nursery planted nearby. An inspection of the nursery revealed abundant pseudothecia on the overwintered leaves (Fig. 6) (Keitt et al. 1941a), and apparently those leaves were the source of the ascosporic inoculum that caused the disease gradient in the Wealthy orchard. The leaf litter in the nursery received a ground eradicant treatment in the spring, 1939, and apparently that treatment eliminated the nursery as a major source of ascosporic inoculum for the Wealthy orchard. This is seen in Fig. 6 by the close correlation between the percentage reduction in pseudothecia and the percentage reduction in fruit lesions at harvest in the treated Wealthy orchard, i.e., both were reduced 98% compared with measurements of pseudothecial density and scab incidence in an untreated Wealthy orchard 91 m away (Orchard #2, Fig 5).



**IMPORTANT POINTS:**

The incidence of lesions on scabbed fruit was reduced 75% over 91 m of orchard.

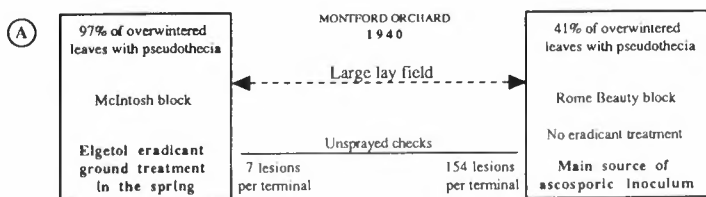
The eradicant treatment reduced the pseudothecial density and the amount of scab correspondingly in 1937 and 1939. In 1938, the eradicant treatment was similarly effective in reducing the pseudothecial density, but more lesions developed than expected. The lesion gradient suggest that ascospores blown from the unsprayed nursery accounted for the increased scab incidence in 1938.

The unsprayed trees can be considered "biological spore traps;" thus, the lesion gradient reflects the gradient of ascospore dispersal from the nursery during 1938.

Fig. 6. The gradient of scab severity on Wealthy fruit in an unsprayed orchard treated with an eradicant chemical the previous autumn. The orchard was less than 100 m from an unsprayed nursery with abundant pseudothecia of *Venturia inaequalis* that apparently was the source of the ascosporic inoculum that caused the disease gradient. Developed from data reported by Keitt et al. (1941). Reprinted, by permission, from MacHardy (1994)

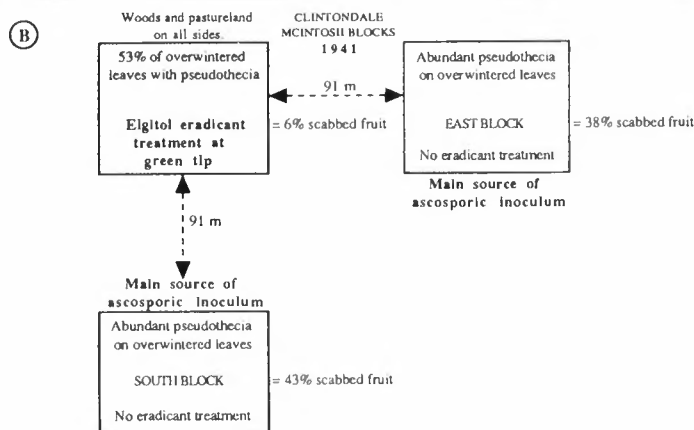
In two other situations (Fig. 7), the dispersal of ascospores from a severely scabbed block (Montford orchard) across a large hay field to a treated block and from two severely scabbed blocks (Clintondale orchard) across 91 m of woods and pastures to a treated block was apparently inconsequential in that no scab developed in the treated blocks that could be attributed to ascospores dispersed from the untreated "source" blocks (Keitt et al. 1941a). At the Montford orchard, the ground eradicant treatment in the McIntosh block reduced ascospore discharge >90% and the lesion severity on extension shoot leaves 95% compared with ascospore discharge and lesion severity in the nontreated Rome Beauty block. The untreated and treated blocks at the Montford orchard were planted with different cultivars, but that does not appear to account for the difference in the amount of scab that developed in the two blocks, because both cultivars are highly susceptible to scab and both

had been severely scabbed the previous year. Rather, the difference is thought to be caused by the eradicator treatment and the lack of significant dispersal of ascospores from the treated to the untreated block. Approximately 41% of the overwintered leaves in the untreated block had pseudothecia, indicating that the ascosporic inoculum must have been many-fold greater than would be expected in an orchard in which scab had been well managed, and probably comparable to an unsprayed or abandoned orchard. The 154 lesions per extension shoot that developed in this block support this idea. Yet, only 7 lesions per extension shoot developed in the treated block. At the Clintondale orchard, the 38% and 43% scabbed fruit in the two untreated blocks indicates that they must have been a large ascosporic inoculum, and the development of only 6% scabbed fruit in the treated block less than 91 m away indicates that a low proportion of the ascospores discharged in the two untreated blocks must have dispersed to the untreated block. The results suggest that if the ascosporic inocula in the untreated blocks had been at the levels usually found in commercial orchards today, the incidence of scabbed fruit in the treated block would have been negligible.



**IMPORTANT POINTS:**

Laboratory tests showed that Elgetol applied to infected leaves reduced ascospore discharge from 90% to 100%. Thus, the main source of inoculum for the McIntosh block treated with Elgetol was from the Rome Beauty block. The 95% fewer lesions per terminal in the McIntosh block suggest that the McIntosh block received no identifiable influx of ascospores from the Rome Beauty block.



**IMPORTANT POINTS:**

All blocks received fungicide sprays in 1941, but the fungicide program was not very effective as evidenced by 43% and 38% scabbed fruit in the South and East blocks. The 6% scabbed fruit in the treated block, which received the same fungicide treatment, indicates an insignificant influx of ascospores or conidia from the two poorly sprayed blocks with high ascosporic inoculum.

Fig. 7. The buildup of apple caused by *Venturia inaequalis* in high inoculum and low inoculum (eradicant-treated) orchards separated by a large hay field (A) or by 91 m of woods and pasture (B). Developed from data reported by Palmiter (1646). Reprinted, by permission, from MacHardy (1994)

Evidence that the number of ascospores dispersed up to 200 m from a high inoculum source can be managed with little or no need for fungicide treatment is seen in a study in New Hampshire, in which 1000 severely scabbed leaves were placed 200 m upwind of an orchard that had received an eradicant treatment of benomyl in the autumn (Gadoury & MacHardy 1984a). Twenty scab infection periods, predicted according to the criteria of Mills (Mills 1944, Mills & LaPlante 1951), occurred during the next growing season; yet, ascospores discharged from the 1000 scabbed leaves caused only 2.0% scabbed fruit on McIntosh, Cortland, and Delicious trees that had been left unprotected with fungicide and only 0.3% and 0.7% scabbed fruit on trees that had been sprayed with captan from one side or from both sides, respectively.

#### **Orchards separated by approximately 0.5 km**

In some chemical eradicant studies in Wisconsin (Keitt et al. 1941a) and New York (Palmiter 1946) the treated and untreated orchards were separated by approximately 0.5 km, and the results were similar to those discussed above for orchards that were closer to one another. The ascosporic inoculum and the number of scab lesions in the treated orchards were each reduced over 90%, indicating that ascospores dispersed from the untreated orchard to the treated orchard did not cause a buildup of scab lesions in the treated orchard that was greater than expected.

#### **SUMMARY**

Ascospores of *V. inaequalis* are dispersed by wind, and the dispersal gradient is steep. Most ascospores are deposited in the orchard in which they are discharged, and most of the remaining ascospores are deposited within 100 m from the source. With respect to making management decisions, the question is not how far ascospores are dispersed from a source; rather, the question is how many ascospores must be dispersed to an orchard for a fungicide treatment (or some other management practice) to be recommended. Based on this review of published studies that have information pertaining to the dispersal of ascospores of *V. inaequalis*, there is no evidence that ascospores dispersed from a high inoculum source (e.g., an abandoned or poorly-managed orchard) throughout the primary (ascospore) season will cause a detectable amount of scab in an orchard >200 m downwind, and quite possibly as near as 100 m downwind. No studies were found that had investigated the amount of scab that would be caused by ascospores dispersed from a high inoculum source during one infection period, but considering the results of the studies reviewed, it is not unreasonable to suggest that the ascospores dispersed only 50 to 100 m downwind will not cause a detectable increase in scab if the orchard is not protected with fungicide for that rainy period. A study is needed that is designed to determine the relationship between a known ascospore dose and the buildup of scab in trees left untreated with fungicide at different fruit bud development stages, or for different infection periods, in an orchard a known distance downwind. The study should include orchards that span the range of high density and low density plantings, as well as the range of tree heights and shapes, common in the region. Such a study is well-suited for an international research project, because many more cultivars, orchard types, ascosporic inoculum levels, weather patterns, and dispersal distances would be included each year.

LITERATURE

- Aylor, D.E. & S.L. Anagnostakis 1991. Active discharge distance of *Venturia inaequalis* ascospores. *Phytopathology* 81:
- Burchill, R.T. 1966. Air-dispersal of fungal spores with particular reference to apple scab (*Venturia inaequalis* [Cooke] Winter). Proc. 18th Symp. Colston Res. Soc., Univ. Bristol. Butterworths Scientific Publications, London.
- Burchill, R.T. & K.E. Hutton. The suppression of ascospore production to facilitate the control of apple scab (*Venturia inaequalis* [Cke.] Wint.). *Ann. Appl. Biol.* 56: 285-292.
- Hirst, J.M. & O.J. Stedman 1962. The epidemiology of apple scab (*Venturia inaequalis* [Cke.] Wint.) III. The supply of ascospores. *Ann. Appl. Biol.* 50: 551-567.
- Kaplan, J.D. 1986. Dispersal gradients and deposition efficiency of *Venturia inaequalis* ascospores and their relationship to lesion densities. Ph.D. dissertation. University of New Hampshire. 198 pages.
- Keitt, G.W., C.N. Clayton & J.D. Moore 1941. Experiments with eradicant fungicides in relation to apple-scab control. (Abstr.) *Phytopathology* 31: 13-14.
- Keitt, G.W., C.N. Clayton & M.H. Langford 1940. Eradicant fungicidal treatments in relation to apple-scab control. (Abstr.) *Phytopathology* 30: 13.
- Keitt, G.W., C.N. Clayton & M.H. Langford 1941. Experiments with eradicant fungicides for combating apple scab. *Phytopathology* 31: 296-322.
- Keitt, G.W. & J.D. Moore 1943. Experiments with eradicant and protectant sprays for apple scab control in 1942. (Abstr.) *Phytopathology* 33: 6.
- Keitt, G.W. & D.H. Palmiter 1937a. Eradicant fungicides in relation to apple scab control. (Abstr.) *Phytopathology* 27: 133.
- Keitt, G. W. & D. H. Palmiter 1937b. Potentialities of eradicant fungicides for combating apple scab and some other plant diseases. *J. Agric. Res.* 55: 397-436.
- Keitt, G.W. & E.E. Wilson 1927. A possible reorientation of aims and methods for apple scab control. (Abstr.) *Phytopathology* 17: 45.
- Keitt, G.W. & E.E. Wilson 1928. Fall applications of fungicides in relation to apple scab control. (Abstr.) *Phytopathology* 18: 146.
- Keitt, G.W. & E.E. Wilson 1929. Third progress report of studies of fall applications of fungicides in relation to apple scab control. (Abstr.) *Phytopathology* 19: 87.

MacHardy, W.E. 1994. A "PAD" action threshold: the key to integrating practices for managing apple scab. Norwegian J. Agric. Sci. Supplement No. 17: 75-82.

Palmiter, D.H. 1940. Eradicant treatments as an aid in the control of apple scab. (Abstr.) Phytopathology 30: 18.

Palmiter, D.H. 1946. Ground treatments as an aid in apple scab control. N. Y. Agric. Expt. Stn. Bull. 714. 27 pp.

Palmiter, D.H. & G.W. Keitt 1936. Progress of studies of eradicant fungicides in relation to apple scab control. (Abstr.) Phytopathology 26: 103-104.

Wilson, E.E. & G.A. Baker 1946. Some aspects of the aerial dissemination of spores, with special reference to conidia of *Sclerotinia laxa*. J. Agric. Res. 72: 301-327.

# Chemical control of apple scab - status quo and future

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One goal of IFP programs is a reduction in the number of fungicide sprays applied for the control of apple scab and other tree fruit diseases. This goal can be reached best if fungicides are applied "as needed", after an infection has already occurred. Fungicides used for the control of apple scab can be divided into two different classes, the multi-site or non-specific protectants and the site-specific systemics. Both classes have advantages and disadvantages. Non-specific protectants are confined to the cuticular surface and can only halt the disease before the spores have penetrated through the cuticle. After-infection activities of protectants are for principle reasons very short. Site-specific systemic fungicides exhibit long after-infection and presymptom activities, but they are prone to the development of resistance. In the control of apple scab, resistance has been encountered with dodine and the benzimidazoles. The systemic sterol demethylation inhibitors are currently in wide use, and widespread resistance has not compromised their performance. Successful anti-resistance strategies developed for these inhibitors are the reduction of disease pressure through sanitation and mixtures with a protectant fungicide, the decrease in selection cycles per season through avoidance of season-long application, and the decrease in frequencies of resistant strains through high application rates.

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The goal of disease control with fungicides is to protect plants from disease without harming the host plant, the applicator, the consumer and the environment. Obviously, this goal is challenging, but considerable progress has been made over the last few decades. Improved chemical control measures have also been developed for the control of apple scab, the most challenging disease of tree fruits. The previous reliance on the relatively rigid use of fungicides as the primary and often only tool of scab management has been replaced by the concept of Integrated Pest Management (IPM) or Integrated Fruit Production (IFP). The mission of IFP is to integrate all available measures of pest and disease control, including insecticides and fungicides; the goal of IFP is to optimize the economical production of fruits with minimal side effects on the consumer and the environment.

The definition of an IFP optimum remains flexible and is different from country to country. For example, the economy of fruit production will depend on the level of government subsidies and on the level of market competition. Safety aspects of fresh and processed fruit are largely determined by the regulatory definition of tolerated pesticide residues. These residue levels are not only determined by the toxicological property of a particular pesticide, but also by the level of "negligible risk" defined in national regulations. In particular the oncogenic (tumor-producing) dietary risk originating from pesticide residues has triggered legislative action but also controversial discussions (Gold *et al.* 1992). Because decisions are not only based on scientific facts but also on societal acceptance of "negligible" risks, it is not surprising that some pesticides can be used in one country but are banned in another.

The safety risk inherent to natural chemicals as part of our diet has received less legislative and public attention in the past. However, questions regarding the relative safety of chemicals, man-made on one side and natural on the other, have emerged (Ames *et al.* 1990a, 1990b), and future IFP concepts might have to adapt to changing definitions of chemical safety. The examples demonstrate that IFP is not a static concept. It will have to adapt to changing regulatory decisions, and IFP recommendations will have to continuously incorporate novel avenues of pest and disease control, including new chemical control agents as they become available. There are no rational reasons to believe that the improvement of chemical disease control agents toward high specificity and low or no side effects on non-target organisms has reached its limits (Köller 1992a).

Regardless of future perspectives and developments, the health risk of fungicide residues in food has received considerable public attention in the past, and it is not surprising that the reduction of the amount of fungicides used in fruit production is envisioned as one of the major current driving forces of IFP. One aspect rarely addressed is the qualitative difference between fungicides, and how characteristic fungicide properties are better suited than others to be utilized in IFP programs.

Part of the continuous progress made over the last two decades is the reduction in the amount of fungicides necessary to control scab. Protectant materials introduced in the 1950s have to be applied at a rate of 5,000 - 10,000 g/ha for control of scab over one season; seasonal rates are reduced to 50 - 100 g/ha for some of the newer systemic compounds introduced in the 1980s. Another part of recent progress is our increased understanding of why and how fungicides act in controlling scab and other diseases. The technical advancements made in the life sciences have facilitated a better understanding of the physical and chemical modes of fungicide action, the mechanisms of resistance and how they relate to the risk of resistance development. Knowledge about fundamental aspects of fungicide action allows us to understand the benefits but also limitations of certain classes of fungicides, and it helps us to select and apply fungicides based on their benefits but also limitations.

If a fungicide is to inhibit the growth of a fungus, it has to interfere with vital processes inside the fungal cell. The term mode of action describes, which processes are blocked in the presence of a fungicide. From a mode of action point of view, the currently available fungicides can be divided into two distinctly different groups, the non-systemic multi-site inhibitors, and the class of site-specific fungicides, which are mostly systemic and, thus, can be applied after infection has occurred. While the non-systemic compounds



are not prone to resistance development, site-specific inhibitors are, and the development of field resistance has necessitated the development and implementation of resistance prevention and management schemes. The goal of this overview is to describe and discuss these benefits and limitations for the fungicides most widely used for the control of apple scab, and to outline the currently emphasized anti-resistance strategies for the site-specific inhibitors.

## MULTI-SITE INHIBITORS

The early history of fungicides is intimately connected with inorganic sulphur. Still widely used for the control of powdery mildew on many crops, apple scab can only be controlled to a certain extent and at higher rates. Sulphur is active on the leaf and fruit surface as an inhibitor of spore germination. Surprisingly, the question of how it works is yet not fully answered, although all mode of action models suggest a non-specific activity. One of the more plausible explanations is that sulphur oxidizes to sulphur dioxide, which converts in the presence of water to sulphonic acid, the actual agent inhibiting spore germination. This process, which is similar to the generation of acid rain from sulphur dioxide as one of the major industrial pollutants, is also responsible for the acidification of soil in orchards where sulphur has been used for a long period of time. Moderate levels of scab control, the potential necessity of soil quality management and the risk of phytotoxic effects at high rates (sulphur burns) explains why apple growers quickly accepted organic fungicides after they were introduced in the late 1940s and early 1950s. However, sulphur has experienced a revival, because it can be used in organic fruit production. Although the quality of sulphur formulations has improved over recent years, many of the former restrictions still apply.

Metalloorganic fungicides such as phenylmercury acetate introduced in 1914 can be viewed as the transition from inorganic to organic fungicides. For toxicological and environmental reasons, use of mercury-containing compounds has been banned in most countries. The first representatives of organic fungicides were the dithiocarbamates such as ferbam and thiram, followed by the ethylene bisdithiocarbamates (EBDCs) such as zineb, maneb, mancozeb and propineb. Another early class of organic fungicides containing an essential *N*-trichloromethylene thio-(or closely related) group was introduced in the early 1950s. Representatives of this class are captan, captafol, folpet, tolylfluanid and dichlofluanid. Dithianon as a single compound also belongs to this class of related fungicides. All compounds are still in wide use for the control of apple scab, although use restrictions mandated by toxicological properties apply. These restrictions are different from country to country.

All of the compounds listed above share a more or less common mode of action (Buchenauer 1990). A great number of enzymes in all classes of organisms contain a SH-(cysteine) group as an essential component of their biocatalytic activity. These SH groups are highly reactive, and they are chemically modified by either the fungicide itself, or by a highly active conversion product originating from the fungicide. The chemical modification leads to the desactivation of the respective enzyme, and the network of interconnected enzymatic reactions within a fungal organism is disrupted at several crucial points. Consequently, all of the compounds have a multi-site or non-specific mode of action.

Damage to the fungal cell is severe and leads to death (fungitoxic action).

Unfortunately, SH-containing enzymes are abundant in nature and not restricted to fungal organisms. A non-specific mode of action therefore implies that not only fungal but also plant enzymes are prone to inhibition, and that plant tissue would be severely damaged if these non-specific fungicides would enter the plant. Fungicide entry is prevented by the plant cuticle, the hydrophobic layer shielding and protecting the epidermal cell layer of all green plant tissues (Köller 1991a). Because phytotoxic effects are unacceptable in crop production, all non-specific fungicides must be restricted to the cuticular surface. Consequently, they exhibit protective activity. They only can interfere with the pathogen before it becomes established underneath the cuticle and thus has escaped the inhibitor confined to the cuticular surface. Consequently, the stages of scab development accessible to inhibitor action are spore germination, germ tube elongation and appressoria formation. The principle limitation of protective fungicides is immediately apparent. They cannot stop disease development after the cuticle has been penetrated and after subcuticular mycelium is established. It is not surprising that, in the past, these scab materials have been applied according to a more or less static protective schedule, with weekly sprays beginning with the appearance of green susceptible tissue in spring.

One of the IFP goals is the avoidance of unnecessary fungicide sprays and the application of fungicides on an "as needed" schedule, a concept dependent on scab warning schemes. This concept also requires that fungicides are applied subsequent and not prior to an infection. Naturally, the question of how long after the start of an infection period these non-specific and protective fungicides can be applied has become important. Helpful in the discussion of this question is the classification of fungicide activities according to Szkolnic (1981):

#### *Protection*

Protection refers to the ability of a fungicide residue to prevent spores from cuticle penetration. Therefore, the residue must be already on the leaf or fruit before the infection is initiated.

#### *After-infection activity*

After-infection activity refers to the ability of a fungicide to completely prevent growth and development of the pathogen, if applied within a given period after infection has occurred. It is expressed as the period of time within which the fungicide must be applied after the beginning of an infection period. A different term frequently used is kick-back activity.

#### *Presymptom activity*

Presymptom activity is defined as an extension of after-infection activity. When applied following an infection period, but beyond the time limits of its after-infection activity, a fungicide with presymptom properties will allow small chlorotic lesions to develop. However, it will inhibit or greatly reduce the production of conidia from those lesions. An alternative term frequently used is curative action.

#### *Postsymptom activity*

Postsymptom activity refers to the ability of a fungicide, when applied to an actively

sporulating scab lesion, to prevent or greatly inhibit the continued production of spores from that lesion. As with presymptom activity, this has the obvious effect of reducing the spread of secondary scab. Eradicative action is often used as alternative term. Because secondary inoculum was already produced, a postsymptom treatment should be followed by an after-infection spray as soon as a new infection occurs.

As stated above, non-specific fungicides are confined to the plant surface, and the invading scab spore has escaped the action of a protective fungicide as soon as subcuticular mycelium develops. This stage is preceded by germination, germ tube elongation and appressoria formation. With conidia of *Venturia inaequalis*, the process of appressoria formation is completed after 24 h of germination at 20°C. The development of visible subcuticular mycelium and, thus, the escape from a non-specific inhibitor confined to the surface, is initiated after 24 h and completed after 48 h (Köller *et al.* 1991a). This time course implies that, at 20°C and after 24 h, a good portion of infective conidia have already escaped the action of a non-specific inhibitor. This time frame has been confirmed in greenhouse studies. At 20°C, captan and mancozeb were virtually inactive when applied in a 24 h after-infection mode (Szkolnic 1981).

According to the "Mills Table", infection is slower at low temperatures. The formation of appressoria from germinating ascospores or conidia follows this trend. Turner *et al.* (1986) listed as time required for the formation of 10% appressoria from germinating conidia 6.1, 6.2, 6.9 and 12.5 h at 20, 15, 10 and 5°C, respectively. For ascospores, this process took 2-4 h longer. The data indicate that the after-infection activity of a protective fungicide is approximately doubled at 5°C.

What level of scab control can be achieved if protective fungicides are applied in a presymptom mode? Two major parameters will determine the level of disease control achieved under such conditions. After-infection activities are commonly given as "hours after the start of an infection". In particular with long infection periods and at low temperatures, spores released at the beginning of an infection period will be in a different "fungicide escape" stage than those released at the end, and "late" infections could still be prevented. Moreover, protective fungicides exhibit relatively good retention on leaf and fruit surfaces and are not excessively and rapidly washed off by rain. The portion of residue that is washed off during rain redistributes and protects new growth. This protection of new growth through redistribution is more pronounced for fruits, which are locally fixed, than for leaves at rapidly expanding terminals at the outside of the canopy. Non-specific fungicides applied in a presymptom mode will not substantially inhibit the development of lesions from the preceding infection period. They will, however, act protective against the next following infection.

In regions, where "regular" scab warnings are issued every other 7-10 days, a presymptom application of a protective fungicide will provide adequate control in most cases, not through its presymptom activity, but rather through its protective activity toward the next following infection. Only the formation of lesions originating from the very first infection period would not be prevented, but all subsequent infections would be covered under conditions of more or less "regular" and frequent infection conditions. In particular in orchards with low potential ascospore doses, the first seasonal spray can be omitted without compromising scab control over the entire season (MacHardy *et al.* 1993). Under these conditions, protective fungicides applied in a "pseudo" presymptom mode can be

expected to provide sufficient control. However, the reduction in the number of sprays will be minimal.

Restrictions to a "presymptom" application of protective and non-specific fungicides apply. If intervals between infection periods are long, the required protection of new tissue by residues redistributed by rain will decline, because the redistributed residue would be diluted over a larger surface area. The dose per surface area would be low, in particular if the compound was applied at low rates. Therefore, control failures are likely to happen, if protective fungicides are regularly applied in a presymptom mode with long intervals between infection periods. These theoretical considerations based on the physical mode of action of non-specific fungicides are confirmed by results obtained from trials performed at an experimental orchard at the New York State Agricultural Experiment Station (Köller & Wilcox, unpublished results). Mancozeb at a low rate was applied three times during the primary scab season of 1992 with a moderate disease pressure (five infection periods). The first spray (tight cluster) was applied in a presymptom mode 10 days after the first and 3 days after the second infection. The second spray (full bloom) was applied 3 days, the third spray (petal fall) was applied 8 days after infection. The more relevant protective intervals were 12 days for the first, 4 days for the second and 3 days for the third application; spray intervals were 17 and 13 days between the second and third spray. Control levels achieved at the end of the primary season were 69 % for fruits and 46 % for leaves. This example shows that some control can be obtained with an apparent "presymptom" application of mancozeb; but it also exemplifies the dangers inherent to long spray intervals combined with low rates of a protectant fungicide.

#### SINGLE-SITE INHIBITORS: THE STATUS QUO

The principle disadvantage of non-specific fungicides is, as outlined above, their confinement to the plant surface and their lack of long after-infection activities. More desirable from an "as needed" point of view would be the availability of fungicides that penetrate into the plant tissue and thus would prevent the development of a disease even after colonization of the tissue has been completed. Because the inhibition of only one essential process in a fungal cell would be sufficient for the inhibition of fungal growth, single-site inhibitors can penetrate the plant tissue as long as their activity is fungal-specific. All of the compounds described below fulfil this requirement. They are highly fungal-specific and lack activity toward plant target sites, and they are systemic with more or less pronounced after-infection, presymptom and sometimes postsymptom activities.

The advantages offered by the class of site-specific and systemic compounds with their lower rates and longer spray intervals is substantial, in particular in view of IFP programs. However, it became apparent that site-specific inhibitors are prone to the development of resistance, a phenomenon never experienced with the class of non-specific protectants (Köller 1991b). It is generally accepted that low frequencies of fungal genotypes with resistance to any given site-specific fungicide are already present in orchard populations, before the fungicide has ever been applied. Under the selective force of the fungicide, they become more competitive, and their frequency will increase until control of the disease is no longer warranted. Over the last decade, it has also become apparent that the speed of

resistance development is not identical for all systemic compounds (Köller 1991b). Understanding these differences and, more importantly, understanding how these differences translate into preventive and counteractive resistance management strategies is the subject of ongoing research.

## DODINE

The first systemic fungicide introduced in the late 1950s for the control of apple scab was dodine (Gilpatrick 1982). As shown by Szkolnic (1981), dodine exhibits very good protective and excellent pre- and postsymptom activity. This clear improvement over protective fungicides makes it understandable that dodine was quickly accepted and widely used soon after its introduction, in particular in the Eastern United States and in Canada. Use in Europe was less widespread because of fruit russeting problems experienced with certain apple cultivars under the climatic conditions typical for some European countries.

The mode of action of dodine was investigated in the early 1960s, and evidence was presented that the inhibitor acts, as a "cationic detergent", on the integrity of fungal membranes, including those of mitochondria. Consequently, one of the measurable effects was the inhibition of respiration (Buchenauer 1990). Most of the mode of action studies were performed with fungi relatively insensitive to dodine. However, results obtained with *Monilinia fructicola*, a pathogen highly sensitive to dodine, did not entirely confirm this "detergent" mode of action. Much higher inhibitor concentrations were required for the inhibition of respiration (membrane effect) than for the inhibition of mycelial growth (Brown & Sisler 1960). Studies employing *V. inaequalis* as dodine's major target organism have never been forthcoming, and the precise mode of action remains elusive.

The dissolution of biological membranes as the widely accepted mechanism of dodine action has to be rated as non-specific. The question of why this non-specific mode of action would not also dissolve plant membranes subsequent to systemic penetration into plant tissue has not been answered. Regardless of these uncertainties, a non-specific mode of action should not allow the development of resistance. This was not the case. Control failures due to field resistance occurred after 10-12 years of extensive use, first in the apple growing region of Western New York in the late 1960s, and some years later in other parts of the United States and in Canada (Gilpatrick 1982). The initial report on resistance to dodine has to be rated as the first case of fungicide resistance under field conditions. Confirmation of dodine resistance was provided by tests of dodine sensitivities of germinating ascospore populations collected from orchards with either uncurtailed dodine performance, or sites where dodine had failed to control the disease (Gilpatrick & Blowers 1974). The germination sensitivities from the latter group of orchards were less sensitive to dodine by a factor of two to three. As noted by the authors, however, the test procedure employed in this study was not very precise, and results were not always consistent with field performance.

During the season of 1990 and 1991, we adapted a sensitivity test originally developed for the monitoring of population sensitivities to the group of sterol demethylation inhibitors described later (Smith et al. 1991, Köller et al. 1991b). The principles of this monitoring procedure are relatively simple. Tests are based on the isolation of single ascospores or

conidia and growth of mycelial colonies. This genetically homogenous mycelium serves as material for subsequent sensitivity tests based on the inhibition of mycelial colony growth at one discriminatory dodine dose. Sensitivity tests are performed with 40-50 isolates, and sensitivities are recorded as relative growth (RG = percent of colony diameter at the discriminatory dose). Low values reflect highly sensitive, and high values reflect less sensitive or resistant isolates.

The baseline sensitivity distribution of dodine derived from 250 isolates collected from orchards never exposed to dodine is shown in Fig. 1. The distribution of sensitivities was continuous and normal in character, with a broad spectrum of individual sensitivities ranging from RG-values of 8 - 95, which is equivalent to ED<sub>50</sub>-values (= 50% inhibition of mycelial growth) of 0.02 to 2 mg/L, with 0.2 mg/L most frequently found (Köller et al. 1993). Comparison of sensitivity distributions established for orchards where dodine had provided poor scab control during the early season of 1991 were clearly different and are shown in Table 1. Isolates with RG-values from 91-100 had increased from 0.5% in baseline populations to 33% in orchards with control failures. They had been selected in the presence of dodine and thus have to be rated resistant. The 66-fold increase in the frequency of resistant isolates in orchards with inadequate scab control indicates the threshold at which control failures will become likely.

Table 1: Dodine sensitivity distribution of scab populations in baseline and threshold orchards

Sensitivity <sup>1)</sup>	RG-Range <sup>2)</sup>	Scab control	Frequency of isolates (%)		Factor of change
			Baseline	Threshold	
S	0-70	easy	97	51	0.5
RS	71-90	difficult at times	2.5	16	6
R	91-100	difficult	0.5	33	66

<sup>1)</sup> S = sensitive; RS = reduced sensitive; R = resistant

<sup>2)</sup> Relative growth at 0.2 mg/L dodine

A survey of 21 orchards in Eastern and Western New York in 1991 (Köller et al. 1993a) confirmed that the monitoring procedure provided sufficient precision, and that the threshold level given in Table 1 is indicative for control failures due to population shifts toward dodine resistance. The survey results can be summarized as follows:

- (a) All orchards where dodine was recently used with good results had sensitivity levels below, all orchards with recent control failures had sensitivity levels above the threshold.
- (b) Resistance levels in old orchards, where dodine resistance was confirmed in the 1970s and where dodine had not been used for 20 years remained high. This result demonstrates that dodine resistance, once it is established, does not decline rapidly.
- (c) Orchards where dodine had been used once or twice (first seasonal sprays) for more than 25 years were at or close to baseline levels, and the respective growers had not noticed a decline in performance.

In summary, dodine was the first fungicide with a documented history of resistance development. As noted by Gilpatrick & Blowers (1974), the speed of resistance development was highest in regions, where dodine had been used season-long and at low doses. In orchards, where dodine has not been used excessively, the compound remains a valuable component in scab management. The restriction of dodine to scab control and its pre- and postsymptom activity makes it a valuable compound in IFP programs, in particular during the early scab season prior to bloom. Such an application should also eliminate problems experienced with fruit russetting. However, dodine can only serve as an "as needed" fungicide with postsymptom activity in orchards, where levels of resistance have not reached prohibitive levels.

## BENZIMIDAZOLES

The class of benzimidazoles, with carbendazim, benomyl and thiophanate-methyl was introduced in the early 1970s (Delp 1987) and was quickly accepted by apple growers. The systemic fungicides provided control of scab, powdery mildew and some of the summer diseases. For the control of scab, benomyl was found to exhibit fair protective and after-infection, and very good pre- and postsymptom activities (Szkolnic 1981).

The biochemical mode of benzimidazole action is the specific binding to fungal tubulin. Tubulin is a small protein and polymerizes to microtubuli, which are involved in the intracellular movement of large cell structures such as dividing chromosomes. Binding of benzimidazoles to the tubulin monomers inhibits their polymerization, and nuclear and thus cell division is blocked. In resistant mutants, which can be easily generated in the laboratory, tubulin had lost its capacity to bind the inhibitor, and functional tubulin polymerization was not inhibited. Resistance could thus be explained by the mutational change of the target site (Davidse 1987).

Development of field resistance of the scab fungus to the benzimidazoles was more rapid than for dodine. In orchards where benomyl had been used intensively, resistance became a problem after 3-4 years (Gilpatrick 1982). As with dodine before, benomyl resistance occurred first in the Western New York, where benomyl was used season-long. Benomyl use in this region was discontinued in the early 1980s. In the Hudson Valley located in Eastern New York, where benomyl was restricted to pre-bloom applications, no field resistance was reported up to 1981 (Gilpatrick 1982). In this region, benomyl is still used as scab material during the primary season and/or in cover sprays for the control of summer diseases. After control failures had occurred in other parts of the United States, benomyl was not used as a single compound but rather in mixture with a protectant.

During our orchard survey in 1991, we tested the current status of benomyl resistance in representative orchards from both regions. The test procedure was similar to the one used for dodine, the determination of RG-values of 40-50 monoconidial isolates at one dose of benomyl. The result shown in Table 2 can be summarized and discussed as follows:

- (a) The level of resistance (RG = 81-100) in Western New York, where benomyl had not been used for over 10 years, remains high. However, resistance was equally pronounced in the Eastern part, where the restricted use of benomyl in mixture with a protectant is still common. Considering the high levels of resistance determined in

these orchards, scab control relies largely on the protectant partner, and the benomyl contribution has become less pronounced. Consequently, all restrictions inherent to non-specific protectants employed in "as needed" IFP programs also apply to benomyl-protectant mixtures, whenever benomyl resistance has reached such high levels.

- (b) The sensitivity distribution found for benomyl (Table 2) is very different from the one established for dodine (Fig. 1). Sensitivities are not continuous in character (RG-values from 1 to 59 are not found), and the resistant population (RG = 81-100; no or only slight inhibition at the discriminatory dose) is widely separated from the sensitive population (RG = 0; full inhibition).

Table 2. Level of benomyl resistance in New York State Orchards

RG <sup>1)</sup>	Frequency of isolates (%)				
	WC 1 <sup>2)</sup>	WC 2 <sup>2)</sup>	WC 3 <sup>2)</sup>	HV 1 <sup>3)</sup>	HV 2 <sup>3)</sup>
0	29	71	65	51	59
60-80	0	0	4	2	2
81-100	71	42	31	47	39

<sup>1)</sup> Relative growth at 0.5 mg/L

<sup>2)</sup> Orchards in Western New York (Wayne County)

<sup>3)</sup> Orchards in Eastern New York (Ulster County)

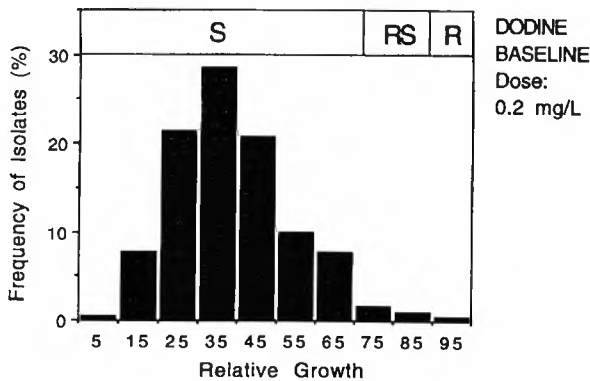


Fig. 1. Baseline sensitivity for dodine

As frequently demonstrated (Jones et al. 1987), growth of highly resistant phenotypes is not affected at concentrations of 50 mg/L and higher, and the term "insensitive" would be more appropriate than "resistant" to describe the phenomenon and to differentiate between the benomyl and dodine case. This difference is important. The typical dodine concentration in dilute sprays is 400 mg/L. With ED<sub>50</sub>-values of 1-10 mg/L for dodine resistant isolates, it is likely that these isolates are still controlled to a certain extent, and although

sporulating lesions might develop, they will be smaller and thus will contain less conidia than in the absence of dodine. The lesion size will become larger, and relatively more resistant conidia will develop if dodine doses are low, on leaves with insufficient spray



coverage (= lower dodine concentration after systemic distribution) and under conditions of very long spray intervals. All three conditions can be expected to increase the speed of selection and resistance development, because more resistant conidia will be generated during one disease cycle.

For benomyl, the typical dilute spray concentration is 100 mg/L. With no inhibitory effect on mycelial growth of resistant or better insensitive isolates at this dose, it is apparent that the development of sporulating lesions is not inhibited under orchard conditions. Moreover, increasing the dose, optimal spray coverage and shorter spray intervals would have very little effect on the development of these insensitive lesions. The difference between dodine and benomyl - residual and dose-dependent control of resistant phenotypes with dodine, but no inhibitory effect on insensitive isolates in case of benomyl - is a likely explanation for the different speeds of resistance development.

The initially proposed and widely adapted strategy of benzimidazole resistance management was the use of benzimidazoles mixed with a protectant (Delp 1987). It had been noted that control failures were not reported from regions where such mixtures had been used from the beginning (Gilpatrick 1982), and computer models had indicated that resistance development would be delayed by mixtures. The data presented in Table 2, however, demonstrate that resistance development was not prevented. Severe control failures were not observed because of the performance of the protective mixing partner.

A novel approach to the management of benomyl resistance appeared to emerge in the early 1980s. It was discovered that a group of phenylcarbamates was negatively cross-resistant to the benzimidazoles. Only growth of benomyl-resistant isolates was inhibited, but benomyl-sensitive isolates reacted insensitive (Ishii 1992). A combination of both compounds controlled both genotypes and was effective at sites, where benomyl alone had failed to control the disease.

A combination product of this type was introduced for the control of *Botrytis* rot. However, the strategy was not without problems. One drawback was economical in nature, because the rate of the combination product was double the rate of benomyl alone. A second and even more severe restriction was the rapid development of resistance to the combination product (Köller 1991b). For the scab fungus, it was discovered early that not all benomyl-resistant phenotypes were sensitive to the phenylcarbamates (Jones *et al.* 1987). Naturally, these isolates resistant to both inhibitors would have been selected even in the presence of the combination.

The molecular basis for this different phenotype response has been clarified recently (Koenraad *et al.* 1992). A single base pair mutation in the tubulin gene results in the substitution of only one amino acid, a small change sufficient for benomyl resistance. However, more than one base pair mutation is possible. Four different mutations were identified in field isolates of *V. inaequalis*, but only two resulted in sensitivity to phenylcarbamates.

In summary, the usefulness of benzimidazoles in the control of scab remains limited. Mixtures with protectants have clear advantages over protectants alone if resistance levels are low. However, resistance will eventually build up even under these conditions, and a critical evaluation of the benzimidazoles contribution to the mixture performance should be encouraged, if such mixtures have been used for a long period of time.

## STEROL DEMETHYLATION INHIBITORS

The group of sterol demethylation inhibitors (DMIs), with currently over 20 commercial products available for a broad spectrum of diseases and several other DMIs still under development, was introduced in the early 1970s (Köller 1992b). The first DMI available to apple growers was triadimefon for the control of powdery mildew but not scab. Products currently available for the control of scab and, for most compounds, also powdery mildew are: bitertanol, penconazole, cyproconazole, difenoconazole, fenarimol, flusilazole, hexaconazole, myclobutanil, nuarinol, pyrifenoxy, and triflumizole. Some other DMIs with good scab and powdery mildew activity will be added to this list in the near future. In scab control, DMIs have excellent after-infection (72 h) and presymptom (5 d) activities. Postsymptom activities are weaker, and some of the compounds such as bitertanol and fenarimol lack good protective activities (Szkolnic 1981, Schwabe et al. 1984). The excellent after-infection and presymptom activity is desirable in IFP programs. Taking full advantage of these DMI properties, Wilcox et al. (1992) have developed a reduced-spray program for the control of primary apple scab in orchards with low potential ascospore doses. The program is independent of infection periods and coincides with the integrated application of insecticides or acaricides. Although only tested and implemented under the climatic conditions of New York, the underlying concept should also apply to other regions.

Although the chemical structures of DMIs are relatively diverse - they contain a triazole, imidazole, pyrimidine or pyridine as essential group - they all share a common mode of action in the inhibition of fungal sterol biosynthesis (Köller 1988, 1992b). The first sterol formed in the biosynthetic pathway is lanosterol, which is converted through several enzymatic reactions to the end product ergosterol. This end product fulfils the structural requirements for its vital function in fungal membrane integrity. During the course of lanosterol modification, three methyl groups have to be removed from the precursor sterols. The DMIs strongly inhibit the removal of one of the three methyl groups, precursors with the methyl group attached accumulate and are incorporated into membranes. Because the structure of these precursors is "wrong", membrane integrity is disturbed and growth is inhibited.

Although DMI-resistant mutants can be easily generated in the laboratory, field resistance developed slower than with the benzimidazoles, and the speed and general pattern of resistance development resembled dodine rather than benomyl. Currently confirmed cases of DMI field resistance are powdery mildew of cereals and grapes and, although not yet widespread, apple scab (Köller 1988, 1991b). Apple powdery mildew appears to be not yet affected (Schulz 1992). In response to first indications for shifts of *V. inaequalis* populations toward DMI resistance, an anti-resistance recommendation was issued in 1988 (Scheinflug 1988). It was recommended that DMIs should be used in combination with protectant fungicides, a strategy very similar to the benzimidazoles.

The first failure of scab control with the DMI bitertanol with evidence for the development of DMI field resistance was experienced in an experimental orchard in Nova Scotia, Canada (Hildebrand *et al.* 1988); the shift of the *V. inaequalis* population toward DMI resistance was recently confirmed (Braun & McRae 1992). At the experimental orchard, bitertanol applied at rates lower than recommended had performed worse than a standard protectant program from 1984 to 1986, with a complete failure in 1987. DMIs had

been extensively tested at this site for 12 years. It has to be emphasized, however, that the performance of other DMIs applied at high rates continued to provide sufficient control of scab (Köller et al. 1993b).

Alarmed by the reports of declining scab performance of DMIs, our program has been engaged in the monitoring of DMI sensitivities of *V. inaequalis* populations, and in the development of resistance management strategies. The first step was the determination of the spectrum of DMI sensitivities residing in wild-type populations of *V. inaequalis* never exposed to any DMI fungicide (Smith et al. 1991). The next step was the simplification of the monitoring procedure, based on the relative growth at one DMI concentration (Köller et al. 1991b). The baseline sensitivity distribution for fenarimol, based on tests with 450 single conidia isolates collected from several orchards in New York never exposed to DMIs, is presented in Fig. 2.

The sensitivity distribution was normal and continuous and resembled the distribution found for dodine (Fig. 1). The sensitivity classification into sensitive (S), reduced sensitive (RS) and resistant (R) as specified in Table 3 was derived from a comparison of the baseline sensitivity distribution with sensitivities determined for isolates collected from the experimental orchard in Nova Scotia, where problems with DMI performance had been encountered (Braun & McRae 1992, Köller et al. 1993b). The sensitivity distribution at this site crosses the threshold level of scab control. Isolates with RG-values of 81-100 had increased 35-fold in frequency and thus have to be rated resistant.

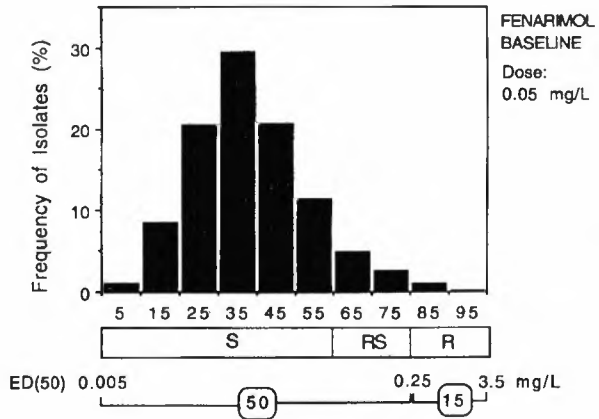


Fig. 2. Baseline sensitivity distribution for the DMI fenarimol

Table 3. Fenarimol sensitivity distribution of scab populations in baseline and threshold orchards

Sensitivity <sup>1)</sup>	RG-Range <sup>2)</sup>	Scab control	Frequency of isolates (%)		Factor of change
			Baseline	Threshold	
S	0-60	easy	91	39	0.4
RS	61-80	difficult at times	8	22	2.8
R	81-100	difficult	1	39	35

<sup>1)</sup> S = sensitive; RS = reduced sensitive; R = resistant

<sup>2)</sup> Relative growth at 0.05 mg/L fenarimol

Based on computer models and experimental confirmation of predicted results, Milgrom and Fry (1988) presented three general principles of anti-resistance strategies. Resistance development is delayed by (a) reducing the disease pressure, (b) reducing the selection time and (c) reducing the frequency of resistant isolates. Experimental evidence indicates that all three principles are valid for the delay of DMI resistance development in populations of *V. inaequalis*.

(a) *Reducing disease pressure*: Comparison of disease data, such as incidence of leaf scab on non-treated check trees and the sometimes poor performance of standard treatments, indicated that the disease pressure at the Nova Scotia site, where problems with resistance arose after 12 years of DMI use (Braun & McRae 1992), was much higher than at a similar experimental site at the New York State Agricultural Station. This difference in disease pressure is most likely explained by longer periods of seasonal temperatures above 25°C in New York. These high temperatures represent conditions of very slow lesion development (Tomerlin & Jones 1983) and, thus, decrease the number of infection cycles. Contributing to a high disease pressure at the Nova Scotia site was the sometimes high incidence of leaf scab even on treated trees, and a large number of heavily diseased non-treated trees. Both have negative impact on the potential ascospore dose during the next primary scab season (MacHardy et al. 1993).

A comparison of DMI sensitivities of isolates collected from the New York orchard with those determined for the Nova Scotia site confirmed that the high Nova Scotia disease pressure had substantial impact on the speed of resistance development. After 12 years of DMI use not different from the resistant Nova Scotia site (40 % of the orchard treated with DMIs), the New York orchard had remained at baseline level, whereas the Nova Scotia population had shifted already to the threshold level of resistance (Köller *et al.* 1993b). At an older experimental orchard in New York State, where DMIs had been used for over 20 years, DMI sensitivities had shifted slightly, but they remained well below threshold, and a flusilazole program provided good scab control. The orchard comparison clearly indicates that low disease pressure will slow the development of DMI resistance. All non-chemical factors decreasing disease pressure such as sanitation and less susceptible cultivars will thus have positive impact on the development of DMI resistance. For example, good control of late season leaf scab and therefore a low potential ascospore dose during the following primary scab season will have a resistance-delaying effect through lowering the potential ascospore dose (MacHardy et al. 1993).

The use of DMIs in mixture with a protectant can also be classified as an anti-resistance strategy acting through the reduction of disease pressure. As discussed above, non-specific protectants are confined to the surface and inhibit spore germination. DMIs have very limited effect on spore germination and penetration, even if they are used in a protective mode, and scab development is inhibited underneath the cuticle. The activities of the partners in a DMI-protectant mixture are therefore separated in space, and the scab population that escapes the protectant's activity will be exclusively exposed to the DMI. The frequency of DMI resistant and selectable phenotypes will not be lower, but the total number of conidia eventually exposed to the DMI will decrease, because a high proportion will be controlled by the protectant on the leaf surface. Consequently, the disease pressure exerted on the DMI will be lower. Preliminary data from an orchard trial at the New York State Agricultural Experiment Station clearly indicate that the speed of resistance

development was indeed slower for a mixture of fenarimol with mancozeb than for fenarimol alone (Köller & Wilcox, unpublished results).

(b) *Reducing the selection time:* This second component of anti-resistance strategies is immediately apparent. The speed of resistance development measured in years is proportional to the number of disease cycles per season controlled with a DMI. The goal of prolonging the "useful lifetime" of DMIs would call for the limitation of DMI sprays per season and the control of scab with alternative fungicides. For example, early season scab could be controlled with alternatives, and DMI applications could be limited to periods where additional powdery mildew control is needed. As discussed above for dodine, season-long use led to field resistance after 15 years, whereas shifts toward resistance remained small and well below threshold levels for over 25 years, where the use of dodine was restricted to the first two seasonal sprays.

(c) *Reducing the frequency of resistant isolates:* A lower disease pressure has no effect on the frequency of resistant isolates. Although the total number of infective spores would be smaller, the proportion of resistant isolates and thus their frequency would not be affected. As discussed above, higher application rates of benomyl would have no effect on the control of resistant (= insensitive) phenotypes, and the proportion of these phenotypes would not be altered. This is not the case with the DMIs, which closely resemble dodine rather than the benzimidazoles. The designation of "DMI-resistant" shown in Fig. 2 and Table 3 was based on the fact that the frequency of isolates with  $RG > 81$  had sharply increased in frequency (from 1% to 35%) and thus had been selected by the DMIs. An  $RG$ -value of 81 corresponds to an  $ED_{50} = 0.25$  mg/L for fenarimol; the most resistant isolates ( $RG = 100$ ; no inhibition) collected from Canadian, Japanese and European orchards with prolonged DMI histories had  $ED_{50}$ -values of 3-4 mg/L (personal communication). Resistant isolates are thus, in contrast to benzimidazoles but similar to dodine, not insensitive to the DMIs. Growth can still be inhibited, although at concentrations considerably higher than those required for sensitive isolates. Furthermore, sensitivities within the resistant subpopulation are different by a factor of 10 to 15.

Under field conditions, high doses can be expected to prevent isolates with lower levels of resistance from sporulation and thus selection. Indeed, resistant isolates with  $RG$ -values from 81 to 95 for fenarimol had increased by a factor of 25 at the resistant site in Nova Scotia, whereas the more resistant isolates with  $RG$ -values  $> 95$  had increased by a factor of 84 (Köller *et al.* 1993). This differential increase in frequencies shows that the "highly" resistant isolates were allowed to produce more selectable conidia than the "less" resistant isolates. "Less" resistant isolates were apparently more frequently controlled than their "highly" resistant counterparts.

From these sensitivity data it can be concluded that, with increasing application rates, an increasing proportion of the resistant subpopulation is either prevented from sporulation, or that lesion development is slowed down and a smaller number of selectable conidia is produced. Consequently, increasing the application rate would decrease the proportion of selectable and thus resistant isolates and would decrease the speed of resistance development. To the contrary, decreasing the application rate would increase the frequency of selectable isolates and would speed up the development of resistance.

Preliminary results from orchard trials conducted in 1992 at the New York State Agricultural Experiment Station confirmed the validity of this concept (Köller & Wilcox,

unpublished results). The sensitivity of isolates collected from trees treated with fenarimol at half of the recommended dose had shifted significantly toward DMI resistance over one primary scab season. No significant shift was detected for isolates from trees treated with the full dose of fenarimol. High DMI doses can thus be rated as a preventive anti-resistance strategy.

The detrimental effect of low application rates on the speed of resistance development is also evident from circumstantial evidence reported for dodine, a compound very similar to the DMIs. Dodine field resistance was first discovered in Wayne County, New York. The history of resistance development was described as follows by Gilpatrick & Blowers (1974): "Wayne County growers during 1959-68 (especially in certain dry years of that period) reduced the dosage and number of applications recommended for near-complete control of scab control; consequently, moderate levels of scab often occurred on foliage even though scab on the fruit was at commercially acceptable levels."

Theoretical considerations, results from orchard experiments and circumstantial evidence from previous experience with dodine strongly suggest that decreasing the frequency of resistant isolates through high application rates decreases the speed of resistance development.

It has to be considered, however, that a "high" rate is defined by the manufacturers of the various DMIs. Evidence exists, that DMIs can differ with regard to their "safety margins" toward resistance isolates, even if they are applied at high rates. For example, the mean ED<sub>50</sub>-values of five resistant isolates collected in Nova Scotia were 0.3 mg/L for flusilazole, 0.7 mg/L for fenarimol, and 1.2 mg/L for myclobutanil (Köller *et al.* 1993). The typical field rates recommended by the manufacturer are 130-times higher for flusilazole, 40-times higher for fenarimol, and 70-times higher for myclobutanil. If this comparison of intrinsic activities with application rates is legitimate, which has not been tested yet in comparative greenhouse studies, the definition of "high" recommended rate would be different for the different DMIs, and the impact of "low" rates on resistance development could differ from DMI to DMI. More experimental work is required to critically evaluate this aspect.

In summary, several components of DMI anti-resistance strategies have been shown to be effective. A simple and immediately plausible countermeasure is the reduction in selection time. As stated by Gilpatrick & Blowers (1974), dodine resistance became a problem after 10-12 years of season-long dodine use, corresponding to 80 - 100 disease cycles exposed to the selecting agent. If only 2-3 instead of 8-10 disease cycles per season would have been controlled with dodine and the rest with alternative fungicides, resistance would not have been a problem for 40-50 years. There are no experimental data indicating that a restriction of dodine and, most likely, DMI use per season has an additional effect on the time delay of resistance build-up. However, a restriction of DMI sprays will undoubtedly prolong the usefulness of these fungicides if measured in number of years.

The two remaining countermeasures, reduction of disease pressure and the reduction in frequencies of DMI-resistant phenotypes through high application rates are both effective countermeasures, and they are both interconnected. The reduction of disease pressure can be accomplished, among several other measures, by good control of leaf scab into the late season (= reduction of potential ascospore dose during the next primary scab season) and by tank-mixing a DMI with a protectant fungicide. Reduction in frequencies of resistant

phenotypes can be accomplished with high DMI doses. Both countermeasures translate into good control of scab, be it on fruit or on leaves.

It has to be emphasized that the consequences of poor control and high levels of leaf scab during one season are different for protectant fungicides and the DMIs (or dodine). In case of non-specific protectants, the "mistake" can be corrected over the next season; in case of DMIs (or dodine), the incidence will have increased the speed of resistance development, a phenomenon not easily corrected.

A question frequently addressed relates to mixtures of DMIs with protectants. Should these mixtures be counted as "DMI applied" or as "alternative" applied. The answer is complex, but according to the principles discussed above, it can be answered. The resistance-delaying effect inherent to such DMI-protectant mixtures is twofold by decreasing the disease pressure toward the following infection cycle (protectant) and by decreasing the frequency of resistant isolates (DMI). Both effects are dose-dependent. If the mixture is applied in a DMI-typical presymptom mode, the resistance-delaying effect of the protectant will be low, if the protective activity toward the next following infection period is low (long spray intervals and low rates of the protectant). If the DMI rate in these mixtures is also kept low, the frequency of resistant isolates will increase and resistance will develop faster. With low-rate mixtures, the positive effect of lowering the disease pressure accomplished by the protectant can be outbalanced by the counteractive effect of increasing resistance frequencies at low DMI rates. In such cases, mixtures should be counted as "DMI applied". Our preliminary data from orchard trials indicate, that the resistance-delaying effect of a high DMI dose was indeed superior to a mixture of the same DMI with mancozeb, both at half rates of the single compounds (Köller & Wilcox, unpublished results).

Adherence to the principles described above - maintenance of low disease pressure through good control of scab and mixtures of DMIs with protectants, use of alternative scab materials and application of DMIs at high rates - comprise promising anti-resistance strategies. Disease pressure is not only mandated by fungicide use patterns but also by climatic conditions. Consequently, anti-resistance measures must be more stringent and multi-faceted in regions with high natural disease pressure throughout the entire growing season.

#### SINGLE-SITE INHIBITORS: THE FUTURE.

For many years, no new classes of fungicides for the control of apple scab had been introduced. All new materials belonged to the class of DMI fungicides, which are cross-resistant to the representatives introduced earlier. The situation has changed recently. Perhaps most important has been the introduction of the new class of methoxyacrylates or strobilurines (Beautement *et al.* 1991). The discovery history of these compounds is interesting and differs from previous fungicides. All synthetic representatives are derivatives of strobilurin, a natural antifungal product produced by the basidiomycete *Strobilurus tenacellus*. In contrast to many other natural products, the chemical structure of strobilurin was relatively simple and accessible to chemical synthesis and variation. The mode of action of strobilurin A was clarified in 1981 (Becker *et al.*). The natural product acts as specific inhibitor of respiration by binding to a particular cytochrome (cytochrom bc<sub>1</sub>)

involved in the transport of electrons through the mitochondrial membrane. For the first time, the mode of action of a new class of fungicides was already known, before first representatives were introduced.

The first two synthetic derivatives in advanced stages of field development - BAS 490 F and ICIA5504 - were introduced in 1992 (Ammermann *et al.* 1992, Godwin *et al.* 1992). Both compounds have protective, after-infection and presymptom activity against apple scab; powdery mildew activity was reported for BAS 490 F.

The resistance risk of this new class of site-specific fungicides has not been investigated in detail. In yeast, however, it has been shown that the mutational exchange of a single amino acid in the cytochrome b target site confers resistance (di Rago *et al.* 1989, Geier *et al.* 1992). This resistance type resembles the mechanism of benomyl resistance and would indicate a high risk of resistance. However, extensive work with plant pathogens will be required to assess this potential risk under field conditions. Although these systematic studies are still lacking, chances are high that preventive anti-resistance strategies will be developed and tested before the compounds are available to growers.

A second experimental fungicide under development for the control of apple scab is the anilino-pyrimidine pyrimethanil (Neumann *et al.* 1992). The mode of action of pyrimethanil has not been clarified, although its systemic properties indicate a specific target site. Indications exist that the compound might act as an inhibitor of enzyme excretion (Neumann *et al.* 1992). Since enzymes like cutinases and cell-wall degrading enzymes are functionally involved in pathogenicity (Köller 1991a), a target site in the secretion of these enzymes would represent a novelty. The impact of this mode of action, if correct, on the risk of resistance cannot be predicted at the present time.

## OUTLOOK

All scab fungicides in use have, regardless of their non-specific or site-specific mode of action, a target site within the fungal cell. Pyrimethanil with its potential action on enzyme secretion would represent an example for a different type of chemical interference, not with metabolic steps but rather with processes crucial to pathogenicity. Modern systemic and site-specific inhibitors are all fungal-specific; otherwise they would be toxic and phytotoxic. Not all fungal organisms, however, are plant pathogens. Within the ecosystem they also thrive as soil saprophytes, phylloplane inhabitants and harmless or even beneficial endophytes. The very specific interference with crucial steps in pathogenicity rather than fungal growth would therefore increase the specificity level of antifungal inhibitors from fungal-specific to pathogen-specific. Progress made in answering the question of "what makes a fungus a plant pathogen?" will open novel avenues of chemical interference with crucial steps in pathogenicity, without effects even on fungal metabolism. Opportunities for the development of such novel disease control agents do exist (Köller 1992b). Success, however, will not only be determined by the scientific progress made, but also by the general future acceptance of chemicals as an integral part of modern agricultural production systems.



REFERENCES

- Ames, B.N., M. Profet & L.S. Gold 1990a. Dietary pesticides 99.99 percent all natural. Proceedings of the national academy of sciences USA 87: 7777-7781.
- Ames, B.N., M. Profet & L.S. Gold 1990b. Nature's chemicals and synthetic chemicals toxicology. Proceedings of the national academy of sciences USA 87: 7782-7786.
- Ammermann, E., G. Lorenz, K. Schelberger, B. Wenderoth, H. Sauter & C. Rentzea 1992. BAS 490 F - A broad-spectrum fungicide with a new mode of action. Brighton crop protection conference - Pests and diseases: 403-410.
- Beautement, K., J.M. Clough, P.J. de Fraine & C.R.A. Godfrey 1991. Fungicidal  $\beta$ -methoxyacrylates: from natural products to novel synthetic agricultural fungicides. Pesticide science 31: 499-519.
- Becker, W.F., G. von Jagow, T. Anke & W. Steglich 1981. Oudemansin, strobilurin A, strobilurin B and myxothiazole: new inhibitors of the bc<sub>1</sub> segment of the respiratory chain with an E- $\beta$ -methoxyacrylate system as common structural elements. FEBS-Letters 132: 329-333.
- Braun, P.G. & K.B. McRae 1992. Composition of a population of *V. inaequalis* resistant to myclobutanil. Canadian journal of plant pathology 14: 215-220.
- Brown, I.F. & H.D. Sisler 1960. Mechanism of fungitoxic action of n-dodecylguanidine acetate. Phytopathology 50: 830-838.
- Buchenauer, H. 1990. Physiological reactions in the inhibition of plant pathogenic fungi. In: Chemistry of plant protection, Vol. 6, G. Haug & H. Hoffmann, eds., Springer-Verlag, Berlin, 217-292.
- Davidse, L.C. 1987. Biochemical aspects of benzimidazole fungicides - action and resistance. In: Modern selective fungicides - properties, applications, mechanisms of action. H. Lyr, ed. Longman, London, 245-258.
- Delp, C.J. 1987. Benzimidazoles and related fungicides. In: Modern selective fungicides - properties, applications, mechanisms of action. H. Lyr, ed. Longman, London, 233-244.
- Di Rago, J.-P., J.-Y. Coppée & A.-M. Colson 1989. Molecular basis of resistance to myxothiazol, mucidin (strobilurin A), and stigmatellin. Journal of biological chemistry 264: 14543-15548.
- Geier, B.M., H. Schaeffer, U. Brandt, A.M. Colson & G. von Jagow 1992. Point mutation in cytochrome b of yeast ubihydroquinone cytochrome c oxidoreductase causing myxothiazol resistance and facilitated dissociation of the iron sulphur subunit. European

journal of biochemistry 208: 375-380.

Gilpatrick, J.D. 1982. Case study 2: *Venturia* of pome fruits and *Monilinia* of stone fruits. In: Fungicide resistance in crop protection, J. Dekker & S.G. Georgopoulos, eds., Pudoc, Wageningen, 195-206.

Gilpatrick, J.D. & D.R. Blowers 1974. Ascospore tolerance to dodine in relation to orchard control of apple scab. *Phytopathology* 64: 649-652.

Godwin, J.R., V.M. Anthony, J.M. Clough & C.R.A. Godfrey 1992. ICIA5504: A novel, broad spectrum, systemic  $\beta$ -methoxyacrylate fungicide. Brighton crop protection conference - Pests and diseases: 435-442.

Gold, L.S., T.H. Slone, B.R. Stern & N.B. Manley 1992. Rodent carcinogens setting priorities. *Science* 258: 261-265.

Hildebrand, P.D., C.L. Lockhart, R.J. Newbery & R.G. Ross 1988. Resistance of *Venturia inaequalis* to bitertanol and other demethylation-inhibiting fungicides. *Canadian journal of plant pathology* 10: 311-316.

Ishii, H. 1992. Target sites of tubulin-binding fungicides. In: Target sites of fungicide action, W. Köller, ed., CRC Press, Boca Raton, 43-52.

Jones, A.L., E. Shabi & G. Ehret 1987. Genetics of negatively-correlated cross-resistance to a *N*-phenylcarbamate in benomyl-resistant *Venturia inaequalis*. *Canadian journal of plant pathology* 9: 195-199.

Koenraad, H., S.C. Somerville & A.L. Jones 1992. Characterization of mutations in the beta-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other plant pathogenic fungi. *Phytopathology* 82: 1348-1354.

Köller, W. 1988. Sterol demethylation inhibitors: Mechanism of action and resistance. In: Fungicide resistance in North America, C.J. Delp, ed., APS Press, St. Paul, 79-88.

Köller, W. 1991a. The plant cuticle - A barrier to be overcome by fungal plant pathogens. In: The fungal spore and disease initiation in plants and animals, G.T. Cole & H.C. Hoch, eds., Plenum Press, New York, 219-246.

Köller, W. 1991b. Fungicide resistance in plant pathogens. In: CRC handbook of pest management in agriculture, 2nd edition, Volume 2, D. Pimentel, ed., CRC Press, Boca Raton, 679-720.

Köller, W. 1992a. Target research in the discovery and development of antifungal inhibitors. In: Target sites of fungicide action, W. Köller, ed., CRC Press, Boca Raton, 255-310.

- Köller, W. 1992b. Antifungal agents with target sites in sterol functions and biosynthesis. In: Target sites of fungicide action, W. Köller, ed., CRC Press, Boca Raton, 119-206.
- Köller, W., A.L. Jones & K.L. Reynolds 1993a. Detection and quantification of population shifts of *Venturia inaequalis* to dodine. Plant disease: manuscript submitted.
- Köller, W., P.G. Braun & K.L. Reynolds 1993b. Detection and quantification of population shifts of *Venturia inaequalis* to sterol demethylation inhibitors. Phytopathology: manuscript submitted.
- Köller, W., D.M. Parker & C.M. Becker 1991. Role of cutinase in the penetration of apple leaves by *Venturia inaequalis*. Phytopathology 81: 1375-1379.
- Köller, W., D.M. Parker & K.L. Reynolds 1991. Baseline sensitivities of *Venturia inaequalis* to sterol demethylation inhibitors. Plant disease 75: 726-728.
- MacHardy, W.E., D.M. Gadoury, & D.A. Rosenberger 1993. Delaying the onset of fungicide programs for control of apple scab in orchards with low potential ascospore dose of *Venturia inaequalis*. Plant disease 77: 372-375.
- Neumann, G.L., E.H. Winter & J.E. Pittis 1992. Pyrimethanil: A new fungicide. Brighton crop protection conference - Pests and diseases: 395-402.
- Milgrom, M.G. & W.E. Fry 1988. A simulation analysis of the epidemiological principles for fungicide resistance management in pathogen populations. Phytopathology 78: 565-570.
- Scheinflug, H. 1988. Resistance management strategies for using DMI fungicides. In: Fungicide resistance in North America, C.J. Delp, ed., APS Press, St. Paul, 93-94.
- Schulz, U. 1992. Sensitivity of apple powdery mildew to triadimefon. Brighton crop protection conference - Pests and diseases: 195-200.
- Smith, F.D., D.M. Parker & W. Köller 1991. Sensitivity distribution of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole: Baseline sensitivity and implications for resistance monitoring. Phytopathology 81: 392-396.
- Szkolnic, M. 1981. Physical modes of action of sterol-inhibiting fungicides against apple scab. Plant disease 65: 981-985.
- Tomerlin, J.R. & A.L. Jones 1983. Effect of temperature and relative humidity on the latent period of *Venturia inaequalis* in apple leaves. Phytopathology 73: 51-54.
- Turner, M.L., W.E. MacHardy & D.M. Gadoury 1986. Germination and appressoria formation by *Venturia inaequalis* during infection of apple seedling leaves. Plant disease 70: 658-661.

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Wilcox, W.F., D.I. Wasson & J. Kovach 1992. Development and evaluation of an integrated, reduced-spray program using sterol demethylation inhibitor fungicides for control of primary apple scab. *Plant disease* 76: 669-677.

# Tolerance of *Venturia inaequalis* to ergosterol biosynthesis inhibiting fungicides in South Africa

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The first ergosterol biosynthesis inhibiting (EBI) fungicides became available in the 1970's for the control of scab and mildew on apples. Since then a large number of this group of chemically-related fungicides have been released, and are being widely used by apple and pear producers.

EBI fungicides are characterized by an excellent curative, but limited protective scab control action. Furthermore, most of these fungicides are highly effective mildewicides. These exceptional properties of EBI fungicides provide a high degree of flexibility to the control programme of pome fruit diseases. As a result, losses due to scab and mildew have virtually been eliminated in South Africa.

However, these favourable qualities of the EBI's have led to excessive usage in the field. This could lead to selection of EBI-tolerant scab strains, in which case the effective use of this remarkable group of fungicides would drastically be shortened.

An investigation was initiated to determine the presence of any scab strains tolerant to EBI's in local apple orchards as well as the degree of such tolerance.

Scab-infected leaves from orchards with different EBI histories were collected. Conidial suspensions were prepared and used for the inoculation of potted MM109 rootstock apple trees, unsprayed and sprayed with sub-lethal dosages of the EBI bitertanol. Infected leaves from trees sprayed with sub-lethal bitertanol dosages were used for subsequent inoculation trials. In the case of one fungus strain, infected leaves from unsprayed trees were used for the preparation of inoculum for successive trials.

After seven greenhouse generations on potted trees some fungus strains were used to demonstrate cross-tolerance. Protective and curative greenhouse trials were carried out.

After the first and second generations on potted trees, none of the fungus strains showed any reduced sensitivity to EBI fungicides. The first indication of a change in sensitivity of three fungus strains collected from orchards with an EBI history was shown after the third greenhouse generation. This phenomenon became more prominent after fungus strains were exposed more frequently to sub-lethal dosages of bitertanol.

The presence of cross-tolerance was demonstrated by protective as well as curative trials. Two fungus strains used in protective trials showed cross-tolerance to bitertanol,

etaconazole, fenarimol, flusilazol and pyrifenoX. One fungus strain also demonstrated cross-tolerance to triforine.

In curative trials where only one fungus strain was tested with bitertanol, fenarimol, flusilazol, penconazole and pyrifenoX, cross-tolerance was demonstrated to all test EBI's.

This investigation indicated clearly that excessive use of EBI's leads to a selection of apple scab strains with reduced sensitivity to this group of fungicides. The reduction in sensitivity was not as impressive as that experienced with benzimidazole fungicides in the 1970's. However, even a slightly reduced sensitivity such as was experienced with dodine in the USA and South Africa, had serious economic implications.

The presence of cross-tolerance of scab strains to various EBI's complicates the magnitude of the problem. Excessive use of even one EBI will result in an increase of levels of tolerant scab populations in orchards. Consequently, none of the wide range of EBI's can any longer be used with confidence for effective curative scab control.

To ensure the longest possible use of the highly effective EBI fungicides, excessive use in the field has to be avoided.

# Development and decline of a population of *Venturia inaequalis* resistant to sterol-inhibiting fungicides

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A population of *Venturia inaequalis* resistant to sterol-inhibiting fungicides (SIs) developed in an apple orchard used for fungicide trials. SI fungicide testing began in 1978 and a complete loss in apple scab control with bitertanol occurred in 1987. Statistical analysis revealed that in 1989, two distinct, normally distributed, populations of *V. inaequalis* were present with mean ED<sub>50</sub> values for myclobutanil of 0.045 mg/L for the sensitive population and 0.46 mg/L for the resistant population. The resistance factor (mean ED<sub>50</sub> value of resistant population/mean ED<sub>50</sub> value of sensitive population) in 1989 was 10.2. Only broad-spectrum protectant fungicides have been used since 1989 and the resistance factor in 1992 has declined to 3.5, but two distinct, normally distributed, population of *V. inaequalis* are still evident.

Key words: Apple, bitertanol, fungicide, myclobutanil, *Venturia inaequalis*.

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Sterol-inhibiting fungicides (SIs) have characteristics which make them attractive for managing apple scab caused by *Venturia inaequalis* (Cke.) Wint. SIs are effective at low rates and have a long post-infection activity (Szkolnik 1981). SIs are site-specific fungicides which theoretically should be quickly overcome by resistance in the target organism (de Waard & Fuchs 1982). Yet, reports of *V. inaequalis* with reduced sensitivity to SIs are rare and only appeared after many years of SI use (Fiaccadori *et al.* 1987, Nowacka 1991, Parisi *et al.* 1990, Stanis & Jones 1985, Thind *et al.* 1986). Hildebrand *et al.* (1988) were the first to report a complete loss of control of apple scab in the field with the SI fungicide, bitertanol.

To facilitate the development of antiresistance strategies to preserve this important group of fungicides for disease management in apple, more information on the resistance development is required (Köller & Scheinflug 1987). The purpose of this paper is to review the development of SI resistance in *V. inaequalis*, characterize the resistant population, monitor its decline when the selection pressure was removed and examine its parasitic fitness.

## MATERIALS AND METHODS

### Field, fungicide trials

Thirteen SI and nine protectant fungicides were tested, at various times, in a block of apple trees, cv MacIntosh and Cortland, over a ten-year period, 1978-1988. The SIs included: bitertanol, cyproconazole, diniconazole, etaconazole, fenarimol, flusilazol, hexaconazole, myclobutanil, penconazole, pyrifenoxy, triflumizol, and triforine. They were applied season long, alone, tank-mixed with a protectant fungicide or alone during the primary scab infection period with protectant fungicides applied for the remainder of the season. The fungicides were tested in single tree plots replicated four times per cultivar and arranged as a balanced incomplete design. Each year about 21% of the trees in the block remained non-treated and treatments were re-randomized each year. The fungicides were applied to run-off with a handgun operated at 2100 kPa on a 7-14 day schedule beginning at green-tip.

The incidence of apple scab lesions was quantified by examining 200-500 leaves per tree after the end of the ascospore discharge season and 100-500 leaves and fruit per tree at harvest. Treatments were compared by analysis of variance on the arcsin transformed, per cent incidence of apple scab on leaves and fruit (Steele & Torrie 1980). The MacIntosh and Cortland data are similar and therefore only data for the more susceptible cultivar, MacIntosh, are presented.

In an attempt to contain and possibly eradicate the SI resistant population of *V. inaequalis* the Research Station orchards were rigorously treated with broad spectrum fungicides applied at 7-10 day intervals during the scab season from 1989 to 1992.

### Fungicide ED<sub>50</sub> determinations

Leaves infected with *V. inaequalis* were collected from Research Station orchards at the end of July, 1989-1992 and from commercial growers in 1989. Monoconidial isolates of *V. inaequalis* were obtained by streaking individual lesions from leaves on 2% water agar (Bacto-agar, Difco Laboratories, Detroit, MI) containing 50 µg/mL each of chloramphenicol, streptomycin, and tetracycline (Sigma Chemical Company, St. Louis, Mo; Smith *et al.* 1991). Conidia were incubated overnight at 18°C in the dark and a single germinated conidium from each isolate was transferred to potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) and incubated in the dark at 18°C for 4 wk.

One-cm-diameter disks were cut from the margins of the monoconidial colonies with a sterile cork borer (Braun & McRae 1992). The disk was transferred to a 2 x 15 cm culture tube containing 6 mL of sterile distilled water (SDW) and homogenized briefly with a 12-mm-diameter probe of a Kinematic® tissue homogenizer (GmbH Kreins, Luzern, Switzerland) to give a homogeneous spore/mycelial suspension. The probe was sterilized between samples with 70% ethanol. Two, 10-µL drops of spore/mycelium suspension were placed in each petri dish of 20 mL PDA non-amended or amended with 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 µg/mL of technical grade myclobutanil. Each 9 x 1.5 cm dish accommodated three rows of four drops for a total of six isolates with two drops each. The dishes were sealed with Parafilm® (American National Can, Greenwich, CT) and incubated at 18°C in darkness for 14 days. The density and diameter of colony growth was rated on a scale of 1 to 5, where 1 equals growth equivalent to growth on non-amended medium, 2 equals 3/4 growth, 3 equals 1/2 growth, 4 equals 1/4 growth and 5 equals no growth.



ED<sub>50</sub> values were calculated by regressing the rated growth value against the log<sub>10</sub> fungicide concentration using a maximum likelihood program (MLP, Ross 1987) and substituting a value of 3, for 50% growth, in the regression equation. ED<sub>50</sub> values for all the isolates collected in a year were fitted to a hierarchical series of normal distribution models: single, then doubles with equal population proportions and variances, unequal populations proportions with equal population variances, and unequal population proportions and variances (Ross 1987). The significance of each parameter was tested with a  $\chi^2$  statistic with one degree of freedom for each step.

### Parasitic fitness

SI fungicide resistant and sensitive isolates of *V. inaequalis* were compared for growth on PDA at five different temperatures, spore size, spore production on PDA, and spore production on apple leaves. Lawn-like cultures of SI resistant and sensitive *V. inaequalis* were prepared by transferring two, 1-cm-diameter agar disks cut from the margin of a growing colony to a culture tube containing six mL of SDW. The agar disks were macerated as described above and one mL of the suspension was transferred to 49 mL of molten PDA (45°C). The spores and mycelium were evenly distributed in the PDA by gently rotating the bottle and three dishes of about 15 mL of PDA were poured immediately. The dishes were sealed with Parafilm® and incubated in the dark at 18°C for 20 days.

The size and number of spores produced per cm<sup>2</sup> of PDA was estimated by transferring a one-cm-dia disk from the various isolates to five mL of SDW in a culture tube. The disks were macerated as described above and the number of spores counted with a haemocytometer. The volume of the spore suspension and the surface area of a disk were accounted for in calculating the number of spores produced per cm<sup>2</sup> of PDA. The length and width of ten spores from each isolate were measured with an ocular micrometer and spore length and width were multiplied for analysis. The experiment was repeated once.

Growth rates of ten resistant and ten SI sensitive isolates of *V. inaequalis* were compared at five different temperatures. Two, five-mm-diameter disks were placed mycelium side down on PDA and incubated in the dark at 5, 10, 15, 20, and 25°C for 16 days. Colony diameters were measured in two directions, the original diameter of the disk subtracted and the resultant growth divided by 16 to give an estimated rate of growth per day. The experiment was repeated once.

Inoculum for inoculating apple leaves was produced on Difco® Malt Agar. Two hundred  $\mu$ L of a conidiospore suspension which had been passed through cheesecloth to remove mycelial fragments was spread evenly on the surface of 10 mL of malt agar. Cultures were incubated in the dark at 18°C for 12-14 days. The spores were collected by flooding the dish with SDW and rubbing the colony surface with a bent glass rod. Since not all *V. inaequalis* isolates sporulated in culture, four sensitive and four SI resistant isolates which produce spores in culture were selected for fitness studies on apple seedlings. For each isolate, five, 4-wk-old apple seedlings (open pollinated cv Beautiful Arcade) were inoculated by applying an even spray of a 50,000 spore/mL suspension to the adaxial side of all leaves (Lalancette et al. 1987). Control plants received only a spray of SDW. The four youngest unfurled but not necessarily fully expanded leaves were tagged. Inoculated seedlings were placed in a mist chamber at 18°C for 48 h and then transferred to a growth chamber at 18°C with a 14 h photoperiod. The plants were watered from below. After 20

days the four tagged leaves were removed and the spores dislodged from the colonies by wetting and gently rubbing the leaves. The number of spores produced per leaf was estimated by counting the number of spores with a haemocytometer. The leaf area was estimated with a leaf area meter and the number of spores produced per cm<sup>2</sup> of leaf was estimated by dividing the number of spores collected by the leaf area.

Parasitic fitness data were subjected to analysis of variance procedures (Genstat 5, Payne 1988).

## RESULTS

### Field, fungicide trials

A reduction in efficacy by bitertanol was first observed on fruit in 1982 and on foliage in 1984 in the fungicide test block at the Kentville Research Station (Table 1 & 2). SI fungicides were tested in this orchard block from 1978 to 1988. Bitertanol was as effective or more effective than the standard protectant fungicide on foliar control until 1983. Fruit scab incidence in the bitertanol treatment was significantly ( $P=0.05$ ) higher in 1978 and from 1982 to 1987. Incidence of fruit scab was high in 1983 for both the protectant and bitertanol treatments because long rainy periods and poor conditions for spraying resulted in longer than recommended intervals between fungicide applications. A complete loss of scab control was experienced in 1987 when 67.1 and 99.4% of the leaves and fruit treated with bitertanol were infected compared to 6.9 and 1.0% for the protectant treatment, respectively.

Table 1. Incidence of fruit scab caused by *Venturia inaequalis* on MacIntosh apples not treated, treated with bitertanol, or a protectant fungicide at 7-10 intervals beginning at green-tip

Year	Bitertanol rate (ppm)	Incidence of fruit scab (%) <sup>1)</sup>		
		Bitertanol	Protectant <sup>2)</sup>	Check
1978	125	3.9	1.3	60
1979	93.5	9.6	7.8	100
1980	39	8.3	10.4	100
1981	39	25.7	38.4	99.6
1982	37.5	15.5	6.7	100
1983	37.5	57.2	66.8	100
1984	37.5	59.1	10.9	100
1985	37.5	82.0	22.5	100
1986	37.5	62.9	10.4	100
1987	37.5	99.4	1.0	100

<sup>1)</sup> Incidence values determined by examining 100-500 apples/tree from four single tree blocks per treatment

<sup>2)</sup> Protectant fungicides used were captan from 1978 to 1985 and mancozeb for 1986 and 1987 at recommended rates

Table 2. Incidence of foliar scab caused by *Venturia inaequalis* on cv MacIntosh trees not treated, treated with bitertanol or a protectant fungicide at 7-10 intervals beginning at green-tip

Year	Bitertanol rate (ppm)	Incidence of foliar scab (%) <sup>1)</sup>		
		Bitertanol	Protectant <sup>2)</sup>	Check
1978	125	0.1	0.8	20.2
1979	93.5	0.7	11.0	81.1
1980	39	4.4	5.7	67.5
1981	39	5.1	15.3	89.8
1982	37.5	3.0	6.3	67.3
1983	37.5	15.8	28.3	71.1
1984	37.5	10.2	6.8	77.4
1985	37.5	31.8	7.9	57.0
1986	37.5	21.0	19.2	68.9
1987	37.5	67.1	6.9	84.9

<sup>1)</sup> Incidence values determined by examining 100-500 apples/tree from four single tree blocks per treatment

<sup>2)</sup> Protectant fungicide used were captan from 1978 to 1985 and mancozeb for 1986 and 1987 at recommended rates

Disease pressure was high in the fungicide test block, ranging from 57.0 to 89.8% and 99.6 to 100% for foliage and fruit scab, respectively, except in 1978 when the incidence of foliar and fruit scab was 20.2 and 60%, respectively. There were an average of 19 scab infection periods determined by monitoring temperature and leaf wetness duration, during the years of 1978 and 1987 with a range of 13 to 24 infection periods. An average of eight fungicide applications were made in a season.

From 1978 to 1987 the incidence of trees treated with a class of fungicide was 28% with SI's alone, 24% with SI's tank-mixed with protectant fungicides, 27% with fungicides with modes of action different than the SI's and 21% of the trees were non-treated (Table 3). From 1989 to 1993 the fungicide test block was sprayed with broad-spectrum protectant fungicides on a 7-10 schedule from green tip to the end of the ascospore discharge period after which the spray interval was increased to 14 days until the end of July or early August.

### Fungicide ED<sub>50</sub> values

Plotting the frequency distribution of myclobutanil ED<sub>50</sub> values for 1989 to 1992 resulted in two separate peaks in each year (Fig. 1). When fitting the 1989 data to normal models of population frequency distribution, a double normal population model with unequal proportions but equal variances was a significantly better fit ( $P=0.031$ ) than a single normal population or double normal with equal proportions and equal variances. The 1991 data fit a double normal population model with unequal proportions and variances significantly better ( $P<.005$ ) than the other models while the 1990 and 1992 data fit a double normal model with equal proportions and variances significantly better ( $P<.005$ ) than other models. The population mean ED<sub>50</sub> values for the myclobutanil sensitive and resistant populations for 1989 to 1992 were; 0.045 and 0.46, 0.05 and 0.39, 0.102 and 0.804, 0.017 and

0.059, respectively (Fig. 1).  $ED_{50}$  values ranged from a low of 0.005 ppm in 1991 and 1992 to a high of 2.0 ppm in 1991. The resistance factors (mean  $ED_{50}$  of resistant population/ mean  $ED_{50}$  of sensitive population) for 1989 to 1992 were 10.2, 7.8, 7.9, and 3.5 ppm, respectively.

Table 3. Per cent of trees in fungicide test orchard treated with sterol-inhibiting fungicides alone, with protectant fungicides used alone after the primary scab period (split), tank-mixtures of SIs and protectants, protectants and fungicides of other modes of action or non-treated check trees

Year	Alone	Split	Mixed	Others	Check
1976	7 <sup>1)</sup>	-	-	71	22
1977	7	-	13	57	23
1978	26	-	26	19	29
1979	52	-	13	13	22
1980	45	-	13	19	23
1981	46	-	7	26	21
1982	13	19	19	32	17
1983	32	7	13	26	22
1984	39	7	13	12	29
1985	45	7	26	13	9
1986	32	7	39	7	12
1987	19	26	32	7	16
1988	-	7	32	57	23

<sup>1)</sup> Per cent of trees in fungicide test orchard treated

### Parasitic fitness

The SI resistant isolates grew significantly more quickly on PDA than the sensitive isolates ( $P=0.001$ , Table 4) at each of the five temperatures tested. The mean growth rates for the SI resistant and sensitive isolates were 0.51 and 0.45 mm/day, respectively. The optimum growth temperature was 20°C for both the sensitive and resistant isolates. The sensitive isolates, however, produced more spores/cm<sup>2</sup> on average than the SI resistant isolates;  $1.4 \times 10^6$  and  $0.4 \times 10^6$  spores/cm<sup>2</sup>, respectively ( $P < .001$ ). Also, the SI sensitive isolates produced larger spores than resistant isolates, with mean values of 171 and 153  $\mu\text{m}^2$  (length x width in  $\mu\text{m}$ ), respectively.

There was no significant difference between SI sensitive and resistant isolates of *V. inaequalis* in the number of trees infected, the number of leaves infected or the number of spores produced per cm<sup>2</sup> of leaf tissue ( $P=0.05$ , Table 5).

### DISCUSSION

A complete loss of control of apple scab with bitertanol occurred only ten years after SIs were first used and only five years after the first sign of resistance. Loss of control and the swiftness with which it occurred were not anticipated in light of predications in the literature (Fuchs & Drandarevsky 1976, Georgopoulos & Skylakakis 1986). Also, SI resis-

tance developed in spite of the unintentional use of several recommended antiresistance strategies (Delp 1980); a mixture of susceptible and more resistant host crop plants (cv MacIntosh and Cortland), mixtures and alternations with fungicides which have different modes of action, and leaving untreated areas as refuges for sensitive organisms which compete with resistant organisms. The conditions which may have contributed to resistance development were; a highly susceptible host (cv MacIntosh), frequent fungicide applications with a highly efficient group of fungicides (SIs), and a high disease pressure from an organism with numerous infection cycles per season and an effective sexual state which may have allowed resistant isolates to acquire increased fitness (Georgopoulos 1988, Skylakakis 1985).

The development of an SI resistant population of *V. inaequalis* with a mean  $ED_{50}$  only ten times higher than the sensitive population and overlapping with the sensitive population is consistent with the observations and predictions of Wolfe (1982) and Skylakakis (1985). In 1989, the mean  $ED_{50}$  value for the sensitive population from the fungicide test block and the population not exposed to SIs collected from growers orchards were similar, 0.045 and 0.055  $\mu\text{g/mL}$ , respectively (Braun & McRae 1992). Shifts in the mean  $ED_{50}$  values for populations in 1991 and 1992 are not understood. The mean  $ED_{50}$  value for a sensitive isolate, used as a standard, for each of the four years was similar in all years,  $0.0245 \pm 0.0165$  (data not shown). The  $ED_{50}$  values for both sensitive and resistant isolates appear to shift together in the same direction by the

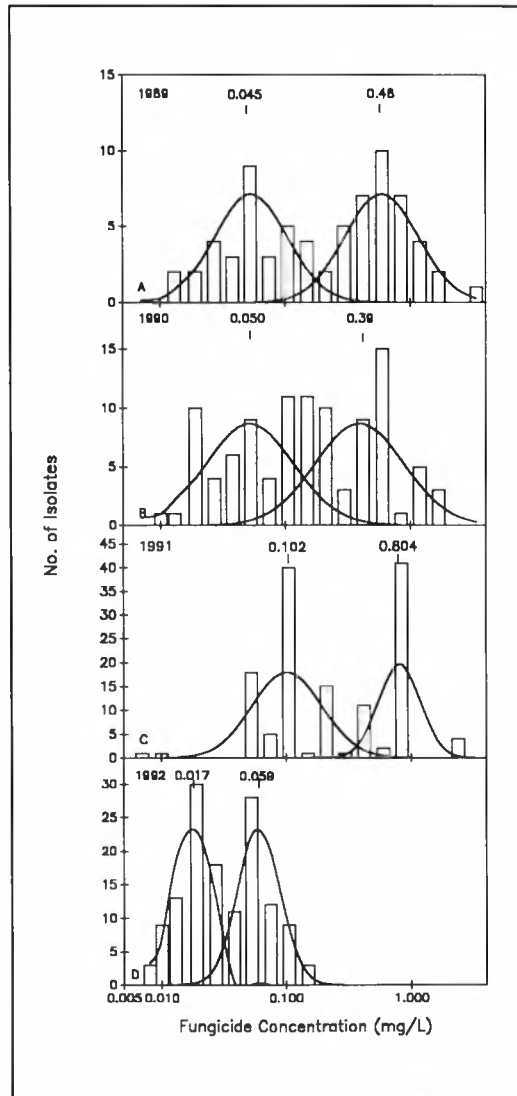


Fig. 1. Frequency distribution of the myclobutanil  $ED_{50}$  values for isolates of *Venturia inaequalis* collected from a fungicide test orchard at the Kentville Research Station exposed to various sterol-inhibiting (SI) fungicides over a 12 year period (1978-1989). Only broad-spectrum protectant fungicides have been used for apple scab control after 1989. The mean  $ED_{50}$  values for the populations are presented above the lognormal distribution curves

same amount and thus resistance factors are not affected.

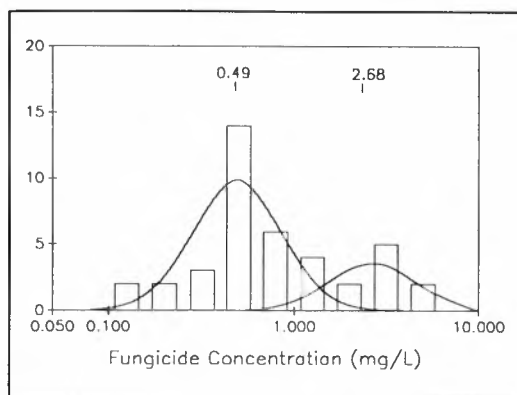


Fig. 2. Frequency distribution of fenetrazole  $ED_{50}$  values for isolates of *Botrytis cinerea*, taken from Elad (1992). The two, normally distributed populations with different population proportions but similar standard deviations, have significantly different means ( $P = 0.05$ ). The mean  $ED_{50}$  values for the populations are given above the lognormal curves

Fitness tests on SI sensitive and resistant isolates of *V. inaequalis* demonstrated that while resistant isolates grew faster on PDA over a range of temperatures the optimum was the same for both. Sensitive isolates produced more and larger spores than SI resistant isolates on PDA. It is difficult to say, however, which characteristics demonstrated *in vitro* are correlated with greater fitness *in vivo*. Stanis and Jones (1985) suggested that since SI resistant isolates of *V. inaequalis* were isolated from sporulating colonies on leaves, grew well and sporulated in culture, and successfully completed the sexual process, they must be considered fit. There was no difference in the incidence of trees infected, leaves infected or in spore production abilities on apple seedlings infected with SI resistant and sensitive isolates in this study. In

addition, the presence of SI sensitive and resistant populations of similar proportions in an orchard three years after the selection pressure was removed suggests that the resistant population is fit. However, the decrease in the mean  $ED_{50}$  value over the three years may be the result of a slightly lower fitness level than the wild-type population.

Table 4. Comparison of *Venturia inaequalis* isolates resistant or sensitive to sterol-inhibiting fungicides. Growth rate, density of spores produced on potato dextrose agar (PDA), size of spores produced on PDA and the number of spores produced per  $cm^2$  of leaf area from infected apple leaves were compared

Isolates	Growth rate on PDA (mm/day)	Spore density on PDA (no./ $cm^2$ ) ( $\times 10^6$ )	Spore size <sup>1)</sup> (l x w) ( $\mu m^2$ )	Spore density on leaves (no./ $cm^2$ ) ( $\times 10^6$ )
Resistant	0.51	0.37	153.8	1.74
Sensitive	0.45	1.43	171.4	1.49
P	0.001	0.001	0.01	0.05
LSD	0.057	0.176	16.8	0.94
df	119	80	208	32

<sup>1)</sup> Spore size for comparison purposes was determined by multiplying the spore length by the spore width measured in  $\mu m$ .

Table 5. Comparison of *Venturia inaequalis* isolates resistant or sensitive to sterol-inhibiting fungicides. Incidence of trees infected, leaves infected, and the number of spores produced per cm<sup>2</sup> of leaf area from infected apple leaves were compared

Isolates	Incidence of trees infected	Incidence of leaves infected	Spore density on leaves (no./cm <sup>2</sup> ) (x 10 <sup>6</sup> )
Resistant	3.25 <sup>1)</sup>	2.00 <sup>2)</sup>	1.74
Sensitive	3.50	2.30	1.49
P	0.05	0.05	0.05
LSD	2.42	2.12	0.94
df	6	6	32

<sup>1)</sup> Number of trees infected out of five inoculated for each of four SI sensitive and resistant isolate of *V. inaequalis*

<sup>2)</sup> Number of leaves infected out of four marked leaves on each tree

The existence of two populations, one sensitive and one resistant to SI fungicides in the same orchard was unexpected, and in particular, that in 1990 and 1992 the populations existed in equal proportions. It appears that Wolfe (1982) was suggesting that, stable equilibria between two populations are unlikely and that one population must disappear. Georgopoulos and Skylakakis (1986) show the development of resistance in SIs as a gradual shift of the entire, normally distributed, population to higher levels of insensitivity. However, plotting the frequency distribution of the fenetrazole ED<sub>50</sub> values reported for 40 isolates of *Botrytis cinerea* by Elad (1992) also results in two normally distributed populations similar to those reported in this paper.

It is not clear, why SI resistance developed so rapidly in the fungicide test block at the Kentville Research Station. Fungicide usage patterns were not inconsistent with various antiresistance strategies. Also, SI fungicide tests have been conducted at the Geneva NY Research Station under similar conditions and yet after 12 years of SI usage a resistant population was not evident (Smith *et al.* 1991). Is it possible that the large number of non-treated trees in the Kentville orchard not only provided a refuge for sensitive isolates to survive and compete with resistance isolates but are also provided a situation in which resistant organisms could increase their fitness through mating with wild-type organisms and increase their numbers to provide initial inoculum for the following spring? The absence of a selection pressure in non-treated trees would then make it possible for two populations with nearly equal fitness levels to survive together as stable populations. Perhaps, leaving non-treated trees as a refuge for wild-type organisms as part of an antiresistance strategy for this particular host/pathogen system is not analogous to the insect systems and should be researched further before being adopted.

## SUMMARY

Complete loss of control of apple scab with bitertanol, a SI fungicide, occurred after ten years of SI use. An average of eight SI applications were made annually and the first sign of reduced efficacy appear on the fruit after five years. Reduce efficacy against foliar scab appeared after seven years. In the tenth year 99.4% of the fruit treated with bitertanol were infected with *V. inaequalis*. In the tenth year, ED<sub>50</sub> analysis for myclobutanil revealed that two populations of *V. inaequalis* were present. The mean ED<sub>50</sub> of the one population was similar to that of a population not exposed to SI fungicides while the mean ED<sub>50</sub> of the other population was 10.2 times higher and considered resistant. After three years with no exposure to SI fungicides and intensive scab control with broad-spectrum protectant fungicides the mean ED<sub>50</sub> of the resistant population was only 3.5 times higher than the sensitive population. Tests to determine parasitic fitness on apple seedlings were unable to differentiate between SI sensitive and resistant isolates of *V. inaequalis*. The reduction in the mean ED<sub>50</sub> of the SI resistant population in the field, however, suggests that the resistant population is slightly less fit than the wild-type population. SI resistance developed in spite of the use of several antiresistance strategies indicating that more research is required to test the theory behind some antiresistant strategies.

## REFERENCES

- Braun, P.G. & K.B. McRae 1992. Composition of a population of *Venturia inaequalis* resistant to myclobutanil. *Can. J. Plant Pathol.* 14: 215-220.
- Delp, C.J. 1980. Coping with resistance to plant disease control agents. *Plant Dis.* 64: 652-657.
- de Waard, M.A. & A. Fuchs 1982. Resistance to ergosterol-biosynthesis inhibitors II. Genetic and physiological aspects. In: *Fungicide Resistance in Crop Production*. J. Dekker & S.G. Georgopoulos (Eds.) Pudoc: Wageningen. pp 87-100.
- Elad, Y. 1992. Reduced sensitivity of *Botrytis cinerea* to two sterol biosynthesis-inhibiting fungicides: fenetrazole and fenethanil. *Plant Path.* 41: 47-54.
- Fiaccadori, R., A.J. Gielink, & J. Dekker 1987. Sensitivity to inhibitors of sterol biosynthesis in isolates of *Venturia inaequalis* from Italian and Dutch orchards. *Neth. J. Pl. Path.* 93: 285-287.
- Fuchs, A. & C.A. Drandarvevski 1976. The likelihood of development of resistance to systemic fungicides which inhibit ergosterol biosynthesis. *Neth. J. Pl. Path.* 82: 85-87.
- Georgopoulos, S.G. 1988. Genetics and population dynamics. In: *Fungicide Resistance in North America*. C. J. Delp (Ed.) APS Press, St. Paul, Minnesota. pp 652-657.



- Georgopoulos, S.G. & G. Skylakakis 1986. Genetic variability in the fungi and the problem of fungicide resistance. *Crop Prot.* 5: 299-305.
- Hildebrand, P.D., C.L. Lockhart, R.J. Newbery & R.G. Ross 1988. Resistance of *Venturia inaequalis* to bitertanol and other demethylation-inhibiting fungicides. *Can. J. Plant Pathol.* 10: 311-316.
- Köller, W. & H. Scheinpflug 1987. Fungal resistance to sterol biosynthesis inhibitors: A new challenge. *Plant Dis.* 71: 1066-1074.
- Lalancette, N., K. D. Hickey & H. Cole Jr. 1987. Parasitic fitness and intrastain diversity of benomyl-sensitive and benomyl-resistant subpopulations of *Venturia inaequalis*. *Phytopathology* 77: 1600-1606.
- Nowacka, H. 1991. A decrease of *Venturia inaequalis* (Cke) Aderh. sensitivity to fenarimol. *Fruit Science Reports.* 18: 139-142.
- Parisi, L., J. Guillaumes, & J.M. Olivier 1990. Variability in the curative effects of sterol demethylation inhibitors towards *Venturia inaequalis*. *Agronomie.* 10: 573-579.
- Payne, R.W. 1988. *Genstat 5*. Genstat 5 Committee, Statistics Department, Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ.
- Ross, G.J.S. 1987. Maximum Likelihood Program. The Numerical Algorithms Group Limited. 190 pp.
- Skylakakis, G. 1985. Two different process for the selection of fungicide-resistant subpopulations. *EPPO Bulletin* 15: 519-525.
- Smith, F.D., D.M. Parker & W. Köller 1991. Sensitivity distribution of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole: Baseline sensitivity and implications for resistance monitoring. *Phytopathology* 81: 392-396.
- Stanis, V.F. & A.L. Jones 1985. Reduced sensitivity to sterol-inhibiting fungicides in field isolates of *Venturia inaequalis*. *Phytopathology* 75: 1098-1101.
- Steele, R.G.D. & J.H. Torrie 1980. *Principles and Procedures of Statistics*. Second edition. McGraw Hill Book Co., Toronto, Ontario.
- Szkolnik, M. 1981. Physical modes of action of sterol-inhibiting fungicides against apple diseases. *Plant Dis.* 65: 981-985.
- Thind, T.S., M. Clerjeau, & J.M. Olivier 1986. First observations of resistance in *Venturia inaequalis* and *Guignardia bidwelli* to ergosterol-biosynthesis inhibitors in France. In: *Proc. Brit. Crop Prot. Conf.- Pest and Dis.* Vol. 2: 491-498.

Wolfe, M.S. 1982. Dynamics of the pathogen population in relation to fungicide resistance. In: Fungicide Resistance in Crop Protection. J. Dekker & S.G. Georgopoulos (Eds.) Pudoc, Wageningen. pp 139-148.

# New curative fungicide families to control scab on pome fruits

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The strobilurine analogues and the anilino-pyrimidines are two new fungicide families which have showed a large spectrum of disease control in several crops. On pome fruits they are highly active against scab (*Venturia* spp). Both groups provide new biochemical modes of action and show no cross resistance to existing fungicides. Because of their curative properties they can give a new supply to the DMI-fungicides for which less sensitive strains are already present in the field. The first results on beneficials are very hopeful and we expect that they can be used without restrictions in the Integrated Pest Management strategy. Preliminary studies indicate that the toxicity and the soil persistence is very low.

Key words : Anilino-pyrimidines, curative control. scab, strobilurine analogues.

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The strobilurine analogues represent a new synthetic fungicide family derived from the natural fungal metabolites belonging to the strobilurines. Strobilurine A, a secondary metabolite of the Basidiomycete *Strobilurus tenacellus*, is structurally the simplest natural molecule of this class (Anke *et al.* 1977).

The strobilurines belong to the chemical group of the  $\beta$ -methoxyacrylates. The natural products are characterized by a photochemical instability and a high volatility.

The fungicidal activity is due to the inhibition of the mitochondrial respiration (Brandt *et al.* 1991).

The acute toxicity is very low and until now, according to the available data there are no teratogenic effects.

The products have systemic properties, offering the potential of post infection control. In contrast with highly systemic DMI-fungicides, disease control is retained at the zone of application, without a rapid accumulation at the leaf border and leaf tip (Godwin *et al.* 1992). Our first results show that, in contrast with the DMI-fungicides, the efficacy is less

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influenced by the climatological conditions during and after the application. At the last Crop Protection Conference in 1992 in Brighton, two fungicides of this group were presented, namely BAS 490 F (Ammerman et al. 1992) and ICIA5504 (Godwin et al. 1992). Our research station has had most experience with BAS 490 F.

The anilino-pyrimidines are also broad-spectrum fungicides. At this moment there are two products in development: one with the common name pyrimethanil (Neumann et al. 1992) and the other with the code number CGA 219417 (proposed common name: cyprodinil). Preliminary results indicate that the mode of action is based on an inhibition of the secretion of enzymes necessary in the pathogenesis process. In the case of *Venturia* the compounds are highly active on the subcuticular penetration process. The anilino-pyrimidines have no effect on spore germination.

For pyrimethanil, with the trade name Scala, the registration in Belgium is expected for the following year.

## MATERIALS AND METHODS

The treatments were applied in the different spray schedules in two replicates of 10 trees of the cultivars Jonagold and Golden delicious. The trees were planted at a distance of 1.5m in and 3.5m between the rows. The fungicides were atomized with a knapsack sprayer (Stihl SG 17) at 100 l per m tree height and per ha. The applied water quantity for an adult fruit orchard is 300l/ha.

For BAS 490 F the results presented in this article are obtained with the 50 DF formulation; by pyrimethanil a 400 SC formulation was used.

Presence of scab was in general evaluated in the early summer. In each object, four times hundred leaves (or fruits) were examined for the scab infestation. Each leaf (fruit) was quoted with a value from 0 to 3 according to the degree of infestation:

0 = no scab spots

1 = 1 to 2 spots

2 = 3 to 5 spots

3 = completely infected

The infection degree ( $TH_3$ ) and efficacy (Abbott value) were calculated according to the formula of Townsend-Heuberger and Abbott.

$$\text{Townsend-Heuberger formula: } TH_{v_{\max}} = \frac{\sum (n \times v)}{v_{\max} \times N} \times 100$$

$TH_{v_{\max}}$  = infection degree in per cent

$v$  = infection classes (0,1,2,3)

$v_{\max}$  = highest infection class

$n$  = number of leaves (fruits) in each class

$N$  = total number of examined leaves (fruits)

$$\text{Example : TH}_3 = \frac{(n_0 \times 0) + (n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3)}{3 \times N} \times 100$$

$$\text{Abbott formula: ABB} = \frac{C - T}{C} \times 100$$

C = infection degree in the untreated object  
 T = infection degree in the treated object

The statistical analysis (Fisher Least Significant Difference) was made after the logarithmic transformation of the TH<sub>3</sub>-value (TH<sub>3</sub> + 1). Efficacy values followed by the same letter are not significantly different at P = 0.05.

The calculation of the influence on predatory mites was based on the formula of Henderson-Tilton.

$$\text{Henderson-Tilton} = \left( 1 - \frac{K_1 \times P_2}{K_2 \times P_1} \right) \times 100$$

K<sub>1</sub> = number of predatory mites before treatments in the untreated plots  
 K<sub>2</sub> = number of predatory mites after treatments in the untreated plots  
 P<sub>1</sub> and P<sub>2</sub> = number of predatory mites before and after treatments in the treated plots.

## RESULTS AND DISCUSSION

### **Strobilurine analogues**

The strobilurine analogue BAS 490 F showed a very good control of scab in different spray schedules. In 1992 the climatological conditions were extremely favourable for scab infections. According to the table of Mills we registered 23 infections during the periode of ascospore discharge.

Especially in the beginning of the season at bud burst and in the period with the strongest release of ascospores, from pink bud to petal fall, the weather conditions were very wet. The first infections resulted in a lot of scab spots on the sepales which lead to secondary infections on the calyx of the fruits.

In the different trials with BAS 490 F, the treatments were applied on a regular base and according to the scab infections. In the schemes with treatments every 10 till 14 days (Table 1), the efficacy of BAS 490 F was comparable to or even better than our best standard scheme in which the DMI-fungicide difenoconazol was mixed with captan. The addition of a classic protectant to the DMI-fungicide improved the activity to a high extent. This strategy to combine DMI-fungicides with classic protectants was already recommended in 1986 (Creemers 1986). Also in a preventive scheme with treatments every 7 till 10 days the best scab activity was obtained with the strobilurine analogue (Table 2).

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Table 1. Scab control with BAS 490 F in a 10 till 14 days spray-interval. Golden delicious: 8 treatments between 24/03-12/06. Jonagold : 9 treatments between 24/03-26/06/92

Fungicide	Dose (g) a.i./ha	Leaves		Fruits	
		Golden	Jonagold	Golden	Jonagold
Untreated % attack		92.5	82.5	97.2	92.5
BAS 490 F	75	98.8 a	93.1 b	98.8 a	96.6 a
BAS 490 F	100	98.4 a	97.6 a	97.4 ab	97.4 a
Bitertanol	187.5	21.5 d	21.4 d	68.5 d	70.1 d
Bitertanol + Captan	187.5 + 900	90.9 bc	74.7 c	91.1 c	89.4 b
Difenoconazol	37.5	85.3 c	70.4 c	94.5 bc	91.6 b
Difenoconazol + Captan	37.5 + 900	98.9 a	92.4 b	98.0 a	95.9 a
Flusilazol	45	68.8 c	59.1 c	75.7 d	80.1 cd
Flusilazol + Captan	30 + 900	95.2 b	67.5 c	95.1 c	86.5 bc

Table 2. Scab control with BAS 490 F in a 7 till 10 days spray-interval. Golden delicious: 8 treatments between 23/03-11/06. Jonagold : 9 treatments between 23/03-26/06/92

Fungicide	Dose (g) a.i./ha	Leaves		Fruits	
		Golden	Jonagold	Golden	Jonagold
Untreated % attack		92.5	82.5	97.2	92.5
BAS 490 F	75	98.8 ab	98.1 a	99.6 a	98.8 a
BAS 490 F	100	99.3 a	97.6 a	99.9 a	99.1 a
Captan	1660	94.8 c	92.3 b	96.5 b	94.9 b
Dodine	650	96.7 abc	97.1 a	99.7 a	99.0 a
Mancozeb	2400	96.3 bc	85.8 c	97.4 b	96.6 b

The strong curative activity of the compound is proved by the results in Table 3 were the treatments were applied 4 or 6 days after the infection with a preventive period of 5 days included. In this trial the efficacy of the different products was always better in the curative 6 days spray program. The differences between both curative schemes were most pronounced in the plots where the DMI-fungicides were applied alone.

In the curative 4 days program the first treatment was carried out in bad climatological conditions as the weather was very windy and rainy. At that moment the phenological stage was tight cluster. For the treatments in the curative 6 days scheme the weather conditions were always good without rain within two hours after the application. The activity of the strobilurine analogue was less influenced by the climatological conditions during and after the treatment. In contrast with highly systemic DMI-fungicides, disease control with the strobilurine-analogues is retained to the zone of application without rapid accumulation at the leaf border and leaf tip (Godwin et al. 1992).

Table 3. Scab control with BAS 490 F applied 4 and 6 days after an infection on the apple cultivar Jonagold. Curative 4 days: 8 treatments between 25/03-23/06/92. Curative 6 days: 8 treatments between 27/03-25/06/92

Fungicide	Dose (g) a.i./ha	Leaves		Fruits	
		4 days	6 days	4 days	6 days
Untreated % attack		82.5	82.5	92.5	92.5
BAS 490 F	75	93.2 a	94.9 b	98.3 a	98.8 ab
BAS 490 F	100	92.9 a	97.3 a	98.2 a	99.0 a
Flusilazol	45	29.9 c	72.6 c	88.1 c	95.3 c
Flusilazol + Captan	650	65.0 b	89.0 b	92.3 bc	96.9 bc
Difenoconazol+	37.5	90.3 a	97.2 a	96.9 ab	96.8 c
Captan	900				

### **Anilino-pyrimidines**

The anilino-pyrimidines also showed curative properties. This was clearly demonstrated on Jonagold in 1990 in a trial comparing pyrimethanil with several classic fungicides. The first treatment was applied after the first infections occurred.

In the pyrimethanil plots no typical scab lesions on the leaf whorls were found (Table 4). In the plots, where classic fungicides were applied, good sporulating scab lesions were present. The symptoms in the pyrimethanil treated plots were very typical; the lesions we found were restricted to small necrotic spots without sporulation. The observed symptoms are totally different from the chlorotic leaf spots after curative treatments with DMI-fungicides.

Table 4. Scab control with pyrimethanil on Jonagold. First treatment delayed till tight cluster: 9 treatments between 02/04-13/06/90

Fungicide	Dose (g)a.i./ha	Rosette leaves	Terminal shoot leaves	Fruits
Untreated % attack		81.3	94.0	96.0
Pyrimethanil	300	99.0 a	96.5 a	98.3 a
Pyrimethanil	450	99.5 a	96.9 a	98.1 a
Pyrimethanil	600	99.7 a	97.7 a	99.0 a
Captan	1660	93.1 b	95.8 ab	98.0 a
Mancozeb	2400	87.6 c	82.0 c	93.3 b
Dodine	650	89.3 bc	90.3 b	97.0 a

In general the scab activity of pyrimethanil is better in comparison with classic protectants (Table 5). Pyrimethanil can also be used in combination with a DMI-fungicide. The dose rate will be 450 g/ha when it is used alone and at least 300 g/ha in combination with a DMI-fungicide (Table 6). By enhancing the dose rate till 900 g/ha, pyrimethanil has given also a good scab control at longer spray intervals (Table 7).

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Table 5. Scab control on leaves with pyrimethanil in a 7 till 10 days spray-interval  
1991 Golden: 13 treatments between 28/03-19/07. Jonagold: 11 treatments between 05/04-03/07  
1992 Golden : 11 treatments between 23/03-11/06. Jonagold : 13 treatments between 23/03-03/07

Fungicide	Dose (g)a.i./ha	Golden		Jonagold	
		1991	1992	1991	1992
Untreated % attack		95.5	92.5	52.0	82.6
Pyrimethanil	450	98.5 a	97.8 a	98.5 ab	97.9 a
Captan	1660	97.1 a	94.8 a	99.8 a	92.3 cd
Mancozeb	2400	75.0 b	96.3 a	96.9 b	85.8 d
Dodine	650	99.3 a	96.7 a	98.5 ab	97.1 ab
Dithianon	562.5	91.9 a	79.0 b	99.8 a	94.3 bc

Table 6. Scab control with pyrimethanil in combination with DMI-fungicides. Golden: 8 treatments between 24/03-12/06/92. Jonagold: 9 treatments between 24/03-26/06/92

Fungicide	Dose (g)a.i./ha	Golden		Jonagold	
		leaf	fruit	leaf	fruit
Untreated % attack		92.5	97.2	82.5	92.5
Pyrimethanil + Fluquinconazol	300	97.6 a	98.0 a	94.2 a	96.0 ab
Mancozeb + Fluquinconazol	1200	89.9 c	91.9 cd	82.7 b	96.8 a
Mancozeb + Fluquinconazol	1800	96.1 b	97.3 ab	94.6 a	96.9 a
Fluquinconazol	75				
Fluquinconazol	75				
Pyrimethanil + Pyrifenox	300	58.9 d	86.5 d	42.9 d	81.2 c
Mancozeb + Pyrifenox	57.6	94.2 bc	94.8 bc	78.4 c	82.2 c
Mancozeb + Pyrifenox	1600	93.3 bc	93.0 cd	81.4 bc	93.4 b
Captan + Difenconazol	900	98.9 a	98.0 a	92.4 a	95.9 a
	37.5				

Table 7. Scab control with pyrimethanil alone and in combination with DMI-fungicides on Golden delicious. Trial 1: 10 treatments between 29/03-19/07/91. Trial 2: 7 treatments between 18/04-21/06/91

Fungicide	Dose (g)a.i./ha	Trial 1	Trial 2	
		leaf	leaf	fruit
Untreated % attack		95.5	84.5	90.8
Pyrimethanil	900	98.8 a	98.4 a	98.4 ab
Pyrimethanil + Fluquinconazol	300	90.8 bcd	99.1 a	95.7 bcd
Mancozeb + Fluquinconazol	1200	87.9 d	88.8 a	99.0 ab
Captan + Fluquinconazol	1000	97.5 ab	91.5 a	98.1 abc
Pyrimethanil + Pyrifenox	300	89.0 cd	94.0 a	94.8 cd
Fluquinconazol	75			
Captan + Difenconazol	900	65.5 e	47.2 b	92.6 d
	37.5	95.4 ab	92.4 a	99.5 a



In a curative spray program we found that pyrimethanil can improve the curative activity of DMI-fungicides (Table 8).

Table 8. Curative scab control with DMI-fungicides in combination with pyrimethanil on Jonagold. Treatments 4 days after an infection (8 treatments between 25/03-23/06/92)

Fungicide	Dose (g)a.i./ha	Leaf	Fruit
Untreated % attack		82.5	92.5
Pyrimethanil +	300	84.6 a	96.8 a
Fluquinconazol	75		
Mancozeb +	1200	29.0 c	85.9 b
Fluquinconazol	75		
Pyrimethanil +	300	71.0 ab	92.5 ab
Pyrifenox	57.6		
Captan +	900	45.7 c	88.3 b
Pyrifenox	57.6		
Flusilazol	45	29.9 c	88.1 b
Captan +	900	65.0 b	92.3 ab
Flusilazol	30		

### Side effects

The crop tolerance with BAS 490 F and Pyrimethanil has been good. Also they have no negative effects on the predatory mite *Typhlodromus pyri* (Table 9).

Table 9. Side effect on the predatory mite *Typhlodromus pyri*. 2 treatments: 4 and 11/08/92

Fungicide	Dose g.(a.i.)/ha	Henderson-Tilton
BAS 490 F	1000	15.2
Pyrimethanil	300	-6.0
Pyrimethanil	600	10.1
Captan	1867	15.4
Dithianon	562.5	1.8

The dose rate of BAS 490 F was 10 times higher than necessary for scab control. Even at this dose rate the fungicide was harmless according to the IOBC classification. Also the anilino-pyrimidine Pyrimethanil was not toxic for the predatory mite *Typhlodromus pyri*.

### CONCLUSIONS

To control scab in an integrated protection program, there is a need for curative fungicides. With the DMI-fungicides it is possible to control the scab fungus till 4 days after the infection.

The last years however, different articles were published reporting on scab strains with a lesser sensitivity for DMI-fungicides (Creemers *et al.* 1988). In practice too scab control failed after the application of DMI-fungicides. The link with resistance was made several times during the workshop in Lofthus.

The fungicide families presented in this report can be a good supplement to the DMI-fungicides in the strategy to avoid the extension of less sensitive strains. Both groups provide new biochemical modes of action with no cross resistance to existing fungicides. The strobilurine analogues have strongly curative properties. The activity seems less influenced by the climatological conditions during and after the application. In addition, disease control is retained to the zone of application, without rapid accumulation to the leaf tip.

The curative activity of the anilino-pyrimidines is shorter, but there is also a possibility to use them in post-infection control programs. They are characterized by a relatively high vapour phase. Therefore it will be better to preserve the anilino-pyrimidines for the beginning of the season when temperatures are lower than to use them later on during the summer months. The preventive activity can be decreased by the evaporation at higher temperatures.

Further investigations are needed to determine the exact period of curative activity and this in relation with temperature.

Besides the curative activity, both fungicide families have the advantages of low persistence and low acute toxicity. Till now they didn't show any terratogenic effects.

The first trials on beneficials, particularly on the predatory mite *Typhlodromus pyri*, showed no negative effects; this factor is a *conditio sine qua non* for the use of these fungicides in an IPM program.

## SUMMARY

The strobilurine analogues and the anilino-pyrimidines represent two new fungicide families which are highly active against scab on apple and pear. Because of their curative properties and the different biochemical modes of action on the fungus, they can give a new supply to the DMI fungicides.

The anilino-pyrimidines have a shorter curative effect than the strobilurine-analogues. Preliminary studies indicate that both fungicide families are selective for beneficials which are important in an Integrated Pest Control.

## REFERENCES

Ammermann, E., G. Lorenz, K. Schelberger, B. Wenderoth, H. Sauter & C. Rentzea 1992. BAS 490 F - a broad-spectrum fungicide with a new mode of action. Brighton Crop Protection conference Pests and Diseases: 403-410.

Anke, T., F. Oberwinkler, W. Steglich & G. Schramm 1977. The strobilurines - new anti-fungal antibiotics from the basidiomycete *Strobilurus tenacellus*. *The Journal of Antibiotics* (30) 806-810.

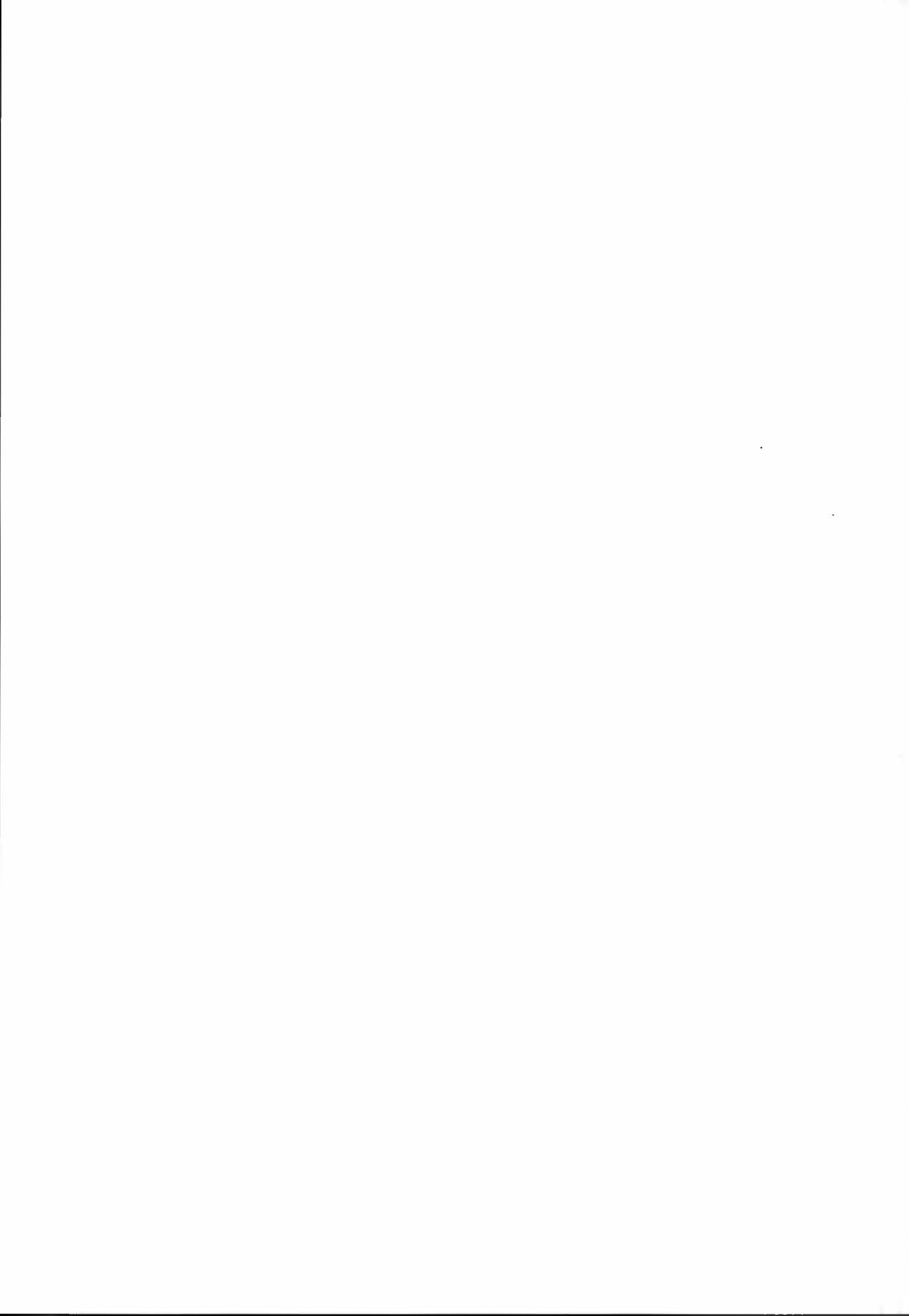
Brandt, U. & G. von Jagow 1991. Analysis of inhibitor binding to the mitochondrial cytochrome C reductase by fluorescence quench titration. *European Journal of Biochemistry* (195) 163-170.

Creemers, P. 1986. New strategies for control of apple scab. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* (51/2b) 659-665.

Creemers, P., J. Vandergeten & A. Vanmechelen 1988. Variability in sensitivity of field isolates of *Venturia* spp. to Demethylation inhibitors. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* (53/2b) 577-587.

Godwin, J.R., V.M. Anthony, J.M. Clough, C.R.A. Godfrey 1992. ICIA5504 : a novel, broad spectrum, systemic  $\beta$ -methoxyacrylate fungicide. Brighton Crop Protection Conference Pests and diseases: 435-442.

Neumann, G.L. & E.H. Winter 1992. Pyrimethanil: a new fungicide. Brighton Crop Protection Conference Pests and Diseases: 395-402.



# Side-effects of fungicide and insecticide sprays on phytoseiid mites in apple orchards

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Various number of applications of 15 fungicides at normally recommended dosages, and 1 application of 6 insecticides at low dosages, were tested on 5 phytoseiid species dominantly present on apple trees. Small differences were found between treatments. On the trees treated with 1-5 fungicide applications in addition to 2-5 routine sprays, the predatory mites survived and increased to a high level, often 100-200 phytoseiids per 100 leaves. At this predator density, the phytophagous mites diminished greatly, and were reduced far below the damage threshold during the experimental period. All the phytoseiid species were efficient predators and seemed to react very similarly to the various treatments. If the high density of predatory mites on the treated trees is due to immigration from unsprayed or low-sprayed trees, the growers may take advantage of this by the use of mixed plantings.

**Key words:** Biological control, dosages, fungicides, integrated control, migration, orchard pests, pesticide tolerance, predatory ability, predatory mites, spray frequency.

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Integrated fruit production (IFP) is largely based upon sound integrated management of pests, diseases and weeds with minimum use of agrochemicals. In such IPM-programmes, important key natural enemies, eg. phytoseiid mites, are often preserved, and use of acaricides against fruit tree red spider mite, *Panonychus ulmi* (Koch) and apple rust mite, *Aculus schlechtendali* (Nalepa) can then be reduced or omitted.

It is well known that many pesticides are very harmful to phytoseiid mites and other natural enemies, when applied at normally recommended dosages (Boller et al. 1989). Some products are selective, causing no harm to the beneficials, while certain insecticides can be made selective by lowering the dosages (Edland 1976).

The side-effects on predatory mites have been widely studied in many countries, using a range of different pesticides at different rates on various developmental stages (eg. Van Zon & Van der Geest 1980; Tuovinen 1992). However, greatly conflicting results and statements have been reported. For instance, in England the acaricide cyhexatin was found to be fairly harmless to predatory mites and was therefore recommended at low rates to

suppress *P. ulmi*, even in IPM-programmes, though it may result in starvation of the phytoseiids through 'overkill' of the prey (Cranham 1979). In contrast, Dutch studies have shown that cyhexatin acts insidiously, since it is barely harmful to phytoseiid adults but very toxic to eggs and larvae (Gruys et al. 1980).

There is general agreement that most organophosphates are very detrimental to phytoseiid mites, except strains which have developed OP-resistance. Some fungicides, eg. dinocap and lime-sulphur, are known to be toxic to the phytoseiid mites (Cranham 1979). Likewise, repeated use of dithiocarbamates and sulphur may have detrimental effects. Therefore, the European guidelines for IFP require restrictions in pesticide use. For sulphur, the limitations are a maximum dose rate of 3 kg/ha per application and maximum three sprays per season (Dickler & Schaefermeyer 1991).

In Norway, the phytoseiid fauna on deciduous trees has been investigated for several years. In commercial apple orchards these predators normally occur very sparsely, while in unsprayed or low-sprayed orchards several phytoseiid species are sometimes present at high densities. However, predatory mites are frequently absent, even on trees which during the previous few years have only received fungicide sprays.

In 1987-88, an OP-resistant strain of *Typhlodromus pyri* Scheuten was introduced from HRI, East Malling, UK, and successfully established in a commercial apple orchard in Norway. This predator has proved more efficient than any acaricide sprays in keeping *P. ulmi* below the damage threshold in this country. Phytoseiid mites that tolerate important pesticides which sometimes are needed to control certain pests are of great benefit in the efforts to produce large yields of high quality apples.

Experiments performed in 1991 and 1992 were designed to clarify which fungicides, as well as some insecticides at very low dosages, can be safely used in Norwegian fruit orchards, without having significant detrimental effects on predatory mites.

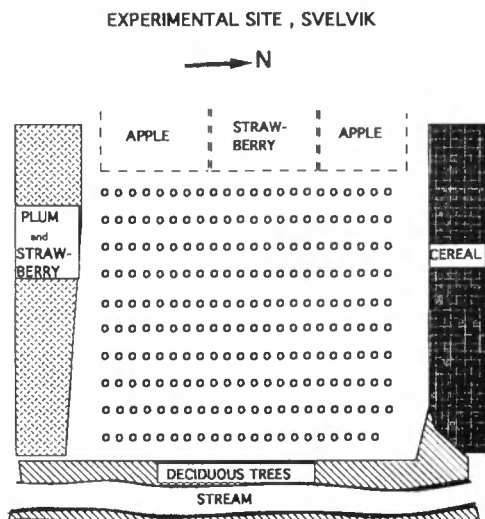


Fig. 1. The apple trees, CV. 'Lobo' on rootstock MM 106, were planted in 1984, plant spacing 4.8 x 3 m and grown as spindle-trees

## MATERIALS AND METHODS

### Experimental site

The experiments were carried out in a 0.32 ha 'Lobo' orchard at Svelvik, on the western side of the Oslofjord, at latitude 59° 36'. The spindle-trees, about 2.5 m high and 1.5 m wide, were planted in 1984. They were all on MM 106 rootstocks and spaced 3.0 m apart in the rows, with 4.8 m between rows. The surrounding vegetation is shown in Fig. 1.

### Mite fauna

The orchard had a serious outbreak of *P. ulmi* in early summer 1990, after having received a spray of an organophosphate

against lepidopterous pests the previous year. In June, dicofol was applied against both spider mite and rust mite, giving very poor effect. However, a few weeks later the spider mite population diminished and reached an acceptable level in August. Leaf samples collected from each of 30 individual trees during the autumn 1990 showed a dense and even distribution of phytoseiid mites on nearly all trees, with an average of 2.5 mites/leaf (Fig. 2). From each sample (25 leaves per tree) approximately 10 specimens were selected from different leaves and mounted for microscopic identification. The most common species were as follows:

*Euseius finlandicus* (Oudemans)  
*Paraseiulus soleiger* (Ribaga)  
*Paraseiulus triporus* (Chant & Shaul)  
*Phytoseius spoofi* Oudemans  
*Typhlodromus pyri* Scheuten

Surprisingly, each species generally occurred alone or was clearly dominant on each tree, making it possible to study the side-effects on the different phytoseiid species.

At bud burst each year, small samples of twigs were collected for investigation of the tree fauna. These showed that some trees were also inhabited by the species *Anthoseius caucasicus* (Abbasova) and *Amblyseius masseei* (Nesbitt).

### Treatments

Alternate trees in the orchard were selected as experimental trees. For each treatment, the pesticides were applied to four random trees (replicates). All sprays were applied by hand, using a backpack sprayer, which gave a volume of spray mixture equivalent to approximately 1500 l/ha.

In both years, a total of 15 different fungicides were applied during the season, at periods and at dosages commonly recommended and used in practice against scab, mildew, *Monilia*, storage rots, etc. (Hermansen 1993). In 1992, six different insecticides at 1/10 of the normally recommended dosages were also included in the experiments. These products and dosages are often used in IPM-programmes for controlling commonly occurring pests in our orchards. The different pesticides and the application rates used in the experiments are shown in Figs. 3-10.

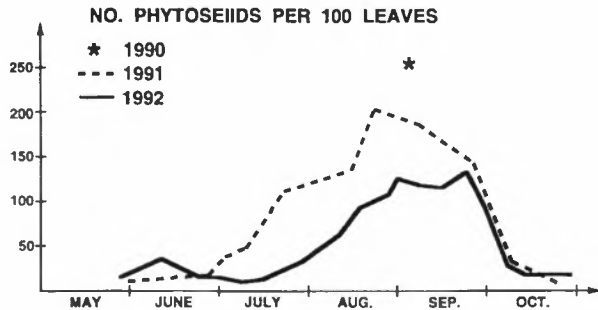


Fig. 2. Average number of phytoseiid mites recorded on all the experimental trees in early September 1990, and at different intervals in 1991 and 1992

Fig. 3.

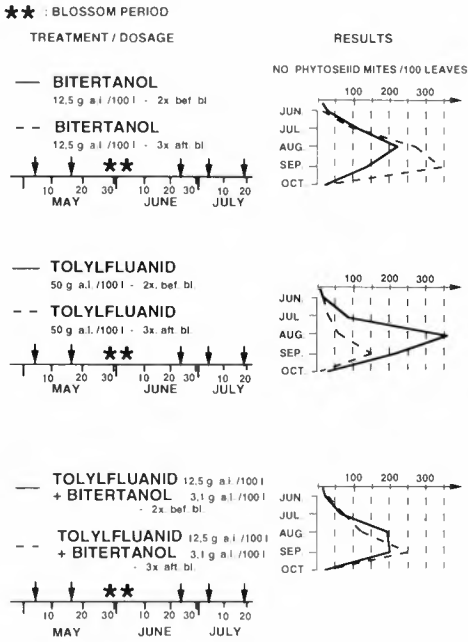


Fig. 4.

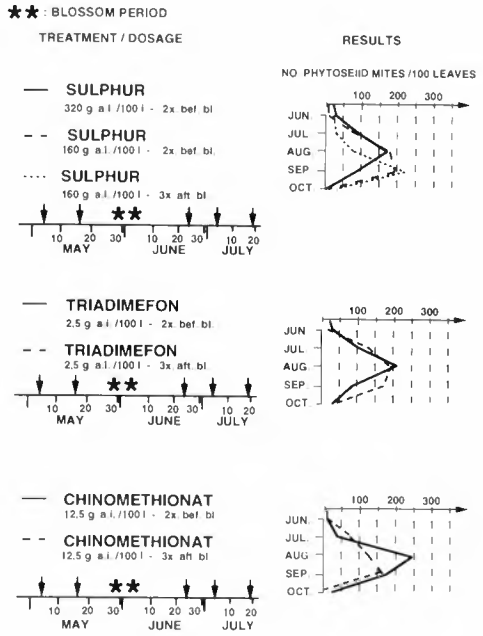


Fig. 5.

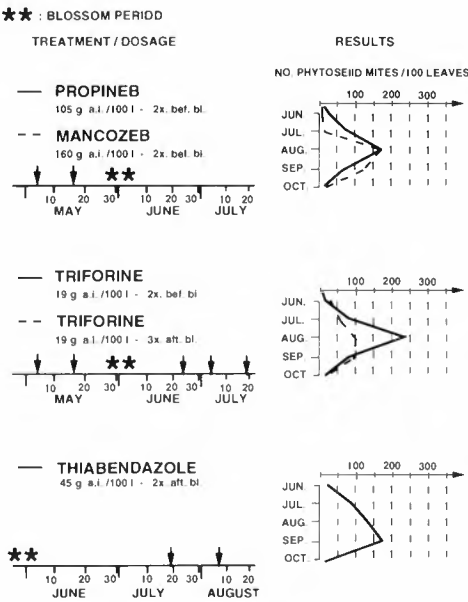
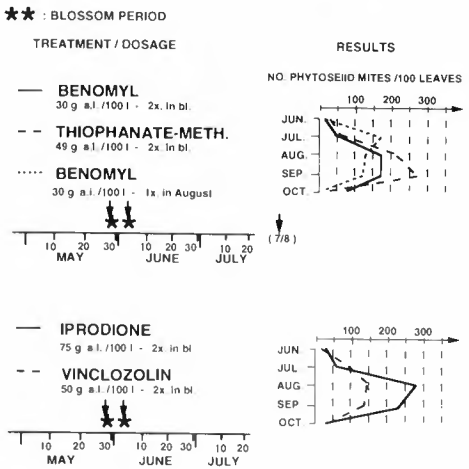


Fig. 6.



Figs 3-6. Effects of different fungicide treatments on phytoseiid mites in 1991. The fungicides, dosages and number of applications are shown and the number of phytoseiid mites recorded at different intervals during the season



Fig. 7.

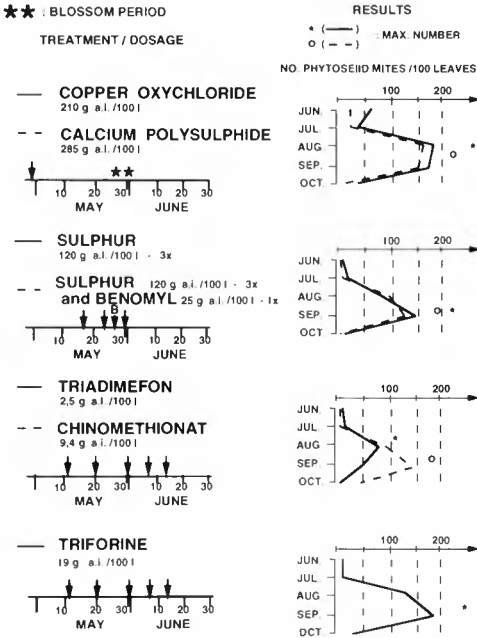


Fig. 8.

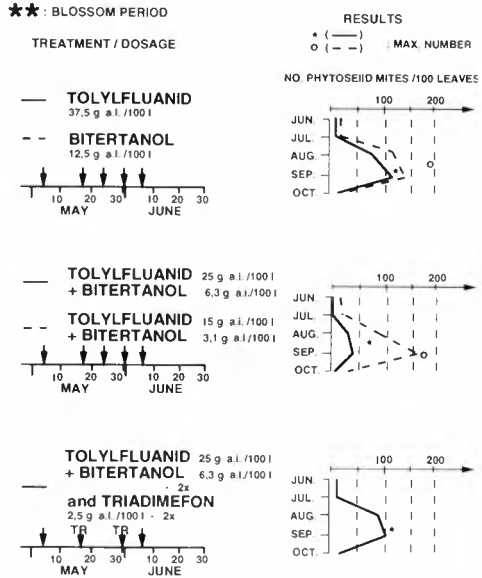


Fig. 9.

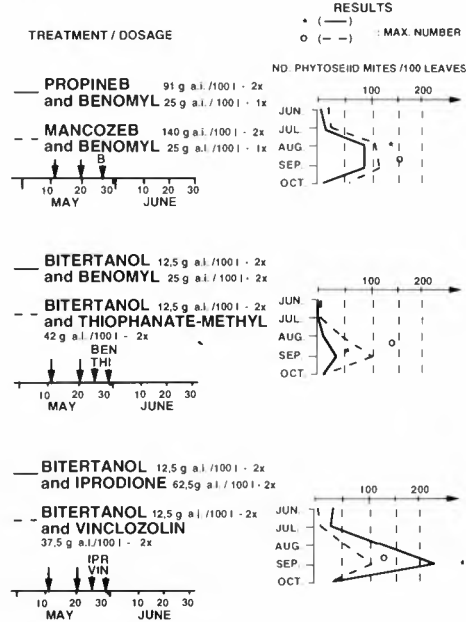
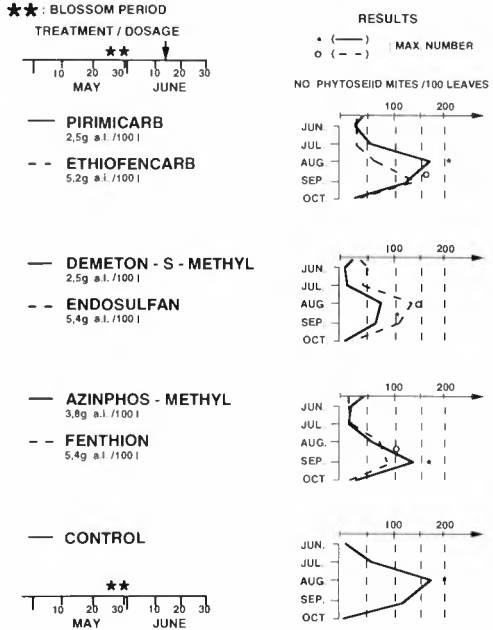


Fig. 10.



Figs. 7-10. Effects of different fungicides, used alone or in combination, and some insecticides applied at low dosages in 1992. The chemicals, dosages and number and period of applications are shown and the number of phytoseiid mites recorded at different intervals during season

In addition to the experimental treatments, the whole orchard received a routine spray programme both years: in 1991, this program was limited to two post-blossom sprays, one on June 9 with a mixture of dodine (624 g a.i./ha) and triadimefon (40 g a.i./ha), and another on June 17 with triadimefon (40 g a.i./ha), both against diseases. In 1992, five sprays were applied, two pre-blossom with dodine (624 g a.i./ha) on April 28 against scab and with copper oxychloride (3.36 kg a.i./ha) the following day against canker. The three post-blossom sprays were triadimefon (40 g a.i./ha) on May 29 a mixture of dodine (520 g a.i./ha) and triadimefon (40 g a.i./ha) on June 13, against diseases, and pirimicarb (40 g a.i./ha) on June 24 against aphids.

For the routine sprays a tractor-drawn air-mist sprayer delivering 400 l/ha was used.

### **Application periods**

To clarify whether any fungicides act differently on the phytoseiid mites at different periods of the season, the treatments in 1991 were applied as follows: before blossom (2 appl.), during the blossom period (2 appl.), after blossom (3 appl.) and in late summer (1-2 appl.). The dates of applications in 1991 are shown in Figs. 3-6.

In 1992, each treatment consisted of 1, 3, 4 or 5 sprays, all of them applied in spring - early summer (April 29 - June 14). For some treatments, the same fungicide was used for all applications. For others, two or three different products were combined. The six insecticides tested in 1992 were all applied on June 14, at the small fruitlet stage (Figs. 7-10).

### **Sampling**

Assessments of the phytoseiid density were normally performed at 1-3 week intervals from May until October. 25 leaves were collected from each tree or 100 from each treatment, and the number of living phytoseiids (adults and large nymphs) were removed from the leaves using a washing method or a brushing machine. The mites were counted under a stereomicroscope. During the whole sampling season some phytoseiids (eg. 1-30 specimens/sample) were selected at certain intervals and prepared for microscopic identification.

## **RESULTS**

In both experimental years the phytoseiid mites occurred at a low level early in the season. In 1991, they increased rapidly in number during July and reached a peak in August. In 1992, there was a great increase in the population and the mites occurred at a maximum during September. In both years the phytoseiids seemed to develop very similarly on treated and untreated trees, but in July-Sept. the number was significantly higher in 1991 than in the following year. The average number of phytoseiids recorded from 100 leaves/sample for all treatments is shown in Fig. 2.

### **1991 experiment**

The numbers of phytoseiid mites recorded per 100 leaves from different treatments during the 1991 season are shown in Figs. 3-6. Even though the number of these predatory mites varied somewhat between different treatments, no fungicide application caused drastic

effects. The lowest maximum number, approximately 100 phytoseiids/100 leaves, was recorded on trees treated with triforine with three post-blossom applications (Fig. 5). This number, however, was high enough to keep phytophagous mites below the injury level. For most treatments the maximum number was about 200 phytoseiids/100 leaves.

As expected, bitertanol showed no harmful effect. On the trees treated with tolylfluanid, the phytoseiid number per 100 leaves was more than 350 after two pre-blossom applications and approximately 150 after three post-blossom sprays. During the sampling, more spider mites were found on the former treatment, and the excess of available food may explain the difference. The mixture of bitertanol and tolylfluanid in low dosages did not result in greater number of phytoseiids than did the two products used alone at higher dosages (Fig. 3).

High dosages of sulphur before or after blossom did not prevent phytoseiids from developing well later in the season. However, the post-blossom applications delayed the increase of the populations, which was not the case on trees treated with triadimefon.

Surprisingly, chinomethionat did not seem to cause much injury to the phytoseiid mites. However, three post-blossom applications did not allow the mites to reach as high levels as most fungicides did (Fig. 4).

The dithiocarbamates, tested as two pre-blossom applications, also appeared to be harmless to the predatory mites. Both products allowed a maximum of approximately 170 phytoseiids/100 leaves. The delayed increase in mite number for mancozeb compared to propineb may be caused by differences in available food (Fig. 5).

In this experiment two applications during the blossom period indicated that thiophanate-methyl and iprodione are more favourable to the predatory mites than are benomyl, thiabendazole and vinclozolin. However, all of these allowed the phytoseiids to reach a level of at least 150 mites/100 leaves (Fig. 6).

### **1992 experiment**

The various treatments with fungicides and insecticides (1/10 dosages) in 1992 also did not harm or disturb the development of predatory mites significantly. The results are given in Figs. 7-10. In addition to the curves showing the number of phytoseiids recorded as a total from the four experimental trees for each treatment, the maximum number recorded from a single replicate is given.

Normally, copper oxychloride is regarded as harmless to phytoseiid mites, while lime sulphur (calcium polysulphide) is known to be very detrimental. In this experiment there were no differences between them. After a pre-blossom application, the predatory mites increased to a level of more than 150 mites/100 leaves during August (Fig. 7).

Sulphur alone, or in mixture with benomyl, also had no noticeable adverse effect on phytoseiids in 1992. The number of predatory mites on trees treated with triadimefon was again lower than expected (ca 75/100 leaves), while five applications of chinomethionat gave high densities of predatory mites; on one replicate approximately 180 phytoseiids/100 leaves were recorded. Five applications of triforine also allowed the phytoseiids to develop to a high level (nearly 200/100 leaves) (Fig. 7).

Tolyfluanid and bitertanol were again tested, separately at normal dosages and in mixture at low dosages. The higher dosages of the mixture resulted in low densities of predatory mites, but lack of available food may be the main reason for the low number

(approximately 40 phytoseiids/100 leaves) (Fig. 8).

On trees treated twice with mancozeb and once with benomyl, the predatory mites developed slightly better than on trees treated with propineb and benomyl. Two applications of benomyl during the blossom period had an adverse effect on predatory mites (50 phytoseiids/100 leaves), whereas two applications of thiophanate-methyl at the same time resulted in twice as many. In 1992, iprodione again allowed survival of a significantly higher level of phytoseiid mites (about 230/100 leaves) than did vinclozolin, which had a maximum density less than half of this number (Fig. 9).

Among the insecticides tested in this experiment (1/10 dosages), pirimicarb showed the best result. The number of predatory mites with this treatment was similar to the control, which received the routine spray program only. Ethiofencarb, endosulfan and azinphos-methyl also gave favorable results, with more than 100 phytoseiids/100 leaves. Demeton-S-methyl and fenthion did not allow the predatory mites to reach such levels, at only 70-80 mites/100 leaves. However, it is unknown if lack of available food may have had an influence on the number of predatory mites. On all the experimental trees, both *P. ulmi* and *A. schlechtendali* were reduced to a very low level late in the season (Fig. 10).

### Phytoseiid species

During the experiments, more than 10,500 specimens were identified to species level, 3,500 in 1991 and approximately 7,000 in 1992. Five species represented 96,4% of the total number: of these, *P. soleiger* was the most abundant species (37.2%), followed by *T. pyri* (26.5%), *E. finlandicus* (13.9%), *Phytoseius* spp. (11.4%) and *P. triporus* (7.5%). As shown in Fig. 11, *P. soleiger* and *T. pyri* increased in relative number during the experimental period, whereas the other species decreased.

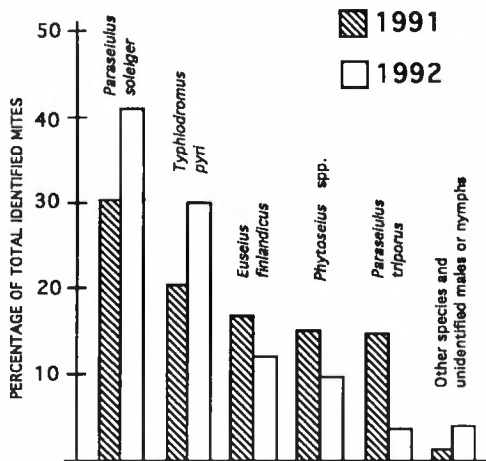


Fig. 11. Distribution of the 10,500 specimens of phytoseiid mites identified in 1991 and 1992

The genus *Phytoseius* was present as at least two different species. The most abundant was obviously *P. spoofi* (= the European form, close to the American *P. macropilis* (Banks)), but on certain trees another form, which very well fits the description of *P. ribagai* Athias-Henriot, was frequently recorded.

Since all the abundant species occurred very dominantly on certain trees during the whole experimental period, the results indicate that all of them reacted to the different treatments very similarly.

## DISCUSSION

As shown in Fig. 2, the density of phytoseiid mites decreased significantly from 1990 to 1992. At the same time the density of their prey, *P. ulmi* and *A. schlechtendali*, also decreased dramatically. The number of *P. ulmi* was much lower in 1992 than in 1991, especially early in the season. Thus, lack of available food may explain why the phytoseiids did not reach such a high level during 1992.

In 1991, when most treatments consisted of two or three applications in addition to the two routine sprays, no fungicide showed clear adverse effects against the predatory mites. Even two or three applications of sulphur, at dosages of 4.8 and 2.4 kg/ha/per application, respectively, allowed the phytoseiids to produce 170-220 mites/100 leaves later in the season.

To clarify whether a higher number of applications per year would cause more damage to the mites, the number of sprays in 1992 was doubled. That year most of the treatments consisted of 4-5 applications in addition to five routine sprays. Surprisingly, the phytoseiids developed well in all treatments, including chinomethionat, which is a commonly used acaricide in Norway.

In Finland, Tuovinen (1992) studied the effect of various fungicides on phytoseiid mites and found that four sprays of triforine or dichlofluanid, applied in June and July, reduced the number of mites significantly: however, on the triforine treated trees the number of phytoseiids increased again later in the season. In the present study, five sprays of triforine in May and June seemed to be rather harmless to the predatory mites. Also tolylfluanid, closely related to dichlofluanid, showed no or insignificant adverse effects, compared to untreated trees. The minor differences recorded between trees treated at different periods are, as already mentioned, probably due to varying access to available food for the predatory mites.

On all the experimental trees both *P. ulmi* and *A. schlechtendali* were reduced to very low levels in the late part of the 1992 season, regardless of which phytoseiid species was dominant. This indicates clearly that the predatory ability must be very similar for the five dominant species.

The small differences found between the tested fungicides are in contrast to results achieved in other countries, and it has been suggested that a high migratory activity may be a reasonable explanation. It is well known that phytoseiid mites are spread by wind. In the experimental plot at least alternate trees received the routine sprays only and might have served as reservoirs for phytoseiid mites. However, since each phytoseiid species occurred dominantly on most trees during the whole experimental period, a large migratory activity would have resulted in a mixed population at the ends of the seasons. Thus, it is unlikely that the high density of predatory mites recorded in August and September were mainly initiated by mites that immigrated from the neighbouring trees.

However, if such migration from unsprayed to treated trees does take place in orchards, this activity should be utilized in practical fruit production. Mixed plantations of susceptible and partially resistant cultivars could make it possible to omit many sprays on the latter types. Thus, the unsprayed or low-sprayed trees could serve as a very valuable resource of phytoseiid mites and greatly reduce the need for acaricide treatments.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- Boller, E., F. Bigler, M. Bieri, F. Häni & A. Stäubli 1989. Nebenwirkungen von Pestiziden auf die Nützlingsfauna landwirtschaftlicher Kulturen. Schweiz. Landw. Fo. 28(1): 3-63.
- Cranham, J.E. 1979. Managing spider mites on fruit trees. Span 22: 28-30.
- Dickler, E. & S. Schäfermeyer 1991. General principles, guidelines and standards for integrated production of pome fruits in Europe, and procedures for endorsement of national or regional guidelines and standards. IOBC/WPRS Bulletin 1991/XIV/3: 1-67.
- Edland, T. 1976. Effectiveness of insecticides at different concentrations on aphids and natural enemies (Eng. summary). Forsk. og forsøk i landbr. 27: 683-699.
- Gruys, P., D.J. de Jong & H.J. Mandersloot 1980. Implementation of integrated control in orchards. In: A.K. Minks & P. Gruys (eds.), Integrated control of insects pests in the Netherland. Pudoc, Wageningen 1980: 11-17.
- Hermansen, A. (ed.) 1993. Plantevern - Kjemiske og biologiske midler. SFFL, Ås & Landbruksforlaget, Oslo 215 pp.
- Tuovinen, T. 1992. Predatory mites in Finnish apple orchards. Acta Phyt. Ent. Hungarica 27 (II): 609-613.
- Zon, A.Q. van & L.P.S. van der Geest 1980. Effect of pesticides on predacious mites. In: A.K. Minks & P. Gruys (eds.), Integrated control of insects pests in the Netherland. Pudoc, Wageningen 1980: 227-230.

# Ascospore discharge in *Venturia inaequalis*

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Literally hundreds of reports have been published containing information on the discharge of ascospores of the apple scab pathogen, *Venturia inaequalis*. The purpose of this review is not to provide an exhaustive survey of the literature, but to focus upon some of the significant research of the last 25 years that has advanced our knowledge of the process of ascospore discharge, and the effects of various internal and external factors on that process. Those interested in a more comprehensive treatment of the earlier literature should consult the recent book by MacHardy (1994).

## THE STRUCTURE OF THE ASCOCARP AND THE PROCESS OF ASCOSPORE DISCHARGE

*Venturia inaequalis* (Cke.) Wint. forms an ascostroma within the internal tissues of infected apple leaves shortly after leaf abscission. The ascostroma is commonly called a pseudothecium due to its morphological resemblance at maturity to a perithecium. A locule forms within the pseudothecium during overwintering and the bitunicate asci which typify the subclass Loculoascomycetidae form shortly thereafter. The bitunicate ascus is composed of a relatively thick-walled and rigid exoascus; and a thin-walled, extensible endoascus.

When the leaf tissue bearing the pseudothecium is wetted, water moves into the ascocarp either through the ostiole or through the porous ascocarp wall, and thence into the asci through osmotic potential (Aylor & Anagnostakis 1991). Physiologically mature asci (Gadoury et al. 1992) then swell and protrude through the ostiole as the exoascus tip ruptures. The endoascus extends further until the entire ascus is approximately twice its original length, and extends approximately 40  $\mu\text{m}$  beyond the ostiole (Brook 1969a). Following the rupture of the endoascus tip, the uppermost spore is forcibly ejected. Each successive spore within the ascus is discharged after pausing briefly at the ascus tip. Because of the steadily decreasing pressure potential within the ruptured ascus, each successive spore released is propelled with less force (Aylor & Anagnostakis 1991). Results of early research on the force of ascospore discharge were undoubtedly confounded by turbulence and convection currents of unstable air (Aderhold 1896; Wallace 1913; Wiesmann 1932; Gadoury et al. 1992; Knoppien & Vlasveld 1947). Aylor & Anagnostakis

(1991) were the first to measure the force of discharge within a still-air system. In their study ascospores were discharged 0.1 to 13.2 mm. The mean distance traveled by discharged ascospores was 3.0 mm, and 75% of all spores were projected less than 4.1 mm. The hydrostatic pressure within a turgid ascus was estimated to be approximately 13 dynes, which was sufficient to propel the apical ascospores the distances observed in their study.

The movement of the ascus from within a closed, protective, and opaque ascocarp into the atmosphere nearly 40  $\mu\text{m}$  above the leaf surface is a significant feature of the process of ascospore release, and creates an opportunity for several interactions between the ascus and the external environment. Furthermore, the physiological nature of the mechanism of ascospore discharge means that the process is likely to be affected by low temperatures indirectly through the effect of such temperatures on membrane permeability and biochemical processes.

#### EFFECTS OF LIGHT ON ASCOSPORE DISCHARGE

Light is often second only to the presence of free water in regard to the magnitude of the effect upon ascospore discharge. Brook (1969a) was the first to demonstrate that light, specifically far red light (1969b), would stimulate ascospore release in *V. inaequalis*. He later described a similar phenomenon in four other fungi with bitunicate asci (Brook 1975). Since these early reports, diurnal periodicity of ascospore release has been described in a number of field studies (MacHardy & Gadoury 1986; Aylor & Sutton 1992). Evidence of diurnal periodicity of ascospore release and the suppression of ascospore release during darkness was also found by MacHardy & Gadoury (1986) and in earlier reports by Hirst & Stedman (1962) and Frey & Keitt (1925). Generally, the suppression of ascospore release during darkness is not absolute. During 31 continuous rain and leaf wetness events over a 4-year period, MacHardy & Gadoury (1986) trapped from 91-100% of all ascospores between 0700 and 1800 h Standard Time (ST). Aylor & Sutton (1992) reported a similar degree of suppression during darkness in 19 of 20 events during which the rate of rainfall was unsteady. In the remaining event, rainfall began at 9 PM and ceased at 5 AM (Aylor & Sutton 1992), which possibly inflated the percentage trapped during darkness. Warner & Braun (1992) reported on ascospore release in a research orchard in Ontario, Canada. In 1991, 69% of the ascospores released were trapped between 0700 and 1800 h, and 87% were trapped between 0500 and 2000 h ST (the times of sunrise and sunset at the research site). Warner & Braun (1992) suggested that the 0700-1800 h daytime interval described by MacHardy & Gadoury (1986) be extended from 0500 to 2000 for eastern Canada, and further studies may support this modification. However, the somewhat higher proportions trapped during darkness by Warner & Braun (1992) can be explained by the temporal distribution of rainfall at their research site. Night rainfall followed by no or little rain during the subsequent day will result in an underestimation of the potential release for a given interval of time, and will inflate the percentage trapped during darkness. Such abbreviated rain events are evident in some of the data reported by Warner & Braun (1992), can not be compared to data reported by MacHardy & Gadoury (1986), and should not be analyzed as evidence of diurnal periodicity. MacHardy & Gadoury (1989) have suggested that Mills's (1944) criteria for predicting apple scab infection periods be revised



to reflect the low proportion of ascospores released during darkness and the low inoculum dose that typifies modern commercial orchards (Gadoury & MacHardy 1986).

Brook (1969b) reported that light within the waveband of 710-730 nm was responsible for the stimulation of ascospore release, but made no mention of the intensity required for this stimulation. Palm (1988) reported that few ascospores of *V. inaequalis* and *V. pirina* are trapped in the field when light intensity is below 2000 lux, a light intensity that may correspond to approximately 2% of maximum sunlight, and which occurs at a time between 0500 and 0600 ST at Geneva, NY during the season of ascospore release. Darpoux et al. (1975) reported stimulated ascospore release in *V. pirina* at 600-950 lux. The actual intensity of the sources in the stimulatory wavelengths identified by Brook (1969b) is unknown. Field studies indicate that some stimulation of ascospore release by light occurs shortly before sunrise, but the maximum rate of release is not reached until several hours later (MacHardy & Gadoury 1986). For example, the maximum of the distribution of ascospores trapped over a 4-year period in New Hampshire, USA, was between 11 AM and 12 M (noon) ST (MacHardy & Gadoury 1986), 5-6 hours after sunrise. However, in laboratory studies, Hirst & Stedman (1962) reported that most release occurs within 3 h of initial wetting; and Gadoury et al. (1992) observed nearly complete exhaustion of the ascospore supply in 90 min. In general, the time required to harvest the total ascospore supply from small leaf samples in laboratory tests has been less than one-half the time required under comparable field conditions. Furthermore, in several unpublished and at least two published reports (Gjærum 1954; Warner & Braun 1992), the pattern of diurnal periodicity of ascospore release and the suppression of ascospore release by darkness has not been reproduced in the laboratory. Thus, while the field experiments were remarkably consistent worldwide and over a period of several decades, the results of laboratory experiments were less consistent and less convincing.

#### EFFECTS OF TEMPERATURE ON ASCOSPORE DISCHARGE

Temperatures between 0 and 5°C appear to reduce both the rate of ascospore release and the absolute number of ascospores released during a wetting event, the effect becoming most pronounced near 0°C. However, above 10°C, there may be little or no significant effect on the rate of release or productivity (Seem et al. 1979). Hirst & Stedman (1962) and MacHardy & Gadoury (1986) both observed cumulative distributions of ascospore release were shifted in time by approximately 2-3 hours when temperatures during natural rain events were below 10-12°C, as compared to the distributions of ascospore release above 10-12°C. In laboratory tests, the peak rate of ascospore release occurred after 4.8, 3.0, 1.8, and 1.3 h of wetting at 0, 5, 10, and 20°C, respectively; while the total spore release was 48, 2624, 2217, and 4822, respectively (Hirst & Stedman 1962).

#### ASCOSPORE RELEASE DURING NATURAL RAIN

The threshold of detection of the most commonly used instruments for measurement of rainfall is approximately 0.2 mm, and there are numerous reports of ascospore discharges of various magnitudes following this minimal amount of rain (MacHardy & Gadoury 1986;

Hirst & Stedman 1962; Brook 1966; Warner & Braun 1992). Recent findings by Aylor & Sutton (1992) indicate that discontinuous rain at minimal rates ( $< 0.25$  mm/h) is highly correlated with maximum spore densities in orchard air above an inoculum source, and that minimal rain is more favourable than heavy rain for transport and deposition of ascospores. Once leaf tissue is sufficiently wet to lead to ascospore release, and rainfall is sufficient to maintain leaf wetness, there appears to be little additional benefit in an increased rate of rainfall with respect to the rate of ascospore release. Considering the myriad of factors that can influence the potential ascospore release from an inoculum source, of which rainfall is only one, it is not surprising that total rainfall or rainfall rate are not highly correlated with ascospore release and airborne ascospore dose (Seem et al. 1979). Distribution of the rain with respect to day and night, temperature during the rain event, and maturity of the population can all interact with rain to modify the effect on ascospore release.

Time-course studies of ascospore release during continuous daytime rains at temperatures above  $10^{\circ}\text{C}$  provide a description of the pattern of ascospore release independent of the confounding effects of darkness and intermittent or premature drying of the leaf litter. In detailed studies by Hirst & Stedman (1962) and Gadoury & MacHardy (1986), ascospore release began within minutes after detectable rain, and the rate of spore release increased steadily for 3-6 h. The rate of release gradually declined to a minimal level after approximately 8 h. In an analysis of 15 rain events which began before 0700 ST and continued until 2400 h, MacHardy & Gadoury (1986) trapped 90% of the ascospores in a 6 h period between 0700 and 1300 h. The distribution of ascospore release was symmetrical and normal, with 50% of the ascospores trapped by approximately 11 AM. Ascospore release during rain events that last for two or more days, given that conditions on the first day are favourable for ascospore release, is typified by a major release as described above, followed by a similar pattern of release, but at a reduced airborne dose, on subsequent days. The releases on subsequent days are usually at least one order of magnitude below that observed on the first day of the rain event. Degree-day accumulation during 2-day rain events is usually too low to allow maturation of a significant percentage of the ascospore population (Gadoury & MacHardy 1982). In regard to the control of apple scab, the practical implication of the foregoing is that the bulk of the matured ascospore supply is released during the first 6 hours of a rain event, unless delayed by darkness or low temperature.

#### ASCOSPORE RELEASE DURING DEW

The extremely small proportion of the matured inoculum that is released by dew has made the detection and quantification of such releases difficult. Dew occurs during darkness, which suppresses ascospore release. Furthermore, dew formation is associated with clear skies and consequently with cooler night temperatures, which could also slow the rate of ascospore release. Wiesmann (1932) was not able to detect ascospore release during dew. MacHardy & Gadoury (1986) detected airborne ascospores only during dew periods that followed rain. They furthermore analyzed data on ascospore release during dew periods reported by Hirst & Stedman (1962) and Miller & Waggoner (1958) and often found a similar association between rain followed by dew formation and ascospore release. Low

numbers of airborne ascospores have been trapped following dews not associated with rain events (Brook 1969a; Hirst & Stedman 1962), and Moore (1958) frequently found ascospores within the dew film upon overwintered leaves on the orchard floor. Thus, the wetting of leaf litter by dew is sufficient to cause release. However, for reasons that are poorly understood, airborne ascospores are rarely detected following dew unless the dew period follows a rain event. Brook (1969a) noted that the ascus tip did not protrude through water films in excess of 40  $\mu\text{m}$ , and ascospores were thus released into thick water films rather than into the air above the leaf. The potential for the development of thick water films in the relatively still environment during dew formation may be an additional factor affecting ascospore discharge into the orchard air.

When detected in the orchard air following dew periods, the ascospores have been present at less than 1% the levels observed during rain events at the same site. A single recent exception to previous studies was briefly described by Stensvand et al. (1993) from two sites in Norway, in which 15 and 27%, respectively, of the seasons total ascospore supply was trapped following dew periods. A more complete analysis of this data is not presently available.

#### RECENT STUDIES ON ASCOSPORE RELEASE AT GENEVA

Substantial differences have been observed between ascospore release under lab and field conditions. As previously noted, the suppression of ascospore release during darkness appears to occur consistently in orchards worldwide (Brook 1969a; Hirst & Stedman 1962; MacHardy & Gadoury 1986; Warner & Braun 1992; Aylor & Sutton 1992). Nonetheless, in a number of unpublished laboratory and greenhouse studies, and in the study reported by Warner & Braun (1992), the ascospore supply of leaves exposed to wetting in complete darkness has been nearly exhausted. To better understand the reasons for the differences observed between laboratory and orchard studies, we constructed an apparatus in which we could accurately recreate the environment of an orchard during a rain event. It included a wind tunnel and spore trap in which we could exercise control over rainfall, temperature, light intensity and quality, humidity, and air flow.

A cross section of the apparatus is shown in Fig. 1. Unlike a typical suction trap, the tunnel is under a positive pressure. Compressed air is forced through a column of water to adjust the humidity and temperature to the desired level. The air then enters the trap at the rate of 20 L/min, passes over a platform bearing the leaf sample, and exits the trap through a 2 X 14 mm orifice. The temperature of the leaf sample is monitored by a thermistor imbedded in the sample platform and coupled to an electronic datalogger. Light is directed onto the leaf sample through a fiber-optic bundle coupled to a 150 W quartz-halogen lamp. The quality and intensity of the light within the tunnel was determined with a multispectral radiometer and was adjusted through the use of various color and neutral density filters. Simulated rain was supplied through a fine spray nozzle approximately 50 cm above the leaf sample. The temperature of the simulated rain, as well as that of the walls of the trap itself, was controlled by passing the water supply through a coil within a controlled-temperature bath. Simulated rain was supplied at the rate of 50-75 ml/min, evenly distributed over a circular area 10 cm in diameter (ca 40-60 mm of simulated

rain/hr). Excess water exited the tunnel through a drain and was collected in a sealed carboy. The apparatus has maintained temperatures as low as  $1.0^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$  for up to 9 hours, and could operate for up to 24 hours continuously.

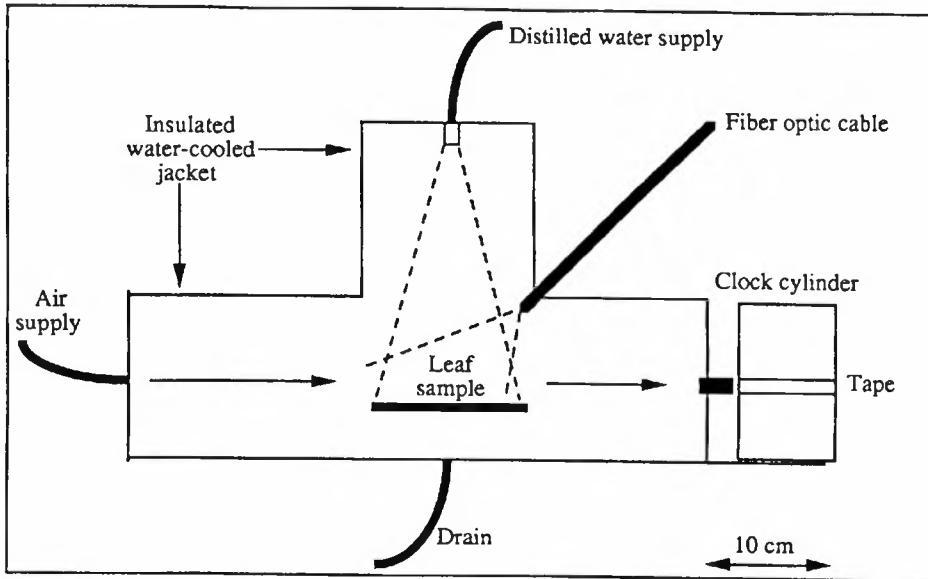


Fig. 1. Diagram of the wind tunnel and spore trap used in studies of *Venturia inaequalis* ascospore release at Geneva, New York. The trap is constructed of polyvinyl-chloride pipe. Compressed air is pumped through a water column to adjust the temperature and humidity, and then enters the trap at 20 L/min. Air flow over a leaf sample borne on a platform near the center of the tunnel and exits the trap through a 2 X 14 mm orifice at the opposite end. Spores entrained in the air stream impact on a transparent tape mounted on a clock cylinder that turns one revolution every 6-24 h. Simulated rain is applied to the leaf sample through a fine mist nozzle 30 cm above the leaf sample. Excess water is drained to a sealed carboy. Light is transmitted to the leaf sample through a fiber optic bundle. Light quality and intensity from a 150 W quartz-halogen lamp are adjusted with neutral density and colour filters. Temperature of the unit is controlled by an insulated water-jacket.

In the course of several experiments using the above described wind tunnel, we determined that the relative humidity of the air supply used in laboratory tests could have a substantial effect upon the release of ascospores. In heated laboratory buildings, the ambient relative humidity (RH) is often near 25%, and is sometimes as low as 10-15%. When non-humidified ambient laboratory air was used as the air supply for the wind tunnel, the normal suppression of the ascospore release during darkness did not occur, and ascospore release during darkness proceeded at a rate comparable to that observed in light (Fig. 2). Humidifying the air supply to a level that would be expected during a natural rain event (ca 90%) resulted in a response to darkness and light similar to that observed under orchard conditions (Fig. 2). It is possible that the extremely dry air typical of heated lab buildings may damage the asci as they emerge from the pseudothecium, and thereby cause premature ascospore discharge during darkness. It is unlikely that this phenomenon occurs under

natural conditions, since high RH and rainfall are coincident. However, this factor might largely explain some of the aberrant patterns of ascospore release (Gjærum 1954; Warner & Braun 1992) observed in lab or greenhouse studies.

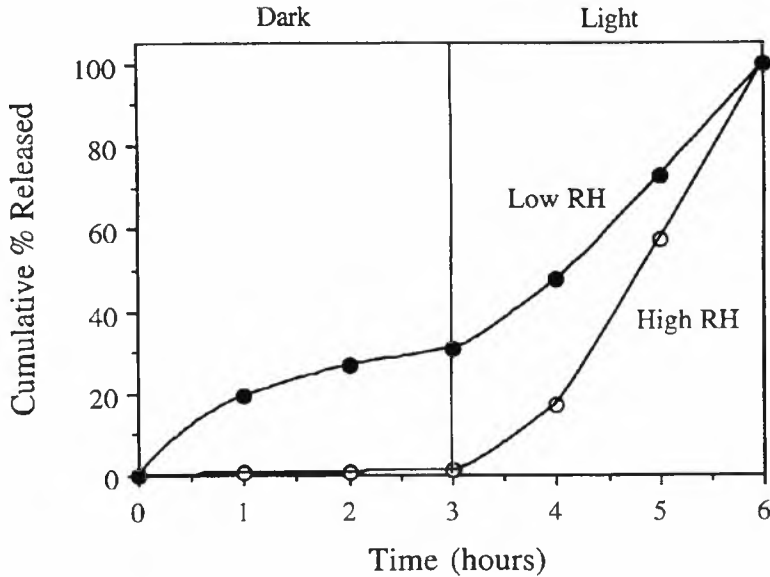
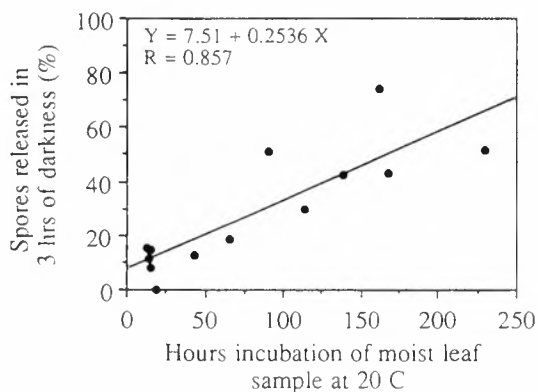


Fig. 2. Effect of relative humidity (RH) upon the suppression of ascospore release by darkness in *Venturia inaequalis*. Duplicate leaf samples were exposed to simulated rain at 20°C for 3 h in darkness followed by 3 h of daylight-balanced artificial light. RH in the low humidity test was ca 20-30% over the course of the experiment. RH during the high humidity test was maintained at 95% ± 4%.

Incubating leaf samples for 300-400 degree-days (base = 0°C) under moist conditions results in maturation of nearly the entire ascospore population (Gadoury & MacHardy 1982). We found that protracted incubation at 20°C results in maturation and perhaps senescence of a significant portion of the population of pseudothecia on apple leaves, and that the length of the period of incubation was correlated with the percentage of ascospores released during darkness (Fig. 3). Moist leaf samples were incubated at 20°C for 1-10 days and were then exposed to simulated rain for 3 h in complete darkness followed by 3 h in light. With each successive day of incubation, a larger proportion of the total release occurred during darkness (Fig. 3). This pattern was due to maturation of the population and possibly senescence, and not due to the physical accumulation of ascospores within pseudothecia. When we purged the leaf samples of ascospores 24 h before a test, and then retested the sample, we obtained the same pattern of ascospore release depicted in Fig. 3. Protracted incubation of leaf samples prior to laboratory tests, in particular during the exponential phase of ascospore maturation (Gadoury & MacHardy 1982) may result in maturation and senescence of the population of pseudothecia, and consequently large

releases of ascospores during darkness. This phenomenon may also have been a factor in the results of greenhouse tests reported by Warner & Braun (1992).

Fig. 3. Effect of incubation of leaf samples upon the suppression of ascospore release by *Venturia inaequalis* during darkness. Leaf samples collected at bud break were incubated moist, but without surface wetness, at 20°C, for 1-10 days. The samples were then exposed to simulated rain for 3 h in darkness followed by 3 h of daylight-balanced artificial light



In 1992, we observed a similar relationship between maturity of the population and the suppression of ascospore release during darkness. Scabbed leaves stored under 6.5 X 6.5 m canopies were sprinkler irrigated at 3, 6, or 9-day intervals. The irrigation was always initiated at 9 PM ST and continued through 3 PM the following day. We observed that as the season progressed, an increasing proportion of the ascospores was released during the predawn hours (Fig. 4). In general, ascospore release appeared to be stimulated by light throughout the season. However, the suppression of ascospore release by darkness was greatly reduced in the last 10-20% of the population (Fig. 4). Although this relationship was statistically significant, this relationship was not observed in similar field studies in 1993 in which a low-volume irrigation system was used. The irrigation system used in 1993 was less uniform and often resulted in intermittent wetting of the leaf litter. We do not yet fully understand the impact of the quantity and perhaps quality of simulated rainfall (irrigation) on ascospore release, but the differences observed between the 1992 and 1993 field studies indicate that the effects may be substantial. In our laboratory studies, the creation of an aberrant environment resulted in equally aberrant patterns of ascospore release. The discontinuous and non-uniform wetting provided by the low-volume irrigation system used in 1993 is not typical of natural rain events, and may have produced the atypical patterns of ascospore release observed in 1993.

As mentioned previously, the rate of ascospore release increases immediately after sunrise under orchard conditions, but the peak rate of release is not recorded until nearly 3-6 hours later. Is this because the light level needed for the maximum rate is relatively high? Does the increasing quantity of light between dawn and noon result in an increased rate of release? We found that the level of light that stimulates ascospore release is relatively low. At the 725 nm wavelength in the band identified as stimulatory by Brook (1969b), we have observed increased rates of release at an intensity of 0.5  $\mu\text{W}/\text{cm}^2$  (Fig. 5). During rain events at our location in Geneva, New York, this level of light intensity is reached between 5 and 6 AM ST during the month of April (Fig. 6). Light intensity increases rapidly above the threshold level of 0.5  $\mu\text{W}/\text{cm}^2$ , and reaches 4  $\mu\text{W}$  by 7 AM,

and 12  $\mu\text{W}$  by 9 AM (Fig. 6). In our lab tests, increasing the intensity of light from 0.5  $\mu\text{W}$ , which would occur before 6 AM, to 26  $\mu\text{W}/\text{cm}^2$ , which would occur near 11 AM (Fig. 6), did not change the rate of ascospore release (Fig. 5). Therefore, at some very low level, light may be a rate determining factor; but it does not operate in this manner under natural conditions after dawn. Light levels are increasing rapidly at dawn, and in real time in the orchard environment, the minimum level that we have found to stimulate ascospore release (0.5  $\mu\text{W}/\text{cm}^2$ ) is rapidly exceeded. If 0.5  $\mu\text{W}/\text{cm}^2$  is not precisely the minimum level that stimulates ascospores release, that precise intensity probably occurs within 15 min. of the time that 0.5  $\mu\text{W}$  is exceeded under natural conditions.

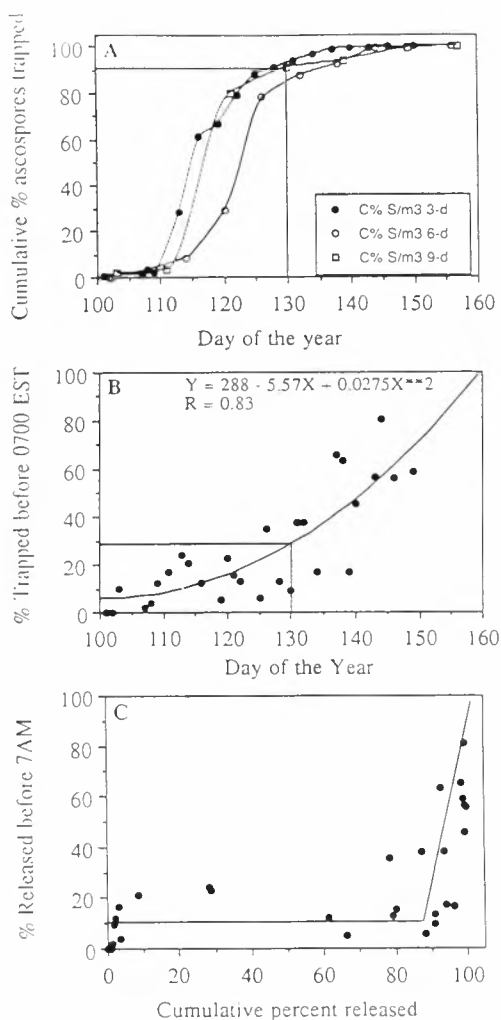


Fig. 4. Effect of maturation and senescence of field populations of *Venturia inaequalis* on suppression of ascospore release by darkness. Leaves enclosed in 6.5 x 6.5 m pens were wet by irrigation beginning at 9 PM and continuing until 3 PM ST the following day, at 3, 6, and 9-day intervals. (A) The cumulative percentage of ascospores trapped when leaves were irrigated at 3, 6, or 9-day intervals. Approximately 90% of the season's total release had occurred by day 130. (B) Relationship between time and the percentage of ascospores trapped before 7 AM during a wetting event that began at night. On day 130, the mean release before 7 AM of all treatments was ca 30%. (C) Relationship between the suppression of ascospore release by darkness and senescence of populations of *V. inaequalis*. "Broken-stick" regression models fit to the data indicated a change in the pattern of ascospore release at some time between the date of 80 and 90% release.

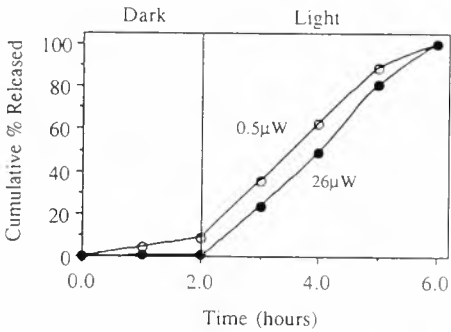


Fig. 5. Pattern of ascospore release by *Venturia inaequalis* at low and high light intensity. Duplicate leaf samples were exposed to simulated rain at 20°C for 2 h in darkness followed by 4 h of daylight-balanced artificial light. Intensity was measured at 725 nm. Linear regression equations indicated that lines fit to the data over the range from 2-6 h were parallel, with equal y-intercepts at  $P = 0.05$ .

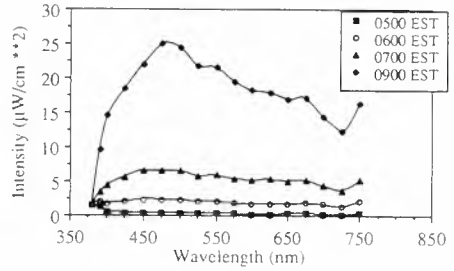
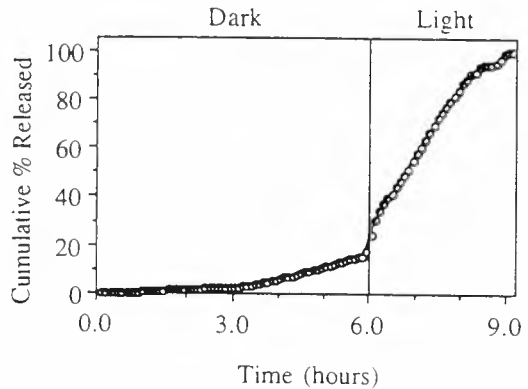


Fig. 6. Light intensity and quality during rain from 0500-0900 h ST on 24 May 1992 at Geneva, New York, USA.

We also found from our lab tests that with all other factors held constant, the rate of ascospore release often increases through time over a period of 3-6 h (Fig. 7). When we exposed leaf samples to simulated rain in darkness, the rate of release gradually increased over a 6-h period (Fig. 7). Even in complete darkness, more spores were trapped in each successive hour for the first 4-6 h of the wetting period. Once light was available, the rate of release was substantially increased. Therefore, the rate of ascospore release may increase through time coincidentally, but independently of increasing light intensity during morning hours. Leaves bearing populations of pseudothecia in different stages of maturity do not respond identically under the above described conditions, and the relationship between time and the rate of ascospore release can vary from linear to exponential.

Fig. 7. Increase in the rate of ascospore release by *Venturia inaequalis* over time during darkness and further stimulation of the rate of release by light. The leaf sample was exposed to simulated rain at 20°C for 6 h in darkness, followed by 3 h in daylight-balanced artificial light





Rainfall during darkness may serve as a form of preconditioning that allows pseudothecia to respond more quickly to light. Fig. 8 depicts the patterns of ascospore release from two identical leaf samples subjected to 6 h of simulated rain at 20°C. When light was supplied from the beginning of the test, there was a 1 h lag period during which few ascospores were detected, followed by a linear rate of ascospore release. When the first 2 h of wetting occurred during darkness, there was an immediate and substantial increase in the rate of release once light was provided. The timing and duration of a rain event may therefore affect the shape of the distribution of ascospore release.

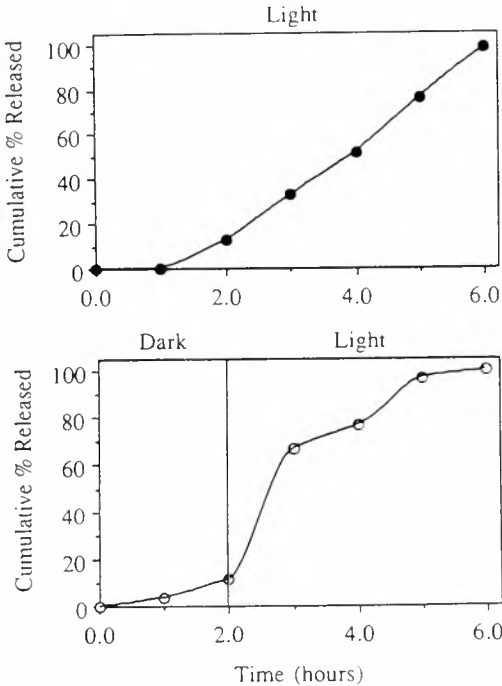


Fig. 8. Lag in the response to simulated rain in dry leaf samples. Upper graph depicts the pattern of ascospore release by *Venturia inaequalis* when leaf sample was exposed to simulated rain and light simultaneously. Lower graph depicts ascospore release from a duplicate sample exposed to 2 h of wetting during darkness followed by 4 h of wetting during daylight-balanced artificial light.

Our studies of the effects of temperature on ascospore release confirmed the major findings of earlier work by others (Hirst & Stedman 1962; MacHardy & Gadoury 1986; Seem et al. 1979), i.e., temperatures above 10°C appear to have little effect on the rate of ascospore release. However, we found that in addition to decreasing the total ascospore release, temperatures of 2-4°C also appear to lengthen the lag phase in release before which few ascospores are detected (Fig. 9). Although substantial progress has been made in quantifying the effects of temperature on the infection process (Mills 1944; Turner et al. 1986), the direct impact of temperature upon the availability of inoculum has been largely ignored in warning systems. Ascospore release is delayed at low temperatures and a relatively low proportion of the total potential release occurs during extremely cold rain. The combined effect of the delay of release and reduced dose may partially explain why a number of lab studies on ascospore infection at low temperatures generally show minimum times for infection that are substantially less than those reported by Mills (1944).

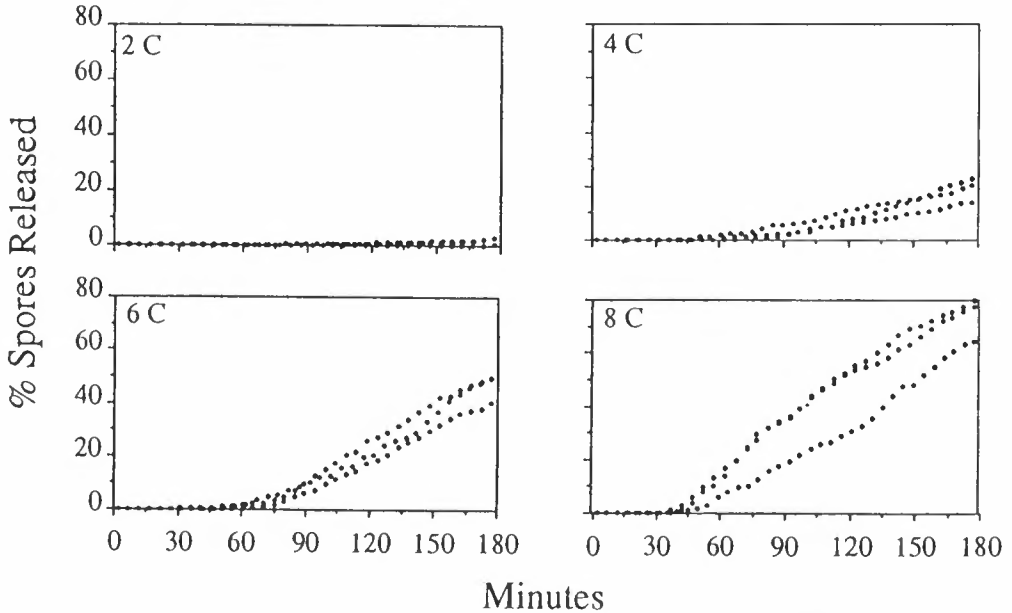


Fig. 9. Effect of temperature on the pattern of ascospore release by *Venturia inaequalis*. Three replicated leaf samples were exposed to simulated rain and daylight-balanced artificial light at the indicated temperatures for 3 h. Total potential release from leaf samples was then determined by raising the temperature to 20°C for an additional 3 h.

#### RESEARCH ON ASCOSPORE DISCHARGE AND MANAGEMENT OF APPLE SCAB

The earliest studies of ascospore release demonstrated that infection from ascospores occurs primarily after rain. Although some ascospores have been detected in orchard air during fair weather (MacHardy & Gadoury 1986) and following dew (Moore 1958; Stensvand et al. 1994) in subsequent work, their role in the epidemiology of apple scab is of unknown importance. Mills (1944) provided the first practical means to use basic information on rainfall and ascospore discharge to time fungicide applications to control apple scab. Based upon the research of Brook (1969a), apple growers in New Zealand began in the 1970's to ignore infection periods initiated by night rains, if the wetting interval after dawn alone was not sufficiently long to allow infection. MacHardy & Gadoury (1989) suggested similar modifications to Mills criteria to account for the suppression of ascospore release during darkness. Warner & Braun (1992) suggested that the revisions proposed by MacHardy & Gadoury (1989) were not applicable to orchards in eastern Canada. It is important to note that discussions of the patterns of ascospore release in *V. inaequalis* have largely involved discussions of percentages and proportions, and that the threat posed by ascosporic inoculum is determined by ascospore dose. The revisions to Mills criteria proposed by MacHardy & Gadoury (1989) were intended for use in commercial orchards. Such orchards generally harbor relatively small populations of *V. inaequalis* as compared to research orchards (Gadoury & MacHardy 1986). If there is general acceptance of the claim that the number of ascospores released by night rains is a small (ca 5-10%) part of the potential release (Brook 1969b; MacHardy & Gadoury 1986; Aylor & Sutton 1992), then the

important question, in regard to control of apple scab, is: does 5-10% of the maximum airborne ascospore dose in a well-managed commercial orchard represent a threat to the crop? MacHardy & Gadoury (1989) provided a numerical example to support the position that significant fruit infection would not occur if trees were left exposed to the maximum airborne ascospore dose that is likely to occur in a well-managed commercial orchard at night. Further evidence of the low risk of infection from similar low airborne ascospore doses is provided in a 6-yr study by MacHardy et al. (1993), in which trees in low-inoculum commercial orchards were not sprayed for the first 1-5 infection periods, without regard to timing of the wetting events and without significant increases in fruit infection. Of course, where potential ascospore dose is relatively high, the levels of infection that could result from night-released ascospores could cause serious damage in orchards. For this reason, the modifications to Mills criteria proposed by MacHardy & Gadoury (1989) should be used as they were originally intended: in well-managed commercial orchards. A potential ascospore dose of 1000 ascospores/m<sup>2</sup> has been suggested as a threshold level of inoculum for the purpose of delaying the first fungicide spray for apple scab (MacHardy et al. 1993). A similar level might be adequate as a threshold level for the use of the modified criteria proposed by MacHardy & Gadoury (1989).

#### LITERATURE

Aderhold, R. 1896. Die Fusicladien unserer Obstbaume. Teil I. Landw. Jahrb. 25: 875-914.

Aylor, D.E. & S.L. Anagnostakis 1991. Active discharge distance of *Venturia inaequalis* ascospores. Phytopathology 81: 548-551.

Aylor, D.E. & T.B. Sutton 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: relationship to spore transport and deposition. Phytopathology 82: 532-540.

Brook, P.J. 1966. The ascospore production season of *Venturia inaequalis* (Cke.) Wint., the apple black spot fungus. New Zealand Journal of Agricultural Research 9: 1064-1069.

Brook, P.J. 1969a. Effect of light, temperature, and moisture on release of ascospores by *Venturia inaequalis* (Cke.) Wint. New Zealand Journal of Agricultural Research 12: 214-227.

Brook, P.J. 1969b. Stimulation of ascospore release in *Venturia inaequalis* by far red light. Nature 222: 390-392.

Brook, P.J. 1975. Effect of light on ascospore discharge by five fungi with bitunicate asci. New Phytologist 74: 85-92.

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- Darpoux, H., A. Lebrun & B. de la Tullaye 1975. Action de traitements sur la formation des perithèces et la production de l'inoculum primaire de *Venturia inaequalis* (Cooke) Wint. et de *Venturia pirina* Aderh. *Phytiatrie-Phytopharmacie* 24: 3-14.
- Frey, C.N. & G.W. Keitt 1925. Studies of spore dissemination of *Venturia inaequalis* (Cke.) Wint. in relation to seasonal development of apple scab. *Journal of Agricultural Research* 30: 529-540.
- Gadoury, D.M. & W.E. MacHardy 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. *Phytopathology* 72: 901-904.
- Gadoury, D.M. & W.E. MacHardy 1986. Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. *Phytopathology* 76: 112-118.
- Gadoury, D.M., R.C. Seem, D.A. Rosenberger, W.F. Wilcox, W.E. MacHardy & L.P. Berkett 1992. Disparity between morphological maturity of ascospores and physiological maturity of asci in *Venturia inaequalis*. *Plant Disease* 76: 277-282.
- Gjærum, H.B. 1954. Ascospore maturity, dissemination and infection by apple scab. *Norwegian Plant Protection Institute Bulletin* 13. 48 pp.
- Hirst, J.M. & O.J. Stedman 1962. The epidemiology of apple scab (*Venturia inaequalis* (Cke.) Wint.). II. Observations on the liberation of ascospores. *Ann. Appl. Biol.* 50: 525-550.
- Knoppien, P. & W.P.N. Vlasveld 1947. Vier Jaren Voortgezet Onderzoek over de Schurft van Appel en Peer. *Tijdschrift over Plantenziekten* 53: 145-180.
- MacHardy, W.E. 1994. *Apple Scab: Biology, Epidemiology, and Management*. APS Press. St. Paul, MN. (In press).
- MacHardy, W.E. & D.M. Gadoury 1986. Patterns of ascospore discharge by *Venturia inaequalis*. *Phytopathology* 76: 985-990.
- MacHardy, W.E. & D.M. Gadoury 1989. A revision of Mills's criteria for predicting apple scab infection periods. *Phytopathology* 79: 304-310.
- MacHardy, W.E., D.M. Gadoury & D.A. Rosenberger 1993. Delaying the onset of fungicide programs for control of apple scab in orchards with low potential ascospore dose of *Venturia inaequalis*. *Plant Disease* 77: 372-375.
- Miller, P.M. & P.E. Waggoner 1958. Dissemination of *Venturia inaequalis* ascospores. *Phytopathology* 48: 416-419.

Mills, W.D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab. Cornell Ext. Bull. 630. 4 pp.

Moore, M.H. 1958. The release of ascospores of apple scab by dew. Plant Pathology 7: 4-5.

Palm, G. 1988. Untersuchungen über den Einfluß der Belichtungsstärke für den Askosporenausstoß des Schorfpilzes (*Venturia inaequalis*, *Venturia pirina*). Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft 245: 420.

Seem, R.C., J.D. Gilpatrick & M. Szkolnik 1979. Quantitative effects of microclimate on spore development and dispersal systems of apple scab. pp. 135-137 in: Proc. Symp. IX Int. Cong. Plant Prot., Washington, D.C.

Stensvand, A., T. Amundsen & L. Semb 1994. Apple scab (*Venturia inaequalis*) patterns of ascospore release in Norway. Proc. 3rd Workshop on Integrated Control of Pome Fruit Diseases. 31 May - 4 June 1993, Lofthus, Norway. Norwegian Journal of Agricultural Sciences. Suppl. No. 17: 49-54.

Turner, M.L., W.E. MacHardy & D.M. Gadoury 1986. Germination and appressorium formation by *Venturia inaequalis* during infection of apple seedling leaves. Plant Disease 70: 658-661.

Wallace, E. 1913. Scab disease of apples. N.Y. Agric. Exp. Stn. Bull. 335. 83 pp.

Warner, J. & P.G. Braun 1992. Discharge of *Venturia inaequalis* ascospores during daytime and nighttime wetting periods in Ontario and Nova Scotia. Canadian Journal of Plant Pathology 14: 315-321.

Wiesmann, R. 1932. Untersuchungen über die Überwinterung des Apfelschorfpilzes *Fusicladium dendriticum* (Wallr.) Fckl. im toten Blatt sowie die Ausbreitung der Sommersporen (Konidien) des Apfelschorfpilzes. Landw. Jahrb. Schweiz 36: 620-679.



# Evaluation and eradication of the primary inoculum of apple diseases

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A thorough knowledge of the survival of fungal pathogens enables: 1. A better judgement of the epidemiological development of a disease in spring. 2. The eradication of pathogens at the site of their survival. 3. The specific control of primary infections.

Tables 1 and 2 concern the sites of survival of some of the most important apple diseases and the possibilities for control. In table 3 the situation for apple scab is displayed.

Table 1. Sites of survival of pathogens of apple in South Germany and their importance

Pathogen	Surviving (mostly overwintering) on/in:				
	Apple trees (1)	Litter* (2)	Soil (3)	Other hosts (4)	Other sites** (5)
<i>Venturia inaequalis</i>	++	++	o	o	o
<i>Podosphaera leucotricha</i>	++	o	o	(+)	o
<i>Monilinia fructigena</i>	++	(+)	o	(+)	o
<i>Gloeodes pomigena</i>	++	o	o	++	o
<i>Schizothyrium pomi</i>	?	o	o	++	o
<i>Nectria galligena</i>	++	+	o	(+)	o
<i>Phytophthora cactorum</i>	+	+	++	(+)	o
<i>Monilinia laxa f. mali</i>	++	(+)	o	o	o
<i>Pezicula alba</i>	++	(+)	o	+?	o
<i>P. malicorticis</i>	++	+?	o	(+)	o
<i>Penicillium expansum</i>	++	+	(+)	(+)	+?
<i>Botrytis cinerea</i>	+	++	o	+	o
<i>Mucor piriformis</i>	o	+	++	(+)	+
<i>Fusarium spp.</i>	++?	+	o	(+)	o
<i>Alternaria alternata</i>	++?	++?	o	(+)	?

++ = very important; + = moderately important; (+) = survival possible but normally without importance; o = no survival; \* Fallen leaves, fallen fruits, prunings; \*\* fruit bins, packhouses, stores. (1)-(5) see Table 2

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Table 2. Methods of controlling/eradicating primary inoculum of apple diseases in relation to the sites of survival (numbers in brackets) presented in Table 1

Pathogen	Methods					
	Biological	Physical	Chemical	Other		
<i>Venturia inaequalis</i>	++ (2)	+ (1/2)	++ (1/2)	o		
<i>Podosphaera leucotricha</i>	o	++ (1)	+? (1)	o		
<i>Monilinia fructigena</i>	+ (2)	++ (1)	+? (1)	o		
<i>Gloeodes pomigena</i>	o	o	+? (1)	+ (4)		
<i>Schizothyrium pomi</i>	o	o	+? (1)	+ (4)		
<i>Nectria galligena</i>	+ (2)	++ (1/2)	o	o		
<i>Phytophthora cactorum</i>	o	++ (1/2)	o	+ (3)		
<i>Monilinia laxa f. mali</i>	+ (2)	++ (1)	+? (1)	o		
<i>Pezicula alba</i>	o	o	++ (1)	+? (4)		
<i>P. malicorticis</i>	o	+ (1/2)	++ (1)	+? (4)		
<i>Penicillium expansum</i>	o	o	+? (1)	+ (5)		
<i>Botrytis cinerea</i>	o	+ (2)	+? (1)	+ (5)		
<i>Mucor piriformis</i>	o	o	o	+ (5)		
<i>Fusarium spp.</i>	o	o	+? (1)	o		
<i>Alternaria alternata</i>	o	o	o	o		

++ = very important; + = moderately important; o = not important or not possible or not yet approved. (1)-(5) see Table 1.

Table 3. Overwintering sites of *Venturia inaequalis* and the possibilities of control different kinds of disease

Sites		Control method*			
		B	P	C	O
Apple trees: (parasite phase)	Twig (wood) scab	o	+	+	o
	S-scab**	o	o	+	+
	Shoot base scab***	o	+?	o	++
	Bud scale scab	o	o	+?	++
	Leaf scab (very seldom ?)	o	+++?	o	++
Litter: (saprophytic phase)	Leaf scab	++	++	+	o
	Fruit scab (very seldom ?)	?	?	?	?

++ = very important; + = moderately important; o = not important or not possible or not yet approved

\* B = biological; P = physical; C = chemical (including the application of fertilizers); o = other methods

\*\* Superficial scab on shoots, normally macroscopically invisible

\*\*\* Developing from buds just after bud break

The investigation, evaluation and eradication of the primary inoculum of phytopathogenic fungi in apple orchards are important in developing integrated control strategies.

Parts of the data shown here are preliminary but should serve as an encouragement for further studies.



# A moisture controlled variable power supply for operating Rotorod samplers

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A moisture-activated circuit was designed and constructed to control switching of electrical power during wetting periods. The circuit was developed to control Rotorod samplers for monitoring the discharge of ascospores of *Venturia inaequalis* in apple orchards. The integrated circuit, a modification of the transistorized-based circuit developed by C.G. Small (1978, *Plant Disease Reporter* 62:1039-1043), utilizes a low power CMOS Schmitt Trigger to produce quick and crisp on/off transitions. It requires a 12-volt power source and provides a regulated output voltage of 0 to 9.6 volts with a current draw up to 1.5 amperes. Other features include a moisture sensor with two interspace coils threaded on plastic tubing; low sensor current drain; and a potentiometer for varying the sensitivity of the sensor.

Key words: Power switch, spore trap.

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Many electronic sensors and circuits have been developed to automatically control moisture dependent sampling and data collection (Davis & Hughes 1970, Gillespie & Kidd 1978, Lomas & Shashoua 1970, Melching 1974, Schurer & van der Wal 1972, Small 1978, Smith & Gilpatrick 1980). These units are used to control spore traps or to record leaf wetness duration data for use in disease management programs. The circuit developed by Winters & Small (1934) was used extensively in New York State and later in Michigan for activating Rotorod samplers in monitoring programs for ascospores of the apple scab fungus *Venturia inaequalis* (Small 1978, Sutton & Jones 1976). A Rotorod sampler (Sampling Technologies, Inc, 2380 Wycliff Street, St. Paul, MN 55114) consists of plastic spore collector rods which are rotated in the air by a constant speed dc motor. This circuit was initially constructed with radio tubes (Winters & Small 1934), then modified and improved with transistor technology (Small 1978). The transistor-based circuit has been used in field monitoring programs in Michigan since the mid 1970's. This manuscript describes the construction with integrated circuits of a moisture controlled power supply designed after the circuit described by Small (1978).

## CIRCUIT DESCRIPTION

The schematic diagram for this circuit is shown in Figure 1. The moisture sensor, consisting of two wires spaced about 2 mm apart on a plastic tube (Small 1978), is plugged into phone jack JP3 (Fig. 2). The analog signal from the moisture sensor is converted to a digital signal by the voltage divider consisting of resistors R1 and R2, a potentiometer (labeled SPAREPOT), and a CMOS Schmitt trigger inverter (74C14N). When the sensor is dry, voltage  $V_x$  is approximately equal to voltage  $V_{cc}$  and the output of the first inverter is at logic low. This voltage is also equal to the  $V_{gs}$  voltage of the MOSFET IRF511 N-channel power transistor. Being lower than the threshold voltage, the transistor is off and there exists an open circuit in the current path of the motor. When the sensor is wet it is a virtual short circuit, making the input to the Schmitt trigger low enough to produce a high output. The signal is fed into the gate of the IRF511 power MOSFET, thus turning on the transistor and completing the current path to output jack JP2. A regulated 9 volts is supplied to the output jack by a LM317T variable voltage regulator. The voltage can be varied from 0 to 9.6 volts by adjusting the 5k $\Omega$  potentiometer (R4). Current drain is kept to a minimum by cutting off the supply voltage to the regulator when the sensor is dry. This is done by feeding the output of the first inverter to a second inverter, whose output represents ground for biasing the regulator.

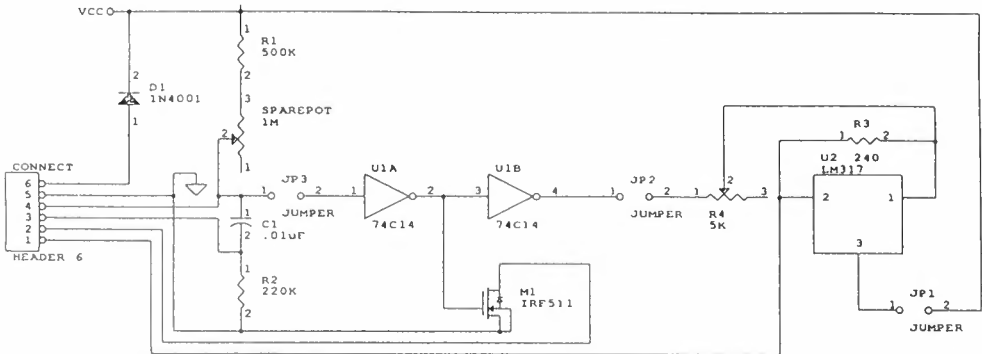


Figure 1. Basic circuit diagram for the moisture-activated variable power supply. The components used in this instrument include: U1 - 74C14N CMOS Schmitt Trigger Hex Inverter; U2 - LM317T variable voltage regulator; Q1 - IRF511 N-channel power MOSFET; D1 - 1N4001 rectifier diode; R1 - 390 K $\Omega$  resistor; R2 and R3 - 220 K $\Omega$  resistors; R4 - 5K $\Omega$  potentiometer; R5 - 500 K $\Omega$  potentiometer (SPAREPOT); and C1 - 0.01  $\mu$ F disc capacitor

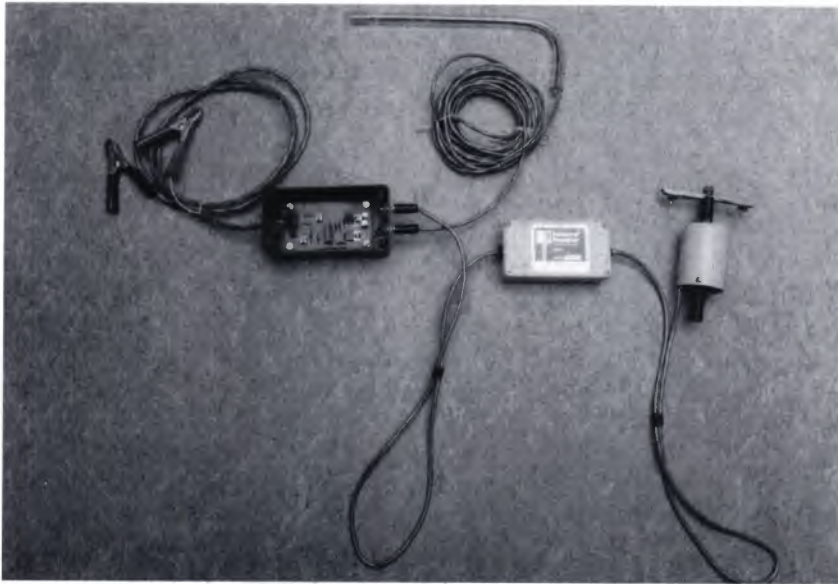


Figure 2. Electronic control unit with moisture sensor and Rotorod sampler attached. The unit has been opened to expose the printed circuit board used for easy assembly and increased reliability

The sensitivity of the moisture sensor can be altered by varying the resistance of the potentiometer, or the potentiometer can be replaced with a short circuit and R1 replaced with a fixed resistor of the value required for the desired sensitivity. If output voltage of 12 volts is desired, it may be achieved by using Vcc as the positive terminal and the source of the MOSFET as the negative (or ground) terminal. The components used in constructing this circuit were selected to give a current drain of less than 3 amperes or the maximum amperage needed to operate four Rotorod samplers. If more current is desired than the IRF511 power MOSFET should be replaced by one with a higher current rating.

The circuit requires a 12-volt dc power supply such as a 12-volt, high amperage, automobile-type battery or a ac-operated power supply with regulated 12-volt dc output. Protection from reverse supply voltage is provided by a IN4001 silicon rectifier diode (D1). A capacitor (C1) is connected across the sensor terminals to protect the CMOS inverter from spurious noise and static discharges. With no load, the circuit draws <1 microamperes during dry conditions and 4 milliamperes during wet conditions. With a model 82 Rotorod sampler connected, the unit draws approximately 130 milliamperes.

## DISCUSSION

This moisture-control power supply has been used for over 35 years in conjunction with Rotorod samplers to measure ascospore dose during entire wetting periods. It was initially used by extension personnel in western New York State (Small 1978) and later by extension

personnel in Michigan (Sutton & Jones, 1976). It continues to be a practical monitoring procedure in grower advisory programs for apple scab in Michigan.

A sensitivity control for the moisture sensor is included in the circuit to allow the user to detect different levels of wetness. Although the sensor can be adjusted to detect dew formation, lowering the sensitivity of the sensor to ignore dew but not rain is adequate for detecting periods of ascospore discharge. If the sensitivity is set too high, the Rotorod samplers operate too frequently for optimum collection of scab ascospores. Frequent operation discharges the battery and causes the sampling surface of the Rotorod sampler to collect unwanted debris.

Because a single sensitivity setting is used in this circuit, the circuit should be modified when used in electronic disease predictors for detecting leaf wetness. When used in electronic apple scab predictors, it is better to utilize a microprocessor programmed with instructions to detect specific kinds of wet periods (Jones et al. 1980).

## SUMMARY

A moisture-control power supply with an integrated circuit was developed to control Rotorod samples during wet periods. An advantage of this circuit is low power drain across the moisture sensor. The sensitivity of the moisture sensor can be adjusted to ignore fog or dew, thereby restricting sampling of ascospore of *Venturia inaequalis* to wet periods from rain when spore discharge is most likely. Although the circuit is used primarily to facilitate practical monitoring procedures for scab ascospores, there are many other possible uses for the circuit.

## REFERENCES

- Gillespie, T.J. & G.E. Kidd 1978. Sensing duration of leaf moisture using electrical impedance grids. *Canadian Journal of Plant Science* 58: 179-187.
- Jones, A.L., P.D. Fisher, R.C. Seem, J.C. Kroon & P.J. van deMotte 1984. Development and commercialization of an in-field microcomputer delivery system for weather-driven predictive models. *Plant Disease* 68: 458-463.
- Lomas, J. & Y. Shashoua 1970. The performance of three types of leaf-wetness recorders. *Agricultural Meteorology* 7: 159-166.
- Melching, J.S. 1974. A portable self-contained system for the continuous electronic recording of moisture conditions on the surface of living plants. United States Department of Agriculture ARS-NE-42. 13 pp.
- Schurer, K. & A.F. van der Wal 1972. An electronic leaf wetness recorder. *Netherlands Journal of Plant Pathology* 78: 29-32.

Small, C.G. 1978. A moisture-activated electronic instrument for use in field studies of plant diseases. *Plant Disease Reporter* 62: 1039-1042.

Smith, C.A. & J.D. Gilpatrick 1980. Geneva leaf-wetness detector. *Plant Disease* 64: 286-288.

Sutton, T.B. & A.L. Jones 1976. Evaluation of four spore traps for monitoring discharge of ascospores of *Venturia inaequalis*. *Phytopathology* 66: 453-456.



# First experiences with an improved apple scab control strategy

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Two different apple scab control strategies were compared in field trials 1991/92: The current strategy in Integrated Production (IP) and an improved strategy in an Integrated Disease Management (IDM). With the IDM strategy no protective fungicide application was made at bud-burst. In the primary season the apple scab warning system was improved by including the biological parameters "ascospore release" and "availability of susceptible tissue". A curative fungicide treatment was advised only if these parameters were fulfilled in addition to the micro-climatic parameters of temperature and leaf wetness. In the secondary season an intervention threshold was used to decide if further treatments were necessary instead of maintaining a protective fungicide coating of the trees. The IDM strategy can be successful if the following conditions are fulfilled: 1) low inoculum pressure, 2) no overwintering of the fungus in the asexual state, 3) full efficiency of the sterol biosynthesis inhibiting fungicide and 4) no excessive growth of the trees and timely cessation of shoot growth. In the field trials in 1991/92 at Strickhof site these assumptions were fulfilled and apple scab control with the IDM strategy was successful. It was possible to keep the amount of scabbed fruits below 0.5 % in both years and with both strategies even if, with the IDM strategy, only four (1991) or three (1992) fungicide treatments were applied (without final treatments). On the other hand, the IDM strategy was not successful at two other sites in 1992, because some assumptions were not fulfilled. At Wädenswil, the inoculum pressure was high (in the previous year scab incidence was over 7 % on leaves and over 10 % on fruits). At Gudo, there was an enormous gradient of the inoculum pressure inside the orchard, coming from overwintered leaves, which were all blown to the fence at one side of the orchard in the previous autumn.

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Apple scab (*Venturia inaequalis*) remains one of the most important plant diseases in Switzerland. A considerable number of specific fungicide treatments against scab are applied every year. For example, in 1991, among farmers in canton Zürich, registered as using an apple scab control strategy in Integrated Production, an average of 10.8 scab specific fungicide treatments were applied (not including those needed for apple

conservation). A reduction of the number of treatments is required both for environmental and for food safety reasons.

To avoid scab infection the most commonly used control strategy in Switzerland in *Integrated Production (IP)* is as follows. At bud-burst, trees are treated with a protective fungicide. Several applications of sterol biosynthesis inhibiting (SBI) fungicides, mixed with a protective fungicide, are then applied during the primary season (since 1992 the SBI fungicides in Switzerland were restricted to four to five applications per season). The decision to apply a SBI fungicide treatment is made only if a potential infection period according to Mills and Laplante (1951) has occurred. During the secondary season, regularly scheduled protective applications are made to maintain a fungicide coating of the fruits and the leaves. However, this strategy in *Integrated Production* often leads to unnecessary treatments. The following considerations show that with an improved strategy a further reduction in the number of fungicide treatments is possible:

- 1.) If there was little or no scab in the orchard in the previous year, then the amount of overwintering fungus in the asexual state inside buds or shoots is likely to be low. Thus at least the first treatment of the season at bud-burst, when ascospores are usually not yet ejected, would not be necessary (MacHardy et al., 1993).
- 2.) Because of modern scab control, overwintering inoculum in the sexual state is low (Gadoury and MacHardy, 1986; Gadoury et al., 1989), and ascospores are not always ejected in high and constant quantities, especially early in the season. Susceptibility of leaves decreases with age (Schwabe, 1979) and leaf growth is temperature dependent. The Mills table (Mills and Laplante, 1951) postulates that large numbers of ascospores and susceptible leaf tissue are present. An improved scab warning system in the primary season must include quantitative data on the biological parameters "ascospore release" and "availability of susceptible tissue" (Schloffer, 1990; Triloff, 1990a and 1990b).
- 3.) If scab attack is low during the primary season, disease development and dissemination in the secondary season will be so low that a critical infection level will not be reached at harvest. With the introduction of an intervention threshold (van der Scheer, 1987 and 1992) it is possible to omit superfluous fungicide treatments in the secondary season.

In this paper we present results of field trials where the current apple scab control strategy in *Integrated Production* is compared with an improved strategy in *Integrated Disease Management*, which involves the above mentioned parameters. Our objective in the present study was to show the conditions, under which a further reduction of the number of fungicide applications against apple scab is possible.

## MATERIAL AND METHODS

### **Improved apple scab control strategy**

In 1991 and 1992 field trials were carried out to compare the current apple scab control strategy in *Integrated Production (IP)* with an improved strategy in an *Integrated Disease Management (IDM)*. Following the IDM apple scab control strategy, no protective treatment is made at bud-burst. The spraying programme starts if i) a massive ascospore



release has been observed (by means of an ascospore trap that will be described later); ii) at least one and a half unfolded leaves are present; iii) the micro-climatic conditions (temperature and leaf wetness) are adequate to fulfil a potential infection period. The scab warning system in the primary season is therefore improved with the important biological parameters "ascospore ejection" and "presence of susceptible tissue". In the secondary season, an intervention threshold is used to decide if further treatments are necessary instead of maintaining a fungicide coating on the trees (van der Scheer, 1987): If scab incidence at the beginning of July is below 2 % of infected shoots (one or more scabbed leaves per shoot from examination of 200 long shoots), fungicide application can be suspended until the beginning of August. If, at the beginning of August, scab incidence is below 7 % of infected shoots, no more fungicide application is necessary until harvest (except final treatment of cultivars used for long-term storage).

### **Characterization of the orchard sites**

Trials were carried out in three different locations in Switzerland. Two were north of the Alps (at the Cantonal Agricultural School "Strickhof" at Eschikon/Lindau and at the Federal Research Station at Wädenswil) and the third was in the canton Tessin, south of the Alps (at the Frutteto Demanio Agricolo at Gudo). Data were collected in 1991 and 1992 from Strickhof, but only in 1992 from the other two sites (Tab. 1).

The orchard at Strickhof was chosen because scab incidence had been low for several years due to efficient control, whereas at Wädenswil there had been a high scab incidence in the previous year (1991). The third location at Gudo was chosen because in this part of Switzerland, there are unusual conditions, characterised by long periods of rain and long periods of drought. The average rainfall in the canton Tessin in the last 30 years was 1844 mm, with a maximum of 218 mm in May.

The apple cultivar available at Strickhof was Maigold; cultivar Golden Delicious was used at the other two sites. Both cultivars are considered susceptible to scab to a similar high level.

### **Weather monitoring and determination of infection periods**

The weather data were recorded either with a Campbell logger (Strickhof 1991 and 1992) or with a KMS-P apparatus (Wädenswil 1992 and Gudo 1992). The following weather data were monitored: temperature, leaf wetness, radiation, relative air humidity and amount of precipitation.

Apple scab infection periods were determined using temperature and leaf wetness duration data. The calculation of an infection period was based on the polynomial equations MacHardy and Gadoury described in 1989: In the primary season, the equation of the Mills/a-curve and in the secondary season that of the S. Africa/c-curve was used. If a wet period was interrupted by a dry period shorter than six hours, the calculation was resumed after the end of the dry period. If the dry period was longer than six hours, a new infection period calculation was started.

Table 1: Characterization of the orchard sites

	Strickhof 1991	Strickhof 1992	Wädenswil 1992	Gudo 1992
cultivar, root stock	Maigold, M9	Maigold, M9	Golden Delicious, M9	Golden Delicious, M9
scab susceptibility	high	high	high	high
year of planting	1986	1986	1984	1978
distances between rows and trees	4 m x 1.8 m	4 m x 1.8 m	4.5 m x 1.75 m	4 m x 1.8 m
orchard size	2 rows with 92 trees	2 rows with 92 trees	9 rows with 35 trees	14 rows with 76 trees
untreated control plots	no	10 trees	2x10 trees (C1, C2)	2x12 trees, at both sides of 3 rows (C1, C2)
scab incidence in the previous year	?	low, leaves: 1 % fruits: <0.5 %	high, leaves: 7 % fruits: > 10 %	?

### Spore deposit

Heavily infected leaves of unsprayed Golden Delicious were collected in autumn and overwintered in each orchard in wire mesh trays.

### Ascospore trap

The spore trap used was constructed by Peter Triloff. At the beginning of the season heavily infected leaves, picked up in the orchard in the previous autumn, are put on a holding plate and covered with a wire netting. Eight microscope slides are held 3 mm above these leaves. After rain, the ejected ascospores are trapped passively on the microscope slides. The slides were changed after each rain period. Four of the slides (those that had the most ascospores at the first noticeable ejection of the season) were prepared for examination under the microscope using four droplets of a 10:1 (v/v) mixture of lactic acid and cotton blue. The trapped ascospores were counted under the microscope by making five complete scans at a magnification of 100x (Wädenswil and Gudo) and 150x (Strickhof) along the long axis of the four slides.

### Foliage growth assessment

The foliage growth was measured every three to four days by marking the youngest completely unfolded leaf on at least 20 newly grown terminal shoots and counting the number of new grown leaves since the last marking.

### **Indicator trees**

Golden Delicious trees, grafted on M29 rootstocks, were grown in 200 mm pots in the greenhouse. For every rain period, four indicator trees were placed in the orchard, and returned afterwards to the greenhouse for incubation. Overwintered leaves with scab were placed around the indicator trees in the field to ensure an infection even in an orchard with low inoculum density. A string tied to the youngest unfolded leaf on every tree marked the susceptible leaves at the time of the rain period. Scab development was assessed weekly.

### **Fungicide application**

Fungicides were applied in concentrations according to the "Pflanzenschutzempfehlungen für den Erwerbsobstbau", using an air-blast sprayer and usually 400 l/ha (Tab. 2).

### **Scab assessment**

Scab incidence in the field was determined every three to four weeks. At Strickhof in 1991, one shoot per tree was examined and the total number and the number of scabbed leaves was counted (90 trees per plot). At Strickhof in 1992, 40 trees per plot, at Wädenswil 16 trees per plot and at Gudo 50 trees per plot were examined, together with 10 (Strickhof, Wädenswil) or 12 trees (Gudo) in the untreated control plots. On every tree, 4 terminal shoots, exposed to different directions and at different heights, were marked early in the season. For each scab assessment these marked shoots were examined and the percentage of scabbed leaves was determined. At harvest, the percentage of scabbed apples in kilograms of the total production of 90 trees (Strickhof), or 20 trees (Wädenswil), was calculated. At Gudo, the percentage of the numbers of scabbed fruits of 50 trees (examination of 20-40 fruits per tree) was determined on the 8th of September.

## **RESULTS AND DISCUSSION**

At Strickhof, the IDM scab control strategy was successful for two years. In 1991, eight fungicide treatments were made with the IP strategy, and only four with the IDM strategy; in 1992, nine treatments with the IP strategy were reduced to only three with the IDM strategy (Tab. 2). Nevertheless, there was no significant difference in scab incidence at harvest (Fig. 3). The fruit scab incidence in fact was very low with both control strategies and in both years: In 1991, in the IP plot: 0.13 %, in the IDM plot 0.38 %; in 1992, in the IP plot 0.15 % and in the IDM plot 0.3 % scabbed fruits at harvest (calculated as the percentage in kilograms of the total fruit production).

With the improved apple scab warning system of the IDM strategy, it was possible in 1992 to avoid the first and in 1991 the first two fungicide applications in the primary season, because no or only sparse ascospore ejection was observed and/or only little susceptible leaf tissue was available during potential infection periods. In the secondary season, in both years, the intervention threshold was never exceeded, therefore no more fungicide treatments (except the final treatments) were made (Tab. 2).

In 1992/1993 it was possible to check the validity of the IDM strategy not only in preventing scab infections during fruit maturation, but also for fruit conservation. Of all the apples that were refrigerated, 0.02 % (calculated as the percentage in kilograms) originating

from the IDM plot came out with an additional apple scab lesion. No scab was found on the apples originating from the IP plot (Fig. 3).

At Wädenswil, a higher level of scabbed apples was recorded at harvest (4.1 and 7.5 % following IDM and IP strategies respectively). In these trials, a modification was introduced: an additional copper treatment was applied at bud-burst. Comparative experiments were carried out with or without this additional application. There was no significant difference (Wilcoxon's U-test, Mann and Whitney:  $P = 0.05$ ) in the scab incidence at harvest between the IP plot without the copper treatment at bud-burst (6.6 %) and both IDM plots, with and without the copper treatment (9.8 % and 7.5 % respectively).

Table 2. Dates of fungicide applications

1)	Strickhof 1991		Strickhof 1992		Wädenswil 1992			
	IP	IDM	IP	IDM	IP (+Cu)	IP (-Cu)	IDM (+Cu)	IDM (-Cu)
1.	30.03.91 R O <sup>2)</sup>	01.05.91 R, O <sup>3)</sup>	11.04.92 D	24.04.92 R	26.03.92 Cu	08.04.92 R	26.03.92 Cu	21.04.92 R
2.	24.04.91 R, O	03.05.91 R, O <sup>1)</sup>	24.04.92 R	05.05.92 R	08.04.92 R	21.04.92 R	21.04.92 R	30.04.92 R
3.	06.05.91 R, O	13.05.91 R, O	05.05.92 R	22.05.92 R	21.04.92 R	30.04.92 R	30.04.92 R	27.05.92 R
4.	13.05.91 R, O	29.05.91 R, O	22.05.92 R		30.04.92 R	27.05.92 R	27.05.92 R	12.06.92 R
5.	29.05.91 R, O	10.06.91 R, O	02.06.92 R		27.05.92 R	12.06.92 R	12.06.92 R	30.06.92 Pc
6.	10.06.91 R, O		13.06.92 D, Pt		12.06.92 R	30.06.92 Pc	30.06.92 Pc	15.07.92 Pc
7.	21.06.91 R, O		27.06.92 D, Pt		30.06.92 Pc	15.07.92 Pc	15.07.92 Pc	
8.	16.07.91 R, O		13.07.92 Pc		15.07.92 Pc			
9.			31.07.92 Pc					
1.	08.08.91 P	08.08.91 P	18.08.92 O	18.08.92 O	03.08.92 O	03.08.92 O	03.08.92 O	03.08.92 O
2.	22.08.91 P	22.08.91 P	10.09.92 O	10.09.92 O	25.08.92 O	25.08.92 O	25.08.92 O	25.08.92 O
3.	16.09.91 P	16.09.91 P			07.09.92 O	07.09.92 O	07.09.92 O	07.09.92 O

1): Number of treatments and final treatments

2): Cu: copper (copper-oxichloride 50 %, 1.6-3.2 kg/ha), D: Delan (dithianon 75 %, 0.8 kg/ha), O: Orthozid 83 (captan 83 %, 2.4 kg/ha), P: Phaltozid (folpet 80 %, 1.6-2 kg/ha), Pt: Pallitop (nitrothal-isopropyl 48 % + metiram 3.2 %, 1-1.2 kg/ha), Pc: Pallicap (captan 55.3 % + nitrothalisopropyl 16.7 %, 3.2-4 kg/ha), R: Rondo (60 % + pyrifenoxy 5 %, 1.6 kg/ha). (Fungicide concentrations according to the "Pflanzenschutzempfehlungen für den Erwerbsobstbau", usually 400 l/ha.)

3): During the treatment it started to rain. The fungicide application was repeated on 03.05.91.

There was a significant difference when the copper treatment at bud-burst was used with the IP strategy, where it was possible to reduce scab infection at harvest to 4.1 %. In the untreated control plots, we observed a scab incidence at harvest of 93.8 % if overwintered leaves were present (control plot 2 with the spore deposit). If such leaves were not present (control plot 1), the percentage was reduced to 64.4 % (Fig. 3).

The analysis of the weather conditions between the copper treatment on March 26 and the first curative fungicide treatment with Rondo on April 8 indicates that a potential infection period had occurred only between the 4th and the 6th of April. Therefore, only the effect of two applications together could prevent a scab infection in the period of the 4th to the 6th of April. The significantly different results obtained with the IP strategy with or without the copper treatment can be explained only if the application of Rondo on the 8th of April (three days after the potential infection period was fulfilled) was not fully effective, perhaps because there were populations of the apple scab fungus with a decreased sensitivity to Rondo in this particular orchard.

Comparing the IDM and the IP plots, we can see that an infection did take place in the period 4th to the 6th of April. The IDM plots were not treated, because the observed ascospore ejection was very low (0.8 % of the total seasonal ascospore ejection) and also because there was only little susceptible leaf tissue present at this time (phenological stage C3). Since a significant infection did actually take place, either the ascospore ejection was underestimated (with the spore trap used, the ascospore ejection in an orchard cannot be quantified, but the approximate development can be determined) or the infection resulted not from ascospores but from conidia which overwintered in buds or which came from superficial scab (see also Becker et al. 1992 and Moosherr 1990). The last hypothesis is the most probable also because no symptoms were recorded on the indicator trees, which were exposed in the orchard during the infection period 4th to the 6th of April, even though they were surrounded by leaves with abundant pseudothecia. Moreover, the observation that, during the previous year, there was a heavy scab infection (about 7 % leaf scab on 16.08.1991 and over 10 % fruit scab on 01.10.1991) seems to confirm this hypothesis. The trials at Wädenswil show that the IDM strategy cannot be applied successfully, when there is the risk, that scab can overwinter in the asexual state and that the SBI fungicide is not fully effective.

At Gudo, the interpretation of the results is difficult because the ascospore catches in the spore trap were too low, due to an incorrect leaf collection in the preceding autumn.

The results show that there was an enormous gradient in the inoculum pressure inside the orchard (Fig. 4). In the untreated control plot 1, which was located at one end of three rows, beside the IDM plot, fruit scab incidence on 08.09.1992 was 77.1 %. In the other untreated control plot 2, located at the other end of the three rows, beside the IP plot, fruit scab incidence was only 20.4 %. An explanation for this phenomenon could be found in the following spring. Cold dry winds, blowing in the winter usually from the Alps down the valley, led to the formation of leaf heaps on the fence at the side of the orchard, where the control plot 2 and the IDM-plot were located. Only a few leaves remained on the mountain side of the fence. Therefore this leaf deposit could explain the large difference that we found between the two control plots. For the same reason, we have to bear in mind that the trees in the IDM plot, located close to the most heavily infected control plot, were under a higher infection pressure than the trees in the IP plot.

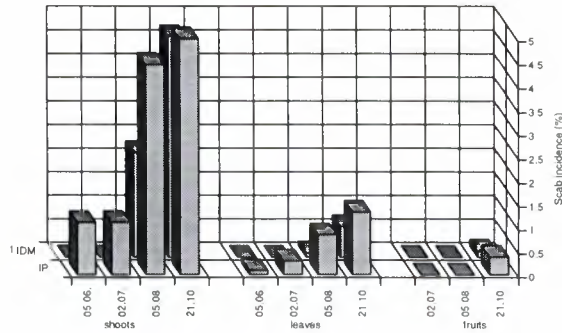


Fig. 1. Scab incidence at Strickhof 1991

IP: Current apple scab control strategy in Integrated Production (Eight fungicide treatments, without final treatments)

IDM: Apple scab control strategy in Integrated Disease Management. In the primary season fungicide applications were made after the following criteria: fulfilled infection period according to MacHardy and Gadoury (1989), ascospores ejected and susceptible tissue available. In the secondary season fungicide applications were made only when the intervention threshold was exceeded (beginning of July > 2 %, beginning of August > 7 % of infected shoots). (Four fungicide treatments, without final treatments)

Six replicates with 15 trees were made in each plot

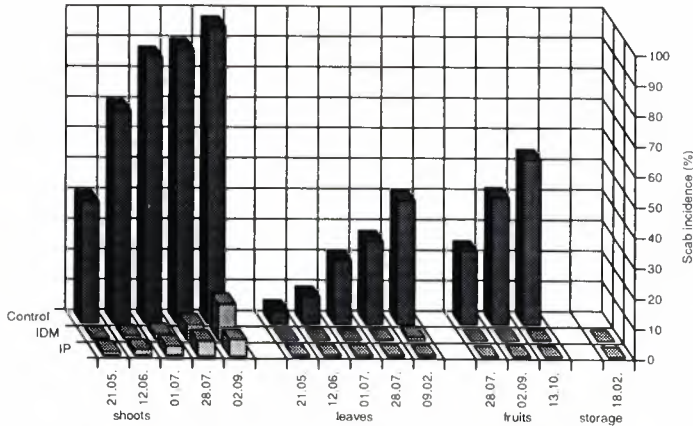


Fig. 2. Scab incidence at Strickhof 1992

IP: Current apple scab control strategy in Integrated Production (Nine fungicide treatments, without final treatments)

IDM: Apple scab control strategy in Integrated Disease Management. In the primary season fungicide applications were made after the following criteria: fulfilled infection period according to MacHardy and Gadoury (1989), ascospores ejected and susceptible tissue available. In the secondary season fungicide applications were made only when the intervention threshold was exceeded (beginning of July > 2 %, beginning of August > 7 % of infected shoots). (Three fungicide treatments, without final treatments)

Control: Untreated control plot

Six replicates with 15 trees were made in the IP and IDM plot. Ten trees remained untreated as a control for disease development

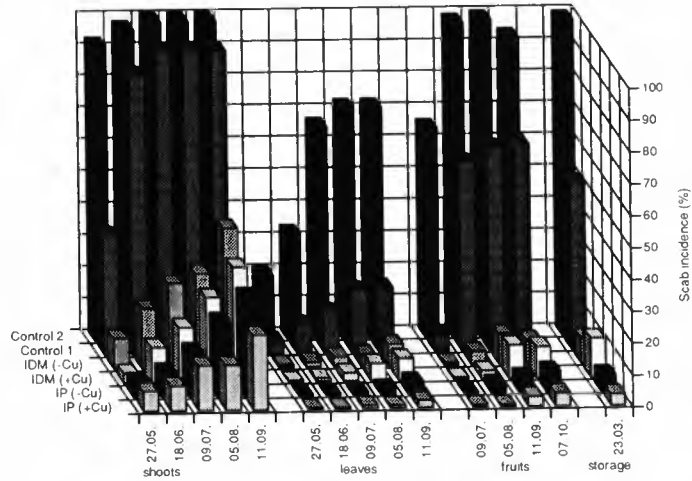


Fig. 3. Scab incidence at Wädenswil 1992

**IP (+Cu):** Current apple scab control strategy in Integrated Production (Eight fungicide treatments, without final treatments)

**IP (-Cu):** Current apple scab control strategy in Integrated Production without a protective copper treatment at bud-burst (Seven fungicide treatments, without final treatments)

**IDM (+Cu):** Apple scab control strategy in Integrated Disease Management, including a protective copper treatment at bud-burst. (Seven fungicide treatments, without final treatments)

**IDM (-Cu):** Apple scab control strategy in Integrated Disease Management. In the primary season fungicide applications were made after the following criteria: fulfilled infection period according to MacHardy and Gadoury (1989), ascospores ejected and susceptible tissue available. In the secondary season fungicide applications were made only when the intervention threshold was exceeded (beginning of July > 2 %, beginning of August > 7 % of infected shoots). (Six fungicide treatments, without final treatments)

**Control 1:** Untreated control plot

**Control 2:** Untreated control plot, including a deposit of heavily scabbed overwintered leaves.

Four replicates with five trees were made in the IP and the IDM plots. Ten trees remained untreated in each of the two control plots

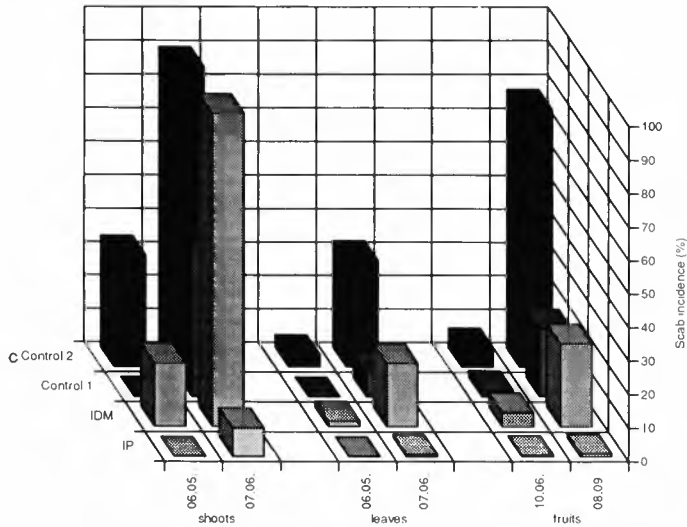


Fig. 4. Scab incidence at Gudo 1992

IP: Current apple scab control strategy in Integrated Production

IDM: Apple scab control strategy in Integrated Disease Management. In the primary season fungicide applications were made after the following criteria: fulfilled infection period according to MacHardy and Gadoury (1989), ascospores ejected and susceptible tissue available. In the secondary season fungicide applications were made only when the intervention threshold was exceeded (beginning of July > 2 %, beginning of August > 7 % of infected shoots)

Control 1: Untreated control plot, located at the end of the three rows, besides the IP plot

Control 2: Untreated control plot, located at the end of three rows, besides the IDM plot

Ten replicates with 21 trees were made in the IP and IDM plots. Twelve trees remained untreated in each of the two control plots

## CONCLUSIONS

In general, the apple scab control strategy in Integrated Production is not intended for an ecological production aimed to reduce fungicide applications to a minimum. It is designed, in effect, as a "worst" case strategy and is routinely adopted. The apple scab control strategy in Integrated Disease Management on the other hand attempts to minimise fungicide application but is most successful when applied in the "best" cases, leading to a consistent reduction of the number of fungicide applications needed.

In 1991, among 75 orchards in canton Zürich registered as IP orchards, 35 (46 %) had a fruit scab incidence above 1 % and 19 (25 %) had a scab incidence above 5 %. Apple scab was either not noticed before July or August or was not noticed at all in 62 % of the IP orchards (C. Gessler, personal communication). These data suggest that an improved apple scab control strategy could be successfully applied in more than half of the IP orchards.



As already mentioned, there are at least four major features to which one should pay attention in order to successfully apply the IDM strategy: 1) there must be no overwintering of the fungus in the asexual state; 2) the ascospore potential must be low; 3) the SBI fungicides employed must be completely effective and 4) the foliage growth must be regular and shoot growth should stop in time and not start again in the autumn. The IDM strategy fails when one of the prerequisites is not fulfilled. It is necessary to know the history of the orchard and also the scab susceptibility of the planted trees.

During the primary season the IDM strategy requires a careful observation of ascospore ejection and of foliage growth (the latter is less important in the warmer areas where a continuous growth is certain) to determine whether the conditions for fungus development are favourable. This allows the avoidance of one or even two fungicide applications in the early season. Later, during the secondary season, only two surveys are needed to determine whether the intervention threshold is reached or not. Following these criteria it is possible to considerably reduce the number of fungicide sprays without increasing the scab incidence both at harvest and during fruit conservation.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- Becker, C.M., T.J. Burr & C.A. Smith 1992. Overwintering of conidia of *Venturia inaequalis* in apple buds in New York orchards. *Plant Disease* 76: 121-126.
- Gadoury, D.M. & W.E. MacHardy 1986. Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. *Phytopathology* 76: 112-118.
- Gadoury, D.M., W.E. MacHardy & D.A. Rosenberger 1989. Integration of pesticide application schedules for disease and insect control in apple orchards of the northeastern United States. *Plant Disease* 73: 98-105.
- MacHardy, W.E. & D.M. Gadoury 1989. A revision of Mills's criteria for predicting apple scab infection periods. *Phytopathology* 79: 304-310.

MacHardy, W.E., D.M. Gadoury & D.A. Rosenberger 1993. Delaying the onset of fungicide programs for control of apple scab in orchards with low potential ascospore dose of *Venturia inaequalis*. *Plant Disease* 77:372-375.

Mills, W.D. & A.A. Laplante 1951. Diseases and insects in the orchard. *Cornell Extension Bulletin* 711: 21-27.

Moosherr, W. 1990. Untersuchungen zu superfiziellem Schorf und Knospenschorf (*Venturia inaequalis* (Cke.) Wint.) beim Apfel. Thesis, University of Hohenheim, Germany.

van der Scheer, H.A.Th. 1987. Supervised control of scab and powdery mildew on apple. *Obstbau Weinbau* 9: 249-251.

van der Scheer, H.A.Th. 1992. Management of scab and powdery mildew on apple with emphasis on threshold values for control of both diseases. *Acta Phytopathologia et Entomologica Hungarica* 27: 621-630.

Schloffer K. 1990. So kann IP-gerechte Schorfbekämpfung aussehen. *Besseres Obst* 8: 4-5.

Schwabe, W.F.S. 1979. Changes in scab susceptibility of apple leaves as influenced by age. *Phytophylactica* 11: 53-56.

Triloff, P. 1990a. Tage und Millimeter? Schorfbekämpfung heute und morgen (Teil 1). *Besseres Obst* 8: 8-10.

Triloff, P. 1990b. Tage und Millimeter? Schorfbekämpfung heute und morgen (Teil 2). *Besseres Obst* 9: 4-7.

# Control of apple scab according to warning equipment

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The practicability of controlling scab by means of a warning system has been investigated on a large scale. All apple and pear plantings of The Department of Pomology were treated according to scab infection periods in 1992. Ascospore release was recored by a spore trap. Control of scab was generally good, but scattered lesions on spur leaves were found. Spread of disease was prevented by continued treatments. Omission of treatments in August-September allowed infections on leaves and fruits and the development of storage scab in some cultivars. The failure to prevent primary infection may have been due to inadequate fungicide coverage in late April-early May, when most ascospores were released.

Key words: Apple, ascospore release, climate, scab, storage diseases, warning equipment,.

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In Integrated Fruit Production (IFP), guidelines demand that the risk of damage by scab must be estimated before any treatments are applied. In the Department of Pomology scab control in 15 apple and pear orchards has been attempted by means of a scab warning system since 1991, in order to evaluate its usefulness in practical fruit growing. Data on weather, ascospore discharge, infection periods, fungicide sprays and scab infections on fruits for 1992 is presented and discussed.

## MATERIALS AND METHODS

Infected leaves from unsprayed plots were collected autumn 1991 and over-wintered in an orchard in a wire-mesh tray. The leaves were placed in a spore trap in March 1992; the construction and operation of the trap were described by Hesjedal (1991). Ascospores were counted after rain only, but counting were sometimes delayed a few days after rain. The scab warning equipment KMS-P (A. Paar, Austria) recorded rainfall per hour and hourly averages of air temperature, relative humidity and leaf wetness and calculated infection periods according to Mills & Laplante (1951). Degree-day accumulation (base = 0°C) was

started on the day of the first ascospore release.

Fungicides were applied with a mist blower, using approximately 150 litres of spray solution per ha (10 x concentrated). Fungicides and dose rates used were: Baycor 25 WP (bitertanol 25%) 0.8 kg/ha; Cadol (dithianon 25%) 4.5 l/ha; Captan (captan 83%) 2.3 kg/ha; Rubigan (fenarimol 12%) 0.4 l/ha; Euparen M (tolyfluanid 50%) 1.5 kg/ha. (The low rate of Euparen M was used in order to spare predatory mites).

Flowering occurred between May 15 and May 30. Ripening was early, 'Cox's Orange' being picked around September 15. Fruit samples for examination of scab were collected from trees 5-10-years-old of nine cultivars grown as spindles (rootstocks M9 and M26). Counts of scab and other fungal diseases on fruits were made after cold storage at 3°C (6 samples of 50 fruits per cultivar/treatment).

## RESULTS

The main release of ascospores in 1992 took place from early April until mid May, i.e. between green tip and beginning of flowering, figure 1. Very few spores were found after a shower in early June. When rain resumed in early July after a long dry period, leaves in the spore trap had completely broken down.

Daily average temperature and rainfall for April, May and June are shown in figure 2. Weather was cold in mid April with night frosts, but May and June were unusually warm and dry.

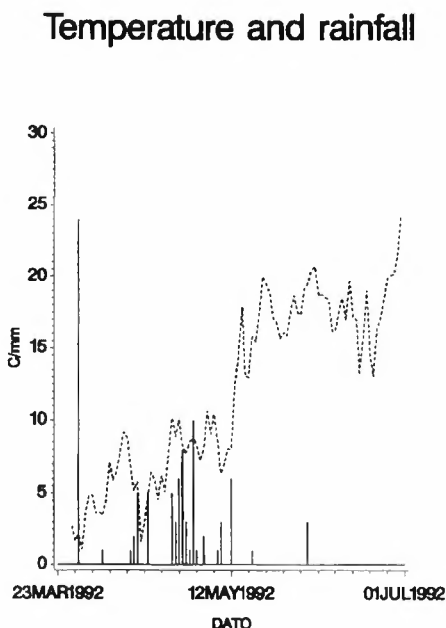
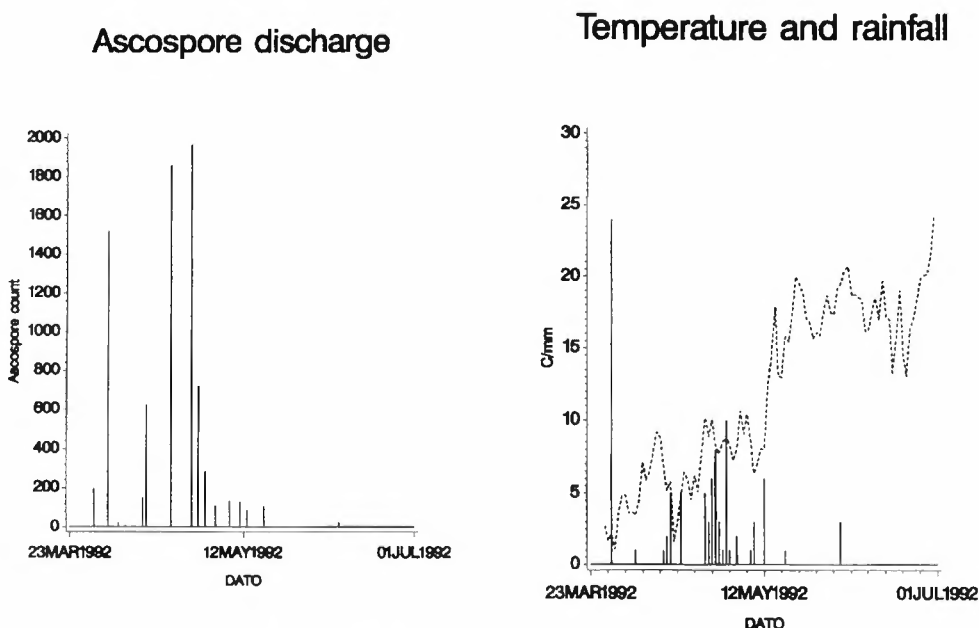


Fig. 1. Release of ascospores in spore trap after rainfall 1992

Fig. 2. Daily average temperature (°C) and daily rainfall (mm) during the primary season 1992

Accumulated ascospore discharge plotted together with accumulated degree days show that most spores were released well before the number of degree days reached 500 (Gadoury & MacHardy 1982), figure 3. The pattern of ascospore discharge agrees well with accumulated rainfall, figure 4.

### Ascospores and temperature

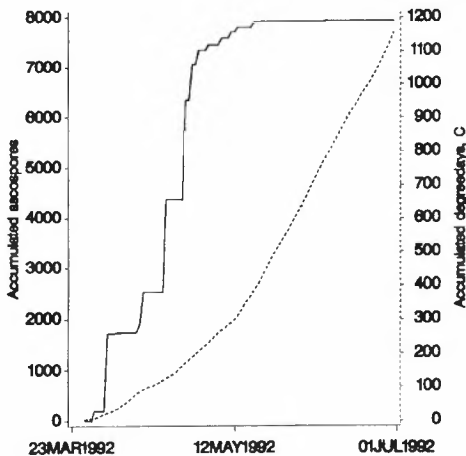


Fig. 3. Accumulated ascospore discharge and accumulated degree-days (base 0°C). Solid line: ascospores. Dotted line: degree-days

### Ascospores and rainfall

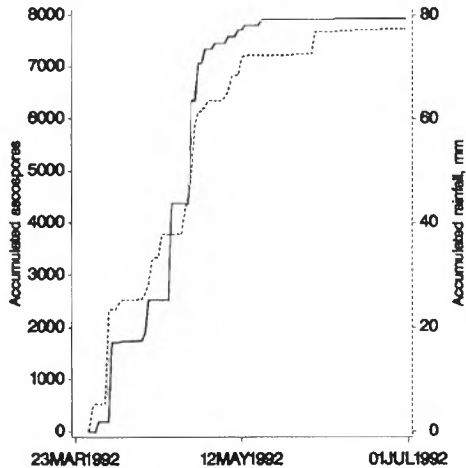


Fig. 4. Accumulated ascospore discharge and accumulated rainfall. Solid line: ascospores. Dotted line: rainfall

The first major discharge of ascospores occurred on April 2, but infection conditions were not fulfilled and no sprays were applied. Large releases of ascospores were associated with rain in the middle and last part of April and beginning of May. Several infection periods occurred and sprays were applied on April 17 and 27 and May 8 and 13 1992, table 1.

Around May 18, a few newly-infected spur leaves were found in some plantings and new infections occurred sporadically later on. So, in order to prevent infection of fruits and new leaves curative sprays were applied according to infection periods until late July, table 1. During the secondary season, weather was warm and rainy with many infection periods, especially in the second half of August (figure 5).

Three captan sprays were applied to protect against storage rots, table 1. Fruit samples of a number of cultivars were examined for fungal attack after storage, table 2. 'Mutsu', 'Elstar' and 'Jonagold' had some fruits with scab, particularly storage scab. The orchard with 'Mutsu' has a "history" of scab infections, probably due to a nearby infection source. The fact that fruits were not protected during the last 10 days of August table 1, may explain the damage due to scab in the cultivars mentioned. Attacks of other diseases were minimal except for *Gloeosporium* on 'Aroma', a cultivar very susceptible to this pathogen (Bergendal 1978).

Table 1. Infection periods and fungicide sprays 1992. (A protective captan spray was applied at bud-break, March 19)

Date of infection period	Date of spray	Fungicide
April 16	April 17	Captan
April 25,26,28, May 1	April 27	Cadol
May 7	May 8	Captan
May 13	May 13	Captan
June 4	June 4	Baycor
July 2,5	July 2	Rubigan
July 12,14,17	July 13	Rubigan
July 22	July 20	Rubigan
July 27	July 28	Euparen M
August 2,12,14,17,18	August 11	Captan
August 21,23,25,26,27,29		
August 30	August 31	Captan
Jonagold, Mutsu and Gloster:	September 11	Captan

### Temperature and rainfall

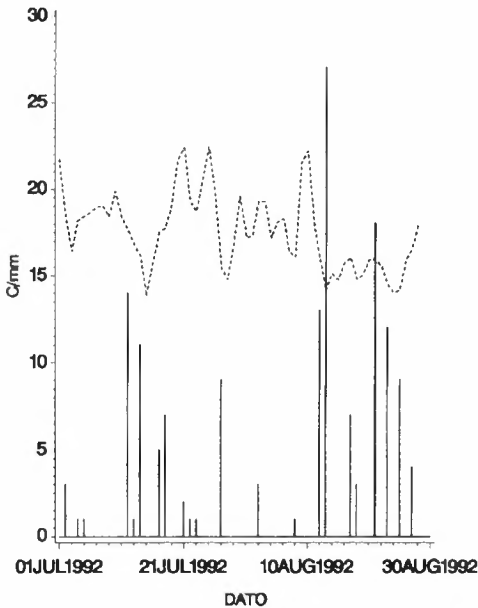


Fig. 5. Daily average temperature and daily rainfall during the secondary scab season 1992

In a separate trial no fungicide treatments were applied in August and September. In one orchard this led to only minor attacks of *Gloeosporium* on 'Elstar' and 'Jonagold', while in another orchard omission of treatments resulted in severe attack of scab on 'Jonagold' and 'Mutsu', table 3. In this field two treatments with either captan or tolylfluanid were clearly not sufficient to prevent storage losses. In the untreated plot scab infections on shoot leaves were frequent.

### DISCUSSION

The weather in the 1992-season was characterized by a very mild winter followed by a very warm summer with a long dry period in May and June. Most ascospores were released already when degree-day accumulation had reached about 300, i.e. within the variation of maturity prediction by Gadoury & MacHardy (1982). All ascospores were released with 400 degree-days. In a similar experiment

in 1991 all ascospores were released when degree-day accumulation reached 550 (Grauslund 1992). The failure to protect leaves from primary infection may have been due to inadequate fungicide coverage in late April - beginning of May. A spray interval of 10 days was thought to be justified since temperatures were low with little leaf growth. But ascospore discharge was high in this period. Spread of infection to shoot leaves and fruits was prevented by subsequent treatments, but omission of treatments in August-September allowed infections to proceed at least in some orchards.

Table 2. Counts of scabbed fruits and fruits with fungal diseases after storage

Cultivar	% fruits with				Time of count 1993
	Scab	Storage scab	Gloeosp. rot	Other rots	
Cox's Orange	0	1	0	0	January
Ingrid Marie	0	1	2	2	January
Aroma	0	0	9	0	January
Spartan	0	0	0	0	February
Gloster	0	0	0	0	March
Jonagold	1	4	0	2	March
Elstar	1	7	3	0	March
Mutsu	7	10	1	1	March
Golden Delicious	0	0	0	0	March

Table 3. The effect of two treatments, on August 12 and September 1, with Captan or Euparen M on the incidence of fungal diseases after storage

Cultivar and treatment	% fruits with			
	Scab	Storage scab	Gloeosp. rot	Other rots
Field 1 Elstar				
No treatment	0	1	3	0
Captan	0	0	1	0
Euparen M	0	0	0	0
Field 1 Jonagold				
No treatment	0	1	5	0
Captan	0	0	3	0
Euparen M	0	0	0	1
Field 2 Jonagold				
No treatment	5	14	1	1
Captan	3	3	0	1
Euparen M	3	3	0	1
Field 2 Mutusu				
No treatment	14	18	0	1
Captan	5	5	0	0
Euparen M	9	7	0	0

However, in 12 out of 15 orchards control of scab was good, but storage scab was a problem in some cultivars in three orchards.

Using a scab warning system in practical fruit growing will meet with difficulties when there are many infection periods and prolonged rainy weather, which make timely applied treatments difficult or impossible. This was particularly true in 1991 (Grauslund 1992). Poor control of scab in the orchard may increase storage scab dramatically. Control of *Gloeosporium* and other storage diseases was generally good.

#### REFERENCES

Bergendal, P.O. 1978. Beskrivningar av Frukt- och Bärsorter. Balsgård, Verksamhetsberättelse 1976-77: 35-41.

Gadoury, D.M. & W.E. MacHardy 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. *Phytopathology* 72: 901-904.

Grauslund, J. 1992. Bekæmpelse af svampesygdomme 1991. *Frukt og Bær* 21: 184-186.

Hesjedal, K. 1991. Bruksrettleiing for Triløff-ascosporefeller. Personal communication.

Mills, W.D. & A.A. Laplante 1951. Diseases and insects in the orchard. Cornell Extension Bulletin 711, rev. 1951.



# Ventem™ - a computerised apple scab warning system for use on farms

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Butt, D.J. & X.-M. Xu 1994. Ventem™ - a computerised apple scab warning system for use on farms. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 247-251. ISSN 0802-1600.

A new PC-based apple scab (*Venturia inaequalis*) warning system called Ventem™, developed at Horticulture Research International, East Malling, was commercially released as version 3.1 in January 1992. Weather data are recorded using a Metos<sup>R</sup> weather station. Ventem™ gives two levels of information: alerts to infection periods and forecasts of scab intensity. Firstly, a dynamic model driven by weather variables simulates infection by both ascospores and conidia and calculates the Infection Efficiency (IE) of inoculum; IE values (0-100%) alert growers to the extent that weather conditions favour infection. Ventem™ next forecasts scab intensity specific to individual orchards and cultivars by taking into account cultivar susceptibility and quantity of inoculum. In orchard tests over 4 years, Ventem™ gave alerts to actual infection periods in spring which were not detected by a system using Mills' criteria.

Key words: Apple scab, disease forecasting, infection warning, model, *Venturia inaequalis*.

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Several dedicated electronic instruments introduced in recent years register warnings of apple scab (*Venturia inaequalis*) infection periods on the basis of Mills' criteria (Mills & La Plante, 1954). These criteria indicate for various temperatures the minimum length of time apple leaves must remain wet for ascospores to infect at three levels of risk: light, moderate and severe. Understanding of the biology and epidemiology of this disease has advanced considerably in recent years, and the review by MacHardy & Gadoury (1989) led to their revision of Mills' criteria for infection.

Most Mills-based scab warning systems only indicate the favourability of weather for infection, ignoring the facts that apple cultivars differ in susceptibility to scab and blocks (orchards) on the same fruit farm may differ in levels of both primary and secondary inoculum. Ventem™ (Venturia East Malling), a new PC-based apple scab warning system, takes into account the weather, host and pathogen in providing infection alerts and scab intensity forecasts that help growers make prudent control decisions at the level of

individual block and cultivar. Ventem<sup>TM</sup> version 3.1 was commercially released in January 1992 (Butt et al. 1992) and is discussed in this paper.

In developing Ventem<sup>TM</sup>, many growers and advisors were consulted. As a result, Ventem<sup>TM</sup> is designed to be user-friendly and practical (Lovelidge 1992). Guideline documents and help facilities are in Ventem<sup>TM</sup> and the User's Manual.

## HOW VENTEM<sup>TM</sup> WORKS

Ventem<sup>TM</sup> runs on IBM<sup>R</sup> PCs and Personal Computer AT<sup>R</sup>, PC/XT<sup>TM</sup> and 100% IBM-compatible computers.

The infection model in Ventem<sup>TM</sup> is fundamentally different from Mills' criteria for infection and the revised version (MacHardy & Gadoury 1989). It is driven by temperature, relative humidity, rainfall and surface wetness. When a certain threshold amount of rain is recorded, the infection model in Ventem<sup>TM</sup> releases viable conidia and discharges viable ascospores and lands them instantaneously on wet young leaves of a susceptible apple cultivar. The infection process begins immediately spores land, and is simulated dynamically and separately for ascospores and conidia. Spore mortality takes place when periods of surface dryness interrupt the infection process. The infection model calculates the Infection Efficiency (IE) of inoculum; this is the percentage of landed spores that has established a parasitic relationship with the host; the final IE in each infection period quantifies the favourability of the weather for infection and alerts users to infection periods.

Ventem<sup>TM</sup> converts every IE value into a scab intensity by taking into account cultivar susceptibility and quantity of inoculum (ascospores and/or conidia). The scab intensity, expressed relative to a given tolerance level, is specific to individual orchards and/or cultivars chosen by the user.

## SPECIAL FEATURES

Ventem<sup>TM</sup> has several important features:

- (1) The infection model is dynamic and simulates several sub-stages from spore deposition to the establishment of the parasitic relationship.
- (2) The rates of infection and mortality depend on weather factors; the rate of spore mortality depends also on the infection sub-stage.
- (3) Concurrent infection by ascospores and conidia is possible; the type of inoculum is regulated by the user.
- (4) Ascospores and conidia infect at different rates.
- (5) The perithecial discharge mechanism is sensitive to light.
- (6) Scab intensity is predicted, takes into account cultivar susceptibility and inoculum level and is specific to individual blocks and cultivars.

VALIDATION OF THE LEAF INFECTION MODEL

In orchard tests over four years, Ventem™ correctly identified infection periods early in the growing season which were not detected by a Mills-based UK warning system driven by the same weather data. In 1989 (Fig. 1), for example, a major infection period (IE c. 80% for both ascospores and conidia), detected by Ventem™ on 5th April, accounted for the earliest occurrence (in May) of scab colonies. A "Ventem" period on 5th June accounted for the outbreak of lesions in late June; the first Mills' Period was not registered until early July! Reliable warnings early in the growing season are essential for the successful management of scab epidemics.

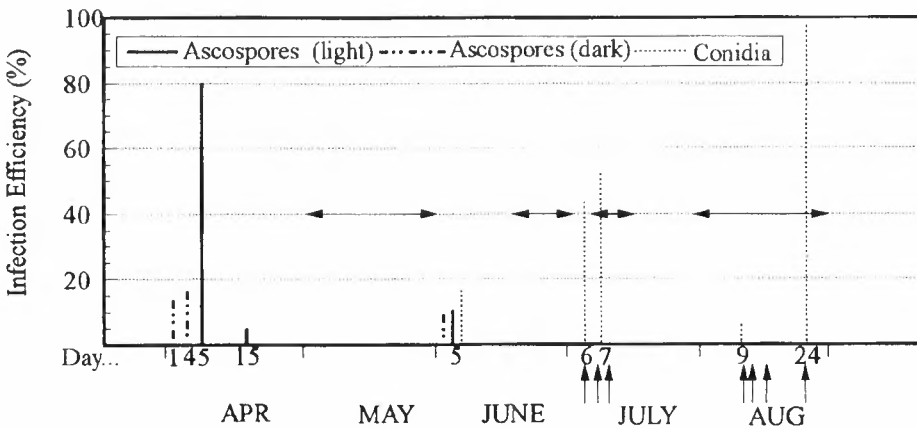


Fig. 1 Ventem's infection periods (vertical bars) and Mills' Periods (vertical arrows) in 1989. Light/dark: ascospores discharged during daytime and night time, respectively. Horizontal arrows: generations of new scab colonies

WEATHER MONITORING

Ventem™ version 3.1 only runs with weather data recorded automatically at 12-minute intervals on Metos<sup>R</sup> instruments manufactured by Gottfried Pessl, Weiz, Austria. Transfer of data from the farm-based weather station to the PC can be by direct connection or by memory (RAM) card, portable computer or other means. Ventem™ contains a facility for handling weather data.

RUNNING VENTEM™

There are three operational steps:

## 250 *An apple scab warning system*

- (1) Transfer data from the weather station to the PC.
- (2) Append (using Ventem™) the transferred weather data to a databank file specific to the weather station.
- (3) Run the scab model to obtain infection alerts and scab intensity forecasts.

### OUTPUT

A graph of IE values alerts users to infection periods on leaves. Tables of scab intensity forecasts and warnings (Table 1), for blocks and cultivars named by the user, prompt questions about each infection period.

Table 1. Example table of relative scab intensity produced by Ventem™  
Cultivar: Granny Smith (High Susceptibility); Orchard: North

Time when spore landed	Time elapsed since earliest infection	Relative scab intensity (T.L. = 1) <sup>a</sup>	Warning <sup>b</sup>
8-Jun 21:00 hrs	1 days 11 hrs	3.66	*
7-Jun 09:00 hrs	2 days 23 hrs	18.72	**

a): T.L. = Tolerance Level

b) Warning is shown as \*, or \*\*, or \*\*\* according to the scab risk level

- (1) Is the relative scab intensity above or below my action threshold?
- (2) If the scab risk is unacceptable, do I expect deposits from my last fungicide spray to have protected this block or cultivar at the time inoculum landed?
- (3) If not, is there time to apply a curative treatment?
- (4) What is the order of priority for applying curative fungicides to the cultivars or blocks at risk?

Ventem™ does not give advice on the choice of fungicide because of large regional and national differences in approved scab control recommendations.

### UPDATES

Ventem™ version 3.1 is being updated to version 4.0 for release in 1994. The new version will give infection alerts and scab forecasts for both leaves and fruits. The new fruit scab model is based on data supplied by Dr. W.F.S. Schwabe. A spray diary will be included for recording the usage of all agrochemicals; Ventem™ will use this diary to identify infection periods when trees were not protected by a previous scab spray. The new version of Ventem™ will control communication between the Metos<sup>R</sup> automatic weather station and the PC for data transfer and other functions: also the new version will be able to use data from other makes of weather station providing the data are in Dbase format. Both mouse and keyboard will be supported.

## ACKNOWLEDGEMENTS

This research project is funded by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Apple and Pear Research Council (APRC). G. van Santen made a major contribution to Ventem™ version 1 (van Santen & Butt 1992). We thank K.B. Stone, HRI software engineer, for implementing the new scab model into Ventem™ as a commercial product. We thank Dr. W.F.S. Schwabe, Stellenbosch Institute for Fruit Technology, South Africa, for generously providing data on fruit scab infection conditions.

## LITERATURE

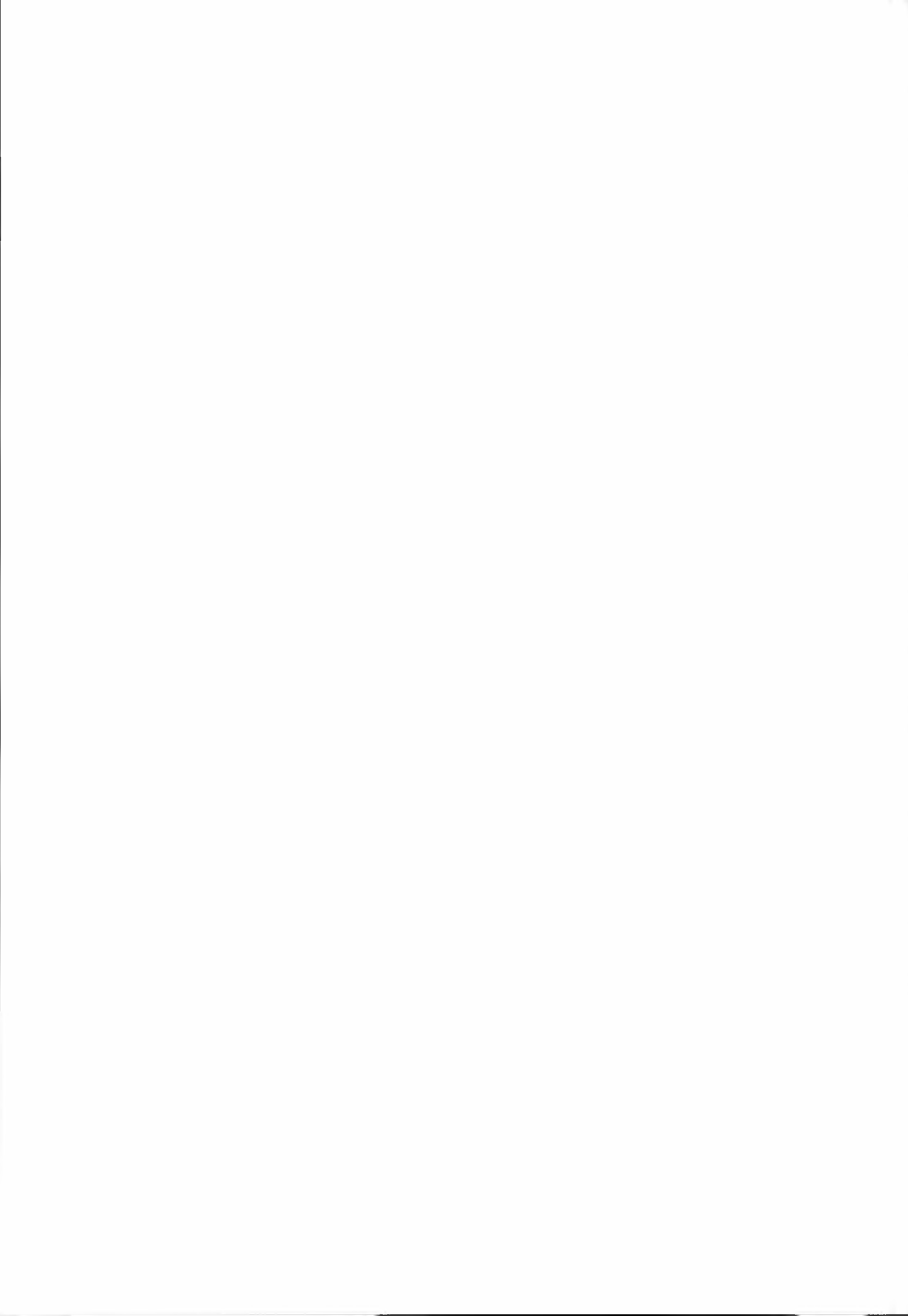
Butt, D.J., G. van Santen, X.-M. XU & K.B. Stone 1992. Ventem™ - an apple scab (*Venturia inaequalis*) infection warning system, version 3.1. Computer software and manual. HRI - East Malling, UK.

Lovelidge, B. 1992. Warnings by computer. *Grower*, February 20, pp. 7-8.

Mills, L.D. & A.A. La Plante 1954. Diseases and Insects in the Orchard. Cornell Extension Bulletin 711: 20-22.

MacHardy, W.E. & D.M. Gadoury 1989. A revision of Mills's criteria for predicting apple scab infection periods. *Phytopathology* 79: 304-310.

Santen van G. & D.J. Butt 1992. The East Malling scab model version I. *Acta Phytopathologica et Entomologica Hungarica* 27: 565-570.



# BIOMAT - a smart tool for apple scab control

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Hofmaier, C. 1994. BIOMAT - a smart tool for apple scab control. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 253-256. ISSN 0802-1600.

The BIOMAT, an easily-to-handle solar powered weather station, is provided with models monitoring the epidemiology of apple scab and other fungal diseases. Logged and calculated data can be seen directly on a LCD display or recorded on an integrated printer. Data transmission to other units (network) can be through several interfaces (2 RS 232, 1 RS 422). LDCS (long distance check-up service) via modem by the manufacturer (Berghof, Germany) supports maintenance. Models can be initialized on-line by modem or RAM card at any time. Collected data can be stored in memory up to one year and data are secure for 10 years.

Key words: Data logging, infection periods, monitoring, orchard diseases, *Venturia inaequalis*, warnings.

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Since the BIOMAT was one of the first electronic devices for monitoring the epidemiology of apple scab more than a decade ago, impressive progress in electronics has been made.

By the valuable support of customers (i.e. farmers, institutes), Berghof has developed BIOMAT to be a very helpful and easy-to-operate tool, predicting several plant diseases.

The BIOMAT can be set up outdoors (figure 1) or indoors but with the sensors outside. For the outdoor mode electronic power supply is guaranteed by a solar panel; for the indoor mode by AC-converter, battery or a combination of both.

Each standard version of the BIOMAT is equipped with sensors recording leaf wetness, temperature, relative humidity, precipitation and light-/dark-recognition.

Another 16 channels are for measuring wind speed, wind direction, ground temperature, leaf wetness and other variables are also integrated as a standard, together with one port for the connection of an acoustic alarm.

Concerning apple scab, the following settings are possible:

- a) ascospores: as described by Mills, MacHardy/Gadoury
- b) conidia: as described by Schwabe and Jones
- c) combinations of (a) and (b).

The user can switch from one prediction model to another at any time and without losing data.



Figure 1. Solar-powered BIOMAT for field use

The BIOMAT calculates the beginning of a light, medium or severe infection period from the duration of leaf wetness and temperature. Readings are taken every minute and calculated. LCD display shows the severity of infection and current sensor values. Daily data stored over a period of one year can be printed out in an easily understandable layout using the unit's own integrated printer. The left part of the printed record shows the weather data and the right the epidemiological consequence (see figure 2). On account of precipitation at midnight leaf wetness has occurred until 10.00 a.m. during which a light infection has been registered (9.00 a.m.).

***** BIOMAT - record date: 27.6.93 *****										date
t	temp	rh	pr	lw	l	m	s			
h	°C	%	mm		%	%	%			
1	N 21.5	85	11	60	6	4	3			
2	N 21.5	86	5	58	16	12	8			development of a
3	N 22.7	86		60	30					light (l),
4	N 22.7	86		60	43	33				medium (m),
5	N 22.7	86	2	60	57	43	28			severe (s)
6	N 22.7	86	7	60	70	53	35			infection in per cent
7	23.1	88		55	83	63	41			100% = infection
8	24.3	84	3	58	94	72	47			
9	25.4	85		60	INF!					light infection
10	25.4	85		5	1h	86	56			has occurred
11	27.0	66			2h	86	56			
12					3h	86	56			
13	31.9	67			4h	86	56			
14	33.3	64			5h	86	56			
15	33.3	58			6h					time in hours (h) since
16	32.3	58			7h	86	56			light infection has occurred
17	28.6	58			8h	86	56			
18	29.3	63			9h	86	56			
19	26.8	65			10h	86	56			
20			3	10	2					registration
21	25.0	79	1	56	13	10	6			of new infection
22	N 23.5	81		60	24	18	12			
23				60	36	27	18			
24	N 21.7	83		60	50	38	25			
-----										
temperature, minimum	tmin: 21.5°C	t0: 170°C	t10: 110°C							Σ temperatures
temperature, middle	tmit: 25.9°C	t5: 245°C	TW: 97°C							base 10°C
temperature, maximum	tmax: 33.5°C	t8: 174°C							Σ temperatures	
-----										
date	date: 18.5.93									Σ temperatures
	scap-model:									base B°C
	according to Mills/Schwabe									ascospore-/conidia mode
										according to Mills/Schwabe

Figure 2. BIOMAT record from the integrated printer



Data transmission to a PC or printer is achieved by two RS 232 interfaces (see figure 3). An RS 422 interface enables connection to a modem. This interface also can be used for data transmission up to 1000 m from the BIOMAT to a building (modem, PC). For about two years, data from BIOMAT to the South Tyrolian advisory board (Italy) have been transmitted by radio. Data transmission by RAM-Card is possible.

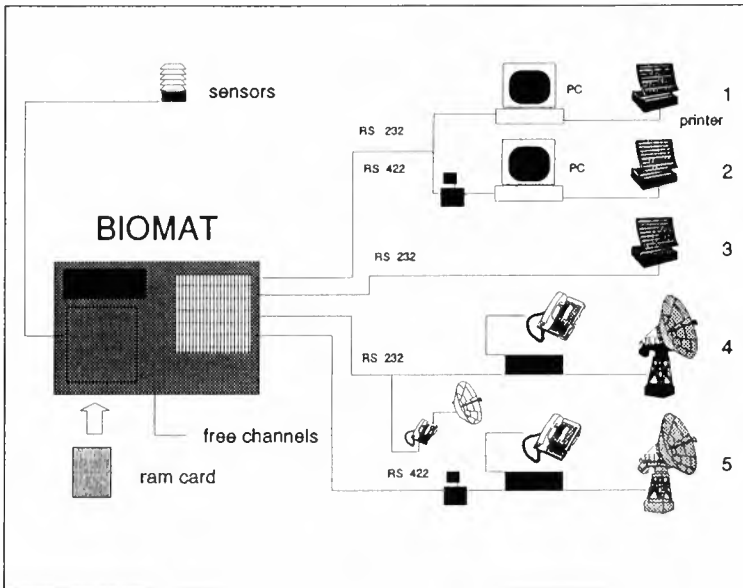


Figure 3. BIOMAT data transmission: 1. to PC and printer (RS 232, maximum 2m); 2. to PC or printer (RS 422; maximum 1000m); 3. to printer (RS 232; maximum 2m); 4. to modem (RS 232, maximum 2m); 5. to modem (RS 422; maximum 1000m).

In the case of data transmission by telephone (modem), the BIOMAT can be programmed for automatic dialing in certain times or it can be called on request by the user's PC.

Data transmission can be done in either direction i.e. logged data can be read into a PC or data from the PC can be sent to the BIOMAT. In the latter case advisors have the opportunity to send notes to users. Moreover, Berghof can initialize the software of the BIOMAT. This can also be done by the user using a RAM-Card.

Concerning after-sales service, Berghof is offering a worldwide long distance check-up service via modem (LDSC). Certain parameters of the sensors will be controlled periodically. If necessary, a message like 'control wetness sensor', for example, will be left in the user's BIOMAT.

If there should be an interruption of the power supply data is secure for up to 10 years.

Transmitted data can be read as an ASCII file for use in other models or it can be visualized by the BID program, **Berghof Infection Dagnosis** (see figure 4).

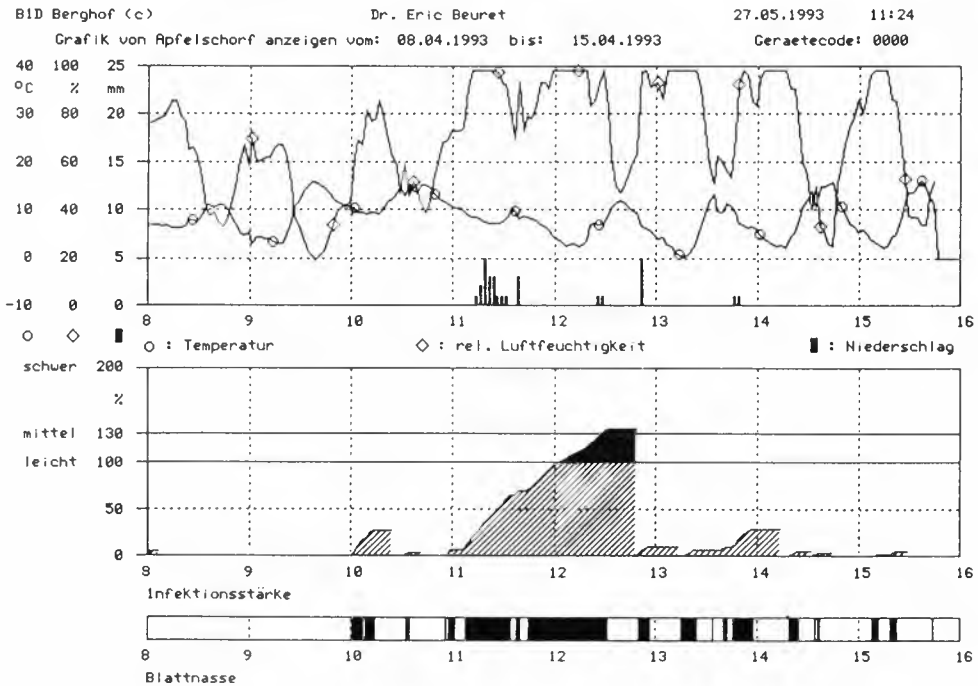


Figure 4. Graphic display using BID (Berghof Infection Diagnosis)

The upper part of figure 4 represents important weather data like temperature, relative humidity and precipitation. The lower part will show the development of scab infection, depending on leaf wetness duration. Likewise, these data can be shown in tabular form. Entering a certain ID-code into each BIOMAT helps to make an easy distinction between users on a network. This year a network is being installed in Switzerland.

The concept of the BIOMAT enables apple growers to get immediate information about apple scab development in their own orchards. Advisors can get information about apple scab development in areas by using networks.

# A logger network to monitor, transfer and evaluate meteorological data for scab warning in Baden-Württemberg

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Dölz, A. & P. Galli 1994. A logger network to monitor, transfer and evaluate meteorological data for scab warning in Baden-Württemberg. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 257-259. ISSN 0802-1600.

For the promotion of the plant protection service in fruit growing, particularly scab warning, 33 small meteorological stations electronically monitoring relevant data have been set up in Baden-Württemberg in the years 1989 to 1992. In Baden-Württemberg (South West Germany) apples are produced intensively in an area of about 11,000 ha.

Key words: Apple scab, data loggers, environment monitoring, forecasting, infection periods, surface wetness.

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## MONITORING METEOROLOGICAL CONDITIONS

Most of the loggers are installed in commercial orchards, but some are working for research institutes. Parameters continuously measured are air temperature, relative humidity, leaf wetness, rainfall and in some cases light intensity. The data are stored at intervals, some of 1 hour (hour means), some of 12 minutes (single values). The devices are supervised by growers assisted by the staff of the plant protection service. All the meteorological stations are electronic scab warning instruments, manufactured by the firms *Paar* (A-8054 Graz, model KMS-P; 1989), *Pessl* (A-8160 Weiz, model METOS D; 1990), *Berghof* (D-7412 Eningen, model BIOMAT; 1991) and *Lufft* (D-7012 Fellbach-Schmiden, model HP-100; 1991/92).

## TRANSFER OF METEOROLOGICAL DATA

Usually, the growers keep the data and inform their advisory office by phone; in some cases the advisors collect the data with the help of memory (RAM) cards (BIOMAT, KMS-P, HP-100 solar). Most devices facilitate the transfer of the meteorological data to a PC

by memory (RAM) cards or by a serial port (METOS, HP-100).

In addition, twelve advanced devices (HP-100 net version) provide the possibility to transfer files of weather data from the data logger on the farm to the advisors' PC by a direct link by modem. For electronic data transmission via telephone the data logger is installed in an office or another farm building, whereas the measuring unit with the sensors is situated in a distance of about 40 to 60 m in the orchard. For data transmission the PC program DATTRANS (*Lufft*) is required. The use of MNP5- modems with data compression and incorporated protocol for error-free communication increases the safety as well as the speed of data transmission.

In comparison with reportings of scab infection periods by phone, the electronic data transfer (HP-100 net version) offers considerable advantages:

- 1) Direct gathering of data at any time, independent of the distance.
- 2) Transfer of all data recorded by the HP-100 at 12-minute intervals, including all calculations such as scab infection indices and temperature sums; in order to distinguish between "dew" and "rain" the HP-100 also stores the readings of the LUFFT leaf-wetness sensor as analogue data.
- 3) Unrestricted use of the instrument by the farmer (only during the process of data transfer are the menu keys blocked).

## EVALUATION OF METEOROLOGICAL DATA

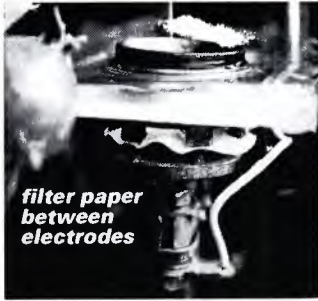
For the electronic evaluation of weather data and for an assessment of scab risk the simulation program SCHORF is employed (*Farmsoftware*, D-7980 Ravensburg-Bavendorf). This program takes into account the specific weather conditions as well as several biological parameters affecting scab infections and provided by the user.

The measurement of the leaf wetness with the HP-100 differentiates between "dew" and "rain" and enables calculation of different scab infestation indices with the help of SCHORF (see fig. 1). SCHORF provides several import modules, so that meteorological data of different devices can be analysed separately and almost homogenously. Thus the advisors - considering additional observations - are able to prepare different regional warnings for their automatic telephone answering service.

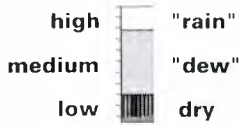
Furthermore, the import of HP-100 ASCII files into a calculation program allows graphic presentations and other evaluations such as comparison of sites, tests of plausibility etc. The main aims of the service are:

- 1) Analysis of scab infection periods.
- 2) Long-term records, including meteorological data.
- 3) Provision of information and facilities for advisory purposes.
- 4) Comparison of sites and scab models.

**HP-100 leaf wetness sensor**



**Analogous measurements of conductivity at 12-minutes-intervals reveal different "levels" of leaf wetness:**



**Interpretation of several 1 day - patterns of analogous HP-100 measurements (with regard to the gradients)**

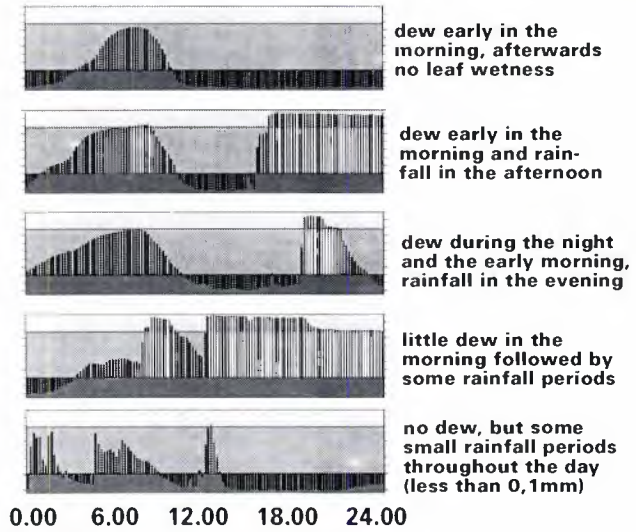


Fig. 1. The recording of surface wetness by the *Lufft* data logger model HP-100



# The influence of integrated and organic spraying programs on the incidence on scab (*Venturia inaequalis* Cooke) on 11 apple cultivars

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Lindhard Pedersen, H & J. Vittrup Christensen 1994. The influence of integrated and organic spraying programs on the incidence on scab (*Venturia inaequalis* Cooke) on 11 apple cultivars. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 261-266. ISSN 0802-1600.

In integrated and organic growing knowledge about the susceptibility of different cultivars is very important in order to minimize the use of pesticides. Eleven cultivars were planted in 1987: 'Aroma', 'Cox's Orange', 'Discovery', 'Elstar', 'Gloster', 'Jonagold', 'Mutsu', 'Belle de Boskoop', 'Ingrid Marie', 'Spartan' and 'Summerred'.

Treatments against scab were: a. Integrated (according to infection-periods) b. Organic (sulphur and copper).

The spraying-schedule, yield, fruit size and results of scab infection on leaves and fruits are shown for 1989 to 1992. The strategy used in the integrated spray program was not satisfactory against scab in all cultivars, because the tolerance level of the cultivars were different from the supposed heavy infection periods appeared autumn 1990 and spring 1991. 'Jonagold' in 1990, 'Summerred', 'Elstar' and 'Ingrid Marie' in 1991 were too much infected. Copper and Thiovit could control scab in years with low infection pressure, but in autumn 1990 and spring 1991 these pesticides were not sufficient. The cultivar 'Summerred' was heavily attacked by scab. The cultivars 'Aroma', 'Discovery', 'Belle de Boskoop' and 'Ingrid Marie' were rather resistant to scab.

Key words: Apple, cultivar, integrated, organic, scab.

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The aim of this study was to compare a number of apple cultivars recommended for growing in Denmark in two production systems: Integrated Fruit Production (IFP) and organic growing. This paper contains results from the first four years.

In IFP scab was controlled with curative and protective fungicides according to infection periods (scab warning). In the organic system protective treatments with copper and sulphur were used.

An important purpose has also been to point out differences in scab susceptibilities between cultivars.

The results of scab control, which is an important part of the system, are reported here.

## MATERIALS AND METHODS

The trees were planted in 1987 at a planting distance of 4.0 x 2.0 m and shaped as spindles. Grass alleyways and 1.25 m mechanical cleaned strips in the row were established.

The two treatments started in 1988: integrated and organic. Each treatment included three blocks of three trees per cultivar. There were three guard trees between blocks.

In the integrated system the trees were treated curative according to heavy infection periods using a KMS-P scab warning system (Mill's infection Table). In 1991 and 1992 the cultivars were divided into three groups. 'Gloster', 'Jonagold', 'Mutsu' and 'Summerred' were treated against scab at light infection. 'Cox's Orange', 'Elstar' and 'Spartan' were treated at moderate infection and 'Aroma', 'Boskoop', 'Discovery' and 'Ingrid Marie' were treated at heavy infection. Fungicides used: Copper (cuprihydroxidchlorid 50 %), Baycor 25 WP (bitertanol 25 %), Euparen M (tolylfluanid 50 %), Captan (captan 83 %), Rubigan (fenarimol 12 %) and Cadol (dithianon 25 %). Dose rates, Table 1.

In the organic treatment Thiovit (sulphur 80 %) was used preventive before rain, and copper (cuprihydroxidchlorid 50 %) only at green tip (Table 1).

Yield and average fruit size were recorded per tree. The level of scab infection on leaves were assessed randomized on branch tendril in June and July on 100 leaves per treatment on each cultivar, according to a scale from 1 to 5 where 1 = no infections and 5 = 10 or more spots per leaf. Fruit scab was assessed on 200 fruits per treatment according to a scale from 1 to 4, where 1 = no infection and 4 = the flecks cover more than 1 cm<sup>2</sup>. These data are used for the  $\chi^2$ -testing only. In table 3 the results are presented as percent of fruits with any attack. The level of leaf infection was calculated according to Townsend & Heuberger (1943).

Data were subjected to analysis of variance using the 'General Linear Model (GLM)' and compared with Duncans test. The level of fruit infection were compared by a  $\chi^2$ -test, where the hypothesis that the scab level in the two treatments were the same, was tested.

## RESULTS

The spraying-schedules are shown in Table 1. The cultivars treated at light and moderate infection periods were treated the same number of times in 1991 and in 1992 the light infection developed one time more than the moderate infection.

The total number of treatments during 4 years in the integrated system varied from 29 to 35 depending on the infection graduation.

In the organic system there were four treatments with copper and 32 treatments with sulphur in four years.



Table 1. Spraying - schedules 1989-92

Year	ORGANIC			INTEGRATED		
	No. of treatm.	Fungicides	kg/l pr. ha	No. of treatm.	Fungicides	Kg/l pr. ha
1989	1	Copper	3.8	1	Copper	3.8
	2	Thiovit	6	9	Baycor 25 WP+Euparen M	0.4 + 0.8
	8	Thiovit	3			
1990	1	Copper	3.8			
	2	Thiovit	6	1	Copper	3.8
	7	Thiovit	3	4	Baycor 25 WP + Euparen M	0.4 + 0.8
1991					<b>Light infection periods</b>	
	1	Bordeaux mixture	3	1	Captan + Baycor 25 WP	2.3 + 0.8
	3	Thiovit	6	1	Rubigan + Euparen M	0.2 + 0.8
	4	Thiovit	3	2	Rubigan + Cadol	0.2 + 2.3
				2	Baycon 25 WP + Euparen M	0.8 + 1.5
				3	Baycor 25 WP	1.2
				2	Captan	2.3
					<b>Heavy infection periods</b>	
				1	Captan + Baycor 25 WP	2.3 + 0.8
				1	Rubigan + Euparen M	0.2 + 0.8
				2	Baycor 25 WP + Euparen M	0.8 + 1.5
				3	Baycor 25 WP	1.2
				2	Captan	2.3
	1992	1	Bordeaux mixture	6		<b>Light/moderate infection periods</b>
2		Thiovit	6	4/3	Captan	2.3
4		Thiovit	3	3	Rubigan	0.4
				1	Baycor 25 WP	0.8
				1	Cadol	1.5
					<b>Heavy infection periods</b>	
				1	Captan	2.3
				1	Baycor 25 WP	0.8
				2	Rubigan	0.4
				1	Cadol	1.5
Number of treatments per year						
	1989		11	10		
	1990		10	5		
				<b>Light</b>	<b>Moderate</b>	<b>Heavy</b>
	1991		8	11	11	9
	1992		7	9	8	5
Total number of treatments						
			36	35	32	29

Light infection periods : 'Summerred', 'Mutsu', 'Gloster' and 'Jonagold'  
 Moderate infection periods : 'Cox's Orange', 'Spartan' and 'Elstar'  
 Heavy infection periods : 'Ingrid Marie', 'Boskoop', 'Discovery' and 'Aroma'

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Yield and average fruit size are shown in Table 2. On average of all years the cultivars 'Boskoop', 'Summerred' and 'Discovery' gave a significant higher yield in the integrated production system, and 'Summerred' had bigger fruits. There was only a very small, but significant difference in fruit size in average of all cultivars and years.

Table 2. Yield (kg/tree) and fruit size (g) in organic and integrated growing of 11 apple cultivars in 1989-92

	Organic	Integrated	Organic	Integrated
Aroma	14.5 a	14.8 a	150 a	151 a
Red Boskoop	9.1 b	13.9 a	221 a	206 a
Cox's Orange	5.3 a	5.8 a	119 a	124 a
Discovery	8.3 b	10.5 a	123 a	120 a
Elstar	14.9 a	14.9 a	134 a	133 a
Gloster	20.2 a	21.4 a	184 a	175 a
Red Ingrid	11.2 a	10.2 a	142 a	135 a
Jonagold	17.6 a	19.0 a	188 a	178 a
Mutsu	15.1 a	13.6 a	252 a	251 a
Spartan	14.5 a	15.3 a	121 a	117 a
Summerred	11.5 b	16.8 a	113 b	117 a
All cultivars	12.9 b	14.2 a	157 a	155 b

Numbers followed by the same letters in rows do not differ significantly for  $P < 0.05$

Most cultivars were only slightly attacked by leaf scab in the integrated plot, whereas organic sprays did not control leaf scab sufficiently in most cultivars (Table 3).

Table 3. Scab infections on leaves (1988-91) and percent fruits with scab at harvest (1989-92) in organic and integrated (IP) growing of 11 apple cultivars

Cultivar	Level of scab infection		Percent fruit scab							
	Organic	Inte- grated	1989		1990		1991		1992	
			Org	IP	Org	IP	Org	IP	Org	IP
Summerred	34.2 a	9.5 b	5.0 a	1.0 a	0.5 a	0.0 a	92.5 a	9.5 a	-	-
Mutsu	11.4 a	0.3 b	-	-	8.7 a	2.0 b	57.0 a	1.5 b	5.5 a	1.1 b
Gloster	9.9 a	1.1 b	0.0 b	0.0 a	11.1 a	2.0 b	58.5 a	1.0 b	2.4 a	0.2 b
Jonagold	14.6 a	1.6 b	5.0 a	0.0 a	7.5 a	5.8 a	55.5 a	0.5 b	2.0 a	0.4 a
Cox Orange	9.4 a	1.8 b	2.0 a	0.0 a	1.8 a	1.1 a	34.5 a	0.5 b	0.0 a	0.0 a
Spartan	11.9 a	1.6 b	1.0 a	1.0 a	0.0 a	0.0 a	33.5 a	2.0 b	1.3 a	0.2 a
Elstar	10.5 a	4.3 b	1.0 a	0.0 a	0.5 a	0.0 a	32.5 a	4.5 b	0.0 a	0.0 a
Ingrid Marie	2.7 a	0.8 b	0.0 a	0.0 a	0.0 a	0.0 a	14.5 a	4.5 b	0.3 a	0.0 b
Boskoop	1.7 a	1.0 a	2.0 a	0.0 a	0.5 a	0.5 a	7.5 a	1.0 b	0.0 a	0.0 a
Discovery	1.5 a	0.2 b	0.0 a	0.0 a	0.0 a	0.0 a	9.0 a	0.0 b	0.3 a	0.2 a
Aroma	2.7 a	0.1 b	0.0 a	0.0 a	0.0 a	0.0 a	3.0 a	0.5 a	0.0 a	0.0 a
Average	10.0 a	2.0 b	1.6 a	0.2 b	2.7 a	1.0 b	36.2 a	2.7 b	1.2 a	0.3 b

Numbers followed by the same letter in rows within years do not differ significantly for  $P < 0.05$

In 1990 a scab infection occurred on fruits in the secondary season on the late cultivars: 'Mutsu', 'Gloster' and 'Jonagold' (Table 3).

During the four year period severe attack of fruit scab occurred in 1991. In the organic treatment most cultivars were very severely attacked, up to 92 percent of the fruits of 'Summerred' (Table 3). Scab infection at fruits in the integrated system was only low compared to the organic system. But scab control was in 1991 not satisfactory in 'Summerred' even in the integrated system where 9.5 percent of the fruits were attacked. The graduation of treatments according to infection severity did not work satisfactory in 'Elstar' and 'Ingrid Marie' 1991, 4,5 percent of the fruits were infected by scab. 'Elstar' were treated 11 times against scab and 'Ingrid Marie' 9 times. Neither of the numbers of treatments were satisfactory (Table 3).

## DISCUSSION

Yield and fruit size were not essentially different in the two treatments. Ruger (1985) and Rais (1989) found that alternative production systems where light soluble fertilizer and chemical synthetic plant protection pesticides not were used (Organic and biodynamic), reduced yield and fruit size compared to traditional (conventional and integrated growing).

Organic growing is a risky method in years with favourable weather conditions for scab. There is a need for other and more effective fungicides for organic growing. Staub und Kienzle (1992) studied two biological fungicides named Ulmacud and Mycosan. These fungicides are based on stonemeal and different plant extracts and had an effect against scab on leaves and fruits at the same level as cobber (0.05 %) or sulphur (0.5 %).

'Aroma' and 'Discovery' were in this study only a little attacked by scab. This was also found by Hansen & Andersen (1985). Whereas Norton (1981) found that 'Discovery' were able to get a rather heavy scab attack.

'Ingrid Marie' and 'Boskoop' were found to be low to medium sensitive to scab, as also found by Blazek et al. (1977), Cimanowski et al. (1988) and Hansen & Andersen (1985).

Aldwinckle (1974), Hansen & Andersen (1985) and Lindhard Pedersen et al. (1993) also found that 'Summerred' and 'Mutsu' were very susceptible to scab, but Norton (1981) found that 'Mutsu' was low to medium susceptible.

Jonagold was severe attacked by scab in 1991. Aldwinckle (1974), Hansen & Andersen (1985) and Norton (1981) found that 'Jonagold' was medium sensitive to scab.

## CONCLUSION

'Summerred' is very susceptible to scab. Treatments at light infection did not give satisfactory control of scab in 1991.

In three out of four years copper and sulphur controlled scab on 'Aroma', 'Ingrid Marie', 'Discovery', 'Elstar', 'Spartan', 'Boskoop' and 'Cox's Orange'.

REFERENCES

- Aldwinckle, H.S. 1974. Field susceptibility of 51 apple cultivars to apples scab and apple powdery mildew. *Plant Disease Reporter* 58: 625-629.
- Blazek, J., J. Kloutvor, & J. Vondracek 1977. The susceptibility of the important apple cultivars to scab *Venturia inaequalis* (Cke) Wint. *Weddecke Prace Ovocnarske* 6: 61-79.
- Cimanowski, J., W. Dzieciol & B. Kowalik 1988. Evaluation of susceptibility of 22 apple varieties to apple scab/*Venturia inaequalis* (Cooke) Aderh/and apple powdery mildew/*Podosphaera leucotricha* (ell et. ev) salm/. *Fruit Science Reports* 15: 81-84.
- Hansen. P. & K.K. Andersen 1985. Æblesorter og skurvmodtagelighed. *Frugtavleren* 14: 182-183.
- Lindhard Pedersen H., J. Vittrup Christensen & P. Hansen 1993. Susceptibility of 15 apple cultivars to major diseases and pests. In preparation.
- Norton R.A. 1981. Field susceptibility of apple cultivars to scab, *Venturia inaequalis* and powdery mildew, *Podosphaera leucotricha* in a cool, humid, climate. *Fruits Varieties Journal* 35: 2-5.
- Rais K. 1989. Vergleich konventioneller und alternativer Apfelproduktion. *Obstbau* 14: 503-508.
- Rüger H. 1985. Möglichkeiten und Grenzen des biologischdynamischen Apfelanbaus. *Obstbau* 10: 212-218.
- Straub M. & J. Kienzle 1992. Die Anwendung von Biologischen Pflanzenbehandlungsmitteln gegen Apfelschorf (*Venturia inaequalis*) und deren Auswirkungen auf Schädlingsbefall an Früchten. 5. Internationaler Erfahrungsaustausch über Forschungsergebnisse zum Ökologischen Obstbau 19. und 20.11. 1992: 59-65.
- Townsend, G.R. & J.W. Heuberger 1943. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter* 27: 340-343.

# Computer applications in the management of diseases in horticultural crops

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Fruit production is becoming more complicated as the principles of integrated fruit production (IFP) are adopted by growers. This complexity is accompanied by an increase in the quality and quantity of information for decision-making. Computers can be used to store, retrieve, organise and interpret information. This information, taken from sources such as technical reports, computer simulations and experts, can be in forms of text, graphics, video images and sound. Research on "artificial intelligence" has led to the emergence of decision-making systems (known as "expert systems"), which use available knowledge to mimic the deductive and inductive reasoning of human experts. This paper describes the general structure and characteristics of several computer-based systems and their applications in plant disease management. Future prospects for computerised decision support systems in disease management are discussed. It is concluded that national and international co-operation between disciplines is essential if a versatile and practical computing system to aid growers' daily decision-making in disease management is to be developed.

**Key words:** Databases, decision support systems, disease management, disease warning systems, expert systems, horticultural crops, integrated fruit production.

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In the last twenty years, computers have begun to play an increasingly important role in plant disease epidemiology and control. The impact has been on areas such as environment monitoring, automated instrumentation, data capture, data analysis and simulation modelling. The understanding of pathosystem dynamics has been considerably advanced by simulation studies. The accelerated research and development of integrated fruit production (IFP) in the past decade has led to a sudden expansion of knowledge about fruit disease management. Fruit growers are increasingly experiencing difficulties in making day-to-day decisions, but the available technical knowledge is not adequately organised in a manner to assist growers and advisors with their decision-making. A challenge facing plant pathologists is to deliver this knowledge effectively and efficiently to users. Computers provide the means to capture, utilise and disseminate this knowledge for practical application.

Several types of computer software have been developed to capture and organise knowledge and assist with decision-making; each type has its advantages and limitations. This paper reviews several computer-based systems and their use in the management of diseases on horticultural crops.

## NEEDS OF THE HORTICULTURAL INDUSTRY

To be successful, horticulturists must be efficient managers, have a broad-based knowledge and be familiar with new technologies. At present, fruit production requires considerable usage of insecticides, acaricides, fungicides, herbicides, growth regulators and fertilisers. The growing public concern about the effects of agrochemicals on health and the environment have forced growers to adopt IFP principles, whereby crops are grown with minimal chemical input.

To follow IFP guidelines, growers must have access to knowledge about many diverse subjects, including cultivar performance, orchard husbandry, disease, insect and weed management, harvesting and storage conditions and market requirements. These subject areas can be further sub-divided into several components. Disease management, for example, includes (a) monitoring and diagnosis, (b) forecasting, (c) assessing control needs, (d) decision on control methods and (f) action (Fig. 1). Each of these components can include several sub-components; for example, disease control methods include the use of cultivar resistance and cultural, chemical and biological procedures.

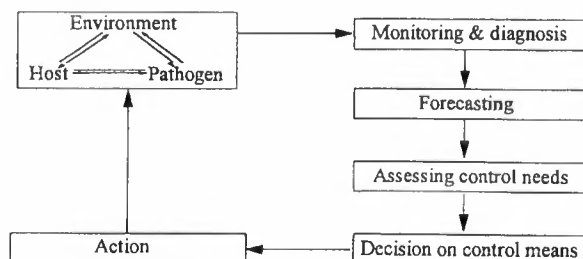


Fig. 1. Diagram of rational management

Growers often use the expertise of horticultural specialists to assist them in daily decision-making because much of the information from research, technical reports and expert opinion is not readily available or understood. Unfortunately, the number of specialists is reducing whilst consultation is becoming more necessary as the complexity of fruit production increases. To alleviate this problem, computer-based decision support systems are urgently needed to provide access to information, to integrate and organise information, to interpret information and to provide solutions to problems.

Much experience of IFP has been derived from simulation studies, but the results are often neither accessible nor usable by growers. Furthermore, much of the present knowledge on IFP is qualitative and retained by specialists. Little effort has been devoted to collecting and assimilating the knowledge and the experience of experts. As a result, the knowledge held by experts can be lost.

Given that it is possible to access information, growers also need to integrate the pertinent elements of this information in order to solve each problem. Computer-aided systems can help growers to identify the precise nature of a problem through interactive dialogue, select the relevant information from a database, request additional information and organise it into a coherent framework for solving the problem.

Once the relevant information is available and organised to solve a problem, the grower has to interpret and use the information to make a decision. Problems often encompass several disciplines, and it is impossible for a grower to be expert in all relevant subjects. A computer system is needed, therefore, to help growers with the interpretation of knowledge and/or to provide the solutions to problems.

To ensure that growers make full use of these opportunities, the interface of the computer systems must be user-friendly. The dialogue and/or information generated by these systems must be easy to understand and should guide growers towards IFP.

#### TYPES AND CHARACTERISTICS OF COMPUTER-BASED SYSTEMS

Several types of computer-based system have been developed to support decision-making, each with unique characteristics and functions (Fig. 2). These systems have evolved through several levels of complexity from simple data processing to advanced decision-making. There are fundamental differences between these systems in their structure, function and application. This review provides discussion of those systems with an application in plant disease management. General references on the development, structure and application of these systems are available (Bennett, 1983; Bonczek et al., 1981; Buchanan & Smith, 1988; Davies, 1986; Payne & MacArthur, 1990; Vasta, 1985; Vazsonyi, 1978).

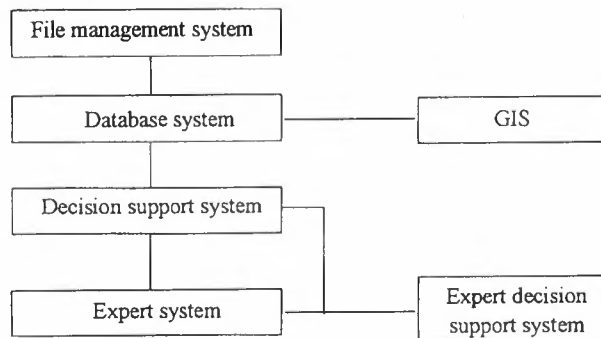


Fig. 2. Development of computer systems for aiding decisionmaking

In Fig. 2, the file management system (FMS) is shown at the first level. An FMS organises data into separate files and is concerned with the organisation and manipulation of data in each file. It was developed to deal with pre-specified questions and for use by individuals in the same knowledge domain as those who developed the system. The information stored and/or generated is generally not interpretable or usable by a non-expert, i.e. by those outside the system domain.

Following on from this early development, the database management system (DBMS) was developed to accommodate information from diverse but inter-related disciplines. A DBMS integrates data from files developed for different purposes with its emphasis on the consolidation of multiple data sets into a common database. Consequently, the efficiency of the storage, retrieval and manipulation of data is greatly increased over FMSs. The interface between the database and the user is via a control program. Some DBMSs also provide 'report-oriented' methods for extracting, manipulating and summarising data into specified formats.

Recent developments in multimedia technology, which enables the integration of text, graphics, sound and video images, may have considerable impact on the structure and application of DBMSs. A DBMS with a multimedia facility would illustrate and present knowledge more logically than by the texts and graphics used in traditional DBMSs.

DBMSs may also include a geographic information system (GIS), capturing, storing, managing, analysing, mapping, and displaying spatial descriptive data. A GIS consists of the following components: (a) input module for collecting and processing the data, (b) a database to store and retrieve data, (c) analytic tools to interpret data and (d) output to display maps and reports. GISs allow users to compile and manipulate the spatially referenced data and to visualise the spatial structure. Geostatistics enable the spatial and temporal relationships to be modelled.

Both FMSs and DBMSs were developed mainly for archiving information and not to assist directly with decision making. The decision support system (DSS) was developed to use disparate information in management decision processes. The DSS is an interactive system designed to help the decision-maker use information; it contains analytic models to solve specific problems. Users of a DSS do not need to be experts in the subject domain. A DSS can define, retrieve, process and organise information in a way that is relevant to users' problems. A DSS contains parts of the above systems, especially the data extraction and summarisation capabilities of the DBMS.

DSSs consists of the following parts (Fig. 3): (a) DBMS, (b) control program, (c) model base and (d) user interface. The model base may include various types of simulation model or application programs. The control program helps users to frame questions through a series of prompts and consequently defines the exact nature of the problem; the control program then identifies and activates the pertinent elements in the model base and database.

Although DSSs represent a significant advance in computer-based problem solving, their output still requires interpretation by users. A DSS does not make decisions but provides users with information pertinent to their specific problems. The effectiveness of a user's interpretation depends on the person's experience and knowledge and this is a shortcoming of the DSS.

The expert system (EXS) has been developed in order to overcome the shortcomings of the DSS (Fig. 2). The EXS stores the knowledge of a domain expert, including the



heuristic reasoning process used by the expert for solving problems. An EXS helps a user to frame the exact problem/question, asks for whatever additional information may be needed to solve the problem and provides a solution. Furthermore, an EXS can provide the logical basis of the solution.

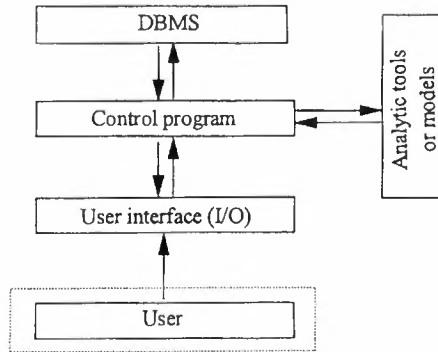


Fig. 3. Structure of a decision support system (DSS)

The major components of EXSs are: (a) knowledge base, (b) inference engine and (c) user interface (Fig. 4). At the centre of an EXS is the knowledge base, which contains facts and heuristics in the domain of the subject. Facts are knowledge that is generally available to and accepted by experts in the domain, whereas heuristics are mostly privately held by individuals and include rules-of-thumb, judgements and experience-based guesses that characterise decision-making by human experts. The knowledge base is assembled by interviewing one or more experts. A special analyst, the knowledge engineer (Fig. 4), may be involved in interviewing or observing the experts and encoding their knowledge into the knowledge base. The knowledge base is further substantiated by information from other sources such as technical reports and simulation studies. The knowledge base contains the system's task-specific information and relationships among the elements in the domain. The knowledge is structured into a network which can be traversed when solving a problem. The knowledge base is often expressed as rules in the form of IF-THEN statements, though other structures are also used, such as frames and semantic nets.

Rules in the knowledge base are processed and interpreted by the inference engine. The inference engine contains the general approach to problem-solving. It selects, accesses and executes the appropriate rules in the knowledge base and determines when an acceptable solution to a problem has been found. The inference engine may employ one of two principle strategies, namely forward and backward chaining (Fig. 5). With forward chaining, the inference engine starts with the appropriate IF clauses and works forward until a desired solution is found; with backward chaining, the inference engine starts with a desired goal or result and scans the rules to find those whose consequent actions implicitly achieve the goal. In practice, an EXS may employ both strategies. Users interact with the system through an interface, which presents them with questions concerning the problem and offers advice generated by the inference engine.

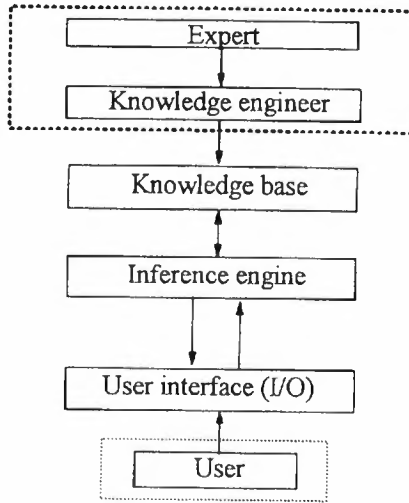


Fig. 4. Structure of an expert system (EXS)

<b>Forward Chaining</b>	<b>Backward Chaining</b>
If A, then B (Rule 1)	<b>Find out about C</b> (Problem)
If B, then C (Rule 2)	If B, then C (Rule 1)
<b>A</b> (Data)	If A, then B (Rule 2)
<u>so C</u> (Solution)	<u>so if A, then C</u> (Implicit rule)

Fig. 5. Two strategies used in the inference engine on an EXS

A feature distinguishing EXSs from conventional programs is that the inference engine is separate from the knowledge domain making it easy to modify, update and enlarge the knowledge base. An EXS is normally reserved for problems for which algorithmic solutions do not exist; heuristic searching is therefore required and in consequence the solutions are often less than optimum. Generally, an EXS does not perform extensive mathematical computations; these are best handled by conventional algorithmic programs called for by the inference engine.

## COMPUTER-BASED APPLICATIONS FOR DISEASE MANAGEMENT IN HORTICULTURE

Computer-based systems can help growers make rational disease management decisions by providing the latest information, diagnosing diseases, alerting them to the risk of disease, evaluating their control needs and recommending and planning action. Of the four types of computer systems discussed above, FMSs and DBMSs were not developed specifically for growers. The major purpose of both these systems is to store research findings and technical data. Nevertheless, a few DBMSs have been developed specially for growers to access and/or store information. Only a few DSSs have been developed to aid in practical disease control. The two most common computer applications developed for disease management are the diseases warning system (DWS) and the EXS.

Computerised DWSs provide warnings of the risk of disease using various combinations of information on weather, host and pathogen. DWSs may be classified as a simple type of DSS. Weather drives the development of both host and disease, and so the recording of weather conditions in or near crops is a prerequisite for a DWS operating on a farm. This paper includes only those systems designed for use in horticulture. Table 1 lists some examples.

### **Database systems**

DBMSs provide growers with the means of storing and/or accessing information and are not very useful in tactical decision-making where decisions largely depend on the dynamic coincidence of weather, pathogen and crop. Nevertheless, readily available information on symptom diagnosis, pathogen biology, disease control, fungicide activity and legislation is important for growers and advisors. Also some DBMSs, such as spray diaries, help growers to keep clear records; these are useful for evaluating past actions and also satisfy the requirements of IFP guidelines. The system developed by Kahrer (1993) is an example of a DBMS: the database provides general information on all aspects of disease and pest control on vegetables and field crops, including diagnosis, biology, control measures (chemical and non-chemical) and their side-effects, and legislation.

Recent advances in multi-media technology will undoubtedly have considerable impact on the development of database systems. The archiving and integration of crop protection texts and pictures on compact disc (CD) is under development (Grøntoft 1993). The potential value of DBMSs in plant disease management depends largely on the degree of integration with other systems, for example, DWSs and EXSs.

A GIS is a useful tool for studying the distribution and development of plants, pathogens and pests. With the incorporation of weather and altitude data, disease development can be predicted over large areas. However, the application depends on the availability of high-resolution spatial data.

Table 1. Selected computer systems developed for plant disease management

Host	Function	References
Database		
Field crops and vegetables	Biology and diagnosis of pathogens, disease control, pesticides, legislation etc.	Kahrer 1993
	CD-ROM storage of crop protection information and pictures	Grøntoft 1993
Disease warning system		
Apple	Dedicated instruments forecasting apple scab infection periods based on weather	Jones et al. 1980; Nielsen & Schacleg 1990
Apple	MARYBLYT - a weather based fireblight warning system	Lightner & Steiner 1990 Steiner 1990
Apple	VENTEM - a scab warning system based on weather, cultivar susceptibility and inoculum level	Butt et al. 1992
Grape	Dedicated instrument for forecasting black rot	Ellis et al. 1986
Potato	BLITECAST - dedicated late blight warning instrument	MacHenzie et al. 1981
Decision support system		
	PHYTOMET - weather data monitoring and collection over time space and data manipulation and reporting	Juhel et al. 1993
Expert system		
Apple	POMME - orchard management for pests and diseases	Roach et al. 1987
Apple	Diagnosis of pests, diseases and disorders	Kemp et al. 1989
Apple	PSAOC - orchard insect and disease management	Travis et al. 1992
Peach	CALEX/Peaches - diagnosis of 120 disorders in California including insect, disease and cultural problems	Plant et al. 1989
Grape	GrapES - management of vineyard pests	Saunders et al. 1987

### Disease warning systems

A computerised DWS predicts an outbreak or increase of disease by using models and analytical tools to examine one or more combinations of weather, host and pathogen data. The number of these components used by a DWS depends on factors such as the targeted stage of the disease cycle, the area of grown crop, whether the DWS is designed for regional or on-farm use and the extent of biological and epidemiological knowledge. Many DWSs do not forecast *per se*: they warn of weather conditions that have favoured infection.

An ideal DWS enables a grower or advisor to determine whether, when, where and how to control disease.

To be operational, a DWS has to be 'packaged' in the context of integrated crop management. The system can be designed for regional or on-farm use. Regional warnings and/or advice are communicated by mail, telephone, radio, telex links and computer networks. Regional systems giving warnings of apple scab have been developed in the United Kingdom (Adam & Seager 1977), the Republic of South Africa (Schwabe 1980) and Canada (Coulombe and Jacob 1981). Multi-disease regional systems have been developed for apple (Hesjedal & Edland 1992) and grapevine (Maurin & Fricot 1993).

A regional system has several advantages: growers do not have to operate the system; the output of the model is interpreted by experts; the warning system can be easily updated. However, there are several limitations: weather data are often not representative of local conditions; the dissemination of information may be slow; site-specific factors such as cultivar susceptibility and inoculum level are often not considered.

Dedicated stand-alone electronic instruments have been developed as DWSs for use on farms. Each unit has environment sensors and a controlling microprocessor producing a disease warning. These instruments monitor local weather and their warnings of disease may, therefore, be more reliable than regional warnings. Such instruments have been developed for apple scab (Jones *et al.* 1980; Nielsen & Schadeegg 1990), fireblight on pome fruit (Garrett 1989; Germann 1989) and grape black rot (Ellis *et al.* 1986). They are easy to operate but the disease models used in these instruments cannot be easily updated and weather records are not stored indefinitely and are unavailable for other purposes. Furthermore, cultivar susceptibility and inoculum potential are often not considered by these systems.

Recently, DWSs have been developed for use on PCs. These systems can use local weather data to drive the model when they are available; weather data stored on the PC are available for other purposes. Information on cultivar susceptibility, inoculum potential and other field/orchard-specific factors can be used as necessary. Examples of such PC-based DWSs are Ventem™ for apple scab (Butt *et al.*, 1992) and Maryblyt™ for fireblight of pome fruit (Lightner & Steiner, 1990). Ventem™ first alerts the user to the favourability of weather for infection and then predicts scab intensity in specific orchards of a farm by taking account of cultivar susceptibility and inoculum (ascospores, conidia) levels.

DWSs are usually for a single disease, but growers often face problems caused by several diseases simultaneously. DWSs for multiple diseases of horticultural crops are needed.

### Expert systems

The number of EXSs developed for disease management has increased considerably over the last few years. Only a few examples are given to illustrate what EXSs can offer.

POMME (Roach *et al.* 1987) provides information about apple diseases, insects and orchard management. It provides growers with information on fungicides, insecticides, cold and drought damage and non-chemical control options. It can diagnose diseases, predict infections, suggest solutions and recommend chemicals. An apple scab infection model is incorporated in the system.

An EXS developed by Kemp *et al.* (1989) provides growers with diagnostic advice

on apple damage caused by insects, diseases and nutrient imbalance. Through interactive dialogue with users the system produces a list of potential causes for the given problem.

PSAOC (Travis *et al.*, 1992) is an EXS for the management of eight diseases and seventeen insects in apple orchards. Weather data are used to assess disease potential, persistence of pesticide deposits and potential phytotoxicity. Recommendations are based on biological, cultural and chemical control options. A chemical management module is also available to select chemicals, rates, spray intervals and to check recommended chemicals for compatibility and restrictions on usage.

A major difficulty in developing EXSs is the complexity and duration of the task. Also, the subject domain is often geographically restricted because plant diseases and methods of their control are often sensitive to local climate, cultivars and legislation.

## DISCUSSION AND CONCLUSION

Although there have been numerous research studies on disease epidemics and their control, only a limited amount of the available knowledge is used by growers. This information gap between research and the horticulture industry has recently received more attention, and researchers are now delivering previously unused knowledge to growers and advisors in the form of computer software. Understanding between growers and researchers is essential if such computerised systems are to be successfully adopted by the industry and their introduction must be accompanied by adequate training programmes.

Current work on computerised systems for applications in plant disease management is often disparate, isolated and sometimes duplicated. Many of the systems produced so far deal with simple situations and are far from the complexity of the agro-ecosystems experienced by growers. An ideal computer system for a horticultural enterprise/crop would cover every aspect of practical disease management and include the following components.

- A DWS giving warnings and risks of all important diseases; the DWS should be driven by weather data recorded on the site and should take into account other factors affecting crop health. Synoptic weather forecasts should be incorporated in order to provide users with advanced warnings of disease development.

- An EXS assisting growers with disease diagnosis; the EXS should use multimedia technology to integrate pictures, texts, video images and sounds. The terminology describing pathogens and diseases should be internationally standardised so that information can be shared.

- An EXS assessing control needs by recommending a type of control measure(s) (e.g. class of fungicide; removal of primary inoculum source) and detailing information on locally acceptable treatments of the recommended type. For example, locally approved fungicides should be listed together with information on rate of active ingredient, spray volume, side effects, warnings of fungicide resistance, application technique, costs and legislation on safe usage. Crop loss and economic models should be included to predict the financial benefits of the treatment.

- A DSS or an EXS assisting growers to plan and optimise the execution of the proposed treatment.

The overall structure of such a comprehensive system could be developed through

international cooperation. Components such as the DWS, disease diagnosis, cost/ benefit assessment and the type of disease control method would comprise the central system to be applicable universally; locally acceptable treatment options would be developed by local experts to be added to the central system. The links between these two parts would have to be internationally agreed before building the system. Clearly, close collaboration between researchers in several disciplines, growers, chemical companies and relevant government bodies will be needed at international level.

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#### LITERATURE

Adams, P.B. & J.M. Seagar 1977. Agrometeorological use of the synoptic data bank in plant disease warning services. *Meteorological Magazine* 106: 112-116.

Bennett, J.L., ed. 1983. *Building decision support systems*. Readings, Mass: Addison-Wesley.

Bonczek, R.H., C.W. Holsapple & A.B. Whinston 1981. *Foundations of decision support systems*. New York: Academic.

Buchanan, B.G. & R.G. Smith 1988. *Fundamentals of expert systems*. *Annu. Rev. Comp. Sci.* 3:23-58.

Butt D.J., van G. Santen, X.-M. Xu & K.B. Stone 1992. *Ventem<sup>TM</sup> - an apple scab (*Venturia inaequalis*) infection warning system, version 3.1. Computer software and manual*. Horticulture Research International - East Malling, UK.

Coulombe, L.J. & A. Jacob 1981. Epidemiology and control of apple scab, *Venturia inaequalis*, from 1972 to 1979, at Frelighsburg, Quebec. *Phytoprotection* 62: 44-52.

Davies, R. 1986. Knowledge-based systems. *Science* 231: 957-963.

Ellis, M.A., L.V. Madden & L.L. Wilson 1986. An electronic grape black rot predictor for scheduling fungicides with curative activity. *Plant Diseases* 70: 938-940.

Garrett, C.M.E. 1989. Fireblight warning systems: problems and progress. In 'Proceedings of the Workshop on Integrated control of pome fruit diseases', Volume II, Brissago, Switzerland, 1988, WPRS Bulletin, pp 26-36.

Germann, K. 1989. Electronic monitoring and interpretation of microclimates. In 'Proceedings of the Workshop on Integrated control of pome fruit diseases', Volume II, Brissago, Switzerland, 1988, WPRS Bulletin, pp 37-36.

Grøntoft, M. 1993. An electronic picture data base in plant protection. EPPO Bulletin 23: 653-656.

Hesjedal, K. & T. Edland 1992. Warning systems for fruit pests and fruit diseases in Norway. Acta Phytopathologica et Entomologica Hungarica 27: 277-280.

Jones, A.L., A.L. Lillevik, P.D. Fisher & T.C. Stebbins 1980. A microcomputer-based instrument to predict primary apple scab infection periods. Plant Disease 64: 69-72.

Juhel, A., G. Froidefond, M. Clerjeau & J. Virolleaud 1993. PHYTOMET - a control system for agrometeorological data. EPPO Bulletin 23: 673-680.

Kahrer, A. 1993. Information system for plant protection in vegetables and field crops. EPPO. Conference on Computerized Advising Systems for Plant Protection; Eslöv, Sweden, November 1992. Abstracts of Papers.

Kemp, R.H., T.M. Stewart & A. Boorman 1989. An expert system for diagnosis of pests, diseases, and disorders in apple crops. New Zealand Journal of Crop and Horticultural Science 17: 89-96.

Lightner, G. & P.W. Steiner 1990. Computerization of a blossom blight prediction model. Acta Hortic. 273: 159-162.

MacKenzie, D.R. 1981. Scheduling fungicide applications for potato late blight with BLITECAST. Plant Disease 65: 394-399.

Magnus, H.A., A. Ligaarden & K. Munthe 1993. An integrated PC environment for the dissemination of plant protection warnings in Norway together with weather reports and forecasts. EPPO Bulletin 23: 669-671.

Mariun, G. & L. Fricot 1993. Météopro: an aid to decision-making in plant protection. EPPO Bulletin 23: 615-618.

McKinion, J.M. & H.E. Lemmon 1985. Expert systems for agriculture. Computers and Electronics in Agriculture 1: 31-40.

Nielsen, S.L. & E. Schadegg 1990. Testing electronic warning equipment together with the curative fungicide bitertanol for control of apple scab (*Venturia inaequalis* (Cooke) Winter). Tidsskr. Planteavl 94: 527-532.



Payne, E.C. & R.C. MacArthur 1990. Developing expert systems. New York: Wiley. 401 pp.

Plant, R.E., F.G. Zalom, J.A. Young & R.N. Rice 1989. CALEX/Peaches, an expert system for the diagnosis of peach and nectarine disorders. HortScience 24: 700.

Roach, J., R. Virkar, C. Drake & M. Weaver 1987. An expert system for helping apple growers. Computers and Electronics in Agriculture 2: 97-108.

Saunders, M.C., C.W. Haeseler, J.W. Travis, B.J. Miller, R.N. Coulson, K.D. Loh & N.D. Stone 1987. GRAPES: an expert system for viticulture in Pennsylvania. AI Appl. Nat. Res. Mgnt. 1: 13-20.

Steiner, P.W. 1990. Predicting apple blossom infection by *Erwinia amylovora* using the Maryblyt model. Acta Hort. 273: 139-148.

Schwabe, W.F.S. 1980. Epidemiology and control of apple scab in South Africa. Phytophylactica 12: 219-222.

Travis, J.W., E. Rajotte, R. Bankert, K.D. Hickey, L.A. Hull, V. Eby, P.H. Heinemann, R. Crassweller, J. McClure, T. Bowser & D. Laughland 1992. A working description of the Penn State apple orchard consultant, an expert system. Plant Disease 76: 545-554.

Vasta, J.A. 1985. Understanding data base management systems. Belmont, Calif: Wadsworth.

Vazsonyi, A. 1978. Information systems in management science. Interfaces 9:72-77.



# Integrated control of apple diseases in an expert system

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The Penn State Apple Orchard Consultant (PSAOC) expert system was developed on a Macintosh computer and employs a frame-based expert system tool, Pennshell, which was written in the C programming language. Although parts of PSAOC were built directly from the C language, Pennshell is designed as a 'tool box' of often-used functions so that little direct coding is necessary. Each frame in PSAOC stores knowledge about a particular object (the phenology of the orchard, for example). Different components (modules) of the expert system are called by a menu interface. For the IPM module in PSAOC the menu items include orchard profile, scouting, weather, insects, diseases and integrated pest management. Once the menu selection is made, the program executes the module until all the necessary information has been obtained to offer a recommendation. The disease management portion of PSAOC is composed of 3 parts; 1) the orchard profile, which is composed of the variables that describe the orchard, 2) the disease rating modules, which determine the potential for disease development and 3) the chemical management modules, which determine the chemicals that are appropriate for the given circumstances, the rates of those chemicals and the spray interval for the next pesticide application. The compatibility of the chemicals and days-to-harvest limitations are also determined. PSAOC was released for use in commercial orchards in 1990.

Key words: Apple, expert systems, fruit, fruit disease, fungicides, orchard disease.

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## INTRODUCTION

University extension and research personnel are concerned that traditional methods of information delivery (i.e., newsletters, production meetings with growers, production guides) seem inadequate for the delivery of complex dynamic information. In the 160-page Pennsylvania Tree Fruit Production Guide, the traditional means of presenting production information for growers since the mid 1940s, recommendations are made on a statewide basis. Ranges for pesticide application rates and timing are suggested, but rates and timing for specific locations depend on local circumstances. This form of information delivery inhibits implementation of new, site-specific integrated pest management (IPM) strategies

by growers. Changes in pesticide labels and new information occur weekly or monthly, but the production guide can be updated only annually. The need for a more effective decision-support tool includes the capability of incorporating constant change into complex management strategies to provide interpretive, integrated, timely, site-specific recommendations.

### **Expert Systems**

To improve delivery of integrated pest management programs and provide more precise and effective pesticide recommendations, an expert system has been developed for use in IPM decision-making. An expert system is a computer program designed to simulate problem-solving mechanisms that imitate those used by experts in a narrow domain or discipline. An expert system is normally composed of a knowledge base (information, heuristics, etc.), an inference engine that analyzes the knowledge base, and the end user interface which accepts inputs and generates outputs. A powerful attribute of expert systems is the ability to explain reasoning. Because the system remembers its logical chain of reasoning, a user may ask for an explanation of a recommendation. The system will display the factors it considered for a particular recommendation; this enhances user confidence in the recommendation and acceptance of the expert system.

The Penn State Apple Orchard Consultant (PSAOC) was developed on a Macintosh computer and employs a frame-based expert system tool, Pennshell, which was written in the C programming language. However, it can be used on both Macintosh and DOS (IBM)-based computers. Although parts of PSAOC were built directly from the C language, Pennshell is designed as a 'tool box' of often-used functions so that little direct coding is necessary.

Different components (modules) of the expert system are called by a menu interface. The menu items for the IPM portion of PSAOC include orchard profile, scouting, weather, diseases, insects and integrated pest management (IPM). Horticultural modules (leaf analysis for nutrients, tree spacing, irrigation scheduling and weed control) are also available for grower consultation. The user can choose one insect or disease, all diseases, all insects, or receive an integrated insect and disease control recommendation by selecting IPM.

The information needed to assemble a meaningful expert system is derived from many areas, and so a team approach was used to develop the knowledge base. The team included experts from plant pathology, entomology, horticulture, agricultural engineering, agricultural meteorology, agricultural economics and rural sociology. Expert systems are best conceived as a whole but then are divided into smaller units for the actual development. For example, PSAOC covers the range of problems encountered by a fruit grower but was built as a series of modules (pest management, leaf analysis, tree spacing, etc.). Each module may be subdivided several more times to arrive at a point of simplification where the information is manageable. For instance, the pest management module of PSAOC includes lower level modules encompassing potentials for apple scab, powdery mildew and cedar apple rust potentials, insect thresholds, chemical, chemical rate and spray intervals. Modules below these predict infection periods, chemical residue levels, etc. These modules, which were built separately, interact to derive an integrated disease and insect recommendation. The relationship and number of modules in an area is determined by the experts who are designing the system. To efficiently utilize information put into the system by the

user, the system stores the orchard description supplied by the user for use by all modules within the system.

### **System Description**

The pest management portion of PSAOC is composed of three parts: 1) the orchard profile which is composed of the variables that describe the orchard, 2) the pest rating modules, which determine the pests, levels of severity, and beneficial organisms and, 3) the chemical management modules, which determine the chemicals and rates that are appropriate for each circumstance and the spray interval for the next pesticide application. The compatibility of the chemicals and days-to-harvest limitations also are determined.

### **Disease Potential Modules**

#### *Apple Scab*

The module for potential apple scab determines the potential for apple scab in the orchard since the last fungicide application. The disease potential levels are qualitative - none, low, moderate, high, or severe for no disease, low, moderate, high potential, or certainty of disease, respectively. Low postinfection (infection period occurred with low disease potential), or high postinfection (infection period occurred with high disease potential) also are recorded (Fig. 1). The disease potential levels none, low, moderate and high rate the disease potential since the last spray if no new infections have occurred. The disease potential levels low and high postinfection rate the disease potential when there was a possibility of infection since the last application. For apple scab, this determination is based on five factors (Fig. 1): the stage of plant development, cultivar susceptibility, incidence of scab at present and last season, and potential for infection since the last fungicide application. Stage of development is determined from the orchard profile according to a standard classification system contained within the system. The cultivar susceptibility (from the orchard profile data) to apple scab is assigned by the system. Cultivar susceptibility is determined from published information and through personal communication with growers, orchard consultants and university personnel. Incidence of scab this season and last season are supplied by the grower within the orchard profile.

The potential for infection since the last fungicide application is determined in a submodule to the apple scab module (Fig. 1). The factors that make this determination include: the amount of rainfall (<5 cm, >5 cm) since the last fungicide application, application to one or both sides of the tree, days since the last fungicide application that the infection period occurred, and infection period calculations based on Mills and Jones et al. The relationship of these factors to each other determines if an infection period has occurred since the last fungicide application. The output of post infection potential (yes, no) is utilized in the apple scab module to determine disease potential level.

The specific relationship of the factors which describe the apple scab module is displayed in a dependency network which is a type of decision tree. Dependency networks are valuable because they are a method of displaying disease management principles and are the means of communication between disease management specialists and computer programmers. The pest management modules in PSAOC contain 135 dependency networks. For example, 'severe' disease potential may occur as a result of one of two situations (Fig. 1) shown as lines drawn from the two 'and' statements. One situation leading to the

'severe' is shown by the darker lines. In this case a severe disease potential for apple scab can occur from green tip to second cover stages of growth if the cultivar susceptibility is high, the incidence of apple scab this season is greater than 0% and there has been no infection potential since the last fungicide application.

Although the network describing the severe goal is relatively simple, involving only two distinct situations (Fig. 1), some networks have many situations to be displayed.

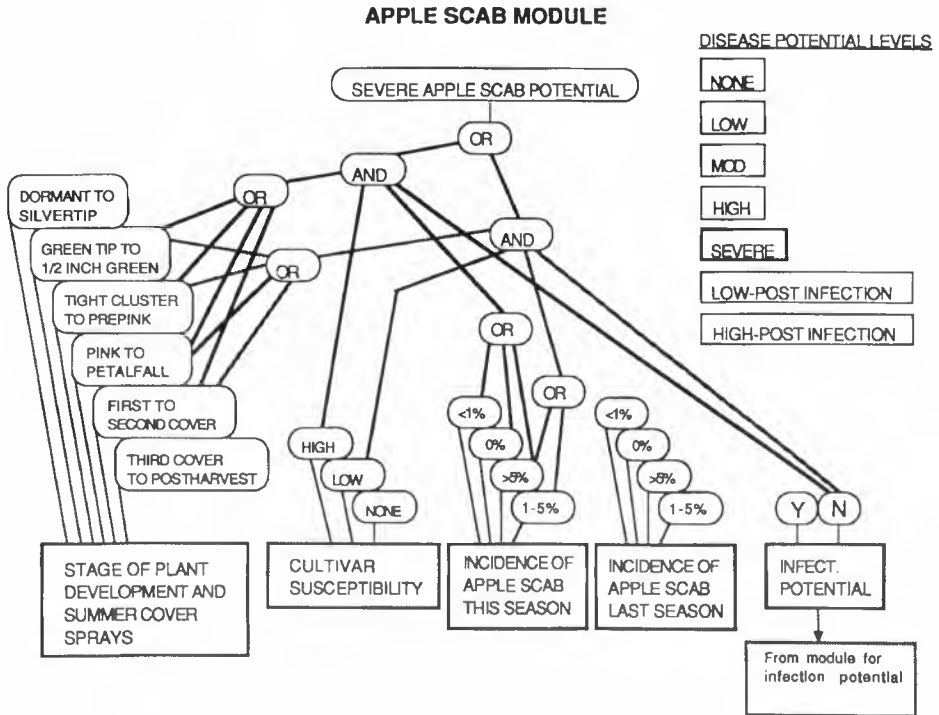


Figure 1. Dependency network of factors which contribute to severe apple scab potential

**Powdery Mildew**

There are three potential levels for powdery mildew in the PSAOC: none, low or high. The fewer disease potential levels for powdery mildew compared with apple scab reflects the lower impact of powdery mildew as an apple pathogen in Pennsylvania orchards. The factors which describe powdery mildew are stage of development, cultivar susceptibility and disease severity during the current and last season. In the orchard profile the grower is asked to indicate the severity of mildew in the orchard, last year's severity if the stage is prior to pink and for this year's severity if later than the pink stage. For example, high powdery mildew potential would be expected from tight cluster stage to second cover with high cultivar susceptibility and more than one terminal per tree showing mildew symptoms. The disease potential scenarios are represented in dependency networks as previously described in the apple scab network.

### **Cedar Apple Rust**

The cedar apple rust potential is also described in three levels - none, low, or high. The factors which describe each level are stage of growth, cultivar susceptibility, infection period and incidence in the recent past. Calculations for infection period are based on Aldwinckle et al. In Pennsylvania orchards, cedar apple rust may be never a problem, occasionally a problem, or frequently a problem. A frequent problem usually indicates that the alternate host (*Juniperus* spp.) is nearby. A 'high' cedar apple rust potential exists at pink to petal fall if the cultivar is highly susceptible, there has been an infection period and the disease was occasionally to frequently a problem in the orchard.

### **Chemical Management Module**

The chemical management module also functions at several levels - i) the chemical selection module, ii) the chemical rate module and iii) the spray interval module.

### **Chemical Selection Module**

The registered insecticides, acaricides and fungicides effective for control of the pests identified as potential problems by the pest modules in the expert system are described within the dependency networks. Critical factors which contribute to the selection of a chemical are: growth stage of trees, the array of insects over threshold, disease levels in the orchard, pathogen infection potential since the last spray, pesticides applied in the last application, known fungicide or insecticide resistance, number of days until harvest of the crop. As an example, appropriate use of benomyl for the control of apple scab is described in a dependency network. Two basic scenarios describe its use pattern: use as a protectant fungicide or use in a post-infection situation. As a protectant, benomyl is recommended from green tip to second cover, when apple scab potential is severe to moderate, there is no infection potential since the last application, a DMI fungicide was not applied in the last application, resistance to benomyl has not been found in the orchard and the harvest date is more than 7 days from the present. The postinfection scenario is the same except that an infection period has occurred within the last 24 hours.

### **Chemical Rate Module**

After the chemicals have been selected the rate of the chemical to be used is determined. For example, practices to control scab early in the season generally include two or more chemicals to prevent resistance and take advantage of multiple modes of action, but late-season fungicides, generally protectants, are recommended alone. A high rate is recommended from tight cluster through prepink, when the apple scab severity level is highest and the recommended chemical used in combination with another fungicide. A table containing rates of each chemical is built into the system, but those for myclobutanil are an exception; myclobutanil is labelled for use according to tree row volume and disease potential. Tree row volume is automatically calculated from information in the profile.

### **Spray Interval Module**

The date of the next pesticide application is determined by stage of growth in the orchard, disease potential rating, occurrence of an infection period since the last spray, grower's spray interval preference, rainfall since the last application and the chemical applied in the

last application. Early in the season, application timing is mandated by requirements to control apple scab. After second cover, requirements for insect control and weather conditions that affect summer diseases of apple dictate timing. Grower preferences are influenced by their ability to spray an orchard at short notice and spray on a regular schedule in order to manage labor. This option allows growers to adjust spray intervals according to pest and weather circumstances or to plan applications based on labor requirements. If a routine schedule is selected, spray intervals will be adjusted if needs for post infection applications occur. The effect of rainfall on chemical residue is based on the rule-of-thumb that less than 2.5 cm of rain does not affect spray residue, 2.5 to 5.0 cm reduces spray residues by one-half and 5.0 cm or more of rain will remove all spray residue. Sometimes the next pesticide application may be recommended within 5 days of the last application in the early season even if the disease potential is low, if there has been no infection potential since the last application, there has been 2.5 to 5.0 cm of rain since the last application, a DMI fungicide was not applied following an infection period and the last application was applied as an alternate side application. All possible scenarios which describe situations which lead to spray intervals are described within dependency networks.

#### **Compatibility and Days-To-Harvest**

After selection, pesticides are checked for compatibility before a recommendation is given. Dependency networks are used to describe which chemicals should not be mixed, and they are checked against the computer's calendar to determine the interval between application and harvest.

#### **A Typical Session with PSAOC**

From the menu on the start-up screen the user can gain access to any module. The pest management program can be initiated either directly from the orchard profile, in which case all profile information will automatically be loaded into the program, or the user will be asked whether a profile needs to be loaded. Typically, an orchard contains many "blocks" or "management units" for which slight differences in management are appropriate. In such a case each block would have its own profile. The user can either choose a previously defined profile or create a new one. The user has the option of looking at an individual pest problem or running the IPM module, which considers the entire orchard block as a system and integrates disease and insect recommendations. For the disease modules, PSAOC first determines the disease potential in the block for apple scab, powdery mildew, cedar apple rust and summer diseases. PSAOC identifies the fungicides that are available to control scab under the circumstances and asks the user to identify preferences. Once the primary scab fungicide is selected by the user, the system lists the compatible fungicides which are recommended to prevent resistance build-up and provide additional control of powdery mildew or rust if necessary. Similar questions are asked about mites, insects and predators.

The recommendation is then given for each chemical selected. In addition, spray incompatibility warnings are displayed. After viewing the recommendation the user has the option of selecting other chemicals, which results in a new IPM recommendation. At this time, the user may also ask for an explanation of how the recommendation was derived. PSAOC then reviews each aspect of the decision making process. The user can also request detailed information about any one of the chemicals or pests included in the recom-



mentation. The user also has the options of printing the recommendation, explanation and profile information.

## LITERATURE

Aldwinckle, H.S., R.C. Pearson & R.C. Seem 1980. Infection periods of *Gymnosporangium juniperi-virginianae* on apple. *Phytopathology* 70: 1070-1073.

Jones, A.L., S.L. Lillevik, P.D. Fisher & T.C. Stebbins 1980. A microcomputer-based instrument to predict primary apple scab infection periods. *Plant Dis.* 64: 69-72.

Mills, W.D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab.

Travis, J.W., E. Rajotte, R. Crassweller, K.D. Hickey & L.A. Hull 1990. Penn State Apple Orchard Consultant. Copyright by The Pennsylvania State University, University Park, PA 16802.

Travis, J.W., R.M. Crassweller, E.G. Rajotte, L.A. Hull, K.D. Hickey, G.M. Greene, J. Halbrecht, M.C. Brittingham, J. Kelly, W. Hock, P. Heinemann, D. Daum, G. Clarke, J. Harper & J.D. Becker 1990. Tree Fruit Production Guide. The Pennsylvania State University, University Park, PA 16802. 160 pp.



# Scab warning beyond the Mills table

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About 70 to more than 90% of chemical input in top fruit growing are fungicides, of which the highest proportion is used for scab control. Checking the present strategies that are applied to control scab there is a high potential to reduce the input of scab fungicides.

In practice scab control is mainly carried out with a calendar spray scheme using more or less fixed spraying intervals. These intervals are on most farms influenced only by a rain gauge. Just a few farms have been using scab warning devices within a decentralized scab warning system. All the devices used are based only on the Mills table or some modified versions. This system, where the scab warning devices are run by the farmers did not significantly reduce the number of sprays against apple scab.

One reason is the Mills table itself because it is just a mathematical correlation of the two physical parameters temperature and duration of leaf wetness, indicating only whether conditions for infection are favourable or not. It ignores the behaviour of host and pathogen completely. Additionally the Mills table is not describing the process of infection correctly. Its safety is derived from calculating too many fulfilled physical conditions for infection that are constantly misinterpreted as successful infections. This is proved by information from South-Tyrol, where only about 20% of the "infections" show lesions. The Mills table's over-reaction is also proved by its modifications in the world that all seem to work properly. This is only possible because all of the present scab warning devices indicate too many infection periods. The results are too many sprays.

A second reason are problems of the farmers in interpreting the results obtained from scab warning devices. This even increases the general trend to spray in case of doubt if assistance by an advisory board is missing.

All these factors led to the idea of operating a new centralized scab warning system that was first introduced in our cooperative in 1989.

## SYSTEM

1. Ascospore release is monitored every single day within every rainy period from bud break until spore release is terminated. Therefore a sporetrap based on microscope slides above heavily infected leaves to have a worst case situation is used.
2. Measurement of leaf growth is done twice a week.
3. Infection conditions are calculated with data from a net of 6 modem-linked CAMPBELL weatherstations maintained only by the advisory board. They measure

air temperature, relative humidity, leaf wetness, rainfall, radiation and windspeed in 12 minute intervals. Data are collected via modem and stored on a computer hard disk. With a separate scab programme infection conditions for ascospores are calculated according the infection curve of MacHardy including day-night periodicity of ascospore release and a minimum rainfall to start spore release. Dew periodes are ignored for ascospores dissemination.

4. An fungicide application within this system is necessary, if there is more increase in leaf area since the last spray than it could protect and a medium or high ascospore release and favourable conditions for infection. These three parameters are checked at any rainy periode from bud burst until ascospore release is terminated.
5. A first orchard check on visible lesions after the end of ascospore release determines the strategy for scab control in the secondary season. A second check is done at the begin of August. These checks follow the principles of van der Scheer. If the threshold levels are exceeded a protectant scheme is applied where protectant fungicides are sprayed shortly before the onset of a rainy period to keep increase in leaf area between spray and begin of rain as little as possible.
6. A scab report is created after every rainy period with information on ascospore release, leafgrowth, infection conditions, an interpretation of these parameters and also advice for the farmer what action he should take. Further information about the actual pest situation and a weather forecast are also added to the report.
7. Reports are mailed by fast electronic information systems (Fax and Videotext). For Videotext the availability of the report in the system is guaranteed from 1300 hours onwards if there is any at a day.
8. Creating and mailing of the report must consume as little time as possible (< 3 hours with Videotext).

### **Restrictions**

All orchards with vigorous growth from heavy pruning and especially regrowth after early summer pruning are excluded from this system because most of these orchards have continuous scab problems. These are mainly caused from infections that occur after harvest on shoot tips that form no terminal bud. Around and after bud burst the lesions on these shoots produce a high amount of conidia close to the new tissue. Therefore infections from ascospores are of minor importance for the epidemiology in these orchards.

### **Requirements**

A major requirement for a sucessfull scab control is a low inoculum orchard. Besides a non-vigorous tree all measures that reduce inoculum in an orchard are fundamental in order to apply new scab strategies resulting in reduced number of applications and lower input of chemicals. Therefore sanitary treatments such as shredding leaves after fall and/or applying liquid cyanamid before bud burst to reduce the ascospore inoculum to the highest possible extent are of basic importance for this new system.

RESULTS

1. In the Lake Constance area ascospore release starts between two and four weeks after bud break and is terminated soon after petal fall. The highest releases are detected between green cluster and full bloom (Fig. 1).

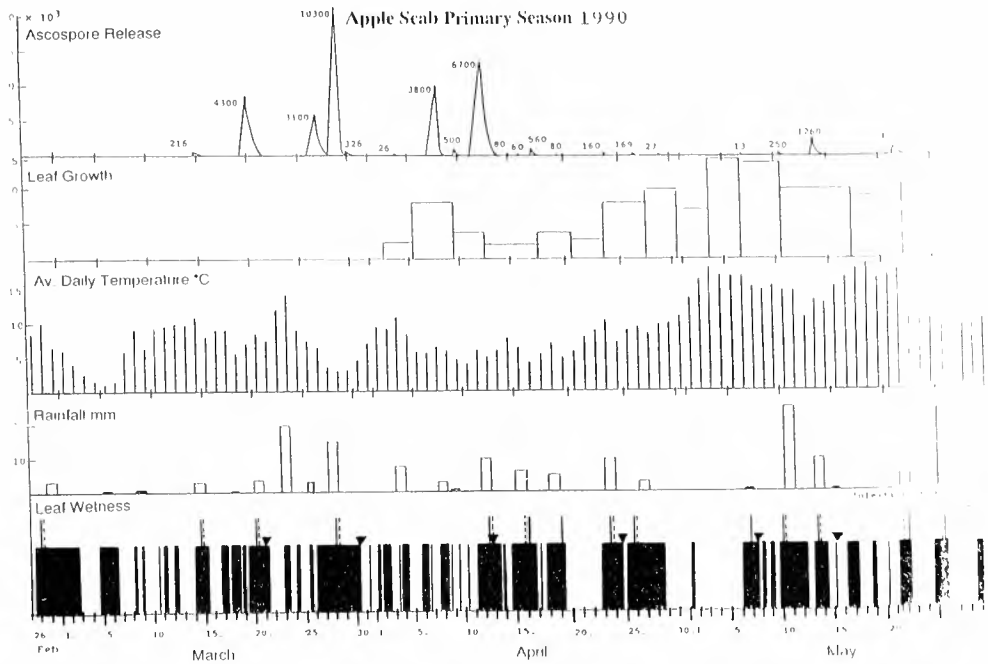


Fig. 1. Records of weather data, leaf growth and ascospore release in 1990

2. High releases are mainly detected after warm periods with heavy leaf growth.
3. After a substantial release at the begin of a rainy period there are only very small releases during a long wetness period.
4. Relative ascospore release is approximately equal in areas with a temporary equal distribution of rainy perodes.
5. There is almost no difference in number and timing of infection conditions from the west to the east of the lake area despite a difference in rainfall of about 800 mm per year. Annual rainfall is about 1.500 mm in the east and 700 mm in the west in the Lake Constance area.
6. With the new system the 15 to 18 scab treatments that are standard in Lake Constance area were cut down to between 4 and 7 applications, depending on the season (Table 1).

Table 1. Input of chemicals

The new system			Conventional		
Spray no.	Date	Products and rates/ha	Spray no.	Date	Products and rates/ha
			1	6.3.	Delan SC 750 500 ml
			2	15.3.	Delan 500 + Rubigan 200 ml
1	21.3.	Delan 350 ml + Benocap 80 g	3	22.3.	Delan 500 ml + Rubigan 200 ml
2	30.3.	Delan 350 ml + Benocap 80 g	4	31.3.	Delan 500 ml + Rubigan 200 ml
			5	9.4.	Delan 500 ml
3	12.4.	Delan 350 ml + Benocap 80 g	6	22.4.	Delan 500 ml
4	24.4.	Delan 350 ml + Baycor 600 g	7	27.4.	Delan 500 ml
			8	5.5.	Dithane ultra 2 kg
5	7.5.	Delan 350 ml	9	12.5.	Dithane ultra 2 kg
6	15.5.	Dithane ultra 1 kg + Benocap 80 g	10	17.5.	Dithane ultra 2 kg
			11	26.5.	Dithane ultra 2 kg
7	1.6.	Delan 350 ml	12	5.6.	Delan 500 ml + Polyram 1 kg
			13	11.6.	Delan 500 ml + Polyram 1 kg
			14	25.6.	Delan 500 ml + Benocap 125 g
			15	7.7.	Delan 500 ml
			16	18.7.	Delan 500 ml
			17	30.7.	Delan fl. 1 l

7. By fast processing and transmission the information is available for the farmer before he starts to spray for safety.
8. Saving of chemicals can reach 80% from a combination of less applications and lower rates per hectare by low volume spraying technique.
9. Financial savings can reach up to DM 1000.- per hectare and year (Table 2).

#### DISADVANTAGES OF THE SYSTEM

1. Calculation of infection conditions with any infection curve is too static. This process needs a dynamic model that can handle varying ascospore releases too.
2. The system in its actual structure is mainly based on curative EBI fungicides that are sprayed after infection. This continuous use of EBIs raises the risk of resistance. The addition of protectants has no influence on the spores that have already infected so that the EBI is the only compound controlling all these spores. Therefore the selection pressure of the EBI is not reduced. The addition of protectants does also not reduce the risk of resistance for the next spore release since after a rainy period bright weather with heavy leaf growth is following. At the beginning of the next rainy period the young new tissue is in most cases no more protected.

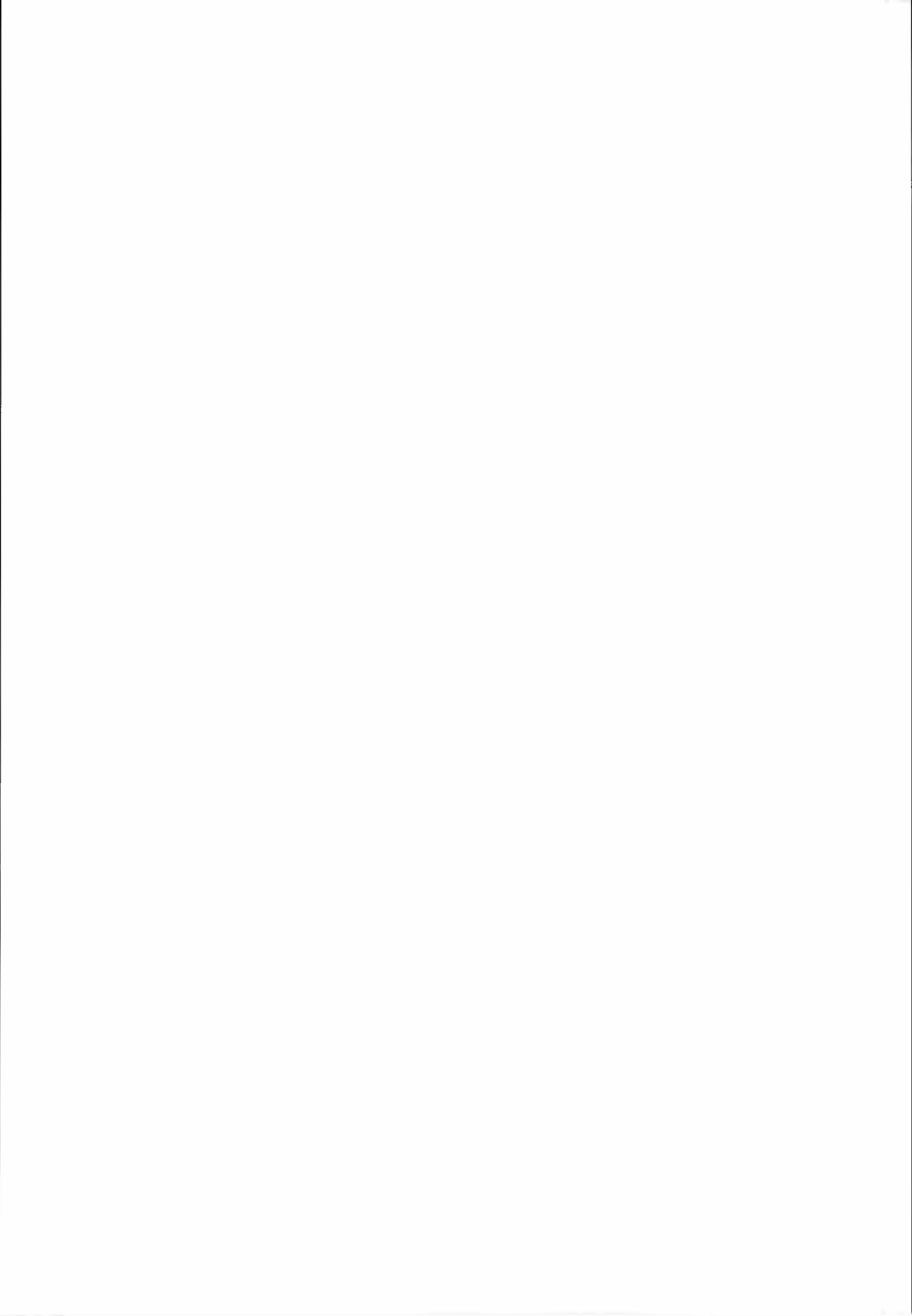
Table 2. The financial and environmental savings (1990)

<b>Treatments</b>	<b>The new system</b>	<b>Conventional</b>
<b>Chemicals/ha/a</b>	<b>7</b>	<b>17</b>
Delan SC 750	2.11	6.01
Delan fl.	-----	1.01
Dithane ultra	1.0 kg	9.0 kg
Polyram Combi	-----	2.0 kg
Benocap	320 g	125 g
Rubigan	-----	800 ml
Baycor	600 g	-----
<b>Total consumption/ha/a</b>	<b>4.02 kg</b>	<b>18.93 kg</b>
<b>Environmental pollution</b>	<b>21%</b>	<b>100%</b>
<b>Costs</b>		
Chemicals	DM 319.24	786.06
Spraying	DM 300.00	900.00
Total Costs	DM 619.24	1686.06
<b>Financial savings/ha</b>	<b>DM 1070.00</b>	

3. A curative spray is the last chance to control a scab infection.
4. The potential of protectant fungicides is only used partly.
5. Bad weather can make it impossible to spray before the curative action period of the EBIs is expired.
6. The system is labour intensive.
7. With the Mills table and all other infection curves prediction is impossible. They just calculate historic data.
8. The system does not work in orchards with scab problems caused by overwintering mycelium on the trips.
9. It is difficult to detect orchards that are not suitable for the system.

## OUTLOOK

Because of the disadvantages, in the future an electronic system needs to be developed that forecast ascospore release in order to replace as many curative sprays as possible by protectants.





# Practical experience with the Ventem™ system for managed control of apple scab in the United Kingdom

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In an unsprayed apple orchard (cv. Cox), the accuracy of the HRI East Malling PC-based apple scab (*Venturia inaequalis*) warning system - Ventem™ - in producing warnings of scab infection periods was compared to the U.K. Mills'-based system and to Smith Periods (U.K. Meteorological office). Disease was monitored weekly in 1991 and twice weekly in 1992, by recording the appearance of new scab lesions on selected apple rosette leaves up to petal fall and thereafter on selected extension shoots. During the early the season from March to May in both 1991 and 1992, Ventem™ proved more accurate than the Mills' system in providing warnings of scab infection periods. The Smith Periods occurred at similar times to Ventem™ warnings, but were more numerous and overestimated risk. Attempts were made to evaluate strategies for making practical use of the scab warnings by comparing scab levels in apple plots sprayed routinely with those sprayed using a managed programme. At harvest, scab levels on the fruit were very low and commercially acceptable under both strategies. In 1991 fewer sprays were applied to managed plots compared to routinely sprayed plots, although in the following year managed plots needed more sprays for scab control. Alternative strategies for managing fungicide use in response to scab warnings are discussed in relation to UK fruit farms.

Key words: Apple scab, disease management, disease warnings, fruit, fungicides, infection periods, orchard diseases.

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The main UK apple varieties Cox (dessert) and Bramley's Seedling (culinary) are susceptible to apple scab (*Venturia inaequalis*). The market requirement for high-quality blemish-free fruit, and the difficulties of scab control once the disease has become established in the orchard, has led to the adoption of routine fungicide sprays from bud burst to May/June at 7-14 day intervals to achieve control. Such schedules, though costly, have proved successful, reliable and simple for farm managers to operate. However, with the increase in public awareness of chemicals and their possible side effects on health and the environment and the increasing costs of chemicals, such routine fungicide programmes

are no longer acceptable. For powdery mildew (*Podosphaera leucotricha*) control it is possible to reduce fungicide use with little risk to fruit quality by matching fungicide rate to the level of secondary mildew assessed in the orchard (Butt & Barlow 1979). In the case of scab, however, the interval between infection and visible colonies can be considerable, particularly during the early part of the season, making assessment of scab levels difficult. However, savings in scab sprays can be made by making use of warnings that conditions have been conducive to infection by *V. inaequalis*, to better time sprays or to omit sprays at times of low risk. At present in the UK, the National Meteorological Office produce regional warnings of apple scab based on Smith Periods, using relative humidity in place of leaf wetness (Preece & Smith, 1961). Such warnings, though useful as a guide, are not local enough to be used reliably in reducing sprays. A few growers make use of apple scab warnings generated by automatic weather stations (e.g. Metos<sup>®</sup>) sited on the farm. These systems at present generally use Mills' model (Mills & La Plante 1954) or MacHardy's modified Mills' system (MacHardy & Gadoury 1989) to generate apple scab warnings. At present in the UK, very few growers make use of apple scab warning systems.

Ventem™, developed at Horticulture Research International (HRI) East Malling is a new PC-based scab warning system designed for operation on fruit farms (Butt et al. 1992; Xu & Butt 1993). The system is best operated with a Metos<sup>®</sup> automatic weather station, manufactured by G. Pessl, Weiz, Austria. For such a system to be adopted by UK growers as an aide to reducing fungicide use, it has to be demonstrated that the system is accurate in identifying scab infection periods. In addition, practical strategies for making use of the scab warnings without putting the apple crop at risk must also be demonstrated. In the UK, size of fruit farms varies considerably but on average is relatively large compared to European farms. Labour inputs are minimised to save costs, such that several days may be required to complete spraying of all orchards. This factor, combined with the unreliable British weather, makes it difficult to operate quick responses to scab warnings. In addition, spraying in response to warnings requires the use of materials with curative properties, which could lead to the over-use of DMI fungicides (e.g. myclobutanil, pyrifenoxy, etc.) and the risk of fungicide resistance or reduced sensitivity, already demonstrated in some parts of Europe for this chemical group (Stanis & Jones 1985; Thind et al. 1986). Thus, making practical use of scab warnings is not without difficulties. The purpose of the study described here was therefore firstly to validate the accuracy of Ventem™ in identifying scab infection periods, and secondly to demonstrate practical ways of making use of such information. The results of the first two years of a longer-term study are described.

## MATERIALS AND METHODS

In replicated large plots (17 x 12 trees) of apple cv. Cox (M9 rootstock), the disease control achieved by a routine programme of fungicide sprays applied from bud burst at 10 day intervals until the end of June (routine programme), was compared with that achieved in plots where the spray interval and fungicide choice were adjusted according to scab warnings generated by the Ventem-Metos System (managed programme). Both treatments were replicated twice in 1991 and three times in 1992 in a randomised complete block design. In the routine-treated plots the fungicides dodine and myclobutanil were used pre-

blossom and myclobutanil, dithianon and captan used post blossom. Choice of fungicide was similar in the Ventem-managed plots as far as possible. The levels of scab on trees were assessed frequently, pre-blossom on rosette leaves and post-blossom on fruitlets and extension shoots. At harvest, 1000 fruit were picked per plot and assessed for scab.

Plots untreated with scab fungicides were also included, although not as part of the experimental design, as a comparison and also to validate the Ventem warnings. The appearance of new scab lesions was recorded during once - or twice - weekly inspection of rosette leaves on selected branches pre-blossom and of leaves on selected extension shoots post-blossom. Using data of temperature x incubation time for scab (Mills 1944), the time of appearance of scab lesions after a given Ventem scab warning was estimated and compared to the time of appearance of new scab lesions in the untreated plots. Scab warnings generated by Mills' system (East Malling) and Smith Periods generated by the nearest national meteorological station at Herstmonceux, East Sussex (45 km away) were also recorded and compared to Ventem.

## RESULTS

Table 1 shows the dates of scab infection periods recorded by Mills' model and the Ventem system at East Malling and Smith Periods recorded at Herstmonceux for March - May 1991, in relation to the estimated and actual time of appearance of new scab lesions on rosette leaves. In 1991, the start of the trial was delayed and first observations were not made until late April. Scab was recorded on the first inspection on 24 April. The single lesion observed was old and had obviously been present for some time. The earliest scab periods recorded as Smith Periods and by Ventem were around 17 March which can be related to the scab observed on 24 April. Mills' system did not record any scab periods in March. Scab periods recorded by all three systems in late April can be related to the new scab lesions recorded on rosette leaves between 10 and 31 May.

Table 1. Dates of apple scab infection periods recorded by Mills (East Malling), Smith (Herstmonceux) and Ventem (East Malling) systems. Estimated and actual appearance of scab lesions on apple leaves March - May, 1991

Date of scab infection periods			Estimated date of symptoms	Date new scab lesions were recorded (number of lesions)
Mills	Smith	Ventem		
---	16-19 Mar	17 Mar	5 April	24 April (1 old lesion)
29/30 April	28-30 April	28 April	16 May	10 May (51) 21 May (59) 31 May (92)

In 1992 (Table 2), the earlier part of the season was much wetter than in 1991 with several scab infection periods recorded. The first scab lesion observed was noted on 21 April, which can be accounted for by infection periods recorded by all three systems on 30 March. Similarly, new scab lesions that were recorded between 1-8 May on rosette leaves can be accounted for by infection warnings generated by all systems on 14-17 April. New scab lesions continued to appear from 11-18 May on rosette leaves and 26 May-1 June on extension shoot leaves. Infection periods were recorded by Ventem and Smith between 24-30 April and 9-13 May, respectively, which could account for the new scab lesions observed. The next infection period recorded by Mills' system was not until 28 May.

Table 2. Dates of scab infection periods recorded by Mills (East Malling), Smith (Herstmonceux) and Ventem (East Malling) systems. Estimated and actual appearance of scab lesion on apple leaves March - early June 1992

Date of scab infection periods			Estimated date of symptoms	Date new scab lesions were recorded (number of lesions)	
Mills	Smith	Ventem			
30 Mar	30 Mar	29/30 Mar	20-23 April	21 April (1 new lesion) 23 April (36) 27 April (23)	Rosette leaves
14 April	14 April	17 April	1-3 May	1 May (36) 5 May (30) 8 May (42)	Rosette leaves
---	24 April	24 April	11-15 May	11 May (47)	Rosette leaves
---	26 April	26 April		15 May (6)	
---	28 April	27 April		18 May (521)	
---	30 April	30 April			
---	9 May	9 May	22 May	26 May (89)	Extension shoot leaves
---	12 May	12 May		29 May (8)	
---	13 May			1 June (7)	
				5 June (0)	

In both 1991 and 1992 scab periods recorded in the later part of the season, in June and July, were more numerous and more difficult to relate to the appearance of particular groups of new scab lesions.

In 1991, the start of the study was delayed such that first fungicide sprays were not applied until 25 April (approximately 3 weeks after bud burst) in the routine plots and 7 May in the managed plots. Apple scab was therefore already established in the plots, with 10-15 per cent of leaf rosettes infected by 15 May (Table 3). Subsequent treatments prevented spread to extension shoot growth and resulted in low incidence of scab on fruit at harvest. Scab developed steadily in the unsprayed plots (Table 3), resulting in 35 per cent of fruit infected at harvest and over 70 per cent of shoots infected prior to leaf fall. Fungicide usage in 1991 (Table 4) was similar for routine and managed plots in the early part of the summer. No further scab fungicides were applied to the managed plots after the

end of July as the scab risk determined by Ventem was minimal. Two additional fungicide sprays were applied to routine plots in late July and August. Therefore in 1991, use of the Ventem system to manage spray frequency resulted in a reduction in fungicide use.

Table 3. Scab incidence (% infected) in 1991 in unsprayed plots and where fungicide sprays were applied either routinely or managed according to spray warnings generated by Ventem

Treatment	Leaf rosettes	Apple shoots	Apple shoots	Fruit	Apple shoots
	15 May	11 July	21 August	26 September	28 October
Routine	10.2	0	0	0.3	37.5
Managed	15.5	0	0	2.4	12.5
Unsprayed	NA*	41.7	44.2	35.4	70.9

\*NA = not assessed

Table 4. Fungicide treatments to routinely sprayed and managed plots in 1991

Fungicide	Active Ingredient	Number of Sprays	
		Routine	Managed
Sythane	myclobutanil	3	2
Sythane + Dithianon	myclobutanil + dithianon	0	4
Sythane + Karamate	myclobutanil + mancozeb	2	0
Dithianon	dithianon	4	1
Total spray applications		9	7

In 1992, scab incidence in the unsprayed plots was higher than in 1991 (Table 5) with over half the fruit infected at harvest. Fewer scab sprays were used during the early part of the season in the managed plots compared to the routine plots, but successive wet days in late June and early July indicated a high scab risk as determined by Ventem, and so two extra sprays were applied to managed plots which were not applied to routine plots (Table 6). Thus in 1992, fungicide usage on managed plots was greater than in routine sprayed plots. Although scab incidence in both the routine and managed plots was maintained at a low level throughout the season, levels were higher in the routine plots.

### 300 Using Ventem™ for apple scab control

Table 5. Scab incidence (% infected) in 1992 in unsprayed plots and where fungicide sprays were applied either routinely or managed according to spray warnings generated by Ventem

Treatment	Trees	Apple shoots	Apple shoots	Fruit	Apple shoots
	8 May	17 June	3 July	14 September	September
Routine	2.2	1.1	0	0.2	6.4
Managed	0	0	0	0.6	2.0
Unsprayed	76.7	43.3	38.4	51.1	91.1

Table 6. Fungicide treatments to routinely sprayed and managed plots in 1992

Fungicide	Active ingredient	Number of sprays	
		Routine	Managed
Radspor	dodine	3	1
Systhane	myclobutanil	3	3
Systhane + Dithianon	myclobutanil + dithianon	2	2
Dithianon	dithianon	0	3
Captan	captan	0	1
Total spray applications		8	10

## DISCUSSION

Compared to the standard Mills' system, Ventem more accurately recorded scab infection periods during the early part of the season in both 1991 and 1992. Smith Periods recorded similar dates for scab infection periods as did Ventem, but the periods recorded were more numerous e.g. seven Smith Periods compared to two for Ventem during March-April 1991. This suggests Smith Periods, which are based on relative humidity rather than leaf wetness as in Ventem and Mills' system, probably over-estimates risk but, early in the season Smith Periods appear to provide better warnings than the existing Mills' system.

When evolving a practical use of a new disease risk assessment system such as Ventem, it is vital that the approach in the early stages of the study is cautious, so that operators become familiar with the system before tackling more ambitious approaches to practical use, thus avoiding loss of confidence in the system by potential users. Thus in the initial part of the study, information generated by Ventem on infection periods was used to adjust the spray interval and fungicide choice. Following this approach resulted in a reduction in fungicide use in Year 1 but justified increased use in Year 2 of the study. Curative spraying according to Ventem risk assessment offers the best strategy for maximum reduction in spray use, but also the greatest risk to the crop and the greatest practical problems. Other strategies with less risk to the crop include routine sprays at identified key times such as bud burst and petal fall, with sprays applied according to Ventem-determined risks at other times. In 1993, this latter strategy of spray management will be compared with a routine protectant strategy and a curative spray strategy.

REFERENCES

Butt, D.J. & G.P. Barlow 1979. The management of powdery mildew: a disease assessment method for growers. Proceedings 1979 British Crop Protection Conference- Pest and Disease, pp.77-86.

Butt, D.J., G. Van Santen, X.M. Xu & K.B. Stone 1992. VENTEM™ - an apple scab (*Venturia inaequalis*) infection warning system, Version 3.1. Computer Software and Manual.

MacHardy, W.E. & D.M. Gadoury 1989. A revision of Mills criteria for predicting apple scab infection periods. *Phytopathology* 79: 304-310.

Mills, W.D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab. *Cornell Ext. Bull.* 630. 4 pp.

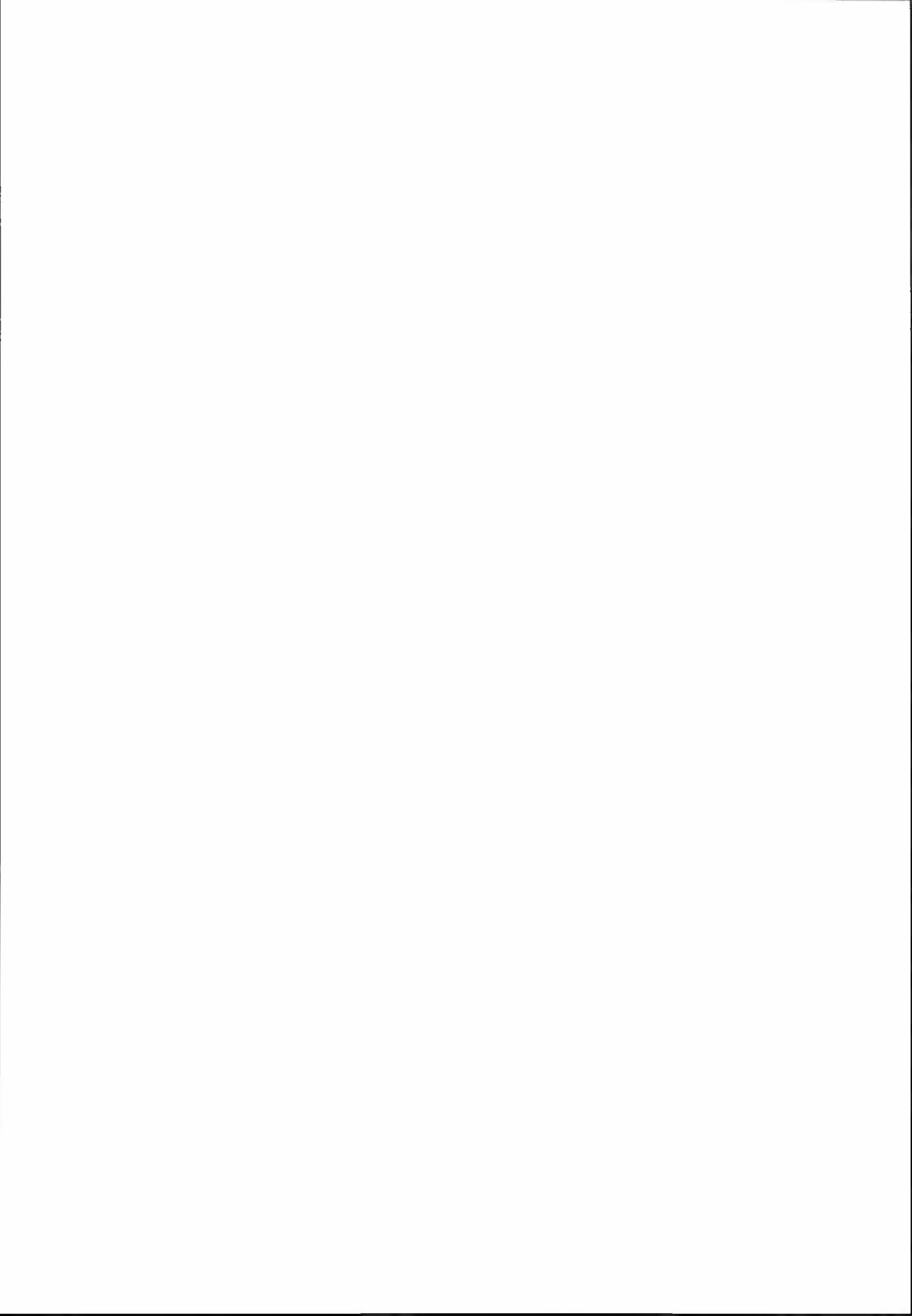
Mills, L.D. & A.A. La Plante 1954. Diseases and insects in the orchard. *Cornell Ext. Bull.* No.711: 20-22.

Preece, T.F. & L.P. Smith 1961. Apple scab infection weather in England and Wales, 1956-60. *Plant Pathology* 10: 43-51.

Stanis, V.F. & A.L. Jones 1985. Reduced sensitivity to sterol-inhibiting fungicides of field isolates of *Venturia inaequalis*. *Phytopathology* 75: 1098-1101.

Thind, T.S., M. Clerjeau & J.M. Olivier 1986. First observations on resistance in *Venturia inaequalis* and *Guignardia bidwellii* to ergosterol-biosynthesis inhibitors in France. In: Proceedings British Crop Protection Conference - Pest and Disease, 4C-1 p. 491-498.

Xu, X.M. & D.J. Butt 1993. PC-based disease warning systems for use by apple growers. *EPPO Bulletin* 23: 595-600.





# Canker causing fungi of apple wood in Pennsylvania

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The purpose of this research was to identify the causal agents responsible for the increased number of cankers in Pennsylvania apple orchards. Cankered wood was collected from orchards throughout the state. Both *Botryosphaeria obtusa* (black rot) and *B. dothidea* (white rot) were isolated from the cankers. In 1990, standard apple trees were inoculated using both of the pathogens, however, cankers were slow to development and canker size was small. In 1991-92, naturally occurring cankers of different types were observed on dwarfed, trellised trees at the Horticultural Research Farm. A *Phomopsis* and *Cytospora* species and an unidentified fungus were isolated from these cankers. Field inoculations on trellised trees using four of the fungi isolated from cankers were performed from 1991-92. Results indicate differences in cultivar susceptibility to the fungi, however, the growth stage of the tree did not affect the development of the cankers for any of the fungi inoculated. Our research indicates that several fungi are involved in the canker-complex and that the initiation and development of cankers is probably dependent upon environmental conditions and tree health.

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In 1989, as a result of grower concern, we began investigating the cause of an increased incidence of apple wood cankers in Pennsylvania orchards. Initially, these cankers were referred to as "black rot" cankers which are usually found on older trees and seem to originate from injuries such as pruning wounds. They normally begin as a tip-dieback which progresses down the branch and eventually girdles and kills the branch. Growers were very concerned because they were seeing these cankers on younger, more vigorously growing trees.

"Black rot" cankered wood was collected from problem orchards throughout Pennsylvania. Isolations were made and the pathogens were identified. Results indicate that black rot, *Botryosphaeria obtusa*, as well as white rot, *B. dothidea*, were present in cankered wood (5). Black rot and white rot cankers look identical in the field and it is impossible to determine which pathogen is responsible for infection. Positive identification can only be made by laboratory isolations and conidial morphology. To determine when

infections occur and how these fungi spread, field inoculations using both pathogens were conducted in 1990.

## MATERIALS AND METHODS

Field inoculations were made on 20-year old standard apple trees which included the Delicious cultivars Redspur and Topred. Trees were wounded by pruning cuts. Spore suspensions of each pathogen were sprayed onto each cut surface. A total of 260 limbs were inoculated throughout the growing season. After one year of observations, cankers caused by both pathogens had formed on wood, but cankers were small (1.3 cm), and they were not typical of the cankers observed on trees in the orchards (6). Many of the inoculated pruning wounds had callused over and healed.

In 1991, isolations were made from naturally occurring cankers on dwarfed, trellised trees at the Horticultural Research Farm. These cankers were atypical of the cankers caused by the white rot or black rot pathogens observed in orchards.

In 1991-92, dwarf apple trees were inoculated with black rot, white rot, and other fungi isolated from the naturally occurring cankers. Our objective was to inoculate apple trees throughout the year with canker causing fungi to determine if trees are more susceptible to canker initiation and elongation at certain times of the year. The remainder of this paper describes the 1991-1992 research project.

### **Isolations from naturally occurring cankers**

Isolations were made from naturally occurring cankers on several cultivars and at various locations in the orchard at the Horticultural Research Farm at Rock Springs, Pennsylvania, beginning in May, 1991. The cankers were on the trunks and scaffold limbs of trees. They were not associated with pruning wounds or any other visible injury. The predominate type of canker observed was salmon in color, elliptical to irregular in shape, and as they aged, the bark around the margin of the canker became rough and cracked which eventually peeled off. This type of canker we referred to as "rough bark." A second type of cankered area was observed around bark inclusions on the tree. These were dark, raised, cankered tissue in various stages of development. A third type of canker was found on large dead or dying scaffold limbs. These cankers began as a die-back, were large, covering most of the scaffold limb beginning at the tip, extending almost to the trunks of the trees, and often were orange in color.

Isolations from all canker types were made from several trees of three cultivars. The cultivars included Reinette du Canada (a yellow apple) and Jonathan which were planted in 1975 on M9 rootstock and Golden Delicious planted in 1968 on EM IX rootstock. Cankers on Reinette du Canada were of the rough bark type. Those on Jonathan were of the bark inclusion type and those on Golden Delicious were of the third canker type characterized by a large, dead area on scaffold limbs. Several isolations from each canker were made. Wood pieces were taken from the canker surface near the center, near the margin, and one inch beyond the visible canker in presumably healthy tissue. When bark was peeled from cankered areas, diseased wood, characterized by dark brown, necrotic tissue, was observed. Isolated wood samples were taken back to the laboratory and surfaced

sterilized with a chlorox solution and/or 70% alcohol to eliminate contaminants. These were then placed in plates containing the artificial media cornmeal agar. All plates were placed in a growth chamber at 26°C under continuous lighting. When fungal growth and sporulation was observed, the fungi were identified.

### Results of isolations

Two different fungi with distinct spore types were isolated from the large cankered limbs and from the cankered bark inclusion areas of the cultivar G. Delicious. *Cytospora* spores were isolated from the large, dying limbs. The *Cytospora* conidia measured 5 x 1.3 microns. *Phomopsis* spores were isolated from the bark inclusion areas with conidia measuring 7.5 x 3.8 microns. From the cultivars Jonathan and Reinette du Canada, on the rough bark cankers, a fungus which produced a very small, oval spore type measuring 5 x 2.5 microns was consistently isolated. This fungus was referred to as Unknown I because it has not been identified as of this writing. This unknown fungus produces a distinct gray-black, shiny fruiting body on the surface of cornmeal agar. Pure cultures of all isolates were obtained and used for future field inoculations. *Cytospora* was not included in the inoculations. Since it was recovered from a dead limb, it may not have been the causal agent of the limb death.

### Field inoculations over time

Preliminary field inoculations were conducted in May, 1991 on one tree replications of Redchief and Jonathan. These cultivars were inoculated with black rot and white rot. Additional inoculations began in June 1991 and were terminated on May 1992. To follow canker development over time, three tree replications of three cultivars each were inoculated with the unknown I fungus isolated originally from the rough bark cankers, *Phomopsis* obtained from the bark inclusion type cankers and black rot and white rot isolates obtained from growers orchards. Cultivars chosen were Jonathan, Golden Delicious, and Red Delicious (Redchief). All were planted in 1975 and were on M9 rootstocks. At each inoculation date, 3 trees of each cultivar were inoculated with each pathogen.

Inoculations consisted of cutting a V-shaped wound approximately 4 mm x 4 mm in size and 2 mm deep, into side branches. Branch diameter was approximately 5 cm to 7 cm. A mycelial plug of each pathogen was placed within the wound. A control consisting of water agar alone was also placed in a wound area. The wound was wrapped with parafilm and the parafilm was additionally wrapped with tape to ensure that moisture would remain within the wounded area. Twenty days after inoculations, the wrappings were removed. At 7, 10, 13, and 16 week intervals, measurements on canker development were taken. After 16 weeks, measurements were taken on a monthly basis.

### Results-field inoculations

Preliminary inoculations made in May of 1991 resulted in larger cankers than at any other inoculation time, including the May 1992 inoculations (Fig. 1). Jonathan was more susceptible to black rot and white rot fungi in 1991, than it was in 1992. The same trend was observed on Redchief inoculated with *B. obtusa*. Inoculations made each month over 1992 resulted in cankers that were slow to expand over the 12 months (Fig. 2). This was

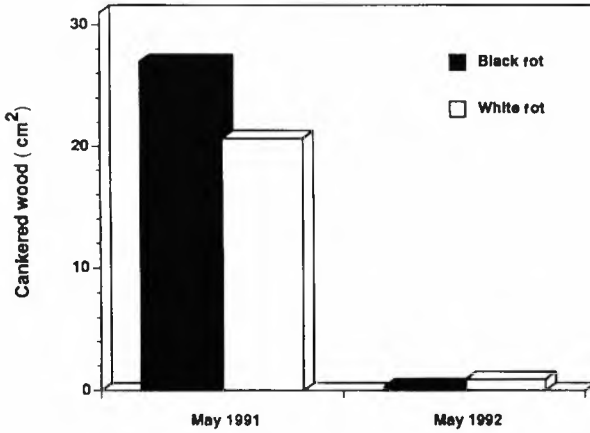


Fig. 1. Canker development on Jonathan by black rot and white rot 1 year after inoculations were made on May of 1991 and May of 1992

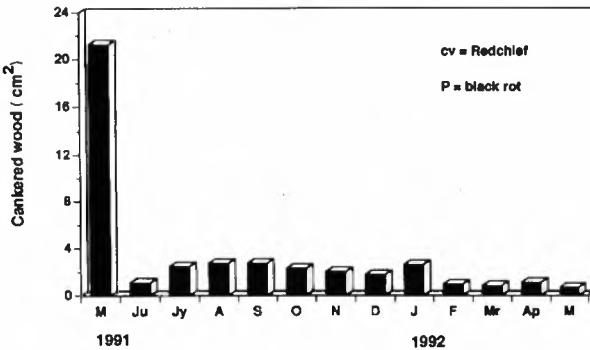


Fig. 2. Canker development 1 year after various inoculation dates

observed for all cultivars and for the four pathogens over the year-long observation period.

**Results-cultivar susceptibility**

The susceptibility of the three cultivars inoculated with four of the canker-causing fungi was evaluated in June (Fig. 3). Results varied between cultivars with regard to pathogen susceptibility. Jonathan was very susceptible to white rot, intermediate in susceptibility to the Unknown I fungus, and least susceptible to black rot and *Phomopsis*. Delicious was most susceptible to *Phomopsis*, followed by white rot, Unknown I, and least susceptible to black rot. Golden Delicious was most susceptible to white rot followed by black rot, and least susceptible to Unknown I and *Phomopsis*.

**DISCUSSION AND SUMMARY**

Cankers developed on standard and dwarfed apple trees from field inoculations of several fungi. Cankers were not typical

cankers of apple wood cankers observed in orchards. In May 1991 inoculations, cankers expanded quickly, but for all inoculations made in 1992, cankers were small.

Several factors may have affected the results. First, all inoculations which initiated cankers were made with cultured fungi using an artificial wounding technique. During inoculations, one of the pathogens was placed within the wounded area. Under natural field conditions, an interaction of other organisms within the wound area may be occurring. Every time isolations were made from naturally occurring cankers, more than one organism, such as the common saprophytes, were also isolated. Possibly these saprophytes play some role in canker initiation or inhibition.

The most probable explanation for the results observed is the severe drought that occurred in Pennsylvania in 1991 and the extremely wet conditions which existed in 1992.

Soil moisture and temperature regimes during these two growing seasons were entirely different. These differences can have dramatic effects on tree physiology and, as a result, probably influenced canker development. Drought conditions cause trees to be stressed and in some cases, this can increase susceptibility to wood rotting fungi (1, 2, 3, 4).

In conclusion, all fungi isolated from naturally occurring cankers in the field, caused cankers on all cultivars tested when trees were inoculated throughout the year. No significant differences in canker elongation on trees infected at different times of the season were observed. As a result of this research, we conclude that natural infections can occur anytime during the year. The initiation and development of cankers caused by various fungi are probably most influenced by tree health.

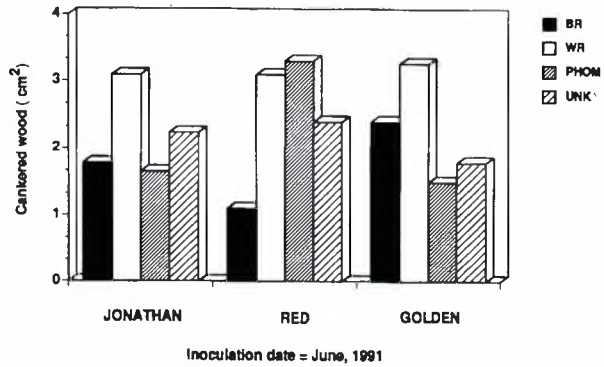
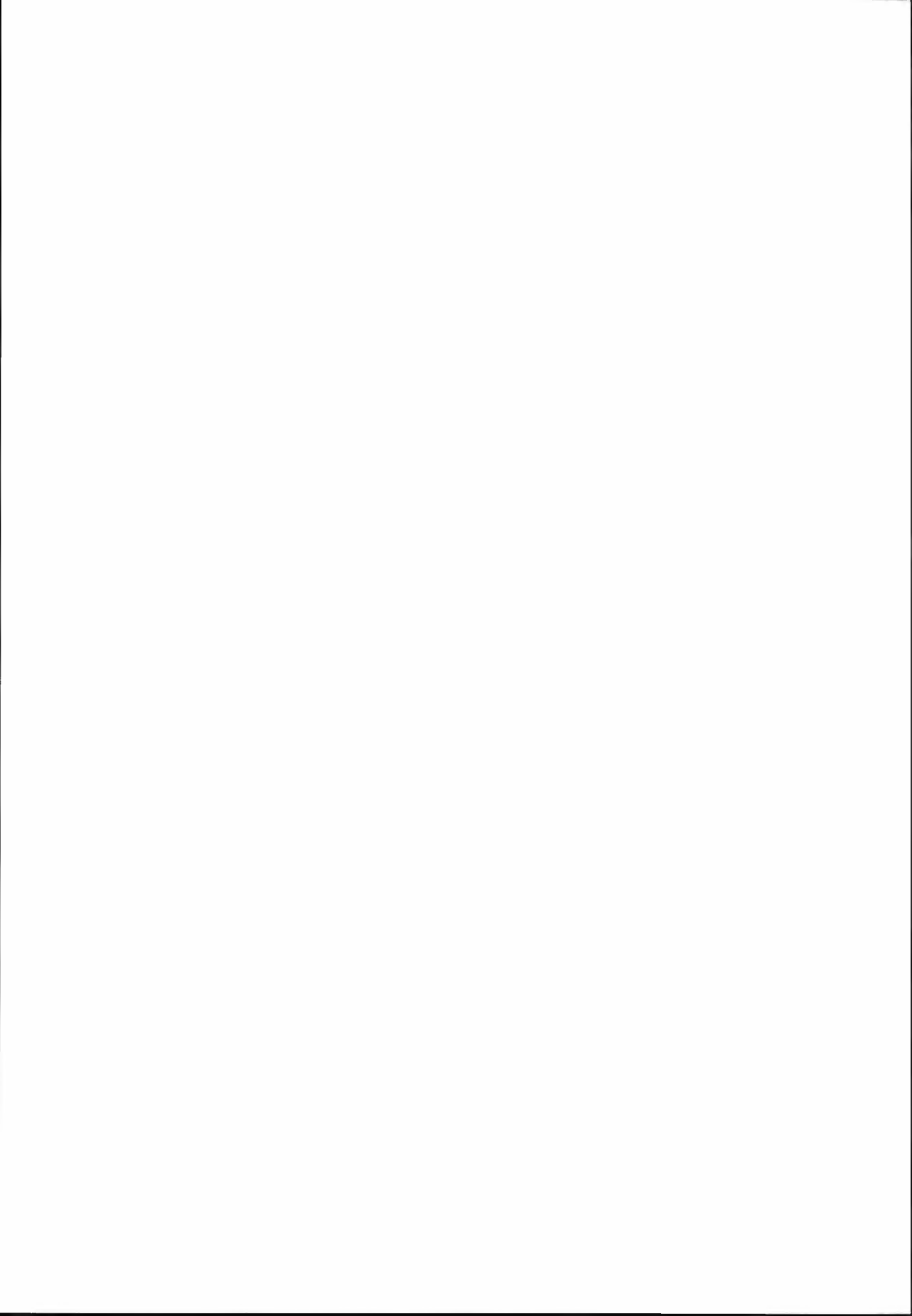


Fig. 3. Canker development by Black rot (BR), White rot (WR), Phomopsis (Phom), and Unknown 1 (Unk) on 3 cultivars one year after inoculations

## REFERENCES

- Brown, E.A. & F.F. Hendrix 1981. Pathogenicity and histopathology of *Botryosphaeria dothidea* on apple stems. *Phytopathology* 71(4): 375-379.
- Crist, C.R. & D.F. Schoeneweiss 1975. The influences of controlled stresses on susceptibility of European White Birch stems to attack by *Botryosphaeria dothidea*. *Phytopathology* 65:369-373.
- Holmes, J. & A.E. Rich 1969. The control of black rot cankers on apple trees in New Hampshire. *Plant Disease Reporter* 53(4): 315-318.
- Pusey, P.L. 1989. Influence of water stress on susceptibility of nonwounded peach bark to *Botryosphaeria dothidea*. *Plant Disease* 73:1000-1003.
- Travis, J.W., K.D. Hickey & J.L. Rytter 1990. Pathogenicity of the black rot fungus, *Botryosphaeria obtusa*, on apple wood. 1989 Research Progress Reports, Pennsylvania Fruit News 70(2): 53-54.
- Travis, J.W., J.L. Rytter & K.W. Hickey 1991. Factors that effect black rot canker development on apple wood. 1990 Research Progress Reports, Pennsylvania Fruit News 71(2): 46-49.



# Release of *Nectria galligena* spores from apple cankers

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Patterns of ascospore flight and conidial release in relation to the onset and duration of wetting of perithecia and sporodochia under various light/dark regimes were recorded in two controlled environment (CE) cabinets, using detached cankers from orchard trees of apple cvs Cox and Spartan. In each cabinet, airborne ascospores and water-borne spores (conidia and ascospores) were trapped using a Burkard suction trap and a fraction collector, respectively. Ascospores were caught within an hour of wetting irrespective of wetting duration and light/dark conditions. After a short wetting period followed by drying in the light, an ascospore discharge peak occurred after 1-4 h; when drying proceeded in darkness after a short wetting period the discharge peak was either delayed by 2-3 h or was prolonged. When cankers were wetted for 12 h, ascospores were caught throughout the wetting period under both light and dark conditions. The number of ascospores discharged sometimes increased in the light period (drying) that followed the end of a dark wetting period, with a peak 4-7 h after the onset of light. Both ascospores and conidia were present in water flowing from cankers within 15 min from the onset of wetting and were released throughout the wetting period.

Key words: Apple canker, ascospore discharge, conidial release, European canker, *Nectria galligena*, orchard disease, spore dispersal.

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European canker of apple, also referred to as *Nectria* canker and apple canker, is caused by the fungus *Nectria galligena*, which also attacks pear and many other hardwood trees (Seemüller, 1988). The disease occurs in many countries (Butler & Jones, 1949) and has been recognised as a serious problem for decades. The pathogen enters the host through either natural or artificial wounds and lenticels. The fungus also infects apple fruits causing *Nectria* fruit rot (eye rot).

Cankers produce perithecia which discharge ascospores and sporodochia which release conidia. Spore production and discharge/release are dependent on rainfall (Swinburne, 1975). Conidia are mainly water-splashed (Munson, 1939); ascospores are both water-borne and airborne after rainfall (Dubin & English, 1975). There are considerable discrepancies however between results reported from various studies on the time and pattern of ascospore

discharge (Lortie & Kuntz, 1963; Swinburne, 1971).

To improve disease control, a *Nectria galligena* warning system is being developed (Xu & Butt, 1993). As part of that project, it was necessary to study spore dispersal in relation to environmental conditions so that the start of each infection period can be timed. This paper reports results from these studies.

## MATERIALS AND METHODS

Spore dispersal was studied in six experiments (Table 1) using two controlled environment (CE) cabinets (Conviron S10h). Short lengths of branch, each with a single canker at a similar sporulation potential, were cut from orchard-grown apple trees of cvs Cox's Orange Pippin and Spartan. A sample of perithecia from each canker was squashed and observed microscopically to determine maturity; sporulation potential was indicated by the perithecial maturity and the presence of sporodochia.

Table 1. Details of spore dispersal experiments

Experiment		1	2	3	4	5	6
Factors studied	Wetting period (h)	0.5 <sup>a</sup>	0.5	0.5	12	12	12
	Light/dark regime	No	Yes	Yes	Yes	Yes	Yes
CE cabinet settings	Relative humidity	80%	80%	80%	98%:70% <sup>b</sup>	98%:70% <sup>b</sup>	70% <sup>b</sup>
	Temperature (°C)	15	15	15	15	15	15
	Light/dark regime (h)	16/8	12/12	12/12	12/12	12/12	12/12
Spores sampled	Water-borne conidia	No	No	Yes	Yes	Yes	Yes
	Water-borne ascospores	No	No	Yes	Yes	Yes	No
	Airborne ascospores	Yes	Yes	Yes	Yes	Yes	Yes

a): A hand sprayer was used to thoroughly wet the canker.

b): Two CE cabinets were used. Experiment 4: cabinet 1 - 98% r.h., light 0900 - 2100; cabinet 2 - 70% r.h., light 2100-0900. Experiment 5: cabinet 1 - 98% r.h., light 0900 - 2100; cabinet 2 - 70% r.h., light 0900-2100. Experiment 6: cabinets 1 & 2 - 70% r.h., light 0900 - 2100.

Both CE cabinets were equipped with a Burkard 7-day volumetric spore sampler and a rotary fraction collector. A branch bearing a single canker was clamped horizontally and level with the orifice of a spore sampler. In experiments 4, 5 and 6 a branch bearing a single canker was suspended in a simple wind tunnel comprising a plastic tube (length 30 cm, diameter 10 cm) wide at one end and narrowing at the end attached to the trap orifice. In experiments 2 to 6, rain was simulated by dripping water at a controlled rate onto the canker from a horizontal perforated plastic tube. Water flowing from the canker (passing through a hole in the wind tunnel), was funnelled to the rotary fractional sampler and collected during the wetting period into 13 ml plastic tubes in 15 min periods (experiment 3) or 1 h periods (experiment 4). After the simulated rain period, the remaining water



flowing from the canker was caught in a single tube. Every plastic tube contained 5  $\mu$ l phenol to prevent spore germination. In each experiment, the canker was temporarily wetted 72 h before treatments started.

The tape from the Burkard spore sampler was cut into lengths (48 mm) equivalent to the 24h sampling period, mounted and scanned microscopically for *Nectria* ascospores. Ascospores were counted with the aid of a squared graticule (central square 0.25 x 0.25 mm). The ascospore count in each traverse of the tape was adjusted to give an estimate of the number of ascospores trapped per hour over the trapping period. The water collected in each plastic tube was removed, centrifuged for 15 min at 5000 rpm and spores were counted using a haemocytometer.

## RESULT

### Ascospores

Number of trapped ascospores varied considerably between cankers and between different wetting treatments of the same canker. For example, in one experiment there was a seven-fold difference in the number of ascospores discharged from two cankers subjected to the same environment; a six-fold difference was associated with two wetting periods on one canker. This variation is not unexpected because the number of ascospores discharged also depends on factors not controlled in these experiments such as the canker age, number of perithecia, orientation of perithecia in relation to the trap orifice and the interval for recovery between two discharges. The pattern of ascospore discharge over time, however, was similar for given treatment combinations in these experiments. The emphasis of this report is, therefore, on the time and pattern of discharge rather than on number of ascospores.

Perithecia discharged ascospores if wetted for 0.5 h or less (experiments 1, 2 and 3). Fig. 1 shows an example of the discharge pattern in response to 0.5 h wetting (experiment 2). The graph shows that ascospores were caught within 1 h of the onset of wetting and peak discharge was 3 h after the onset of wetting when the canker was wetted and then dried in the light. When cankers dried in the dark, irrespective of the light/dark condition during wetting, the first significant catch of spores was delayed until 3-4 h after the onset of wetting and reached a peak at 5 to 6 h. For a few wetting treatments in experiment 3, however, drying in darkness prolonged the discharge but did not delay the discharge (Fig. 2).

The effect of r.h. on ascospore discharge pattern was insignificant, although in few cases a low r.h. considerably increased the number of ascospores caught. When the same canker was wetted for 12 h and then dried under two different r.h. levels, the two discharge patterns were similar (Fig. 3) in experiment 5.

Following 12 h wetting periods, continuous light or darkness over 24 h (including the wetting period) produced similar discharge patterns (Fig. 4) in experiment 6, although the peak discharge in darkness was more prominent.

Fig. 5 shows the discharge patterns of two cankers subjected to 12 h wetting; in this experiment (4), r.h. levels and light/dark factors were confounded. Two types of pattern were detected: in one, the main peak occurred 3-4 h after the onset of wetting with minor

peaks a few hours into the drying period; in the other, a minor peak was observed 5 h after the onset of wetting but major peaks occurred several hours into the drying period. The first type was associated with cankers wetted in light and drying in darkness and the second type with cankers wetted in darkness and drying in light.

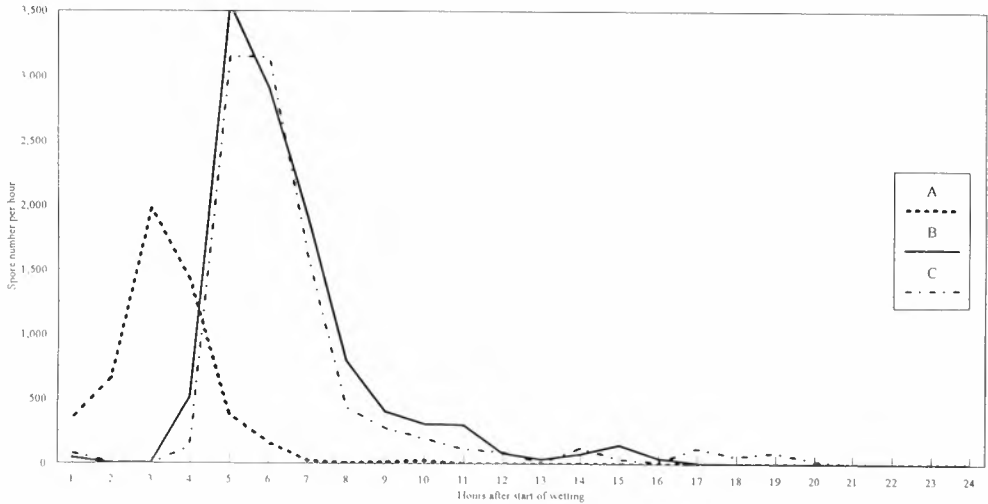


Fig. 1. Ascospore discharge patterns from a single canker in response to 0.5 h wetting (experiment 2) A: wetting and drying in the light; B: wetting and drying in the dark; C: wetting in the light and drying in the dark

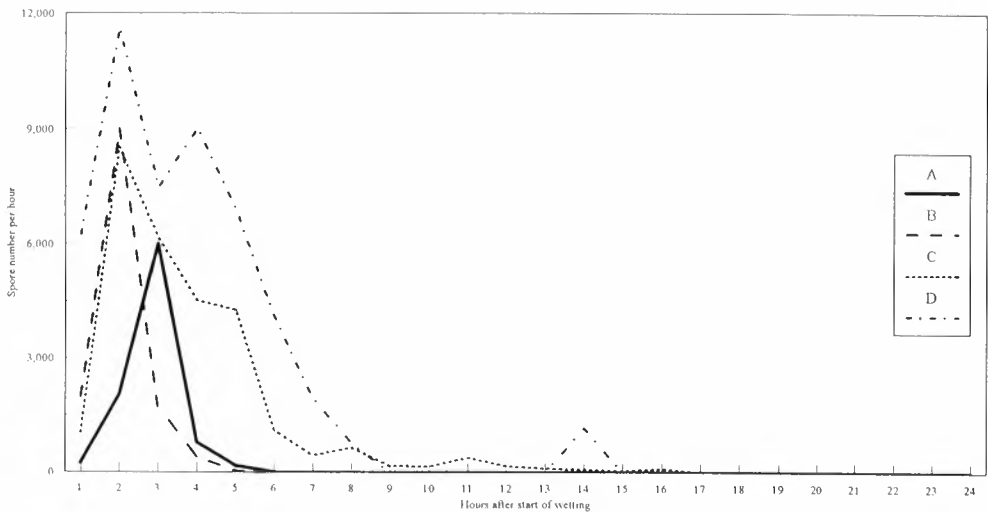


Fig. 2. Ascospore discharge patterns from a single canker in response to 0.5 h wetting (experiment 3) A: wetting and drying in the light; B: wetting in the dark and drying in the light; C.: wetting in the light and drying in the dark; D: wetting and drying in the dark

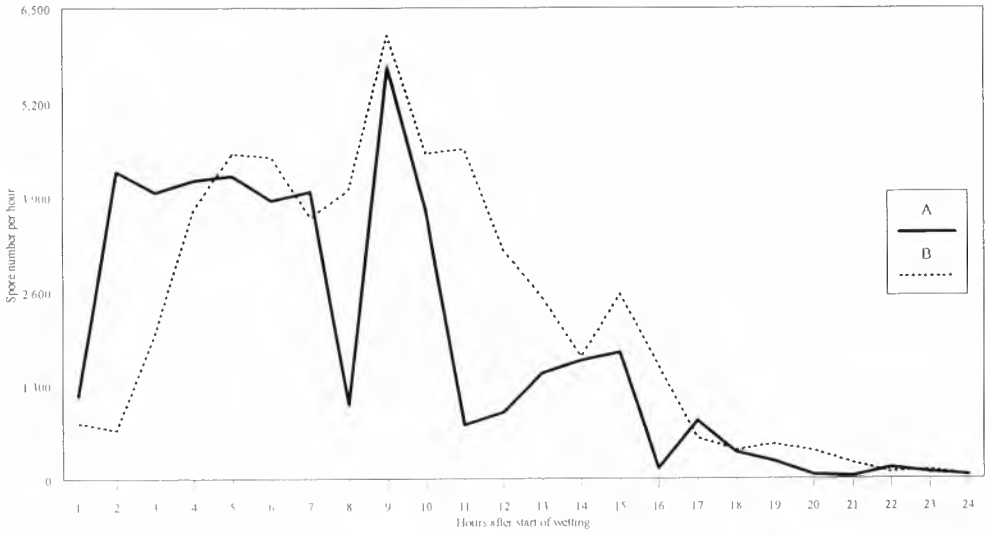


Fig. 3. Ascospore discharge patterns from a single canker in response to 12 h wetting (experiment 5) A: wetting in the light and drying in the dark at 98% r.h.; B: as A except at 70% r.h.

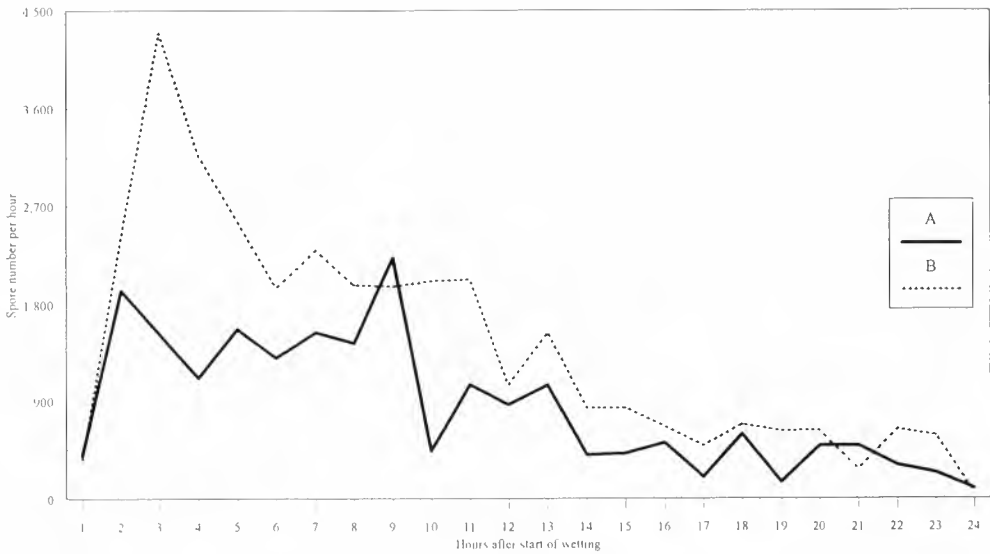


Fig. 4. Ascospore discharge patterns from a single canker in response to 12 h wetting (experiment 6) A: wetting and drying in the light at 70% r.h.; B: as A but with wetting and drying in the dark

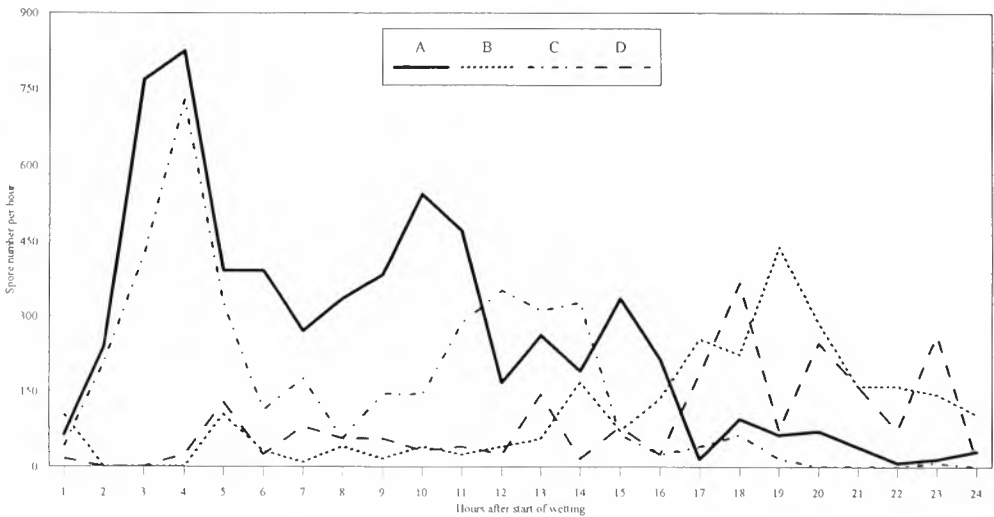


Fig. 5. Ascospore discharge patterns in response to 12 h wetting (experiment 4)

A: wetting in the light and drying in the dark at 98% r.h. (canker 1); B: as A but with 12 h wetting in the dark and drying in the light at 70% r.h.; C: wetting in the light and drying in the dark at 98% r.h. (canker 2); D: as C but with 12 h wetting in the dark and drying in the light at 70% r.h.

Water-borne ascospores were present 15 min after the onset of wetting and were thereafter present in samples throughout the wetting period (data not shown). Change in the number of water-borne ascospores with time was small. There was no evidence that light/dark conditions and r.h. affected the presence of water-borne ascospores.

### Conidia

No airborne conidia were observed. Water-borne conidia were present within 15 min after the onset of wetting and were thereafter caught throughout the wetting period. The number of conidia released tended to decline with time, although this varied between cankers (Fig. 6, A & B vs C & D). There was no evidence for an effect of light/dark regime on conidial release (A vs B in Fig. 6). Overall, the number of conidia present in water samples was larger than the number of water-borne ascospores.

### DISCUSSION

Although the present study was conducted under a limited range of environments using only a few cankers, interesting results were obtained. It has been confirmed that ascospore discharge and conidial release are both triggered by rainfall and that ascospores are not only released into the air but also into the water flowing over cankers which simultaneously releases conidia (Lortie & Kuntz, 1963; Munson, 1939; Swinburne, 1971). The results are, however, at variance with some previous reports.

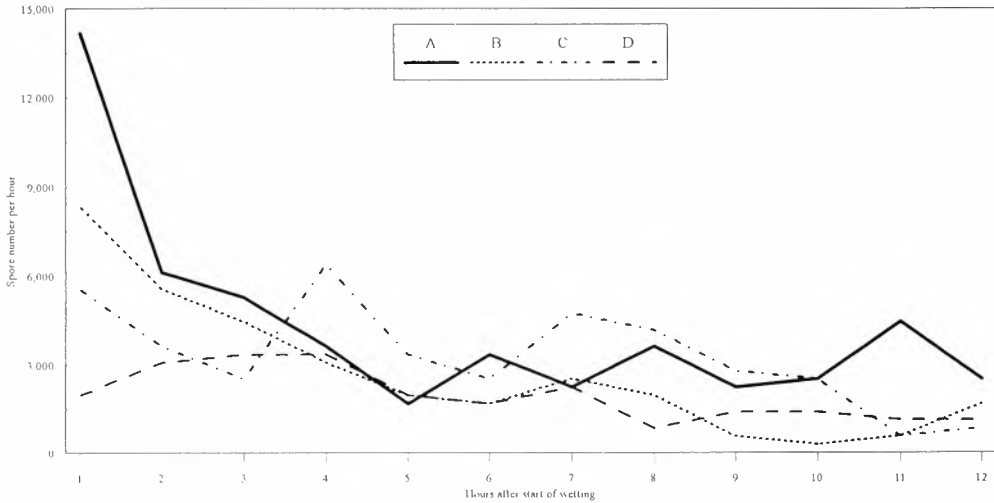


Fig. 6. The pattern of release of water-borne conidia in response to 12 h wetting

A: wetting in the light at 98% r.h. (canker 1, expt. 4); B: as A but wetting in dark at 70% r.h.; C: wetting in the light at 98% r.h. (canker 2, expt. 4); D: as C but at 70% r.h.

Lortie & Kuntz (1963) found that a 2-3 h drying period was required for considerable numbers of ascospores to be discharged after a wetting period lasting 10-30 min. However, the present study indicates that a drying period is not necessary for ascospore discharge if cankers are wetted for 12 h. The present study shows that considerable numbers of ascospores are discharged 1-4 h after the onset of wetting, irrespective of the duration of wetting. The longest wetting period studied by Lortie & Kuntz (1963) was 30 min, which is too short to observe any significant level of ascospore discharge during the wetting period.

The observation by Swinburne (1971) that long wetting periods inhibited ascospore discharge is difficult to explain because in the present results the majority of ascospore discharge patterns were similar, with peaks 1-7 h after the onset of wetting, irrespective of the length of wet period. In some cases, the hourly rate of ascospore discharge was relatively low throughout the long wetting period in darkness (B & D in Fig. 3), but the rate increased in the following drying period in light.

In the majority of cases, drying in darkness following a short wetting period delayed ascospore discharge by 2-3 h (Fig. 1); drying in light following a long wetting period (12 h) in dark increased the number of ascospores discharged in the drying period. This may be due to the light/dark effect although other causes are equally possible, such as the state of canker development (sporulation). More studies are needed before conclusions on the effects of light/dark regimes on ascospore discharge can be drawn.

These results indicate that notable ascospore discharge occurs 1-4 h after wetting begins, depending on light/dark conditions. In practice, however, an infection period at infection sites in the vicinity of mother cankers begins almost immediately rainfall starts because water-borne conidia and ascospores are almost immediately dispersed in the water

flowing, splashing and dripping from cankers. These results have been used in the design of a PC-based *Nectria galligena* warning system, currently under development at HRI - East Malling (Xu & Butt, 1993).

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#### LITERATURE

- Butler, E.J. & S.G. Jones 1949. Plant Pathology. pp 224-228. Macmillan and Co. Ltd., London.
- Dubin, H.J. & H. English 1975. Effects of temperature, relative humidity, and desiccation on germination of *Nectria galligena* conidia. *Mycologia* 67: 83-88.
- Lortie, M. & J.E. Kuntz 1963. Ascospore discharge and conidial release by *Nectria galligena* Bres. under field and laboratory conditions. *Canadian Journal of Botany* 41: 1203-1210.
- Munson, R.G. 1939. Observations on apple canker. I. The discharge and germination of spores of *Nectria galligena* Bres. *Annals of Applied Biology* 26: 440-457.
- Seemüller, 1988. *Nectria galligena* Bresad. In: Smith, I.M., R.A. Lelliot, D.A. Phillips & S.A. Archer (eds.) 'European handbook of plant diseases', Blackwell Scientific Publications. pp 280-282.
- Swinburne, T.R. 1971. The seasonal release of spores of *Nectria galligena* from apple cankers in Northern Ireland. *Annals of Applied Biology* 69: 97-104.
- Swinburne, T.R. 1975. European canker of apple (*Nectria galligena*). *Review of Plant Pathology* 54: 787-799.
- Xu, X.-M. & D.J. Butt 1994. The biology and epidemiology of *Nectria galligena* and an infection warning system. Papers presented in 3rd IOBC Workshop on integrated control of pome fruit diseases, Lofthus, Norway.

# The biology and epidemiology of *Nectria galligena* and an infection warning system

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This paper reviews the biology and epidemiology of *Nectria galligena*, causal agent of European apple canker and *Nectria* fruit rot, and describes a prototype of a predictive model for a PC-based *N. galligena* infection warning system for use on farms. When *N. galligena* cankers are wetted by rain, the model immediately releases abundant viable conidia from sporodochia and instantaneously lands them on the wet surface of fresh pruning cuts, fresh leaf scars and fruits of a very susceptible cultivar. The infection process begins on these sites immediately conidia land, the rate of infection depending on the type of site and temperature during the period of surface wetness. Spores may die when a period of surface dryness interrupts the infection process, the rate of mortality depending on the relative humidity and temperature in the dry period. The output from this first part of the model alerts growers when weather has favoured infection. The model next predicts the incidence of canker and fruit rot (percentage of sites infected) near sporulating cankers in the orchard, taking into account the ages of pruning cuts and leaf scars.

**Key words:** Apple canker, disease forecasting, infection warning, model, *Nectria galligena*.

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European canker of apple, also known as *Nectria* canker and apple canker, is caused by the fungus *Nectria galligena*, which also attacks pear and many other hardwood trees (Seemüller, 1988). The disease occurs in Europe, Australia, New Zealand, Canada and the United States (Butler & Jones, 1949) and has been recognised as a serious problem for centuries.

*N. galligena* is a wound parasite and enters the host through either natural or artificial wounds. Important entry sites include leaf scars (Crowdy, 1952; Dubin & English, 1974), pruning wounds (Bulit, 1957), fruit scars due to detachment of fruits (Burmeister & Kennel, 1967) and lesions caused by *Venturia inaequalis* (Wiltshire, 1922). The mycelium grows through the bark and wood. When a canker girdles a twig or branch, the supply of water and nutrients is disrupted, causing the distal part to wither. *N. galligena* also infects

fruits through the calyx end, and through lenticels, scab lesions and wounds (Berrie, 1989; Burchill & Edney, 1972). In recent years, losses due to *Nectria* fruit rot in stored Cox have increased in the UK (Berrie, 1989).

An infection warning system designed to alert growers of the risk of infection by *N. galligena* leading to canker and fruit rot in orchards, would enable control decisions to be made in accordance with the principles of integrated fruit production. For example, the warning system would help a grower to decide at harvest whether to store fruits or sell immediately, and whether to apply a post-harvest fungicide treatment. A PC-based infection warning system for *N. galligena* is being developed at Horticulture Research International (HRI) - East Malling.

This paper reviews the biology and epidemiology of *Nectria galligena* used in constructing the model and describes the structure and operation of the prototype version.

## BIOLOGY AND EPIDEMIOLOGY OF *NECTRIA GALLIGENA*

### **Sporulation and dispersal**

Young cankers usually produce only sporodochia, perithecia developing in the year after the lesion forms (Loughnane & McKay 1959; Saure 1962). Thereafter, conidia and ascospores can be produced from a canker throughout the year. Perithecial development may be at the expense of the production of sporodochia (Bulit 1957).

Spore production is dependent on rainfall and so varies with climate. In Europe, conidial production begins in June (Bennett 1971; Kennel 1963; Saure 1962) or even earlier in the wetter conditions of N.W. Europe (Swinburne et al., 1975). Conidia can be released continuously throughout the summer months and reach maximum numbers from August to October or November (Kennel 1963; Swinburne 1971a). Although conidia can be wind-dispersed (Swinburne 1971a), they are mainly dispersed in rain water (Munson 1939). Conidia are released from sporodochia soon after cankers are wetted and the concentration of conidia in the water flowing from the canker reaches maximum within 1 h of wetting (Butt et al., 1993). The dispersal distance is normally less than 10 m (Taylor & Byrde 1954).

In European studies there are differences in the time of maximum ascospore production. In the UK, Munson (1939) showed that perithecia discharge ascospores throughout the year, with a peak in February and a minimum in September. In contrast, Cayley (1921) observed that perithecia develop in winter and discharge in spring. In Northern Ireland, peak ascospore production in spring and early summer was followed by a minor peak in autumn (Swinburne 1971a). In France, maximum peak discharge was recorded in autumn (Bulit 1957), whereas in Germany the time of perithecial formation varied between late summer and late autumn (Saure 1962). Ascospores are dispersed by both rain and wind (Dubin & English 1975), and have been found in surface water flowing from cankers very soon after wetting (Butt et al. 1994). Lortie & Kuntz (1963) noted that after a wetting period of 10-30 min, a period of 2-3 h dry atmosphere was necessary for the discharge of air-borne ascospores. A recent study at East Malling, however, showed that the discharge of ascospores was not dependent on a dry period after wetting; in light, the aerial discharge began shortly after the onset of wetting and peaked about 3 h later,



irrespective of the duration of wetting (Butt et al. 1994). In darkness, the aerial discharge peaked about 5-6 hours after the onset of wetting.

### **Infection**

The infection process by single spores of pathogens like *Venturia inaequalis* (apple scab) and *Podosphaera leucotricha* (apple powdery mildew) are divided into sub-stages based on events before and after penetration; specialised structures to overcome the host barrier such as germ tubes, appressoria and penetration pegs are produced and there is clear termination of the infection process, as indicated, for example, by the production of the first haustorium. In the case of a parasite like *N. galligena*, the infection process into wounds by a population of spores leading to a single canker at the site cannot be divided into easily recognised sub-stages based on specialised structures. It is theoretically possible, however, to divide the infection process into three sub-stages, namely germination, invasion and colonisation. When hyphae from germinated spores have invaded the wound the host is infected; colonisation leads to the cankers and their expansion. There is no simple physical transition between these sub-stages.

The incidence of canker or fruit rot is the result of interaction between host, pathogen and environment. Among the many factors affecting the infection process are the host cultivar, the type of infection site, the amount of inoculum and weather conditions.

In terms of canker size, apple cultivars differ greatly in susceptibility to *N. galligena* (Borecki & Czyncyk 1984); this was confirmed by van de Weg et al. (1992) but they did not find difference in canker incidence following inoculation of wounded leaf scars. Recent studies at HRI East Malling showed that differences in cultivar susceptibility to *N. galligena*, expressed as canker incidence, depended on the type of inoculation site: cultivar differences in susceptibility were less obvious following inoculation of pruning cuts than leaf scars. Furthermore, site and cultivar interacted such that the classification of cultivar susceptibility depended on the site (Butt et al., unpub.).

Natural and artificial wounds become progressively more resistant to *N. galligena* as they age (Crowd 1952; Dubin & Englis 1974; Krähme 1980; Saur 1962; Wilson 1966). The length of time the sites are reported to be susceptible varies between studies. Dubin & English (1974) reported that leaf scars remained susceptible for 10 days, whereas Wilson (1966) showed that leaf scars remained susceptible for 28 days. Krähmer (1980) suggested that resistance to infection of old wounds was due to rate of suberisation, a process dependent on temperature.

Regarding the dose of inoculum needed to produce a canker or a fruit rot, a lower number of conidia/site are needed on pruning cuts (Butt *et al.*, unpub.) compared with the number necessary on leaf scars (Butt et al., unpub.; Dubin & English 1974) and fruits (Butt et al., unpub.; Swinburne 1971b).

For a period following inoculation, free water is essential for infection. Several experiments have shown the relationship between duration of surface wetness at various temperatures and the incidence of canker or fruit rot. For disease to develop, the minimum duration of surface wetness at a given temperature depended on the type of inoculation site; the minimal surface wetness duration on fresh pruning cuts was considerably shorter than on fresh leaf scars, and mature fruits required a longer period of wetting (Butt et al., unpub.): fresh leaf scars needed a minimal 6 h wetness, and 69% became cankered follow-

ing a 72 h wet period at 15°C (Wilson 1966). Dubin & English (1974) reported that at 13°C about 10 and 36% of fresh leaf scars became cankered after wet periods of 6 and 24 h, respectively.

### **Disease development**

Several factors affect the incubation period and canker growth. An effect of tree growth on canker development was shown by van de Weg (per. comm.); when trees were growing fast at the time of inoculation, a short wet period resulted in a shorter incubation period and higher canker incidence than when the trees were growing slowly. A similar relationship between shoot growth and canker development was also observed by Heinrich (1984).

Inoculum dose is negatively correlated with the length of incubation period and positively correlated with disease incidence for canker and fruit rots (van de Weg, pers. comm.; Butt et al., unpub.). Johnson et al. (1982) observed that the number of cankers/tree was related to the total number of cankers on the eight neighbouring trees.

Van de Weg et al. (1992) reported that the temperature in the first 5 days of incubation (when the inoculated leaf scar was covered with vaseline) affected canker size but not canker incidence; canker size decreased with increasing temperature from 15 to 29°C. Dubin & English (1975) observed that cultivars differed in the length of incubation period. Cankers grow faster at high r.h. than at low r.h.; the disease was latent at low temperature but cankers appeared when the temperature increased (Krähmer, 1981). The disease may remain latent for a long time before external symptoms are visible (Crowdy, 1952; Dubin & English, 1974; Wilson, 1966).

## **AN INFECTION WARNING SYSTEM FOR *Nectria galligena***

The philosophy of model building at HRI East Malling and the structure of apple disease warning systems emerging from the research program have been described elsewhere (Xu & Butt 1993). One of these systems will give a warning when weather conditions favour an attack by *Nectria* conidia and forecasts the incidence of canker and fruit rot in specific orchards and cultivars. Ascospores are omitted from the model because they lag behind conidia in their time of discharge and dispersal activated by rain, and because most experiments on infection conditions have been reported for conidia. A simple flow diagram of the system is shown in Fig. 1.

### **Spore dispersal**

The model assumes that cankers have sporodochia and that viable conidia are released, dispersed by rain-splash, drips and in surface water, and instantaneously landed on wet infection sites (fresh leaf scars, fresh pruning cuts and near-mature fruits) on a susceptible cultivar, near each sporulating canker, when a certain threshold of rainfall is recorded. This immediate release and dispersal is based on the finding that considerable numbers of conidia are present in water flowing from a canker very soon after it is wetted (Butt et al. 1994). Dew is discounted as a dispersal agent for conidia because most conidia would not be dispersed far from the mother canker and so a minimal inoculum dose for infection at many sites would be unlikely.

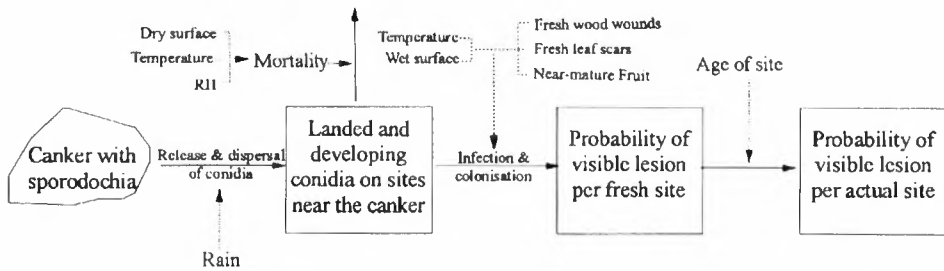


Fig. 1. Simple flowchart of a system giving warnings of apple canker and fruit rot caused by *Nectria galligena*

**Infection**

*N. galligena* can enter through any wounded site and through fruit skin lenticels, but only three types of site are included in the model: fresh leaf scars, fresh pruning cuts and near-mature fruits. The infection conditions on other natural wounds are assumed similar to those for leaf scars and on other injuries similar to those on pruning cuts.

The incidence of canker and fruit rot depends on the temperature in the wet period and the type of inoculation site. This relationship was derived from limited data collected for susceptible cultivars under few environmental conditions, and so it is not justified to use Boxcar techniques to simulate the above relationship. Instead, the incidence of disease at each type of site is calculated from the cumulative duration of surface wetness and the average temperature in the wet period.

Conidial mortality takes place when dry periods interrupt an infection period. There are no field measurements of spore mortality, and so the mortality rate used in the model is derived from a laboratory study (Dubin & English, 1974). In the model, the rate of mortality depends upon the r.h. and temperature during dry periods.

**Disease prediction**

The infection model predicts incidence of disease on *fresh* leaf-scars and pruning-cuts and *near mature* fruit. The actual disease incidence depends upon cultivar susceptibility, the dose of conidia and the age of site at the time of inoculation. These variables should be included to improve accuracy of the forecast.

Evidence of differences in cultivar susceptibility to canker refers to canker size rather than canker incidence. Furthermore, cultivar differences in susceptibility interact with inoculation sites; data on this interaction is very limited. The sporulation capacity of cankers varies with their age, and estimation of the inoculum potential of an orchard is not practical. The quantitative relationship between fruit age/size and rot incidence is unknown. For these reasons, the effects of cultivar susceptibility, quantity of conidia, and age of fruits on actual disease incidence are not included in the warning system.

Dates of pruning, leaf fall, June drop and harvest are include in the warning system in order to predict the actual disease incidence. These variables allow the calculation of the age of site available at the time of each potential infection period, and hence adjustment of their susceptibility. These dates will be combined with the output from the infection model to produce a cultivar/orchard-specific forecast of the percentage of visible disease on each type of inoculation site within the vicinity of every mother canker.

## FUTURE DEVELOPMENT

Research is needed on the biology and epidemiology of *N. galligena* if the warning system is to be made as comprehensive as Ventem™, the HRI East Malling apple scab warning system (Butt *et al.*, 1992). Further information is needed on the following topics:

- (a) Sub-stages of the infection process.
- (b) Mortality of infecting spores in dry periods.
- (c) Susceptibility of wounds and fruit in relation to their age.
- (d) Latent infections.
- (e) Prediction of the duration of the incubation period.
- (f) Conditions for infection by ascospores.
- (g) Interaction between cultivar susceptibility and inoculation site.
- (h) Spore dispersal gradients in relation to canker sporulation intensity and weather.

## ACKNOWLEDGEMENTS

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## LITERATURE

- Bennett, M. 1971. A comparison of a copper and mercury spray programme against apple canker (*Nectria galligena* Bres.). *Plant Pathology* 20: 99-105.
- Berrie, A.M. 1989. Storage rots of apple and pear in South East England 1980-88: incidence and fungicide resistance. In: Gesseler, Butt & Koller (eds): *Integrated control of pome fruit diseases* Vol. II, 229-239.
- Borecki, Z. & A. Czynczyk 1984. Susceptibility of apple cultivars to bark canker diseases. *Acta Agrobotanica* 37: 49-59.
- Bulit, J. 1957. Contribution a l'étude biologique de *Nectria galligena* Bres. agent du chancre du Pommier. *Annales de l'Institut de Recherches Agronomique de Roumanie, Serie C* 8: 67-89.
- Burchill, R.T. & K.L. Edney 1972. An assessment of some new treatments for the control of rotting of stored apples. *Annals of Applied Biology* 72: 249-255.
- Butt, D.J., N.D. Gunn & X.-M. Xu 1994. Release of *Nectria galligena* spores from apple cankers. Papers presented in 3rd IOBC Workshop on integrated control of pome fruit diseases, Lofthus, Norway.

- Burmeister, P. & W. Kennel 1967. Infektionsversuche mit *Nectria galligena*, *Gloeosporium perennans* und *G. album* an fruchtansatzstellen des apfels in verbindung mit chemischen Bekämpfungsversuchen. Zeitschrift für Pflanzenkrankheiten, Pflanzenpathologie und Pflanzenschutz 74: 615-626.
- Butler, E.J. & S.G. Jones 1949. Plant Pathology. pp 224-228. Macmillan and Co. Ltd., London.
- Cayley, D.M. 1921. Some observations on the life history of *Nectria galligena* Bres. Annals of Botany 35: 79-92.
- Crowdy, S.H. 1952. Observations on apple canker. III The anatomy of the stem canker. Annals of Applied Biology 36: 483-495.
- Dubin, H.J. & H. English 1974. Factors affecting apple leaf scar infection by *Nectria galligena* conidia. Phytopathology 64: 1201-1203.
- Dubin, H.J. & H. English 1975. Effects of temperature, relative humidity, and desiccation on germination of *Nectria galligena* conidia. Mycologia 67: 83-88.
- Heinrich, E. 1984. Development of a test method for the examination of fungicides against *Nectria galligena* on detached shoots of the apple tree. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 91: 449-458.
- Johnson, D.L., J.L. Doust & G.W. Eaton 1982. The effect of European canker and its spatial pattern on four apple cultivars in British Columbia. Journal of Applied Ecology 19: 603-609.
- Kennel, W. 1963. Zur pathogenese des obstbaumkrebses (*Nectria galligena*) an apfel. Gartenbauwissenschaft 28: 29-64.
- Krähmer, H. 1980. Wundreaktionen von Apfelbäumen und ihr Einfluß auf infektionen mit *Nectria galligena*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 87: 97-112.
- Krähmer, H. 1981. Regenerationsvorgänge an Schnittwunden von Apfelbäumen und deren Anfälligkeit für *Nectria galligena*-infektionen. Angew. Botanik 55: 429-439.
- Lortie, M. & J.E. Kuntz 1963. Ascospore discharge and conidial release by *Nectria galligena* Bres. under field and laboratory conditions. Canadian Journal of Botany 41: 1203-1210.
- Loughnane, J.B. & R. McKay 1959. Perithecial stage of apple canker on current season shoots. Plant Pathology 8: 113.

Marsh, R.W. 1939. Observations on apple canker. II. Experiments on the incidence and control of shoot infection. *Annals of Applied Biology* 26: 458-469.

Munson, R.G. 1939. Observations on apple canker. I. The discharge and germination of spores of *Nectria galligena* Bres. *Annals of Applied Biology* 26: 440-457.

Saure, M. 1962. Untersuchungen über die Voraussetzungen für ein epidemisches Auftreten des Obststamkrebsses (*Nectria galligena* Bres.). *Mitt. OVR Alten Landes, Beiheft 1*: pp. 74.

Seemüller, 1988. *Nectria galligena* Bresad. In: Smith, I.M., R.A. Lelliot, D.A. Phillips & S.A. Archer (eds.) 'European handbook of plant diseases', Blackwell Scientific Publications. pp 280-282.

Swinburne, T.R. 1971a. The seasonal release of spores of *Nectria galligena* from apple cankers in Northern Ireland. *Annals of Applied Biology* 69: 97-104.

Swinburne, T.R. 1971b. The infection of apples, cv. Bramley's Seedling, by *Nectria galligena* Bres. *Annals of Applied Biology* 68: 253-262.

Swinburne, T.R. 1975. European canker of apple (*Nectria galligena*). *Review of Plant Pathology* 54: 787-799.

Swinburne, T.R., J. Cartwright, N.J. Flack & A.E. Brown 1975. The control of apple canker (*Nectria galligena*) in a young orchard with established infections. *Annals of Applied Biology* 81: 61-73.

Taylor, R.E. & R.J.W. Byrde 1954. Control of *Nectria* eye-rot of apple by an eradicant fungicide. *Plant Pathology* 3: 72.

Weg, van de W.E., S. Giezen & R.C. Jansen 1992. Influence of temperature on infection of seven apple cultivars by *Nectria galligena*. *Acta Phytopathologica et Entomologica Hungarica* 27: 631-635.

Wiltshire, S.P. 1921. Studies on the apple canker fungus. I. Leaf scar infection. *Annals of Applied Biology* 8: 182-192.

Wilson, E.E. 1966. Development of European canker in a California district. *Plant Disease Reporter* 50:182-186.

Xu, X.-M. & D.J. Butt 1993. PC-based disease warning systems for use by apple growers. *EPPO Bulletin* 23: 595-600.

Xu, X.-M., D.J. Butt & G. van Santen 1994. A dynamic simulation model of apple leaf infection by *Venturia inaequalis*. Submitted to *Plant pathology*.

# Early detection and control of fireblight

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Fireblight, a destructive disease of apple and pear, is on the move in Europe. Many countries have during the last few years spent great resources attempting to eradicate the disease, but few have experienced success. To control the introduction of fireblight in a new area, early detection and establishment of an eradication programme is of crucial importance. Planting of highly susceptible host plants should be avoided. Weather-based fireblight risk assessment systems may be of great value to avoid establishment of the disease.

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Fireblight, caused by the bacterium *Erwinia amylovora*, is regarded as one of the most destructive diseases of pome fruits. It rapidly kills blossoms and shoots, and may spread further into branches and the trunk, whereby the tree usually dies. Trees may be killed in one season. The incidence and severity of fireblight is highly dependent on weather. The most commonly affected hosts are pear, apple, hawthorn (*Crataegus* spp.), *Cotoneaster* spp., particularly the larger species, *Sorbus aria* and *Pyracantha* spp. Fireblight has been known in North America since 1780, it appeared in New Zealand in 1919, in 1957 in England, and during the next 35 years it became established in many other European countries. Up to 1993 fireblight is known in 30 countries (EPPO 1992).

Fireblight may cause serious losses in apple and pear orchards, both in the current years crop, and in the subsequent years production by killing fruit spurs, branches, and whole trees. In the U.S.A., pear cultivation has been largely abandoned in some states because of the disease. In parts of Europe with warm conditions at first flowering (18-24°C), frequent periods of rain or high humidity, and highly susceptible pear cultivars, severe losses have been reported. In Greece fireblight appeared in 1985, and until now nearly 1000 ha of sensitive, traditional pear varieties have been destroyed (Psillakis 1993).

Fireblight is usually not of major economic importance to apple and pear production in northern Europe in many years, however, in occasional years damage could be severe. In the cider and perry orchards of South West England losses have been high. Indirect losses to growers because of export difficulties as a result of quarantine measures are also of considerable importance.

## CONTROL MEASURES

Even if fireblight has been known for about 200 years, there is still no completely satisfactory and reliable control measure. The present knowledge of the disease cycle and the many factors that affect disease development demonstrate that there is no single, easy answer to fireblight control. Successful preventive measures are restrictions on the importation of susceptible hosts from countries where the disease occurs, eradication and containment campaigns to stop or limit spread soon after the introduction of fireblight, and orchard management of susceptible hosts to minimize the effects of infection, including encouragement of the use of cultivars that are resistant or have low susceptibility. Rootstocks M 9, M 26, M 27 and MM 106 are more susceptible than other rootstocks (Huet & Michelesi 1990). Among apple cultivars, 'James Grieve' is very susceptible, 'Ingrid Marie', 'Gravenstein', 'Summerred' and others are moderately susceptible. Among pear cultivars 'Clara Frijs' and 'Herzogin Else' are susceptible (Thibault & Le Lezec 1990).

Fireblight is difficult to control with known chemicals. None of them are curative, and must therefore be used to prevent entry and establishment of the pathogen. Copper compounds and antibiotics have been used with some success, but they both have severe disadvantages. Some new chemicals have been introduced recently, showing promising results in controlling fireblight, but they need further investigation under a range of conditions. Among other approaches are the use of biological control agents, which again need much testing before they can be released commercially.

Chemical and biological control have had some success when used in conjunction with a fireblight risk assessment system (Billing 1992, Steiner 1990).

## ERADICATION OF FIREBLIGHT IN NORWAY

No country has yet managed to completely eradicate fireblight, but several countries have had success in containing the disease, at least for some years. Others have given up at an early stage, usually because of lack of money for eradication campaigns, and very rapid spread of the disease.

In Norway fireblight was detected for the first time in 1986 (Sletten 1990). The focus of infection was in and around the city of Stavanger on the south west coast of the country. Diseased plants were found in private gardens, around public buildings, in recreation grounds, along roads, and in rural areas. In this district there is no commercial fruit-growing, however, many nurseries are situated there. Spring is often dry and cold, but in the summer temperatures can be high, with maximum temperatures well above 20°C. Rainfall in July, August and September is usually high, with frequent storms.

*Cotoneaster bullatus* and *C. salicifolius* were the two most important hosts, but the disease also occurred on other *Cotoneaster* spp., as well as on *Sorbus aria* and *Pyracantha coccinea*.

A Government funded eradication campaign was set up in 1986, with the aim to protect important fruit-growing districts about 40 km north of the infection site, and nurseries 5 km to the south. It has been continued each year, with a total expenditure up



to 1992 of about NOK 5 million. So far no compensation for the removal of diseased plants has been paid.

A quarantine area of about 700 km<sup>2</sup> was established around the focus of infection. Within this area the production and sale of all common fireblight hosts are prohibited, and such plants must not be removed from the area. Plants susceptible to fireblight can be removed from private and public grounds, regardless of health condition. Protective zones of about 500 m were established around fruit orchards and nurseries. Moving of beehives is restricted.

The Plant Protection Institute, the Plant Health Inspection Service, and the local County Agricultural Advisory Service were in charge of the eradication campaign. In the growth season between 20 to 40 persons were engaged in the tracing, cutting and removal of diseased plants. Roots were killed with glyphosate or imazapyr. In 1989 the strategy was changed. To reduce the possible build-up of inoculum on *C. bullatus* and *C. salicifolius*, these two species were completely removed from about 300 km<sup>2</sup> of the quarantine area. More than 30.000 private gardens were searched, in addition public areas, recreation grounds, and rural districts. The efficiency of removing and destruction of plants was greatly increased when we instead of burning plants used a transportable wood chipper, grinding shoots, branches and stems quickly into fine chips, which were decomposed in heaps for a year. By voluntary agreement all nurseries in the quarantine area stopped their production of the most common hosts to fire blight, and they destroyed their stocks of *C. bullatus* and *C. salicifolius*.

These drastic measures could not have been accomplished if the public awareness of the disease and its destructive potential had not been raised at an early stage of the campaign. Information about fireblight and which measures that were to be taken were given in leaflets distributed to the public by post, and in "growers bulletins". Newspapers and local radio stations gave regular reports about the progress of the campaign.

Fireblight was detected at around 2.000 locations during the years 1986-1992. Disease incidence increased during the first years, but from 1990 there was a decrease in numbers of new outbreaks. Fireblight also became limited to the two main hosts. Only a single diseased *C. bullatus* was detected in 1992, and 1993 will probably be the last year with organized removal of plants. But the surveillance of the quarantine area will have to be continued for several years. Nevertheless, the eradication campaign must so far be considered successful. In spite of weather conditions favourable to the development of fireblight, and the common occurrence of very susceptible host plants, the prospects of declaring the quarantine area free from fireblight is good. It has been of advantage that there was no commercial fruit-growing in the district, and that the disease did not enter nurseries. The removal of the main hosts greatly reduced the build-up of inoculum, and made the surveillance work easier.

#### FIREBLIGHT RISK IN LOFTHUS-AREA

In collaboration with Dr. Eve Billing weather data for the years 1990-1993 from Lofthus, Norway, an important fruit-growing area about 150 km north of the fireblight outbreak in Stavanger, were analyzed with Billing's revised system for fireblight risk assessment

(Billing 1992). We had incomplete data for primary and secondary blossom, and no knowledge about damage to young tissue from frost, hail, wind or insects. Further studies are necessary, but some preliminary assessments on fireblight risk can be made.

If inoculum had been present, there was a post-bloom risk in early June 1990, when late flowers might be present. The weather was warm and dry. In July there was a high risk for secondary blossom infection, and possibly for young shoots, if they had been damaged for instance by insects or by periods of heavy rain and storm.

In 1991 there was no spring or early summer risk, but in early July and early August high risk for secondary blossom and possibly for young shoots. There could also be a risk of overlap of secondary blossom and cotoneaster blossom and so, spread between hosts if either was diseased.

In 1992 there was a slight pre-bloom risk. Blossom risk started 22 May, but there was no recorded rain until 7 June. If there had been mist or dew during the warm period, risk of blossom blight might be high. There was a high risk in mid and late June for young shoots, especially if there had been storm damage.

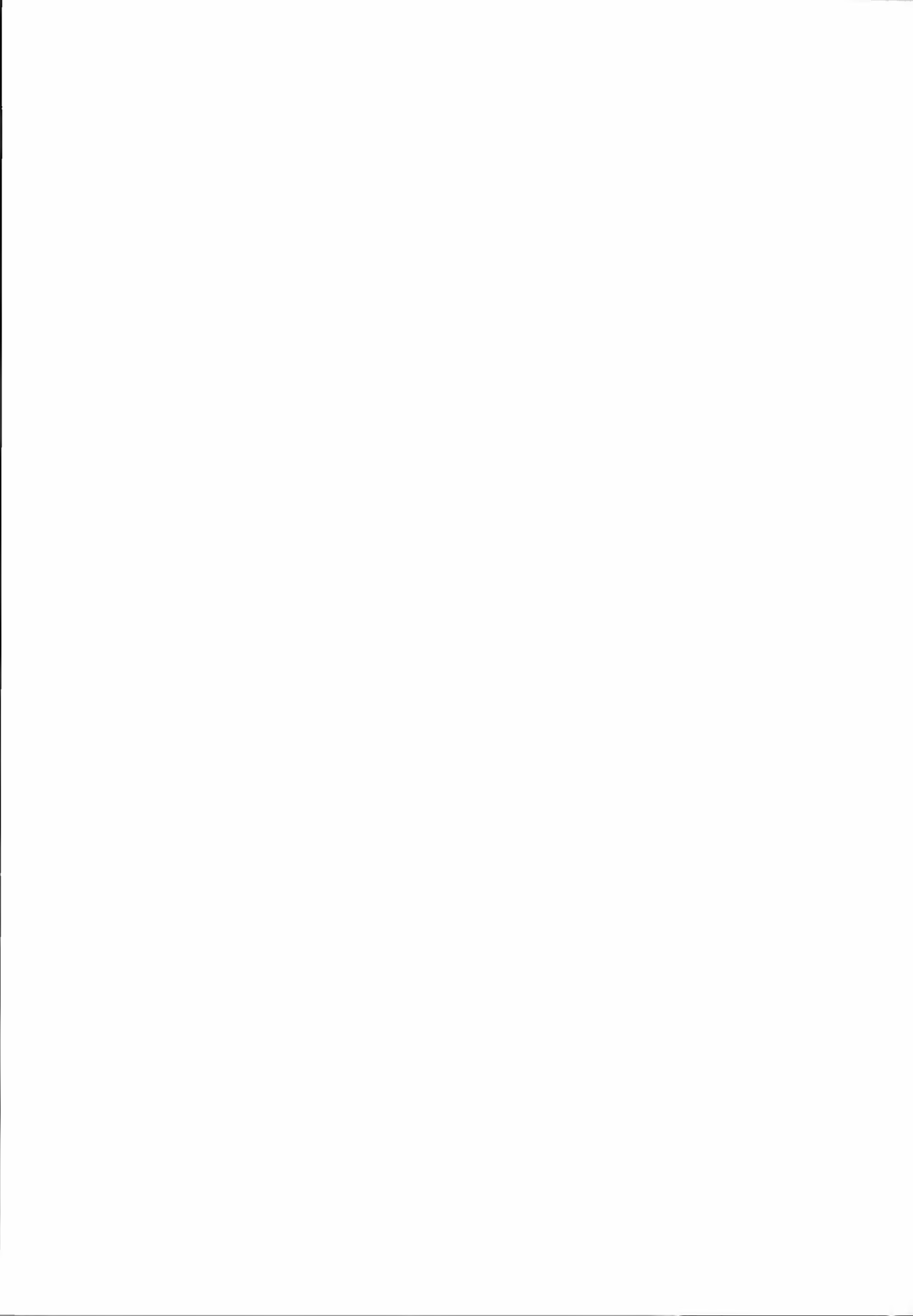
These results indicate that weather in this area could be favourable for fireblight. However, other factors than weather are also of importance. Among the most important can be mentioned the presence of susceptible cultivars, insect activity, tree age, cultural conditions, soil moisture, bud break, blossom periods and shoot extension growth rates. Alternative hosts, (like *Cotoneaster*, *Crataegus* and *Sorbus*), near orchards will be of importance, in particular if there is an overlap of flowering times with pear (primary and secondary blossom) and apple.

## CONCLUSIONS

During the 35 years since the first introduction of fireblight in Europe many attempts have been made of eradication and containment of the disease, with variable results. Among the most important factors for the success of a campaign is the detection of fireblight at an early stage of its introduction in a country. However, a low level of infection is not easy to detect, even with extensive surveys and trained personnel. It may go on unnoticed for some time, especially if weather conditions are unfavourable for disease development. The outbreak in Norway most likely had its origin at least a couple of years before it was detected. Once detected, it is of crucial importance that strict measures for eradication start as soon as possible if success of the campaign is to be expected. Many countries have experienced that the failure of eradication is not caused by insufficient legislation and organization of the campaign, but by the common distribution of very susceptible host plants, and in particular if some of these are wild growing. Infections in ornamentals in build-up areas and cities are usually extremely difficult to control, and they may be an important reservoir of inoculum for further spread of the disease.

REFERENCES

- Billing, E. 1992. Billing's revised system (BRS) for fireblight risk assessment. EPPO Bulletin 22: 1-102.
- EPPO 1992. EPPO Distribution List of *Erwinia amylovora*. EPPO Reporting Service, Report No. 527.
- Garrett, C.M.E. 1990. Control of fire blight. CEC Agrimed research programme. Fire blight report (1990), EUR 12601.
- Huet, J. & J.C. Michelesi 1990. Sensibilité au feu bacterien des principaux porte-greffe du poirier et du pommier utilisés en Europe. CEC Agrimed research programme. Fire blight report (1990), EUR 12601.
- Psillakis, N. 1993. Opening address at the 6th International Workshop on Fire Blight (*Erwinia amylovora*). Acta Horticulturae, in press.
- Sletten, Arild 1990. Fire blight in Norway. Acta Horticulturae, 273: 37-40.
- Steiner, Paul W. 1990. Predicting apple blossom infections by *Erwinia amylovora* using the *Maryblyt* model. Acta Horticulturae 273: 139-148.
- Thibault, B. & M. Le Lezec 1990. Sensibilité au feu bactérien des principales variétés de pommier et de poirier utilisées en Europe. CEC Agrimed research programme. Fire blight report 1990, Eur 12601.
- van der Zwet, T. & S.V. Beer 1991. Fire blight - its nature, prevention and control: A practical guide to integrated disease management. U.S. Department of Agriculture, Agriculture Information Bulletin No. 631, 83pp.



# The position of fireblight (*Erwinia amylovora*) avoidance and resistance amongst pear breeding priorities

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Concorde and Doyenné du Comice, pear varieties which crop regularly tend to be free from secondary blossoms and thereby avoid infection from fireblight in the cooler growing regions, despite having high tissue susceptibility. In some areas high levels of tissue resistance are essential. Resistant selections from the cultivated pear provide the most promising sources of tissue resistance. At HRI selections are chosen on the basis of fruit and cropping characteristics before being assessed for resistance in the greenhouse, using a measured drop inoculation technique. New scion and rootstock varieties with satisfactory fireblight avoidance and high levels of tissue resistance are being produced.

Key words: Disease resistance, *Erwinia amylovora*, Pears, Plant Breeding, Pome Fruits

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Early regular heavy crops of high quality long storing fruit, with a long shelf life, are the main aims of most pear breeding programmes (Alston 1983a). Poor productivity in the years immediately following planting is common in all pear growing areas. It particularly threatens the survival of pear growing in cooler growing regions, such as north west Europe, where low temperatures at flowering, leading to poor fruit set, have a further detrimental effect on cropping. Careful selection for precocity, tree habit and flowering date in scion varieties can lead to major improvements, providing care is taken to combine such features with good fruit quality. Breeding new rootstocks which can induce precocity and semi-dwarf tree habit in established varieties is another approach to this problem.

In those warmer pear growing regions where *Erwinia amylovora* is prevalent, such as south-western France, fireblight is the most serious factor limiting pear production. It has had the most severe effects on the industry of mid-west U.S.A., where fireblight resistance is necessary for survival in both the scion and the rootstock.

### Fireblight avoidance

Secondary blossoms, which can appear up to 5 months after the normal flowering time, provide the main infection sites in most regions. Selection for the absence of secondary blossoms provides a means of avoiding serious fireblight damage. However, direct selection for the absence of secondary blossoms can prolong breeding programmes, since they rarely appear amongst fruiting progenies in the first 7 years from germination (Thibault 1980). Fortunately, a low incidence of secondary blossoms can be achieved concurrently during selection for precocity, high yield and regular cropping (Alston 1989). A tendency to produce secondary blossoms is frequently associated with poor fruit set, often as a result of low temperatures at flowering time, and thus more frequently observed in early flowering varieties. The late flowering variety Doyenné du Comice, which is slow to crop, produces only moderate, but consistent, crops; despite its high tissue susceptibility it consistently avoids serious damage from fireblight. It seems that late flowering, which can be regarded here as a component of regular cropping, might then be indirectly associated with fireblight avoidance in pears. Flowering time in pears is correlated with chilling requirement (Spiegel-Roy & Alston 1979), which presents early selection possibilities in young progenies, on the basis of bud break less than one year after germination,

### Fireblight resistance

Initially derivatives of *Pyrus ussuriensis* and *P. serotina* appeared to present the greatest potential as sources of very high levels of stable resistance under simple genetic control (Alston, 1973), but associated with small fruit size and astringent stony flesh. Before commercial varieties, including this resistance, become available a lengthy series of backcrosses to the cultivated pear is necessary. Work started at HRI using a first backcross derivative, Purdue 77-73 (*[P. ussuriensis* 76 x William's] x Comice), selections from a third backcross progeny, Beurré Hardy x (Conference x Purdue 77-73) are now close to fruiting.

Meanwhile, the most promising resistant varieties are derived from the cultivated pear, *P. communis*, and combine resistance with the good fruit qualities normally associated with commercial pears (Kappel & Quamme 1987). Varieties of this type, with moderate to high tissue resistance form the basis of most programmes. This resistance appears to be under polygenic control, progenies usually producing a low proportion of highly resistant seedlings. Much of this work is based on resistant selections from north American breeding programmes, although satisfactory levels of resistance have been identified amongst European varieties (Alston 1983b, Thibault et al. 1987b, Zwet & Bell 1990). Clara Frijs and Louise Bonne each have a high level of resistance, while Pierre Corneille and Conference both show a moderate level.

### Selection for resistance

Inoculating young seedlings within 1 month of germination either as intact plants in the greenhouse (Thibault *et al.* 1987a) or detached shoots in the laboratory (Alston, 1983b) is imprecise and too severe. It is not possible at this stage to satisfactorily discriminate between the various levels of resistance or to make choices which take into account the main aims of most breeding programmes, productivity and fruit quality.

Tests on pot-grown maiden trees, needle inoculated close to the growing point and

maintained in an isolation greenhouse, gave very promising results showing good discrimination between the differing degrees of resistance known in a range of varieties (Alston 1983b). These results agree well with those obtained after field tests on trees in an orchard in south west France where a number of young shoots on each tree were similarly needle inoculated (Thibault *et al.* 1987b). Consequently, a routine selection procedure was established at East Malling using this method on selections previously chosen for superior yield and fruit characters.

Very little variation has been observed in the relative response of pear varieties to various isolates of *Erwinia amylovora* leading to the conclusion that isolates should be chosen for inoculum on the basis of high general aggressiveness alone (Quamme & Bonne 1982, Bell *et al.* 1990). In such conditions dosage control is critical. Since we found it difficult to standardise dose levels through needle inoculation, we recently adopted a measured drop inoculation technique.

### Screening advanced selections

The results of a screening experiment serve to illustrate the basis of decisions necessary in the course of a breeding programme aimed at improving the productivity and profitability of pear growing.

Four potted maiden trees, worked on Quince C, of each of three varieties and 17 scion and rootstock selections, chosen for good fruit and agronomic characters, were inoculated in a gauze house. A measured drop of a suspension of a highly virulent isolate was applied to an exposed petiole scar close to the growing point of the leading shoot of each tree. The number of infected internodes was recorded after 3 weeks (Table 1).

The intermediate level of resistance (Conference) and high susceptibility (Comice) of the control varieties was clearly demonstrated. Both quince rootstock selections, QR193/2 and QR193/16, showed some resistance. Of these, QR193-16, semi-dwarf and an improvement on quince C (Browning & Watkins 1991), is being developed commercially. All the pear rootstock selections showed susceptibility, except QR517/9. The high resistance of Old Home was not apparent amongst its QR708 derivatives. However, QR708-36 will continue to be developed; orchard trials suggest that it could be a valuable semi-dwarf rootstock, it also roots easily, a rare combination amongst *Pyrus* rootstocks. Of the scion varieties, the three most resistant, P448/9, P384/39 and P384/52 each have the highly resistant selection from Illinois, 13B83 ([Comice x Farmingdale] x Maxine) as a parent, chosen also for good fruit appearance and quality and late maturity. P384-52 looks particularly promising, giving a valuable combination of productivity, good fruit quality and fireblight resistance. The new HRI variety Concorde (Alston *et al.* 1989) showed high susceptibility. Neither this variety nor Doyenné du Comice are seriously threatened by fireblight in the cooler growing areas. Concorde is a precocious and heavy regular cropper, Doyenné du Comice is slow to crop but irregular; both are late flowering and produce very few secondary blossoms and thereby avoid the disease. The susceptible selection P414-22 appears promising for north west Europe since it combines the precocity, high productivity and regular cropping potential of P39-13 (Beurré Hardy x William's) and the good fruit quality of Comice.

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Table 1. Reaction of advanced pear scion and quince and pear rootstock (QR) selections to fireblight

Selection	Parentage	Mean No. infected internodes/ shoot	95%* confidence limits
QR517-9	Open pollinated Ankara pear	0	(0,0.8)
P448-9	13B83 x PB28-19	0.3	(0,2.5)
P384-39	Conference x 13B83	2.2	(1.1,4.4)
P384-52	Conference x 13B83	3.5	(2.0,6.0)
QR193-2	Quince C51 open pollinated	4.5	(2.8,7.3)
P384-49	Conference x 13B83	5.7	(3.8,8.8)
Conference		6.7	(4.6,10.0)
QR193-16	Quince C51 open pollinated	9.7	(7.0,13.5)
QR708-23	B13 x Old Home	11.2	(8.3,15.2)
P459-26	P152-8 x Conference	13.4	(8.9,20.1)
QR311-7	Packham's Triumph x C8	14.7	(11.3,19.2)
QR708-63	B13 x Old Home	14.7	(11.3,19.2)
P414-22	Doyenné du Comice x P39-13	15.5	(11.9,20.0)
P15-157	William's x Conference	15.7	(12.2,20.3)
QR708-36	B13 x Old Home	16.0	(12.4,20.6)
Concorde	Doyenné du Comice x Conference	16.7	(13.0,21.5)
QR708-13	B13 x Old Home	17.8	(13.5,23.5)
P446-7	Packham's Triumph x P55-54	17.9	(10.9,29.5)
QR107-2	Open pollinated <i>P. longipes</i>	18.7	(14.8,23.7)
Doyenné du Comice		19.7	(15.7,24.8)

\* Calculation based on a Poisson distribution for the number of infected internodes per shoot.

## DISCUSSION

New varieties with good quality, satisfactory levels of cropping and the ability to avoid serious damage from fireblight are becoming available. However, where climatic conditions are conducive to the disease over much of the growing season, trees can be infected through primary blossoms and injured shoots. In such regions a high level of tissue resistance is required, both in the scion and the rootstock. Reliable regular cropping remains so important in most areas that new varieties with such features are likely to be preferred, whether or not they are resistant, in all but the most severe fireblight situations. Such an approach is likely to be very successful in cool growing regions like south east England, where fireblight incidence in pear orchards is sporadic (Billing 1980).

In some parts of north west Europe, however, planting varieties which can avoid serious fireblight damage may not be sufficient in some seasons. In such cases, where the spread of the disease is limited but not eliminated chemical or antibiotic control may be necessary (Deckers *et al.* 1987). Most of the chemicals used can be phytotoxic and there are inherent dangers from antibiotics in that their use encourages the development of resistance to antibiotics amongst bacteria (Manceau *et al.* 1987). The use of resistant varieties should provide the most efficient and environmentally safe means of controlling this disease. Tissue resistance must be combined in new varieties with heavy regular cropping



and good fruit quality. The current HRI breeding programme seeks to achieve this and will thus combine two biological means of fireblight control, avoidance and tissue resistance.

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#### SUMMARY

Early regular crops of high quality long storing fruit with a good shelf life remain the main aims of most breeding programmes. A strong emphasis is placed on selecting for precocity and productivity leading to regular heavy crops. Such varieties tend to be free from secondary blossoms, the main fireblight infection sites in most regions, and thereby often avoid infection, even where plant tissues are highly susceptible. Where climatic conditions are conducive to the disease over much of the growing season, trees can be infected through primary blossoms and injured shoots. In such regions high levels of a tissue resistance in both the scion and the rootstock are an important prerequisite. At East Malling the main source of resistance is the cultivated pear, *Pyrus communis*, resistant parents are chosen also on the basis of their fruit and cropping characteristics. Selections are made first for these features and subsequently young trees of such advanced selections are assessed for resistance in the greenhouse using a measured drop inoculation technique. Advanced quince and pear rootstocks are assessed at the same stage. New varieties with satisfactory fireblight avoidance and high levels of tissue resistance will be produced from this programme. While moderate levels of resistance were detected amongst advanced quince rootstock selections, an outstanding degree of resistance was recorded in a pear rootstock selection. There are thus good prospects of achieving satisfactory control of the disease, without resource to copper or streptomycin sprays, whilst improving levels of productivity and fruit quality.

#### REFERENCES

- Alston, F.H. 1973. Pear Breeding at East Malling. Proceedings of ISHS Fruit Section Working Group on Pear Symposium, Angers, 1972, 41-50.
- Alston, F.H. 1983a. Pear breeding progress and prospects. Proceedings of the 21st International Horticultural Congress, Hamburg, 1982, 127-137.
- Alston, F.H. 1983b. Fireblight (*Erwinia amylovora*) resistance in the East Malling pear breeding programme. Bulletin SROP/WPRS, 6: 165-170.
- Alston, F.H. 1989. Breeding pome fruits with stable resistance to diseases. 2. Selection

techniques and breeding strategy. IOBC Working Group on Integrated Control of Pome Fruit Diseases, Brissago, 1988. WPRS Bulletin XII/6: 90-99.

Alston, F.H., J.R. Stow & G. Browning 1989. 'Concorde' the new Malling pear variety. *Plantsman*, 2: 130-131.

Bell, R.L., T. Zwet & W.G. van der Bonn 1990. Environmental and strain effects on screening for fireblight resistance. *Acta Horticulturae*, 273: 343-347.

Billing, E. 1980. Fireblight in Kent, England, in relation to weather 1955-76. *Annals of Applied Biology*, 95: 341-364.

Browning, G. & R. Watkins 1991. Preliminary evaluation of new quince (*Cydonia oblonga* Miller) hybrid rootstocks for pears. *Journal of Horticultural Science*, 66: 177-181.

Deckers, T., J. Geenen & W. Porreye 1987. Strategy for fireblight control (*Erwinia amylovora* (Burr.) Winslow *et al.*) under natural infection conditions. *Acta Horticulturae*, 217: 203-210.

Kappel, F. & H.A. Quamme 1987. Processing quality of pear selections in the Harrow breeding programme. *Fruit Varieties Journal*, 41: 136-140.

Manceau, C., L. Gardan & J.P. Paulin 1987. Use of antibiotics to control fireblight in France: environmental hazards and established legislation. *Acta Horticulturae*, 217: 195-202.

Quamme, H.A. & W.G. Bonn 1982. Virulence of *Erwinia amylovora* and its influence on the determination of fireblight resistance of pear cultivars and seedlings. *Canadian Journal of Plant Pathology*, 3: 187-190.

Spiegel-Roy, P. & F.H. Alston 1979. Chilling and post-dormant heat requirement as selection criteria for late flowering pears. *Journal of Horticultural Science*, 54: 115-20.

Thibault, B. 1980. Etude de la transmission de quelques caracteres dans des descendes de poirier. *Proceedings Eucarpia Tree Fruit Breeding Symposium*, Angers, 1979, 47-58.

Thibault, B., P. Lecomte, L. Hermann & A. Belouin 1987a. Comparison between two methods of selection for resistance to *Erwinia amylovora* in young seedlings of pear. *Acta Horticulturae*, 217: 265-269.

Thibault, B., P. Lecomte, L. Hermann & A. Belouin 1987b. Assessment of the susceptibility to *Erwinia amylovora* of 90 varieties or selections of pear. *Acta Horticulturae*, 217: 305-309.

Zwet, T. van der & R.L. Bell 1990. Fireblight susceptibility in *Pyrus* germplasm from Eastern Europe. *HortScience*, 25: 566-568.

# Biology and biotechnology in strategies to control apple scab

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The interaction between commercial apple cultivars and the scab pathogen *Venturia inaequalis* is controlled by genes for resistance in the host and corresponding genes for virulence in the pathogen. This type of interaction occurs with most old and some new cultivars and it is postulated therefore that the composition of the pathogen population is due to selection by host resistance genes. The consequences for breeding strategies and for orchard planting to minimise fungicide input are discussed as well as the use of genomic markers to help in adopting the proposed strategies.

Key words: Apple, biotechnology, breeding, *Malus*, marker, scab, *Venturia*.

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"If the histories of varieties of fruits could be written from the natural-history side, I fancy that many of our notions would be upset" (Bailey 1892).

Apple scab is the most important disease of its host in many countries, while in others (England) it is second only to powdery mildew. Aderhold (1899) first described the biology of the fungus and most of the important aspects of its interaction with the host. The first recommendations for controlling the pathogen (Blair 1899, Clark 1891, Chester 1895, Crandall 1909, Fairchild 1894, Goethe 1888, Goff 1889, Frank 1901, Aderhold 1902, see Wallace 1913) concerned chemical treatment with copper-lime and later with Bordeaux mixture. Ten pounds of apples were enough to pay for the recommended three treatments. Aderhold (1902) cites trials where, with three treatments, 2112 pounds of apples per tree of a good quality (price 51.2 cts) were harvested compared to 760 pound at 12.5 cts for untreated trees. The cost/benefit ratio (1/193) was already highly in favour of treatment. Today the situation is little different. Fungicide application is still the economically optimal solution and the best insurance for maximum production of a high quality product.

However, consumer and environmental concerns require a new orientation of production systems. The main objective up to now has been maximal production regardless of sustainability and use of resources, dependent only on financial optimisation which was

often altered artificially by political decisions. Agricultural production is now, or should be, oriented towards environmental safety, sustainability, low input and maintenance of floral and faunal diversity. Moreover, in particular regions, farming has to interact with and to take responsibility for the desires of the general public regarding recreational aspects of the landscape, wildlife conservation and the maintenance of local cultural traditions.

Policy makers today direct their attention towards replacement of agrochemicals, in particular pesticides and fertilisers, and the introduction of environmentally safer and sustainable technologies. Since its foundation, the "International Organisation for Biological and Integrated Control of Noxious Animals and Plants" (IOBC) has directed its efforts in this direction. A Commission was charged with defining Integrated Production, and also to describe the underlying strategy and to establish technical guidelines and standards for implementation (see El Titi et al. 1993). Integrated Production is defined as a farming system that produces high quality food and other products by using natural resources and regulating mechanisms to replace polluting inputs and to secure sustainable farming. Emphasis is placed on the agro-ecosystem, particularly on natural resources and regulation mechanisms, to achieve maximum replacement of off-farm inputs. Biological diversity is regarded as the backbone of the natural regulation factors, ecosystem stability and landscape quality.

Such concepts are translated into local IP guidelines, which postulate that natural limiting factors, such as resistance of the cultivars, choice of site, natural enemies and appropriate culture systems should be fully exploited. Only when all of these resources have been used and the risk is still too great or a tolerance level has been passed, can pesticides be applied to control the target organism. The control of pests and diseases should be by need rather than by routine and then only through the use of environmentally safe methods. The use of resistance is considered highly advantageous for farmers, environment and consumers, but durability of the resistance is crucial. Johnson (1981) defines "durable" disease resistance as resistance that lasts at least the period during which the resistant cultivar is widely cultivated and e.g. successful on the market (Hogenboom 1993). Durability of resistance is greatly influenced by its management (Wolfe 1983) and may also include resistances that have already been overcome (Hogenboom 1993). In this paper I will try to suggest strategies to use the many ephemeral resistances known in *Malus*, either in breeding or in orchard planning.

## OBSERVATIONS ON INTERACTIONS IN ORCHARDS

Aderhold, even though he described chemical treatments as highly cost efficient, was aware that other factors could be used to reduce scab, e.g. the elimination of leaf litter. More practical was the recommendation to use cultivars with higher resistance against the disease. However, Aderhold questioned this tactic. He observed that even if particular cultivars seem to be more resistant than others, the observations were not consistent. He mentions examples where a cultivar defoliated by scab in one year was almost scab-free in another; under the same conditions another cultivar behaved in exactly the opposite way. Aderhold explained these facts by the growth habit of a single tree, pointing out that often one cultivar grows slowly, taking a long time to produce new leaves and is therefore prone to

scab, while other cultivars in the same year will complete their vegetative growth rapidly and thus escape scab infection. However, he also mentioned observations which put this explanation in doubt. Not only does the resistance/susceptibility of a single cultivar change with years, but also with the site and soil type. He collected data on susceptibility/resistance from various reports and concluded that the susceptibility of a cultivar is different at different sites. Some cultivars may have behaved uniformly but others such as the white Winter-Cavill, Goldenzeugapfel, Morgenduftpfel (Rome Beauty), Osnabrücker Reinette and Winter Goldparmäne were sometimes described as susceptible, and at other sites as quite resistant.

To Aderhold these discrepancies seemed to be due not to the cultivar nor the site, but rather to the fungus itself. He experimented with *Fusicladium pirinum*, the pear scab pathogen, and observed that infections were most successful using inoculum from the same cultivar and most difficult using inoculum from another cultivar. If this were true also for the apple scab fungus, Aderhold explains, then one can understand why a cultivar can stay almost scab-free for a long time near to scabbed trees of a different cultivar. However, once it has been severely infected, all trees of the same cultivar in the proximity will be severely scabbed. In another region, where the fungus has not yet accommodated to the host, this cultivar was still scab-free. This led Aderhold to recommend avoidance of the first infection on a particular cultivar and for that reason he insisted that the nurserymen keep their trees scab-free with Bordeaux-mixture so that they delivered only healthy material. His exhortation to the German Pomological Society to consider the question of resistance and susceptibility not only from the practical but also from the scientific point of view, was prophetic. He proposed that investigations should be made on the behaviour of various cultivars at various sites not only once but over several years. He concluded that until more is known, the best practice is still to collect all leaf litter and to treat with Bordeaux-mixture in winter and early summer (Aderhold 1900). Today we may not rely on the squirt-gun, rather on a turbo-blower, but the concept remains the same.

Wallace (1913) from Cornell, in a review, concluded that the use of resistant cultivars offers little promise as a means of control. The results available gave little hope, since varieties known to be fairly resistant changed to susceptible over the years. Wallace suggested that, in such orchards, particular strains adapted to a particular cultivar were bred by natural selection. He saw no reason to expect that any variety on which the fungus can grow, no matter to how small an extent, would remain resistant indefinitely if it was allowed to select and multiply those strains of the fungus capable of attacking it.

Wiltshire (1915) affirmed that it was well-known that one variety may be heavily attacked one year and remain almost immune the next, despite the presence of the fungus, but he found it unprofitable to formulate hypotheses to account for these phenomena in the absence of definite facts.

Even more recently, cultivars have been classified completely differently in particular regions. For example, from field trials in central Italy, Govi (1966) classified Gravenstein as free from scab, Golden Delicious, Cox's Orange and Jonathan as resistant, and Starking and Starkinson as susceptible, whereas in Germany and Switzerland, Golden Delicious was recognised as highly susceptible, Gravenstein as susceptible (Bavendorf) or as highly susceptible (Switzerland), and Cox's and Jonathan as intermediate (Götz and Silbereisen 1989, Aeppli et al. 1983).

Bailey (1892) was also prophetic when he wrote "enemies often progress or develop as rapidly as do the host plant. I imagine that by the time we are able to breed scab-proof varieties our scab-fungus will have developed a capability to attack more uncongenial hosts. For my generation, at least, I must pin my faith to the squirt-gun."

#### EXPERIMENTATION TO UNDERSTAND THE DIFFERENTIAL INTERACTION

Johnstone (1931) suggested from his results the existence of biological specialisation of strains of the fungus to certain groups of varieties. Wiesmann (1931) confronted the problem scientifically. Unfortunately he had no infection success with monoconidial isolates, so he used conidial suspensions from naturally infected leaves to demonstrate differential reactions of the cultivars Boiken apple, Wellington Reinette, Gravenstein and Virginia Rose-apple. His experiments showed 'more/less' responses rather than clear 'yes/no' differences. This could have been for several reasons, for example, that the differential resistance genes gave a quantitative response, allowing some colonisation by an inoculum that consisted of sub-populations of different races in which the respective compatible race was most frequent (Gessler 1989). Wiesmann (1931) can be credited with the first clear results showing differential reaction between different resistances of apple varieties and specific virulences of the scab pathogen.

Palmiter (1934), working with monoconidial cultures, was able to show that particular varieties showed differential reactions towards scab isolates. A group of 5 varieties, Yellow Transparent, McIntosh, Dudley, Missouri Pippin and Hubbardston Nonsuch, differentiated the isolates clearly by allowing either infection and sporulation or no symptoms. From the gene-for-gene concept, each of these cultivars has at least one resistance gene giving complete resistance and certain races have the corresponding gene or genes for virulence. The author mentioned that other varieties (Ingram and Haraldson) were severely infected by some isolates but only slightly by others. A third group of varieties was infected more or less equally by all isolates. Keitt and Nusbaum demonstrated differential interaction between particular isolates of scab and particular cultivars generally regarded as susceptible. Keitt & Nusbaum (1936) and Nusbaum & Keitt (1938) pointed out that isolate 22a parasitized Yellow Transparent vigorously and Fameuse moderately, while isolate 17a behaved in exactly the opposite way.

Schmidt (1936b) tested the interactions of monoconidial isolates from various sites and varieties, confirming the existence of differential reactions and therefore of specific virulences and resistances. His results are much more complex. The isolates collected at a single site (a variety collection) not only showed a consistent ability to attack the cultivar from which they were isolated, but also to attack many if not most other hosts tested. The hosts in the differential set were, in turn, resistant to at least one isolate except for the cultivar Jacob Lebel which was susceptible to all. No isolate and no variety behaved in the same way, indicating resistances overcome by specific isolates in 13 of the 14 varieties tested, involving 13 functionally distinct resistances. None of the isolates was a simple race but each had a complex range of virulences, probably due to selection and recombination on the cultivar collection. The isolates which originated from other sites were less complex. Unfortunately, the characteristics of the orchard (other cultivars and cultivar frequency)

from where the isolates originated are not given. But we can still observe that complex races were present, and that their virulence composition varied greatly, partly dependent on the site so that, for example, the virulence overcoming the resistance of Coulon Reinette was present in isolates from only two out of five sites.

Dufrenoy (1936) suggested that the "immunologique relations" have to be defined for each apple cultivar against each "race" of *V. inaequalis* to which it is exposed. He based his statement on the assumption that the "mycological specie" *V. inaequalis* consists of a number of races characterised by a particular degree of virulence toward a particular apple cultivar. He stated that these studies guide the geneticists (breeders?) from the USA, Germany and Switzerland in selecting resistant cultivars. In this respect the breeders of Münchenberg were well aware that true physiological specialisation existed and that it is necessary to test a new scab resistant apple selection with strains from quite different areas or to expose those selections to natural infection in different regions (Schmidt 1938).

The first genetic approaches to the analysis of virulence were made by Keitt and co-workers (Keitt and Palmiter 1938, Keitt and Langford 1938), thanks to the development of a technique allowing formation of perithecia and viable ascospores under controlled conditions. They were able to isolate and grow singly in culture the eight ascospores from a single ascus. The virulence of the parents was unknown because the perithecia used were formed after natural infection on a Dudley apple leaf. Virulence on Yellow Transparent and McIntosh was present in four of the eight ascospores but absent in the others; virulence on Missouri Pippin, Hubbardston Nonsuch and Fameuse was present in all. From these studies we also know that the latter four varieties are not generally susceptible but are capable of differentiating pathogen strains. Further studies with parent strains with known virulences (Keitt et al. 1943; Keitt et al. 1948) showed the presence of two genes (or four genes but closely linked as two pairs) governing virulence/avirulence toward the cultivars, respectively, Haraldson and Wealthy, and Yellow Transparent and McIntosh. The two loci were not linked. Boone and Keitt (1957) demonstrated the presence of a total of 7 virulence/avirulence genes. From the gene-for-gene relationship (Flor 1942) therefore, the cultivars possess one to several resistance genes (Table 1) and the pathogen isolates used by Boone and Keitt, and presumably those present in nature, are complex, carrying several virulences. Since these virulences are mostly inherited independently they may be recombined during each sexual cycle. These findings led to the statement by Boone and Keitt that there is little point in classifying lines of *V. inaequalis* into physiologic races with respect to the resistance genes present in commercial apple cultivars.

Boone (1971), reviewing the genetics of *V. inaequalis*, assembled data showing the presence of 19 genes for virulence against corresponding genes for resistance (some of which were identified but most were speculative) in commercial cultivars (see Table 1), in selections used for breeding, or in other *Malus* species. Even though corresponding data for European cultivars was not included, the previously mentioned papers of Wiesmann, Rudloff and Schmidt indicate that many more resistance genes occurred in Europe. We may assume that the gene-for-gene hypothesis holds for all of these genes since Boone noted that if two or more pathogenicity (virulence) genes controlled virulence on one variety or selection, then the avirulence allele at one locus was epistatic to any virulence allele at other loci, i.e. for a compatible interaction all resistance alleles in a single cultivar have to be matched by their corresponding virulence alleles.

Table 1. Hypothetical resistance genes present in commercial apple cultivars (after the data from Boone and Keitt 1957)

Cultivar / resistance gene	1	2	3	4	5	6	7
McIntosh	+		+				
Yellow Transparent	+						
Wealthy		+					
Beacon		+					
Prairie Spy		+					+
Northwestern Greening		+					
Rome Beauty		+					
Red Astrachan/			+				
Haraldson		+		+			
Hyslop				+	+		
Grimes Golden						+	

Shay and Williams (1956) noted that few, if any, commercial apple varieties are resistant to a sufficient proportion of the total U.S. or European population of biotypes (pathotypes, races) of *V. inaequalis* to warrant their use as resistant parents in apple improvement. The attention of breeders was directed, therefore, towards scab resistance from other *Malus* species and that of pathologists towards the identification of virulence against those resistances. Shay and Williams (1956) used the resistant varieties from *Malus baccata*, Dolgo, Bitter Crab and Alexis, the variety Geneva (open pollinated descendant from *M. pumila* var. *niedzwetzkyana*) and certain segregates of a Russian variety of *Malus pumila* R12740-7A, to identify three pathogen "races". They defined as race 1 all isolates giving only necrotic or chlorotic lesions with no sporulation on these differential hosts, race 2 as isolates that overcame all resistances and race 3 as isolates that overcame the resistance of Geneva. From the segregation data on *V. inaequalis* they deduced that race 2 was a complex race with virulence based on three different genes, each specific for one of the differential varieties. Race 3 had a single gene for virulence overcoming the resistance of Geneva, but at a different locus from the gene of race 2 for virulence on Geneva. This fact remains unexplained. Race 1 therefore includes the world-wide population of the scab pathogen except for those isolates that can overcome the resistance of one or more of these differential hosts. Since then, three more "races" have been named: race 4 is the same as race 1 except that it can attack a particular segregant of the Russian variety R12740-7A



different from the ones mentioned above that can be overcome by race 2 (Shay Williams and Janick 1962); race 5 is specific for the resistance in *M. micromalus* 245-38 and *M. atrosanguinea* 804 pit type reaction (Williams & Braun 1968). Most recently described is race 6 that can overcome the Vf resistance from *M. floribunda* (see Parisi et al. 1993 and 1994). Still open is the denomination of the race overcoming the original *M. floribunda* (see Roberts et al. 1994). Denomination of complex races simply by following their order of detection may be impractical and lead to errors. On the other hand, denominating the races by the resistances which they can overcome, even if this seems most logical, presents problems since, as shown above, a cultivar may often have more than one resistance and the same resistance can be present in more than one cultivar or *Malus* species.

## RESISTANCE IN MALUS

Commercial cultivars have never been analysed for segregation of genes for differential resistance. Because of the variability of the scab population and the high frequency of virulence genes overcoming specific resistances of the commercial cultivars, breeders opted to use material resistant against all strains of *V. inaequalis* present at their location. This included other *Malus* species, often from other continents (Asia). For example, *M. baccata* Borkh. originates from the Himalayas, Siberia and eastern Asia. *M. floribunda* is known only as cultured species from Japan, probably originating from interspecies crosses from *M. Kaida* x *M. baccata*, *M. ringo* x *M. spectabilis* x *M. baccata* or others (Hegi 1963). *M. micromalus* comes from China.

Resistances from other *Malus* species were studied intensively indicating the existence of a large pool of resistances (Williams and Kuc 1969). The available data allows various interpretations, for example, to assign the Vf resistance of *M. floribunda* 821 to a single gene (Williams and Kuc 1969, page 226), or to suggest that the original level of resistance in *M. floribunda* 821 was due to a group of closely-linked quantitative genes. Alternatively, resistance was due to a qualitative gene giving a class 3 reaction type, closely linked with one or more quantitative genes always inherited to the modified backcross progeny and due to a gene giving a class 1 reaction, not detected any more in the backcross progenies (Williams and Kuc 1969, page 227). Crosby et al. (1992) reviewing the success of breeding apple for resistance against scab, list 48 cultivars resistant to scab of which 37 carry the Vf-resistance. They hypothesised, as have Rousselle *et al.* (1974) and Gessler (1989), that the phenotype of the Vf resistance is enhanced or augmented by additional genes. The fraction of a segregating population carrying the Vf gene from a cross between a Vf carrier and a susceptible parent show continuous gradation of the resistance reaction from no symptoms to restricted sporulation and even to full susceptibility.

To my present knowledge, 19 independent resistance loci have been described (Bagga & Boone 1968). So far, only six resistances at different loci from 6 *Malus* species are named (Vf, Va, Vb, Vbj, Vr and Vm). As Dayton & Williams (1967) noted, it is still not clear whether all genes are truly different or whether some are identical genes that were transferred to non-homologous chromosomes (or loci) by aberration during the evolutionary development of *Malus*. On the other hand, there may be as many mechanisms of resistances as there are resistance genes present (Bagga & Boone 1968) and, correspondingly, the same

number of different virulence genes. This can be established only after the corresponding virulences have been found and the resistance carriers tested accordingly.

The Vm resistance is different from the other single resistances as it is overcome by race 5. Some of the 25 single gene resistances found in 41 crab-apples by Bagga must be functionally different from each other because some remained resistant to an inoculum consisting of different isolates (Williams & Kuc 1969, page 228). Again we cannot deduce that the 7 crab-apple selections which were resistant to the isolate used by Bagga but susceptible to the undefined inoculum used by Williams, had the same, or up to 7 different resistance genes. Similarly, we cannot deduce that the remainder had the same functional resistance but at different loci. Other examples where interpretation is impossible can be found in the literature. Recently (Gessler et al. 1993, Sierotzki et al. 1994) it was shown that three popular cultivars considered to be susceptible, have differential and ephemeral resistances, each being active against populations of the pathogen collected from the other cultivars. We may now assume that the speculation that a gene-for-gene relationship exists (Boone 1971) is also correct and valid for these unrecognised resistance genes in the commercial cultivars. The total number of functionally different resistances, from wild *Malus* species and from cultivated commercial varieties, almost all recognised as ephemeral, may be many times greater than expected.

The data described indicate that many different ephemeral resistance genes are present in old cultivars and other *Malus* species. A corresponding number of pathotypes is probably present in the world population of the scab pathogen, with the limitation, often found in other host-pathogen systems, that a number of the resistance genes have the same function or occur at the same loci, so that the number of different virulences may be less.

#### STRATEGIES TO IMPROVE THE DURABILITY OF EPHEMERAL RESISTANCE

By incorporating any resistance (or part of the immunity) into *Malus domestica* (for example, the Vf resistance) we exert selection on the scab pathogen which leads to the emergence of a specific race (or races) as Parisi et al. (1994) and Roberts et al. (1994) showed. Substituting susceptible cultivars by cultivars with Vf, or any other single resistance, may not be a long-term strategy as long as we maintain the concept of monoculture; we can predict that the resistance will be completely overcome and the cultivars will then be dependent only on their background resistance.

Populations of pathogens are genetically variable and therefore respond to selection. This is the basic concept of evolution. Mankind changed the natural environment drastically by uniting single genomes (i.e. cultivars) into units larger than single trees, creating large and dense, genetically uniform, host areas (monoculture). Seemingly, the Greeks propagated apple clones by grafting and the Romans cultivated particular varieties in orchards and had disease problems with them. Until the beginning of this Century, the planting system was mostly single trees in meadows and not arable land, most of the trees originating from seedlings. The intensive orchard forms planted with a limited number of cultivars which we know today, was adopted only during this Century mostly thanks to the systematic selection of root stocks with particular growth characteristics (Hatton 1939). Parallel to the disappearance of the sparse planting system of trees of unequal genome, the

disease situation worsened. With the introduction of dense orchards chemical disease control was introduced. Even today we can observe that from single standing trees of "older" cultivars (cultivars not popular anymore), scab does not cause a total loss, on the other hand in the same situation scab causes in an unsprayed intensive orchard of a single cultivar total or near total loss. Even if the destabilizing effect of increasing aggregation of plants with the same genome is poorly substantiated (Zadoks 1993) we can postulate that this change shifted drastically the relationship between the host (now a single entity) and the parasite. Previously, variability was required to enable the pathogen to infect more than a single entity (by sexual ascospores) followed by asexual reproduction from a successful infection; now, however, uniformity gives an advantage to the pathogen. Scab would now damage trees to such an extent that trees would be defoliated early and some individuals would no longer be able to survive competition from other trees, leading to a thinning of the close stand. However, mankind again intervened by reducing pathogen damage through protection by pesticides. As efficacy of the pesticides and of pesticide scheduling improved, host density was maintained or increased.

At this stage, the pathogen population also responds to the fungicides and resistant strains or sub-populations are selected. We are now fixed to such an extent on the use of pesticides to maintain an equilibrium between pathogen and host that we have forgotten how this situation arose; the current unnatural situation is now considered natural.

Can we return to the earlier system of dispersed trees of various cultivars (described for apple above)? I am not advocating this as it is unrealistic. Can we continue on our current road? Again this is unrealistic or at least short-sighted. Few classes of fungicides have been developed and the probability of finding further new products is diminishing. Moreover, even with the best strategy of use, fungicide resistance is likely to emerge. Indeed, by definition, no chemical strategy is sustainable because of either resistance or of toxicity to man or other organisms.

In the co-evolution of a host and pathogen in a particular environment, the main stabilising factors are, to different extents, distances between hosts, inter-cropping and genetic variability of the host population. As the first two factors cannot be altered the last remains.

One way is shown (Wolfe 1983, Blaise and Gessler this volume) by cultivar mixing, in which each cultivar needs to have a different resistance. A simple mixture of only three cultivars greatly limits the production of primary lesions, but we have now to prove or better demonstrate the validity of the theory for this host-pathogen system. However, we do not know the resistance gene composition of all cultivars and old cultivars do not correspond to our needs, therefore modern cultivars need to be studied accordingly or new cultivars need to be bred.

Breeders have two principal options. The first is to breed a set of cultivars each having a functionally different resistance. These unrelated cultivars, each with one or more resistances, may differ so much that adaptation of the fungus is difficult if not impossible. In other words, super races may be much less likely in appropriate cultivar mixtures than in a cultivar with the same total number of resistances even without considering the possibility of stabilising selection *sensu* Vanderplank (1982) (see Schaffner et al. 1992 for similar data on the response of populations of barley mildew to large scale use of variety mixtures). The recognition of functional different resistances is possible if the resistances

are ephemeral and only through the use of differential races. Such a selection scheme would be very cumbersome. An alternative would be to transfer the genome segment carrying the resistance from one cultivar into the target cultivar. The target cultivar could be a popular commercial cultivar. By incorporating functionally different resistances, the concept of near-isogenic lines (NIL) could be adopted. Even if this cannot yet be done, it may well be possible in the foreseeable future, although it is not clear whether this will be acceptable to consumers. Moreover, by incorporating the resistances into a single genetic background, we may simplify the problem for the pathogen of overcoming the resistances (Wolfe 1993) and thus select a fit super-race (a single race able to overcome all mixture resistance components) even in a resistance mixture concept.

The second option is to breed varieties that contain the greatest possible number of resistance genes (pyramiding). The presence or absence of each gene could be recognised by serial testing of the progenies of correctly selected parents with inoculum of races carrying the appropriate virulences. Alternatively, the races could be mixed in the inoculum to reach the same conclusions in fewer tests except that genes giving incomplete resistance may be missed. This system could again be used only for resistances known to be ephemeral, i.e. if we have the corresponding virulent races which would allow identification of a second (or third?) resistance. However, selection for super-races may be stronger than in the NIL concept, particularly for a parasite such as *V. inaequalis* which reproduces sexually each year. There has been much discussion on the advantage or disadvantage of either strategy (Wolfe 1993), mostly by experts on annual plants. The choice of strategy of multi-cultivar mixture, NIL or pyramiding resistance genes may not be relevant to the apple system because the constraints on success may be of a completely different nature.

A more elegant approach than selecting resistance in the glasshouse or field, is to identify or mark the genome segment carrying the resistance information and to select progeny by the presence of such markers. The success of such a method is based on the assumption that resistances at different loci are also functionally different (or least that this is highly probable).

Since the resistance genes are often not easily identified, it may be better, to concentrate on marker-assisted breeding, particularly, because of its high feasibility and cost-benefit ratio.

Techniques to find DNA-markers started with the discovery of RFLPs (Lander and Botstein 1989). More recently, a method has been introduced which can be more accurate for large-scale screening, that is, the use of Random Amplified Polymorphic DNA (PCR-RAPD) (Williams et al. 1990). Specific probes can be developed from RAPDs and the routine use of these in screening can help to eliminate problems of inconsistency that sometimes occur with RAPDs.

Molecular genetic markers closely linked to a desired trait can now be found readily by bulked segregant analysis (Michelmore et al. 1991) in which bulks of DNA from individuals of a progeny which possess or lack, for example, a desired resistance are compared for the associated presence or absence of a linked RAPD band on a gel. Since each band represents a specific amplified genomic DNA segment (Williams et al. 1990), differences between the discriminated bulks are most probably markers linked to the disease resistance genes. Such a marker can then be analysed in a larger progeny for segregation

with the trait and to find further markers. Linked traits and markers will segregate together and distance can be calculated by appropriate methods from the number of cases of dissociation.

The availability of linked markers will allow solution of an additional problem of back-crossing. Repeated back-crossing and selection removes the wild parent chromosomes not linked to the desired gene (by a factor of two) and allows recombination to remove wild parent segments which are linked to the target gene. Linked wild-type segments may persist longer or be removed faster than expected (Stam & Zeven 1981; Zeven et al. 1983; Young & Tanksley 1989). The probability of removal of such segments falls to very low values with their increasing proximity to the target gene. Moreover, breeding perennials poses several additional problems, not least that back-crossing is not true back-crossing because the recurrent parent changes from generation to generation (Wolfe & Gessler 1993). A genetic marker method that allows us to recognise progeny individuals with minimal linked segments can reduce considerably the number of progeny selected at an early stage in the breeding programme. Once a marker (in the absence even of the trait itself) or extremely close to the desired trait together with linked markers at a reasonable distance on each side, have been found, individuals of the progeny of selected Vf x susceptible crosses can be screened for that particular marker (e.g. for the resistance phenotype) and for the absence of the flanking markers.

This method was successfully used (Michelmore et al. 1991) in lettuce to find markers linked to a gene for resistance to downy mildew and to find additional markers linked to a known marker (Giovannoni et al. 1991) in tomato. The authors suggest that the technique should be used to construct a saturated genetic linkage map around a desired area. On the other hand the availability of markers (RAPD, RFLP or others) for several differential resistance genes, including the unrecognised resistance genes of older cultivars, will allow selection of individuals with several resistance genes in an appropriate progeny. Moreover, recognition of ephemeral resistance genes in commercial cultivars is greatly facilitated by a set of DNA-probes able to detect those differential resistance genes. It would be possible to provide each cultivar with a key denoting its resistances which in turn would facilitate the choice of optimum partners for a cultivar mixture at a particular site. This information is fundamental to the use of the concept of apple cultivar mixtures as proposed by Blaise and Gessler (this volume).

## CONCLUSIONS

Requirements of consumers and environmental concern impose strong pressure to change production systems toward systems with lower pesticide inputs. Resistance breeding is regarded as highly relevant since widespread adoption of disease resistant cultivars would solve most of the concerns expressed by environmental movements and consumers. However, a crucial question for the success of resistant cultivars is the faith of the producer in the marketing quality of the cultivars and in the superiority of the cultivar because of their resistance. If the producer is not convinced of the durability of the resistance it will be difficult to substitute well-known susceptible cultivars with unfamiliar resistant cultivars. The Vf resistance was therefore tested carefully over 40 years in scab favourable situations

all over the world and breakdown of the resistance was not expected (Crosby et al. 1992). However, it occurred (Parisi et al. 1993) and left breeders and producers in doubt of the relevance of resistant cultivars. As documented by over 100 years of observations and research, breakdown of resistances in apple is a natural and common phenomenon, so common (specific resistances in commercial cultivars) that we do not notice it except in special cases (e. g. the Vf resistance). With modern genomic markers, breeding strategies can be envisioned which can select individuals with several and/or different functional resistances. With the right cultivar combination, planting strategies can then be devised which will impede the pathogen from reaching intolerable epidemic levels. Only through co-ordinated breeding and later correct use of the selected cultivars can fungicide input be reduced permanently. Natural, successful "planting systems" are usually based on genetic variability; our conception of orchards for the next Century should follow this lesson.

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#### LITERATURE

Aderhold, R. 1896. Die Fusicladien unserer Obstbäume, I Teil. Thiels Landwirtschaftliche Jahrbücher 25: 875-914.

Aderhold, R. 1899. Auf welche Weise können wir dem immer weiteren Umsichgreifen des *Fusicladium* in unseren Apfelkulturen begegnen und welche Sorten haben sich bisher dem Pilz gegenüber am widerstandsfähigsten gezeigt? Pomologische Monatshefte: 266-272.

Aderhold, R. 1900. Die Fusicladien unserer Obstbäume, II Teil. Thiels Landwirtschaftliche Jahrbücher 29: 541-588.

Aderhold, R. 1902. Aufforderung zum allgemeinen Kampf gegen de *Fusicladium* - oder sog. Schorfkrankheit des Kernobstes. Keiserliches Gesundheitsamt. Biologische Abteilung für Land- und Forstwirtschaft. Flugblatt Nr. 1 Februar 1902. 4 pp.

Aeppli, A., U. Gremminger, Ch. Rapillard & K. Röthlisberger 1983. 100 Obstsorten. Verlag Landwirtschaftliche Lehrmittelzentrale Zollikofen CH. 259 pp.

Bailey, L.H. 1892. Scab-proof apples. Garden and forest 5: 442.

Boone, D.M. 1971. Genetics of *Venturia inaequalis*. Ann. rev. of Phytopathology 9: 297-318.

- Boone, D.M. & G.W. Keitt 1957. *Venturia inaequalis* (Cooke) Winter XII. Genes controlling pathogenicity of wild-types lines. *Phytopathology* 47: 403-409.
- Crosby, J.A., J. Janick, P.C. Pecknold, S.S. Korban, P.A. O'Connor, S.M. Ries, J. Goffreda & A. Voordeckers 1992. Breeding apples for scab resistance 1945-1990. *Fruit Varieties Journal* 46(3): 145-166.
- Dayton, D.F. & E.B. Williams. Independent genes in *Malus* for resistance to *Venturia inaequalis*. *Amer. Soc. for Horticultural Science* 92: 89-94.
- Dufrenoy, J. 1936. Etudes épidémiologiques relatives a la tavelure du pommier. *Revue de Microbiologie appliquée à l'agriculture, à l'hygiène. à l'industrie* 2: 4-20.
- El Titi, A, E.F. Boller & J.P. Gendrier 1993. Integrated Production. Principles and Technical Guidelines. *IOBC/WPRS Bulletin* 16: 97 pp.
- Flor, H. 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32: 653-669.
- Gessler, C. 1989. Genetics of the interaction *Venturia inaequalis* - *Malus*: the conflict between theory and reality. In: Integrated control of pome fruit diseases II (Eds. Gessler, Butt & Koller) *OILB-WPRS Bulletin* XII/6: 168-190.
- Gessler, C., M. Eggenschwiler, H. Sierotzki & B. Koller 1993. La resistenza di varietà di meli ritenuti suscettibili contro la ticchiolatura. *Rivista di Frutticoltura* 9: 49-53.
- Gessler, C., M. Eggenschwiler & H. Sierotzki 1993. Vertikale Resistenz gegen Schorf in anfälligen Apfelsorten. *Schweiz. Landw. Fo.* 32(3): 401-410.
- Giovannoni, J.J., R.A. Wing, M.W. Ganai & S.D. Tanksley 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping population. *Nucleic Acid Research*, 19: 6553-6558.
- Götz, G. & R. Silbereisen 1989. *Obstsorten-Atlas*. Eugen Ulmer Verlag Stuttgart Germany 365 pp.
- Govi, G. 1966. Sensibilità varietale del melo alle infezioni di *Venturia inaequalis*. *Phytopath. med* 5: 145-146.
- Hatton, R.G. 1939. Rootstocks work of East Malling. *Sci. Hort.* 7: 7-16.
- Hegi, G. 1963. *Illustrierte Flora Mitteleuropas (1905-1931)*. Carl Hansen Verlag, München. Vol IV/2: 745-754.

- Hogenboom, N.G. 1993. Economic importance of breeding for disease resistance. In Jacobs, Th. & J. E. Parleviet (eds.). Durability of disease resistance, Kluwer Acad. Publ.: 5-10.
- Johnston, R. 1981. Durable resistance: Definition of, genetic control, and attainment in plant breeding. *Phytopathology* 71: 567-568.
- Johnstone, K.H. 1931. Observation on the varietal resistance of the apple to scab (*Venturia inaequalis*, Aderh.) with special reference to its physiological aspects. *J. Pomol. and Hort. Sci.* 62: 805-347.
- Keitt, G.W. & C.J. Nusbaum 1936. Cytological studies of the parasitism of two monoconidial isolates of *Venturia inaequalis* on the leaves of susceptible and resistant apple varieties. *Phytopathology* 26: 97 Abstract.
- Keitt, G.W. & M.H. Langford 1938. Heterothallism and Variability in *Venturia inaequalis*. *American Journal of Botany* 25: 338-344.
- Keitt, G.W. & D.H. Palmiter 1938. Heterothallism and segregation for pathogenicity in *Venturia inaequalis*. *Phytopathology* 28: 12 Abstract.
- Keitt, G.W., M.H. Langford & J.R. Shay 1943. *Venturia inaequalis* (Cke.) Wint. II Genetic studies on pathogenicity and certain mutant characters. *Am. J. Botany* 30: 491-500.
- Keitt, G.W., C. Leben & J.R. Shay 1948. *Venturia inaequalis* (Cke.) Wint. IV Further studies on the inheritance of pathogenicity. *Am. J. Botany* 33: 334-336.
- Kellerhals, M. 1989a. Breeding of pome fruits with stable resistance to diseases. In: Integrated control of pome fruit diseases II (Eds. Gessler, Butt & Koller) OILB-WPRS Bulletin XII/6. 116-129.
- Kellerhals, M. 1989b. Breeding disease resistant apple cultivars in Switzerland. In: Integrated control of pome fruit diseases II (Eds. Gessler, Butt & Koller) OILB-WPRS Bulletin XII/6. 130-136.
- Lander, E.S. & D. Botstein 1989. Mapping Mendelian Factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121: 185-199.
- Michelmore, R.W., I. Paran & R.V. Kesseli 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in a specific genomic region by using segregating population. *Proc. Natl. Acad. Sci. USA.* 88: 9828-9832.
- Nusbaum, C.J. & G.W. Keitt 1938. A cytological study of host-parasite relation of *Venturia inaequalis* on apple leaves. *J. Agr. Res.* 56: 595-618.



- Palmiter, D.H. 1934. Variability in monoconidial cultures of *Venturia inaequalis*. *Phytopathology* 24: 22-47.
- Parlevliet, J.E. 1993. What is durable resistance, a general outline. In Jacobs, Th. & J. E. Parlevliet (eds.). *Durability of disease resistance*, Kluwer Acad. Publ.: 23-40.
- Parisi, L., Y. Lespinasse, J. Guillaumes & J. Krüger 1993. A new race of *Venturia inaequalis* virulent to apples with resistance due to the Vf gene. *Phytopathology* 83: 533-537.
- Parisi, L. Y. Lespinasse, J. Guillaumes & J. Krüger 1994. A new race of *Venturia inaequalis* overcomes apples resistance due to the Vf gene. *Norwegian Journal of Agricultural Sciences. Suppl. No. 17*: 95-104.
- Roberts, A.L. & I.R. Crute 1994. Apple scab resistance from *Malus floribunda* 821 (Vf) is rendered ineffective by isolates of *Venturia inaequalis* from *Malus floribunda*. *Norwegian Journal of Agricultural Sciences. Suppl. No. 17*: 403-406.
- Rousselle, G.L., E.B. Williams, & L.F. Hough 1974. Modification of the level of resistance to apple scab from the Vf gene. In *Proceedings of the XIXth International Horticultural Congress, Vol III Warsaw, Int. Soc. Hort. Sci.* 19-26.
- Schaffner, D., B. Koller, K. Müller & M.S. Wolfe 1992. Response of populations of *Erysiphe graminis* f. sp. *hordei* to large-scale use of variety mixtures. In Zeller F.J. & G. Fischbeck (eds.). *Cereal rusts and mildews. Vorträge für Pflanzenzüchtung Heft 24*: 317-319.
- Schmidt, M. 1936a. *Venturia inaequalis* (Cooke) Aderhold. IV. Weitere Beiträge zur Rassenfrage beim Erreger des Apfelschorfes. *Gartenbauwiss.* 10: 364-389.
- Schmidt, M. 1936b. *Venturia inaequalis* (Cooke) Aderhold. VI. Zur Frage nach dem Vorkommen physiologisch spezialisierter Rassen beim Erreger des Apfelschorfes. Erste Mitteilung. *Gartenbauwiss.* 10: 478-499.
- Schmidt, M. 1938. *Venturia inaequalis* (Cooke) Aderhold. VIII. Weitere Untersuchungen zur Züchtung schorf widerstandsfähiger Apfelsorten. *Der Züchter* 10: 280-291.
- Shay, J.R. & E.B. Williams 1956. Identification of three physiological races of *Venturia inaequalis*. *Phytopathology* 46: 190-193.
- Shay, J.R., E.B. Williams & J. Janick 1962. Disease resistance in apples and pear. *Proc. A. Soc. Hort. Science.* 80: 97-104.

Sierozki, H. M. Eggenschwiler, J. McDermott & C. Gessler 1994. Specific virulence of *Venturia inaequalis* on "susceptible" apple cultivars. Norwegian Journal of Agricultural Sciences. Suppl. No. 17: 83-93.

Stam, P. & A.C. Zeven 1981. The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30: 227-238.

Tanksley, S.D., J. Miller, A. Paterson & R. Bernatzky 1988. Molecular mapping of plant chromosomes. In Gustafson, J.P. & R. Appels (eds). Chromosome structure and function. Plenum Press, New York, pp. 157-173.

Vanderplank, J.E. 1982. Host-Pathogen Interaction in Plant Disease. Academic Press, New York. 207 pp.

Wallace, E. 1913. Scab disease of apples. N.Y. Cornell Agric.Expt.Sta. Bull. 335: 543-642.

Wiesmann, R. 1931. Untersuchungen über Apfel- und Birnenschorfpilz, *Fusicladium dendriticum* (Wallr.) Fckl. und *Fusicladium pirinum* (Lib.) Fckl. sowie die Schorfanfälligkeit einzelner Apfel- und Birnensorten. Landw. Jahrbuch der Schweiz 35: 109-156.

Williams, E. B. & A. G. Brown. 1968. A new physiological race of *Venturia inaequalis*, incitant of apple scab. Plant Disease Reporter 52: 799-801.

Williams, E.B. & J. Kuc 1969. Resistance in *Malus* to *Venturia inaequalis*. Ann. Rev. Phytopathology: 223-246.

Williams., J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski & S.V. Tingey 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research. 18: 6531-6535.

Wiltshire, S.P. 1915. Infection and immunity studies on the apple and pear scab fungi. Ann appl. biol. 4: 335-350.

Wolfe, M.S. 1983. Genetic strategies and their value in disease control. In: Kommedahl, T. & Ph. Williams (eds). Challenging problems in plant health. The Americ. Phytopathol. Soc. St. Paul, pp 461-473.

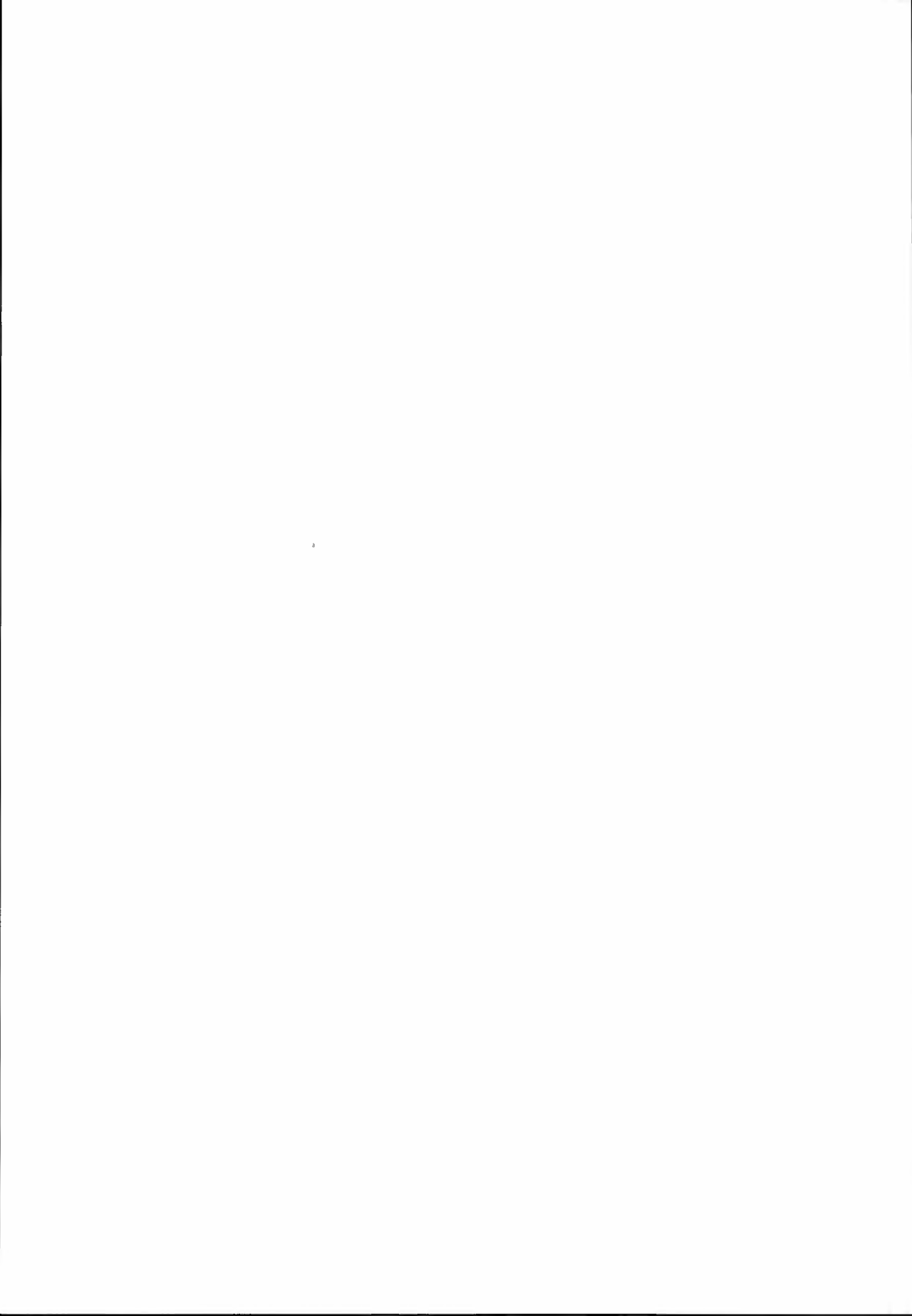
Wolfe, M.S. 1993. Can the strategic use of disease resistant hosts protect their inherent durability? In Jacobs, Th. & J.E. Parleviet (eds.). Durability of disease resistance, Kluwer Acad. Publ.: 83-96.

Wolfe, M.S. & C. Gessler 1992. Resistance genes in breeding: epidemiology. In: Plant gene research. Genes Involved in Plant Defense. (Eds. Boller, T. & F. Meins) Springer Verlag: 3-23.

Young, N.D. & S.D. Tanksley 1989. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. *Theor. Appl. Genet.* 77: 353-359.

Zadoks, J.C. 1993. The partial past. In Jacobs, Th. & J. E. Parleviet (eds.). Durability of disease resistance, Kluwer Acad. Publ.: 11-22.

Zeven, A.C., D.R. Knott & R. Johnson 1983. Investigation of linkage drag in near isogenic lines of wheat by testing for seedling reaction to races of stem rust, leaf rust and yellow rust. *Euphytica* 32: 319-327.



# Cellulases of *Venturia inaequalis*

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Investigations on regulation and characterization of cellulases from *Venturia inaequalis* may reveal mechanisms of the very specific host-parasite interactions which are responsible for ontogenetic and varietal resistance. *In vitro*-culture experiments indicated the specific factors for induction of the enzymes. Synthetic cellulosic sheets and intact solvent-extracted leaves were inductive and no repression or inhibition could be stated. A complex cellulase pattern was obtained after electrophoresis which was conserved in all *V. inaequalis* strains tested. Cellulases were detected on scab lesions of leaves and isolated from diseased plants. The qualitative profile of enzymes from host-parasite interaction was nearly identical with the enzymes produced *in vitro*.

Key words: Biotrophic interaction, cellulase, enzyme induction, pathogenesis, resistance.

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*V. inaequalis* is growing in intimate association with live host tissues from which it efficiently derives nutrients and incites the pathological processes. After penetrating the cuticle, the stroma growth occurs on the outer layer of the epidermal cells. Cellulases are thought to be involved in the degradation of the cellulosic substrate from plant cells. These fungal enzymes may be very important for the biotrophic host-pathogen interactions and for a successful development of this pathogen. Inhibition of production and release of cellulases or the loss of activity of the enzymes caused by substances of the host metabolism could be correlated with ontogenetic and varietal resistance.

## MATERIALS AND METHODS

The effect of cellulosic inducers was tested in static liquid cultures containing potato broth with or without a further carbon source. 19 monoconidial isolates of *V. inaequalis* were used. The various cellulosic substrates were carboxymethylcellulose (CMC), powdered cellulose, cotton wool, filterpaper, cell wall- and cellulose-preparations from apple leaves, intact solvent-extracted leaves (chloroform/methanol 1:2, methanol, ethanol) or native leaves, xylan, cellodextrins and cellulosic sheets. Production of cellulases was achieved by liquid static culture (PDB, Difco) supplemented with 2% cellulosic sheets (Cellophane) or preferably with dialysis tubing (Serva, regenerated cellulose) filled with the medium. Particles of different size from cellulosic sheets were obtained after fractionating with sieves

of a defined mesh size. Relevant sugars of apple leaves were tested for inductive, repressive and inhibitory action.

Cellulolytic activity was detected and quantified with standard viscometry versus CMC. A cup-plate diffusion assay was performed with 0.05 M citric acid, 0.1 M Na<sub>2</sub>HPO<sub>4</sub> (pH 5), 1% CMC (Serva) and 0.8% agarose. A siliconized cup-former was dipped in the hot agarose medium creating cups of about 80  $\mu$ l. For visualization of hydrolysis the plates were flooded with 0.1% Congo red after incubation of test solutions over night at ambient temperature. HPLC- (high-performance liquid chromatography) analyses of the cellulose products and sample cleanup were done as described by Kollar & Seemüller (1990). Enzyme preparations were done by lyophilization of culture filtrate, fractionated ammonium sulphate precipitation (50% and finally 80% for obtaining cellulases), ion-exchange chromatography (Sephadex QAE 50, 100 x 15 mm mm I.D., buffers see below) and preparative isoelectric focusing (IEF; Rotofor, Bio Rad). Determination of molecular weight was done with standard gel filtration on Sephadex G-75 SF (Pharmacia). Analytical IEF was performed on ultrathin gels followed by a zymogram technique. The IEF gels were blotted onto agarose/CMC plates (s.a. without cups) and these were incubated and stained for cellulase activity as described for cup-plate assay.

Cellulase production on plant surface by the pathogen was detected with an agarose overlay technique. Leaves with lesions were blotted on CMC-substrate agar (s.a.) for 20 h and for an additional incubation for 20 h after removing the leaves. Detection of activity was done with Congo red as described for cup-plate assay.

Enzymes produced on leaves were isolated by scraping on single non-necrotic apple scab lesions (about 400), which before were covered with a layer of buffer (0.05 M Tris, 0.1 M NaCl, pH 10). This preparation was loaded directly onto ion-exchange column (s.a.). Unbound substances were eluted with the buffer and cellulases recovered with buffer containing 1 M NaCl.

## RESULTS AND DISCUSSION

All commonly used cellulosic carbon sources failed to enhance cellulase production. Extracellular enzyme production occurred only in media containing cellulosic sheets or solvent extracted leaves. The constitutive amount of cellulases in media without inductor or other cellulose sources was in the range of a 1% compared to an induced culture. A repression of enzyme production by exogenous soluble carbon sources could not be detected even with high quantities of the additional sugars. The yield of enzyme was only correlated with mycelial development and the presence of cellulosic sheets of a defined square dimension (Fig. 1.) or intact solvent-extracted leaves (Fig. 2.). As indicated in Figures 1 and 2 *V. inaequalis* seems to be induced for cellulase production effectively by a combination of topographic and chemotrophic signals. Obviously these were present in artificial and natural substrates as a specific cellulosic surface area. The obtained activities clearly indicated that Wagner et al. 1988 and Koller et al. 1992 investigated the constitutive level or at most a slightly enhanced level of cellulases of the fungal strain used. Valsangiacomo et al. 1989 postulated a production of low amounts of cell wall degrading enzymes bound to the mycelium of *V. inaequalis*. In this investigation, however, cellulases were found to be extracellular with high activities when induction was effective.

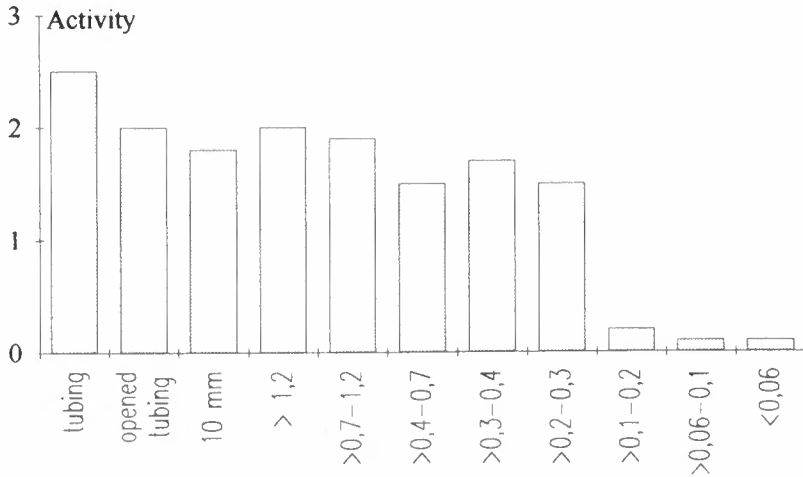


Fig. 1. Cellulosic sheet substrates of different size and shape were tested for production of cellulases in potato dextrose broth. *V. inaequalis* was grown in 40 ml cultures for 40 d and all substrates were at a concentration of 2 %. Cellulase activities of ammonium sulphate preparations equivalent to about 12 ml of culture filtrate were determined in cup-plate assays (mm, zones of hydrolysis). The first two columns are referred to cultures with dialysis tubing as the substrate ("tubing") filled with medium or tubing cut open. Numerical values indicate the range of size (mm) from sieve fractions of milled cellulosic sheets included into the culture media. Particles of cellulosic sheets were obtained by milling cellophane in liquid nitrogen and subsequent fractionating by sieves of a defined mesh size.

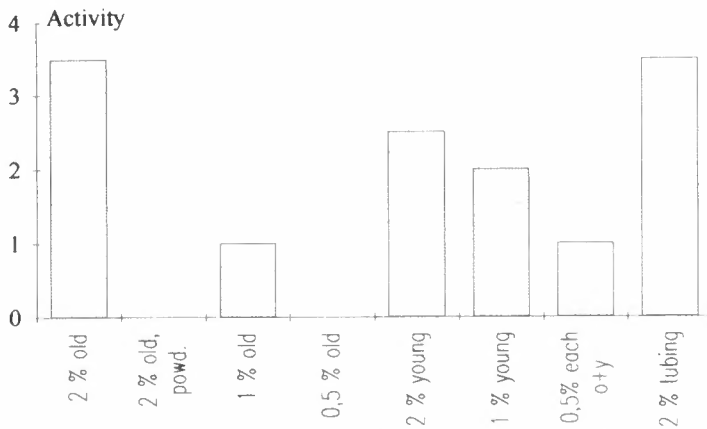


Fig. 2. Solvent-extracted leaf materials were tested as the cellulosic substrate for induction of cellulases in potato dextrose broth (PDB) cultures. Culture conditions and the method for cellulase assay were as described in Fig. 1. The control ("tubing") for an effective cellulase induction was the culture of *V. inaequalis* on a dialysis tubing filled with PDB. The other culture media were supplemented with leaf preparations of mature ("old"), immature ("young"), a mixture of both ("o+y") or with powdered ("powd.") leaf material.

The cellulolytic system was able to macerate the sheets and could also be detected by its CMCase activity. HPLC analyses indicated that activity increased with the degree of polymerisation and revealed the endoglucanase type cleavage. Cellodextrins were degraded to cellotriose and cellobiose.  $\beta$ -glucosidases were mainly associated with fungal hyphae. Optimal pH for cellulolytic activity was at pH 4. Activity increased linearly beginning at

5°C with a distinct rise around 20°C. There was no temperature optimum observed up to 45°C.

Electrophoretic separation (isoelectric focusing) revealed the presence of various components of the cellulase system (Fig. 3.). The isoelectric points (pI's) of the 12 isoenzymes were in the range of 3.7-5.6. The molecular weights were about 60 kD for 5 enzymes and about 25 kD for the 5 more active isozymes (Table 1). The cellulase pattern from the 19 *V. inaequalis* isolates were essentially identical. Differences were restricted mainly to quantitative variability. The cellulases produced *in situ* were detected in the area of leaf lesions with the overlay technique. The cellulase pattern derived from the host-parasite interaction was qualitative nearly identical with the enzymes produced *in vitro*. These data indicate a very specific action and regulation of cellulases within the biotrophic interaction, which could be affected by the host to improve or acquire the factors of resistance.

Fig. 3. Isozyme banding patterns of the cellulases from *V. inaequalis* obtained after isoelectric focusing followed by staining of CMCase activity. The first two lanes show gels with ampholytes generating a pH range from 3-10 and the third lane from 3-6, respectively.

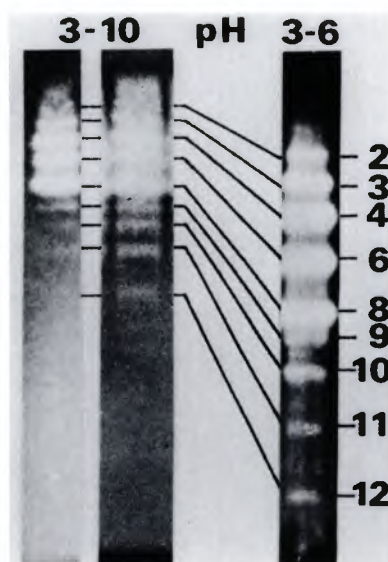


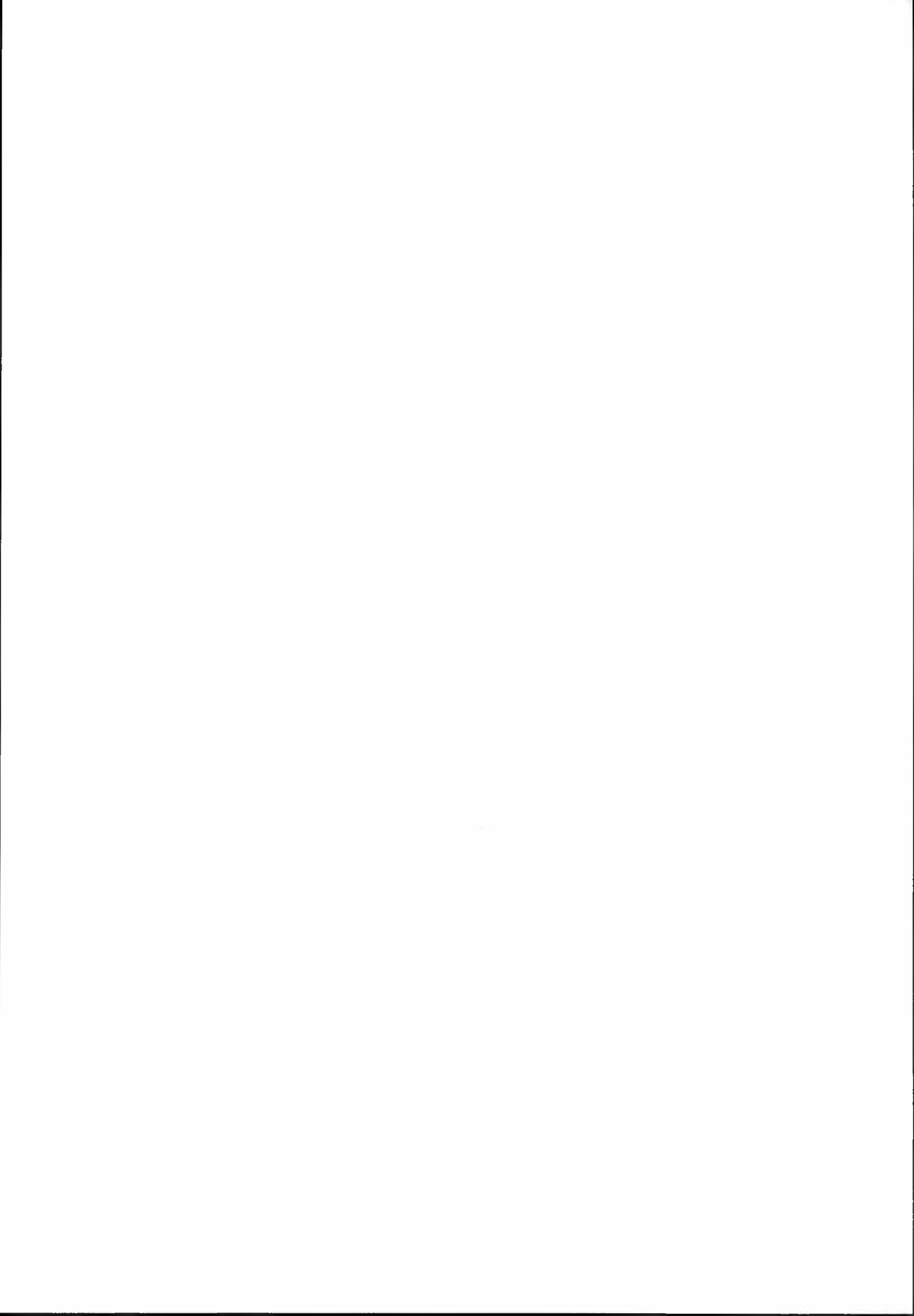
Table 1. Molecular weights and isoelectric points of cellulase isozymes

Isozyme	pI	Molecular weight (kD)
C 1	3,7	25
C 2	3,8	25
C 3	4,0	25
C 4	4,1	25
C 5	4,2	60
C 6	4,4	25
C 7	4,5	60
C 8	4,6	60
C 9	4,8	60
C 10	5,0	60
C 11	5,3	?
C 12	5,6	?



REFERENCES

- Kollar, A. & E. Semüller 1990. Chemical composition of phloem exudate of mycoplasma-infected apple trees. *J. Phytopathology* 128: 99-111.
- Koller, B., M. Müller, C. Valsangiacomo & C. Gessler 1992. Cell wall degrading enzymes and inhibitors involved in the interaction between *Venturia inaequalis* and *Malus domestica*. *Acta Phytopath. et Entomol. Hung.* 27(1-4): 353-359.
- Valsangiacomo, C., K. Wagner, B. Stadler, M. Ruckstuhl, P. Manini-Gessler, M. Michel & C. Gessler 1989. Aspects of host resistance and pathogenesis in the interaction between *Venturia inaequalis* and apple leaves. *Integrated control of pome fruit diseases, Vol. II, IOBC/WPRS Bull. XII/6: 191-204.*
- Wagner, K., L. Hitz-Germann, J.M. Seng & C. Gessler 1988. Cellulolytic ability of the scab fungus, *Venturia inaequalis*. *J. Phytopathology* 123: 217-221.



# Current status of the management of summer diseases of apples

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The summer disease complex of apples in the southeastern United States is composed of eight fungal diseases and one physiological disorder and includes: black rot (*Botryosphaeria obtusa*); Brooks fruit spot (*Mycosphaerella pomi*); sooty blotch (*Gloeodes pomigena*); flyspeck (*Schizothyrium pomi*); bitter rot (*Glomerella cingulata*); white rot (*B. dothidea*); black pox (*Helminthosporium papulosum*); Alternaria blotch (*Alternaria mali*); and necrotic leaf blotch of Golden Delicious (physiological). Knowledge of the biology and epidemiology of the fungi causing this disease complex has increased over the past 15 yr and has enabled the development of models for forecasting some of the diseases. Opportunities for the use of forecasting systems is limited by the number of pathogens involved, the types of damage they cause, quantification of inoculum levels within orchards, and the availability of eradicator fungicides. Forecasters developed for Alternaria blotch and sooty blotch and flyspeck can be integrated into current summer disease management programs.

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Summer diseases of apples are most common during the period from petal fall to harvest and tend to be most important in warm, moist apple growing regions of the world. In the United States, the summer disease complex is most important in the apple growing regions in the Southeast, but can be important in many apple growing regions in the eastern US. Summer diseases of apples are not important in the western US. The summer disease complex is comprised of eight fungal diseases and one physiological disorder and includes: black rot (*Botryosphaeria obtusa* (Schwein.) Shoemaker; anamorph *Sphaeropsis* sp.); Brooks fruit spot (*Mycosphaerella pomi* (Pass.) Lindau); sooty blotch (*Gloeodes pomigena* (Schwein.) Colby); flyspeck (*Schizothyrium pomi* (Mont. & Fr.) Arx; anamorph *Zygothiala jamaicensis* E. Mason); bitter rot (*Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk; anamorph *Colletotrichum gloeosporioides* (Penz) Penz. & Sacc. in Penz. and *C. acutatum* J.H. Simmonds); white rot (*Botryosphaeria dothidea* (Moug.:Fr.) Ces. & DeNot.; anamorph *Fusicoccum aesculi* Corda); black pox (*Helminthosporium papulosum* A. Berg); Alternaria blotch (*Alternaria mali* Roberts); and necrotic leaf blotch of Golden Delicious (a physiological disorder). Necrotic leaf blotch of Golden Delicious will not be considered in this paper.

Losses to these diseases can be extensive and reduce both yield and quality. The rot diseases-bitter rot, black rot, and white rot- have the most significant impact on yield because infected fruit are not marketable and often rot on the tree prior to harvest. Bitter rot is the most important rot disease. Sooty blotch and flyspeck infections downgrade fruit and probably cause greater losses to fresh market fruit than any of the other diseases. Brooks fruit spot and black pox have little impact on yield, but infections downgrade fruit and lesions on fruit are often infection sites for secondary fungi during storage. Fruit infections by *A. mali* are not important on the cultivars commonly grown in the US, but severe defoliation results in reduced yield and quality.

Knowledge on the biology and epidemiology of the fungi causing the summer disease complex has increased significantly over the past 10-15 yr (Tables 1A,B). Overwintering sites and inoculum types involved in primary and secondary infections have been identified for most of the pathogens. The time of infection, environmental conditions which favour infection, and the duration of the incubation and latent periods are also described for many of them. Little information is known about orchard inoculum levels. The epidemiology of black pox is the least understood of the summer diseases.

Table 1A. Summary of current information on some of the biological and epidemiological parameters associated with the summer disease complex of apples

Pathogen-disease	Identity of overwintering sites	Qualification of orchard inoculum levels	Importance of inoculum type			
			Primary		Secondary	
			Sexual	Asexual	Sexual	Asexual
<i>Botryosphaeria obtusa</i> - Black rot	++++ <sup>a</sup>	++	++	++++	+	++++
<i>Mycosphaerella pomi</i> - Brooks fruit spot	+++	++	0	++++	0	0
<i>Gloeodes pomigena</i> - Sooty blotch	++++	++	0	+++	0	++++
<i>Zygothia jamaicensis</i> - Flyspeck	++++	++	+++	+	0	++++
<i>Colletotrichum</i> spp. - Bitter rot	++++	++	+	++++	+	++++
<i>B. dothidea</i> - White rot	++++	++	++	++++	++	++++
<i>Helminthosporium papulosum</i> - Black pox	+++	+	0	++++	0	++++
<i>Alternaria mali</i> - Alternaria blotch	+++	++	0	++++	0	++++

<sup>a</sup>++++ = good information available or inoculum type important;

+ = little information available or inoculum type unimportant;

0 = no spores produced

Table 1B. Summary of current information on some of the biological and epidemiological parameters associated with the summer disease complex of apples

Pathogen-Disease	Time of infection	Quantification of environmental conditions favoring infection	Duration of incubation/latent periods	Cultivar susceptibility	Primary effect of disease	
					Yield	Quality
<i>Botryosphaeria obtusa</i> - Black rot	++++ <sup>a</sup>	++++	+++	+++	++++	0
<i>Mycosphaerella pomi</i> - Brooks fruit spot	++++	+++	++++	+++	+	++++
<i>Gloeodes pomigena</i> - Sooty blotch	++++	++	+++	+++	0	++++
<i>Zygothia jamaicensis</i> - Flyspeck	++++	+++	+++	+++	0	++++
<i>Colletotrichum</i> spp. - Bitter rot	++++	+++	+++	+++	++++	0
<i>B. dothidea</i> - White rot	++++	++++	+++	+++	++++	0
<i>Helminthosporium papulosum</i> - Black pox	++	+	+	+	+	++++
<i>Alternaria mali</i> - Alternaria blotch	++++	++++	++++	+++	+++	+++

<sup>a</sup>++++ = good information available or large effect;

+ = little information available or slight effect;

0 = no effect.

## MANAGEMENT OF THE SUMMER DISEASE COMPLEX

Management of the summer disease complex depends on a combination of cultural practices and a preventative fungicide spray program. Pruning out dead wood and cankers, removal of mummied apples, and burning or flail chopping prunings reduce the inoculum levels of *B. obtusa*, *B. dothidea*, and *Colletotrichum* spp. (Starkey & Hendrix 1980; Miller & Anagnostakis 1980; Drake 1971). Dormant and summer pruning opens the tree canopy allowing the fruit to dry faster and improve pesticide deposition resulting in reduced infection and better control of sooty blotch and flyspeck (Ocamb-Basu *et al.* 1988; Cooley *et al.* 1992a). Removal of reservoir hosts (Baker *et al.* 1977), particularly brambles (*Rubus* spp.), aids in the control of sooty blotch and flyspeck by reducing the primary inoculum level and tissues available for the production of secondary inoculum.

Cultivars do not vary greatly in their susceptibilities to most summer disease pathogens. From a practical standpoint there are no differences in the susceptibility of fruit

to *B. obtusa*, *B. dothidea*, *Colletotrichum* spp., *Z. jamaicensis*, or *G. pomigena*. *Alternaria mali* does not cause significant fruit infections on any of the cultivars grown widely in the US. However, there are significant differences in the susceptibility of the foliage; strains of the cultivar Delicious and Empire are most seriously affected (Filajdic & Sutton 1991). Strains of Delicious are much less susceptible to infection by *M. pomi* than Rome, Stayman, Golden Delicious and Ida Red. Little information is available on the susceptibility of modern cultivars to *H. papulosum*; Golden Delicious and Rome appear more susceptible than Delicious.

Fungicides for control of the summer diseases are applied on a 10- to 14-day schedule beginning at petal fall and continuing until harvest in the southeastern US. Spray schedules of 14 to 30 days are used in more northerly growing regions where the diseases are not so severe. Fungicides commonly used are captan, ziram, and thiram, often in combination with benomyl or thiophanate methyl. Combinations are commonly used because of differences in the activities of the fungicides (Table 2). EBDC fungicides, currently registered in the US for use at reduced rates within 77 days of harvest, are also often used in the early cover sprays.

Table 2. Relative effectiveness of various fungicides on the summer diseases of apples. None of the fungicides listed has any useful activity on *Alternaria* blotch

Fungicide and rate (kg)/ha	Brooks spot	Black rot/White rot	Bitter rot	Sooty blotch	Black Flyspeck	pox
Captan 50W 8.97	++++ <sup>a</sup>	+++++	+++++	++++	++	+++
Ziram 76W 8.97	++++	++	++++	++++	+++	++++
Thiram 65W 8.97	++++	++	++++	++++	+++	++++
Benomyl 50W 0.56	+++++	++++	-	+++++	++++	+
Thiophanate methyl 70W 0.56	+++++	++++	-	+++++	++++	+
EBDC 75DF 3.36 <sup>b,c</sup>	+++	+	++	+	++	?
Captan 50W 8.97 + benzimidazole <sup>d</sup> 0.56	+++++	+++++	+++++	+++++	+++++	++++
Ziram 76W 8.97 + benzimidazole 0.56	+++++	++++	+++++	+++++	+++++	++++
Captan 50W 4.49 + ziram 76W 4.49 + benzimidazole 0.56	+++++	+++++	+++++	+++++	+++++	+++++

<sup>a</sup>+++++ = most effective; + = least effective; - = no useful activity; ? = unknown

<sup>b</sup>Includes mancozeb, maneb + zinc, and metiram

<sup>c</sup>Applied with a 77-day preharvest interval

<sup>d</sup>Benzimidazole = benomyl or thiophanate methyl

## OPPORTUNITIES FOR USE OF FORECASTING SYSTEMS

Opportunities for the use of forecasting systems to aid in the management of the summer disease complex are limited by the number of pathogens involved, the types of damage they cause, the availability of eradicant fungicides, and the difficulty in assessing inoculum levels in orchards.

Because infections can be initiated by most pathogens involved in the summer disease complex throughout the summer growing season, fungicides targeted for one pathogen cannot be made without regard to the others. Forecasting systems for *Alternaria* blotch, and sooty blotch and flyspeck described below are examples of how fungicides targeted for specific pathogens can be integrated into programs for the management of the other summer diseases.

The nature of the damage to the fruit greatly influences the utility of a forecasting system. Because the three rot diseases result in loss of fruit, there are fewer opportunities for using a forecasting system, and protectant fungicide spray schedules are usually necessary where these diseases are a problem. *Alternaria* blotch affects only leaves, and unless defoliation is extensive, fruit quality and yield are unaffected. Colonies of sooty blotch and flyspeck grow epiphytically on the cuticle of the fruit, and do not result in any loss of yield. Minor infections can often be washed off fruit in the packing line or removed by chlorine bleach (Hendrix 1991). Infections by *M. pomi* and *H. papulosum*, result in small shallow lesions, and if not too extensive, fruit can be used for processing or juice.

There are few fungicides available that have any significant eradicant activity against the summer disease pathogens. Brown and Sutton (1993) and Rosenberger *et al.* (1990) found that applications of a benzimidazole fungicide, applied after symptoms of sooty blotch and flyspeck appeared, arrested further symptom development and demonstrated some eradicant activity. Arauz and Sutton (Arauz & Sutton 1990) examined the eradicant activity of flusilazole, mancozeb, tebuconazole, triflumizole and benomyl against *B. obtusa* on detached fruit and found that tebuconazole and benomyl reduced the severity of black rot when applied 96 hr after infection or earlier. However, in studies conducted in the orchard, they did not find any significant difference between the non-treated check and fruit treated with these fungicides. Parker and Sutton (1993) examined the eradicant activity of these same fungicides against *B. dothidea* and did not find any significant eradicant activity.

Lack of information on how to quantify inoculum levels within orchards and relate it to disease intensity is a major limiting factor to the development of more efficient management strategies. There is little or no information on measures of differences in inoculum levels from orchard to orchard for most of the pathogens; consequently, the same management level often is imposed on orchards with low and high inoculum levels. The amount of dead wood in trees (for rot diseases) and proximity to reservoir hosts (sooty blotch and flyspeck) are used as general indicators of differences among orchards.

## EXAMPLES OF FORECASTING SYSTEMS

### **Alternaria blotch**

Over the past 6 yr, *Alternaria* blotch has become severe on strains of the cultivar Delicious

throughout much of the apple growing region of the southeastern US (Filajdic & Sutton 1991). The disease is characterized by circular necrotic spots on the leaves; severely affected leaves abscise resulting in up to 80% defoliation by harvest. In 1991 and 1992, fruit from severely affected trees had significantly less soluble solids and yield was reduced 73% compared to control plots (Filajdic & Sutton unpublished).

Management of the disease was initially complicated because there were no fungicides registered for its control in the United States. Iprodione was granted a Section 18 Specific Exemption by the Environmental Protection Agency for use in the 1992 growing season. Three applications are allowed per year with at 30-day preharvest interval; consequently, proper timing of fungicide applications is very important. If applications are made too early in the epidemic, they may be wasted and additional applications cannot be made later due to the three-spray limit on the label. Use of iprodione when not warranted may also lead to the development of less sensitive strains of *A. mali*. Less sensitive strains have been reported in Japan (Sakurai & Fujita 1978). In addition, iprodione is expensive, and use when not needed, reduces grower profits. Management of Alternaria blotch is based on two models: one model indicates when first symptoms are likely to occur and when monitoring should begin and the second indicates if and when iprodione applications should be made.

The first model, developed in South Korea (Kim et al. 1987), uses degree-day accumulations from the phenophase tight cluster and occurrences of rainfall events to predict the first occurrence of the disease. In NC, over a 3-yr period, the model predicted first symptom appearance within a week of actual orchard observations. Once the model indicates that symptoms are likely to occur, growers begin monitoring twice a week to plot the progress of the epidemic and begin utilizing the second model.

The second model is based on the relationship between incidence and severity (Filajdic & Sutton 1992) and their relationship to yield and quality parameters. The disease incidence is usually correlated with severity throughout the season and both incidence and severity in June are closely correlated to final defoliation. An incidence of 65% has been established as a threshold for making the first application of iprodione. This level was selected because (i) 65% incidence is an average for iprodione-sprayed plots, (ii) there is no yield loss associated with 65% incidence in June, (iii) many orchards with light to moderate problems will never exceed this level until much later in the season, (iv) initial applications will be delayed as late as possible in order to conserve the three applications of iprodione permitted by the label. Furthermore, by limiting the use of iprodione, selection of resistant strains may be less likely. The model is currently being refined to include the effect of various environmental parameters on disease progress and the influence of arthropods on defoliation (defoliation is much greater when moderate to high levels of mites are present). The model will be written for MS DOS in TURBO PASCAL and will be made available to growers on diskette.

### **Sooty blotch and flyspeck**

Sooty blotch and flyspeck are chronic diseases in the southeastern US and affect 5 to 20% of the fruit annually in North Carolina. To more effectively control the diseases, growers include a benzimidazole fungicide in their spray program from first or second cover until harvest, resulting in six to eight benzimidazole applications each year.

Two approaches have been used to reduce the number of benzimidazole applications



used to manage these two diseases in North Carolina: (i) applications of protectant fungicides + a benzimidazole are initiated after petal fall only when first symptoms of sooty blotch and flyspeck are observed in the orchard (post-symptom approach) and (ii) benzimidazole applications are made prior to predicted symptom appearance (presymptom approach). Both approaches rely on the ability of benzimidazole fungicides to arrest symptom development.

#### *Post-symptom approach*

In a series of studies conducted in 1987, 1989, and 1990, Brown and Sutton (Brown & Sutton 1993) controlled scab and powdery mildew through petal fall with fenarimol, but did not make subsequent fungicide applications until first symptoms of sooty blotch and flyspeck were observed. They found that applications of captan or mancozeb + a benzimidazole fungicide applied after first sooty blotch and flyspeck symptoms appeared resulted in consistently greater but not significantly different disease severities from standard protectant treatments at harvest. However, disease incidence was generally significantly greater than the standard protectant treatments. Two to five fungicide applications were saved each season utilizing this approach; however, because fruit quality decreased according to US grade standards, this approach was not a viable option for fresh fruit production, but may be useful for fruit used in the processing or juice market.

In this same study, Brown and Sutton (1993) addressed the problem of the presence of other summer diseases during the period from petal fall until fungicides were applied for control of sooty blotch and flyspeck. There was generally more black rot, white rot and bitter rot in eradicant treatments which included benomyl than in protectant treatments, but differences usually were not significant. There was, however, significantly more Brooks fruit spot and black pox in eradicant treatments than the protectant ones.

#### *Presymptom approach*

A second approach to control sooty blotch and flyspeck is to apply a fungicide such as captan on a standard schedule after petal fall to control other summer diseases (as well as any secondary scab) and then add a benzimidazole fungicide before symptoms of sooty blotch and flyspeck become visible.

The incidence and severity of sooty blotch and flyspeck is dependent on temperature and moisture conditions (Baines & Gardner 1932; Kirby 1954; Sharp & Yoder 1985, E. Johnson, unpublished). Brown and Sutton (unpublished) correlated several measures of leafwetness over 5 yr with observations of first symptoms of sooty blotch and flyspeck and developed an empirical model to predict the first symptom appearance. If hours of leaf wetting of 4-hr duration or greater were accumulated beginning 10 days after petal fall, first symptoms were visible after an average of 293 hr of wetting (range 265-328 hr) and appeared as early as 4 June and as late as 16 July. Based on the model predictions, they suggest that first benzimidazole applications should be timed at 200 to 250 hr of leafwetness, depending on forecasts of weather over the next week. Utilizing this approach, one to three benzimidazole applications could be saved yearly over the standard spray schedule in which a benzimidazole fungicide is included in all sprays beginning at first or second cover. In areas of the eastern United States with a dryer growing season than the Southeast, additional sprays could be saved. Cooley et al. (1992b) examined the model

under conditions in the New England growing area and found that model predictions of first symptoms and orchard observations are in good agreement. Hartman (J. Hartman, University of Kentucky, personal communication) evaluated the model under conditions in Kentucky and found that symptoms were visible earlier than predicted by the model. However, he used a different leafwetness sensor and differences in wetting duration as detected by the sensors may explain model discrepancies.

## REFERENCES

- Arauz, L.F. & T.B. Sutton 1990. Protectant and after-infection activity of fungicides against *Botryosphaeria obtusa* on apple. *Plant Disease* 74:1029-1034.
- Baines, R.C. & M.W. Gardner 1932. Pathogenicity and cultural characteristics of the apple sooty blotch fungus. *Phytopathology* 22: 937-952.
- Baker, K.F., L.H. Davis, R.D. Durbin & W.C. Snyder 1977. Greasy blotch of carnation and flyspeck of apple: diseases caused by *Zygophiala jamaicensis*. 67: 580-588.
- Brown, E.M. & T.B. Sutton 1993. Time of infection of *Gloeodes pomigena* and *Schizothyrium pomi* on apple in North Carolina and potential of an eradicant spray program for their control. *Plant Disease* 77: 451-455.
- Cooley, D.R., C. Telgheder, W.A. Autio, & J. Gamble 1992a. Using summer pruning to reduce flyspeck and sooty blotch of apple in the Northeast. (Abstr.) *Phytopathology* 82: 1075.
- Cooley, D., W. Autio, & J. Gamble 1992b. A new look at flyspeck and sooty blotch. *LISA Apple Newsletter* 3(1): 4-5,19.
- Drake, C.R. 1971. Source and longevity of apple fruit rot inoculum, *Botryosphaeria ribis* and *Physalospora obtusa*, under orchard conditions. *Plant Disease Reporter* 55: 122-126.
- Filajdic, N. & T.B. Sutton 1991. Identification and distribution of *Alternaria mali* on apples in North Carolina and susceptibility of different varieties of apples to *Alternaria* blotch. *Plant Disease* 75: 1045-1048.
- Filajdic, N. & T.B. Sutton 1992. The influence of temperature and wetting duration on infection of apple leaves and pathogenicity of different isolates of *Alternaria mali*, *Phytopathology* 82: 1279-1283.
- Hendrix, F.F., Jr. 1991. Removal of sooty blotch and flyspeck from apple fruit with a chlorine dip. *Plant Disease* 75: 742-743.

Kim, C., W. Cho & S. Kim 1987. An empirical model for forecasting *Alternaria* leafspot on apple. Korean Journal of Plant Protection 26: 221-228.

Kirby, R.S. 1954. Relation of rainfall to occurrence of apple scab and sooty blotch. (Abstr.) Phytopathology 44: 495.

Miller, P.M. & S.L. Anagnostakis 1973. Piles of apple prunings as sources of conidia of *Physalospora obtusa*. Phytopathology 63: 1080.

Ocamb-Basu, C.M., T.B. Sutton & L.A. Nelson 1988. The effects of pruning on the incidence and severity of *Zygothiala jamaicensis* and *Gloeodes pomigena*. Phytopathology 78: 1004-1008.

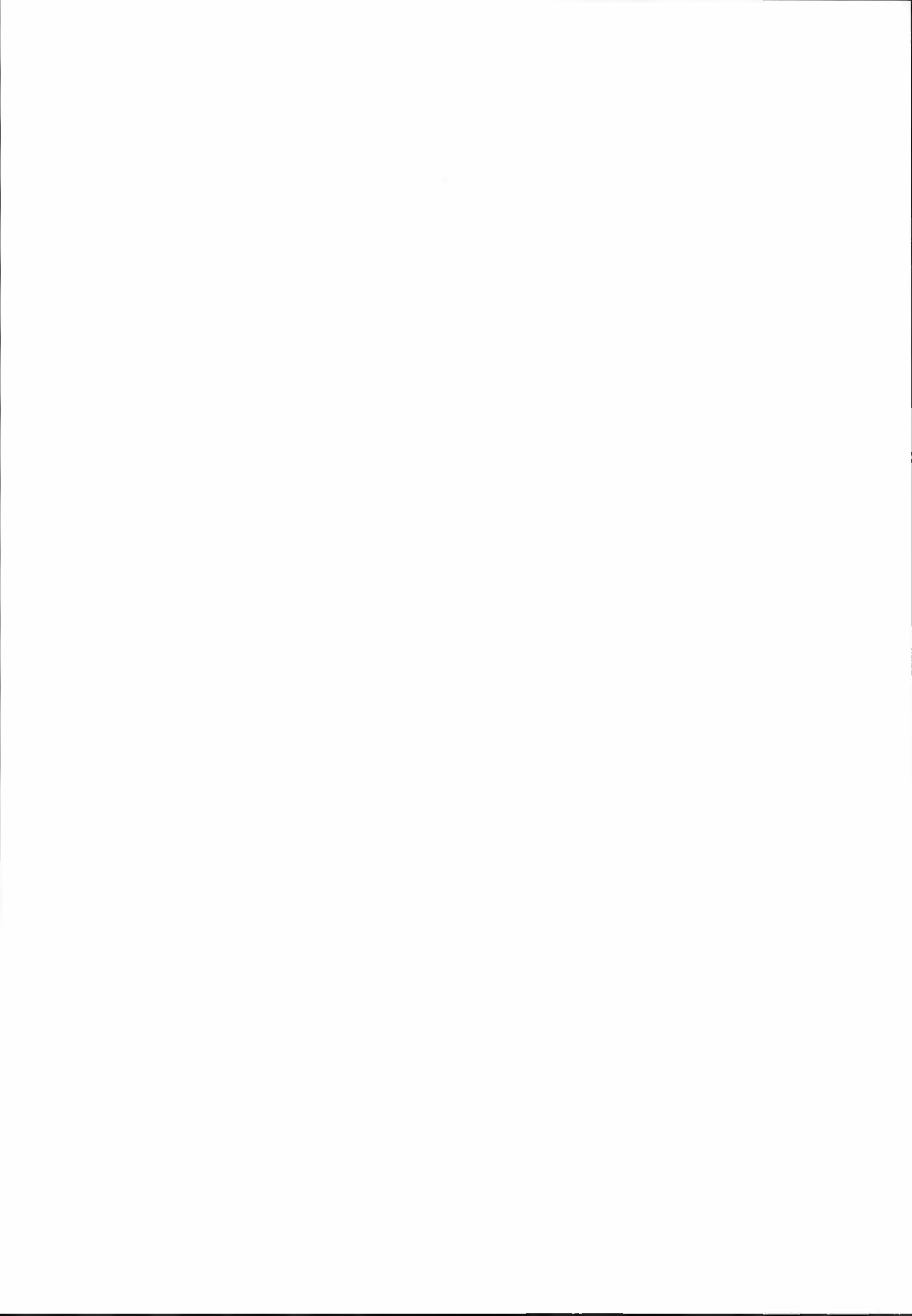
Parker, K.C. & T.B. Sutton 1993. Effect of temperature and wetness duration on apple fruit infection and eradicant activity of fungicides against *Botryosphaeria dothidea*. Plant Disease 77: 181-185.

Rosenberger, D.A., S.M. Rondinaro & F.W. Meyer 1990. Presymptom eradication of sooty blotch and flyspeck on apples. (Abstr.) Phytopathology 80: 123.

Sakurai, H. & S. Fujita 1978. The antifungal activity of polyoxin B and iprodione against pathogenic fungi isolated from diseased plants in Japan. Pesticide Science 9: 207-212.

Sharp, W.L. & K.S. Yoder 1985. Correlation between humidity periods and sooty blotch and flyspeck incidence in Virginia apple orchards. (Abstr.) Phytopathology 75: 628.

Starkey, T.E. & F.F. Hendrix, Jr. 1980. Reduction of substrate colonization by *Botryosphaeria obtusa*. Plant Disease 64: 292-294.



# Determining factors for storage scab incidence in apple

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In 1962, the South Tyrolean Advisory Service for Fruit- and Wine-Growing established an apple scab warning service. In this fruit-growing area there are 6-8 moderate or severe Mills Periods every year, only two or three of which are followed by lesions. In the years 1991 and 1992 the situation was different. In most districts the primary scab infections were more severe than usual and there were unusually long wet periods immediately before and during harvest. On the basis of the field record books, the spray treatments and the grading of the apples answers are sought for the following questions:

- \* Were the post-infection sprays correctly timed?
- \* Was the amount of fungicide applied correct for the size of the orchard, the planting system and tree height?
- \* Was the spraying equipment adequate?
- \* What was the percentage of shoots with lesions at the end of ascospore discharge?
- \* Did the interval between the last spray and harvest influence storage scab incidence?
- \* Is there a relationship between storage scab and IP-guidelines?

Key words: Apple scab, ascospore discharge, Mills Periods, scab management, scab warning system, spray schedules, storage scab.

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Years with a high level of storage scab are an exception in the South Tyrol. Oberhofer (1985) considers that in the last 100 years only 8 stood out because of severe scab attacks. The last of those 8 "scab years" was 1985, which was decisive for the further improvement of the scab warning system, which has been running since 1962.

## SURVEY OF THE INFECTION CONDITIONS 1965-1993

Oberhofer (1986) observed that from 1965 to 1985 only 40 out of 213 Mills Periods caused visible primary infections (= 19%). Between 1986 and 1993 81 Mills Periods were recorded, 28 of which (= 35%) were responsible for lesions (Table 1).

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Table 1. Mills Periods and lesions 1965-1985 and 1986-1993

Years	light	moderate	severe	total	with lesions	%
1965-85	52	59	102	213	40	19
1986-93	16	30	35	81	28	35

In this area, light Mills Periods do not generally result in lesions. In 1991 and 1992 there were more Mills Periods with ascospore discharge than usual. Furthermore, in both years there were extremely long leaf wetness periods and a high amount of precipitation in September and October. In 1991 these circumstances favoured storage scab, especially on Golden Delicious harvested late and from orchards with leaf scab. In 1992, not only Golden Delicious but also Rome Beauty, Winesap and in some cases Granny Smith and Jonagold were affected.

#### EXTREME CONDITIONS IN 1991 AND 1992

In 1991, 4 light and 1 moderate Mills Periods were recorded, all without ascospore discharge as well as 5 severe ones which were always accompanied by ascospore discharge. We could differentiate 3 infection periods (Table 2). In the very hot summer there were a lot of short leaf-wetting periods. From the end of September to mid-October there were 3 longer precipitation periods with 103, 62 and 96 hours of leaf wetness and 144 mm precipitation in total. According to Schwabe (1984) each of them could lead to fruit scab.

Table 2. Mills Periods (MP), ascospore release (AS), lesions and recommended number of sprays, "Burggrafenam" 1986-1993

Year	light MP		moderate MP		severe MP		MP with lesions	rec. sprays in spring
	AS		AS		AS			
	no	yes	no	yes	no	yes		
1986	1	0	0	3	0	4	4	6
1987	4	0	0	3	1	1	2	4
1988	3	0	4	5	1	1	3	7
1989	2	0	4	0	1	4	3	6
1990	1	0	1	0	2	2	1	4
1991	4	0	1	0	0	5	3	4
1992	0	1	1	2	2	4	8	9
1993	0	0	0	6	1	6	4	8
Total	15	1	11	19	8	27	3.5 (average)	6 (average)

In 1992, the primary scab situation broke all records (Table 2). There were 6 severe, 3 moderate and 1 light Mills Period registered. The lesions recorded in abandoned orchards could be attributed to 8 different Mills Periods. In 1992 9 post-infection sprays were recommended in spring.

Eight long (more than 2 days) leaf wetness periods with a total of 80 mm precipitation in September and 123 mm in October were responsible for the spread of fruit scab.

Because of the difficult weather conditions one application of Ziram was recommended for orchards with leaf scab at the end of October where fruits (Golden Delicious and Rome Beauty) were to remain on the trees for 10 days or longer before harvest.

## METHODS FOR MONITORING APPLE SCAB

### **Mills Periods**

Meteorological conditions for Mills Periods are recorded with the help of 65 automatic weather stations. Leaf dryness is visually ascertained.

### **Ascospore discharge**

Since 1986 the time of ascospore maturity is checked every February/March on leaf samples from different locations. Ascospore discharge is monitored by two VPPS 2000 traps at Lana and Terlan.

### **Infection**

After each incubation period abandoned orchards are inspected for lesions and shoots are recorded for the position of infected leaves.

### **Leaf scab control**

Since 1990 the success of the primary scab management programme in spring is assessed by the method of Van der Scheer (1990), counting the percentage of shoots with lesions.

### **Evaluation of the grading data**

A number of packhouses in the Burggrafenamt made their grading results of the harvested crops available in 1991 and 1992. Each lot containing scabbed fruit was marked. The percentage of storage scab was calculated by subtracting the average loss from that of the indicated lot. This assessment is based on about 2,000 tons of apples.

### **Field book control**

The field book is a valuable aid to discovering errors in the timing of the treatments. The records allow a comparison between recommendations and the actual spray treatments. Furthermore, they contain information about intervals between sprays and also the fungicide usage in each area.

## SPRAY EQUIPMENT

Since May 1992 there have been 3 check-points for sprayers (Lind system) in the South Tyrol. Three specially trained advisers advise the orchardists before and after the sprayer test. If the results of the test are available they are taken into consideration in assessing errors in scab management.

## RESULTS

**Mills Periods and ascospore discharge 1986-1993**

As described by Gadoury & MacHardy (1982), in the last 8 years ascospore discharge finished after 600 degree-days (base 0°C) under the conditions in the Etsch Valley (Table 3).

Table 3. Ascospore release (AS) and degree-days (DD) 1986-1993 at Lana

Year	start AS-release date	max. AS-release date	end AS-release date	DD
1986	9/4	28/4	24/5	-
1987	3/4	3/5	17/5	636
1988	7/4	2/5	11/5	542
1989	3/4	13/4	25/4 <sup>1)</sup>	240
1990	4/4	6/4	15/5	603
1991	23/3	2/5	10/5	503
1992	24/3	15/4	4/5	457
1993	24/3	6/5	14/5	665

<sup>1)</sup> A year with a low level of infection

During light Mills Periods ascospore discharge was recorded only once, in 1992. The remarkable thing was that the highest density of ascospores per m<sup>3</sup> air and per hour since 1986 was recorded during a light Mills Period (63 ascospores/m<sup>3</sup>/h). In rare cases ascospore discharge occurred at night, e.g. on April 26th 1993 at 0400 a.m.

**Leaf scab occurrence in June in 1991 and 1992**

In the year 1991, in about 30% of all orchards in the district Burggrafenamt more than 2 shoots out of 100 had scab lesions at the beginning of June.

In 1992 in some places nearly 50% of the orchards had more than 2% shoots with lesions (Table 4).

Table 4. Scab incidence June 1992

location	% orchards with 0-2 % infected shoots	% orchards with > 2 % infected shoots
1	50	50
2	69	31
3	54	46



**Storage scab 1991**

The analysis of fruit scab incidence in a co-operative in the Burggrafenamt showed that 14.6% of the members had delivered lots with an economically significant percentage of scabbed fruit. Seventeen of the members were willing to help analyse the problem. Table 5 shows that all of them had omitted or delayed one or more of the 6 post-infection sprays recommended in spring.

Table 5. Causes of storage scab in 1991

Number of orchardists	out of 6 recommended post-infection sprays
1	all sprays on time
3	1 spray left out
7	2 sprays left out
1	3 sprays left out
4	4 sprays left out
1	5 sprays left out
6	fungicide amount/ha too low

Six out of the 17 orchardists had used too little fungicide per ha for the tree height and the planting system. An analysis of the grading results of another packhouse supported this finding.

What is the relationship between the sprays in spring and storage scab incidence? Figures 1 and 2 show the Mills Periods from March to September. Figure 1 illustrates four examples of lots with storage scab. It is seen that scheduled sprays had been ignored or delayed in spring. Figure 2 contains examples of lots without scab; in these orchards the spray schedule had been observed for the most part.

What are the risks fruits in orchards with leaf scab are exposed to, if the fungicide residue is washed off and further leaf wetness periods that last longer than 2 days follow? Figure 3 shows the storage scab incidence on Golden Delicious lots that were delivered to a packhouse by an orchardist in October 1991. Captan had been applied for the last time between 2nd and 5th September. Precipitation (70 mm) seems to have washed off the fungicide residue by the end of September.

Lots which were stored after 15th October all showed more-or-less severe scab lesions in March/April 1992. The lots originate from 5 different orchards. The interval between the last fungicide application and harvest did not have a great influence on storage scab incidence where the leaves had been free of scab. As shown in Fig. 4, there are examples from 1991 when orchardists allowed up to 50 days between the last spray and harvest without the development of a notable level of storage scab.

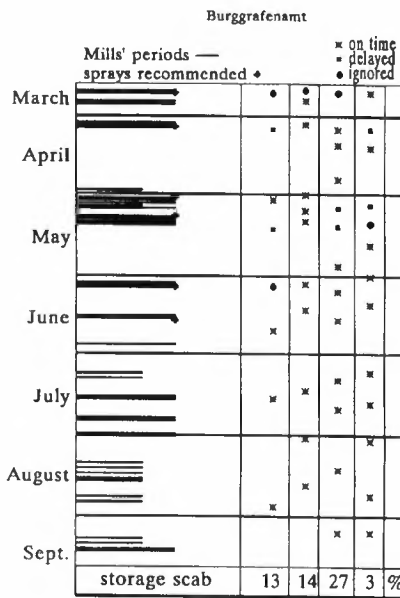


Figure 1. Mills Periods, Spray Dates and Storage Scab in 1991

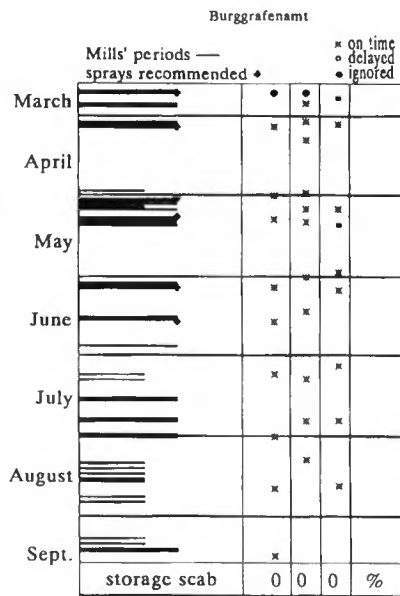


Figure 2. Mills Periods, Spray Dates and Storage Scab in 1991

### Golden Delicious

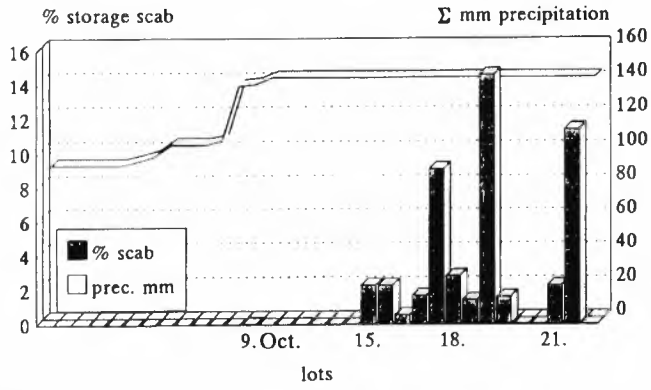


Figure 3. Precipitation and Storage Scab in 1991

### Interval Last Spray - Harvest

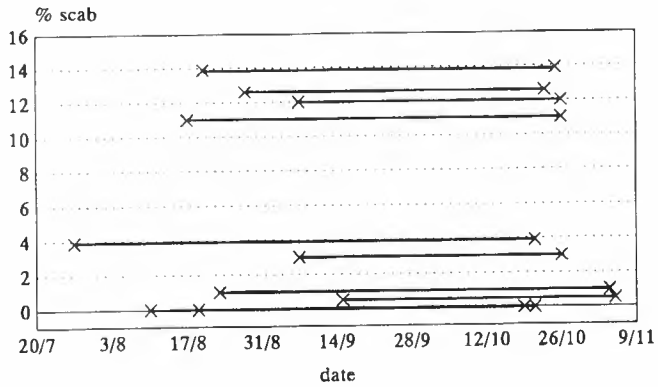


Figure 4. Storage Scab in 1991

**Storage scab 1992**

*Influence of the spray date*

Part of the 1992 harvest will remain in the packhouses until mid-July 1993 and so only a preliminary report can be given here. It is certain, however, that storage scab affects mainly Golden Delicious, less often Rome Beauty or Winesap and rarely Granny Smith and Jonagold. Moreover, all the scabbed fruits come from orchards where the scab control programme had not been conducted faultlessly (more than 2 % infected shoots in June). The fruits concerned had all been harvested after 10th October 1992 and had therefore been exposed to at least one infection of 78 hours without the protection of a fungicide, since there had been 60 mm or more precipitation after the last fungicide application.

Fig. 5 shows 4 orchards the fruits of which showed an economically significant level of storage scab at the time of grading in March 1993. In all these orchards, one or more of the recommended post-infection sprays had been delayed or infection periods had been ignored. Fig. 6, on the other hand, illustrates 4 examples of orchards where, on the whole, all infection periods had been taken into consideration. Two orchards are situated in a late location, where the vegetation was still dormant during the first Mills Period in 1992.

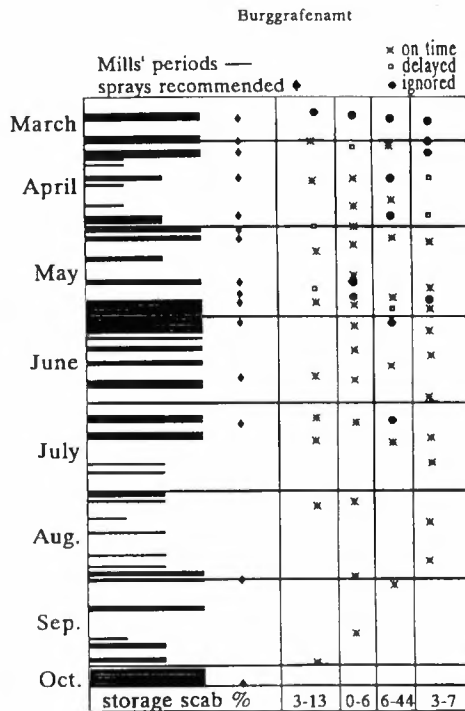


Figure 5. Mills Periods, Spray Dates and Storage Scab in 1992

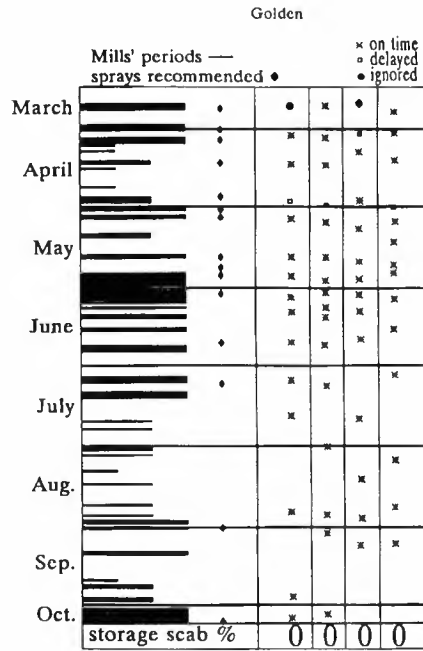


Figure 6. Mills Periods, Spray Dates and Storage Scab in 1992

**Influence of the spray equipment**

Only a few orchardists have so far had the opportunity to test their sprayers at the check-points. In some cases, however, the failure of scab management in 1992 was without doubt due to a defective sprayer. In a Golden Delicious orchard on seedling stock there were 33% shoots with lesions in June 1992. During investigation before the sprayer test it turned out that the amount of fungicide per ha was 40% too low. The test showed clearly that the distribution of the spray volume was faulty (Fig. 7).

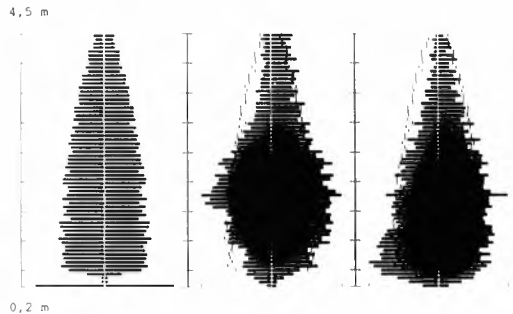


Fig. 7. Distribution of the Spray Volume

**Importance of the production method**

Conventional and integrated operations receive similar recommendations regarding apple scab management. The only difference is that IP-orchards must not use more than 4 dithiocarbamate treatments per season.

Are IP-guidelines to blame for storage scab occurrence in the last two years ? Figures 8 and 9 demonstrate that orchards treated according to IP-guidelines were less often affected by the problem of storage scab. Fig. 8 contains the grading results of 87 lots from IP-orchards and of 9 lots from conventional orchards harvested in 1991. The loss due to storage scab was significantly higher in conventionally-produced lots.

The data for the year 1992 are based on 689 lots from IP-orchards, 80% of which were free of scab at grading (Fig. 9). Out of 108 lots from conventional orchards, only 53% were free of scab at grading.

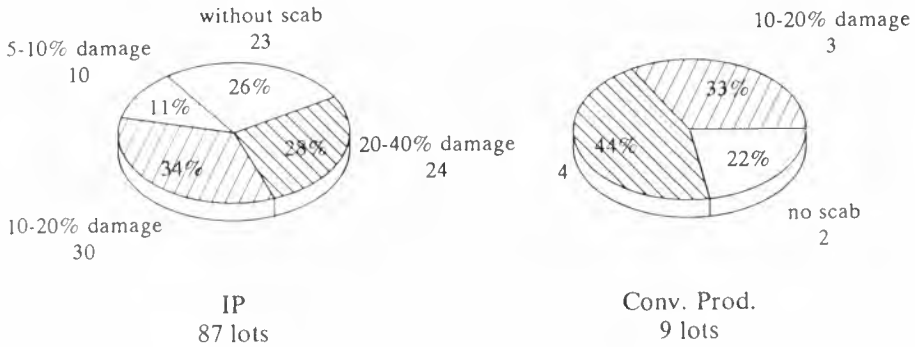


Figure 8. Storage Scab and IP, delivered 17th-24th Oct. 1991, graded 1st half of Febr. 1992

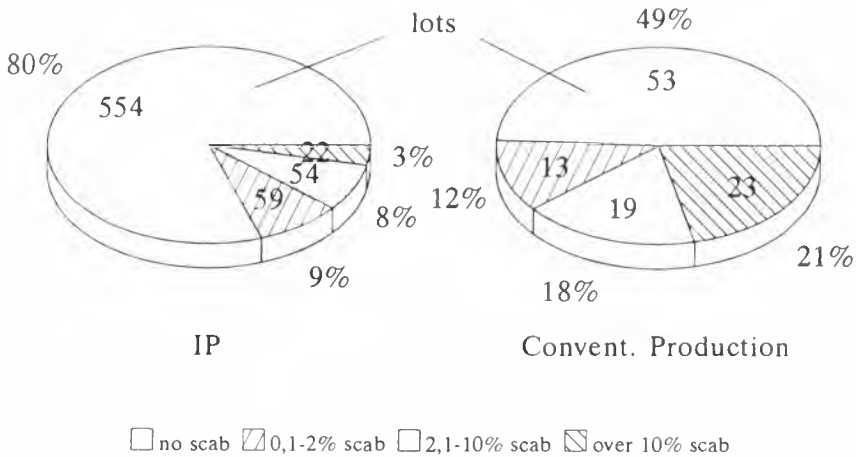


Figure 9. Storage Scab and IP in 1992, Golden Delicious delivered from 1st Oct. 1992 onward, graded until 3rd Febr. 1993

### **Late sprays before harvest**

Whether a late fungicide treatment, in October, in Golden Delicious orchards with leaf scab was able to prevent storage scab completely or partly cannot be answered at this time, as insufficient data are available; the few cases evaluated so far are contradictory.

### **DISCUSSION AND CONCLUSIONS**

- \* Apples from orchards without leaf scab in June 1991 and 1992 were not affected by storage scab, even under extremely favourable infection conditions.
- \* Many orchardists do not succeed in timing sprays correctly, according to infection periods. Some Mills Periods are ignored, especially the first in the year.
- \* Deficiencies in the control of primary infections may often be due to fungicide amounts per ha that are too low for the tree height or planting system.
- \* Poor performance of the spraying equipment represents an additional risk.
- \* The examination of the percentage of scab shoots with scab lesions is a valuable aid in assessing the risk of storage scab.
- \* In orchards that are free of scab at the end of the primary infection period, harvest intervals of up to 50 days are possible under South Tyrol conditions without increasing the risk of storage scab. In orchards with leaf scab, however, a last application of captan is advisable about 20 days before the first picking date. On the basis of experiences in 1992, this fungicide appears to have a tenacity to rainfall of 60-70 mm.
- \* If the fungicide residue washes off after the treatment, the orchardist has the possibility, within legal waiting periods, to apply ziram until 10 days before harvest. This treatment is an emergency measure and at this moment it is not certain if it can prevent storage scab.
- \* In 1991 storage scab was mainly in the variety Golden Delicious, but in 1992 also in Rome Beauty and Winesap, rarely in Granny Smith and Jonagold.
- \* The South Tyrolean IP-guidelines, which require slightly longer harvest-interval periods (captan, ziram, dodine: 20 days), cannot be held responsible for the storage scab problems in 1991 and 1992.

### **ACKNOWLEDGEMENTS**

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REFERENCES

Gadoury, D.M. & W.E. Machardy 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. *Phytopathology*, 72: 901-904.

Oberhofer, H. 1985. Der Apfelschorf. Lebensweise und Bekämpfung. Südtiroler Beratungsring für Obst- und Weinbau, 23: 12-22.

Oberhofer, H. 1986. Die Infektionsbedingungen des Apfelschorfes. *Obstbau\*Weinbau* 23: 12-15.

Scheer Van Der, H. 1990. Geleid Bestrijding Schurft/Meeldouw Rijp voor practijc 1990. *De Fruitteelt* 80: 32-33.

Schwabe, W.F.S., A.L. Jones & J.P. Jonker 1984. Changes in the susceptibility of developing apple fruit to *Venturia inaequalis*. *Phytopathology* 74: 118-121.



# The importance of *Botrytis cinerea* as a storage rot of apple cv. Cox and pear cv. Conference

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Berrie, A. 1994. The importance of *Botrytis cinerea* as a storage rot of apple cv. Cox and pear cv. Conference. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 383-389. ISSN 0802-1600.

In the U.K., *Botrytis cinerea* (Botrytis rot) is the most important cause of rotting in stored Conference pears, accounting for over 70 per cent of rotting and causing significant losses in pears from most orchards in most seasons in the absence of fungicide treatment. Most of the rotting appears to be secondary, originating from damage to pears at harvest, although some Botrytis rot does occur at the stalk and calyx ends of the fruit, indicating a possible origin from orchard infections. Until recently, Botrytis rot in U.K. apples was considered of minor importance compared to rotting due to *Nectria galligena*, *Gloeosporium* spp., *Phytophthora syringae* and *Monilinia fructigena*. However, the significance of Botrytis rot in Cox has steadily increased over the past few seasons until it has become the most important cause of rotting in the last two seasons. Two distinct types of Botrytis rot are apparent on Cox - secondary infection, and primary infection arising at the fruit calyx from previous orchard infection at blossom time. Reasons for the increased incidence in Cox are not clear and are discussed. Methods of evaluating Botrytis rot in the orchard in order to develop a rot-risk assessment system for both Cox apples and Conference pears, and thereby determine need for fungicide treatment, are also discussed.

Key words: *Botrytis cinera*, fruit rots, fungicides, pome fruit, post-harvest treatments, storage diseases.

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Since the 1970s, post-harvest treatments, mainly of benzimidazole fungicides, have been used in the UK for control of storage rots of apple and pear. These treatments proved so effective that they were adopted by the fruit industry as routine and used regardless of need. In addition, all further research into storage rots ceased, apart from limited surveys of rotting in treated stored apples and pears to monitor rot incidence and resistance to fungicides (Berrie 1989). Despite the advantages of post-harvest fungicide treatments compared to alternative fungicide control measures, with respect to efficacy and environmental contamination (Berrie 1992), such treatments are not favoured by consumers and consequently the market. This is partly because of the higher fungicide residues present on fruit treated post-harvest (Berrie 1992), although these are usually below the maximum

residue level (MRL) permitted. Thus, the U.K. fruit industry was faced with the prospect of no longer being able to use post-harvest treatments, but having no alternative strategy for rot control. The aims of the current research programme are, therefore, to develop an alternative rot-control strategy. Initially, a survey of rotting in apples and pears treated with fungicides and untreated post-harvest, was carried out to establish the size of the rot problem and to identify the main fungi responsible for losses.

#### SURVEY OF ROTTING IN COX APPLES 1991/92

A previous survey of rotting in Cox apples in the 1960s (Preece, 1969) identified *Gloeosporium* spp. as the main rot, with up to 30 per cent losses in some stores. In the first year of the current survey, losses due to rotting in treated apples were on average 2.3 per cent and increased to 4.5 per cent in untreated apples (Table 1). The main fungal rots identified (Table 2) were *Phytophthora syringae* in fruit marketed pre-Christmas and *Nectria galligena* and particularly *Botrytis cinerea* in late-stored fruit. *Gloeosporium* spp. were present but at low incidence. The high incidence of *Botrytis cinerea* was a particular contrast to the earlier survey where this rot was very much considered of minor importance. The survey was repeated in 1992/93 with fruit from the same orchards, so that the effect of seasonal variation in weather could be examined. Higher rainfall in August 1992 compared to 1991 was reflected in a high incidence of rots by *Nectria galligena*, but preliminary results again indicated a high incidence of *Botrytis cinerea*. A similar increase in incidence of *Botrytis* rot in stored Cox apples was also indicated in surveys of rotting in drenched fruit in 1982-91 (Berrie, 1992).

Table 1. Mean per cent losses due to rots in Cox apples fungicide-treated or untreated pre-storage 1991/92

Month graded 1992/92	Orchards sampled	Mean % loss in treated fruit (range)	Mean % loss in untreated fruit (range)
November	1	3.3	5.0
December	7	1.3 (0.5-3.0)	2.9 (0.5-9.8)
January	11	1.4 (0.5-4.1)	2.2 (0.8-5.1)
February	14	2.5 (0.5-6.3)	4.7 (1.3-11.8)
March	12	3.1 (0.8-6.6)	4.8 (1.0-8.6)
April	2	3.8 (3.5-4.0)	4.7 (4.3-5.1)
Total	47	2.6	4.1

Table 2. Rot types - mean rot incidence per 100 rotted Cox apples 1991/92

Fungal rot	Treated fruit	Untreated fruit
Botrytis	21.4	24.0
Brown rot	6.4	9.5
Gloeosporium	4.3	5.5
Nectria	19.8	16.7
Phytophthora	30.3	31.1
Mucor	0.2	1.4
Penicillium	11.9	8.0
Other	5.3	4.1

### SURVEY OF ROTTING IN CONFERENCE PEARS 1991/92

Losses due to rots in treated pears were on average 2-3 per cent and increased to 5 per cent in untreated fruit, although actual losses varied considerably from orchard to orchard (Table 3). Preliminary results from 1992/93 survey indicate similar losses. In both seasons, 70-80 per cent of the rotting was accounted for by *Botrytis cinerea*. Previous surveys of rotting in Conference pears treated with fungicides post-harvest (1980-91) also confirmed the importance of *Botrytis* as a storage rot in pears (Berrie 1992).

Table 3. Mean per cent losses due to rots in Conference pears fungicide-treated or untreated pre-storage 1991/92

Month graded 1991/92	Orchards sampled	Mean % loss in treated fruit (range)	Mean % loss in untreated fruit (range)
November	4	0.9 (0.3-1.3)	1.3 (0.5-2.3)
December	4	1.5 (0.7-2.1)	2.3 (1.2-4.2)
January	13	1.4 (0.1-4.2)	2.6 (0.8-5.7)
February	3	0.7	4.1 (0.9-7.4)
March	3	4.0 (1.5-7.9)	6.2 (2.5-11.7)
April	1	4.7	14.6
May	1	2.4	3.9
Total	29	2.2	5.0

### SYMPTOMS OF BOTRYTIS ROT IN CONFERENCE PEARS AND COX APPLES

On Conference pears, *Botrytis* appears initially as a semi-firm, later becoming soft, mid-brown circular rot generally found on the cheek of the fruit. Such symptoms are an indication that the rotting has resulted from damage at harvest. In store, *Botrytis* rot spreads rapidly by contact giving rise to typical nests of rots in the bulk bins. Once out of store the rot soon becomes covered with a grey fungal growth as the fungus starts to sporulate. Occasionally both eye-end rotting and stalk-end rotting can be found on pears, the latter particularly in long-term stored fruit. This symptom could also be associated with wound invasion, but could also be due to primary infection of the fruit in the orchard. *Botrytis* is

rarely seen as a fruit rot in orchards, but can be readily found elsewhere in the orchard e.g. sporulating on dead flower parts of young fruitlets early in the season.

Observations on symptoms of Botrytis rot on Cox during the current survey and previously indicate four distinct types of symptoms. Rotting can occur at the calyx-end, where the rot is mid-dark brown in colour. Such eye-end rots are usually one-sided and restricted or radiate like fingers from the calyx down the apple cheek. Occasionally, Botrytis rot can be found at the stalk end of the fruit where it is mid-brown in colour and distinguishable from Nectria rot by remaining firm rather than collapsing inwards. Symptoms of Botrytis rot were also observed on the cheek as a small often irregular rot mid-dark brown in colour. Such fruit when cut open revealed extensive internal rotting. These three symptom types can be considered as primary rots, possibly resulting from infection at blossom time. Botrytis rot can also occur on the cheek of the fruit as a semi-firm rot varying in colour from pale-gingery brown to dark brown. Often such rots have a darker border and reddening around the lenticels. This symptom is generally associated with wounds or long-term storage of fruit. In the orchard, Botrytis rot may be visible on fruits in the summer as rot/blemish at the calyx end, varying from browning of the calyx or a red blemish at the base of the calyx, to a slight rot. Generally this rot dries and cracks away from the fruit, forming dry eye rot. In some seasons dry eye rots can be common in Cox orchards.

#### POSSIBLE REASONS FOR THE INCREASE IN BOTRYTIS ROT INCIDENCE

*Botrytis cinerea* has always been recognised as an important cause of losses in stored Conference pears and one of the limiting factors in pear storage. It is only recently that the significance of Botrytis rot in Cox apples has been recognised. Reasons for the increase are not clear but could be due to changes in fungicide use in orchards, or increasing use of high humidity fruit cold stores to ensure fruit quality or an increasing incidence of *Botrytis cinerea* isolates which are resistant to benzimidazole fungicides (Berrie 1989). Before the advent of DMI fungicides such as fenarimol, myclobutanil and penconazole, commonly used scab fungicides such as captan and dithianon were considered to be toxic to pollen and therefore likely to reduce fruit set. Consequently, thiophanate methyl was sprayed at blossom time for disease control as it was deemed to be safe, but at the same time appeared to increase fruit set. This treatment may have given some control of Botrytis rot. More recently, DMI fungicides tend to be used during blossom for scab/mildew control in apples and the use of benzimidazole fungicides in orchards was discouraged because of possible harmful effects on predatory mites such as *Typhlodromus pyri* (Childers et al. 1992) and earthworms (Kennel 1989).

#### PROSPECTS FOR CONTROL OF BOTRYTIS ROT

In Conference pears, where Botrytis rot in store appears to be mainly associated with damage to fruit during harvest, adequate control of rotting in store has been achieved in the U.K. by use of a single post-harvest fungicide dip or drench. Initially, benzimidazole

fungicides were used and later vinclozolin, once control with benzimidazole fungicides declined due to the development of resistance. Concern over the use of post-harvest fungicides and the suspension of the approval for use of vinclozolin in the U.K. prompted the search for effective alternative fungicides and evaluation of their use, both as pre-harvest sprays and post-harvest dips for the control of *Botrytis* rot in pears. In a replicated trial in which pear fruits had been inoculated with *Botrytis* rot by placing infected pears (using a benzimidazole-resistant isolate) amongst the healthy fruit, a single post-harvest dip was more effective than a programme of three pre-harvest sprays with the final spray applied two weeks before picking (Table 4). Only iprodione was as effective as vinclozolin in controlling contact rots.

Table 4. Mean per cent *Botrytis* rot in pear cv. Conference treated with fungicides applied either pre-harvest (3 sprays) or as a single post-harvest dip

Treatment	Pre-harvest sprays	Post harvest dip
vinclozolin	30.0	0.5
iprodione	18.0	2.5
thiophanate methyl	39.3	53.3
dichlofluanid 1	51.3	33.5
dichlofluanid 2	36.0	28.8
captan	56.0	70.8
thiram	52.5	57.8
imazalil	58.8	19.5
untreated	50.3	65.0
water dip	---	59.0

With Cox apples, benzimidazole drenches either alone or in mixture with metalaxyl have been used for control of storage rots. Until recently, *Botrytis* has not been the main target rot so that little thought has been given to control. Preliminary trials were conducted in 1992 to examine the effects of orchard sprays of a mixture of captan and benomyl, applied at petal fall and/or pre-harvest (three sprays). Although the final incidence of *Botrytis* rot was low, rotting was reduced by either programme with the greatest reduction achieved by the combined blossom and pre-harvest sprays (Table 5).

Table 5. Effects of orchard sprays of benomyl + captan applied at petal fall and/or pre-harvest on control of *Botrytis* rotting in store 1992

Treatment schedule (numbers of sprays)	Mean wt <i>Botrytis</i> -rotted fruit (kg.)
Petal fall sprays (2)	3.85
Pre-harvest sprays (3)	3.07
Petal fall + pre-harvest sprays (2 + 3)	1.60
Untreated	5.15

## DISCUSSION AND FUTURE RESEARCH

Current research on post-harvest rots in the U.K. is aimed at reducing the use of fungicides, applied either pre- or post-harvest, by developing integrated control methods and a system of rot risk assessment to determine need for treatment (Berrie 1993). Preliminary results indicate good prospects for predicting the incidence of rots caused by *Nectria* and *Gloeosporium*. In preliminary trials where *Botrytis* was the main rot in Cox apples, no correlation was found between incidence of this rot and any of the factors evaluated - fruit mineral composition, rainfall and other orchard factors. Future research must therefore be targeted at identifying the factors that influence *Botrytis* rot both in apples and pears, such that an integrated approach to control can be formulated.

*Botrytis cinerea* is the main cause of fruit losses in most soft-fruit crops. In strawberries, raspberries and blackcurrants the importance of blossom infection to the subsequent development of rotting in the mature fruit has been established (Williamson & McNicol 1986). Infection of apple blossom and calyx-end rot have also been recognised in other apple cultivars (Tronsmo & Raa 1977; Tronsmo et al. 1977) but this was considered unimportant in the epidemiology of the post-harvest rot (Rosenberger 1990). In view of the observations on symptoms in Cox apples, the significance of blossom infection needs investigation. In addition, sources of inoculum in the orchard and store, together with the effect of weather conditions on rot incidence, need evaluation. Once these factors have been established it may be possible to develop a forecasting system to determine need for treatment or at least to target sprays more accurately.

## REFERENCES

- Berrie, A.M. 1989. Storage rots of apple and pear in south-east England. 1980-1988 incidence and fungicide resistance. Integrated control of pome fruit diseases, II, IOBC Bulletin XII/6: 229-239.
- Berrie, A.M. 1992. Alternative strategies for the control of post harvest rots in apples and pears. Proceedings Brighton Crop Protection Conference Pests & Diseases, 301-310.
- Berrie, A.M. 1993. Progress towards integrated control of post harvest rots of Cox apples in the United Kingdom. Acta Horticulturae 347: 107-114.
- Childers, C.C., M.A. Easterbrook & M.G. Solomon 1992. Chemical control of eriophyoid mites in world crop pests. VI.-6. Eriophyid Mites. Their biology, natural enemies and control. Editors: E.E. Lindquist, M.W. Sahlis and W. Helle. Elsevier, Amsterdam.
- Kennel, W. 1989. The integrated use of benzimidazole fungicides to control *Gloeosporium* fruit rot of apple. Integrated control of pome fruit diseases II, IOBC Bulletin XII/6: 247-255.

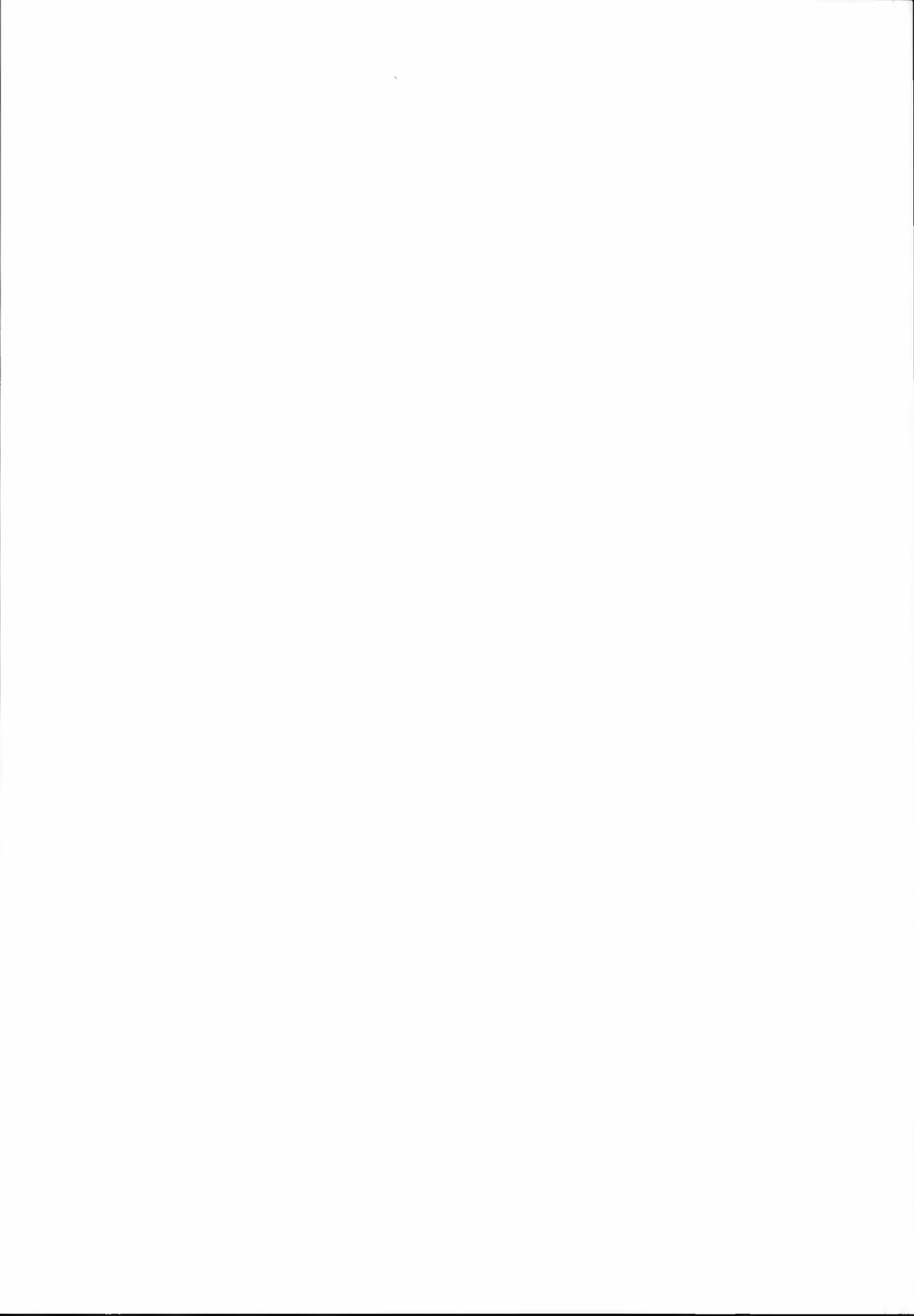
Preece, T.F. 1967. Losses of Cox's Orange Pippin apples during refrigerated storage in England. 1961-65. *Plant Pathology* 16: 176-180.

Rosenberger, D.A. 1990. Grey mould. In: *Compendium of Apple & Pear Diseases*, edited A.L. Jones & H.S. Aldwinckle. The American Phytopathological Society 1990.

Tronsmo, A. & J. Raa 1977. Life cycle of the dry eye rot pathogen *Botrytis cinerea* Perl. on apple. *Phytopath. Z.* 89: 203-207.

Tronsmo, A. & J. Raa 1977. Cytology and biochemistry of pathogenic growth of *Botrytis cinerea* Perl. in apple fruit. *Phytopath. Z.* 89: 208-215.

Williamson, B. & R.J. McNicol 1986. Pathways of infection of flowers and fruits of red raspberry by *Botrytis cinerea*. *Acta Horticulturae* 183: 137-141.





# A supervised control of apple powdery mildew *Podosphaera leucotricha* (Ell. et. Ev.) Salm. on Cortland cv. in Poland

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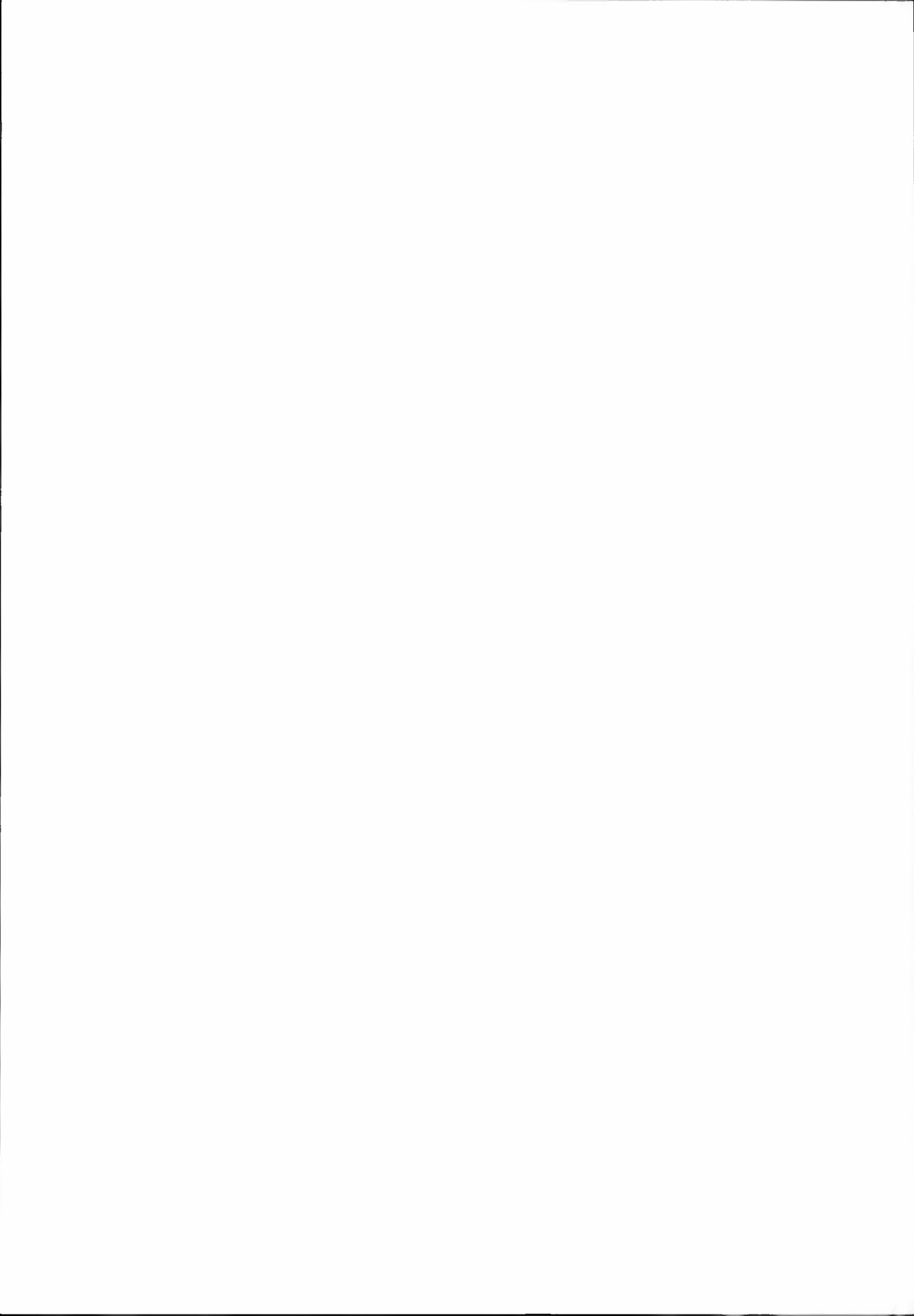
Experiment was carried out in the period 1982-1986 on 156 trees of Cortland cv. grafted on Antonovka, planted 6 x 5 m. Each year only postblossom control with 3 sprayings with sulphur fungicides was applied. The data concerning shoot infection and yield of individual tree were collected. The percentage of infected shoots during primary (May), percentage of infected shoots at secondary infection after 3 sprayings (end of June), and then in August were determined. Eight unsprayed trees were left as check. Control of apple scab was made with fungicides containing dodine and captan.

High infection levels occurred in 1982-1985. Lower numbers of diseased shoots were observed only in 1986. During all the seasons, except 1984 good yield was harvested. Disease level on check trees was usually the greatest, however some sprayed trees were infected at the same extent. All of them were summarized for statistical calculations.

The results showed, that in traditional orchard, infestation up to 5% of shoots at the beginning of bloom (primary infection) did not decrease fruit crop when postblossom control (3 sprayings) was applied. The higher the infestation of shoots at bloom, the lower was the crop. The obtained data indicate that in such orchard, the spraying with antispore fungicides at the beginning of bloom seems to be unnecessary when shoot infection does not exceed 5%.

The data indicate also, that fruit crop was not decreased when during secondary infection, after 3 sprayings with sulphur the number of infected shoots was below 40%. Shoot infestation exceeding this value required additional sprayings for crop protection.

The decrease of yield took place when the disease level in August exceeded 80% of infected shoots.



# Phytophthora crown rot of apple trees: scion/rootstock interaction

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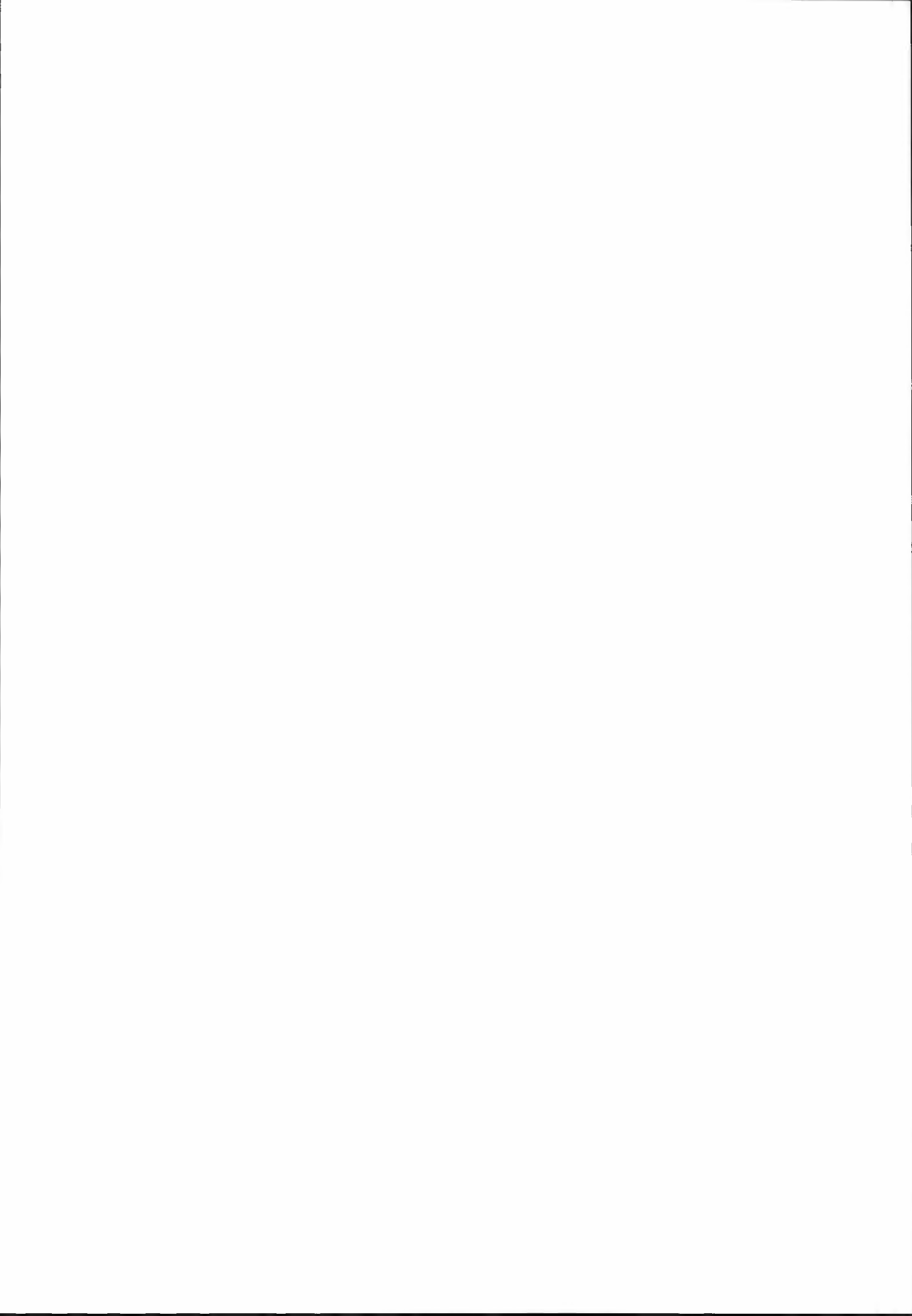
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Bielenin, A. 1994. Phytophthora crown rot of apple trees: scion/rootstock interaction. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 393. ISSN 0802-1600.

The observations were carried out in two commercial apple orchards with crown rot problem. *Phytophthora cactorum* was isolated from necrotic tissue of declining trees. Occurrence of the crown rot on rootstocks M 26, P 1 and P 22 worked to four apple cultivars, Gloster, Idared, Melrose and Jonagold, in the first orchard and on the rootstock MM 106 grafted with three cultivars, Cortland, Lobo and Idared, in the second one was examined. The susceptibility of apple rootstocks to crown rot was strongly influenced by cultivar. It was observed especially clearly in case of very susceptible rootstocks P 1 and MM 106. Significantly higher frequency of incidence of crown rot was recorded on P 1 rootstock in case of Gloster and Idared than Melrose and Jonagold. No crown rot occurred on P 22 rootstock, and only a few trees showed symptoms of the disease in rootstock M 26 worked to Idared. In the second orchard with trees on MM 106 crown rot occurred on Lobo and Cortland cvs at similar level (5,5% and 6,8% of infected trees) whereas no disease symptoms on Idared trees were observed.

Influence of the cultivar on resistance of rootstocks was also observed in naturally infected apple maiden trees produced in containers. Trees of Sampion cv. on P 2 and P 22 rootstocks known as much less susceptible than P 1 and MM 106 showed higher incidence of crown rot than cv. Jonagold.

Also the results of the greenhouse experiments with inoculated rootstocks confirmed the field observations claiming that the frequency of incidence of crown rot depends on cultivar worked to. Lesions were larger on rootstock P 2 grafted with Melrose than with Vista Bella, and on rootstocks P 1 and MM 106 worked to cultivar Gloster than to Jonagold. It suggests that Jonagold cv. lessens susceptibility of rootstocks to *P.cactorum* infections as well under field as under greenhouse conditions.



# Are phenolics and enzyme inhibitors involved in resistance of apple against *Venturia inaequalis*?

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Müller M., H. Sierotzki & C. Gessler 1994. Are phenolics and enzyme inhibitors involved in resistance of apple against *Venturia inaequalis*? Norwegian Journal of Agricultural Sciences. Supplement No. 17: 395-398. ISSN 0802-1600.

Electron-microscopy studies of *Malus x domestica* leaves infected with *Venturia inaequalis* (Cke.) Wint. demonstrated plant cell wall degradation (CWD) at contact sites between well-developed fungal stroma and epidermal host cells. Such forms of stroma were found mainly in young, scab-susceptible leaves. CWD enzymes may thus be involved, directly or indirectly, in mechanisms of susceptibility or resistance to this pathogen. Extracts from *V. inaequalis* showed pectinolytic as well as cellulolytic activity. A protein was extracted from apple leaves that inhibits the polygalacturonase (PG) from *V. inaequalis*. This PG-inhibiting protein (PGIP) was purified and characterized. To investigate the PGIP for physiological significance in the pathogenesis of apple scab, we determined the amounts of inhibitor without and shortly after inoculation in young and old leaves of a scab-susceptible and a scab-resistant apple cultivar.

Other investigations of the mechanism of resistance of *M. x domestica* against *V. inaequalis*, have often concentrated on the phenolic compounds in leaves and peels. Treutter showed, with the help of a chemical detection reaction (CDR), that the amount of flavan-3-ol in apple leaves of different Vf-resistance cultivars is correlated positively with their resistance. For a better understanding of this possible relationship, we determined the flavan-3-ol content with the same CDR in clones resistant and susceptible to apple scab and in progeny of crosses of resistant and susceptible parents of *M. x domestica*. We present the relation of the flavan-3-ols to the Vf-resistance (biochemically as a mechanism or genetically as a marker) and to the ontogenetic resistance (using apple varieties with different resistance sources), and the relation of the amount of flavan-3-ols to the degree of resistance of offspring from susceptible and resistant parents.

## MATERIAL AND METHODS

### **PG- and PGIP-extraction, purification and quantification**

*V. inaequalis* was grown on liquid cultures. PG was extracted from the mycelium and purified by affinity-chromatography. PG-activities were measured with the reducing end-group assay (Nelson & Somogyi).

Apple trees were grown in the greenhouse. Leaves were cut and immediately frozen in liquid nitrogen. The leaves were homogenized in the extracting buffer. The heat-treated, centrifuged, dialyzed and ultrafiltered concentrates were subjected to isoelectric focusing, the gels cut in slices and the PGIP eluted by diffusion. The inhibitory activities of the leaf extracts were quantified.

### Flavan-3-ol extraction and quantification, resistance classification

After extraction with an acetone/water-mixture the flavan-3-ols were detected by a CDR with p-dimethylaminocinnamaldehyde.

The seedlings were inoculated artificially and scored for resistance after 8 days.

## RESULTS

The PGIP-content of leaves of "Florina" (scab-resistant) was independent of age. However, young leaves (group "1, 2") of "Golden Delicious" (scab-susceptible) contained significantly ( $p < 0.05$ ) more PGIP than older leaves (groups "3, 4" and "5, 6"). Comparing the amounts of PGIP in inoculated and non-inoculated leaves, there were no differences, neither in the scab resistant ("Florina") nor in the scab-susceptible variety ("Golden Delicious") (Fig. 1).

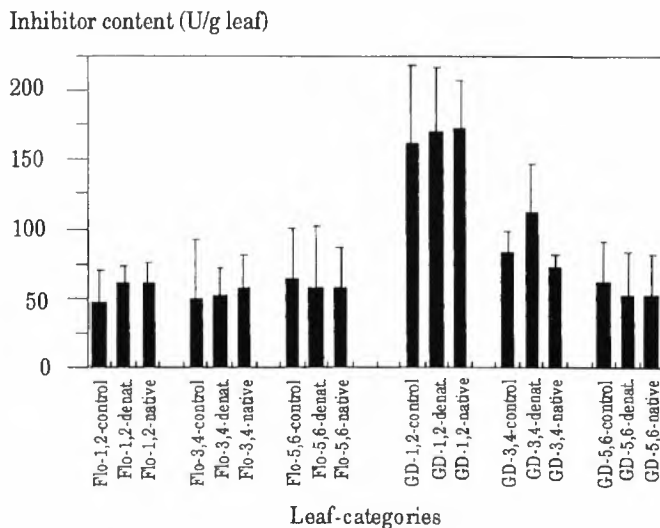


Fig. 1. Amounts of PGIP in non-infected and scab-infected leaves from "Florina" (Flo) and "Golden Delicious" (GD). The first six leaves of one shoot were divided in 3 categories: "leaves 1,2", "3,4" and "5,6". The PGIP-contents of these 3 groups were determined 4 days after the leaves had been covered with water drops (control), heat-denatured conidia (denat.) or live conidia (native) from *V. inaequalis*.

The content of flavan-3-ols in the leaves of apple was highly dependent on the age of the leaf: in all investigated clones the first three leaves had the highest content, with decreasing amounts in older leaves (Fig. 2.)

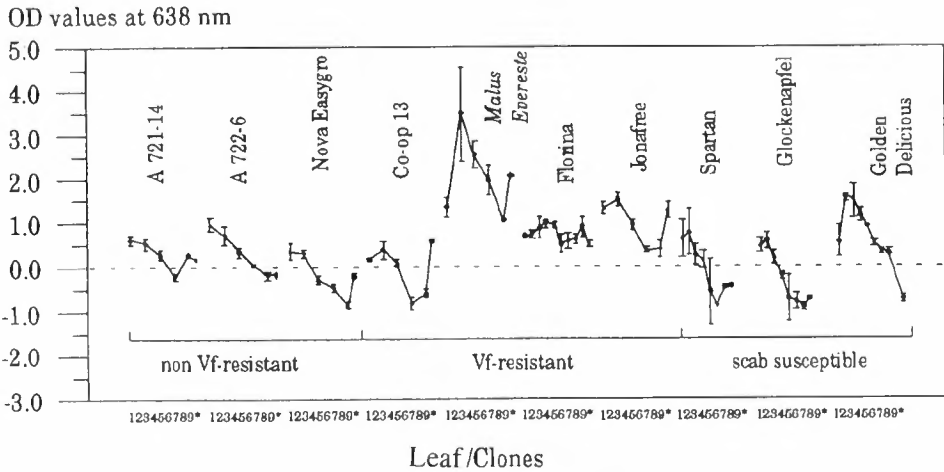


Fig. 2. Flavan-3-ol content (plotted in OD values after reaction with DMACA and subtraction of the ground absorption) of the clones A 721-14 (Vbj-resistance), A 722-6 (Vb-resistance), Nova Easygro (Vr-resistance), Co-op 13 (Vf-resistance), *Malus Evereste* (Vf-resistance), Florina (Vf-resistance), Jonafree (Vf-resistance), Spartan, Glockenapfel and Golden Delicious (susceptible). Numbers below (on the x-axis) indicate the age of the leaf (counted from the first unfolded leaf of a growing shoot; \* stands for the numbers 10, 11 and 12 which are pooled). Standard deviations are calculated from at least three repetitions.

The resistances of Co-op 13 (Vf-resistant), A 722-6 (Vb-resistant), A 721-14 (Vbj-resistant) and Nova Easygro (Vr-resistant) were independent of flavan-3-ol content. Among the seedlings from all crosses there was a wide range of flavan-3-ol content but no direct relation to the degree of resistance (Fig. 3).

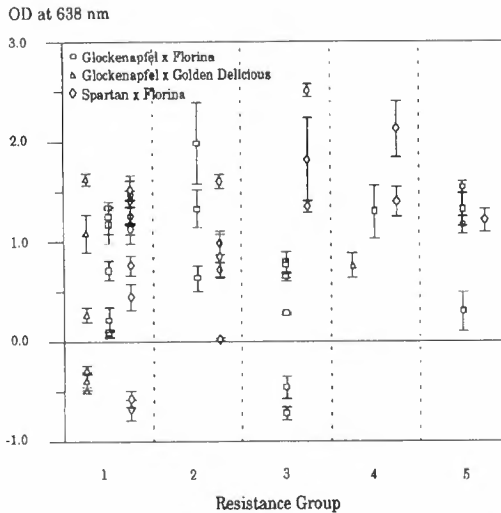


Fig. 3. Relation between flavan-3-ol content (OD values after reaction with DMACA and subtraction of the ground absorption) and the resistance group classification for each seedling from a particular cross. There is no correlation ( $r^2 = 0.06$ ) between flavan-3-ol content and resistance group. Standard deviations are calculated from three repetitions

#### DISCUSSION AND SUMMARY

From the high PGIP-content in scab-susceptible leaves (young leaves of "Golden Delicious") and the lower amounts found in scab-resistant leaves (old leaves of "Golden Delicious" and all leaves of "Florina"), there is no simple correlation (4 days after inoculation) between the resistance of an apple leaf against *V. inaequalis* and the PGIP-content of the leaf. However this does not rule out a pathogenesis-dependent relation of PG and PGIP in the resistance mechanism against *V. inaequalis*.

Pre-formed flavan-3-ols appear to have no obvious relation with scab-resistance (varietal or ontogenetic), neither as biochemical mechanisms nor as markers for genetic investigations. The results give a further indication that constitutive phenolics may not be involved in resistance to scab.



# Impairment of ontogenetic resistance of apple against *Venturia inaequalis*

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The degree of ontogenetic resistance of apple to *Venturia inaequalis* depends on the cultivar and type of plant tissue as well as the incubation time and the inoculum used for infection. For scab control strategies, the highly susceptible leaves and fruits in their earlier stages of development are considered. In the course of a comprehensive epidemic investigation, the vertical distribution of disease along shoots of cv. 'Golden Delicious' at different time intervals was assessed. A breakdown of ontogenetic resistance was determined, which was only detectable in the orchard. This phenomenon may affect disease incidence in autumn, which would then cause an increase of potential ascospore dose for the subsequent growing season.

Key words: Apple scab, epidemiology, incubation, ontogenetic resistance, symptomatology.

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Young, growing apple leaves pass through a stage of high susceptibility to scab but with increasing age acquire a resistance to infection. At the time of full leaf expansion, cv. Golden Delicious (Gessler and Stumm 1984) and apple rootstock MM 109 show almost complete resistance to infection (Schwabe 1979; Kirkham and Hignett 1973). Keitt and Jones (1926) stated that the upper (ventral) surface leads in the speed and degree of development of resistance. Acquisition of resistance on the lower surface is delayed and less, and varies with the cultivar. A more prolonged incubation period, of about 1 to 2 months, led to a development of the fungus characterized as 'sparse and often diffuse and inconspicuous'.

## MATERIALS AND METHODS

'Golden Delicious' seedlings were exposed in the orchard daily or according to potential infection periods. At least 10 seedlings were used per exposure from 24 March to 12 October, 1992. Deposits of scabbed leaves from the previous year were placed around the seedlings in order to increase airborne ascospore inoculum and on and after the 1 June the

seedlings were placed around severely infected trees of cv. 'Golden Delicious'. The exposed seedlings were incubated in the greenhouse and recorded for apple scab up to 2 months later.

In the orchard, cv. 'Golden Delicious' was examined for scab at intervals of about 14 days. Thirty entire shoots were assessed for scab infections and the position of each leaf was recorded. Growth rate of the shoots in the orchard was determined by registering newly- developed leaves within units of time.

## RESULTS AND DISCUSSION

Two severe infection periods by ascospores and at least a further 17 by conidia were detected by the trap plants. The time course of infections in the orchard was comparable with the dates determined by the exposed seedlings. According to the infection periods, the frequency of scabbed leaves revealed two peaks along the shoots (Fig. 1). At least 4 healthy leaves separated these scabbed leaves. These leaves were in a susceptible state at a time when dry weather conditions prevailed with no infection periods. In late August, however, scab symptoms were visible on leaves which thus far had formed the healthy zone on the diseased shoots. At the end of July, the beginning of diffuse mycelial development was observed on the lower surface of leaves carrying the primary infections and on the leaves which up until then had formed the healthy zone. The same was observed on leaves with secondary infections, however with a reduction in symptom expression on the younger leaves. At the end of August typical lesions were detectable over the entire shoot (Fig. 2).

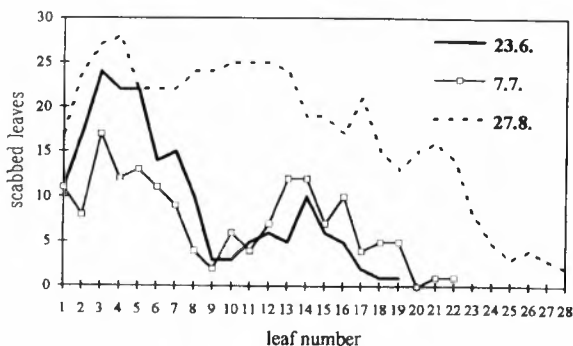


Fig. 1. Disease assessment on shoots of apple cv. 'Golden Delicious'. 30 shoots were collected for each date and the leaves were numbered serially beginning with the oldest. Leaves were classified as scabbed or healthy without counting lesions

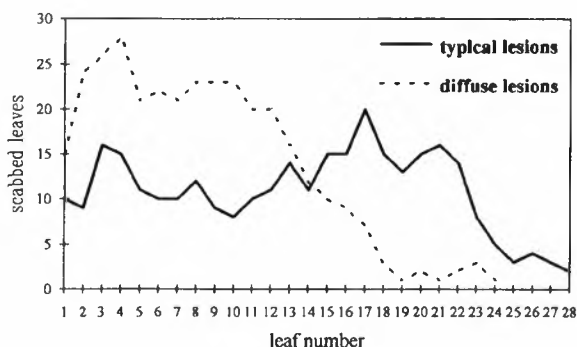


Fig. 2. Disease assessment of shoots as described in Fig. 1 on 27.8.93 with differentiation of typical lesions and diffuse mycelial development. Some data could not be classified clearly because of an intermediate symptom expression and were assessed according to the predominant type

The importance of incubation time as the explanation for these different symptom developments does not seem to be appropriate for the interpretation of this phenomenon. Diffuse symptoms appeared within a rather short time of less than 1 month and showed an inverse gradient in the vertical distribution of disease: normally, infections on the lower surface are more prominent on younger leaves and a reduced infection rate or a prolonged incubation period occurs with increasing age of the leaves. Attributing the type of observed symptoms delayed disease expression after earlier infection periods would give incubation times of 4 months for the oldest leaves, about 2.5-3 months for leaves in the healthy zone and about 2 months or less for the younger leaves. It is inconsistent with this wide range of maximum incubation periods that all additional symptoms appeared in a rather short interval of time. This period of less than 1 month could also be described as the true incubation time. After exposure in the orchard or using standard inoculation procedures with conidia, seedlings only revealed a slight shift of symptoms towards older leaves when incubation times were extended in the greenhouse for more than 2 months. Essentially, the pattern found for extremes of incubation periods were the same as the short incubation times found by other authors (Gessler and Stumm 1984; Schwabe 1979; Kirkham and Hignett 1973) for cv. 'Golden Delicious' and apple rootstock MM 109. The abundant development of disease on the lower surface of even old leaves after prolonged incubation, as noted by Keitt and Jones 1926 with cv. 'Duchess', was not observed with seedlings or infection experiments with trees in the orchard. Considering all results it can be concluded that, in late summer, ontogenetic resistance was rendered ineffective and symptom development was attainable along the entire shoot in the course of the same interval of time. The cause for the loss of ontogenetic resistance may be the physiological status of leaves, which changed for unknown reasons. The only obvious reasons could be environmental factors in the orchard, which are different to the conditions in the greenhouse.

REFERENCES

Gessler, C. & D. Stumm 1984. Infection and stroma formation by *Venturia inaequalis* on apple leaves with different degrees of susceptibility to scab. *J. Phytopath.* 110: 119-126.

Keitt, G.W. & L.K. Jones 1926. Studies of the epidemiology and control of apple scab. *Bull. Wis. agric. Exp. Stn.* 73.

Kirkham, D.S. & R.C. Hignett 1973. Control of the vertical distribution of apple scab disease on shoots of the apple rootstock MM 109, p. 55-66. *In* R.J.W. Byrde and C.V. Cutting (eds): *Fungal pathogenicity and the plant's response*. London: Academic Press.

Schwabe, W.F.S. 1979. Changes in scab susceptibility of apple leaves as influenced by age. *Phytophylactica* 11: 53-56.

# Apple scab resistance from *Malus floribunda* 821 (*Vf*) is rendered ineffective by isolates of *Venturia inaequalis* from *Malus floribunda*

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Roberts, A.L. & I.R. Crute 1994. Apple scab resistance from *Malus floribunda* 821 (*Vf*) is rendered ineffective by isolates of *Venturia inaequalis* from *Malus floribunda*. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 403-406. ISSN 0802-1600.

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Resistance to *Venturia inaequalis* derived from *Malus floribunda* 821 has been used in apple breeding programmes throughout the world for more than 40 years. This resistance, ascribed to gene *Vf*, has been considered to be highly 'durable' (Crosby *et al.* 1992). *M. floribunda* 821 is the primary source of resistance to *V. inaequalis* used in the apple breeding programme at HRI, East Malling.

*M. floribunda* is a species known to be susceptible to scab in Kent. A range of apple varieties with and without *Vf*, and other recognised genes for resistance to *V. inaequalis*, were tested for their response to isolates of the pathogen derived from *M. floribunda*.

## MATERIALS AND METHODS

### *Malus* germplasm

Budwood of *M. floribunda* 821 was obtained from Dr. Korban, University of Illinois, U.S.A. All apple cultivars were obtained from germplasm collections at Brogdale Horticultural Trust, Kent or HRI, East Malling. Potted trees for inoculation were produced on M9 rootstocks.

### *V. inaequalis* isolates

A mass-spore isolate (FL1) of *V. inaequalis* derived from several lesions of a naturally infected *M. floribunda* tree in a local garden was used in most inoculations in comparison with a standard monoconidial isolate (E1) maintained at HRI East Malling since 1949. A monoconidial derivative of FL1 (TR 50) was utilised in some inoculations and expressed a virulence phenotype identical to FL1. Limited tests have also been conducted with a second mass-spore isolate (FL2) derived from a certified *M. floribunda* tree at HRI East Malling.

### Inoculation and assessment

Potted trees were placed in a controlled temperature growth room (16 h photoperiod, 18 °C,  $200 \pm 50 \mu\text{mol/m}^2/\text{s}^{-1}$ ) for 48 h prior to inoculation.

Shoots bearing young leaves were inoculated with a conidial suspension ( $2 \times 10^5$  conidia/ml) of *V. inaequalis* using an atomiser to deliver a fine spray. Inoculated shoots were enclosed in polyethylene bags for a further 48 h under the same growth room regime before being transferred to a cooled glasshouse (18-25 °C).

Symptoms on inoculated leaves began to appear 10-14 days after inoculation and five different interaction phenotypes (IPs) were recognised as follows:

- O - no macroscopically visible symptoms;
- H - small necrotic 'pits' (resembling a hypersensitivity reaction);
- 1 - chlorosis and/or necrosis +/- leaf distortion but no sporulation;
- 2 - chlorosis and/or necrosis accompanying sparse sporulation;
- 3 - sporulating lesions of varying size in the absence of any other symptoms.

### RESULTS

*M. floribunda* 821 and several, but not all, apple cultivars considered to carry gene *Vf* proved susceptible to FL1 (IP of 2 or 3) but, as expected, were resistant (IP 0, H or 1) to E1 (Table 1). Florina and SA 15-4 (an East Malling selection) were among those that remained resistant to both E1 and FL1. *M. floribunda* 821 was also susceptible to a monoconidial isolate (TR 50) originating from FL1 and to a second mass conidial suspension (FL2) from a certified *M. floribunda* (Table 2). Nova Easygro, considered to carry gene *Vr* (from *M. pumila* 'Russian Seedling') was also susceptible to FL1 and TR 50 but resistant to E1.

A few apple cultivars susceptible to E1 (Beauty of Bath, Blenheim Orange and Prairie Spy) were apparently resistant to FL1 (Table 1). Miller's Seedling and Ribston Pippin (a putative parent of Cox) were resistant to both E1 and FL1.

### CONCLUSIONS

A pathotype of *V. inaequalis* has been found which is capable of rendering ineffective the resistance derived from *M. floribunda* 821. The notion that this source of resistance will be 'durable' needs to be reappraised. The resistance to FL1 of most cultivars considered to carry *Vf* suggests that this resistance could be contributed by a gene or genes carried by one or more of the apple cultivars used in the crossing programmes.

A set of *V. inaequalis* isolates with diagnostic potential to discriminate among genes for resistance is required to select efficiently for gene combinations in breeding programmes.

Table 1. Interaction phenotypes observed following inoculation with *V. inaequalis* isolates: E1 and FL1

	Interaction Phenotype	
	E1	FL1
Cox/Queen Cox	3	3
Golden Delicious	3	3
Lord Lambourne	3	3
Beauty of Bath	3	1
Blenheim Orange	3	1
Prairie Spy (R7)	3	1
Jonafree ( <i>Vf</i> )	1	3
Liberty ( <i>Vf</i> )	1	2
<i>M. floribunda</i> 821 ( <i>Vf</i> )	1	3
Macfree ( <i>Vf</i> )	1	3
Novamac ( <i>Vf</i> )	1	3
Priscilla ( <i>Vf</i> )	1	3
Redfree ( <i>Vf</i> )	1	3
Nova Easygro ( <i>Vr</i> )	1	3
Miller's Seedling	1	1
Ribston Pippin	1	1
Florina ( <i>Vf</i> )	H	1
Priam ( <i>Vf</i> )	1	1
Prima ( <i>Vf</i> )	1	1
Sir Prize ( <i>Vf</i> )	1	1
SA 15-4 ( <i>Vf</i> )	1	1

For a description of interaction phenotypes see Materials and Methods

Table 2. Interaction phenotypes observed following inoculation with *V. inaequalis* isolates: E1, FL1, FL2 and TR50

	E1	Interaction Phenotype		TR50
		FL1	FL2	
T31/12	3	3	-	3
Florina ( <i>Vf</i> )	H	1	-	1
<i>M. floribunda</i> 821 ( <i>Vf</i> )	1	3	3	3
Nova Easygro ( <i>Vr</i> )	1	3	-	3
Novamac ( <i>Vf</i> )	1	3	-	3

For a description of interaction phenotypes see Materials and Methods

ACKNOWLEDGEMENT

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REFERENCES

Crosby, J.A., J. Janick, P.C. Pecknold, S.S. Korban, P.A. O'Connor, S.M. Ries, J. Goffreda & A. Voordeckers 1992. Breeding apples for scab resistance: 1945-1990. *Fruit Varieties Journal* 46: 145-166.



# Integrated fruit production in Austria: problems, and aspects of regional strategies of disease management

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Holzer, U. & A Nothnagl 1994. Integrated fruit production in Austria: problems, and aspects of regional strategies of disease management. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 407-411. ISSN 0802-1600.

In 1992 a study of the ascospore-discharge of apple scab (*Venturia inaequalis*) was done at 3 different locations in Austria. In Spitz, this investigation and the use of electronic monitoring led to a regional advisory system. Fungicide treatments according to this system could prevent scab infections. Because of the low number of rainfalls in spring and summer 1992 most spore discharges were followed by calculated scab infections. An investigation of the pathogens causing fruit rots in stored apples is described. The effect of a single pre-harvest treatment 3 weeks before harvest with dichlofluanid 0,075% and 0,2% and benomyl 0,02% was tested. Benomyl and dichlofluanid 0,075% led to a significant reduction of rotted fruits compared with the untreated plot. The higher dose of dichlofluanid improved this control.

Key words: Apple, ascospore-discharge, electronic monitoring, fungicide treatments, orchard diseases, spray timing, storage diseases, *Venturia inaequalis*

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Apple scab, powdery mildew, canker (*Nectria galligena*), *Phytophthora* root- and collar rot (*Phytophthora* spp., mainly *Phytophthora cactorum*), *Mycosphaerella*-fruit spot on cv. 'Idared' (*Mycosphaerella* sp.) and various storage rots are the most important diseases in Austrian apple orchards.

Depending on weather conditions, and storage techniques, various fungal diseases cause high losses of stored fruits. In Austria, post-harvest pesticide treatments are not permitted so that orchard practice has to keep the infection pressure low. In 1992 benomyl was the only permitted pesticide against storage diseases. As it is not allowed in the IFP programme because of its negative effect on *Lumbricus terrestris* and the problem of a likely resistance, an alternative had to be found. Dichlofluanid is known to have a good activity against pathogens, especially *Gloeosporium* sp. In other countries the recommended dosages range from 0,75 to 2 kg/ha: a trial was needed to find the preferable dosage for Austrian conditions. To be able to answer the question of the correct timing for fungicide

sprays (depending on the specific pathogen) as well as the choice of the product, a study was carried out to determine the fungi responsible for the losses.

Associated with the high percentage of farmers participating in the Austrian IFP programme the number of electronic warning systems used in orchards for scab management increased. Apple scab may be epidemic in some years and so the growers tend to apply fungicides more often than necessary. The aim of an investigation was to show that usually only a few infection periods are responsible for the infection caused by overwintering scab ascospores.

## MATERIAL AND METHODS

### **Apple scab study**

In autumn 1991, severe scab-infected apple leaves were collected and overwintered outdoors. They were placed under a net at a copper-treated spot on the orchard ground. In Vienna and Graz a spore trap was run from March 10th 1992 and was operated after each rainfall.

An orchard in Spitz (cv. Gloster) was chosen for the experiment because no scab infections were noticed in 1991. There was minimum risk of primary infections induced by conidia formed on the twigs. The observations started at the same time as in Vienna and Graz. The discharged ascospores were counted after each precipitation until a significant rise in the number of ascospores was recorded. From this time the spores were only checked if a rainfall was followed by an infection period, as indicated by electronic scab monitoring computers. Fungicides were applied curatively, after infection periods.

### **Storage diseases study**

Apples (cv. Golden Delicious) from two orchards with different planting systems were used for the investigations. While orchard 1 is characterized by 15-year-old, tall trees, the apples from the second site (orchard 2) were picked from spindles on which hail caused severe damage early in the season.

The range of tested fungicides included dichlofluanid (0,2%, 0,075%) and benomyl (0,02%) which were applied 3 weeks before harvest. The apples were stored in a cold store developing fruit rots. The infected apples were removed every two weeks and the pathogens identified by a microscopic examination.

## RESULTS AND DISCUSSION

### **Storage diseases**

It was unexpected that the number of rotten fruits of the untreated plot in orchard 2 (with hail damage) was much lower (5%) than the equivalent plot in orchard 1 (36,5%) (Fig. 1). The conditions for the development of the observed fungi seem to be better in big trees.

Because of hail the moisture content of the fruits was reduced and they became wrinkled. This condition did not raise the susceptibility to storage diseases.

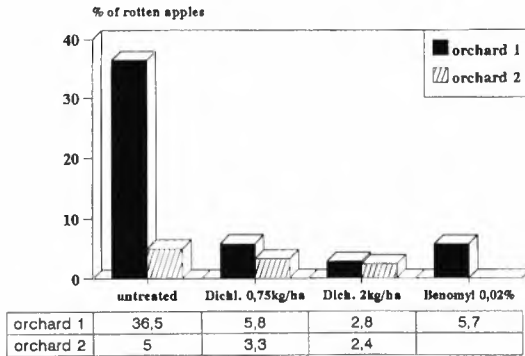


Fig. 1. The total losses of apples caused by pathogens during storage

*Penicillium expansum* was the fungus responsible for 24% of the loss and therefore economically the most important cause of fruit rots (Fig. 2): the main reason for blue mould rot is a lack of hygiene during harvest time.

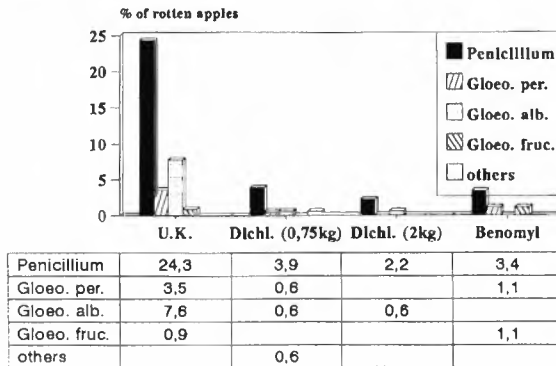


Fig. 2. The effect of different concentrations of dichlofluamid on pathogens of stored apples in orchard 1

In contrast, *Gloeosporium* spp. is present in the orchard during the whole season and may infect the ripening fruits at an early stage. Obviously the incidence of the pathogen on the stored apples cannot be evaluated by a single preharvest treatment.

*Monilinia fructigena* causing brown rot appeared only in orchard 2 until January and could not be dedected later in the storage period (Fig. 3).

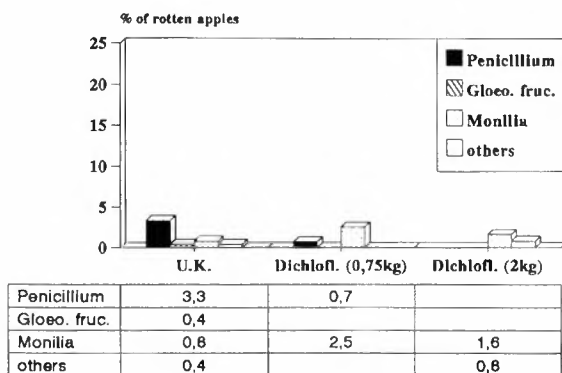


Fig. 3. The effect of different concentrations of dichlofluanid on pathogens of stored apples in orchard 2

Mucor-rot, Phytophthora-fruit rot and grey mould only occurred rarely.

Especially in orchard 1 the number of apples infected by *Penicillium* in the plot with the lower dosage of dichlofluanid was reduced significantly. When the concentration was raised to 0,2%, the results could be slightly improved. In orchard 2 the infection pressure was much lower and therefore the difference between the plots was not obvious. In addition to that it was surprising that the effect of Benomyl 0,02% was not better than that of the lower dosage of dichlofluanid 0,075% (Figs. 2, 3).

### Apple scab

In Vienna the first ascospores released from the overwintered perithecia were trapped on 15th April 1992. Nine days later discharge started in Graz and on 27th April in Spitz (Fig. 4-6). It was a surprising fact that the ascospores developed so much earlier in Vienna compared with Spitz, because both places have similar climate conditions.

The first chemical application using a protective fungicide was sprayed on 26th April because rain was forecast. All other treatments (SBI fungicides) were applied curatively according to scab infection periods.

Dates of treatments: 26.4.92, 1.5.92, 12.5.92, 24.5.92, 4.6.92, 10.6.92

Assessment of leaf scab on May 5th showed that no scab symptoms were present. In June 1st, scab symptoms were recorded in the untreated plot; the fungicide-treated part of the orchard was still disease-free and scab was prevented for the rest of the season.

In 1992, monitoring of ascospore release could not reduce the number of fungicide applications because every rainfall was followed by an infection period. But it was demonstrated that long wet periods do not necessarily intensify the infection period (as

indicated by electronic monitoring systems) because the number of mature ascospores will decrease. Where the first primary infection (by ascospores) is relatively late, management systems that do not consider the availability of inoculum would already have recommended 1-2 unnecessary sprays.

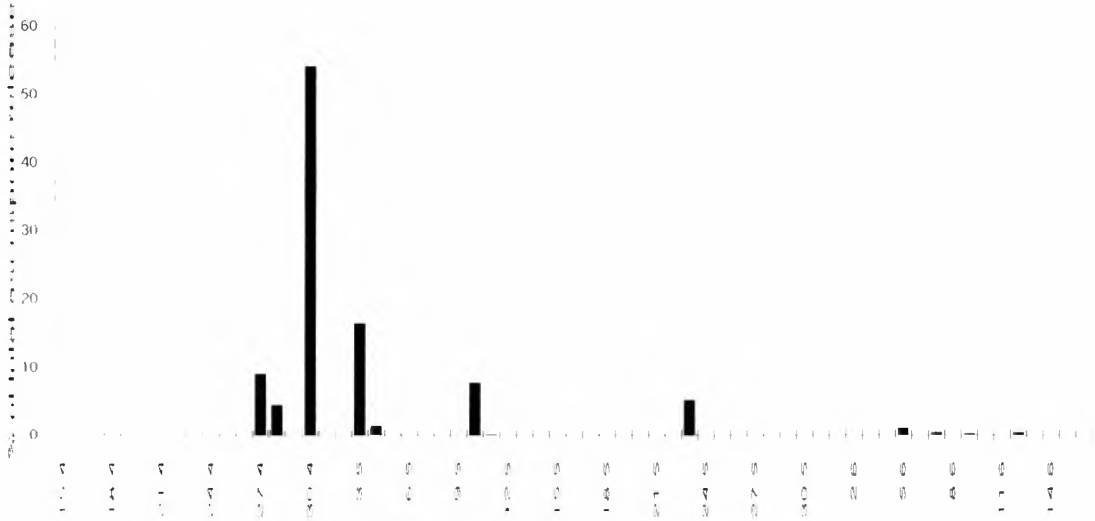


Fig. 4. *Venturia anaequalis*; ascospore release in Graz 1992

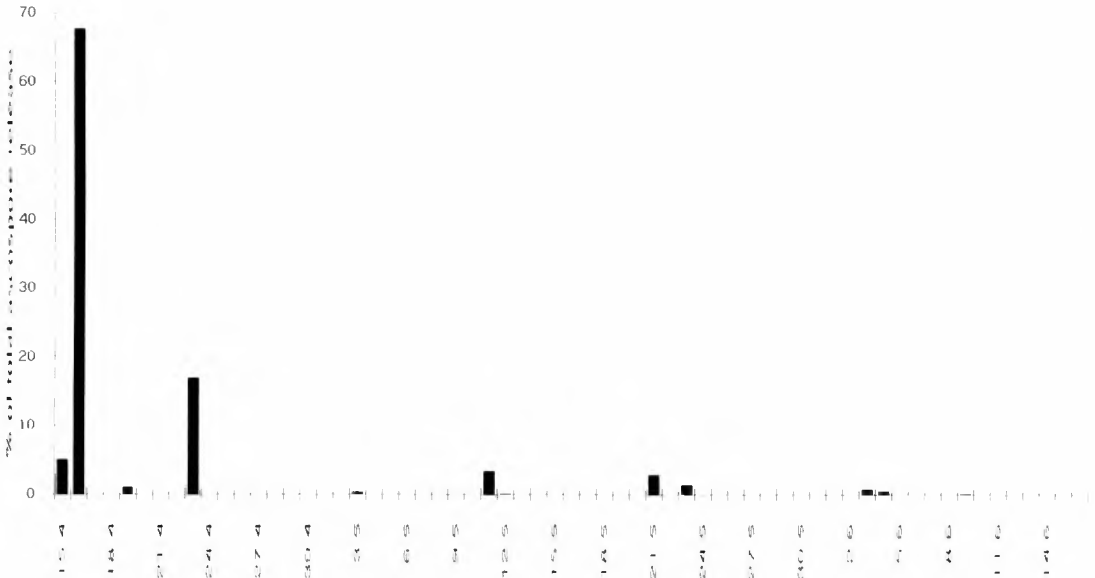


Fig. 5. *Venturia inaequalis*; ascospore release in Vienna 1992

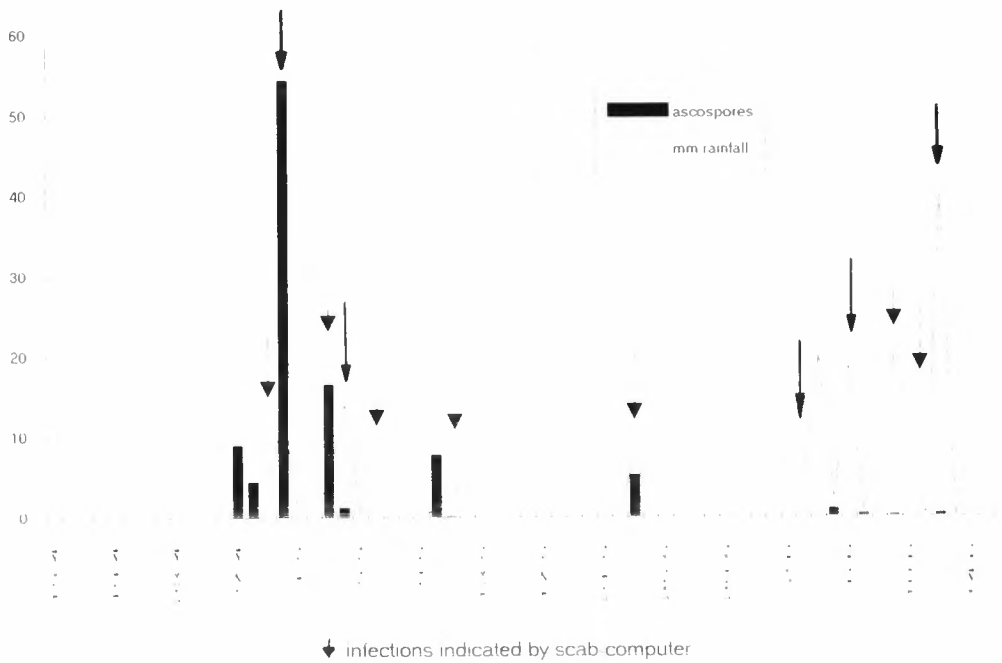


Fig. 6. *Venturia inaequalis*; ascospore release in Spitz 1992

## REFERENCES

- Anagnostakis, S. & D.E. Aylor 1991. Efficiency of Ascospores of *Venturia inaequalis* in Producing Scab Lesions. *Plant Disease* 75 (9): 918-728.
- Bosshard, W.S. & H. Schüepp 1985. Erfahrungen mit Sterolsynthese-hemmenden Fungiziden zur Schorfbekämpfung. *Schweiz. Zeitschrift für Obst und Weinbau* 121: 166-173.
- Gadoury, D.M. & W.E. MacHardy 1986. Forecasting Ascospore Dose of *Venturia inaequalis* in Commercial Orchards. *Phytopathology* 76, No.1: 112-118.
- Hirst, J.M. & O.J. Stedman 1962. The epidemiology of apple scab-Observations of the liberation of ascospores. *Ann. Appl. Biol.* 50: 525-550.
- Palm, G. 1988. Neue Erkenntnisse zur Bekämpfung des Apfelschorfes. *Besseres Obst* 7: 229-234.

# Progress with the apple scab warning system in the South Tyrol

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Bradlwarter, M. 1994. Progress with the apple scab warning system in the South Tyrol. Norwegian Journal of Agricultural Sciences, Supplement No. 17: 413. ISSN 0802-1600.

In 1962, the South Tyrolean Advisory service for Apple- and Wine-Growing started a telephonic scab warning service. Until 1990 there were 30 leaf wetness recorders altogether and 1 or 2 thermohygrographs per district for use with this project. Until 1985 ascospore discharge was recorded with a home-made vacuum-trap at Neumarkt.

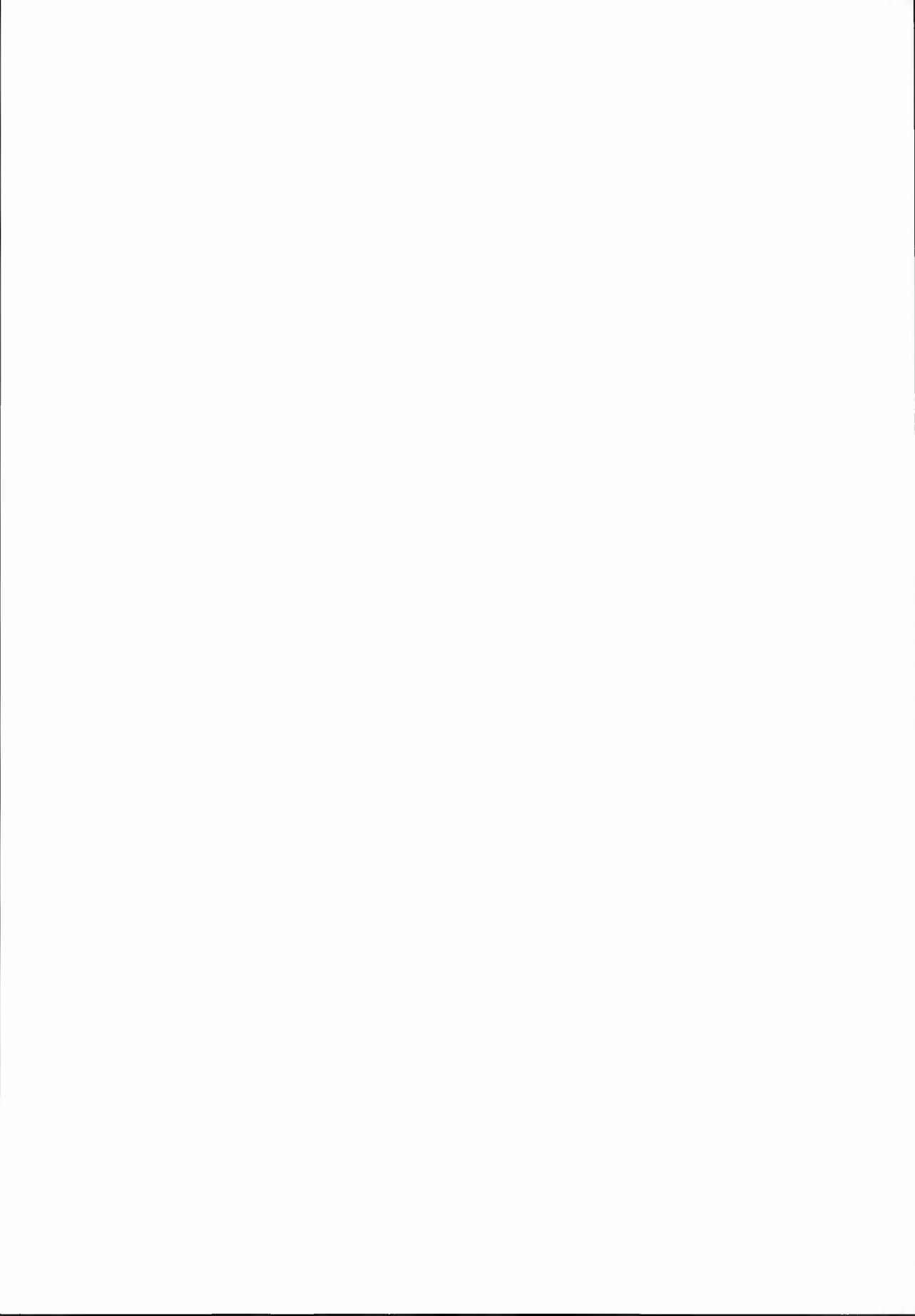
Today there is a network of 65 automatic weather stations altogether, distributed over 6 districts, each fitted with a temperature sensor, a highly sensitive precipitation meter and a wind velocity recorder. Some weather stations are also equipped with air humidity sensors. Three stations are testing especially developed prototypes of a leaf wetness sensor. The whole project has been developed in co-operation with a local engineering firm (Barbieri Electionia, Brixen), according to the special requirements.

The recorded meteorological data are transmitted by radio to the head-office of each district. A computer records, for each station separately, the beginning of rainfall, adds up the amount of precipitation (mm) as well as the duration of leaf wetness (hours) and calculates the mean temperature from the beginning of rainfall. Finally, the programme supplies values of the current temperature, air humidity and wind velocity and direction.

At present there is little confidence in the various leaf wetness sensors, therefore the adviser responsible for the scab warning service has to ascertain visually if there are still some drops of water on the vegetation in the different locations of a district.

The seventh district, Unterland, has opted for the Berghof System with 6 automatic weather stations. Although each of these stations is equipped with a leaf wetness sensor the advisers in charge of the warning service are instructed to visually ascertain leaf wetness.

Since 1986, monitored in February and March has been the level of ascospore maturity, by checking leaf samples from various locations and ascospore discharge has been recorded by means of two ascospore traps (type VPPS 2000). The fact that ascospore discharge in the valley finishes around 15th May, which corresponds to the 600 degree-days according to Gadoury and MacHardy (1984), has confirmed the old saying that "apple scab is no longer dangerous at haymaking time".





# Four years monitoring of primary scab infection on apple and ascospore release of *Venturia inaequalis* in Trentino (Italy)

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Comai, M., R. de Clauser & G. Flaim 1994. Four years monitoring of primary scab infection on apple and ascospore release of *Venturia inaequalis* in Trentino (Italy). Norwegian Journal of Agricultural Sciences. Supplement No. 17: 415-424. ISSN 0802-1600.

The authors report four years of observations (1989-1992) of *Venturia inaequalis* primary infections on 4-year-old potted apple trees and of ascospore release. Ascospore discharge was monitored by a ground-positioned aspirating spore trap. The aim of the study was to evaluate the sensitivity of the spore trap. During the four years the spore trap gave variable results. It was not always capable of indicating the start of ascospore release, while the end was always protracted in time, even though primary infections were no longer detected on the trap plants.

**Key words:** Ascospores, infection periods, monitoring, orchard diseases, spore trap, *Venturia inaequalis*.

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The fungus *Venturia inaequalis*, causal agent of apple scab, is the most serious pathogen in Trentino's fruit orchards. Control sprays are applied mostly during March, April and May which coincide with the maximum release of ascospores. After this period and if no inoculum in the orchard a farmer can consider the problem resolved. Notwithstanding this, the number of treatments needed to control the pathogen is considerable.

Presently, control is based solely on meteorological data and on the phenological stage of the apple tree; ascospore release is not considered. Spraying begins with the first rainy period in March giving favourable climatic conditions according to Mills (1944) and with the green tip phenological stage. It is not known if ascospores are ready to be released from the perithecia at this time; 2-3 sprays may be applied before ascospore release.

For this reason, the performance of a ground-positioned aspirating spore trap was evaluated. The choice of the spore trap was based on observations of colleagues working in other fruit areas and on the ease of operation.

The reliability of the trap was confirmed by comparison with primary infections on potted apple trees placed in the same orchard as the spore trap.

## MATERIALS AND METHODS

Primary infection was determined on four-years-old potted "Golden Delicious" apple trees raised in a greenhouse, protected from rain and not subject to pesticide sprays (Fig.1). Groups of four plants were exposed to rainy periods sufficient to determine a light infection according to Mills. The plants were then put back in the greenhouse and incubated until scab lesions developed. Average percentage of floral clusters and shoots with lesions was recorded.



Fig.1. Potted "Golden Delicious" plants used in the study

The release of *Venturia inaequalis* ascospores was monitored by a ground-based spore trap manufactured by the Marchi Company, Bologna (Fig.2). The trap consists of an aspirating pump, a rotating cylinder turning one rotation per week, and a side-walled container with scabbed leaves. Ascospores released from perithecia are sucked on to vaseline-covered glass slides positioned on the rotating cylinder. After every rainy period the slides were removed and the spores counted with the aid of a microscope (200x). The infected leaves were gathered in the autumn from "Golden Delicious" apple trees not treated with fungicides. During the winter the leaves were kept on the ground between two nets.



Fig. 2. Ascospore trap used in the study

Leaf wetness periods and average temperature were recorded by a Bazier thermohygrograph.

## RESULTS AND DISCUSSION

For each year considered, the tables (Tab. 1 - 1989, Tab. 2 - 1990, Tab. 3 - 1991 and Tab. 4 - 1992) show each rainy period in March, April and May, the phenological stage of the apple trees wetness period, average temperature, number of spores released and percentage of floral clusters and shoots with lesions. The figures (Fig. 3-6) also show the number of ascospores released and the lesions present on the test plants in these years.

The data show that for the four years considered the spore trap did not always indicate the start of ascospore discharge. In 1989 and in 1991, spore release and infection were obtained on the same date, while in 1990 and 1992 scab lesions occurred about 7 days before spore release was noted. Except for 1991, with 751 spores counted at the first release, in general the first discharge involved a limited number of ascospores. This is probably due to a very dry winter, especially in March. In fact, March with little rain coincides with a low number of spores released (10-13).

After spore release started there was usually a certain association between favourable climatic conditions, spore discharge and infection. Some exceptions were noted: 17 April 1989 - with favourable weather conditions (moderate - severe according to Mills) and in the presence of inoculum no infection occurred; 4 April 1991 - in the presence of inoculum but unfavourable weather conditions according to Mills infection occurred. In other cases (18-23 April 1991) unfavourable weather did not result in infection even in the presence of ascospore number.

Ascospore release continued, even after the last primary scab lesions were noted on plants. In 1989 and in 1990, scab attacks ended in April while spore release continued into May; in 1991, spore release seemed to terminate before scab lesion development was finished. In 1992, the end of spore release coincided with the end of primary lesion development. Sometimes spore release continued into June but the number of spores captured was low (data not shown). The number of leaves present in the spore trap was very important with regards to the length of time spores were released. In fact, in the first two years with more than a hundred leaves pressed in the spore trap, spore release continued until the end of May (1990) or even until the first week of June (1989). In the successive years, about fifty leaves were placed in the trap and the spore release period was much shorter.

Tab.1: Infection (%) and number of ascospores released during the rainy periods in 1989

Date	Phen. stage 1)	Wet time	Wet period(h) 2)	Temperature °C 3)	Rain mm	Mills inf. 4)	Spore number	% Infection	
								fl.clust. 5)	shoot
31/3	G3-D			13.8					
1/4				12.8					
2/4		0-11	11	14.8(11.0)	0.2		0	0	0
3/4	E	18-24		13.4	11				
4/4		0-24		8.8	41				
5/4	E-E2	0-24		9.8	21	S			
6/4		0-11	65	9.5(9.2)			13	7	0
7/4				11.0					
8/4	E2	2-24		11.7	10				
9/4		0-11	23	13.6(10.9)	9	M-S	13	9	0
10/4				13.7					
11/4		14-24		13.8	2				
12/4		0-24		11.5	5				
13/4		0-24		10.6	35				
14/4	E2-F	0-24		11.3	1				
15/4		0-6	88	12.7(11.9)		S	278	9	2
16/4	F	21-24		12.5	8				
17/4		0-15	18	10.0(10.0)	16	M-S	42	0	0
18/4				12.0					
19/4	F2	0-24		11.1	2				
20/4		0-12	36	12.1(10.7)	0.2	S	478	0	1
21/4		2-24		10.4	18				
22/4	F2-G	0-24		9.1	12				
23/4		0-10	56	11.0(9.1)		S	555	0	2
24/4				11.3					
25/4		14-24		8.5	5				
26/4		0-24		8.4	32				
27/4	G	0-24		10.8	19				
28/4		0-24	82	10.6(9.8)	7	S	691	0	10
11/5				16.5					
12/5		23-24		17.9	9				
13/5		0-13	14	17.2(15.4)	27	M-S	670	0	0
14/5		3-13	10	16.7(16.0)	8	M-L	529	0	0
15/5				15.4					
16/5		19-24		14.8	0.4				
17/5		0-9	14	15.8(12.2)		M-L	71	0	0
18/5				16.9	0.4				
19/5		22-24		17.9	4				
20/5		0-24		18.4	0.6				
21/5		0-8	34	20.3(17.1)		S	1523	0	0
22/5				21.2					
23/5		0-10	10	18.0(16.8)	16	M-L	0	0	0
24/5				18.4					
25/5				19.1					
26/5		20-24		18.4					
27/5		0-9	13	18.9(14.6)		M-L		0	0

1) Phenological stage according to Fleckinger: C3 = green tip, D = closed cluster,

E = tight cluster, E2 = early pink, F2 = full bloom, G = late bloom

2) Sum of wet hours

3) Average temperature (average temperature while raining)

4) Infection according to Mills: S = severe, M = moderate, L = light, \* = no infection according to Mills

5) % infection on floral cluster leaves

Tab.2: Infection (%) and number of ascospores released during the rainy periods in 1990

Date	Phen. stage 1)	Wet time	Wet period(h) 2)	Temperature °C 3)	Rain mm	Mills inf. 4)	Spore number	% Infection	
								fl.clust. 5)	shoot
27/3	G3-D	14-24		6.6	4				
28/3		0-13/21-24	23	7.0(6.6)	0.6/0.4		0	2	0
29/3		0-13	16	10.2(6.8)	0.2	L	0	0	0
30/3	D-E			12.1					
31/3				9.9					
1/4				11.4					
2/4				11.7					
3/4		6-24		11.7	3				
4/4		0-17/22-24		9.6	4.5				
5/4		0-13	55	11.6(10.2)	0.5	S	11	5	0
6/4	F	12-24		9.4					
7/4		0-24		9.3					
8/4		0-10/17-24	46	11.2(9.4)		S	6	22	0
9/4		0-16	23	8.5(9.3)		M-S	9	5	0
10/4				11.6					
11/4				9.4					
12/4	F2			10.4					
13/4				8.3					
14/4		20-24		8.1	2				
15/4		0-16/20-24		8.8	1.2				0.02
16/4		0-4	32	11.6(8.5)		S	15	2	
17/4	F2-G			10.3					
18/4		3-24		8.4	8				
19/4		0-1/20-24	22	9.5(8.4)	70.8	S	525	0	0.03
20/4		0-12	16	8.2(6.0)	0.2		5	0	0
21/4		1-13	12	9.3(8.4)	0.4				
22/4		18-24		9.6	4				
23/4	G	0-24		7.0	18				
24/4		0-10	40	9.9(7.4)		S	225	6	0.2
25/4				11.7					
16/5				20.2					
17/5		13-24		19.3	4				
18/5		0-9/13-24		16.3	2.5				
19/5		0-9	44	17.4(17)		S	85	0	0
20/5		20-24		16.8					
21/5		0-10	14	20.0(16)	1	M-S	11	0	0
22/5				19.6					
23/5				20.2					
24/5		2-24		17.2	4				
25/5		0-11	33	10.2(17.4)	1	S	41	0	0

1) Phenological stage according to Fleckinger: C3 = green tip, D = closed cluster, E = tight cluster, E2 = early pink, F2 = full bloom, G = late bloom

2) Sum of wet hours

3) Average temperature (average temperature while raining)

4) Infection according to Mills: S = severe, M = moderate, L = light, \* = no infection according to Mills

5) % infection on floral cluster leaves

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Tab.3: Infection (%) and number of ascospores released during the rainy periods in 1991

Date	Phen. stage 1)	Wet time	Wet period(h) 2)	Temperature °C 3)	Rain mm	Mills inf. 4)	Spore number	% Infection		
								fl.clust. 5)	shoot	
22/3	C3-D	6-24		14.4	23					
23/3		4-9/13-24		13.6	16					
24/3		0-9	51	15.1(13.6)		S	751	1.6	0	
25/3		15-24		14.1	8					
26/3		0-15	24	13.3(13.4)	5	S	802	0.7	0	
27/3				14.4						
2/4	D-E			12.4						
3/4		21-24		12.8	0.4					
4/4	E	0-7/17-24	10	12.0	16	*	70	3.9	0	
5/4		0-24		9.9	7					
6/4		0-7	38	9.0(9.6)	0.4	S	966	76	4.4	
7/4				13.4						
16/4	F2			12.2						
17/4	F2-G	10-24	14	4.5(4.3)	10.2					
18/4		5-7	2	5.8	0.2	*	109	0	0	
19/4				6.3						
20/4				5.7						
21/4		17-24		6.3						
22/4	G	0-14	21	6.8(6.4)	0.6	*	722	2.3	4.4	
23/4		7-11/17-23	4/6	6.7	0.2/1.2	*	304	0		
24/4				8.2						
25/4				7.8						
26/4				8.9						
27/4			20-24		11.5	1				
28/4			0-11	15	11.1(10.2)		L	298	0	13
29/4			15-16	1	11.5	0.6		>1000		
30/4					11.4					
1/5					11.0					
2/5		1-24		10.6	9					
3/5		0-23	46	10.0(10.3)	18					
4/5		6-9/17-24	2	9.2	5	S	716	0	96	
5/5		0-11	18	9.9(8.4)	5/9	L	30	0	9	
6/5		0-13	16	9.6(8.6)	4	L	34	0	8	
7/5				10.2						
8/5				10.8	0.2					
9/5		21-24		11.9	1					
10/5		0-24		9.9	16					
11/5		0-15/21-24	42	13.3(10.1)	9	S	65	0	48	
12/5		0-10	13	14.5(11.5)	0.4/0.2	L	0	0	8	
13/5				13.5						

- 1) Phenological stage according to Fleckinger: C3 = green tip, D = closed cluster, E = tight cluster, E2 = early pink, F2 = full bloom, G = late bloom
- 2) Sum of wet hours
- 3) Average temperature (average temperature while raining)
- 4) Infection according to Mills: S = severe, M = moderate, L = light, \* = no infection according to Mills
- 5) % infection on floral cluster leaves

Tab.4: Infection (%) and number of ascospores released during the rainy periods in 1992

Date	Phen. stage 1)	Wet time	Wet period(h) 2)	Temperature °C 3)	Rain mm	Mills inf. 4)	Spore number	%Infection 5)		
								fl.clust.	shoot	
23/3	D	20-24		11.7	10					
24/3		0-16/20-24		7.5	12					
25/3		0-12	40	7.9(7.5)		S	0	0	0	
26/3				7.9						
29/3				8.3						
30/3		9-24		7.0						
31/3		0-24		6.5	43					
1/4		0-11/12-13/15-24		7.4	9					
2/4			0-12	75	8.4	2	S	0	0	0
3/4			14-24		7.3	15				
4/4	0-24			9.5	12					
5/5	0-24			10.3	20					
6/4	D-E		0-24		10.4					
7/4			0-13	97	12.2(10.0)		S	0	2.04	0
8/4					13.5					
9/4	E2		0-12	12	15.5(11.9)	0.8	L-M	0	0	0
10/4					12.0					
11/4				11.7						
12/4				12.2						
13/4		12-13/16-24		10.8	1					
14/4		0-8	20	13.0(10.7)		L-M	12	0	0	
15/4		12-24		9.1	25					
16/4		0-18	30	7.7(8.0)	1.2	M-S	169	15.5	3.23	
17/4				11.4						
22/4	F2			13.7						
23/4		16-24		14.7	0.6					
24/4		0-11	19	16.2(14.0)		M-S	113	0	0.51	
25/4				18.7						
26/4				19.9						
27/4	G	16-17/20-24		18.2	0.2					
28/4		0-10/11-16/20-24		17.0	9					
29/4			0-18	50	12.3(15.7)	17	S	652	0	7.43
30/4			19-20/23-24		14.1	0.4				
1/5			0-7	12	15.8(13.9)		L-M	4	0	0
2/5					15.5					
3/5					19.9					
4/5			16-24		17.1(10.4)	0.2				
5/5			0-9	17	19.0(16.1)	5	S	117	0	6.35
6/5					19.9					
19/5				18.0						
20/5		7-12/14-15		13.8	6,5					
21/5		7-8		15.3	0.2					
22/5		19-24		16.3	1,8					
23/5		0-13	18	17.0(16.3)	9	M-S	860	0	3.74	
24/5				18.8						
25/5				19.0						
26/5		22-24		19.2	3,6					
27/5		0-10/18-24	12	19.0(16.8)	0.2/1.4	M	0	0	0	
28/5		0-8/19-24	14	20.0(17.6)	0.4/1.8	M-S	0	0	0	
29/5		0-10/20-24	15	20.3(17.8)	8	M-S	0	0	0	
30/5		0-9/18-24	13	21.6(19.6)	2/7	M-S	0	0	0	
31/5		0-13/15-24		20.3	6					
1/6		0-11	41	21.0(20.5)	16	S	6	0	0	

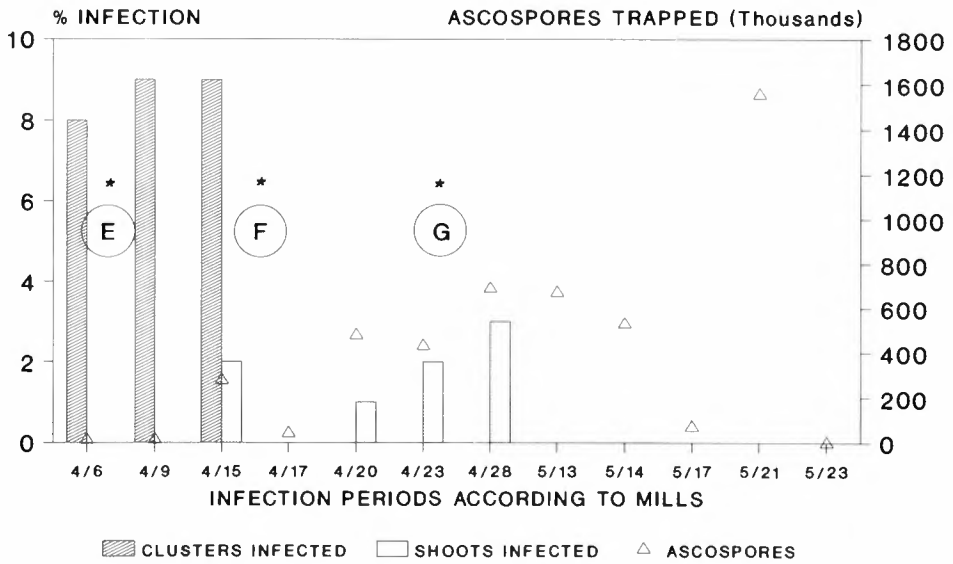
1) Phenological stage according to Fleckinger: C3 = green tip, D = closed cluster, E = tight cluster, E2 = early pink, F2 = full bloom, G = late bloom

2) Sum of wet hours

3) Average temperature (average temperature while raining)

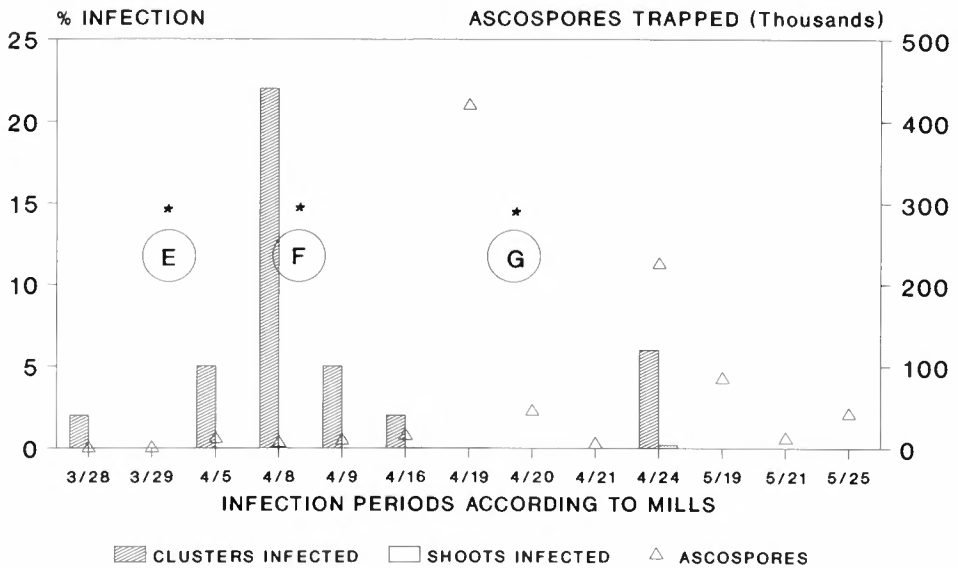
4) Infection according to Mills: S = severe, M = moderate, L = light, \* = no infection according to Mills

5) % infection on floral cluster leaves



(\*) Phenological stage according to Fleckinger

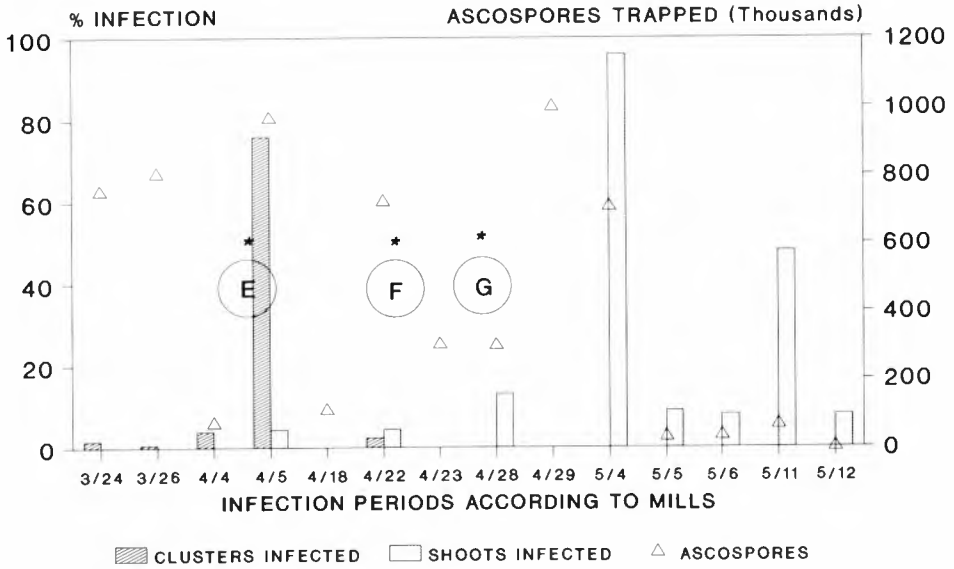
Fig. 3. Scab infection on floral clusters and shoots, ascospore discharges and dates of Mills Periods in 1989



(\*) Phenological stage according to Fleckinger

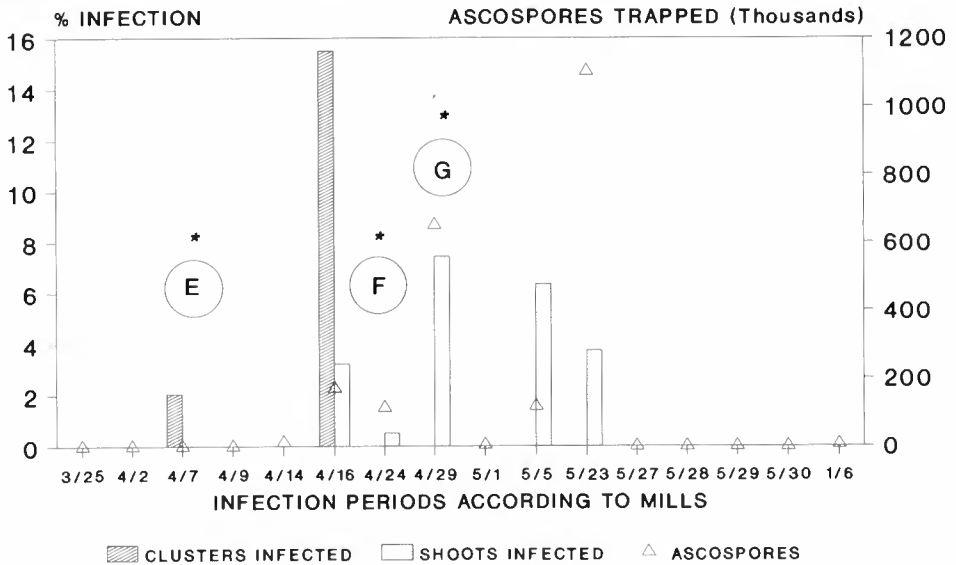
Fig. 4. Scab infection on floral clusters and shoots, ascospore discharges and dates of Mills Periods in 1990





(\*) Phenological stage according to Fleckinger

Fig. 5. Scab infection on floral clusters and shoots, ascospore discharges and dates of Mills Periods in 1991



(\*) Phenological stage according to Fleckinger

Fig. 6. Scab infection on floral clusters and shoots, ascospore discharges and dates of Mills Periods in 1992

## SUMMARY

With respect to the actual conditions present in the orchard the spore trap represented an artificial situation for the determination of *Venturia inaequalis* ascospore release. From the four years of observations it can be concluded that:

- the spore trap was not sufficiently sensitive to indicate the start of spore release from perithecia. In two years out of four the dates did not coincide;
- after the first effusion, a release of *Venturia inaequalis* ascospores was associated with every rainy period;
- spore release seemed to last the whole month of May when weather conditions were favorable, while in general the development of primary lesions had ended;
- there was no obvious relationship between the number of ascospores trapped and the severity of infection.

These results are not sufficient to draw final conclusions on the type of spore trap needed but allow some suggests to be made. The fact that the trap did not always detect the first spores released suggests that volumetric sampling was not sufficient for the area involved.

It may be enough to increase the volume of air passing over the scabbed leaves: considering the advantages of this spore trap with respect to data gathering, it would be better to modify the pump rather than choose another type of spore trap.

## ACKNOWLEDGEMENTS

The authors thank F. Fellin of the local Agricultural Assistance Organization for technical help with the spore trap.

## REFERENCES

Mills, W.D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab. Cornell Extension Bulletin. 630. 4 pp.

# Monitored and forecast weather for use on farm

G. PESSL

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Pessl, G. 1994. Monitored and forecast weather for use on farm. Norwegian Journal of Agricultural Sciences. Supplement No. 17: 425-427. ISSN 0802-1600.

The Metos<sup>®</sup> and the more recent MetosDat<sup>®</sup> have been used for many years for on-farm predictions of apple scab (*Venturia inaequalis*), Tortrix moth (*Adoxophyes orana*) and other pests and diseases. At the beginning very simple and small models like Mills' Table were used. Recently, many sophisticated models have been developed. Programs like Ventem<sup>™</sup> (from HRI East Malling - UK) made it possible predict apple scab far more accurately than the models used before. New microclimate weather forecast systems by means of satellites and consequent incorporation of that information will increase the efficiency of the whole system.

Key words: Computer program, cost savings, datalogger, diseases, forecast, Metos<sup>®</sup>, pests, monitoring, spraying, warning.

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For many years warning of apple scab (*Venturia inaequalis*) has been dependent on a system called Mills' Table, developed by Dr. Mills in the forties. In the beginning of the fifties a scab warning system was provided by plant protection institutes and consultants with data collected by thermohygrographs and mechanical leaf wetness recorders. The weather data were analysed by plant protection specialists and processed manually according to "Mills' Table". Warnings were delivered as written bulletins to growers after several days delay. The entire procedure was very time consuming and liable to error. Also the information was based on macroclimate data and was consequently, in many cases, not very accurate.

## PRESENCE AND FUTURE

In recent years electronic scab warning instruments have found wide acceptance. The Metos<sup>®</sup> electronic warning instrument processes weather data automatically every twelve minutes, will record air temperature, relative humidity, precipitation, duration of leaf wetness, daylight and will calculate the scab infection risk from this data. Additionally, much work has been undertaken to improve the accuracy of warnings, concentrating on sensor efficiency and program modelling. These instruments operate with rechargeable batteries (no mains required) and are dedicated for on-farm warning systems. The advantages for the grower are many, because he need not rely on weather data recorded

many miles away from his orchard but on local data from his his own orchard which will match exactly with his own on-farm microclimate. Better scab control is achieved and the number of chemical sprays considerable cut due to new findings and research on the biology of the fungus together with better hardware and software. After 8 years experience the Pessl Company has been able to develop a new computer program, which enables the user easily to employ weather forecast data in the measurement of the microclimate. New microclimate weather forecast systems using satellites have been developed which provide a high precision forecast for the next 3-4 days. The forecast data can be fed easily into the Metos program and consequently be combined with the locally-recorded microclimate data.

The advantage for the grower is in having accurate on-farm microclimate weather data combined with reliable forecasts; will consequently improve plant protection considerably. In addition, historic and forecast microclimate weather could be used for many other applications, such as pest control and irrigation management.

## HOW IT WORKS

First, the latest weather data must be downloaded from the Metos<sup>R</sup> instrument to the growers PC, using Met8 downloading program. This can be done through online connection, by RS422 (long line), and by telephone modem or via radio.

The user must use the disease and pest warning software Met9 or Ventem<sup>TM</sup>. Running the program the grower will automatically calculate disease risks. He can choose any period from one day to 365 days out of his Met8 database to make disease predictions displayed as tables and graphics (only if using DOS 5.0 or higher).

The program uses a "pull-down" type of menu, the international standard used in major software programs.

The routine "running the program" is executed in various steps:

### *Running one or two weeks*

The user is given an overview of the weather conditions and the spray decisions for the past two weeks.

### *Running and viewing the last 1-3 days*

To see if spraying is necessary the grower will take a closer look at the conditions of the last 1-3 days.

### *Looking into the future*

The grower will enter a future date and will run the program to obtain a prediction of future.

### *Taking decisions*

The grower will be advised the best choice of fungicide for the particular conditions and the approximate efficiency of the same product.

*Monitoring the decision*

Checking forecasted weather data with real historic data the grower can re-run the program in the next days to confirm the decision or eventually correct it.

When the grower is used to operating the program it will not take more than 5 minutes a day to get the best control decision.

CONCLUSIONS

These instruments and programs are management tools and give timely additional information to the grower and advisor. Such tools do not eliminate the knowledge and experience of the grower or his advisor, but assist both by providing more information to make better decisions.



# An agrometeorological network for warnings against scab in Belgium

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Boshuizen, A. & C. Verheyden 1994. An agrometeorological network for warnings against scab in Belgium. *Norwegian Journal of Agricultural Sciences. Supplement No. 17*: 429-441. ISSN 0802-1600.

The actual scab warning system is based upon a mechanic apparatus which is read by the fruit grower-observer. This information is transferred by telephone to the Research Station of Gorsem (Belgium). In order to modernize and automatize this system, a Mety-network was purchased. The functioning of this agrometeorological network is discussed. The mety stations are installed all over the Belgian fruit areas and the central computer in Gorsem collects daily all information from the regional stations and processes them into a warning message against scab. The way in which the warning message can be returned from Gorsem to the regional reporting posts is also explained.

Key words: Agrometeorological network, scab warnings.

*Adri Boshuizen, Bodata, Burgemeester Jaslaan 2, NL-3319 AC Dordrecht, Netherlands.*

The actual scab warning system is based upon a mechanic apparatus, namely Bazier, which is read by the fruit grower-observer. The latter passes the information by telephone to the Research Station of Gorsem. In order to modernize and automatize this system, a Mety-network was purchased. This article is about the functioning of the automatic scab warning system, as it was installed by Bodata in 1991 and 1992 in Belgium. The aim is to give a short explanation about the functioning of the system and not to go further into the numerous technical details.

Chapter two starts with the discussion of the functioning of the mety stations installed all over the Belgian fruit areas. Chapter three goes further into the functioning of the central computer in Gorsem, which collects daily all information from the regional stations and processes them into a warning message against scab. This chapter also discusses in which way the warning message can be returned from Gorsem to the regional reporting posts.

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<sup>1</sup> Research subsidied by I.W.O.N.L. (Institute for the encouragement of scientific research in Industry and Agriculture

### **The Mety-stations in the Belgian fruit areas**

#### *Survey of the Mety stations in Belgium*

In the context of a cooperation project of the Ministry of Agriculture, the Phytopharmaceutical fund, the Research Station of Gorssem and Gom Limburg, 20 Mety stations were installed in Belgium. They can all be read from the Research Station of Gorssem. Figure 1 represents the location of these stations.



Figure 1. Survey of the different Mety stations in Belgium

The Mety stations were installed in such a way that they are representative for the climatological circumstances in the Belgian fruit areas. The experiences with this registration system in the next years will show if a few more posts are necessary.

#### *The Mety stations at the registration posts*

In all registration posts a Mety station (see figure 2) is installed. The Mety stations are equipped with sensors for the registration of temperature, rain fall and leaf wetness. The Mety stations in the experimental stations of Velm, Rillaar, Destelbergen, Rumbeke-Beitem and Gorssem also have a sensor for the registration of the relative humidity.



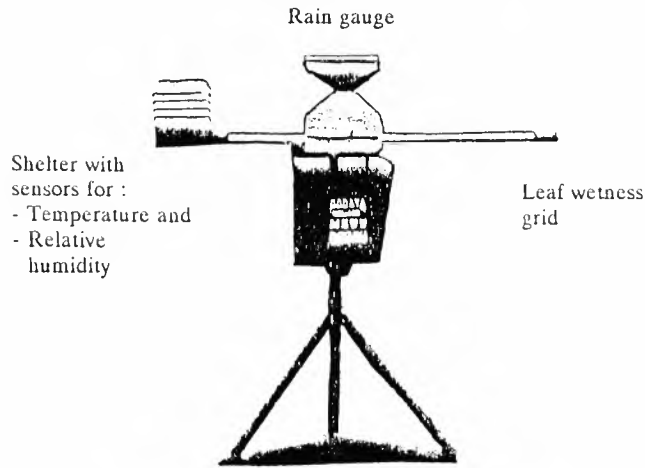


Figure 2. Scheme of a Mety station

In all registration posts, except for Halmaal, there is not only a Mety station installed but also a computer. The experimental stations and the fruit growers did not buy these computers within the scope of the project. Bodata has installed the Mety program on these computers, which are of different brands and dealers. The Mety station in the orchard is connected through a cable with the computer inside. The length of this cable varies from 50 to 250 meters. Via the so called serial RS-232 port of the computer communication is established between computer and Mety station.

#### *Operating the Mety station with the computer on the spot*

The computer can operate the Mety station on the spot. Therefore the Mety program was installed on the computer. When starting the Mety program the main menu appears on the screen (figure 3).

Via option 1 "reading weather station" and option 2 "actual registration values" communication with the Mety station is established. When choosing 2 "actual registration values", the actual registration values appear on the screen (figure 4).

When choosing option 1 "reading weather station", the average hour values stored by the Mety station during the last days, are sent to the computer. Further on, with option 8 "scab calculation" one can see the scab graph on the screen (figure 5). Apart from the presentation in a diagram, this information can also be obtained in a table (table 1). In some registration posts the computer has been installed to start each morning, to get the hourly information from the Mety station and to print table 1.

Mety V1.4 27/09/92


METY STATION MAIN MENU    Menu : 1

<p style="text-align: center; border: 1px solid black; margin: 0;">COMMUNICATION</p> <ul style="list-style-type: none"> <li>1 Read weather station</li> <li>2 Actual calculation values</li> <li>4 Manual registration</li> <li>6 Link with Gaby</li> <li>7 View DAILY information</li> </ul>	<p style="text-align: center; border: 1px solid black; margin: 0;">SURVEYS</p> <ul style="list-style-type: none"> <li>8 Scab calculation</li> <li>9 Temperature sums</li> <li>10 View weather information</li> <li>12 To MS-Dos</li> <li>13 COPY THE INFORMATION</li> <li>14 System maintenance menu</li> </ul>
---	---

- 15 Infections survey
- 16 Annual survey
- 18 Calculation of daily totals
- 20 Calculation of evaporation

Your choice is (Esc) is previous menu

Figure 3. Survey of the Mety program



To print  
F8

Table  
Enter

To stop  
Escape

Latest registration values

Leaf wetness	R.H.	Temperature	Light	Rainfall	Accu
0.0	26.0	14.0	18.0	0.0	14.4
%	%	C	W/m <sup>2</sup>	mm	Volt

Figure 4. Some actual registration values with option 2 "actual registration values"

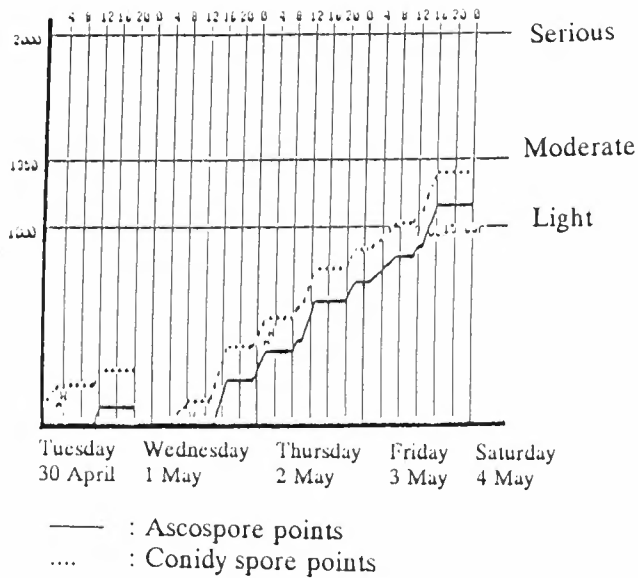


Figure 5. Scab graph

*Communication with Gorsem*

Because of the communication with Gorsem, apart from the computer there is also a relay with a week switch-clock and modem installed. Figure 6 represents this formation in a scheme. Normally, the computer has access to the Mety station via the modem-switch. During ten minutes a day the central system in Gorsem has access to the information in the Mety station. This happens as follows:

The week switch clock is installed in a way so that the modem is powered 10 minutes a day. When the central system in Gorsem calls within these ten minutes, the modem answers the telephone. Due to the contact between the modems, the relay in the modem-switch switches and Gorsem gets access to the information in the Mety station. Further on, the information of the Mety station is sent to Gorsem and the communication is ended.

During the communication with Gorsem the Mety station cannot be read by the computer because the switch-cupboard gives priority to Gorsem. In fact, the computer is not necessary to communicate with Gorsem and can be switched off during the communication with Gorsem or can be used for another purpose.

In most of the registration posts the telephone line is used for ordinary telephone calls during the rest of the day. In Velm (and Alken) during ten minutes a fax line is used. In Roeselare a modem line is used, which can be used by the IBM-AS-400 for the rest of the day. Finally, in Destelbergen there is an extra telephone line for the Mety station.

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Table 1. Scab information of the Mety station in Velm

Date	Hour	Temp in °C	Leaf wetness in %	Rain in mm	R.H. in %	Spores Points this hour	Conidies Total points	Ascospores Points this hour	Total points	
20/06/92	0: 0	11.5	5	0.0	90	0.0	0.0	0.0	0.0	
20/06/92	1: 0	11.6	99	0.8	98	83.3	83.3	83.3	83.3	
20/06/92	2: 0	11.2	97	0.4	100	83.3	166.6	83.3	166.6	
20/06/92	3: 0	11.2	97	0.2	100	83.3	249.9	83.3	249.9	
20/06/92	4: 0	11.4	98	0.4	100	83.3	333.3	83.3	333.3	
20/06/92	5: 0	11.7	99	0.0	100	86.9	420.2	86.9	420.2	
20/06/92	6: 0	12.0	94	1.6	100	86.9	507.2	86.9	507.2	
20/06/92	7: 0	12.1	87	0.6	100	86.9	594.2	86.9	594.2	
20/06/92	8: 0	12.1	83	1.0	100	86.9	681.1	86.9	681.1	
20/06/92	9: 0	12.3	86	0.0	100	90.9	772.0	90.9	772.0	
20/06/92	10: 0	12.4	87	1.2	100	90.9	862.9	90.9	862.9	
20/06/92	11: 0	12.8	86	0.4	100	90.9	953.8	90.9	953.8	
20/06/92	12: 0	16.7	9	0.0	97	111.1	1064.9	111.1	1064.9	L O
20/06/92	13: 0	17.5	20	0.0	89	111.1	1176.1	111.1	1176.1	L O
20/06/92	14: 0	16.4	78	0.8	98	111.1	1287.2	111.1	1287.2	L
20/06/92	15: 0	16.5	80	2.6	99	111.1	1398.3	111.1	1398.3	M
20/06/92	16: 0	17.1	65	1.0	99	111.1	1509.4	111.1	1509.4	M
20/06/92	17: 0	19.4	0	0.0	89	111.1	1620.5	111.1	1620.5	M O
20/06/92	18: 0	19.2	0	0.0	89	111.1	1731.6	111.1	1731.6	M O
20/06/92	19: 0	19.8	5	0.0	83	0.0	1731.6	0.0	1731.6	M
20/06/92	20: 0	19.6	64	0.2	85	111.1	1842.7	111.1	1842.7	M
20/06/92	21: 0	17.2	88	0.4	100	111.1	1953.8	111.1	1953.8	M
20/06/92	22: 0	16.3	83	5.2	100	111.1	2064.9	111.1	2064.9	S
20/06/92	23: 0	15.7	70	3.6	100	111.1	2176.1	111.1	2176.1	S
21/06/92	0: 0	15.0	66	1.6	100	100.0	2276.1	100.0	2276.1	S

Rain                      Saturday 20.4 mm                      Friday 0.2 mm                      This week 0.6 mm

Note: L = light infection; M = moderate infection; S = severe infection; O = Observation must be done by the fruit grower himself

**The central system in Gorsem**

In Gorsem the central computer for storing and processing the information from all Mety stations is installed. This computer has a 80386SX processor and a hard disk of 200 MB. Bodata has provided this machine with an extension card. This card has a modem function (for reading the Mety stations) as well as a fax function (for sending faxes). A week switch-clock makes that the computer and the printer, installed the one next to the other, start automatically every morning at 7 a.m.

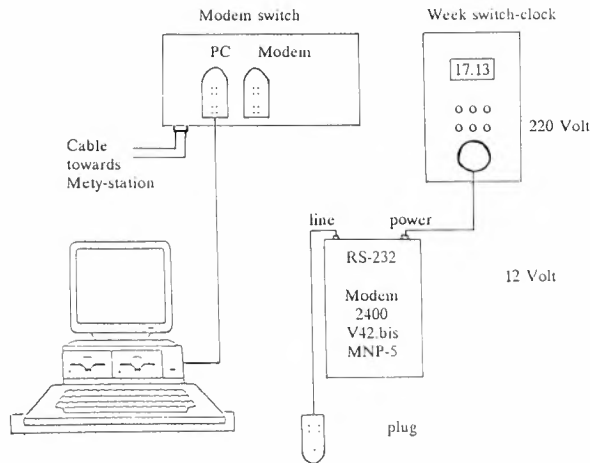


Figure 6. Scheme of the formation of a modem-switch, a week switch-clock and modem

On this computer the programs are installed for the central reading of Mety stations on the registration posts and for storing and processing the registered information. The software on this computer functions under MS-dos 5.0. All furnished programs were developed by Bodata in Dataflex and in Microsoft C. The software installed consists of the following three components :

- software for reading the Mety stations all over Belgium;
- software for processing the registered information;
- software for sending the warning messages.

The following paragraphs describe the functioning of this software.

#### *Software for reading the Mety stations*

The program for reading the Mety stations mainly consists of two components. The first component, "read weather station", calls the Mety station and reads its information. The second component of the program, "Scedy", reads all Mety stations daily on fixed points of time.

To explain the possibilities of the program as simply as possible, chapter Reading weather from the menu describes how the reading of the weather stations from the menu is done. Further, paragraph Daily automatical reading of Mety stations explains how all Mety stations are fully automatically read with "Scedy" each morning between 7 and 8 a.m.

#### *Reading weather stations from the menu*

When the Mety program is started, the main menu appears on the screen (figure 7).

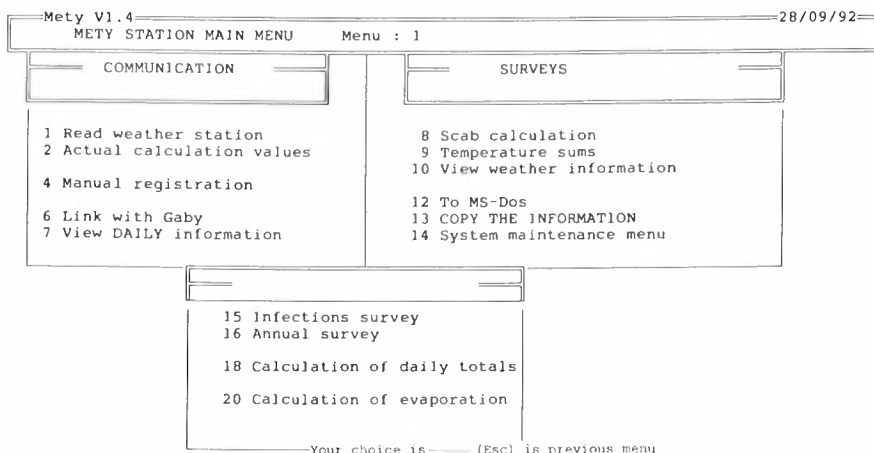


Figure 7. Scheme of the main menu of a Mety weather station

When option 1 "read weather station" is chosen, the intermediary screen appears (figure 8).

Mety V2.0 28/09/92

Read weather station						
Registration post	Telephone	Last communication	Number	Line number	Comprt	Baud rate
1 GORSEM2	N	20/08/92 7:55	1	2	2	9600
2 HALMAAL	J 681305	23/06/92 7: 2	1	1	2	2400
3 VELM	J 691518	19/08/92 7:48	1	1	2	2400
4 GINGELOM	J 485693	20/08/92 7:24	1	1	2	2400
5 HOPPERTINGEN	J 012742373	20/08/92 7:11	1	1	2	2400
6 TONGEREN	J	0, 0	1	1	2	2400
7 KORTISSEM	J 377077	20/08/92 7:28	1	1	2	2400
8 DILSEN	J 089563831	20/08/92 7:17	1	1	2	2400
9 ALKEN	J	23/06/92 7:17	1	1	2	2400
10 NIJWERKERKEN	J 312238	09/08/92 7: 7	1	1	2	2400
11 HALEN	J 013442408	20/08/92 7:3	1	1	2	2400
12 ASSENT	J 013441532	20/08/92 7:15	1	1	2	2400
13 RILLAAR	J 016500240	24/07/92 7:38	1	1	2	2400
14 BRUSSEGEM	J 024601385	20/08/92 7:29	1	1	2	2400
15 VORSELAAR	J 014223449	20/08/92 7:10	1	1		2400

Figure 8. Scheme of an intermediary screen "read weather station" (option 1)

Via this intermediary screen one can now choose a Mety station. Once the choice is made, the program will call this station and the registered information is read.

Daily automatical reading of Mety stations

In principle, when the Mety stations are daily automatically read the same procedure is followed as when the Mety stations are read from the menu. The only difference is that Mety stations are read automatically instead of manually. In the morning a few minutes before 7 a.m. the computer in Gorsem is started by a week switch-clock. Then the following survey with tasks to be carried out appears on the screen (figure 9).

Scedy v1.0 28/09/92

Actual time 7:49 Monday 28th September 1992		
Task	Date and time	Status
READ METY STATIONS	7: 0 Monday 28 September 1992	Started
SCAB REPORT	9:49 Monday 28 September 1992	Started
SEND MESSAGES	10: 0 Monday 28 September 1992	To be executed
READ METY STATIONS	7: 0 Tuesday 29 September 1992	To be executed
SCAB REPORT	7:49 Tuesday 29 September 1992	To be executed

Figure 9. Survey of tasks to be carried out when the Mety-stations are read automatically

Now the computer keeps waiting until it is tuesday 29th September at 7 a.m. As soon as it is 7 a.m. the task "read Mety station" is started. After that the screen appears (figure 10).

Scedy v1.0a 28/09/92

Actual time 7:55 Monday 28th September 1992		
Task	Date and time	Status
READ GORSEM	7:46 Monday 28 September 1992	Started
INTRODUCE INFORMATION	7:49 Monday 28 September 1992	Started
READ HALEN	7: 1 Tuesday 29 September 1992	To be executed
READ NIEUWERKERKEN	7: 4 Tuesday 29 September 1992	To be executed
READ VORSELAAR	7: 6 Tuesday 29 September 1992	To be executed
READ HOEPERTINGEN	7: 9 Tuesday 29 September 1992	To be executed
READ ALKEN	7:11 Tuesday 29 September 1992	To be executed
READ ASSENT	7:14 Tuesday 29 September 1992	To be executed
READ DILSEN	7:16 Tuesday 29 September 1992	To be executed
READ MEIGEM	7:19 Tuesday 29 September 1992	To be executed
READ GINGELOM	7:21 Tuesday 29 September 1992	To be executed
READ KORTESSEM	7:24 Tuesday 29 September 1992	To be executed
READ BRUSSEGEM	7:26 Tuesday 29 September 1992	To be executed
READ NEUFCHATEAU	7:29 Tuesday 29 September 1992	To be executed
READ ROESELARE	7:31 Tuesday 29 September 1992	To be executed

Press TAB to mutate the tasks which are to be executed

Figure 10. Survey of the task "read Mety-stations"

The screen shows which Mety stations will be read at which points of time. One by one the Mety stations are called and read. After about one hour all stations are read and the program for processing the information is started.

### **Software for processing the information**

#### *Processing program for scab calculation*

As soon as the registered information of the Mety stations is processed, the task "scab report" is automatically started. By means of this task a scab calculation is made of all Mety stations. During this operation the scab diagrams of the different registration posts appear on the screen (figure 5).

Further on, a common survey is made and is finally printed. (figure 11).

#### *Other possibilities*

Apart from the scab calculation the Mety program disposes of several other possibilities. To illustrate this we give a survey made by means of the program component "view day information" (figure 12).

Another possibility is the calculation of temperature sums. From a date on, determined by the user, any temperature sum or cold sum can be calculated. Especially for research this possibility for calculation can be interesting (figure 13).

Apart from the two examples given (fig. 12 and 13) even more reports can be made by the Mety program, for instance the evaporation measuring. These and other processing programs are still in development.

### **Software for sending the warning messages**

When the collective survey is ready at about 8.30 a.m., the composition of the warning message can be started with.

Further on, this message can be sent back to the computers at the registration posts and to the regional informers or auctions.

First of all the people of the Advice and Ombudsman Service of Gorsem check whether all Mety stations have been read properly. When it doesn't seem to be the case, they can call again at 9 a.m. to a Mety station that wasn't read.

Consequently, the collective survey is completed with extra information (figure 14).



Registration post	Sunday 16 August 1992				Monday 17 August 1992				Tuesday 18 August 1992				Wednesday 19 August 1992				MM 19 Aug.	MM 18 Aug.	MM Week								
	4	8	12	16	20	0	4	8	12	16	20	0	4	8	12	16				20	0	4	8	12	16	20	
19/08/92 7:53 A 1 GORSEK2 C	813				972				590				654				0.0	0.0	30.6								
23/06/92 7: 2 A 2 HALMAAL C																									0.0	0.0	0.0
19/08/92 7:48 A 3 VELM C					959				735				760				0.0	0.0	30.4								
19/08/92 7:21 A 4 GINGELOK C					770								111 290				0.0	0.2	24.0								
19/08/92 7:13 A 5 HOOPERTINGE C	670				498								392 880				0.0	0.0	25.0								
0: 0 A 6 TONGEREN C																									0.0	0.0	0.0
19/08/92 7:25 A 7 KORTESSEM C	784				650				646				848				0.0	0.0	0.0								
19/08/92 7:18 A 8 BILSEN C	559				885				553				574				0.0	0.0	30.2								
23/06/92 7:17 A 9 ALKEN C																									0.0	0.0	0.0
09/08/92 7: 7 A 10 NIEUWERKERK C																									0.0	0.0	0.0
19/08/92 7: 5 A 11 HALEN C					1298								90				0.0	0.0	22.6								
19/08/92 7:16 A 12 ASSENT C	803				1063				633				770				0.0	0.0	15.6								
24/07/92 7:38 A 13 RILLAAR C																									0.0	0.0	0.0
19/08/92 7:27 A 14 BRUSSEGEN C									1473				977				0.2	0.2	10.2								
19/08/92 7:11 A 15 VORSELAAR C	1087				1231				1164				1063				0.0	0.0	31.4								
19/08/92 7:36 A 16 OESTELBERGE C	936				1708								1344				0.0	0.0	40.4								
19/08/92 7:20 A 17 MEIGEM C					1985				1131				1016				0.0	0.0	39.2								
19/08/92 7:15 A 18 ROESELARE C																									0.0	0.0	0.0
07/08/92 7:31 A 19 NEUFCHATEAU C																									0.0	0.0	0.0

= developing infection   
  = light infection   
  = moderate infection   
  = serious infection

Figure 11. Survey of scab infections (period from 16/08/92 to 20/08/92)

(c) Bodata Mety V2.0

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view DAILY informations 28/09/92 15:32:18

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Demonstration

Date	Rainfall in mm GORSEM2	Rainfall in mm VELM	Rainfall in mm VORSE- LAAR	Rainfall in mm MEIGEM	Rainfall in mm ROESE- LARE
01/07/92	0.0	0.0	0.2	0.2	0.0
02/07/92	0.0	0.0	0.0	0.0	0.0
03/07/92	0.0	0.0	0.0	18.6	25.6
04/07/92	8.8	12.8	23.4	15.6	4.6
05/07/92	6.0	10.8	0.2	0.4	0.0
06/07/92	2.4	2.4	0.6	0.2	0.0
07/07/92	0.0	0.0	0.2	0.0	0.0
08/07/92	0.0	0.0	0.0	0.0	0.0
09/07/92	0.0	0.0	0.0	0.0	0.0
10/07/92	0.6	0.2	2.2	3.2	0.4
Total	17.8	26.2	26.8	38.2	30.6

Figure 12. Survey of the other possibilities of the Mety-program based upon the program component "view daily information"

(c) Bodata Mety V2.0

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view DAILY information 28/09/92 15:46:16

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Demonstration

Selection of 1 GORSEM2  
Starting date 1st january 1992 From 250 to 300 day degrees

Date	Registra- tion va- lues	Temp sum this day	Temp sum 4.0 C
23 May 1992	48	29.4	265.4
24 May 1992	24	15.2	280.6
25 May 1992	24	14.7	295.4
26 May 1992	24	14.9	310.3

Figure 13. Use of the Mety program for the calculation of temperature sums

```
Data bank Process Search Options          TXT          ?
-----
::: = starting infection  ☒ = light infection  ▣ = moderate infection

The message can simply be complemented with extra messages.
These remarks have to be introduced before the message is sent.

IBM DOS Editor (F1=Help) Press Alt to activate menus      | N 00070:057
```

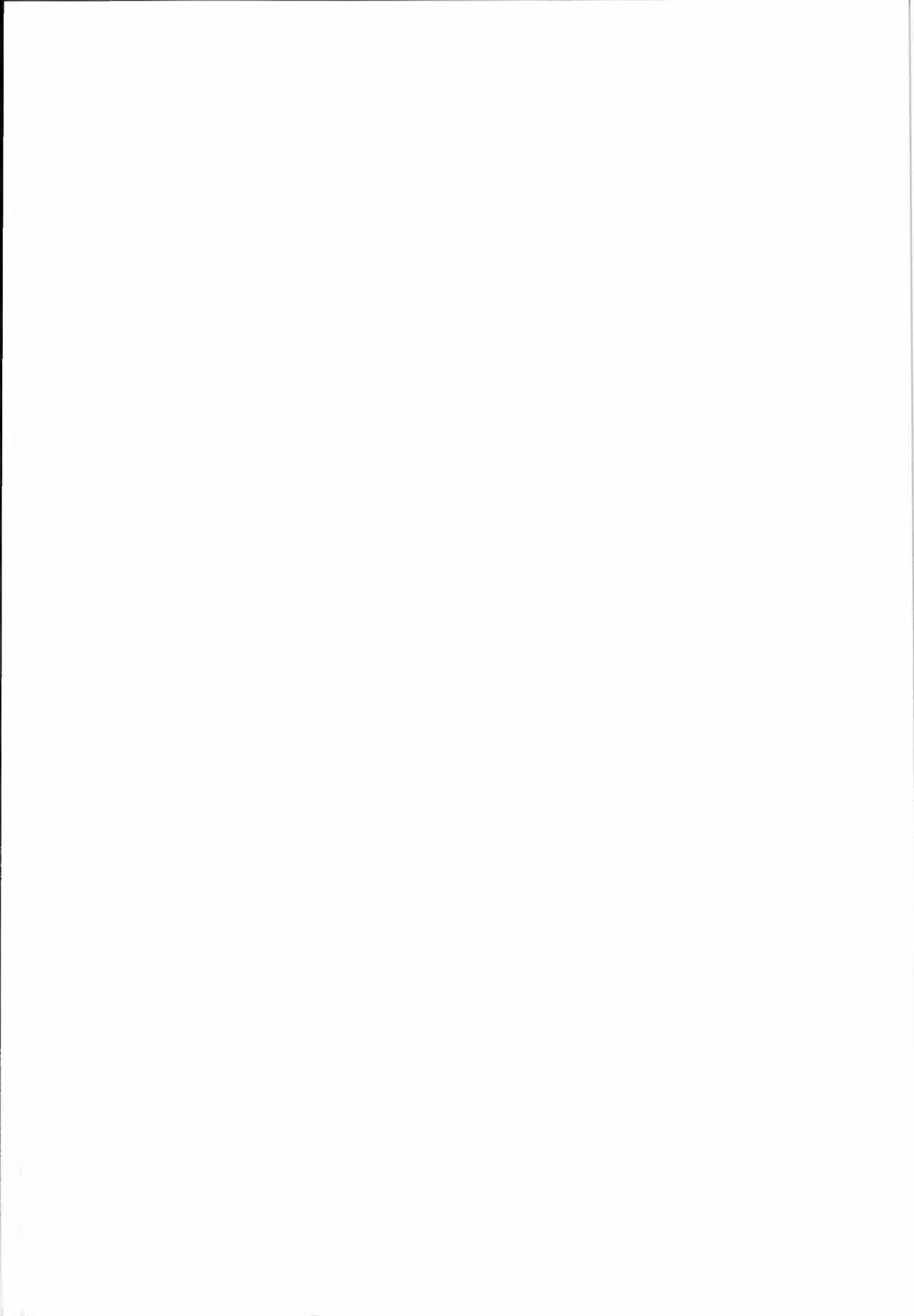
Figure 14. Collective survey (warning message) completed with extra information

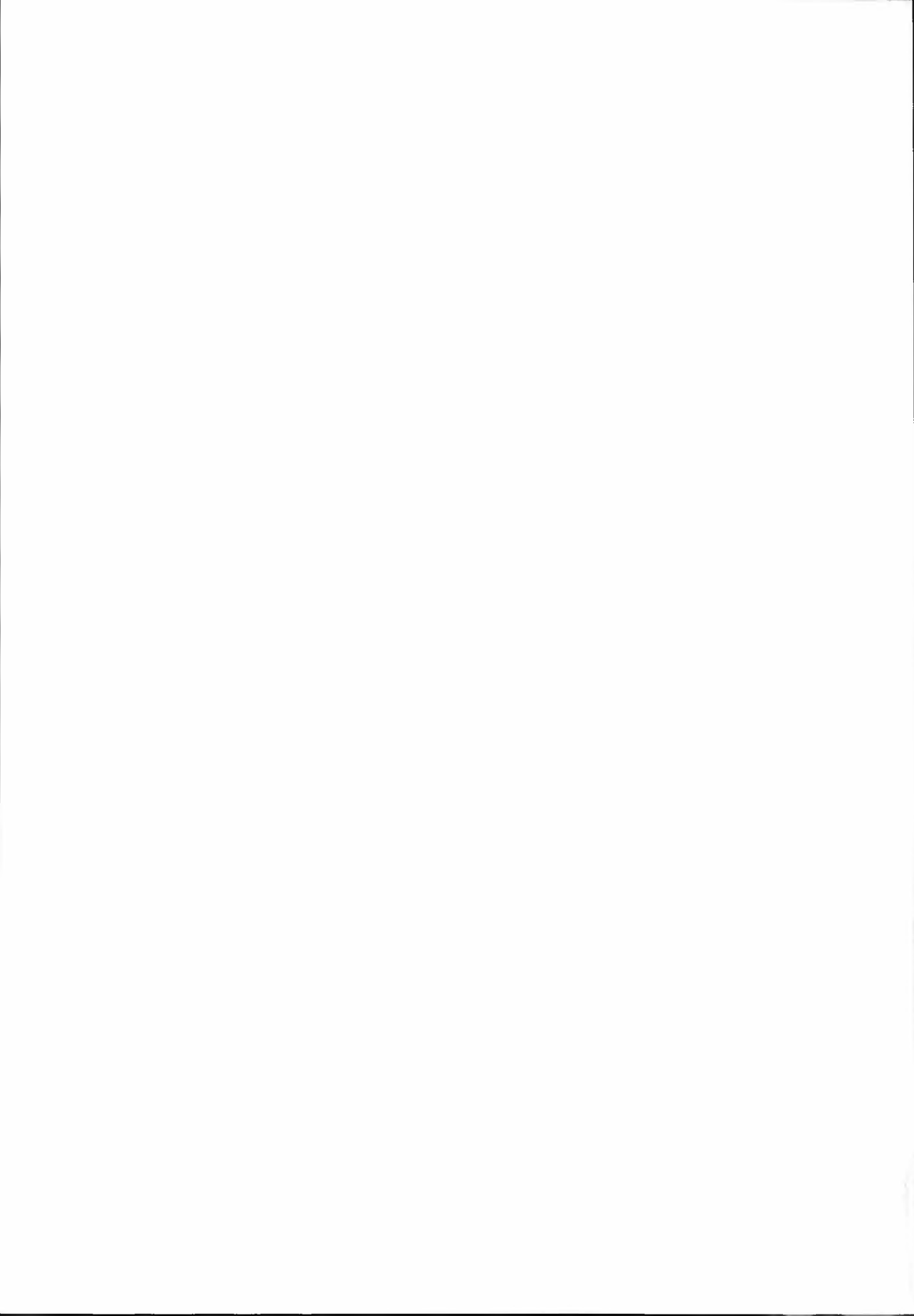
Then the warning message is printed and the contents of the message are checked by the departments of mycology, zoology and bacteriology of the Research Station of Gorsem. When this is done, the message is ready for sending.

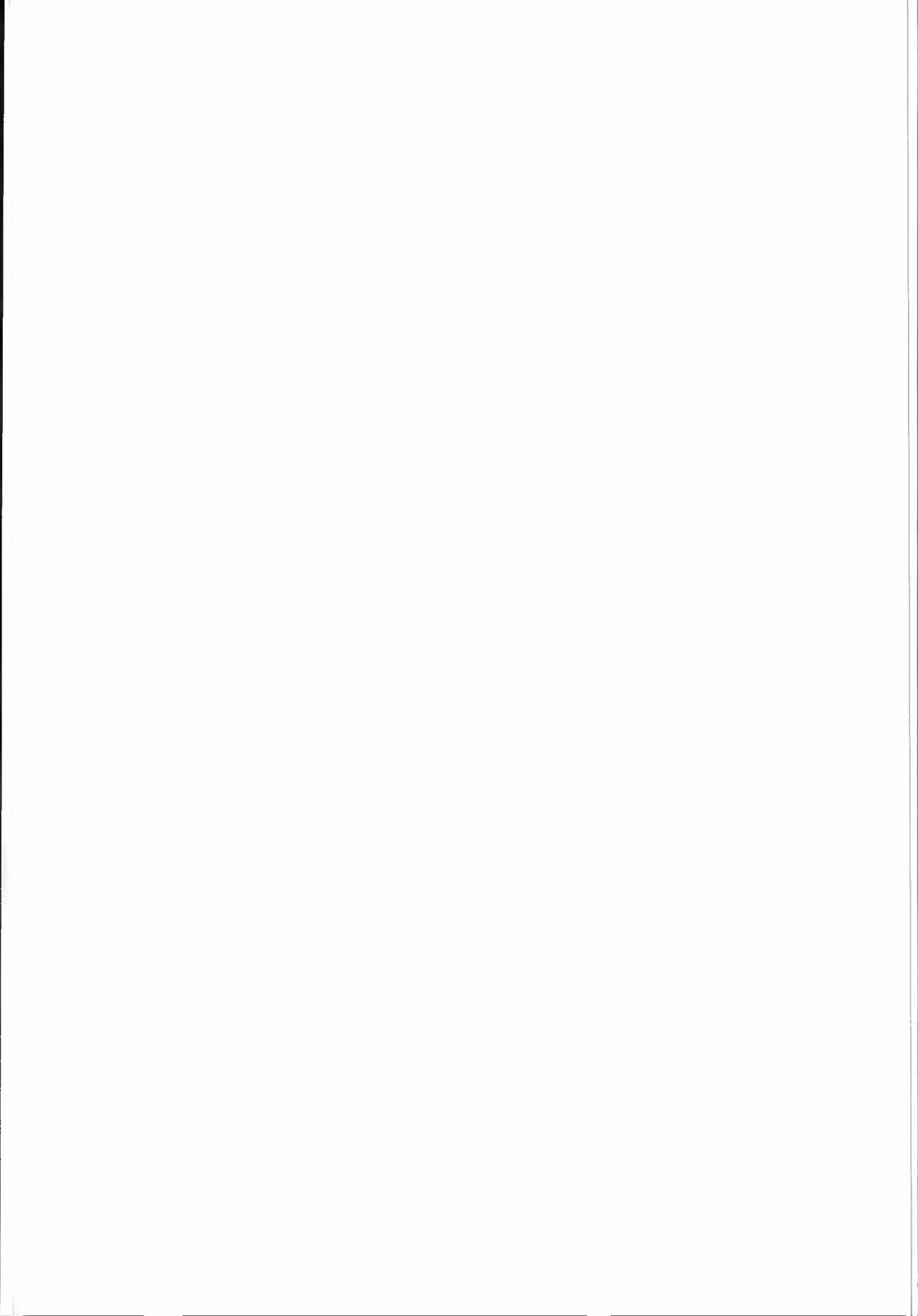
The sending can be done by telecopy as well as by modem. Sending by telecopy is the easiest way, because a telecopy machine is attainable all day long and there is no need to install a baudrate. When sending by modem, arrangements have to be made with the receiver to see at which point of time the receiver is attainable.

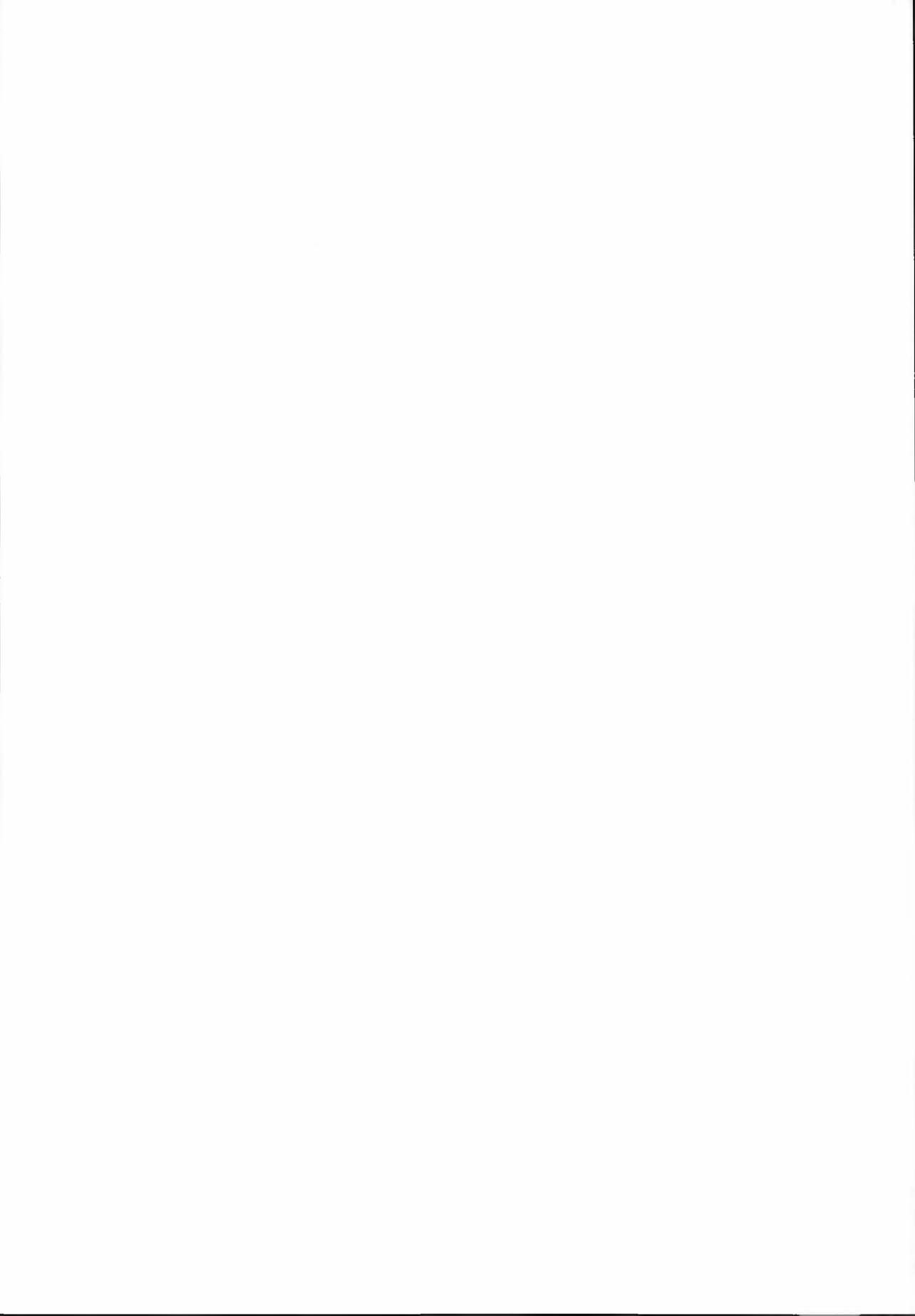
The Research Station of Gorsem hopes that this agrometeorological network will be very usefull to the individual fruitgrower as well as to fruitculture in general.



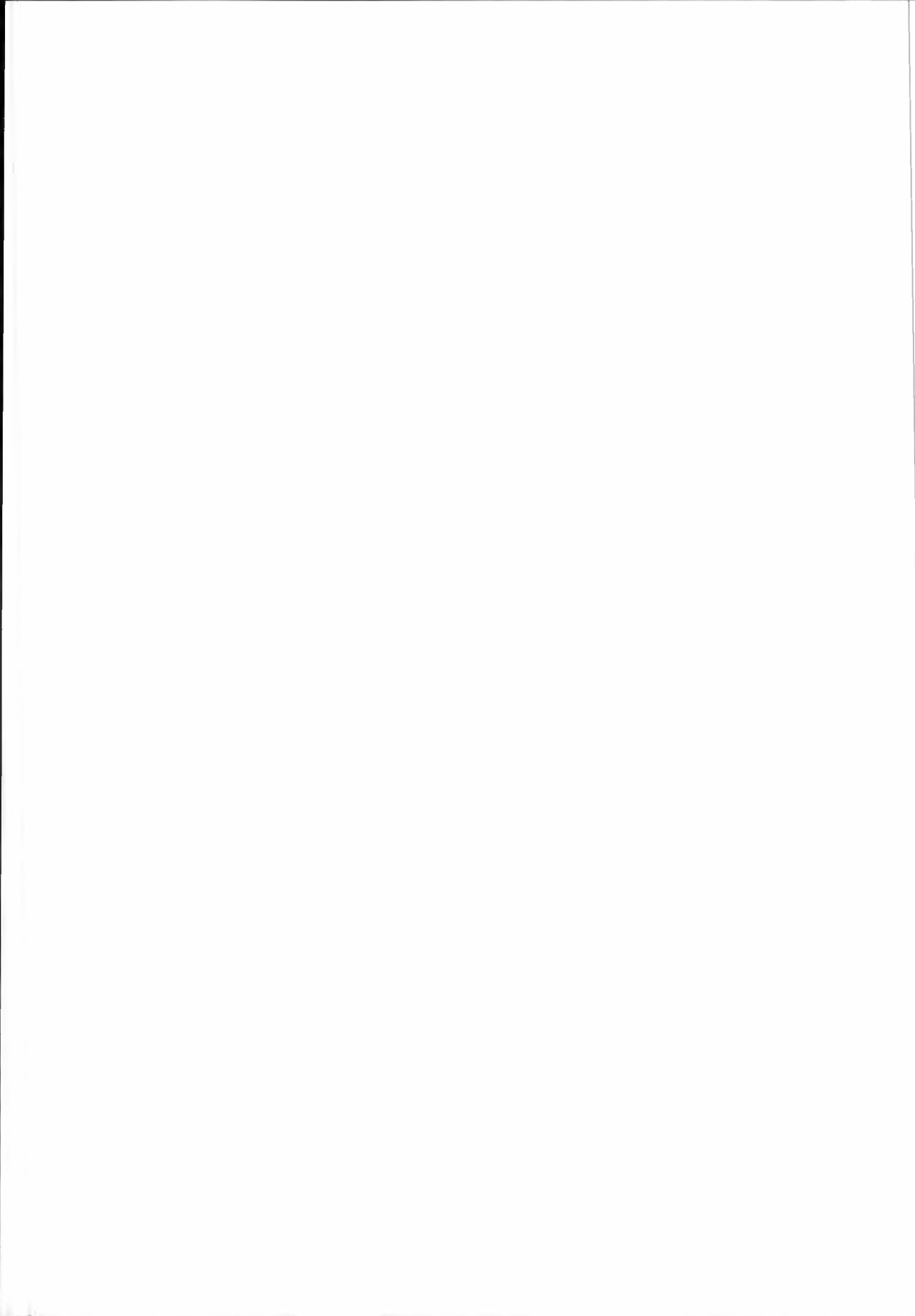


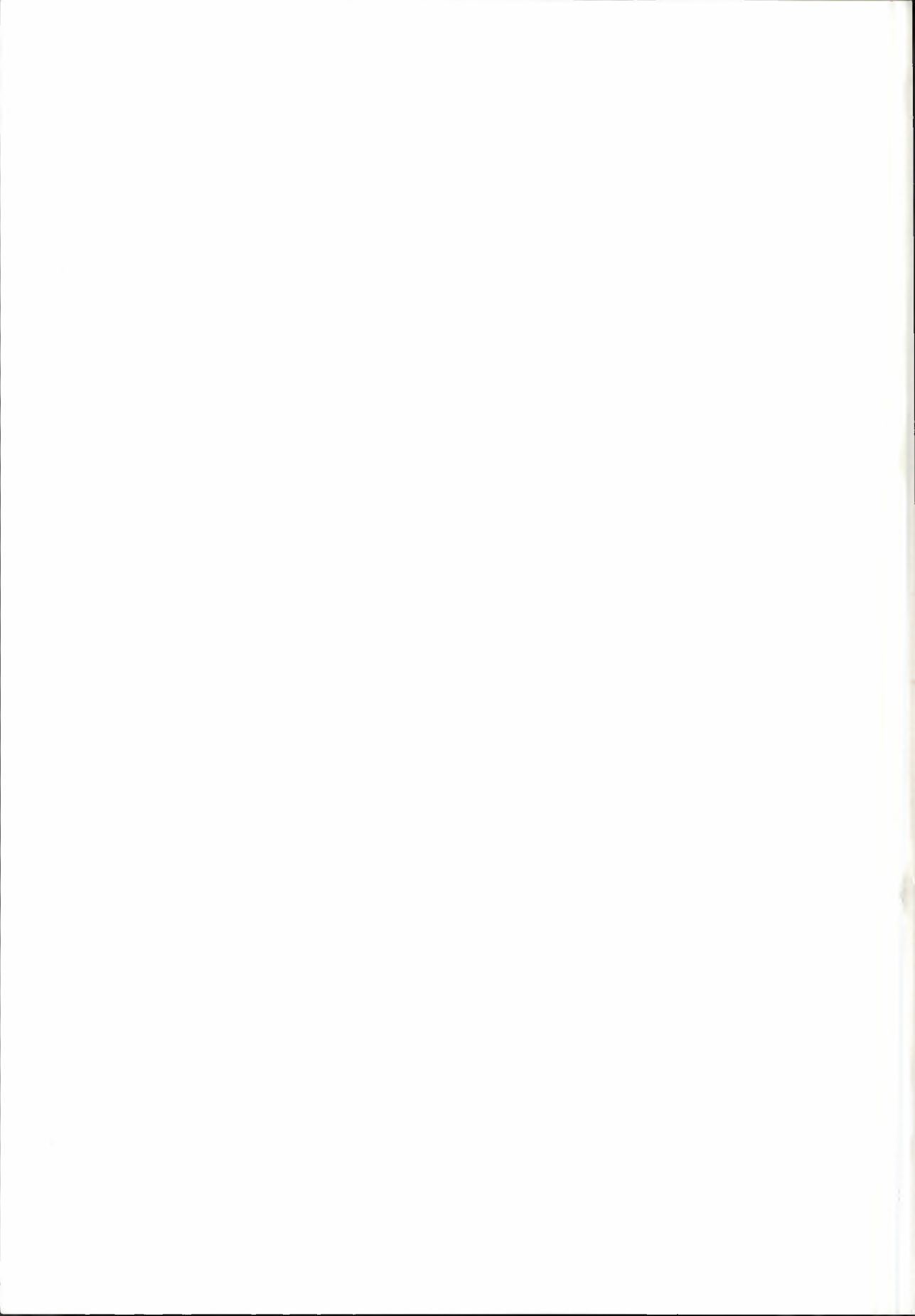












# NORWEGIAN JOURNAL OF AGRICULTURAL SCIENCES

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