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Disease resistance in agricultural crops

NJF-Seminar no. 188

Norwegian Agricultural Advisory Service, Ås, Norway

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Preface

The NJF seminar no. 188, Diaease resistance in agricultural crops, was held at Ås 11-14th of February 1991. The seminar was arranged by NJF-sections II and IV: "Working group for winter survival of agricultural crops" and "Working group for disease resistance in cereals", in connection with the centennial of Plant Protection in Norway. Altogether 31 lectures were given at the seminar, of which 14 are published here. Abstracts of all lectures are published in Nordisk Jordbruksforskning 73 (3), 512-545.

Content

Snow mould fungi in Canada

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Drew Smith, J. 1992. Snow mould fungi in Canada. Norwegian Journal of Agricultural Sciences. Supplement No. 7. 5-12. ISSN 0801-5341.

The range, relative importance, and recent studies on the snow mould fungi found on grasses, winter cereals and other crops in Canada are reviewed. *Microdochium nivale*, *Sclerotinia borealis*, *Typhula incarnata*, *T. ishikariensis* vars. *ishikariensis* and *canadensis*, *Coprinus psychromorbidus*, (in the non-sclerotial (LTB) and sclerotial (SLTB) forms), and the weak pathogens, *Phoma sclerotiodes* and *Acremonium boreale* are currently recognized as common snow moulds of grasses and cereals in Canada. *C. psychromorbidus*, *P. sclerotiodes* and *A. boreale* also occur on forage legumes. *C. psychromorbidus* also causes a storage rot of pome fruit. Snow moulds and other fungi occur in complexes. *Typhula phacorrhiza* and *Acremonium boreale* have been tested as possible biological control agents for snow mould diseases of turfgrasses. Changes in climate may modify the incidence of snow diseases in the prairie region.

Key words: Antagonists, biological control, cereals, climate change, forage legumes, grasses, pathogens, saprobes, winter cereals.

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This paper reviewes the range, relative importance and recent studies on the snow mould fungi found on grasses, cereals and other crops in Canada. The possible influence of climatic changes on the incidence of snow moulds is also discussed.

SNOW MOULDS IN CANADA

Microdochium nivale (Fries) Samuels & Hallet (1983) (syn. *Fusarium nivale* Ces.) with a teleomorph *Monographella nivalis* (Schaffnit) E. Muller (1977) is a highly variable mesophilic fungus (Bennet 1933; Smith 1957, 1983) which is pathogenic on grass and cereal hosts from temperate to boreal regions in Canada and causes fusarium patch disease of turfgrasses. Although mesophilic, some isolates will grow at temperatures below 0°C. However, a snow cover is not a requirement for disease development. Initial pre-nival infections may develop further under the snow. Pink sporodochia produced on infected plants on exposure to light after snow melt give rise to the symptoms of pink snow mould. Canadian isolates from turfgrasses could not be induced to produce ascocarps, but those from cereals did so on wheat straw in artificial culture (Smith 1983).

Sclerotinia borealis Bubak & Vleugel (Vleugel 1917) (synonym *Myriosclerotinia borealis* (Bubak & Vleugel) Kohn is a low-temperature-tolerant pathogen of grasses and

cereals causing sclerotinia snow mould in regions with prolonged, heavy snow cower, particularly north of the Canadian/US border. There are no macroconidia, but spermatia or microspores have been reported in culture by Canadian and Russian workers (Smith 1987). The mould has been found on turfgrasses from Lat. 44°N in southern Ontario, and from Minnesota (Stienstral 1980) to southern Manitoba and Saskatchewan (Lat. 50°N) to the Prince George region in British Columbia (Lat. 54°N) and Alaska (Lat. 60°N). In western Canada two epidemic seasons were noted in 1971/72 and 1973/74 on grasses and winter cereals (Smith 1987). In the drier portions of the Canadian prairies, e.g. in southern Saskatchewan, where seasonal change in temperature is rapid and autumn is often dry, sufficiently long periods of weather favourable for apothecial development are probably rare. Apothecia have not yet been found in Saskatchewan, but have been recorded from southwest Alberta (L. Piening, personal communication) and the more humid climate of the Peace River region of British Columbia (J.G.W. Davidson, personal communication). Contradictory evidence has been obtained on the relative importance of ascospores, sclerotia, or mycelial inoculum in initiating infection, and the epidemiological significance of unfrozen versus frozen ground or leaf tissues is in dispute (Tomiyama 1955; Årsvoll 1975; Sakuma & Narita 1963; Matsumoto & Araki 1982; Smith 1974). The competitive saprophytic ability (CSA) of *S. borealis* is low and its mycelial growth rate is slow. It was shown that *S. borealis* had an optimum temperature for mycelial growth in culture of 0°C, following initial optima of 5-15°C (Ward 1968). Ice crystals in the culture medium retarded, but did not prevent mycelial growth on agar medium (Tomiyama 1955; Ward 1968). Once infection is established a long duration snow cover and declining carbohydrate reserves are necessary for severe epidemics.

T. incarnata (Lasch ex Fries), causing typhula blight or gray snow mould, is widely distributed across Canada, but is found particularly in regions with milder winter temperatures, e.g. in the valleys of southern British Columbia. The pathogen is less common than *Typhula ishikariensis* vars. in regions with colder winter temperatures and a longer duration of snow cover. However, the two species occasionally may occur together in disease complexes (Burpee et al. 1987; Smith & al. 1989). The higher CSA of *T. incarnata* is probably the major factor ensuring its ecological niche in competition with the more highly virulent *T. ishikariensis* (Matsumoto & Sato 1983). *T. incarnata* has a higher optimum mycelial growth temperature than *T. ishikariensis* (Smith 1987) and it will cause turfgrass snow mould with very light, brief snow cover. In Canadian prairies, two varieties of *T. ishikariensis* var. *ishikariensis* and var. *canadensis* (Årsvoll & Smith 1978) are the most common. *T. ishikariensis* var. *idahoensis* has not been recognized. The profuse, weblike, superficial aeriel mycelium in which the small sclerotia of var. *canadensis* are suspended suggestes that the mycelium and sclerotia are significant means of dispersal of this pathogen in this windy region of Canada. Although sclerotia of all varieties of *T. ishikariensis* will produce fertile sporophores under controlled conditions (Årsvoll & Smith 1978) these have not been observed in nature in Saskatchewan in inoculated turf or in pots exposed to autumn weather. It was found that sclerotia of several isolates of *T. ishikariensis* var. *canadensis* and *T. incarnata* produced in culture on sterile rye in 1973 and stored for 16 years in polyethylene bags at -7°C were still viable and pathogenic on *Poa pratensis* turf when used as inoculum in late fall 1989 under conditions unfavourable for sporophore production (Smith unpublished). In Ontario, Burpee et al. (1987, 1990) obtained significant suppression of

typhula blight of creeping bentgrass turf caused by *T. ishikariensis* var. *ishikariensis* by the application of inoculum of a non pathogenic isolate of *Typhula phacorrhiza* Fr. The mechanism involved in suppression was considered to be nutrient competition rather than hyperparasitism or cellular lysis induced by an antibiotic or hyphal contact. However, antibiosis may be involved in the antagonism.

Coprinus psychromorbidus Redhead & Traquair (1981), causing Cottony Snow Mould of grasses, winter cereals, herbage legumes and several other hosts, is a small agaric of the *Coprinus urticicola* complex of the section *Herbicolae*. It was found by Traquair (1980) fruiting on the necrotic crowns of lucerne (*Medicago sativa* L.), dug from the field, subjected to a freezing test then allowed to recover in the greenhouse. It was also found fruiting on stolons in an *Agrostis stolonifera* L. sod, showing symptoms of low-temperature basidiomycete (LTB) snow mould brought into a greenhouse maintained at ca. 22°C (Smith 1987). A mycelial non sclerotial (LTB) and a sclerotial form (SLTB) were shown to be conspecific with *C. psychromorbidus* by di-mon parings (Traquair & Smith 1982). A conspecific mycelial form (FRLTB) causes rot of pome fruits (Spotts et al. 1981). None of the isolates from apples were pathogenic on *Poa pratensis* (Gaudet & Sholberg 1990). Great variation in pathogenicity has been found in isolates from different hosts. The optimum temperature for most isolates on winter wheat is -3°C (Gaudet 1986). The optimum temperature for *in vitro* growth of most LTB strains is 12.5-17.5°C (Gaudet & Sholberg 1990; Smith 1987; Traquair 1980; Ward et al. 1961), but isolate (DAOM 198183) has an optimum growth temperature of 22°C (Traquair 1980). Gaudet & Sholberg (1990) suggest that there are different optimum temperatures for isolates depending on whether growth is parasitic or saprobic. Lower optimal temperatures for parasitic versus saprobic growth in snow mould fungi may depend on the restriction of antagonists at the lower temperatures (Matsumoto & Tajimi 1988).

The sclerotial form (SLTB) of *C. psychromorbidus* has a similar range and distribution in western Canada as the non sclerotial (LTB form). The sclerotia are found in spring on the leaves, culms and sheaths of diseased cereals and grasses, and on forage legumes and many other hosts as well as developing on twigs and other plant debris lying on the substrate. The sclerotia also develop in culture on agar media. SLTB isolates usually have woollier and more adpressed mycelium than LTB isolates and are less virulent than the latter (Gaudet 1986; Traquair & Smith 1982). They generally have lower optimum growth temperatures (10°C to 15°C) than non sclerotial isolates, but the ranges for LTB and SLTB overlap. Some isolates may remain sclerotial in culture for many years (Smith 1987), while others, after several transfers, resemble LTB isolates (Traquair & Smith 1982). Although not proven, it seems possible that the SLTB sclerotia indicate the oversummering stage of the fungus.

A deep snow cover providing fairly constant temperatures -1° and -5°C was found to be most conducive to the development of LTB snow mould in winter wheat. A thin, brief snow cover permitted slight penetration and internal development was possible without a persistent snow cover (Gaudet et al. 1989). As with *Typhula* spp. and *Microdochium nivale* in cereals and grasses (Bruel & Cunfer 1971; Abe 1986; Amano & Osanai 1983) disease resistance to LTB in winter wheat was found independent of freezing resistance (Gaudet et al. 1989).

Date of inoculation may be a significant factor in the development of disease in turfgrasses. Replicated turfgrass plots of mown *Poa pratensis* were inoculated in discrete

specific locations with aliquots of rye cultures of a turfgrass LTB isolate on 12 June, 17 August and 18 October 1989. Eight of ten plots inoculated on 18 October showed LTB symptoms, 1 of 10 inoculated on 17 August was diseased, but none inoculated on 11 June or control plots inoculated with sterilized rye grain checks showed symptoms of the mould disease in spring 1990 (Smith, unpublished).

Phoma sclerotiodes (Preuss ex Sacc.) (synonym *Plenodomus meliloti* (Dearness & Sanford), causing a brown root rot, has a wide host range on plants from regions in the northern hemisphere with severe winters (Smith 1987). In Canada, it is possibly a pathogen, particularly on dormant forage legumes (Sanford 1933; B.D. Gossen, personal communication), or more usually a primary invasive saprobe on plants damaged by low temperatures during the winter quiscent period. Pathogenicity on grasses and cereals has not been proven experimentally. In senescent winter cereals it is often found in complex with the sclerotial form (SLTB) of *C. psychromorbidus* on crowns and roots (Smith 1987; Smith & Piening 1980). It has been found as the dominant fungus on winter-damaged grasses, e.g. on *Festuca rubra* in Alaska (Lebeau & Logsdon 1958) and on *Dactylis glomerata* which is not fully winter hardy at Saskatoon (Knowles & Smith 1981).

Acremonium boreale Smith & Davidson (1979) (telemorph - *Neiria tuberculariformis* (Rehm ex Sacc.) Winter (Samuels et al. 1984) is common on grasses and many other species and plant debris in Canada from Ontario to British Colombia (Smith 1987). It has an arctic/alpine distribution on herbaceous hosts from Norway, the Swiss Alps, Austria, N. Dakota and Colorado (Samuels et al. 1984). Although weakly pathogenic on grasses, its main significance is as a low-temperature-tolerant, slow-growing, primary invasive saprobe. Its sclerotia-like stromata are often collected with sclerotia of *C. psychromorbidus, S. borealis*, and *Typhula* sp. from snow mould damaged turfgrass in western Canada. Immature stromata have been found on green aerial portions of *Bromus inermis* at snow melt and associated mycelium is deep-seated in plant tissues, but has not been shown to be an endophyte. Although slow growing, isolates of the fungus show strong *in vitro* antagonism towards several psychrophilic and mesophilic fungal pathogens (Samuels et al. 1984; Smith & Davidson 1979). It has been field tested as a possible biological control agent against snow mould diseases of *Agrostis stolonifera* turf. Fields tested against *Microdochium nivale* in 1987/88 and 1989/90 and against complexes of *M. nivale* and *C. psychromorbidus* in two field tests in 1989/90 gave inconclusive results. Snow mould incidence in all cases was low (Smith, unpublished). In a fully replicated test under controlled conditions the results suggested that the antagonist might be pathogenic at higher dosages or that the antagonist and the pathogen were antagonizing other psychrophilic pathogens in the turf, e.g. *M. nivale*, which was isolated (Smith & Gossen, in press).

Climatic change and snow mould incidence

The recorded history of the Canadian prairies is patterned with drought cycles. One of the predicted effects which would result from a doubling of the carbon dioxide concentration of the atmosphere, i.e. the "greenhouse effect", is that the frequency and severety of droughts is Saskatchewan over any 20-30-year period would be greater (Williams et al. 1988). On the average, snow contributes approximately one-third of the mean total precipitation at Saskatoon and the mean annual snowfall has declined over the last three decades. This may be one of the causes for the decline in importance of

injury attributable to *C. psychromorbidus*, *T. ishikariensis* and *M. borealis* in grasses and winter cereals which require a long, deep snow cover and the prominence of *M. nivale* and desiccation injury in Saskatchewan (Smith, in press).

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Winter injuries in grasslands in northern Norway caused by low temperature fungi

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Andersen I.L. 1992. Winter injuries in grasslands in northern Norway caused by low temperature fungi. Norwegian Journal of Agricultural Sciences. Supplement No. 7. 13-20. ISSN 0801-5341.

The most serious low-temperature fungi causing winter injuries in grasslands in northern Norway are *Myriosclerotinia borealis* (Bubak & Vleugel) Kohn and *Typhula ishikariensis* Imai. In regions with stable and cold winters with a continuous snow cover of 150-180 days or more, the grasslands are highly exposed to low-temperature fungi. Young timothy plants are less resistant to *M. borealis* and *T. ishikariensis* than older ones. Severe winter injuries caused by low-temperature fungi are usually more frequent in the first year of timothy ley than in older leys. In the eastern part of Finnmark mowing timothy at about the heading stage leads to a higher frequency of winter injuries caused by low-temperature fungi than moving at a later stage. In western parts of Finnmark, weed control in timothy leys using phenoxycarboxylic acid compounds in August and September resulted in severe injuries, caused by both ice-encasement and low-temperature fungi.

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In 1906 Ulander (1910) recorded the first observations of winter injuries to grasslands (cocksfoot) in northern Fennoscandia caused by a low-temperature fungus. The fungal pathogen responsible was *Myriosclerotinia borealis* (Bubac & Vleugel) Kohn. Later, several studies were carried out to obtain more information about the distribution of low-temperature fungi, causing winter injuries in Finland (Jamalainen 1949, 1957; Mäkelä 1981), Sweden (Ekstrand 1947, 1955) and Norway (Andersen 1960, 1966, 1990; Røed 1956, 1960; Årsvoll 1973, 1975). The results of these studies indicated that *M. borealis* and *T. ishikariensis* occurred and caused winter injuries in grasslands in the most northerly part of Finland, Sweden and Norway.

WINTER INJURIES IN GRASSLANDS IN NORTHERN NORWAY CAUSED BY ABIOTIC FACTORS RELATED TO THE WINTER CONDITIONS

The most important fungi causing winter injuries in northern Norway are *Myriosclerotinia borealis* and *Typhula ishikariensis*. Districts with stable winter weather and continuous snow cover are the most exposed to low-temperature fungi. According to Årsvoll (1973), severe attacks of *M. borealis* occur in localities with more than 180 days of snow cover, including 110-120 ice days. The corresponding data for *T. ishi-*

kariensis are 150 and 80 days. The regions most exposed to low temperature fungi in northern Norway are Pasvikdalen, Tana and Alta in Finnmark county, Nordreisa and the surrounding districts of Tromsø in Troms county and the inland districts of Rana, Hemnesberget and Vefsn in Nordland county. A distribution map of *M. borealis* and *T. ishikariensis* for northern Norway is presented in Fig. 1.

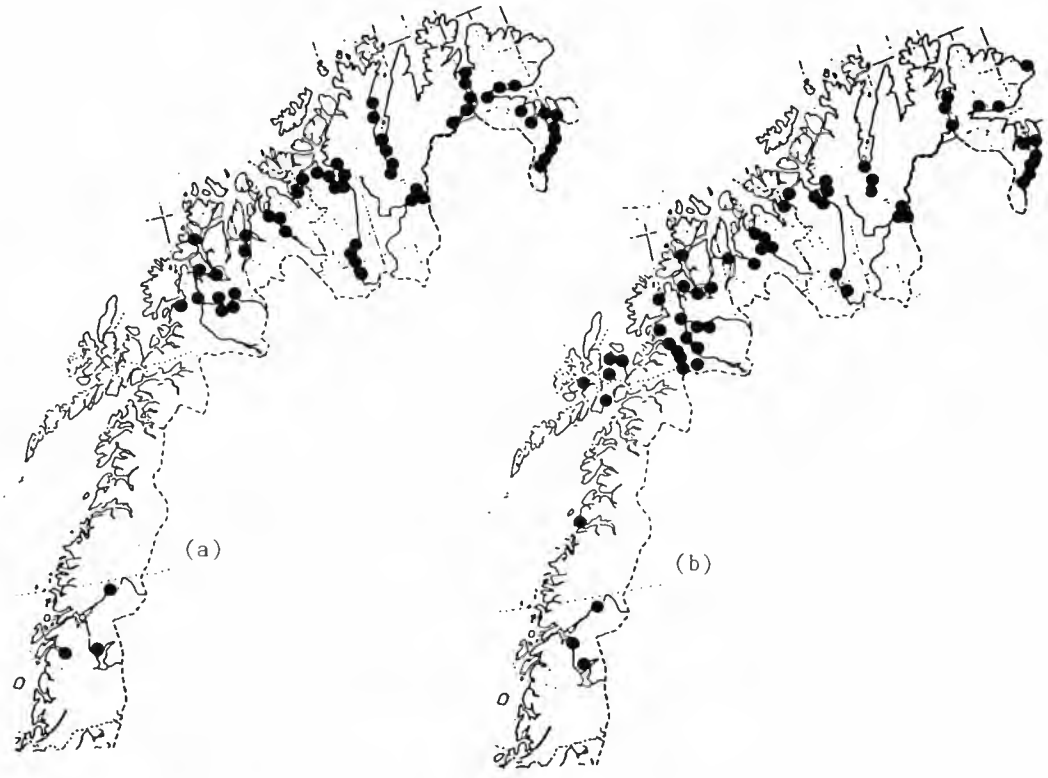

Fig. 1. Distribution maps of *Myriosclerotinia borealis* (a) and *Typhula ishikariensis* (b) for northern Norway. Mainly based on Årsvoll (1975) and Andersen (1990)

The low temperature fungus *Typhula incarnata* (Lasch ex Fries) also occurs frequently in northern Norway. It is found in seven grass species, but in nearly all cases there has been no severe injuries. However, strains of cocksfoot deriving from the southern part of Europe, have been totally damaged by *T. incarnata* in a field experiment at Holt Research Station.

Årsvoll (1975) isolated another eight weak low temperature fungi on grasses in northern Norway. One of the species, *Dactylaria graminicola* Årsvoll, was described and named for the first time by Årsvoll (1975a).

WINTER INJURIES CAUSED BY LOW TEMPERATURE FUNGI IN RELATION TO THE DEVELOPMENT OF TIMOTHY

Mowing of timothy at the heading stage has usually resulted in unsatisfactory overwintering. This is clearly shown in several investigations carried out in northern Norway during the last 40-50 years (Hansen 1946; Larsen 1972; Valberg & Bø 1972; Østgård 1962).

In the period 1922-60 severe winter injuries occurred only every eight years in the eastern part of Finnmark (Andersen 1960). In contrast, severe winter injuries has occurred every 3-4 years during the last 15-20 years in this region. Moving timothy at about the heading stage is believed to have caused a delay in production of new vegetative shoots and to have reduced the condition of the plants before overwintering. Årsvoll (1977) showed that young timothy plants were less resistant to *Myriosclerotinia borealis* and *Typhula ishikariensis* than older ones.

Preliminary investigations have been carried out in Tana and Pasvikdalen to gather more information about the effects of early mowing of timothy on the frequency of years with severe winter injuries (Fig. 2).

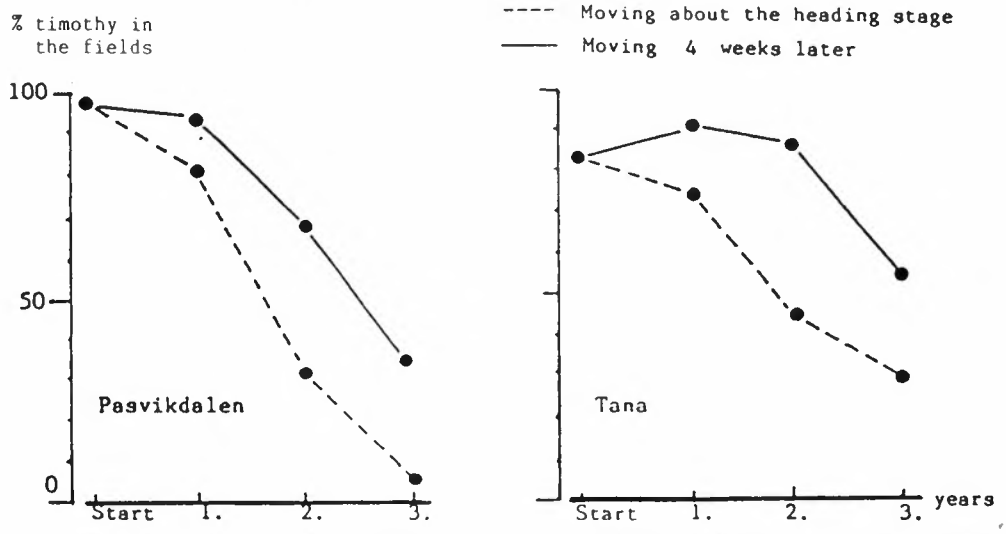

Fig. 2. Effects of mowing timothy at different development stages on the frequency of this species in experiment fields at Tana and in Pasvikdalen

After the second and the third overwintering, mowing the leys at around the heading stage gave significant less timothy in the fields compared with mowing four weeks later. In Pasvikdalen most of the winter injuries were caused by *M. borealis*, while both *T. ishikariensis* and *M. borealis* caused the winter injuries at Tana (Andersen 1986).

Late sowing of timothy in August and the first part of September has frequently led to increasing injury caused by low-temperature fungi (Nissinen & Salonen 1972; Pulli 1986). It is possible that late sowing produces plants that are too young to withstand the winter stress. In Finnmark county (Alta) sowing in the middle part of August 1965 led to severe winter injuries caused by *M. borealis* in 1966 (Fig. 3).

Fig. 3. Sowing timothy in the middle of August in Alta in 1965 led to severe winter injuries caused by *Myriosclerotinia borealis* in 1966

INFLUENCE OF AUTUMN APPLIED MCPA AND MECOPROP ON THE WINTERING OF TIMOTHY LEYS IN WESTERN FINNMARK

Experiments with weed control in timothy leys using phenoxycarboxylic acid compounds (MCPA and Mecoprop) were carried out in western Finnmark. The sprayings which were applied in August and September led to severe winter injuries, as a result of both low-temperature fungi and ice-encasement (Fig. 4).

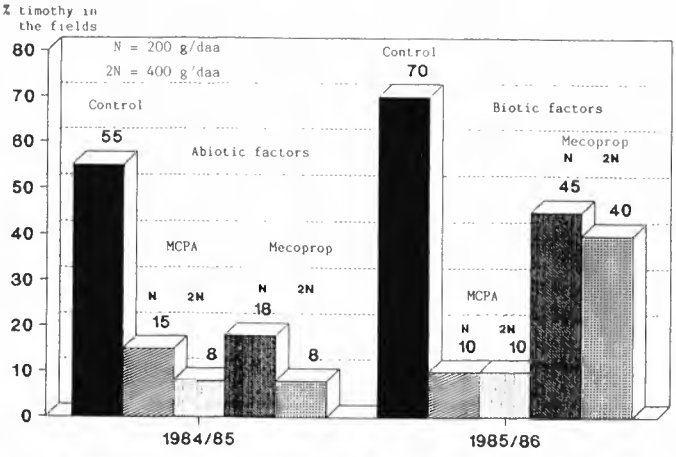

Fig. 4. The histograms indicate the cover of timothy (%) after the winters 1984/85 and 1985/86 for the various treatment (Andersen 1989)

In 1986 similar weed-controlling experiments were carried out in the same region toward the end of July, in August and in September. In these cases too the overwintering capacity was reduced, but less so when the spraying was carried out toward the end of July (Fig. 5).

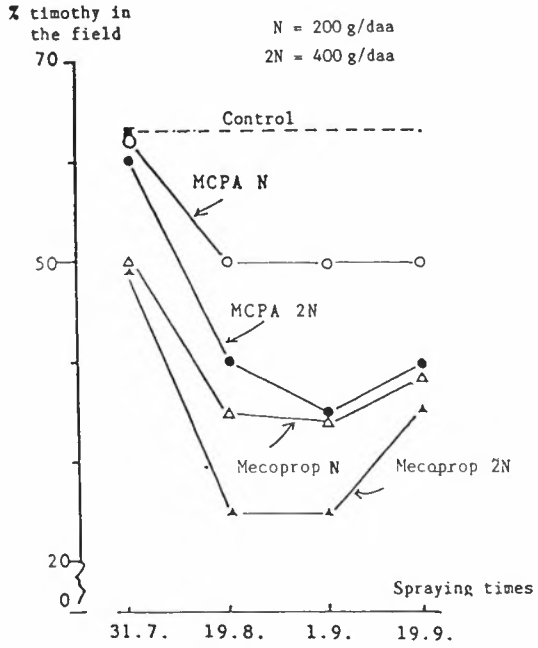

Figure 5. Effects of different amounts and spraying times in 1986 of phenoxycarboxylic acid compounds on the cover of timothy (%) in 1987

Investigations carried out in Canada and the USA showed that small plants of winter wheat, winter rye and cocksfoot became less frost hardy after the addition of phenoxycarboxylic acid compounds (Fowler et al 1958; Freyman & Hamman 1979; Freyman et al. 1982).

Epidermal tissue of grass species was markedly damaged by phenoxycarboxylic acid compounds in investigations carried out by Wortmann (1965). It is quite natural to compare these results with the anatomical and chemical changing of epidermal tissue during the hardening of winter rye as described by Griffith & Brown (1982), Griffith et al. (1985) and Huner et al. (1981).

Phenoxycarboxylic acid compounds can to some degree damage the epidermal tissue of grass leaves, and cause severe winter injury in grasslands when applied in the autumn in regions with long winters. The changes in the epidermal tissue of the plants caused by phenoxycarboxylic acid compounds provide a natural point of comparison with the results presented by Griffith et al. (1985). They concluded as follows: The dramatically thickened cuticle and cell wall of the epidermis and the decreased number of stomata in cold-grown leaves (Huner et al. 1981) may provide protection against penetration and deterioration of the leaf by fungal hyphae. In fact, northern varieties of winter cereals are generally less susceptible to infection by snow moulds than southern varieties (Jamalainen 1974), perhaps as a result of changes in epidermal structure induced by low temperatures.

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Differences between varieties in resistance to snow mould fungi

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In northern Sweden and especially in the inland areas damage caused by snow mould fungi on ley plants is relatively common. In 1962-66 a thorough inventory was made of fungi damage. About 800 trials, with and without quintozene against snow mould fungi, were carried out. The treatment was applied in the autumn in the establishment year and the effect was measured in the first cut in the first year ley. On average, the difference between treated and untreated plots was 15% which gives some idea of the significance of the damage. From analyses of the botanical composition it was obvious that red clover was clearly favored by the quintozene treatment (Vestman 1971).

Varity trials
In the ordinary variety testing, differences can be observed in overwintering capacity between varieties. Usually varieties from southern Sweden suffer most. There is no treatment against snow mould fungi in the variety testing and therefore it is hard to judge to what extent the damage is caused by snow mould fungi. Damage from ice and water is also very frequent.

Varieties with and without quintozene
During the 1970s two experiment series were carried out. Different species and varieties, treated and untreated against snow mould fungi, were tested. One series was carried out at the experimental stations in the district, and all but one were located in the coastland areas. The effect of quintozene was poor. Another series was carried out in the inland areas at the experimental sites Duved, Gunnarn and Pajala. There, the effect of quintozene was more significant.

Experiments at experimental stations
A total of 24 experiments were carried out at the stations. Most of them were harvested over three years and in total there are results from 71 crop years. One-half of each experiment was treated twice a year with 5 kg quintozone per ha around 15 September and 25 October. The treatment was given in the establishment year and in leys I-II. The effect was measured by harvest in leys I-III (See Table 1 for results).

On average the differences between treatments are small. Varieties from southern parts of Sweden seem to sustain more damage from snow mould fungi than those from nothern parts. Thus the timothy variety Vanadis from southern Sweden displays bigger differences between treated and untreated plots than Bottnia II from nothern Sweden. The tendency is the same for the timothy part of the stand according to the botanical

Table 1. Dry matter yield and content of sown variety at the experimental stations. First cut, leys I-III. Average of 71 crop years

	Dry matter yield, kg/ha		Content of sown variety, %	
	With quintozene	Without quintozene (difference)	With quintozene	Without quintozene (difference)
Timothy				
Bottnia II	4 990	-180	94	-5
Vanadis	4 630	-250	88	-7
Engmo	5 120	-370	93	-3
Meadow fescue				
Bottnia II	3 910	-120	85	+1
Mimer	3 740	-230	81	-5
Cocksfoot				
Tammisto	3 570	-140	77	-4
Frode	3 390	-190	69	-5
Reed canary grass				
Commodity	3 870	-60	69	-3
Red clover + timothy				
Bjursele	5 140	-230	17	-1
Ulva	5 090	-330	12	-1
Disa	5 010	-210	12	-1
Hermes	5 020	-360	10	-1

analyses. However, in Engmo from nothern Norway there was a large difference between the treatments. Engmo is regarded as a very hardy variety but it is abviously susceptible to snow mould fungi. It seems to be hard particularly against ice and water, however.

Of the meadow fescue varieties, Mimer from southern Sweden sustained more damage than the north Swedish variety Bottnia II. This is also apparent from the botanical analyses. The cocksfoot variety Frode from southern Sweden was more damaged than the Finnish variety Tammisto. The difference, however, was rather small. There was little difference between the treatments for reed canary grass.

The differences between the red clover varieties were small and difficult to judge. In total yield, red clover + timothy, Bjursele and Disa had the smallest differences between treated and untreated plots. All had a 1 % lower clover content for untreated compared with treated plots. Thus, they all seem to be equally susceptible to snow mould fungi.

On the whole the effect of quintozene was rather small. Since in most experiments there seems to have been very little snow mould fungi, all the trials recorded with snow mould fungi were combined. (See Table 2 for the result). In these experiments the difference between treated and untreated plots was greater. However, the consecutive order between varieties and species was the same.

Experiments in the inland areas

At three experimental sites in the inland area 11 experiments totalling 43 crop years were carried out. The plan was similar to that at the experimental stations but species and varieties were somewhat altered. Treatment with 10 kg quintozene per ha carried out in the autumn of the establishment year and in leys I-III. The experiments were harvested in leys I-IV. The total yield, first and second cut, is given in Table 3. The

	Dry matter yield, kg/ha		Content of sown variety, %	
	With quintozene	Without quintozene (difference)	With quintozene	Without quintozene (difference)
Timothy				
Bottnia II	5 020	-670	96	-11
Vanadis	4 550	-890	90	-20
Engmo	5 250	-1 060	96	-8
Meadow fescue				
Bottnia II	4 200	-570	85	+2
Mimer	3 920	-670	76	-16
Cocksfoot				
Tammisto	3 800	-670	80	-10
Frode	3 610	-730	69	-16
Reed canary grass				
Commodity	3 870	-570	61	-6
Red clover + timothy				
Bjursele	5 290	-640	15	0
Ulva	4 960	-400	9	-1
Disa	4 990	-630	9	-2
Hermes	5 020	-640	7	0

Table 2. Dry matter yield and content of sown variety at the experimental stations. First cut, leys I-III. Average of 16 crop years with snow mould fungi recorded

second cut was usually small and sometimes not harvested at all. The yield of pure sown variety is also shown in the table, i.e. the total yield multiplied by the content of sown grass and clover varieties according to botanical analyses (Hagsands & Landström 1989).

The results are well coordinated with those in the other series, but damage from snow mould fungi was more frequent and the differences between treated and untreated plots were bigger.

The timothy variety Vanadis from southern Sweden suffered more damage from snow mould fungi than Bottnia II. Engmo had, as in the previous series, a big reduction in yield on untreated plots, but according to botanical analyses had a relatively high proportion of the stand also on untreated plots. SvL 0884 from Svaløf, Luleå, thus a variety from nothern Sweden, seemed to be severely damaged by snow mould fungi.

The meadow fescue variety Boris from nothern Sweden was hardier against snow mould fungi than the southern varieties Sena and Mimer. The red fescue variety SvL 01815 was very hardy and not damaged at all, while Reptans suffered the same degree of damage as the meadow fescue varieties.

The differences among the red clower varieties were also small in these experiments, but the consecutive order was the expected one. Bjursele from nothern Sweden was hardier than Björn, which in turn was hardier than Disa from southern Sweden.

Among species, timothy seems to sustain more damage by snow mould fungi than meadow fescue and red fescue.

SUMMARY

This paper deals with two series of experiments carried out in nothern Sweden, one at the experimental stations in the coastland area, and the other at three sites in the inland area. In the experiments, species and varieties are compared, with and without treatment against snow mould fungi.

Table 3. Total dry matter yield and yield of sown varieties in the inland area. First cut + second cut, leys I-IV. Average of 43 crop years

	Total yield, kg/ha		Sown variety, kg/ha	
	With quintozene	Without quintozene (difference)	With quintozene	Without quintozene (difference)
Timothy				
Bottnia II	5 870	-640	4 820	-1 420
Vanadis	5 400	-640	3 940	-1 840
Engmo	5 880	-750	4 910	-1 560
Sv L 0884	6 050	-930	4 950	-1 760
Meadow fescue				
Boris	5 310	-230	4 230	-650
Sena	4 990	-420	3 170	-880
Minter	4 940	-320	3 000	-950
Red fescue				
Sv L 01815	5 040	-40	3 490	-110
Reptans	5 040	-410	3 220	-840
Red clover + timothy				
Bjursele	6 160	-700	610	+20
Björn	6 040	-690	410	-50
Disa	6 100	-860	270	-70
Alsike clover + timothy				
Tetra	6 040	-690	150	0

The results indicate that there are differences in hardiness against snow mould fungi between species and varieties. Varieties from southern Sweden are usually more damaged than varieties from nothern Sweden. In the inland area timothy sustained more damage from snow mould fungi than meadow fescue and red fescue.

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Grass variety reaction to selection for resistance to *Typhula ishikariensis*

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After three to five cycles of selection for resistance to *Typhula ishikariensis* in the main herbage grasses a significant improvement in laboratory survival rate has been achieved. The response to selection is dependent on ploidy and parentage of the original population. Tetraploid populations react more slowly to selection than diploids. Populations of southern origin show a strong improvement in survival rate. A shift in the yield pattern is noted and a higher proportion of the total yield is harvested in the first cut. Natural selection and variation in seed set has reduced the progress of similar selection in material from the northern part of the Nordic countries.

Key words: *Festuca pratensis*, *Lolium perenne*, *Phleum pratense*, snow mould.

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Fungal diseases during the winter rest period constitute an important part of the overwintering complex in the Nordic countries. In perennial grasses these diseases are often the most serious threat to a productive stand (Ekstrand 1955; Mäkelä 1986; Årsvoll 1973).

In South Scandinavia pink snow mould (*Fusarium nivale*) is the most common fungus and may appear also when there is no snow cover. Grey snow mould (*Typhula incarnata*) requires a shorter duration of snow (2-4 months) and is found on perennial grasses in the inner parts of South and Middle Sweden. In the northern part of the Nordic countries speckled snow mould (*Typhula ishikariensis*) and snow scald (*Sclerotinia borealis*) are the most important grass diseases. They require a snow cover during 4-6 months. (Tronsmo 1986.)

In order to resist attacks of these diseases the grass plant has to be well hardened. During the hardening process the carbohydrate content is built up and forms the reservoir to be utilized in the plant respiration during the winter period and at the start of growth in spring. But there are also inherent differences in carbohydrate content, in winter hardiness and in disease resistance (Jamalainen 1974).

A winter hardy plant often ceases growth early in autumn and generally has a larger part of its production in spring and early summer, but less in the autumn regrowth.

Attacks of snow mould can diminish the green matter production quite substantially, especially at the early growth in spring. Ryegrass is the most susceptible herbage grass and may be quite damaged after a winter that is favourable for the snow moulds.

In Table 1 the situation during part of the 1980's is listed and the coefficient of correlation shows that the first cut yield is highly correlated with the degree of snow mould attack as assessed. The winters preceding these harvest years were the coldest during the last decade and there was a permanent snow cover for one or several months also in South Sweden during these winters.

Table 1. Correlation between assessment of snow mould attack in the spring (0-10, 0 = healthy, 10 = dead plants) and green matter yields in the first cut. Diploid perennial ryegrass in the second harvest year at Weibullsholm

Year	No. of plots	Coeff. of correlation	Equation of regression
1982	24	-0.622**	y = 23.5 - 2.19x
1984	20	-0.546*	y = 24.6 - 2.19x
1985	72	-0.109	y = 23.9 - 0.32x
1986	44	-0.836***	y = 44.9 - 3.83x
1987	72	-0.539***	y = 20.8 - 1.29x

For several years now we have made selections for resistance to snow mould in the major herbage species (Jönsson & Nilsson 1986), and the aim of this paper is to demonstrate the differential reaction to selection depending on origin and ploidy of the starting populations.

MATERIAL AND METHODS

The focus of our work was initially on *Fusarium nivale*, but after some years we changed to *Typhula ishikariensis* as this fungus is in many ways easier to handle. There seems to be a good correlation in resistance to these two fungi.

Typhula ishikariensis is isolated from sclerotia collected in nature. The sclerotia are surface sterilized, grown on a malt-yeast-glucose agar and multiplied on autoclaved wheat/oat (50:50) kernels in glass flasks.

The plants to be inoculated are raised in the greenhouse up to 12-16 weeks of age; then they are transferred to a controlled environment chamber and hardened in a temperature down to +2°C for 3-6 weeks. They are supplied with fluorescent light (6 h/day) during the hardening period.

The plants are inoculated by spreading them with the wheat/oat kernels. An aggressive single isolate is used. After inoculation the plants are covered with moist paper and a plastic sheet.

The incubation period varies from 12 to 20 weeks depending on the state of hardening and the susceptibility of the material. The aim is to attain a maximum of 25% and preferably 5-10% surviving plants.

After the incubation period, the plants are left to recover under light and a gradually increasing temperature. Surviving plants are counted and transplanted to the field for seed production. The relative survival rate (RSR) is calculated as the percentage surviving plants in relation to the percentage survival in a standard variety.

This procedure is repeated for up to five cycles. Usually we have a strict pedigree, with the progeny from surviving plants being directly entered in a new cycle of selection

(Fig. 1 gives an example), but sometimes different progenies or material from different selections are mixed in a new cycle of selection. In this way the material is integrated in the conventional breeding material.

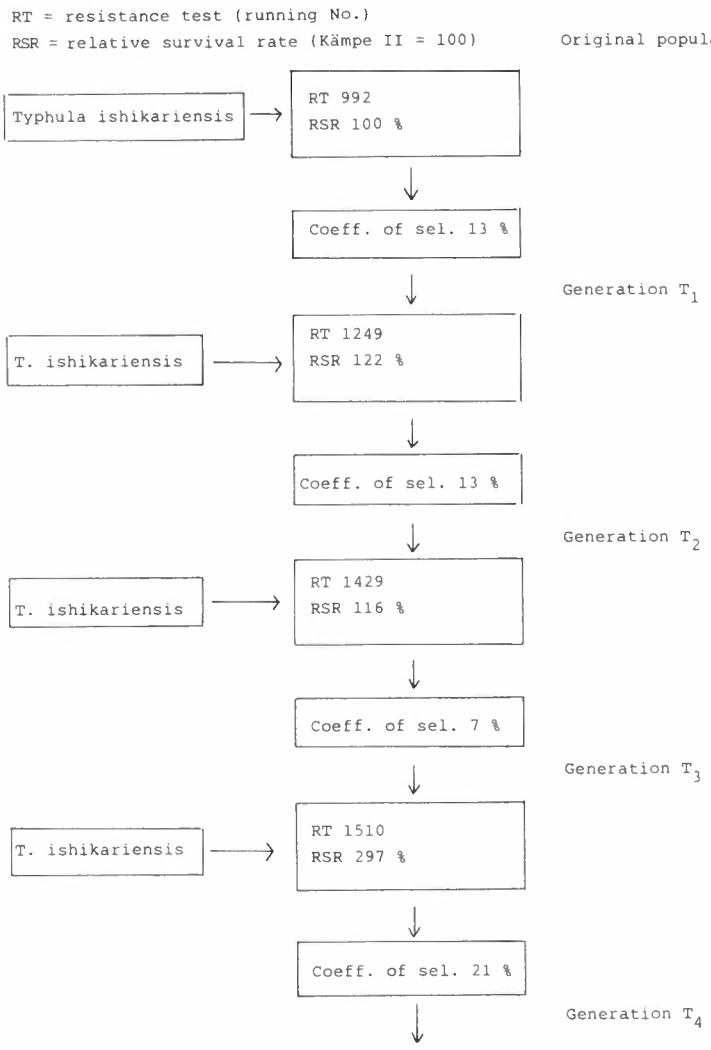

Figure 1. Selection scheme in timothy Kämpe II

RESULTS AND DISCUSSION

Timothy (*Phleum pratense*)

Timothy is considered to be a healthy species with no serious disease problems. In spite of this, we started a selection programme in varieties of both southern and northern

origin. The selection in the southern varieties aimed at an increase in the wintering ability in this material for a utilization further to the north. A still better winter survival in the northern varieties was the primary objective of the selection in that material.

Figure 2 shows the initial negative response to our selection in varieties of both southern and northern origin. Each point is the average of several selections, thus giving a better reliability to the values. Although the result is negative during the first two cycles of selection, the third generation in the southern material shows a fair improvement in survival, and it is expected that the future selections will work in the same direction.

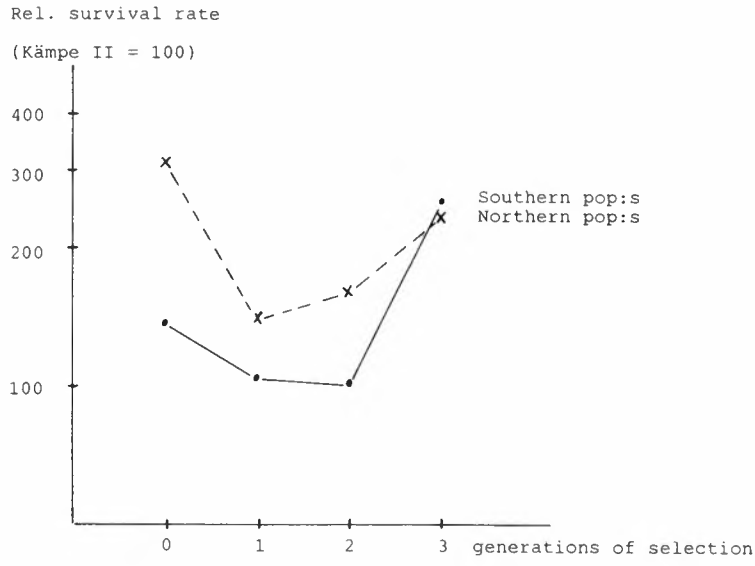

Figure 2. Selection progress in timothy

The northern material starts from a satisfactory position and the decrease in survival rate after one selection is very drastic. Although there has been a steady increase during the next two generations, the level of the unselected material has not yet been reached.

The initial negative response in the southern material is probably due to random effects, but the much stronger negative reaction in the northern material must have another explanation. The selected plants are grown from seed under South Scandinavian conditions (Weibullsholm, 56°N) in spaced plantings. In this way the variation in seed yielding ability can be fully expressed. Plants adapted to more southern conditions and with a higher seed yield will be dominant in the progeny and such plants will not necessarily be the best in snow mould resistance. Thus the seed propagation at Weibullsholm might counteract the selection for snow mould resistance.

Figure 3 presents the more specific picture of how two single varieties have performed during 3-4 cycles of selection. Although the response in the North-Scandinavian Engmo is somewhat variable, the latest selection shows a 30% improvement in survival rate.

The South-Scandinavian Kämpe II demonstrates an excellent rate of improvement after three generations of selection. The slow start of selection effect can be considered typical of a species with high ploidy like the hexaploid timothy.

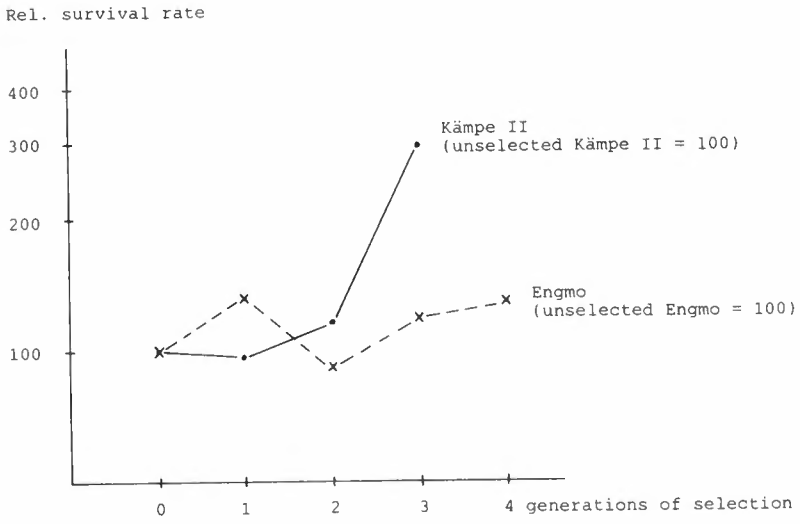

Figure 3. Selection progress in timothy

The yield figures in Table 2 have been collected in Öjebyn in North Sweden (65°N). One generation of selection for resistance to *Typhula* in the variety Kämpe II has decreased the total yield to some extent, but the change in yield distribution is more significant. The selection has a larger first cut yield and a lower regrowth yield. Weibullsholm Plant Breeding Institute in Landskrona (56°N) is the breeder of Kämpe II, and the change in yield distribution is typical for selections in southern material. Such a change has also been demonstrated by Vestman (1986) in North Swedish timothy.

Table 2. Replicated yield trials in timothy

Total dry matter yield Öjebyn	First	Second	Third harvest year
Sown 1987			
Kämpe II, kg/ha	8080	6060	5540
Kämpe II, rel.	100	100	100
Kämpe II T_1, rel.	97	98	89
Sown 1988-89			
Alma, kg/ha	9380	6340	
Alma, rel.	100	100	
Alma T_1, rel.	104	108	
Alma T_2, rel.	99	102	
Percentage distribution of dry matter yield in first/second cut			
Sown 1987			
Kämpe II	47/53	62/38	55/45
Kämpe II T_1	49/51	64/36	60/40
Sown 1988-89			
Alma	55/45	65/35	
Alma T_1	53/47	65/35	
Alma T_2	54/46	66/34	

One and two generations of selection in the Finnish variety Alma have led to a slight yield increase, but the yield distribution is practically unchanged. Alma was bred at the Agricultural Research Institute, Jokioinen, at 61°N.

Selections performed up to the third generation in the variety Engmo (from North Norway, lat. 70°N) have been tested in yield trials in North Sweden (Table 3). The different generations of selection have not been tested in the same trials but are all compared with the control variety Bottnia II. The generations T_1 and T_2 have at least kept the same yield level as the original variety and are also practically unchanged in yield distribution over the growing season.

Table 3. Replicated yield trials in timothy

Total dry matter yield North Sweden	First	Second	Third harvest year
Sown 1979-86			
Bottnia II, kg/ha	8190	7740	6000
Bottnia II, rel.	100	100	100
Engmo, rel.	89	96	102
Sown 1983-84			
Bottnia II, kg/ha	7290	6170	5330
Bottnia II, rel.	100	100	100
Engmo T_1, rel.	97	99	123
Sown 1986			
Bottnia II, kg/ha	7790	5840	7080
Bottnia II, rel.	100	100	100
Engmo T_2, rel.	100	108	103
Sown 1988-89			
Bottnia II, kg/ha	9450	6480	
Bottnia II, rel.	100	100	
Engmo T_3, rel.	81	85	
Percentage distribution of dry matter yield in first/second cut			
Sown 1979-86			
Bottnia II	64/36	75/26	76/24
Engmo	68/32	78/22	81/19
Sown 1983-84			
Bottnia II	54/46	70/30	79/21
Engmo T_1	56/44	75/25	81/19
Sown 1986			
Bottnia II	66/34	82/18	71/29
Engmo T_2	71/29	88/12	76/24
Sown 1988-89			
Bottnia II	58/42	70/30	
Engmo T_3	65/35	67/33	

The third generation (T_3) seems to diverge from this by becoming a more southern type with increased regrowth but a lower first cut yield. Its total yield is also lower than that of the preceding generations.

Meadow fescue (*Festuca pratensis*)

Meadow fescue shows a similar picture as timothy (Fig. 4). The southern varieties start at a lower level but respond much better to selection than do the northern varieties. In

the northern varieties there is also a small negative effect of the first selection in this species.

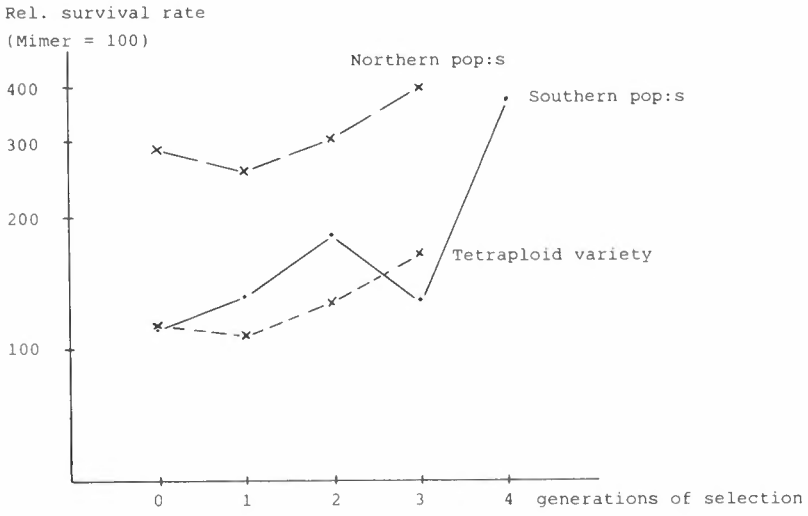

Figure 4. Selection progress in meadow fescue

These meadow fescues are all diploids, which is the normal ploidy level. Selection has, however, also been carried out in a tetraploid variety (Fig. 4). This is a southern type and starts at a similar level as the diploids. As expected the improvement is slower than in the diploids.

When examining in more detail the selections from the two varieties Mimer (southern type) and Salten (northern type) in comparison with their origins (Fig. 5), it is found that the southern variety, like timothy, responds more strongly than the northern variety. The explanation is probably the same.

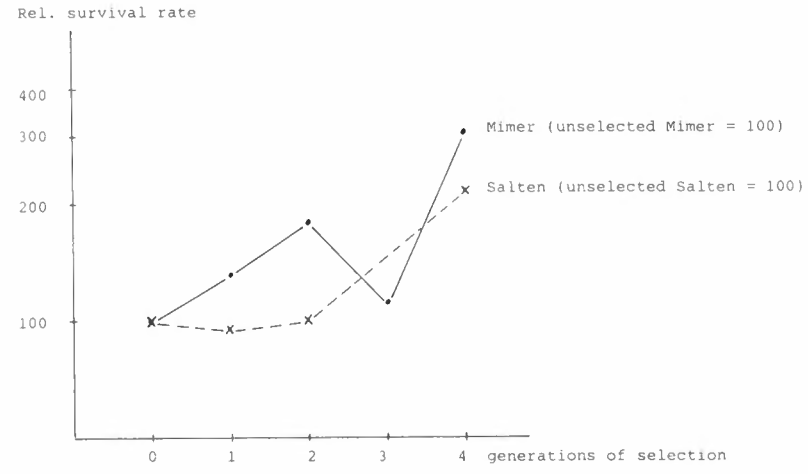

Figure 5. Selection progress in meadow fescue

Two Norwegian varieties of meadow fescue, Løken from Løken Agricultural Research Station lat. 61°N and Salten from Vågønes Agricultural Research Station, lat. 67°N, were examined in more detail (Table 4). In Løken two cycles of selection were performed with *Fusarium nivale* as test fungus, whereas in Salten one selection was carried out with *Fusarium* and a second with *Typhula*, The yield was unchanged or affected only slightly in a negative direction, and the yield distribution was also practically unaffected.

Table 4. Replicated yield trials in meadow fescue

Total dry matter yield North Sweden year	First	Second	Third harvest
Sown 1985-88			
Löken, kg/ha	7930	8290	8130
Löken, rel.	100	100	100
Löken Fus_2, rel.	94	96	97
Sown 1987-88			
Salten, kg/ha	8150	7570	8630
Salten, rel.	100	100	100
Salten Fus_1T_1, rel.	99	100	97
Percentage distribution of dry matter yield in first/second cut			
Sown 1985-88			
Löken	60/40	65/35	64/36
Löken Fus_2	60/40	66/34	63/37
Sown 1987-88			
Salten	65/35	62/38	58/42
Salten Fus_1T_1	65/35	63/37	58/42

Perennial ryegrass (*Lolium perenne*)

Work on perennial ryegrass initially produced a very good response to the selections, as was previously reported (Jönsson & Nilsson 1986). However, when the results of the selections are now summarized, the average response curves (Fig. 6) do not seem so convincing. The improvement is certainly there, both on the diploid and on the tetraploid level, but after the second cycle of selection no further increase in survival rate has occurred. We think this lack of continued increase in survival rate is due to the fact that new, unselected material is introduced not only as new starting populations but also into populations which have already undergone one or several generations of selection. The general performance of the populations is certainly improved by this addition, but the addition also slows down the further increase in survival rate after snow mould inoculation. Another possible explanation is that we have, in the first two selection cycles, exhausted all available genetic variation for the character. This is, however, not very likely when considering the shape of the selection curves in timothy and meadow fescue. In those species the best effect has occurred first in the second or third generation of selection.

The ploidy level influences the selection effect as was shown in meadow fescue. In approximate figures the selections have changed the relative survival rate in the diploids from 100 to 300 but in the tetraploids from only 150 to 200 (Fig. 6).

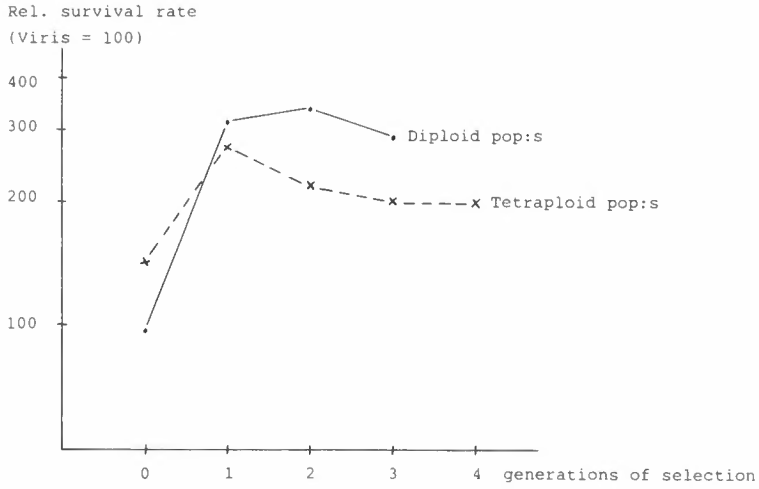

Figure 6. Selection progress in perennial ryegrass

The selection has generally improved the performance during the second harvest year. In Table 5, yield figures are given for two generations of selection (test fungus:*Fusarium nivale*) in three varieties. We had no unselected material of E39 and E40, and Viris is used as standard in this case. The yield distribution has, especially in the second harvest year, shifted to a larger proportion of the total yield being harvested in the first cut. These varieties were developed at Weibullsholm, and the test station Bjertorp, situated in West Sweden at 58°N.

Table 5. Replicated yield trials in diploid perennial ryegrass

Total dry matter yield	First	Second harvest year
Weibullsholm, sown 1985		
Viris, kg/ha	9260	6760
Viris, rel.	100	100
E 39 Fus_1, rel.	96	105
E 39 Fus_2, rel.	98	103
E 40 Fus_1, rel.	108	111
E 40 Fus_2, rel.	110	112
Bjertorp, sown 1985		
E 23, kg/ha	7630	10510
E 23, rel.	100	100
E 23 Fus_1, rel.	99	100
E 23 Fus_2, rel.	95	107
Percentage distribution of dry matter yield in first cut/regrowth cut		
Weibullsholm		
Viris	56/44	40/60
E 39 Fus_1	59/41	47/53
E 39 Fus_2	58/42	52/48
E 40 Fus_1	55/45	41/59
E 40 Fus_2	52/48	41/59
Bjertorp		
E 23	34/66	38/62
E 23 Fus_1	37/63	43/57
E 23 Fus_2	35/65	42/58

CONCLUSION

This work has shown that the survival rate after a snow mould attack may be improved to a great extent by selection in timothy, meadow fescue and perennial ryegrass of South Scandinavian origin. After one or several cycles of selection a larger part of the total yield is harvested in the first cut, i.e. a shift towards a more northern type occurs. Natural selection and variation in seed set have reduced the progress of similar selection in material from the northern part of the Nordic countries. A balance is attained in which the selection for snow mould resistance acts towards a more northern type whereas adaptation to the South Swedish environment and higher seed set of southern material selects towards a more southern type.

ACKNOWLEDGEMENT

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Resistance to snow mould fungi in breeding material of grasses

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Tronsmo, A.M. 1992. Resistance to snow mould fungi in breeding material of grasses. Norwegian Journal of Agricultural Sciences, Supplement No. 7. 35-38. ISSN 0801-5341.

Breeding materials of timothy and cocksfoot have shown wide variation in resistance to snow mould fungi. There is a significant positive correlation between resistance to *Microdochium nivale* and *Typhula ishikariensis*. The heritability of the traits indicates that improvement by selection is attainable.

Key words: Cocksfoot, *Microdochium nivale*, timothy, *Typhula ishikariensis*.

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Since 1983 all the breeding material of grasses in the official Norwegian Grass Breeding Programme have been tested for resistance to the two snow mould fungi *Microdochium nivale* (syn. *Fusarium nivale*) and *Typhula ishikariensis*. The breeding materials have been evaluated for agronomic characters in field trials for three successive years. During the same period, the resistance to snow mould fungi has been artificially tested.

METHOD

The method used for detection of resistance has been inoculation of greenhouse-grown, cold-hardened plants with a mycelial suspension of the fungus, followed by incubation under artificial snow cover for 6-10 weeks before recovery and assessment of resistance. The resistance has been assessed according to a scale from 0 to 9 (Tronsmo 1984).

The aim of the procedure is to minimize the random variation by controlling the environmental conditions. Since our facilities only allow a semicontrolled environment, all genotypes have been tested in four replicates or more, with the replicates separated in time throughout the year.

RESULTS AND DISCUSSION

In both timothy and cocksfoot the collected germ-plasm, which is the starting material for the breeding programme, shows a great variation in resistance (Figs. 1 and 2). There are some genotypes with very low snow mould resistance, and others with very high resistance, much higher than that found in any Norwegian cultivar. The Norwegian

cultivars of cocksfoot all have a moderate snow mould resistance, none are very susceptible, or very resistant. In timothy, however, we have cultivars with high snow mould resistance.

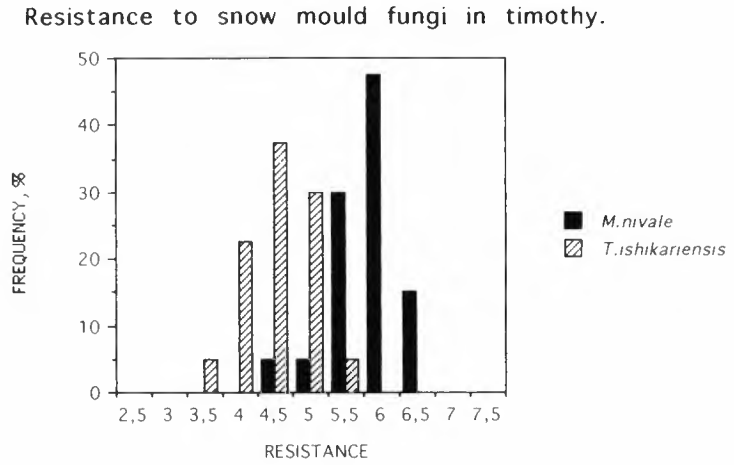

Fig. 1. Frequency distributions for resistance to snow mould fungi in timothy populations (N = 121)

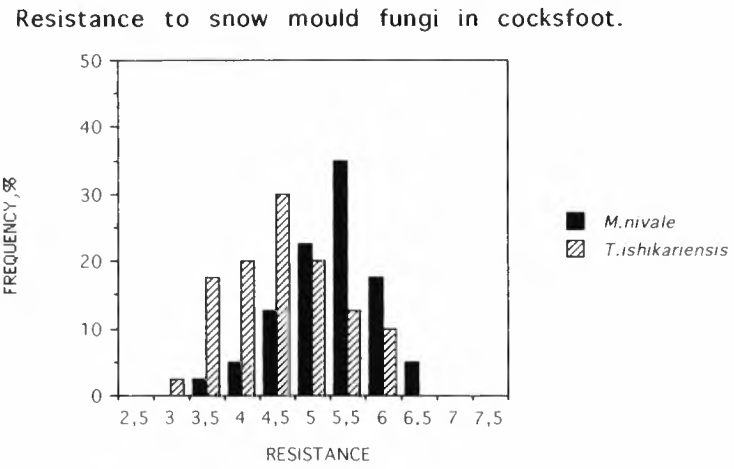

Fig. 2. Frequency distributions for resistance to snow mould fungi in cocksfoot populations (N = 133)

One of the aims of our project is to simplify the procedure for selecting disease resistant cultivars. One of the questions usually asked is: Is there any connection between the resistance to *T. ishikariensis* and resistance to *M. nivale*?

The calculation of the phenotypic correlations for the traits in three different breeding materials (cocksfoot and timothy) revealed a positive, significant correlation (between 0.4 and 0.5) between resistance to *T. ishikariensis* and resistance to *M. nivale* (Table 1).

A selected material, consisting of half sib families from polycrosses of cocksfoot has been further analysed to obtain an estimate of the genetic variation. Anaysis of variance revealed a significant difference between populations, and the components of variation could be used to estimate the broad sense heritability (Falconer 1960).

Plant material	N	R (corr)
Timothy	121	0.42
Cocksfoot	133	0.51
Cocksfoot, half sib families	304	0.43

Table 1. Phenotypic correlations (R) between resistance to *Typhula ishikariensis* and *Microdochium nivale* in grasses

The broad sense heritability is an expression for the proportions between the genotypic and the phenotypic variance, and will give us an indication of any possible improvement by selection.

For resistance to the two snow mould fungi, *T. ishikariensis* and *M. nivale*, the heritability was found to be 0.42 and 0.49 (Table 2). These results indicate good possibilities for progress by selection for snow mould resistance.

Character, Resistance to:	Heritability h
T.ishikariensis	0.42
M.nivale	0.49

Table 2. Heritability (h) for resistance to snow mould fungi

Half sib families from four polycrosses of cocksfoot could be further analysed. The genotypic correlation between the traits «Resistance to *T. ishikariensis*» and «Resistance to *M. nivale*» was calculated from the analysis of covariance of this material. The genotypic correlation was found to be close to 1. The interpretation of this is that resistance to the two snow mould species depends on the same trait. If this is the case in other germ-plasm collections and other species as well, the consequence for the breeding programme should be that selection for resistance to one snow mould fungus should give resistance to both fungi.

This finding also implies that it can be worthwhile to investigate further what mechanisms the resistance is based on. If we are dealing with one trait, not two, a further simplification or improvement of the procedure for testing snow mould resistance could be achived by minimizing the environmental variation by growing, inoculating and incubating the plants *in vitro*. An approach reported by Dr. Huang McBeath, at this seminar, could then be easily applied for screening breeding material.

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Hardening ability of some winter wheat, winter rye and winter barley varieties

Use of conductivity method in evaluating the hardening ability of overwintering crop species

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Hömmö, L.M. 1992. Hardening ability of some winter wheat, winter rye and winter barley varieties. Use of conductivity method in evaluating the hardening ability of overwintering crop species. Norwegian Journal of Agricultural Sciences, Supplement No. 7. 39-50. ISSN 0801-5341.

A study of the hardening ability of 12 winter wheat, 8 winter rye and 5 winter barley varieties was carried out using the electric conductivity method. Leaf samples were collected from the field trial during the hardening period and after frost treatment the conductivity of an elfusate was determined. The amounts of calcium and potassium ions in the leachate were also determined. The injury index and indexes for calcium and potassium were calculated. During the hardening period there was a distinct decrease in the conductivity values indicating the increase in the level of hardening. The same decrease was also found in the amounts of both calcium and potassium ions in the effusate. In most cases a clear negative correlation was found between the index values and overwintering capacity of varieties. The results of this study indicate that the conductivity method can be used in estimating the level of winter hardiness of winter wheat, winter rye and winter barley varieties.

Key words: Cold hardiness, conductivity, frost tolerance, ion leakage, rye, winter barley, winter wheat.

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Since both winter wheat and winter rye reach their northernmost growth limit in Finland, winter barley in Sweden, one of the most important tasks in the breeding work of these species is to enhance their winter hardiness. This, however, is a rather complex undertaking because of the many different kinds of stresses and changes in weather conditions during the winter period. Consequently, there is a great need for laboratory testing methods that enable the breeder to obtain sufficiently reliable estimates on the winter hardiness of varieties and breeder lines of overwintering crops. Laboratory methods are of particular value in the screening of early generations of these crops. To be of any use to practical breeders, these methods should be repeatable, simple, rapid and cheap. To help this situation an internordic project was initiated in 1989 with the aim of developing reliable laboratory testing methods for winter hardiness breeding.

Winter hardiness of an overwintering crop variety depends on its ability to harden during the autumn period. The hardening process in itself includes a very complicated series of plant reactions to changes in the environment (decreasing temperature, shortening of photoperiod) by altering its metaboly on a cellular level. If the plant is unable to make these changes, or if they are delayed, the first frosts in winter will lead to cell damage.

The use of the electric conductivity method, or ion leakage method as it is also called, is based on the idea that during frost treatment the cell membranes are damaged to some degree and this leads to an efflux of ions through the cell membrane to the effusate. The basic assumption is that the greater the damage to the tissue the more ions that are leaked out.

The electric conductivity method was introduced into frost hardiness research by Dexter et al. (1930, 1932), and it has been quite extensively used by many researchers ever since. Flint et al. (1967) studied the frost resistance of ornamental shrubs, strawberry crowns and the roots of apple trees. They also determined different kinds of injury indexes to obtain better evaluations of the amount of cell injury. According to Wilner (1960) the ion leakage method is a very reliable means of determining "both relative and absolute ratings of hardiness of apple trees as well as a means of studying the effect of certain seasonal changes on their (plant) winter survival". Aronsson & Eliasson (1970) found good correlation between frost hardiness of hardy Scots pine tissues and cell injury estimations obtained by conductivity measurements. The seasonal changes in hardening and dehardening of the mountain plant *Diapensia lapponica* were studied with the aid of the conductivity method by Junnila (1985). Sukumaran & Weiser (1972) were able to differentiate *Solanum* species representing a wide range of frost tolerance by using conducti- vity measurements.

Frost hardiness of overwintering cereals has been studied by electric conductivity measurements by e.g. Chen et al. (1983) and Uemura & Yoshida (1984). They found that this method gave reliable estimations of the amounts of damaged or dead cells after frost treatment. Palta et al. (1977a, 1977b) used onion bulb cells in their studies, and they discovered that not only dead, but also reversibly or irreversibly damaged cells, are responsible for the efflux of ions to the effusate.

In this study the state and progress of hardening in field conditions in the autumn time were studied in 12 winter wheat, 8 winter rye and 5 winter barley varieties by the conductivity method. The amount of calcium and potassium ions in the effusate was also determined. On the basis of these measurements injury indexes were calculated and the correlations between the index values and overwintering capacity were studied.

MATERIALS AND METHODS

The field trial from which the leaf samples for conductivity measurements were collected was sown in Jokioinen (60°49'N, 23°29'E) on 27 August 1990. The trial comprised 24 winter wheat, 13 winter rye, 5 winter triticale and 12 winter barley varieties. In these conductivity measurements 12 winter wheat varieties (Linna, Aura, Vakka, Albidom 12, Kharkov, Norstar, Mironowskaja 808, Frederick, Goertzen 5559, Rida, Longbow and Vitus), 8 winter rye varieties (Musketeer, Anna, Voima, Talovskaja

12, Vågonäs höstråg, Jussi, Petkus II and Danko) and 5 winter barley varieties (Borwina, Frost, Lady, Igri and Maris Otter) were included.

The trial entries were sown in one-metre-long rows, and the trial was completely randomized with four replicates.

The leaf samples were collected from the field trial once a week, beginning on 9 October. The last samples were collected on 20 November just before the permanent snow cover.

The first leaves of the plants were cut in the field and put into the plastic bags. The leaves were rinsed quickly with cold tap water, and control samples (10 leaves per variety) were put on ice to keep them cool while being prepared for the conductivity measurements.

The rest of the samples (about 20 leaves per variety) were transferred in the plastic bags into the freezing chamber and the freezing programme was started immediately. At the beginning of the programme the temperature was at about 0°C. The whole programme took 24 hours. During the first eight hours the temperature dropped to -12°C in 1.5°/hour. The temperature was maintained at -12°C and then reset to 0°C after eight hours.

After frost treatment, while preparing the samples for conductivity measurements, the plastic bags with the leaves were covered with ice in a styrox box to minimize any cell injury that might be caused by the rapid changes in temperature.

The samples in all the conductivity measurements comprised five leaf pieces originating from five different plants. The leaf pieces were weighed together to provide a sample of about 100 mg. After this the samples were shaken for about 5 h at room temperature in test tubes containing 20 ml of deionized water. Before immersion in water the five leaf pieces were put in a single-use needle (Terumo nr. 14) to ensure that all the pieces were totally under water.

The shaking period was followed by determination of the conductivity of the effusate. These measurements were carried out using the Radiometer Copenhagen CDM 83 conductivity meter. After the first measurements were taken half of the frost-damaged samples were totally killed by submerging them for five minutes in liquid nitrogen. All samples were shaken in the effusate for one more hour and the final conductivity measurements were carried out.

Finally, all the effusates were frozen in single-use plastic test tubes for later determination of calcium and potassium content.

The effusate was analysed for calcium and potassium content by atomic absorption spectrophotometer (Ca^{2+} by Perkin-Elmer 4000 and K^{+} by Varian techtron 1200).

Based on the obtained conductivity values and values for leaked Ca^{2+} and K^{+} ions, the index of injury and indexes for calcium and potassium were calculated according to the formulae:

INDEX OF INJURY

$$\frac{(B - A) \times 100}{(C - A)}$$

where A = conductivity of effusate of unfrozen sample
B = conductivity of effusate of frost-injured sample
C = conductivity of effusate of frost-injured and then liquid-nitrogen-killed sample

INDEX OF CALCIUM AND POTASSIUM CONTENT IN EFFUSATE

$$\frac{(Ca^{2+}B - Ca^{2+}A) \times 100}{Ca^{2+}C - Ca^{2+}A}$$

$$\frac{(K^{+}B - K^{+}A) \times 100}{K^{+}C - K^{+}A}$$

where $Ca^{2+}A$, $K^{+}A$ = calcium and potassium content in effusate of unfrozen sample
$Ca^{2+}B$, $K^{+}B$ = calcium and potassium content in effusate of frost-injured sample
$Ca^{2+}C$, $K^{+}C$ = calcium and potassium content in effusate of frost-injured and then liquid-nitrogen-killed sample

The overwintering capacity values of varieties used in correlation analyses were based on the means of winter survival percentages of varieties in field trials sown in 17 sites throughout the Nordic countries. These field trials are a part of an internordic winterhardiness project initiated by Internordic Plant Breeding (SNP) in 1989. The trials were sown in autumn 1989 according to the plan described above.

Pearson's correlation analysis (SAS Institute Inc. 1985) was used in studying the relations between overwintering capacity of varieties and various indexes

RESULTS

During the hardening period (9 October-15 November 1990) the state and progress of hardening of winter wheat, winter rye and winter barley varieties were studied using the electric conductivity method. In all species the conductivity of an effusate decreased during the hardening period (Table 1). In winter wheat the values were low at the beginning of the period so the change was not so clear-cut, but in winter rye and in winter barley a very pronounced decrease in ion leakage was observed.

The average winter survival percentages of varieties in winter 1989-90 were calculated by Hjortsholm (1991) on the basis of results from field trials of the Internordic Plant Breeding project (Table 2). These survival percentages were used in this study as overwintering capacity values of varieties.

Table 1. The conductivities of effusates in which frost injured leaf samples of winter wheat, winter rye and winter barley have been soaked. Samples were collected from field trials during the hardening period in 1990. The conductivity values are µS/cm

	October			November		
	9-17	22-25	29.10-1.11	5-8	12-15	19-20
Winter wheat:						
Linna	13.0	8.6	12.5	7.5	13.2	6.6
Aura	13.0	8.4	10.2	12.1	10.1	7.7
Vakka	9.3	8.1	12.3	17.6	10.0	5.8
Albidom 12	10.3	6.3	9.3	11.7	9.8	6.7
Kharkov	7.2	11.4	9.4	12.4	10.6	6.8
Norstar	6.6	6.7	8.8	6.5	8.7	5.9
Mironowskaja 808	30.0	9.4	9.7	9.8	9.1	5.7
Frederick	17.5	10.5	16.3	9.3	12.8	5.5
Goertzen 5559	11.7	7.1	7.1	11.2	9.2	8.2
Rida	16.0	14.3	26.2	18.1	10.3	4.7
Longbow	51.0	13.5	14.1	35.9	14.5	5.7
Vitus				90.9	33.7	8.7
Winter rye:						
Musketeer	14.3	19.1	12.7	4.8	6.0	
Anna	40.5	14.1	11.1	5.3	7.6	
Voima	42.0	19.3	13.1	6.5	7.5	
Talovskaja 12	39.6	6.5	9.1	6.1	5.6	
Vågonäs höstråg	32.4	10.4	10.1	7.5	5.9	
Jussi	39.5	12.4	18.1	9.0	6.5	
Petkus II	79.2	24.8	28.5	5.6	7.3	
Danko	63.9	35.4	11.5	10.5	6.4	
Winter barley:						
Borwina	86.0	78.1	37.4	30.1	25.4	
Frost	95.6	71.3	68.5	14.3	21.3	
Lady	97.6	74.0	72.5	47.8	21.9	
Igri	81.4	58.9	72.9	22.3	31.2	
Maris Otter	97.3	76.7	81.6	11.1	18.9	

Table 2. Winter survival percentages of winter wheat, winter rye and winter barley varieties. Values are based on results from field trials in all the Nordic countries. The mean values are calculated by Hjortsholm (1991)

Winter wheat	Survival %	Winter rye	Survival %	Winter barley	Survival %
Linna	86.3	Anna	83.5	Borwina	52.0
Aura	82.1	Voima	80.0	Frost	38.8
Vakka	81.2	Talovskaja	79.9	Lady	31.7
Albidom 12	78.9	Vågonäs	79.0	Igri	28.3
Norstar	75.8	höstrog		Maris Otter	13.7
Frederick	72.5	Jussi	77.9		
Goertzen 5559	67.4	Petkus II	69.4		
Rida	67.3	Danko	67.3		
Longbow	46.4				
Vitus	2.3				

When studying the correlations between overwintering capacity and index of injury of varieties, a definite negative correlation was found in most cases. There was even statistical significance in some results, although the number of varieties studied was quite low (Table 3).

Table 3. Correlations between overwintering capacity of varieties and index of injury. Index values are calculated on the basis of conductivity values of the effusates in which leaf samples have been soaked

	OVERWINTERING		
	Winter wheat	Winter rye	Winter barley
INDEX 1 (9-17.10)	-0.86** N = 10	-0.76* N = 7	-0.70 N = 5
INDEX 2 (22-25.10)	-0.32	-0.88 **	-0.37
INDEX 3 (29.10-1.11)	0.23	-0.49	-0.76
INDEX 4 (5-8.11)	-0.96 ***	-0.66	-0.12
INDEX 5 (12-15.11)	-0.77 **	0.47	-0.60

At the beginning of the hardening period the amounts of leaked calcium and potassium ions in the effusate varied in winter wheat between 0.00-0.88 mg/l and 1.13-14.91 mg/l for calcium and potassium respectively. The same values were 0.10-3.70 mg/l (calcium) and 3.37-18.96 mg/l (potassium) for winter rye and 1.45-3.65 mg/l (calcium) and 23.1-27.7 mg/l (potassium) for winter barley.

At the end of the test period the values for winter wheat were 0.15-0.81 mg/l (calcium) and 1.32-2.34 mg/l (potassium), for winter rye 0.08-0.38 mg/l (calcium) and 0.63-1.24 mg/l (potassium) and for winter barley 0.64-2.78 mg/l (calcium) and 1.63-7.05 mg/l (potassium).

The amounts of calcium and potassium ions in the effusate decreased very markedly during the hardening period in winter rye and winter barley varieties. In most winter wheat varieties the amounts of both ions in the effusate were already low when the measurements were first carried out and consequently no clear-cut changes were observed (Figs. 1-6).

In all species there was a distinctly negative correlation between the overwintering capacity of varieties and calcium and potassium indexes (Tables 4, 5, and 6). The correlation between the potassium index and overwintering capacity in particular was usually very clear.

DISCUSSION

The ability of plants to react to the lowering of the temperature and shortening of the photoperiod by changing their cell metaboly is the prerequisite to good winter hardiness. In particular changes in chemical properties of cell membranes during acclimation are supposed to be of great importance (Kasamo 1988; Palta 1977a, b; Palta & Li 1982; Steponkus 1984; Uemura & Steponkus 1989; Uemura & Yoshida 1984). It is generally agreed that the plasma membrane is the primary site of freezing injury, but

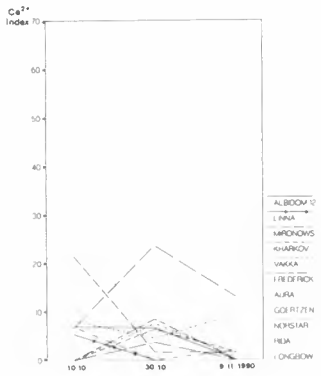

Fig. 1. The changes in the calcium index values of winter wheat varieties during the hardening period in autumn 1990

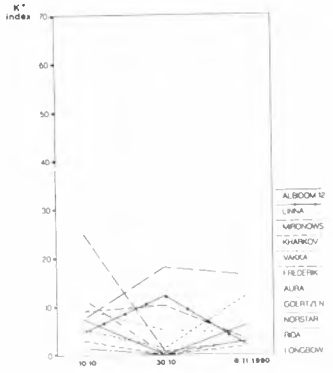

Fig. 2. The changes in the potassium index values of winter wheat varieties during the hardening period in autumn 1990

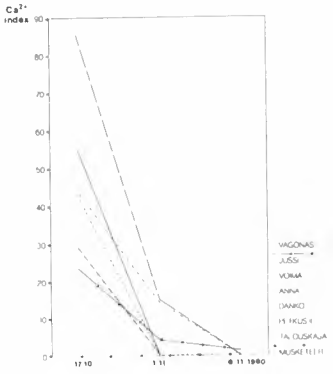

Fig. 3. The changes in the calcium index values of winter rye varieties during the hardening period in autumn 1990

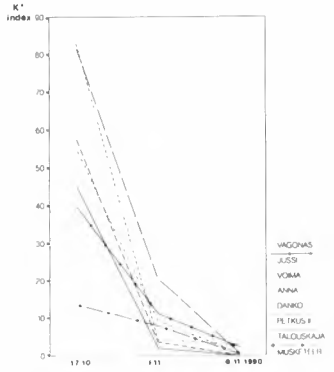

Fig. 4. The changes in the potassium index values of winter rye varieties during the hardening period in autumn 1990

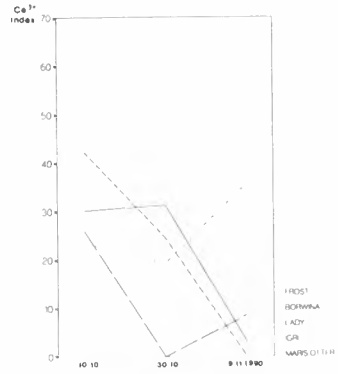

Fig. 5. The changes in the calcium index values of winter barley varieties during the hardening period in autumn 1990

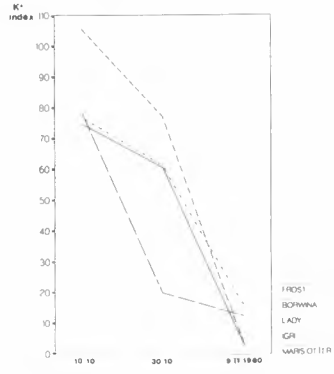

Fig. 6. The changes in the potassium index values of winter barley varieties during the hardening period in autumn 1990

Table 4. Correlation between overwintering capacity of winter wheat varieties and their state of hardening determined by Ca^{2+}- and K^+-contents in the effusate

Date of collecting samples from field		Correlation coefficients Overwintering	
10.10.90	Ca^{2+}	-0.80**	N = 9
	K^+	-0.87**	N = 9
30.10.90	Ca^{2+}	0.01	N = 9
	K^+	0.16	N = 9
6.11.90	Ca^{2+}	-0.97***	N = 9
	K^+	-0.95***	N = 9

Table 5. Correlation between overwintering capacity of winter rye varieties and their state of hardening determined by Ca^{2+}- and K^+-content in the effusate

Date of collecting samples from field		Correlation coefficients Overwintering	
17.10.90	Ca^{2+}	-0.54	N = 7
	K^+	-0.82*	N = 7
1.11.90	Ca^{2+}	0.23	N = 7
	K^+	0.12	N = 7
8.11.90	Ca^{2+}	-0.01	N = 7
	K^+	-0.62	N = 7

Table 6. Correlation between overwintering capacity of winter barley varieties and their state of hardening determined by Ca^{2+}- and K^+-content in the effusate

Date of collecting samples from field		Correlation coefficients Overwintering	
10.10.90	Ca^{2+}	-0.31	N = 5
	K^+	-0.77	N = 5
30.10.90	Ca^{2+}	0.44	N = 5
	K^+	0.78	N = 5
6.11.90	Ca^{2+}	-0.02	N = 5
	K^+	-0.16	N = 5

how this comes about is still not clear. As a result of freezing injury, ions and also sugars are leaked out from damaged cells. The electric conductivity method is based on an assumption that the more the cells of a plant are damaged during the frost treatment, the higher the conductivity of an effusate and the poorer the frost hardiness of a given variety.

In this study the hardening ability of some winter wheat, winter rye and winter barley varieties was studied using the electric conductivity method. According to this method most of the winter wheat varieties were already quite well hardened by the be-

ginning of the test period, and only a slight decrease in conductivity values during the hardening period was observed.

The conductivity values of winter rye and winter barley cultivars were high at the beginning of the test period, but they decreased, especially in rye, very rapidly. The last conductivity values were determined just before the permanent snow cover. On the whole, winter rye varieties had the lowest conductivity values, and thus it is expected that their hardening ability and also winter hardiness will be very good. The conductivity values of winter wheat varieties were also quite low, indicating rather good winter hardiness. In winter barley the conductivity values were still very high in the late autumn, which means that winter barley varieties were inadequately hardened before the snow cover, and the plants will in all probability be damaged to some degree during the winter time.

The injury index was determined in order to take into consideration the amount of ions leaked out of cells of unfrozen samples and also the total ion leakage from samples killed by liquid nitrogen. Usually a very clear negative correlation was found between the overwintering capacity of varieties and the values of the injury index.

The amounts of calcium and potassium ions in the effusate were also determined and the calcium and potassium indexes were calculated in the same way as the injury index. These indexes were used to investigate how well the amount of leaked ions in the effu- sate correlates with the overwintering capacity of varieties, and whether these two ions differ from each other in this respect.

In this study the concentration of potassium in the effusate was about 10 times that of calcium. According to Palta et al. (1977a) the major cation present in the effusate is potassium. They found that when potassium and the same amount of correspondent anion (probably Cl^-) are combined they account for practically all of the conductivity of an effusate. In their onion bulb scale studies they found the amount of leaked potassium ions to be almost 100 times that of calcium.

A pronounced negative correlation was found in most cases between calcium and potassium indexes and overwintering capacity of varieties. The potassium index values seem to give more reliable estimates of the state of hardening of varieties than the calcium indexes. This is also to be expected since the amounts of calcium in the effusate are very small.

There are considerable fluctuation especially in the values of winter wheat varieties during the hardening period. The reason for this could be that winter wheat varieties were already quite well hardened by the beginning of the test period, and the amount of ions leaked out of the cells possibly always varies within some limits. According to Pomeroy et al. (1975) the probability of dehardening during the quick preparation of samples at room temperature is very small, at least as far as winter wheat or winter barley varieties are concerned. Thus the samples entered in the freezing test should have the same level of hardening they had obtained in the field. On the other hand Pomeroy et al. (1975) discovered that winter wheat varieties in particular dehardened quite easily during a prolonged period of slightly higher temperatures.

In Finland the weather in autumn 1990 was quite cold at the end of September and at the beginning of October, so the conditions were very favourable for the hardening of plants. There was a warm period in the middle of October (9-18 October) during which the daily temperatures were above $+10°C$, and night temperatures were also

between 0°C and + 10°C. After this warm period, the temperature began to drop again, the night temperatures in particular going down quite suddenly.

Most of the variation in the conductivity and also in the calcium and potassium values could be explained according to the weather conditions during the hardening period. The long warm period in October could have induced the dehardening process especially in sensitive winter wheat varieties. In dehardened plants the freezing test resulted in an increased incidence of cell injury.

The overwintering capacity values used in this study are based on survival percentages in field trials from the year 1989-90. That particular winter was very mild throughout the Nordic countries, and this could have obscured the results of the correlation analysis to some extent. The survival percentages of winter rye varieties in particular were very high. In these conditions it is difficult to differentiate between varieties with almost the same level of winter hardiness, and this could have caused inaccuracy in the ranking list of varieties.

SUMMARY

According to the results of this study the electric conductivity method seems to give quite reliable estimations of the level of winter hardiness of winter wheat, winter rye and winter barley varieties. Potassium seems to be the main cation leaked out of the damaged cells. A clear negative correlation was found between the amount of K^+-ions in the effusate and the overwintering capacity of varieties.
Since the electric conductivity method is quite simple, reproducible and reliable, it could possibly also be used in practical breeding work when breeding material is screened on the basis of winter hardiness.

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Test for tolerance to grey snow mould (*Typhula spp.*) in winter barley

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Elovson, L. & C. Nilsson 1992. Test for tolerance to grey snow mould (*Typhula spp.*) in winter barley. Norwegian Journal of Agricultural Sciences. Supplement No. 7.51-54. ISSN 0801-5341.

There are wide differences in tolerance to grey snow mould (*Typhula incarnata*) in winter barley. The objective of this project is to find a means of selection for good tolerance to this disease in a breeding material of winter barley. The test method is, with some small modifications, the same as that used for snow mould tests in grasses at our institute, involving artificial inoculation with *T. ishikariensis* and the provision of a simulated snow cover during the incubation period. The results reflect, with few exceptions, a very good consistency between years regarding the reaction of the different varieties. The old six-row variety Fimbul II has a high level and the two-row variety Igri a low level of tolerance to grey snow mould. In the breeding material there is a wide range of reactions. Under the very severe disease pressure in the trials, it was found that the survival rate varies from 0 to 80%. This has provided excellent opportunities for selection.

Key words: *Hordeum vulgare*, snow mould, *Typhula* spp., winter barley.

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Grey snow mould, caused by the fungus *Typhula incarnata,* is the most serious overwintering disease of winter barley in northern Europe (Mielke 1990).

The disease can be controlled by a fungicide treatment if applied just before the onset of winter, but as the treatment must be carried out before the infection rate in the field is known, it is always difficult to deside whether or not to spray. A general treatment also leads to an unnecessary use of fungicides, which is an undesireable situation.

As a complement or a replacement to the fungicide treatment, several plant husbandry measures can be taken to decrease the risk of a severe outbreak of grey snow mould. The most important of these measures are:

1. Apply a sound crop rotation. *T. incarnata* is a facultative parasite and can survive in the field, either as sclerotia in the soil or as mycelium on dead or living plant tissues (Jacobs & Bruehl 1986). As the host range of the fungus includes most of the cultivated and weedy grasses, this measure also includes control of grassy weeds and cereal volunteers, especially barley.

2. Bury the inoculum with deep ploughing.

3. Avoid a too early sowing and too high plant densities.

4. Keep the barley healthy and vigorous. *T. incarnata* can only infect weakened plants and every measure that can keep the winter barley healthy, helps to prevent an attack of grey snow mould (Lehman 1965).

5. Use tolerant varieties. Completely resistant barley varieties do not exist, but there are wide variations in tolerance to *T. incarnata* among the winter barley varieties and this fact forms the background for the work presented here.

With the good selection method against snow mould, primarily developed for the breeding of grasses (Jönsson & Nilsson 1986), it followed that selection work with winter barley, should also be initiated.

MATERIAL AND METHODS

The barley to be tested is sown directly in the soil in plastic boxes, each box containing 11 varieties sown in one row of 10 plants. One of the varieties in each box is always a control. All varieties are sown with 5-10 replications, randomly distributed among the boxes. After emergence, the barley is permitted to grow to the three- to four-leaf stage at low temperature (+10°C).

Therafter the boxes are transferred to a chamber with controlled low temperature for vernalization and hardening for six weeks at a day/night temperature of 5/1°C. The day length decreases during this period from 8 to 4 h to simulate natural hardening conditions.

The hardened barley plants are then infected with sclerotia of *T. ishikariensis*, isolated from sclerotia collected in the field and multiplied on a mixture of wheat and oat kernels. *T. ishikariensis* is used in the test because it is easier to handle and more virulent in the laboratory than *T. incarnata,* and cereals have been shown to react in the same way to the two fungi (Bruehl 1967; Jamalainen 1974). The boxes are then covered with moist paper and black plastic sheets to reproduce the humid and dark conditions prevailing under a snow cover.

In the ideal situation the incubation period should be long enough to kill about 75% of the plants, which means around eight weeks, but this depends on the conditions and susceptibility of the barley plants and on the virulence of the inoculum. To break the incubation period at the right moment is one of the most difficult parts of the test.

After a recovery period of 2-3 weeks in humid conditions and at gradually rising temperatures, the level of tolerance is measured as number or percentage of surviving plants.

An analysis of variance and a Duncan-test have been carried out on the material (Table 1). Data were x-transformed prior to analysis.

RESULTS AND DISCUSSION

The object of this project is to select for tolerance to the grey snow mould in a winter barley breeding material. As it is a very time- and resource-consuming test, the number of tested lines is restricted to around 150/year and consequently the procedure has to be

carried out rather late in the breeding process, in this case in generation F7. Another consequence of this is that very few varieties have been tested for more than one year.

Table 1. Analysis of variance

Source of variation	DF	SS	MS	F
Between varieties	16	5	0.33	4.60***
Between years, within varieties	32	2	0.07	1.26NS
Error	321	18	0.05	

This analysis shows a significant difference between varieties, but no significant difference between years within varieties, and the results from the different trials have therefore been pooled.

The five varieties Frost, Fimbul II, Igri, Sonja and W 51084 have been included in all four tests during the years 1986-89 and their survival rates are presented in Table 2. There is a marked difference in tolerance between the two Swedish six-row varieties, the old Fimbul II and the new Frost, with Fimbul II being the most significantly tolerant. The German two-row variety Igri has a very low tolerance level, while the old two-row variety Sonja is almost as good as Fimbul II. The new two-row line W 51084 displays a superior level of tolerance.

Among new breeding lines, there are examples of both very high and very low levels of tolerance to grey snow mould (Table 2 and 3), but also of lines showing varying results. These inconsistent results call for a certain cautiousness when interpreting the results. The risk of escape has to be considered, as the infection severity is very dependent on the initial status of the plants, which can vary slightly even within the boxes. It is also difficult to provide a 100% even infection.

Table 2. Percentage of surviving plants after infection with *T. ishikariensis*. Means of four trials, 1986-89

Igri	3	a			
Frost	8		b		
Sonja	15		b	c	
Fimbul II	19			c	
W 51084	35				d

Figures followed by the same letter are not significantly different at the 5 % level

Table 3. Percentage of surviving plants after infection with *T. ishikariensis*. Means of two trials, 1988-89

W 09588	2	a				
Banteng	2	a	b			
Igri	2	a	b			
Frost	6	a	b	c		
Sonja	8	a	b	c	d	
W 04086	12			c	d	e
W 22987	20					e
Fimbul II	21					e

Figures followed by the same letter are not significantly different at the 5 %-level

In spite of the objections, however, most of the results are consistent enough to allow a safe judgement of the tolerance level. Aided by this method, it is possible to raise the level of tolerance to grey snow mould in winter barley considerably.

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Chemical control of winter damaging fungi in cereals

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Olvång, H. 1992. Chemical control of winter damaging fungi in cereals. Norwegian Journal of Agricultural Sciences. Supplement No. 7: 55-61. ISSN 0801-5341.

A review of the literature on control of winter damaging fungi in winter cereals has been carried out. In Sweden seed dressing on average increased the yield by 500-550 kg/ha in winter wheat and by 600-700 kg/ha in winter rye for the period 1965-82. The results differ considerably between areas depending on winter conditions, and small differences were found between compounds in their effect on *Microdochium nivale*. Benzimidazole compounds had less effect on *Septoria nodorum* than mercury and guazatine. The effect is dependent on infestation level in the seed, type of preceding crop and on the prevailing winter conditions. Treatment of the growing crop with benomyl increased the yield by approximately 200-225 kg/ha. It was found that the treatment effects are greater when the crop is well developed in the autumn, after cereals as the preceding crop and under more severe winter conditions. Resistance to MBC fungicides was discovered in 1982 and prochloraz and guazatine were tested as substitutes. Prochloraz is very effective against both *M. nivale* and *Pseudocercospoella herpotrichoides*, while guazatine only seems to work effectively on *M. nivale*.

Key words: Foliar treatment, *Microdochium nivale*, *Pseudocercosporella herpotrichoides*, seed-dressing, *Typhula* spp.

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Winter damaging fungi may infect the crop from both seed and soil inoculum. Some fungi can infect in both ways, while others have only one or the other means of infecting. Included among the fungi that are traditionally regarded as the cause of winter damage to winter cereals are *Microdochium* (*Fusarium*) *nivale*, *Typhula* spp. and *Pseudocercosporella herpotrichoides*, but fungi such as *Septoria nodorum* and *Fusarium* spp. from the "roseum group" can also cause damage.

CHEMICAL CONTROL BY SEED-DRESSING

The first attempts to control fungi on the seed go back a long way in time and include drenching in urine or wine. In more modern times copper compounds ($CuSO_4$) and inorganic mercury compounds were in common use. In the 1930s organo-mercury compounds were introduced and in Sweden in the 1950s almost all winter cereal seed

was treated with Hg compounds. The mercury debate triggered a search for less harmful substitutes. The benzimidazole (MBC) fungicides proved to be valuable and at the beginning of the 1970s fuberidazole (Neo-Voronit) and thiabendazole (Sidipreg) became the dominant products on the Swedish market. An estimate of the frequency of MBC fungicides for seed-dressing and for spraying on the growing crop against winter fungal damage based on information from the chemical companies on the sale of the products, is presented in Table 1.

Table 1. Acreage of winter cereals and estimates of use of benzimidazole fungicides for seed dressing and foliar treatment in Sweden 1971/72-1981/82

Year	Acreage, hectares	Seed treatment, % of acreage	Foliar application, % of acreage	
			Autumn	Spring
1971/72	341,000	31	--	--
1972/73	342,000	73	--	--
1973/74	402,000	70	--	--
1974/75	324,000	74	--	--
1975/76	462,000	46	--	--
1976/77	477,000	56	--	1
1977/78	328,000	60	5	2
1978/79	267,000	54	6	9
1979/80	329,000	43	17	17
1980/81	230,000	22	17	26
1981/82	290,000	8	14	19

In the mid-70s guazatine (Panoctine) was introduced for seed-dressing in winter cereals and since the beginning of the 1980s almost all winter cereal seed is treated with this compound. The new non-mercury seed-dressing compounds showed as good an effect as the mercury-based products and in 1979 mercury was banned for treatment of winter cereal. As can be seen from Table 1, the MBC fungicides were introduced for foliar application at the same time as they were withdrawn from use in seed treatment. In 1982 decreased efficiency of MBCs was suspected for the first time in a rye seed-treatment experiment and in 1983 resistance to the fungicides was proven in *M. nivale* both in seed and in the field (Olvång 1984).

Olofsson (1971) and Olofsson & Johnsson (1985) have studied the effect of seed-dressing in Sweden for the period 1966-82. From their reports it seems that seed-dressing of *S. nodorum*-infested seed lots can influence the emergence considerably (Table 2). It is difficult to compare effects obtained in different experiments, but the result is supported by observations in several field experiments that *S. nodorum* strongly reduces the germination rate. There is a lack of Swedish experience from seed infested with *Fusarium* spp. like *F. culmorum* and *F. avenaceum*, as it is difficult to obtain seed with a high and pure infestation. It seems, though, that these fungi cause relatively little damage to the seedlings.

In the experiments, seed lots with varying degrees of infestation have been used. The seed lots in the *M. nivale* series were, on average, infested by 15% (1-46) *M. nivale* and in the *S. nodorum* series the average infestation was 50% (12-72). There have been mixed infestations on the seed. It is difficult in greenhouse tests to distinguish between

Table 2. Percentage of healthy plants in greenhouse tests and yield (kg/ha), plant density in the autumn and spring (0-100) in seed-dressing experiments with winter wheat 1971-82 in Sweden. From Olofsson & Johnsson (1985)

Seed treatment	Healthy plants, %	Plant density, Autumn	Spring	Yield kg/ha
M. nivale-infested seed (1971-82), 45 experiments				
Untreated	47.8	97	84	5194
Mercury [1]	92.8***	99	94***	+462***
Fuberidazole [2]	87.9***	99	95***	+473**
Thiabendazole [3]	86.2***	99	95***	+556**
Guazatine [4]	88.0**	99	93**	+556*
S. nodorum-infested seed (1979-82), 16 experiments				
Untreated	34.0	90	75	4832
Mercury	87.0*	99	87**	+476**
Fuberidazole	69.7*	99	87**	+459**
Thiabendazole	68.0	99	89**	+560**
Guazatine	83.4*	99	88**	+471**

[1] Panogen Metox, methoxi-ethyl-mercury-acetate, 24.8 mg/kg seed
[2] Neo-Voronit, fuberidazole, 1.68 mg/kg seed
[3] Sidipreg, thiabendazole, 4.0 mg/kg seed
[4] Panoctine, guazatine, 700 mg/kg seed

the symptoms of the different diseases and in Table 2 the effect of seed treatment has been given as the percentage of healthy plants.

The corresponding values for rye are summarized in Table 3. *M. nivale* was the dominating fungus in the rye seed. An average of 21% (2-47) of the kernels were infested with *M. nivale* and only a low frequency of *Fusarium* species occured.

Table 3. Percentage of healthy plants in greenhouse tests and yield (kg/ha), plant density in the autumn and spring (0-100) in 45 seed-dressing experiments in winter rye in Sweden 1971-82. From Olofsson & Johnsson (1985)

Seed treatment [1]	Healthy plants, %	Plant density, Autumn	Spring	Yield kg/ha
Untreated	38.9***	94	70	4235
Mercury	88.0***	100**	89***	+497***
Fuberidazole	80.1***	99**	90***	+572***
Thiabendazole	83.0***	99***	93'''*	+635***
Guazatine (33 exp.)	85.5**	100'*	88***	+712***

1). See footnotes to Table 2

In Tables 2 and 3 it can be seen that there are small differences between compounds in the effect on *M. nivale* in the greenhouse tests. The benzimidazoles (fuberidazole and thia-bendazole) had a somewhat smaller effect on *S. nodorum* than mercury and gua-

zatine. However, this difference is not expressed in the field as plant density in the spring and in the yield. This is probably dependent on the systemic transport of the MBCs, which gives a certain protection to the seedlings against soil-borne infection.

The yield increase in the field experiments (Tables 2-3) varied between 450 and 550 kg/ha in winter wheat and 500-700 kg/ha in rye for the different seed-dressing compounds. In an earlier series of experiments (1966-70) the effect in wheat was 500-600 kg/ha and in rye 750-1100 kg/ha (Olofsson 1971). The effect of seed treatment on stand and yield depends on the degree of infestation of the seed, on the winter conditions and on the preceding crop. Johnsson (1976) found a treatment effect in rye of approximately 250 kg/ha at a low infestation level but 750-1325 kg/ha at high infestation. The effect of winter conditions can be seen from experiments in different parts of Sweden. In southern Sweden (M-län) the yield increase from seed-dressing was 0-300 kg/ha in wheat and 200-550 kg/ha in rye, while in central Sweden, where more severe winter conditions prevail, the corresponding effect varied between 700 and 1300 kg/ha (Olofsson 1971; Olofsson & Johnsson 1985). In the above-mentioned experiments it was also found that the seed-treatment effect was lower if the preceding crop was cereals than if it was lay or fallow (Johnsson 1978). This indicates that the soil-borne inoculum may reduce the importance of the protection provided by seed-dressing.

CHEMICAL CONTROL IN THE GROWING CROP

In the 1950s and 60s chemical control of fungal winter damage in the growing crop was investigated by Jamalainen (1964). Several compounds like mercury, arsenic, cadmium, maneb, mancoseb, thiram and pentachlornitrobenzen (PCNB) were tested. They are all protective fungicides and most of them had a week and unreliable effect against the fungi. In order to provide good control of the fungi, they should be applied as late as possible before a permanent snow cover arrives. PCNB was fairly effective against *M. nivale* and was particularly effective against *Typhula* spp. Bengtsson (1971) showed that treatments with PCNB (Brassicol 50%, 15 kg/ha) increased yield by 160 kg/ha in winter wheat and 280 kg/ha in rye when the crop is sown early. Because of the control of "snow moulds" it was possible to sow at an earlier date. However, the method never became popular, probably because the treatment had to be carried out late in the autumn. PCNB has not been allowed in Sweden since 1986.

Since the 1970s MBCs (benomyl, carbendazime, thiabendazole) have been tested as foliar sprays with good results. They control *M. nivale* and *P. herpotrichoides* well, but have no effect on *Typhula* spp.. Cases have even been reported where the *Typhula* attack significantly increased after treatment with benzimidazoles (unpublished).

In sowing-date experiments Bengtsson (1983) demonstrated the same effects of benomyl (Benlate, 0.4 kg/ha) on snow mould and yield as in the earlier experiments with PCNB, although the effects were greater. In both winter wheat and rye the attacks of *M. nivale* were most severe at the recommended date for earliest sowing. Without benomyl treatment the highest yield was obtained at a sowing on the recommended date for the area. With a benomyl treatment the highest yield was obtained at a sowing date 10 days before recommended. In winter rye the average yield increase for 43 experiments was 600 kg/ha at the first sowing date but only 160 kg/ha when the crop was sown one month later. In winter wheat the corresponding values were 290 and 70

kg/ha, respectively. Similar results were obtained in experiments with benomyl (Benlate, 0.3 kg/ha) in commercial fields (Olvång 1987a, 1991). The average yield increase was slightly more than 200 kg/ha in both winter wheat and rye. The effects increased towards the north, where more severe winter conditions prevail. The attacks by snow mould and the effect on yield increased with early sowing. In winter rye the attack by *M. nivale* was 2-3 times higher and the yield increase more than double at sowing before 2 September in Central Sweden or 18 September in the South of Sweden than at sowing 10 days later. The vegetative development of the crop in the autumn influences the results. In winter wheat the yield increase for the treatment was only 71 kg/ha when the plants had less than three leaves but 510 kg/ha when more than four leaves had developed. The effect of the treatments was greater when the preceding crop was cereals (except oats) or lay than after other crops.

The resistance of *M. nivale* to MBCs has increased since its discovery in 1982 (Olvång 1987c). This resistance is more frequent in rye than in wheat. Foliar treatment with MBC fungicides is no longer recommended in rye and in winter wheat except when the seed is healthy and MBCs have been used sparsely in the field. In 1987 the frequency of benomyl resistance in two randomly sampled untreated winter wheat fields was found to be 30% in *M. nivale* (unpublished).

In 1985 the resistance frequency in Sweden of *P. herpotrochoides* to MBC fungicides was expected to be low (Olvång 1987c). However, investigationes by Haegermark (1988) revealed that out of 413 isolates collected in 1987 from farms with intensive cereal production in the South of Sweden 27% were resistant to benomyl. In the southernmost areas the frequency was considerably higher, which probably reflects the use of MBCs in cereal production.

In the search for substitutes for the MBCs, prochloraz (Sportak) and guazatine have been tested. Prochloraz has a good effect of both *M. nivale* and *P. herpotrichoides*, while guazatine only seems to control *M. nivale*. In field experiments both compounds appeared to have less effect on *M. nivale* than on benomyl. Treatment with Sportak (1.0 l/ha) increased yield more than treatment with Benlate, particularly in rye (Olvång 1987a). The effect of prochloraz on *P. herpotrichoides* seems to be approximately equal to that of benomyl. However, autumn treatments with Sportak appear to give a more durable effect against *P. herpotrichoides* than treatments with Benlate, which is probably due to the low mobility of the compound in the plant. The effect of guazatine on yield was less than that of benomyl. The company has stopped testing guazatine for foliar treatments, probably because using it for both seed-dressing and spraying the crop would increase the risk of resistance to the fungicide.

DISCUSSION

In Sweden the yield loss caused by fungal winter damage that can be chemically controlled in winter cereals is estimated to an average of 700-800 kg/ha. The Swedish acreage of winter cereals is approximately 250,000-300,000 ha and with a value of 1 SEK/kg, the loss would amount to 200-300 MSEK per year. An estimate by Sundell (1977) for only seed treatment in winter wheat and rye gave a loss of 370 MSEK per year at a cost of 0.63 and 0.58 SEK/kg for winter wheat and winter rye, respectively.

In Sweden there is a tendency to decrease the chemical control of weeds, diseases and pests. A reduction in the seed-dressing of winter cereals in particular would cause an increase in seed-borne diseases and the risk of increased damage by fungi. In order to minimize this risk the farmers could take certain precautions. In particular, they should avoid extremely early sowing of the winter cereal. The winter cereal should be sown after a suitable crop (oil seed, peas, oats, etc.). It is also necessary to exercise good soil-tilling procedures in order to reduce the amount of infectious plant debris.

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Use of near-isogenic lines in analysing race-specific pathogen interrelations

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The virulence pattern of race-specific plant parasite populations can be studied in detail because of the close gene-for-gene interrelation with genes for resistance in the host. For the inventory, host lines are needed which carry individual genes that by exact matching can identify the corresponding gene for virulence in the pathogen. Such lines should preferably have the same general genetic background, i.e. be isogenic lines. Analysis made on such premises reveal that virulence genes may have a pleiotropic function. Besides the host selection pressure, their prevalence will depend hereupon and how they harmonize with their genetiv background. The latter interaction is favoured by the recombinatory processes in a sexual phase. An asexual propagation will hamper such an adjustment and enlarge the spesific and general gene erosion encountered with the assortative effect of race-specific resistance. A need for host alternation as well as a dikaryotic/diploid constitution favour a preadaptive accumulation of virulence genes. It is evident that "breakdown" of a newly introduced resistance may just as well depend on preadaption as on a mutational event. All these different circumstances explain why the same pathogen may show different patterns in different ecological niches, and different pathogens may behave differently in the same environment. Such knowledge is essential for the proper planning of breeding for race-spesific resistance.

Key words: Isogenic lines, mildew, oats, race-specific resistance, rust, wheat.

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Nature works in a holistic way with the complex interrelation between plant pathogens and their hosts, but plant breeders have difficulties in doing so. They prefer a more analytical approach. From the perspective of the host plant and its diseases, they find it more convenient to distinguish between tolerance, pseudoresistance, non-spesific resistance and race-specific resistance (Mac Kay 1986). Although there are really no sharp demarcation lines between the four systems, they clarify the breeding objectives.

The analytical approach fits best in connection with race-specific resistance. Whenever developed, this type of true resistance is also generally preferred. It works with phenotypically comparative strong genes and generally gives a higher degree of protection than non-specific resistance (Table 1). Each gene has, however, a very

precise, protective function, since there is a gene-for-gene relation between the ability of the pathogen to establish and the host plant to resist (Flor 1942).

Table 1. Lesion area on eight rice cultivars inoculated with four isolates of *Xanthomonas oryzae* by the needle-picking method (after Ezuka et al. 1975; Yamamoto et al. 1977)

Cultivar	Carries race-specific gene	Lesion area in $\sqrt{mm^2}$ caused by isolate T-7174	T-7147	T-7133	Xo-7323
Nikisakae...	-	10	13	13	12
Asahi 1.....	-	23	21	24	21
Pelita I/1..	Xa-1	1	8	7	7
Norin 27....	"	1	26	29	27
TKM 6.....	Xa-1,Xa-2	1	1	16	9
Tadukan.....	" "	1	3	32	39
Nagomasari..	Xa-3	2	2	1	13
Chugoka 45..	"	2	1	2	24

The steplike dividing line separates on the left race-specific reactions, on the right nonspecific reactions with difference between pairs of cultivars but not between isolates.

When the number of gene interrelations in the system is limited, its assortative dynamic will operate. If sufficiently diversified on both sides by evolutionary strokes and counter-strokes, such a mutual provocation will generally and in some kind of stabilizing selection or balanced polymorphism (Person 1966; Van der Plank 1968; Browning 1974).

The reliability of the race-specific type of resistance works satisfactorily in nature where there is diversification on both sides. The reliability became endangered, however, by the introduction of the monoculture as a prerequisite (cf. Donald 1981) for developing productive crop models. Race-specific resistance soon came into disrepute because of too many devastating surprises.

Today it is better understood when breeding for race-specific resistance is suitable. A prerequisite is that the relations must be fully understood and the knowledge thus gained properly applied. Basically, for the needed information to be accumulated it is necessary that the gene for virulence in the pathogen can only be identified by its matching gene in the host plant and vice versa.

GENE-FOR GENE TYPES OF PLANT RESISTANCE

The gene-for-gene relation between a pathogen and its host plant works as an incompatibility system. The direct pattern indicating how individual genes on both sides work against each other is not always the same. It appears that the difference depends upon the type of parasitism involved.

Facultative parasitism can be said to be an extension of the saprophytic alternative. It is generally based on killing the host cell by secreting substances which either dissolve the cell walls or destroy the cytoplasm. Such pathogens work with toxins or enzymes which are either compatible or incompatible with the host genotype. A gene for virulence corresponds to a gene for susceptibility (Lim et al. 1974; Ellingboe 1976, 1980).

An obligate plant parasite does not invade host cells from which it takes its energy supply. The parasite and host cells are separated by a complicated series of menbranes through which nutrients are transported. Under such circumstances, the pathogen cannot work with killing toxin, while the host cell may defend itself by producing a toxin. Such antimetabolites can act either directly, or indirectly by starving the obligate parasite to death as a consequence of self-destruction, a so-called hypersensitivity reaction of the plant. A gene for virulence corresponds to a specific gene for resistance by somehow inactivating or going round the trigger mechanism of the latter (Person 1959).

The two systems for gene-for-gene interrelations can conveniently be named the *Helminthosporium* and the *Melampsora* type of race-spesific resistance, since they were first discovered in relation to these two fungal pathogens. It should be observed that they behave genetically like some kind of mirror image of each other (Table 2).

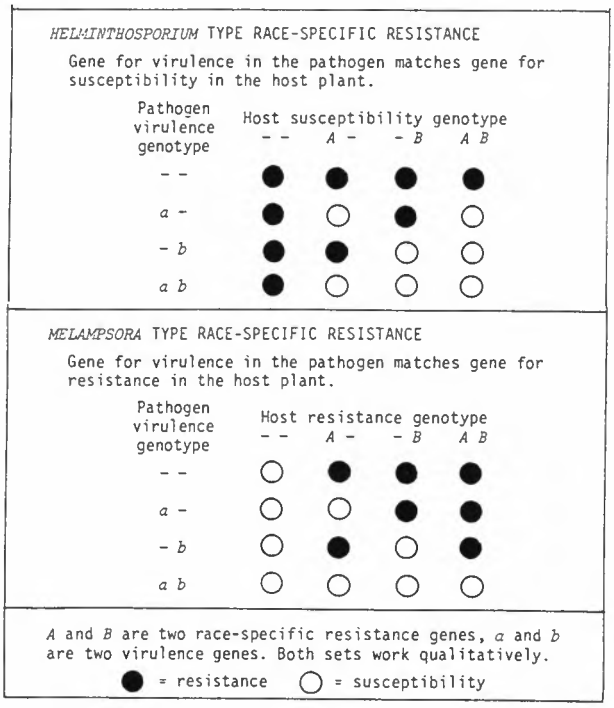

Table 2. Different systems of race-specific host plant:fungal pathogen interrelations

In order to avoid confusion, only the obligate parasitism will be used for demonstration in the following. It behaves exactly like a lock-key system. Only the matching key can open the lock and to open a door with more than one lock, all the matching keys are needed.

PRODUCTION OF ISOGENIC LINES WITH RACE-SPECIFIC RESISTANCE

For an anticipative strategy in connection with race-specific resistance breeding, it is necessary to know the virulence genes present in the concerned pathogen population. It

is also of interest to know whether they tend to accumulate or not and to what extent new genes or new combinations may occur (Mac Kay 1974, 1977). As indicated above, such an inventory is feasible, since a gene for virulence can be identified by overcoming the matching gene for resistance of the host. The existence of complementary, additive or cytoplasmic gene actions does not conflict with this general principle and may only be looked upon as a mere variation on the theme.

The more efficient tools for such an inventory of a pathogen population must be a series of host genotypes carrying only one specific gene for resistance each. Since genes are seldom completely autonomous in their phenotypic expression, it is a clear advantage but not a necessity to have the same genetic background. Such basically uniform lines differing by one unique gene only, constitute an isogenic series.

The advantage of developing series of this kind has become increasingly obvious. In a global perspective, several such series have now been developed or are being developed. They can be produced in different ways:

Intraspecific transfer through repeated backcrosses (Mac Key 1974).

Interspecific transfer through sexual/somatic hybridization, a directed chromosome elimination process and a conclusive transfer through translocation (cf. Knott & Dvorak 1976).

Interspecific transfer through some modern DNA technique (Willmitzer 1987).

Induction de *novo* by mutagenic treatment (cf. Jørgensen & Jensen 1978).

The two last-mentioned methods are more accurate, while the two first-mentioned methods offer problems with linkage. Due to this circumstance, exactly isogenic lines can be difficult to obtain. For the purpose of deciphering pathotypes, near-isogenic lines work almost as well. Additionally transferred genes may, however, give agronomically undesirable features and will somewhat complicate the use of isogenic lines for biochemical gene analyses. This is a new field, where isogenic lines have become very popular as research objects.

The transfer methods mentioned are both time and labour consuming. This is strengthened by the fact that the phenotypic expression of race-specific gene may be difficult to trace, especially with screening methods that are not reliable enough.

Quite another problem is involved in the search for the widest possible set of relevant genes for resistance. Such an inventory, preferably of global dimension, is complicated by the fact that n individual genes may recombine in 3^n different ways, of which 2^n are homozygous.

While the interrelation demonstrates the advantage of thinking in genes rather than in sources of resistance, it also explains the likelihood of stumbling on the same gene(s) over and over again. This will especially occur if there is a paucity of sufficiently discriminating tester races. The possibility of isolating new pathotypes with special, assorting capacity is automatically dependent on number of unique genes for resistance at hand. In other words, there occurs an interdependence in prograss.

The principle of mutual identification can also be applied in attempts to keep control of genes for resistance. A collection of genetically different isolates can be used not simply to discover new unique genes for resistance (cf. Table 3).

CI	Line/Entry	Reaction to crown rust race: North American						Scandinavian					
		264B	264	456	216	321	290	201	203	331	234	240	239
-	Ascencao x Sun II[3]..	-	-	-	-	R	R	R	R	R	R	R	R
-	Landhafer x Sun II[6]..	-	-	-	R	-	-	R	R	-	R	R	R
-	Bondvic x Sun II[6]..	-	-	-	-	-	R	R	R	-	r	R	R
4077	Quincy Red.....	-	-	-	-	R	R	R	R	R	R	R	R
5044	Santa Fe sel.....	-	-	-	R	-	R	R	R	-	R	R	R
8133	Coker 64-35.....	-	R	R	R	R	R	R	R	R	R	R	R
8238	Minn. 65-B2414-2426..	R	-	R	R	R	R	R	R	R	R	R	R
8040	Portal.....	-	R	-	R	R	R	R	R	R	R	R	R
3436	Brazil.....	R	R	R	R	-	R	R	R	R	-	R	R
2766	Alber.....	-	-	R	-	R	-	-	R	R	R	R	r
1175	Beardless Probsteier.	-	-	-	-	R	R	R	R	R	R	R	R
2144	Minrus.....	-	-	-	-	-	R	R	-	R	R	-	R

R = resistant, r = medium resistant (2-X), - = susceptible reaction

Table 3. Access to different races improves identification of unique genes or sources for race-specific resistance as illustrated by the oat:crown rust interrelation

Different pathopypes can also be used to check whether an isogenic line has a narrower resistance spectrum than its donor, indicating that this source must have at least another gene as well. In addition, such a set of pathotypes is useful for detecting different kinds of experimental errors.

Table 4 is an excerpt from such control over 35 near-isogenic lines in both spring and winter wheat using 26 different isolates of wheat powdery mildew (Mac Kay & Leijerstam, unpubl.). The table reveals that Chul and Ulka as donors had only one gene each for race-specific resistance and that a mishap must have intervened, since the 'Ulka x Prins[7] near-isogenic line' proved to carry the Chul gene. The two Halle lines must have more than one gene, one already found alone in Ulka and a new one. The latter happened to be transferred to winter wheat cv. Starke II in both cases but unfortunately in none of the spring wheat cv. Prins isogenic lines. Instead, they carry the gene *Pm2*, missed in the Ulka transfer programme but obtained also from the donor line CI 12633. In this case both the spring and winter wheat programmes missed the gene *Pm6*.

GENIC INVENTORIES OF PATHOGEN POPULATIONS

The recognition of race-specific resistance was originally based on the assortative effect of different cultivars (Stakman & Levine 1922), and all older race keys used such testers. When race inventories took a strictly genic approach, definite gains in simplicity and direct information were achieved. Today's differentials are, however, often still cultivars but known to carry individual genes or known gene combinations. The advantage of working with isogenic or near-isogenic lines, preferably with low non-

Source/near-isogenic line	Gene(s)	Reaction to Scandinavian wheat powdery mildew isolate no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27
Chul	*Pm3b*	R	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
Chul x Prins[7]	*Pm3b*	R	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
Chul x Starke II[7]	*Pm3b*	R	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
Halle 8810-47	*Pm2+?*	S	R	I	R	I	S	R	R	S	R	S	R	R	R	S	R	S	R	S	R	S	R	S	R	S	S
Halle 8810-47 x Prins[7]	*Pm2*	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S
Halle 8810-47 x Starke II[9]	*Pm?*	S	S	S	R	S	S	R	R	S	R	S	R	S	R	S	R	S	R	S	I	S	R	S	R	I	S
Halle 13471	*Pm2+?*	S	R	I	R	I	S	R	R	S	R	S	R	R	R	S	R	S	R	S	R	S	R	S	R	S	R
Halle 13471 x Prins[7]	*Pm2*	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S
Halle 13471 x Starke II[8]	*Pm?*	S	S	S	R	I	S	R	R	S	R	S	R	S	R	S	R	S	R	S	I	S	R	S	R	I	S
CI 12633	*Pm2+6*	R	R	R	I	I	R	R	R	R	R	S	R	R	I	R	R	I	R	S	R	R	R	R	I	S	S
CI 12633 x Prins[10]	*Pm2*	S	R	S	I	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S
CI 12633 x Starke II[10]	*Pm2*	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S
Ulka	*Pm2*	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S
Ulka x Prins[8]	*Pm3b*	R	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
Ulka x Starke II[7]	*Pm2*	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S	S	R	S	R	S	R	S	S	S	S

Prins = spring wheat receptor, Starke II = winter wheat receptor

R = resistant, I = intermediate, S = susceptible reaction

Table 4. Access to discriminating isolates allows identification and control of near-isogenic lines for race-specific resistance as illustrated by the wheat:mildew interrelation. Cf. also text

specific resistance, is not always at hand. Neither were there any isogenic lines at hand at the time when there was an interest in running race inventories in Scandinavia.

The old international race key for oat stem rust offers a good demonstration, however. The four oat cultivars chosen as differentials happen to carry one race-specific resistance gene each, first named *A*, *B*, *D* and *E*, later *Pg2*, *Pg4*, *Pg1* and *Pg3*, respectively. In order to follow the gene-for-gene relation more easily the old denomination will be used and with *a*, *b*, *d* and *e* representing the matching virulence genes in the rust. This denomination also fits from the viewpoint that the genes for resistance are dominant and those for virulence are recessive. It should also be observed that the gene-for-gene interrelation is in this case the simplest possible. Not only the autogamous oat cultivars but also the dikaryotic uredospores must have their respective gene in a homozygous arrangement, in the latter case to have the virulence phenotypically expressed.

Based on the true resistance vs. susceptibility reaction on each differential, it has been possible to distinguish 16 different patterns or physiological races. They were originally numbered in the USA as they were found, and all of them have also been observed in Scandinavia (Mac Key 1980). In addition, 10 more races were described in the USA, since they show a mesothetic reaction on one of the differentials. This mixed and temperature-unstable syndrome is proved to be due to a superimposed cytoplastic effect (Green & Mckenzie 1967). In a true gene-for-gene relation, it should be read as susceptibility, which limits the number of true races observed to 16 (Table 5).

Since four genes of gene duplexes can be recombined in 16 different ways (2^4 = 16), all possible 16 races have apparently been observed. In Table 6, the 16 races are rearranged after the increasing number of genes involved as coming out from their reaction patterns and the gene-for-gene relationship. Since the differentials chosen happened to function in a similar way to isogenic lines, the old races can apparently be dechipered. Already-made race surveys can be used to understand how races are composed.

Differential variety	Res. gene	Reaction of differential variety to oat stem rust race no.: 1	1A	2 5	2A 5A	3	3A	4	4A	6 13	6A 13A	7 12	7A 12A	8 9 10	8A 9A 10A	11	11A
White Tartar	*D (Pg1)*	●	●	●	●	○	○	○	○	○	○	○	○	●	●	●	●
Richland	*A (Pg2)*	●	●	●	●	●	●	○	○	○	○	●	●	○	○	○	○
Sevenothree	*E (Pg3)*	●	●	○	○	●	●	●	●	○	○	○	○	○	○	●	●
Rodney	*B (Pg4)*	●	○	●	○	●	○	●	○	●	○	●	○	●	○	●	○

● = resistant ○ = mesothetic or susceptible reaction

Table 5. Old conventional race key for ost stem rust

Gene for resistance	Pattern of reaction of oat stem rust race number: 1	11	1A	3	2	11A	4	8	3A	2A	7	4A	8A	6	7A	6A
A (Pg2)...	●	○	●	●	●	○	○	○	●	●	●	○	○	○	●	○
B (Pg4)...	●	●	○	●	●	○	●	●	○	○	●	○	○	●	○	○
D (Pg1)...	●	●	●	○	●	●	○	●	○	●	○	○	●	○	○	○
E (Pg3)...	●	●	●	●	○	●	●	○	●	○	○	●	○	○	○	○

Gene for virulence	Combination of virulence genes in oat stem rust race number: 1	11	1A	3	2	11A	4	8	3A	2A	7	4A	8A	6	7A	6A
a.....	–	*a*	–	–	–	*a*	*a*	*a*	–	–	–	*a*	*a*	*a*	–	*a*
b.....	–	–	*b*	–	–	*b*	–	–	*b*	*b*	–	*b*	*b*	–	*b*	*b*
d.....	–	–	–	*d*	–	–	*d*	–	*d*	–	*d*	*d*	–	*d*	*d*	*d*
e.....	–	–	–	–	*e*	–	–	*e*	–	*e*	*e*	–	*e*	*e*	*e*	*e*

● = resistant ○ = susceptible reaction

Table 6. Deciphering as to genic composition of races of oat stem rust identified by the old conventional race key (Mac Kay 1974

As examples, deciphered race spectra for Sweden and the USA are presented in Table 7. The two countries represent total absence and presence, respectively, of host selection pressure. The period 1956-59 had to be chosen as being the only years in which Swedish inventories were made. It also fits well to illustrate the effect of host selection pressure, since the resistance genes *A* and *D* were separately introduced in North America in the late 1940s, and they were combined together or with *B* in the middle of the 1950s (Stewart & Roberts 1970).

The two sets of race spectra clearly demonstrate that prevalence of race-specific gene for virulence is not only determined by host selection pressure. Contrary to the idea of Van der Plank (1969), genes 'unneccessary' for invading the plant host, do not always have to be selected against as a consequence of some kind of negative fitness or inablity to compete.

Besides their specificity, virulence genes may even have some kind of more general or supporting function. As is evident from Table 7, all races vital enough to be recorded over the four years carry gene *e* in the USA but gene *d* in Sweden. The two genes are in their respective geographic areas the most common virulence genes, despite their not

Year	n	Relative prevalence (%) of oat stem rust race number/pathotype:															
		1	11	1A	3	2,5	11A	4	8,10	3A	2A,5A	7,12	4A	8A,10A	6,13	7A,12A	6A,13A
		----	*a---*	*-b--*	*--d-*	*---e*	*ab--*	*a-d-*	*a--e*	*-bd-*	*-b-e*	*--de*	*abd-*	*ab-e*	*a-de*	*-bde*	*abde*
Sweden:																	
1956	32	-	-	-	31	-	-	28	-	6	-	13	-	-	13	3	6
1957	61	-	-	-	7	-	-	7	-	3	-	11	3	-	13	25	31
1958	96	-	-	-	24	-	-	14	-	5	-	20	3	-	15	6	14
1959	133	-	-	-	12	-	-	9	-	7	-	16	5	-	10	21	20
1956-59	322	-	-	-	16	-	-	12	-	6	-	16	4	-	12	15	19
U. S. A.:																	
1956	476	-	-	-	-	16	-	-	15	-	-	66	-	-	1	2	-
1957	522	-	-	-	-	12	-	-	21	-	0	59	-	-	2	6	0
1958	286	-	-	-	-	14	-	-	26	-	-	54	-	-	1	5	-
1959	230	-	-	-	-	7	-	-	11	-	-	59	1	-	10	10	2
1956-59	1514	-	-	-	-	13	-	-	19	-	0	60	0	-	3	5	0

Table 7. Deciphered race spectra of oat stem rust for Sweden and the USA in 1956-59 (/Mac Key 1977)

being needed for overcoming the resistance of the host. With no host selection pressure in Sweden, gene *d* is as unnecessary as all other virulence genes. For obvious reasons, the resistance gene *E* has never been used in North American oat breeding and thus neither should the virulence gene *e* have been challenged to prevail on that continent. Obviously, the gene *e* must otherwise be important in one situation, gene *d* in another.

In addition, it is clear that preservation of virulence genes in a rust population is even more complicated. In spite of absence of a host selection pressure, all Swedish race spectra show a significant tendency toward gene accumulation.

The American oat stem rust behaves differently. The introduction of resistance genes *A* and *D* has, as expected, given the matching genes *a* and *d* a selective advantage. When genes *A* and *D* were brought together along with gene *B*, the matching combinations of virulence genes have been favoured. Complex races do not, however, prevail as in Sweden, indicating that stabilizing selection works.

A similar contradiction has been observed for wheat leaf rust. In Sweden as well as in the whole of East Europe, gene accumulation prevails. In the USA as well as in Portugal stabilizing selection has been proved to function (Mac Kay 1981).

GENERAL FITNESS OF VIRULENCE GENES

Apparently, genes for virulence may show different ability to be preserved over time in rust. Environment will have an effect on this but likely genetic background as well. For safe overwintering in Sweden, rust has to pass the complete cycle including a sexual phase. In North America the more important biotypes rely on constant asexual reproduction at the uredinial stage owing to the efficiency of the so-called *Puccinia* Path (Frey et al. 1977). Separated only by the narrow strait of Gibraltar, the Pyrenean peninsula is likely to be in a similar position. Obviously, the potential for recombination

as a basis for genetic background adaptation must be very different. It is also important to be aware of the fact that the assortative effect of race-specific resistance introduced in a crop plant implies not only a specific but also a general gene erosion. Such an impoverishment will be much stronger, if races are never or seldom recombined.

In addition, a need for host alternation calls for a preservation capacity. This has also been proved for oat stem and crown rust in Israel (Wahl et al. 1964; Wahl 1970) and for oat stem rust in Australia (Luig & Baker 1973). Common oats are infected by 'unnecessarily' complex races. In seasons when annual oats do not grow, other grasses serve as alternative hosts.

If rust shows preadaptation whenever circumstances so allow, it is difficult to see genes for virulence as only responsible for the effect we study. It is also likely that we overestimate mutational events, when we are confronted by a more or less sudden 'breakdown' of a new resistance introduced.

Lines carrying individual genes for resistance can also be useful to illustrate this aspect. Up to 1974, 13 different genes for race-specific oat stem rust resistance were known (Mac Key 1974). Lines carrying one of the genes each, can be laid out as some kind of trap nursery or be checked by a representative number of collected rust samples.

In spite of no host selection pressure, at least 11 of the matching genes for virulence were found to exist in Sweden, even more strongly supporting the pattern of gene accumulation and preadaptation (Table 8). With such a result, it is also easy to understand that a thorough inventory has to be made prior to a non-hazardous planning of breeding for race-specific resistance. The number of virulence genes found was, in fact, very much the same as in the USA where there has been conscious breeding for resistance since the 1940s.

Source	Carries gene	
Lanark, White Russian, Minrus	*Pg1 (D)*	*
Exeter, Richland, Ajax	*Pg2 (A)*	*
Canuch, Jostrain, Roxton	*Pg3 (E)*	*
Rodney, Torch	*Pg4 (B)*	*
CI 2710, RL 524.1, CI 4023	*pg8 (f)*	*
CI 5844	*pg9 (h)*	*
CI 3034	*pg11*	*
Kyto	*pg12*	
Avena sterilis CW490-2	*pg13(m)*	
Garry, Hajira	*(G)*	*
CI 1575	*(G)*	
Milford, Winter Turf	*(N)*	*
Rosen Mutant (*pg9* + ?)		*
Saia, 2x (*Avena strigosa*)		*

* matching gene for virulence already found in the Scandinavian population of oat stem rust.

Table 8. Genes for race-specific resistance against oat stem rust known up to 1974 and the pre-existence of the matching virulence genes in Sweden (Mac Key 1974)

A demonstrated incidence in the USA of virulence genes matching the resistance of *E* (*Pg3*), *f* (*pg8*), *h* (*pg9*) and *m* (*pg13*) but absence of the matching genes for resistance in the host population (Martens et al. 1970) supports the idea of a preparedness.

Wheat leaf rust has been proved to behave in a similar way. East European races may often hold 4-5 or even more 'unnecessary' genes for virulence (cf. Stewart et al. 1967; Ralski 1972; Boskovic & Browder 1976).

Since genes for virulence can only be detected by matching genes for resistance, insufficient knowledge of an already existing preadaption appears thus as plausible as a mutational event when the rust overcomes a newly introduced resistance. It is true that mutants resulting from the high number of spores already at low infection are quite abundant. Parlevliet & Zadoks (1977) give an estimate of ca. 1000 mutants per locus/day/hectare at not too severe a rust infection. Such figures, the above indication of the preparedness and the general experience from rust epedimics indicate that mutation frequency and mutation establishment must be very different. Almost all raw mutants may be unable to survive if not exclusively needed to overcome the resistance of the obligate host. The chances of an 'unnessary' mutant surviving in a monoculture may be almost nil. A subsequent background adaptive process may be a necessary prerequisite. In the dikaryotic rust, this process can proceed stepwise via heterozogosity.

Arguments along this line are supported by studies of a true haploid pathogen with no host alternation. Table 9 presents deciphered race spectra for wheat powdery mildew from an inventory in Sweden before host selection pressure was introduced. Again a fourfold gene interrelation is enough to show that in mildew it is now simple races that prevail in Sweden. It is obvious that if restricted recombination is one case for impaired gene accumulation, low genetic storage capacity as in true haploidy is another.

Year	n	Relative prevalence (%) of wheat powdery mildew pathotype:															
		----	*a---*	*-b--*	*--c-*	*---d*	*ab--*	*a-c-*	*a--d*	*-bc-*	*-b-d*	*--cd*	*abc-*	*ab-d*	*a-cd*	*-bcd*	*abcd*
Sweden:																	
1960	161	42	23	1	6	4	2	8	5	-	-	3	1	1	4	-	-
1961	440	16	10	3	22	3	2	26	3	2	0	7	0	0	6	0	-
1962	358	11	14	3	20	2	3	29	2	2	-	6	2	-	5	-	1
1960-62	959	19	14	3	19	3	2	24	3	1	0	6	1	0	5	0	0

Symbol key: *a* = virulence gene matching *Pm1 (Ml-t)* in Norka,
b " " " *Pm2 (Ml-u)* " Ulka,
c " " " *Pm3a(Ml-c)* " Chul,
d " " " *pm5 (ml-h)* " Hope Selection.

Table 9. Deciphered race spectra of wheat powdery mildew for Sweden in 1960-62 (after Leijerstam 1962, 1965)

CONCLUDING REMARKS

Because of the gene-for-gene relation in connection with race-specific resistance, it has been shown that individual genes for resistance can be used to analyse pathogen population structures. It is evident that a warning against too wide a generalization is appropriate when planning breeing for race-specific resistance. Different populations of

the same *forma specialis* in different situations as well as different pathogens in the same ecological niche may differ as to ability to accumulate and preserve temporarily 'unnecessary' genes for virulence.

It is apparent that the host selection pressure cannot be the sole reason for prevalence of certain genes for virulence. They may have pleiotropic effects, and their interaction with their genetic background may be decisive for their preservation. The efficiency of such an adaptive process as well as risks for specific and general gene erosion are dependent on whether sexual or asexual reproduction prevails.

A preservation capacity of great importance for pathogens with host alternation appears to be proportional with the ability of genetic storage capacity. True haploidy implies here a disadvantage, while the immediate phenotypic exposure favours a more provisional flexibility towards a changing host selection pressure.

SUMMARY

The virulence pattern of race-specific plant parasite populations can be studied in detail because of the close gene-for-gene interrelation with genes for resistance in the host. For the inventory, host lines are needed which carry individual genes that by exact matching can identify the corresponding gene for virulence in the pathogen. Such lines should preferably have the same general genetic background, i.e. be isogenic lines.

In facultative race-specific parasitism, a gene for virulence corresponds to a gene for susceptibility. In obligate parasitism, a gene for virulence overcomes a certain gene for resistance. The two systems are like mirror images of each other and can be studied after similar principles.

Analysis based on these premises reveal that virulence genes may have a pleiotropic function. Besides the host selection pressure, their prevalence will depend hereupon and how they harmoniz with their genetic background. The latter interaction is favoured by the recombinatory processes in a sexual phase. An asexual propagation will hamper such an adjustment and enlarge the specific and general gene erosion encountered with the assortative effect of race-specificity. A need for host alternation as well as a dikaryotic/diploid constitution favour a preadaptive accumulation of virulence genes. It is evident that 'breakdown' of a newly introduced resistance may just as well depend on preadaptation as on a mutational event.

All these different circumstances explain why the same pathogen can show different patterns in different ecological niches and different pathogens can show patterns in the same environment. Such knowledge is essential for proper planning of breeding for resistance.

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Mlo aggressiveness of barley powdery mildew

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A laboratory-derived Mlo-aggressive powdery mildew isolate, and a non-aggressive isolate, and two near-isogenic barley lines with and without the powdery mildew resistance gene *mlo5* were used as a model system to study the effect of Mlo aggressiveness of the pathogen and Mlo resistance of the host. The aggressiveness of powdery mildew was expressed as an approximately 5, 75 and 1000-fold increased ability successfully to infect subsidiary, short and long epidermal plant cells, respectively, supported by a reduced latent period. The Mlo resistance was expressed by reduced, very reduced and virtually no successful infections in the three cell types, respectively, and a substantially extended latent period. The number of mildew colonies formed per 100 viable conida applied was about 25 on non-resistant barley. On Mlo-resistant barley the figures were 0.05 for the non-aggressive, and 3 for the aggressive isolates. The ability of the Mlo-aggressive isolate to grow on non-resistant barley was not statistically significantly reduced. The results are briefly discussed in relation to survey methods and to the possible evolution of Mlo aggressiveness in natural powdery mildew populations.

Key words: Aggressiveness, barley, *Erysiphe graminis* f.sp. *hordei*, *Hordeum vulgare*, powdery mildew, resistance.

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In recent years new high-yielding spring barley varieties with Mlo resistance to barley powdery mildew have become widely grown in Europe (Andersen 1989b, 1991). This resistance is conferred by recessive genes in locus *mlo* on barley chromosome 4. It differs from race specific and partial resistance, and does not conform to the gene-for-gene system (Jørgensen 1987).

The resistance is caused by rapid formation of large callose-containing cell wall appositions at the sites of the infection attempts from the pathogen (Skou et al. 1984; Bayles et al. 1990) that render normal epidermal cells resistant. The subsidiary cells to gard cells at the stomata are only moderately Mlo-resistant (Jørgensen & Mortensen 1977); infections here can give rise to occasional mildew colonies. The mechanism of Mlo resistance has been reviewed recently by Aist & Gould (1987), Bayles et al. (1990) and Jørgensen (1991).

The Mlo resistance is apparently effective against all powdery mildew isolates (Andersen 1991; Jørgensen 1977). However, Schwarzbach (1979) made a selection experiment under laboratory conditions where he maintained a strong and continuous

selection pressure for 48 successive conidial generations (Schwarzbach 1987) on large populations of powdery mildew from isolate 'GE 3' inoculated onto the Mlo-resistant barley line HLN 70-8. The final selected population had increased its pathogenic effectiveness by a factor of about 9 in terms of formation of ESH (elongating secondary hyphae), by a factor of about 150 in terms of number of colonies formed, and about 30 in terms of conidia produced per unit of leaf area. The final population was, however, heterogeneous. The most effective individual isolate selected, 'HL 3', had increased the infection frequency (number of ESH formed) by a factor of about 200, an increase to 8.68 from 0.043 of the original isolate. This is indeed a substantial increase, but since the infection frequency of the original isolate on compatible barley is 8 to 10 times higher (cf. Jørgensen & Mortensen 1977; Mendgen 1984), the increased pathogenic effectiveness of the selected population and isolates has been termed as increased aggressiveness, not virulence, on Mlo barley (Jørgensen 1983, 1984). It has been shown that the increased aggressiveness of powdery mildew isolate 'HL 3' is effective on all Mlo-resistant barleys tested, but unchanged on non-Mlo-resistant barley (log. cit., Portmann 1982; Keller & Mendgen 1984). Some results (Jørgensen 1984; Keller & Mendgen 1984) suggest that the Mlo-aggressive isolate 'HL 3' has a somewhat decreased fitness compared to that of non-aggressive isolates when grown on compatible barley tissue. If this is really the case, it will have a substantial impact on the prospects of the durability of Mlo resistance. Mlo-aggressive mildew that may arise in natural powdery mildew populations would then not spread so easily.

In 1987 to 1989 a Nordic project financed by 'Internordic Plant Breeding' had been undertaken with the aim of elucidating the nature of Mlo aggressiveness, and the potential of Mlo-aggressive mildew to arise and spread in natural powdery mildew populations. This was done by describing Mlo aggressiveness phenotypically by measuring the infection frequency, colony growth, and spore production of Mlo-aggressive and non-aggressive mildew on near-isogenic barley lines differing in Mlo resistance vs. susceptibility. The quantitative nature of Mlo aggressiveness and resistance required methods for quantitative and reproducible inoculation. The methods used here have also been used to assess grades of partial resistance of barley to powdery mildew (Knudsen 1984; Knudsen et al. 1987). Some of the present results have been described briefly elsewhere (Andersen 1989a, 1989b).

MATERIALS AND METHODS

Barley material

A pair of near-isogenic spring barley (*Hordeum vulgare* L.) lines differing in Mlo resistance were used. They were developed from mutant 'Risø 5678' (CI 15219) (CI and NGB numbers are accession numbers at the USDA Small Grains Collection and the Nordic Gene Bank, respectively) with mutant gene *mlo5* from the spring barley variety 'Carlsberg II' (CI 15218) (Jørgensen 1975). The mutant was crossed and backcrossed three times to 'Carlsberg II'; then, gene *mlo5*) was held heterozygous for eight consecutive generations of selfing, and a homozygous resistant and a susceptible line, Risø 5678/3* (F8) Carlsberg II (R) (NGB 9276), and Risø 5678/3* (F8) Carlsberg II (S) (NGB 9277), respectively, were selected (Jørgensen, unpublished). In the present study the two lines are designated R 5678 (R) and R 5678 (S), respectively. The two seed lots

were fractioned by seed size, and only seeds with a diameter between 2.8 and 3.0 mm (1000 grain weight approximately 58 g) were used.

The barley seedlings were grown in K-soil, a commercial peat-rich soil, that was homogenized to ensure an even distribution of nutrients. Polyacrylamide plates (2.5 mm thick, 21 cm long, and 10 cm broad with 90° bending near the middle) were placed in the soil and 18-20 seeds sown in one row in the middle of a square pot (13 x 13 cm) about 2 cm from the plate (Fig. 1). The plants were raised in a growth chamber with a light intensity of about 5000 lux for 12 h and a temperature of about 15°C in the light and about 13°C in the dark. Seven days after sowing and one day prior to inoculation, ten plants in the pot were selected for uniformity, and the first true leaf per plant was fixed to the horizontal part of the polyacrylamide plate with the abaxial side up using two unbleached 1-mm-thick cotton strings placed 5.5 cm apart; 5-cm parts of the leaves between the strings were used for making impression films, colony counting, and assessment of colony development.

Fig. 1. Primary leaves of barley seedlings in square plastic pots being fixed to the horizontal part of a right-angled polyacrylamide plate before inoculation

Powdery mildew isolates

The Mlo-aggressive isolate 'HL 3/5' of the powdery mildew fungus (*Erysiphe graminis* DC f.sp. *hordei* Marchal) selected by Schwarzbach (1979) and isolate 'GE 3' from which 'HL 3/5' was derived were used. They may be considered near-isogenic apart from the genes that confer the differences in Mlo aggressiveness. This is supposed by the identical virulence of the two isolates with respect to 29 different powdery mildew resistance genes (Giese et al. 1990; Jørgensen & Jensen, unpublished). The isolates were taken from the stock collection of powdery mildew isolates at Risø National Laboratory.

Inoculum production

Inoculum production comprised multiplication of the isolates on 7-day-old seedlings of the variety 'Manchuria' in 10 cm diameter pots. The pots were contained in perspex cages 40 x 40 x 35 cm with two sides equipped with spore proof filters. The plants in the cages were incubated in a controlled environment chamber under 14 h of lighting

(4500 lux), a relative humidity of 85-95%, and at 18°C under light and 13°C in darkness. After four days, 5-cm-long leaf segments with an even distribution of about 10 colonies were detached. The selected leaf segments were placed on agar plates with 0.5% water agar with 15 ppm benzimidazole in 12 x 8 x 2 cm polystyrene boxes with the abaxial side up and fixed by plastic partitions to prevent the leaves from curling and drying. The leaf segments were incubated for five days at 14°C and 3000 lux continous lighting. Sixten hours before use the boxes were inverted and tapped gently with a finger to remove old conidia.

Inoculation, incubation and assessment methods

The conidia from 'Manchuria' leaf segments were harvested with a cyclone spore trap (from ERI Machine Shop, Iowa State University, Ames, Iowa 50011) and within 30 min the desires amounts of conidia (in mg) were suspended in fluorochemical FC 43 (in ml). The suspension was sprayed using an 'inoculator' described by Knudsen (1984) onto the horizontally fixed leaves of the barley lines. The inoculum density in conidia per cm^2 leaf area was adjusted to: 100 of isolates 'GE 3' and 'HL 3/5' on line R 5678 (S); and 5000 of 'GE 3' and 700 of 'HL 3/5' on line R 5678 (R). The inoculated plants were incubated in the perspex cages described above and under those environmental conditions. Four pots each with ten leaves were inoculated for each of the four host/pathogen combinations. This complete experiment was replicated twice. Forty-eight hours after inoculation, parlodion impression films were made from one leaf from each pot (see Thordal-Christensen & Smedegård-Petersen 1988). The films were mounted in lactofuchsin (see Jørgensen & Mortensen 1977). They were used to assess the frequensis of spore germination and epidermal cell types, and the frequency of infection by light microscopy. The remaining nine leaves per pot were used to assess the latent period and disease efficiency.

Germination frequency

In each leaf imprint 300 randomly chosen conidia were examined. A conidium was considered germinated if it had a normally appearing appressorial germ tube with at least one lobe, or an elongating secondary hyphae (equal to the types 0, 1 and 2 in Fig. 2b, and to types 1, 2 and 3 in Fig. 2c, respectively, described by Andersen & Torp (1986)).

Frequency of epidermal cell types

Leaf imprints were searched from the leaf edge to the central leaf vein at 125 x phase contrast magnification. For greater detail 200 or 500 x magnification was used. In each imprint 300 to 350 conidia with appressorial germ tubes were scored for position of its primary lobe assuming that the underlying epidermal cell was exposed to an infection attempt by the penetration peg at the central part of the lobe. These epidermal cells were classified as long, short or subsidiary cells (Fig. 2). The frequency of conidia encountering the three different cell types is assumed to represent the percentage of the leaf surface constituted by this cell type.

On the susceptible leaves appressoria normally had only one lobe, wheras on the resistant leaves many appressoria had two or even three lobes. In such cases the challenged epidermal cell was scored as the one encountered by the latest developed lobe.

Infection frequency
In three of the four host/pathogen combinations all the germinated conidia were also scored for the presence or absence of an elongating secondary hyphae (ESH), i.e. whether a functional primary haustorium had been established or not by the fungus (Masri & Ellingboe 1966). The infection frequency in the different cell types was calculated as germinated conidia with ESH as a percentage of all germinated conidia encountering the same cell type. Because of the low frequency of successful infections of the non-aggressive isolate 'GE 3' on the resistant line R 5678 (R), an indirect approach was necessary to assess infection frequency. A multiple number of strips across the leaf imprint were searched at low magnification (78 x), and only the very rarely but easily found appressoria with ESH were scored with respect to infected cell type. From the known area of the strips, inoculum density, frequency of germination and cell types and the infection freqeancy in each cell type were estimated. In this combination the estimated number of germinated conidia was more than 57,000.

Latent period and disease efficiency
On the remaining nine leaves per pot the visible mildew colonies on the 5-cm leaf segments were counted each day between the fourth and eighth day after inoculation on R 5678 (S), and until the eleventh day on R 5678 (R), using a large 10 x magnifying lens. The latent period was calculated to be the first day when the final number of developing mildew colonies became visible. The disease efficiency was calculated to be the percentage of germinated conidia that produced visible colonies.

Statistical analysis
Statistical analysis were carried out using the Statistical Analysis System (SAS). Analyses of variance or general linear model analyses were used. Comparisons between isolate/host combinations were made by 'Duncan's new multiple range test'. An exception was the infection frequency, where 95% intervals of confidence were calculated in accordance with the formula for binomial distribution.

RESULTS

Germination frequency
The germination percentage of conidia was independent of the host genotype, i.e. unaffected by the presence vs. absence of gene *mlo5* in the plants (data not shown). The experimental set-up was not designed for a critical comparison of the germination percentages of the two isolates with and without Mlo aggressiveness.

Frequency of epidermal cell types
The estimates of the percentage of the leaf area constituted by the three cell types are given in Fig. 2. The cells subsidiary to stomata constituted 6%, the short cells 35%, and the long cells 59% of the leaf area. These figures are from estimates on the two barley lines, the susceptible R 5678 (S) and the resistant R 5678 (R). However, as they did not differ, the data were pooled. These figures are close to previous estimates (cf. Koga et al. 1990).

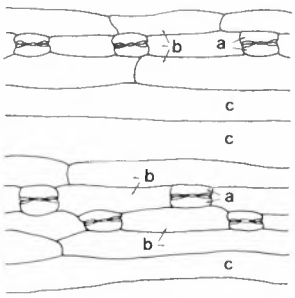

Fig. 2. Epidermal cell types of the abaxial side of the first true leaf of barley

Cell type		Per cent of leaf area
a:	Subsidiary cells in stomata	6
b:	Short cells in contact with stomata	35
c:	Long cells without contact with stomata	59

Infection frequency

The percentage of germinated conidia that successfully infected an epidermal cell, i.e. formed as ESH (Table 1), is practically the same irrespective of cell type and isolate on the susceptible line R 5678 (S), except for isolate 'HL 3/5' in subsidiary cells. This estimate is based on observations of a limited number of conidia, however, and is, therefore, unreliable.

Table 1. Infection frequency in three types of epidermal cells and in the leaf, latent period, and disease efficiency for the Mlo aggressive powdery mildew isolate 'HL 3/5' and the non-aggressive isolate 'GE 3' on primary leaves of the *mlo5* resistant barley line R 5678 (R) and the susceptible line R 5678 (S). Relative values are in parentheses. The recorded number of ESH and germinated conidia are indicated by the figures separated by a slash. Values within each column followed by different letters are significantly different at the 5% level. For columns 1-4 comparisons between columns are also valid

Mildew isolate	Barley line	Infection frequency in percent in subsidiary cells	short cells	long cells	the leaf	Latent period, days	Disease efficiency, percent
GE 3	R 5678 (S)	36.7 ± 10.4 ab 29/79	39.3 ± 5.0 a 146/372	46.5 ± 3.9 a 292/628	43.3 ± 3.0 a (100)	5.44 ± 0.48 a (100)	25.1 ± 6.4 a (100)
HL 3/5	R 5678 (S)	20.4 ± 10.6 b 11/54	43.0 ± 5.4 a 138/321	43.9 ± 4.3 a 219/499	42.3 ± 3.3 a (98)	5.51 ± 0.23 a (101)	23.4 ± 2.7 a (93)
GE 3	R 5678 (R)	0.84 ± 0.32 e 28/3331	0.10 ± 0.05 f 20/20213	<0.003 f 0/33720	0.08 ± 0.02 e (0.2)	9.63 ± 0.33 c (177)	0.05 ± 0.02 c (0.2)
HL 3/5	R 5678 (R)	4.14 ± 3.41 cd 6/145	7.55 ± 1.63 c 77/1020	3.67 ± 1.14 d 39/1062	3.92 ± 0.90 d (9)	7.65 ± 0.51 b (141)	2.93 ± 1.13 b (12)

The effect of the resistance gene *mlo5* is seen in the combination of isolate 'GE 3' and line R 5678 (R) where the infection frequency on the whole leaf is reduced by a factor of about 500. The majority of infections (28) were in subsidiary cells and the rest (20) were in short cells. The subsidiary cells are thus nearly ten times more susceptible than the short cells and they exhibit a moderate level of Mlo resistance only. No successful

infections were seen among nearly 34,000 infection sites in long epidermal cells. In other experiments (unpublished data) a few successful infections have been recorded in long cells. The true value of the infection frequency in long cells of Mlo-resistant barley by non-aggressive powdery mildew may thus be considered to be approximately one per 20-30,000 germinated conidia.

The effect of the aggressiveness of isolate 'HL 3/5' on the Mlo-resistant line R 5678 (R) is seen as an increase in infection frequency on the leaf by a factor of about 50 when compared with that of the non-aggressive isolate 'GE 3'. It is important to note, however, that this highest known level of aggressiveness is still only 10% that in a compatible combination, e.g. by 'GE 3' on R 5678 (S). The Mlo aggressiveness of isolate 'HL 3/5' results from an approximately 5, 75 and 1000-fold increase in its ability to seccessfully infect subsidiary, short and long epidermal cells, respectively.

The latent period

The latent period (Table 1) shows no difference between the two isolates when growing on the susceptible line. The very few colonies developed by isolate 'GE 3' on the resistant line require nearly ten days to reach a size that enables detection. The latent period of the Mlo-aggressive isolate 'HL 3/5' on the resistant line is intermediate between that of the non-aggressive isolate on the resistant line and that of the two isolates on the susceptible line.

Disease efficiency

The percentage of germinated conidia that infect the host and survive until the reproductive stage (Table 1) is about 25 in the two compatible host/pathogen combinations; it is drastically reduced with the non-aggressive isolate, and reduced to about 10% only with the aggressive isolate. The relative values in the four combinations are close to those for infection frequency on the leaf. Calculations reveal that the percentage of successful infections that survive to produce a visible colony is 58, 55, 64, and 75% respectively, for the host-pathogen combinations GE-(S), HL-(S), GE-(R), and HL-(R). These differences are probably due to the experimental set-up. On the susceptible barley line, the number of colonies per square centimetre that had to be counted reached 20 or even 25, so that two or three close-lying colonies may have been counted as one, and some may have repressed by vigorous growth of close-lying colonies. Therefore, we consider these numerical differences invalid, and assume that the true percentage of conidia with ESH that survive to produce a sporulating colony is around 70% in all host/pathogen combinations.

DISCUSSION

The present study describes stages of infection of Mlo-resistant and non-resistant barley by aggressive and non-aggressive powdery mildew. This is required in order to understand the mode of action of Mlo aggressiveness, and to extend our understanding of the effect of Mlo resistance, which recently has become a major source of resistance in European spring barley.

The first stage in the infection, the infection frequency, was between 35 and 45 in all cell types of the susceptible barley line. In contrast to some other studies (e.g.

Andersen & Torp 1986; Jørgensen & Mortensen 1977; Koga et al. 1990), we did not find differences in susceptibility between different epidermal cell types. It is often found that subsidiary cells are very susceptible, short cells are less so, and long cells are the least susceptible, estimated as the percentage conidia that form haustoria in these cells and subsequently elongating secondary hyphae (ESH). The effect of Mlo resistance is virtually complete resistance of long epidermal cells (Table 1) and a high level of resistance of short cells that together constitute nearly 95% of the leaf area. Only the subsidiary cells show relatively frequent infections, close to 1%. This falls in line with the observation that cell-wall appositions are formed infrequently in subsidiary cells (Skou 1985) probably because these cells have a unique physiology and flexible cell walls that enable them to open and close the stomata. The Mlo-aggressive isolate differs from the non-aggressive one primarily in its ability to successfully infect long epidermal cells; the aggressive isolate is about 1000 times more effective than the non-aggressive one. A simple, but rather laborius screening method for Mlo aggressiveness of powdery mildew isolates may be to assess the frequency of infections in long epidermal cells. Averaged over the entire leaf (Table 1) the Mlo-aggressive isolate has an infection frequency close to 4%, a value that is about 10% that in compatible interactions.

The latent period is the phenotypic expression of the degree of success of the numerous secondary, tertiary and subsequent infection attempts, and the resulting amount and density of mycelium, conidiophores, and conidia produced. The substantially prolonged latent period of ordinary powdery mildew on Mlo-resistant barley reflects the delay in these processes. The less prolonged latent period for the Mlo-aggressive isolate is an expression of its higher success rate of infection.

The relative data on disease efficiency (Table 1) are close to those on infection frequency but with an overall reduction to about 70% in numerical values as described above.

We also attempted to assess colony size and spore production in the present and a subsequent experiment, but due mainly to technical obstacles most data turned out to be worthless. The data suggested, however, that neither of these two parameters is applicable for easily assessing grades of Mlo aggressiveness among many isolates. Furthermore, the spore production of the non-aggressive isolate on Mlo-resistant barley was very low, and that of the aggressive isolate so high that it can easily maintain itself on seedlings of Mlo-resistant barley provided that the spores produced are utilized effectively as inoculum, and under the given environmental conditions. Some of the data presented are slightly or somewhat lower than expected (Schwarzbach, pers. comm.) from the Mlo-aggressive powdery mildew isolate 'HL 3/5'. We purified our sample of 'HL 3/5' at the end of the experiments by isolating and aggressiveness-testing single-spore isolates. Only small variations were found. Therefore, we conclude that our data are representative for isolate 'HL 3/5' with perhaps very small admixture of non-aggressive mildew.

The issue of whether the Mlo-aggressive isolate has a reduced fitness when growing on susceptible barley is not resolved by the present experiments. The aggressive isolate has a statistically insignificantly reduced infection frequency and disease efficiency (Table 1). When added to former data (Jørgensen 1984; Keller & Mendgen 1984) it appears that this aggressive isolate may have a slightly reduced fitness on non-Mlo-resistant barley. This issue is important because the fitness of Mlo-aggressive mildew on non-Mlo barley may play an important role when/if Mlo aggressiveness arises and

spreads in natural powdery mildew populations in a region where Mlo- resistant and non-Mlo-resistant barley crops are grown intermixed.

The results from the present and similar studies were derived in the laboratory under environmental conditions optimal for growth and reproduction of powdery mildew on the first true leaf of barley seedlings. Caution must therefore be exercised when using such data to predict the behaviour of this host-pathogen system under field conditions where the environmental conditions are less favourable for powdery mildew and where the host tissue is a less favourable substrate for the fungus. The present results indicate that a substantial increase in Mlo aggressiveness is required for powdery mildew to be able to survive and maintain itself on Mlo-resistant barley seedlings. We anticipate that a level of aggressiveness close to that of isolate 'HL 3/5' is required under field conditions. This level of aggressiveness is unlikely to be attained by a single-step mutation (Jørgensen 1987), and experimental results suggest that Mlo aggressiveness of isolate 'HL 3/5' is conferred by several or many additively acting genes (Schwarzbach 1979; Andersen 1991). The accumulation of Mlo aggressiveness in natural powdery mildew populations is therefore likely to occur via repeated cycles of selection for aggressiveness on Mlo-resistant barley followed by maintenance and multiplication of the selected subpopulation on non-Mlo-resistant barley, from where it serves as inoculum for Mlo-resistant barley. This anticipated process is a mirror image of the process Schwarbach (1979) used to select isolate 'HL 3/5'. During the first 15 cycles of selection, the subpopulations selected by the Mlo-resistant barley line had to be multiplied on non-Mlo-resistant barley in order to maintain it.

Given the above assumptions, we may predict that the evolution of Mlo aggressiveness to self-sustenance on Mlo-resistant barley under field conditions will require on the order of 40 successive cycles of selection on Mlo-resistant barley intermitted by multiplication on non-Mlo-resistant barley. If Mlo aggressiveness is associated with fitness reduction when growing on non-Mlo-resistant barley, more cycles are required. Finally, if aggressiveness of a natural powdery mildew population evolves to self-sustenance, i.e. like that of isolate 'HL 3/5', the amount of disease incited will be on the order of 10% of that incited in compatible interactions. This is deduced from the estimates of relative disease efficiency (Table 1). In conclusion, the Mlo resistance in barley to powdery mildew appears to be a very durable one. If, however, Mlo-resistant spring- and winter barley varieties are grown extensively, it is likeley that the powdery mildew will slowly but steadily evolve increased aggressiveness and gradually cause disease that may approach the threshold level for crop losses.

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Side-effects of the herbicide isoproturon on the severity of powdery mildew in spring barley and winter wheat

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In order to evaluate the effects of the herbicide isoproturon on the severity of powdery mildew (*Erysiphe graminis* DC.:Fr.) disease under Danish climatic conditions, field trials were conducted in 1989 and 1990 in spring barley and in the 1989/90 growing season in winter wheat. In spring barley, the herbicide was applied at the recommended dosage, and in winter wheat at six dosage levels ranging from 0 to 2 times the recommended dosage. In 1989 the herbicide application had no effect on the severity of powdery mildew on barley. In 1990, however, a significant increase in the powdery mildew severity was observed of leaf numbers 2-4. In winter wheat, an increased dosage of isoproturon strongly increased the severity of powdery mildew after ear emergence.

Key words: Disease, epidemic, *Erysiphe graminis*.

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The powdery mildew fungus (*Erysiphe graminis* DC.:Fr.) is a biotrophic pathogen and is therefore dependent on a living host throughout its life cycle. Cultural practices which change the growing conditions and the vigour of crop plants may therefore change the conditions for the fungus and thereby change the rate of reproduction. It has been demonstrated that application of herbicides normally applied to soil or plants to control weeds alters the resistance of a crop to fungal diseases (Altman & Campbell 1977; Katan & Eshel 1972). In German investigations it has been shown that the use of urea- and triazine herbicides can significantly increase the severity of powdery mildew in wheat, while other groups of herbicides appear to have no effect (Ibenthal & Heitefuss 1979a). The effect of the herbicide application on the severity of the powdery mildew attack is ascribed to physiological changes in the host plants (Ibenthal & Heitefoss 1979b). The physiological response is divided into two phases: first there is the shock phase, following immediately after the herbicide application, in which the plants are more resistant to infection by powdery mildew than the untreated control plants. Later in the growing season, the shock phase is replaced by a "recovery phase", where

the treated host plants are more susceptible to powdery mildew infection than the untreated control plants (Ibenthal & Heitefuss 1979a, b).

The use of one cultural practice (herbicide application) may therefore create the need for another cultural practice (fungicide application), thereby increasing the use of chemicals in agriculture. It is therefore important to widen our knowledge of the side-effects of herbicides in order to reduce the use of chemicals.

Thus, in order to investigate whether the use of the urea herbicide isoproturon has an influence on the severity of powdery mildew in grain crops under Danish climatic conditions, and to discover to what extent the effect is dependent on the herbicide dosage, field trials were conducted in spring barley and winter wheat.

MATERIALS AND METHODS

Experimental methods

Field experiments were conducted at the Research Station of the Agricultural University of Copenhagen, which is located at Zealand.

A complete randomized block design with six replications per treatment was utilized with 1.5 x 10.0 m. (15 sq.m.) plots. All plots were separated with guard plots of spring wheat in the barley trials and with winter barley in the winter wheat trial in order to avoid interplot interference. All plots were hand-weeded to avoid differences in the microclimate between the treatments.

The spring barley cultivar Corgi, which is susceptible to powdery mildew under Danish conditions and relatively resistant to leaf rust, was used in both years. In 1989 the experiment was sown on 3 April and nitrogen was supplied at 80 kg N/ha as calcium ammonium nitrate (kas) on 28 April. The herbicide was applied at the recommended dosage (1.0 kg a.i./ha) on 25 May. In 1990 the experiment was sown on 28 March and nitrogen was supplied on 26 April with 100 kg N/ha and the herbicide at the recommended dosage on 10 May.

The winter wheat cultivar Citadel was sown on 21 September 1989 and nitrogen applied at a rate of 120 kg N/ha (kas) on 6 April. The herbicide isoproturon was applied at six increasing dosages ranging from zero to two times the recommended dosage (1.26 kg a.i./ha) on 2 April.

Data collection and analysis

Spring barley

Five times during the growing season 1989, 20 tillers in each plot were collected randomly and the severety of powdery mildew was assessed by counting the number of powdery mildew colonies on the four uppermost leaves on each tiller. In 1990 the disease level was high, and the degree of severity was determined by estimating the proportion of diseased leaf area on leaf numbers 2-4, and by counting the number of powdery mildew colonies on the uppermost leaf (leaf 1) five times during the growing season. For the last assessment, leaf number one equalled the flagleaf. In both years, data from the individual tillers were pooled and analysed by the Area Under Disease Progress Curve (AUDPC) as described by Shaner & Finney (1977). Grain yield (15% water content) and thousand grain weight were determined.

Winter wheat
Twenty tillers per plot were collected randomly six times during the growing season and the number of powdery mildew colonies were counted on the four uppermost leaves. The flagleaf was included from the third assessment date. Data were pooled and analysed with an analysis of variance for the individual assessment dates. Grain yield and thousand grain weight were determined.

RESULTS AND DISCUSSION

Spring barley

In 1989 the isoproturon treatment had no significant effect on the severity of powdery mildew estimated by counting the number of colonies during the growing season (Table 1). In 1990 the level of severity on leaves 2-4 was significantly greater in the treated plots compared with that in the untreated control. The slightly increased disease level on leaf 1 was not significantly different from that on the untreated control plots (Table 1). The increased disease severity did not affect the grain yield and thousand grain weight.

The present results indicate that the use of the herbicide isoproturon in spring barley under certain conditions may have the effect of increasing the powdery mildew severety, but whether the effect is of any practical importance is still an open question. There have been no other reports on the impact of isoproturon on the severity of powdery mildew in spring barley.

		Control	Isoproturon	LSD 5%
1989	leaves 1-4	8203	7418	-
1990	leaf 1	4370	5115	-
	leaves 2-4	1675	1898	192

Table 1. Area Under Disease Progress Curve (AUDPC) for powdery mildew disease after treatment with the herbicide isoproturon in field trials for the spring barley variety Corgy during 1989 and 1990

Winter wheat

The effect of increasing the dosage of the herbicide isoproturon on the average number of powdery mildew colonies from 7 May to 13 June is presented in Fig. 1.

The powdery mildew levels in the different treatments were almost the same up to ear emergence on 21 May, after which a pronounced effect was seen. The drop in number of powdery mildew colonies after 14 May is due to a change in leaf numbers. After this date leaf 1 is the flagleaf.

A clear dosage response pattern can be seen. The highest dosage (twice the normal) resulted in more than five times as much powdery mildew as in the untreated control plots on 29 May and 6 June. Dosaged giving a significantly higher level of disease than the untreated control are indicated in Table 2.

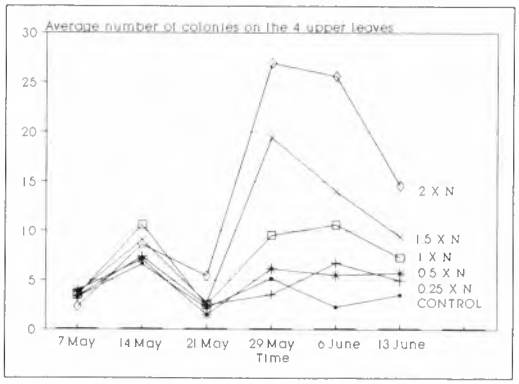

Fig. 1. Average number of mildew colonies on the four upper leaves in a field trial with the winter wheat variety Citadel treated with 0, 0.25, 0.5, 1.0, 1.5 and 2.0 times the normal dosage (N) of the herbicide isoproturon (N = 1.25 kg a.i./ha) on 2 April

Table 2. Dosages of the herbicide isoproturon giving a significantly higher level of disease than the untreated control in winter wheat expressed as $LSD_{.95}$ at the different assessment dates

	Assessment date					
	7 May	14 May	21 May	29 May	6 June	13 June
Dosage	-	1	2	1	$\frac{1}{4}$	1
				$1\frac{1}{2}$	1	$1\frac{1}{2}$
				2	$1\frac{1}{2}$	2
					2	

In winter wheat the increased disease severity after ear emergence is in accordance with German results. Thus, Ibenthal & Heitefuss (1979a) ascribed the response to a physiological change (recovery phase) in the host plant due to the herbicide treatment. However, no shock phase was observed in the present study. This may partly be due to the relatively low disease severity in the trial, and partly an issue of the time between the first appearance of powdery mildew and the spraying time. If the herbicide application takes place around the first powdery mildew appearance, the direct fungitoxic effect of the herbicide isoproturon, as demonstrated by Kaysø & Munk (unpubl.) in greenhouse trials, may be responsible for a decreased disease severity in the first part of the growing season.

It was observed that the highest dosage of the herbicide kept the upper parts of the wheat plants somewhat greener than those of the control plants at the end of the growing season. This "greening effect" may to some extent account for the increased disease severity. The "greening effect" is described by Ibenthal & Heitefuss (1979b), who, in greenhouse trials with spring wheat, demonstrated that the rate of senescence was delayed after herbicide treatment.

The treatment with the herbicide isoproturon had no effect on the thousand grain weight, but the yield was slightly affected by the herbicide treatment (Table 3). The highest dosage caused a significant reduction in the yield. The reduction in yield does not reflect cultural practice since all the plots were hand-weeded, and an increase in yield due to removal of competition from the weeds was eliminated.

Dosage	N[1] X 0	¼	½	1	1½	2
Tgw (g)	39.3	39.0	39.1	39.1	38.2	38.2
Yield hkg/ha	56.6	57.9	57.2	56.3	54.8	50.0

[1]: N = 1.25 kg a.i./ha LSD_{95} Yield = 4.30

Table 3. Grain yield (hkg/ha) and thousand-grain weight in field trials in the winter wheat variety Citadel 1989/90 treated with increasing dosages of the herbicide isoproturon. Dosage multuplied by the recommended dosage (1.25 kg a.i./ha)

In the same winter wheat trial the side-effects on aphids (*Sitobion avenae*, *Rhopalosiphum padi* and *Metopolophium dirhodum*) were recorded on 28 June in the plots treated with 0, 1 and 2 times the recommended dosage (N). It was found that the herbicide treatments at 1 and 2 times N significantly increased the number of aphids. In spring barley, however, where samples were taken during the entire growing season, it was found that there was no effect on the number of aphids (Kristiansen, pers. comm.).

The present investigations were carried out in one variety of spring barley and winter wheat, and the effect from herbicide treatment was much more pronounced in winter wheat than in spring barley for both powdery mildew and aphids. It is not known, however, whether different varieties of spring barley exhibit specific reactions to the herbicide treatment, as has been demonstrated for winter wheat (Kuhlmann & Heitefuss 1987).

CONCLUSION

This is the first report on the impact of herbicides on powdery mildew in spring barley and winter wheat in Denmark. In the first year of field experiments, no effects were found in spring barley. However, during the subsequent year an increase in the severity of powdery mildew on leaves 2-4 was recorded. The present results in spring barley therefore indicate that using the urea herbicide isoproturon under certain conditions may increase the severity of powdery mildew in barley, but further investigations are required.

In winter wheat, the present results from a single season are in accordance with field trials carried out in Germany. It is therefore likely that the side-effects from urea herbicides reported from Germany in winter wheat are valid and of practical importance under Danish climatic conditions. Further research in this subject is needed and forms a natural element in the ongoing debate about sustainable agriculture.

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Resistance to *Septoria nodorum* in Norwegian spring wheat

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The results from artificial field inoculation of breeding material of spring wheat with *S. nodorum* during 1988-90 revealed some variations in resistance between lines, while the commercial cultivars were medium resistant. One short line was almost as resistant as the taller and later CI 12463 line and a few lines had a resistance level somewhere between this and the cultivars. One-third of the lines were significantly more susceptible than the most resistant cultivar. Selection for resistance with this field testing method should make it possible to increase the level of resistance in our breeding population of wheat.

Key words: Glume blotch, inoculation, *Triticum aestivum*.

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Septoria nodorum (Berk.) Berk. (*Leptospaeria nodorum* E. Müller) is a serious pathogen on wheat (*Triticum aestivum* L.) in many parts of the world. In Norway the pathogen occurs frequently, and in rainy summers wheat may suffer heavy attacks of the fungus. For some time now Norwegian wheat breeders have placed great emphasis on resistance to *S. nodorum* in their breeding programmes. Thus, cultivars like Runar have some resistance to the fungus but not enough to sustain a heavy disease pressure. Elen (1981) showed that when Runar was treated with fungicides in a year with heavy attacks, the yield reduction on untreated plots was 39% (1790 kg/ha).

Previously, the breeders relied on natural infection, but occasionally there could be a dry period lasting several consecutive years, e.g. the dry 1970s when there was no disease development in the field. The breeding line, T 69027, was almost released as a new cultivar when we had two rainy summers in 1979 and 1980. According to Strande (1981), 37% of the leaf area of this cultivar was attacked in 1980, while the corresponding value of Runar was 9%. The yield of T 69027 was 19% lower than that of Runar for the same year, while during the previous dry years, T 69027 had exceeded Runar by 10 to 15%.

MATERIALS AND METHODS

In the first, second and third years of a field study, 225, 121 and 144 spring wheat breeding lines and cultivars respectively, including CI 12463 (Coastal) and the four cultivars Runar, Reno, Bastian, and Tjalve, were sown in a lattice design with three replicates. Most of the breeding lines were different from year to year. In 1988 and 1989 each plot comprised a single row with 25 cm between rows, and in 1990 a hill plot with 30 cm between hills. In the first year the plots were inoculated by spraying a spore suspension (10^6 spores ml^{-1}) on the leaves and the heads just after ear emergence and then irrigated for 24 h with an intensity of about 1 mm h^{-1}. In the last two years the inoculum consisted of infected straw from the previous year. The inoculum was spread at the 3-4 leaf stage and the field was irrigated one day a week in the dry periods.

The disease severity was assessed as a percentage of the area on the upper two leaves between growth stage 60 and 70 and on the spike at about stage 85 (coding by Zadoks et al. 1974). In 1989 the straw length was measured. Each year an analysis of variance was performed for all the lines. In the third year the means from 27 cultivars and lines common for the three years were combined for an analysis of variance with the years as replicates.

RESULTS AND DISCUSSION

After inoculation with straw, the disease developed at a very early stage on the lower leaves and then spread upwards during the summer. In contrast, inoculation by spraying reached both leaves and heads at the same time, and the disease developed only at the later stages. It was not possible to detect any great differences between the methods late in the season, perhaps partly because of the small plot size. If the plots had been larger, differences in resistance could perhaps have been easier to detect even between years.

The analysis of variance revealed statistically highly significant differences between lines every year. However, this was partly due to the high number of lines and thus the number of degrees of freedom. The margin of error was quite large and the $LSD_{5\%}$ values varied from about 50% to 135% of the means (Table 1), making it possible to detect only very large differences in resistance between lines.

Table 1. Means of percentage of leaf and spike area infected by *S. nodorum* on all tested lines and cultivars. $LSD_{5\%}$ values in parentheses ( )

	1988	1989	1990
Leaves	34 (24)	35 (18)	18 (13)
Spikes	36 (20)	19 (15)	14 (19)

The average attack of *S. nodorum* on the 27 cultivars and lines tested in all three years is indicated in Table 2. The three-year average did not greatly reduce LSD and the values were between 15.5 and 16.3. However, the means over three years should give a more accurate estimate of the resistance than results from a single year. The wide

variation between years may be due to an interaction between lines and years, but the experimental design did not permit statistical analyses which could clarify this point.

Table 2. Average attack of *S. nodorum* on some cultivars and lines 1988, 1989, and 1990 and straw length 1989

Cultivar/ line	% on leaves	% on spikes	Straw length cm
Runar	35	16	71
Reno	21	23	72
Bastian	21	29	64
Tjalve	25	24	59
T4023	24	27	64
Vo289-71	17	14	81
VoT10413-79	19	22	84
CI 12463	7	1	87
T8041	10	5	53
T1015	31	15	59
T5038	40	22	60
T7010	19	19	66
T7015	28	29	62
T7031	19	19	67
T7034	35	35	65
T7042	13	11	67
T8008	45	27	59
T8016	41	29	61
T8018	40	33	72
T8021	38	33	58
T8022	47	34	57
T8024	42	44	58
T8026	30	18	62
T8027	29	19	67
T8036	49	19	57
T8046	36	27	60
T145-910	18	15	83
$LSD_{5\%}$	16.3	15.5	-

Among the cultivars, Runar was the most susceptible on the leaves, while Bastian was the most susceptible on the spikes. On the other hand, Runar was the most resistant on the spikes and Reno and Bastian on the leaves. There were not, however, any significant differences between the cultivars. CI 12463, T8041, T7042, Vo289-71, and T145-910 were significantly more resistant on the leaves than the most susceptible cultivar and the first three lines mentioned were also more resistant on the spikes. Examination of most of the other breeding lines indicates that there has been little advance in resistance compared with the cultivars. Moreover, nine lines were significantly inferior in resistance compared with the most resistant cultivars.

T 8041 had a remarkably high level of resistance both on leaves and on spikes, despite its being the shortest line. CI 12463 was the most resistant line, and it was also very tall and late, so this may partly explain its resistance. Karjalainen (1985), too, found that CI 12463 was the most resistant bread wheat in an investigation of resistance to *S. nodorum* in Finland. He also found that Runar had a fairly good leaf resistance

compared with that of the Finnish cultivars and lines. Although Runar did not have a good leaf resistance in the present investigation, several lines were considerably more susceptible, so Runar seems to have some leaf resistance to *S. nodorum*.

There is sufficient variation in resistance to *S. nodorum* in Norwegian spring wheat for it to be utilized in wheat breeding. Inoculation and irrigation of the plants in the field will permit a harder selection for resistance to this pathogen. The most susceptible lines will be eliminated and the wheat population will become more resistant, which again will lead to more resistant lines in later generations.

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DNA-transformation technology of phytopathogenic fungi and its applications

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Recent advances in the development of gene transfer systems for fungal plant pathogens are reviewed with illustrations of techniques for transforming a barley pathogen, *Bipolaris sorokiniana*. Up until now, successful transformations have been reported for more than ten fungal genera. The use of versatile dominant drug markers has expanded transformation into genetically poorly characterized fungi. In most fungi, transformation efficiensy is low, but will be considerably improved when transformation procedures are optimized and autonomously replicated sequences introduced into fungal vectors. Transformation technology is the most efficient way to reveal pathogenicity and virulence factors in fungi, and it will offer new ideas for improving disease resistance by genetic engineering. Transformation of specific antifungal genes into biocontrol strains is now technically feasible.

Key words: *Bipolaris sorokiniana*, DNA-transformation, fungal pathogens, pathogenicity.

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Fungi constitute the largest and economically most important group of plant pathogens in northern Europe. However, the precise mechanisms by which fungal pathogens cause disease are poorly understood. The putative role of cell-wall degrading enzymes, such as pectinases and toxins, as pathogenicity factors has often been demonstrated (Yoder & Turgeon 1985), but we still lack genetic evidence. Detailed understanding of the mechanisms behind the disease process is of primary importance in trying to develop novel ways of disease control against such pathogens as certain forest fungi, which are extremely difficult to control by available strategies. Elucidating the genetic weakness of the pathogen may be an important component of future disease control.

Recent advances in recombinant DNA techniques have given considerable hope that the application of these new tools will lead to a more thorough understanding of the pathogenic nature of phytopathogenic fungi (Leong & Holden 1989; Timberlake & Marshall 1989). Some progress has already been made in cloning genes encoding putative pathogenicity factors. For example, Soliday at al. (1984) isolated a cutinase gene, Weltring et al. (1988) cloned the pisatin detoxification gene, and the pectinase gene was also recently cloned (Dean & Timberlake 1989). Two groups have isolated

mating type genes from a maize pathogen (Kronstadt & Leong 1989; Schultz et al. 1990). A putative avirulence gene was also recently isolated from *Fulvia fulva* (Van Kan et al. 1991). The potential role of isolated genes as important components in plant-fungal interaction requires that the cloned gene can be reintroduced at a desired location of a given fungal strain by transformation. DNA-mediated transformation thus plays a crucial role in the progress of understanding the molecular basis of fungal pathogenicity to plants. In this article, we review the recent advances in the development of DNA-transformation of phytopathogenic fungi with special examples of transforming a barley pathogen, *Bipolaris sorokiniana*.

SELECTABLE TRANSFORMATION MARKERS

Transformation of a phytopathogenic fungus was first reported i 1985 (Turgeon et al. 1985). Today DNA-mediated transformation is possible for more than ten genera of phytopathogenic fungi (Table 1). The basic requirement for a successful transformation is a selectable marker, which enables the selection of putative transformants from the background of non-transformed cells. A variety of selection systems are currently used. One of the most common markers can be found in the strains that carry a nutritional auxotrophy that can be rescued by complementation with a corresponding cloned gene. For example, Ballance et al. (1983) used the *N. crassa Pyr-4* gene to complement an *A. nidulans PyrG* mutation. In a similar way an *Arg B* mutant of the rice pathogen *Pyricularia oryzea* was complemented by the plasmid pMA2, which carried the *A. nidulans ArgB* gene that encodes ornithine carbomoyl transferase (Parson & al. 1987). However, this kind of transformation is limited in transforming plant pathogenic fungi because suitable auxotrophic mutant strains do not widely exist, and making mutants for every isolate to be transformed is a laborious task. Therefore gene vectors that do not require corresponding mutant strains are desireable in transforming phytopathogenic fungi.

The *amdS* gene has been cloned from *A. nidulans*, which codes for acetamide (Hynes 1986), which degrades acetamide to acetate and ammonia. This gene allows fungi to grow on a medium with acetamide as the sole nitrogen source. The *amdS* gene has been used to select transformants of some phytopathogenic fungi (Turgeon et al. 1985). However, although this selection marker is suitable for some filamentous fungi (Hynes 1986; Penttilä et al. 1987), it is not attractive to phytopathogenic fungi because their unclear backgrounds make selection of transformants difficult.

Resistance to the fungicide benomyl can be based on a single mutation, and its target molecule is β-tubulin (Orbach et al. 1986). The mutated β-tubulin gene is capable of transforming wild-type *N. crassa* cells to a resistant phenotype (Orbach et al. 1986). Gene vectors based on benomyl resistance have also been used to transform fungal plant pathogens, *Collotoirichum graminicola* and *Gaeumannomyces graminis*, but in both cases transformation frequency remained low (Henson et al. 1988; Panaccione et al. 1988). One way to improve transformation frequency in this system is to clone the own β-tubulin gene of the fungus by using conservative regions of heterologous β-tubulin genes as probes for screening libraries.

The most versatile selection markers in fungal transformation today are dominant antibiotic and drug markers, end especially plasmid vectors containing the hydromycin resistance gene fused to fungal promoters (Wang & Leong 1989). Hygromycin is an

Table 1. Selectable markers used for transformation of phytopathogenic fungi

Fungus	Selectable marker	Reference
	Mutants	
Magnaporthe grisea (*Pyricularia oryzae*)	*ArgB*	Parsons & al. 1987
Nectria haematococca (*Fusarium solani* f. sp. *pisi*)	*ArgB*	Rambosek & Leach 1987
Ustilago maydis (*Phanerochaete chrysosporium*)	*Pyr-3*	Banks & Taylor 1988
	Ade2	Alic et al. 1989
Fusarium oxysporum	*niaD*	Malardier et al 1989
	Acetamide	
Cochliobolus heterostrophus	*amdS*	Turgeon et al. 1985
	Fungicide resistance	
Gaeumannomyces graminis	ben^R	Henson et al. 1988
Colletotrichum trifolii	ben^R	Dickman 1988
Colletotrichum graminicola	ben^R	Panaccione et al. 1988
	Hygromycin B resistance	
Leptosphaeria maculans	$hygB^R$	Farman & Oliver 1987
Cochliobolus heterostrophus	$hygB^R$	Turgeon et al. 1987
Fulvia fulva	$hygB^R$	Oliver et al. 1987
Colletotrichum lindemuthianum	$hygB^R$	Roderiquez & Yoder 1987
Septoria nodorum	$hygB^R$	Cooley et al. 1988
Fusarium oxysporum	$hygB^R$	Kistler & Benny 1988
Ustilago maydis	$hygB^R$	Wang et al. 1988
Ustilago hordei	$hygB^R$	Holden et al. 1988
Ustilago nigra	$hygB^R$	Holden et al. 1988
Cryphonectria parasitica	$hygB^R$	Churchill et al. 1990
Magnaporthe grisea	$hygB^R$	Leung et al. 1990
Alternaria alternata	$hygB^R$	Tsuge et al. 1990
Ophiostoma ulmi	$hygB^R$	Royer et al. 1991

aminoglycoside antibiotic that inhibits protein synthesis in procaryotes and eucaryotes by interfering with translocation and causing misreading. *HygB* resistance genes have been cloned from *Streptomyces hygroscopicus* and *E. coli*. The gene coding for hygromycin B phosphotransferase, inactivates the antibiotic by phosphorylation. The major advantage of using hygromycin B resistance as a selectable marker is that it is suitable for genetically poorly charcterized fungi, and the basic requirement is that the fungal isolate to be transformed is sensitive to hygromycin. Several gene vectors are now available to be used for hygromycin B resistance based transformation, and evidence suggests that promoter elements from *Aspergillus* function well in various phytopathogenic fungi. Recently, a number of pathogenic fungi have been transformed using a variety of fungal vectors containing hygromycin B resistance (Table 1), but it seems that certain basidiomyces are difficult to transform by this system. An alternative dominant antibiotic marker for these fungi might be vectors based on bleomycin and pleomycin resistance (Austin et al. 1990).

GENE VECTORS

Plasmid vectors containing a selectable marker are most often used in fungal transformation, but cosmid vectors which accept large inserts (about 40 kb) are also available (e.g. pAN-7-2). The advantage of using cosmid vectors is that the large size of inserts permits the size of nearly complete genomic library to be relatively small, which makes it easier to find the right clones compared with plasmid-based libraries. Both plasmids and cosmids are typical derivates of pBR plasmids and contain bacterial origins of replication and antibiotic resistance markers, which make them easy to be amplified and manipulated in *E. coli*. Efficient expression of antibiotic markers in fungal transformation often requires that the marker gene is incorporated into a fungal transcriptional promoter (Wang & Leong 1989). For example, Wang et al. (1988) constructed a selectable marker system for *Ustilago maydis* transformation by transcriptional fusion of the coding region of the gene for *E. coli* hygromycin B with a *U. maydis* hsp 70 gene promoter. Later, Tsukuda et al. (1988) modified this vector (pHL1) by inserting autonomously replicating sequences (ARS) from the *U. maydis* genome into pHL1 to generate a new vector pcM43, which self-replicates in fungal cells and yields a very high transformation frequency. A similar strategy was used by Samac & Leong (1989) when the vector pTR1 was constructed by incorporating a *Hind*III fragment with a terminal inverted repeat (1211 bp) from the linear plasmid from *Fusarium solani* f. sp. *cucurbitae* into a unique *Hind*III site adjacent to the hygromycin B gene of the integrative *Ustilago* vector. With this vector, a 21-fold transformation increase was achieved compared with the vector lacking this additional 1211 bp fragment from *Fusarium*.

Fungal promoters were functionally selected by Turgeon et al. (1987) when they ligated DNA-fragments from *Cochliobolus heterostrophus* to the 5' end of the *E. coli hygB* gene, and DNA fragments capable of directing the expression of the fused gene were isolated. However, it seems evident that especially vectors that fuse *Aspergillus* promoters with the *hygB* gene efficiently express resistance to hygromycin B in many phytopathogenic fungi (Table 1), indicating that heterologous expression signals may be widely used in fungal transformation. Our experience in transforming the barley pathogen *Bipolaris sorokiniana* suggests that promoters from *Aspergillus* function more efficiently than promoters from related *Cochlibolus* fungi. Today, vectors that are widely used in fungal transformation are pAN-7-1 and pDH-25 which contain the *hygB* gene whose expression is controlled by *A. niduland gpd* and *trpC* expression signals (Punt et al. 1987; Kistler & Benny 1988).

FACTORS AFFECTING TRANSFORMATION FREQUENCY

The basic requirement for DNA-mediated transformation is that fungal cells are capable of taking up exogenous DNA. In phytopathogenic fungi, this competence is usually achieved by removing cell walls. They are usually digested with the enzyme Novozyme 234, which is a hydrolytic enzyme mixture secreted by the filamentous fungus *Trichoderma harzianum*. The concentration of Novozyme needed and the incubation time vary widely among fungi. For example, it takes about two hours at 30°C (Fig. 1) to digest the cell walls of *Bipolaris sorokiniana* and *Drechslera teres*, while it takes two days to digest the cell walls of *Heterobasidion annosum*, a forest fungus.

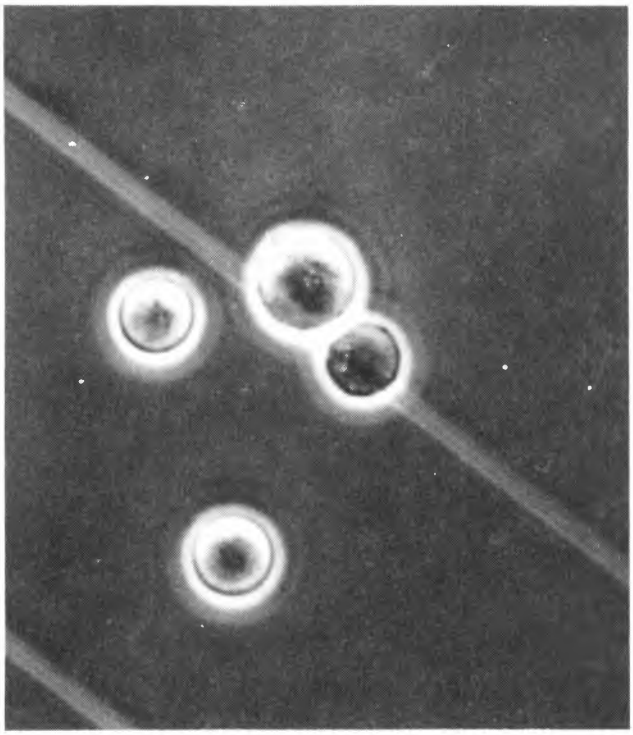

Fig. 1. Protoplasts of *Bipolaris sorokiniana* releases from young mycelium after incubation with Novozyme 234 at 30°C for two hours

In a few cases DNA is also introduced into osmotically sensitive cells by electroporation and particle bombardment (e.g. Timberlake & Marshall 1989; Wang & Leong 1989), but so far these methods do not appear to be as suitable for fungal as for plant transformation.

Several factors affect transformation efficiency, e.g. protoplast quality and incubation temperature of the DNA protoplast mixture (Kistler & Benny 1988; Specht et al. 1988). The concentration and quality of polyethylene glycol and the concentration of $CaCl_2$, polyamines, spermine, and spermamide are also important parameters affecting transformation (Dickman 1988; Kistler & Benny 1988). The quality of vector DNA and its linearization have a strong influence on transformation (Wang et al. 1988). When optimizing transformation strategies for barley pathogens, we found that the optimal timing of adding the selectable agent hygromycin to medium was a critical factor. We achieved the best results when hygromycin was added 24-48 h after plating protoplast-DNA solution on regeneration medium (Fig. 2).

In general, transformation frequency is low, 5-200/µg DNA. However, it has been considerably improved by protocol modifications and vector construction (Wang et al. 1988; Specht et al. 1988; Churchill et al. 1990; Royer et al. 1991). For example, Royer et al. (1991) recently described an efficient transformation system for the forest pathogen *Ophiostoma ulmi*, in which protoplast regeneration was optimized using the linearization of the best vector and inclusion of 2-mercaptoethanol in the transformation reaction. This yielded 4 x 10^3 transformants/µg DNA/10^7 protoplasts.

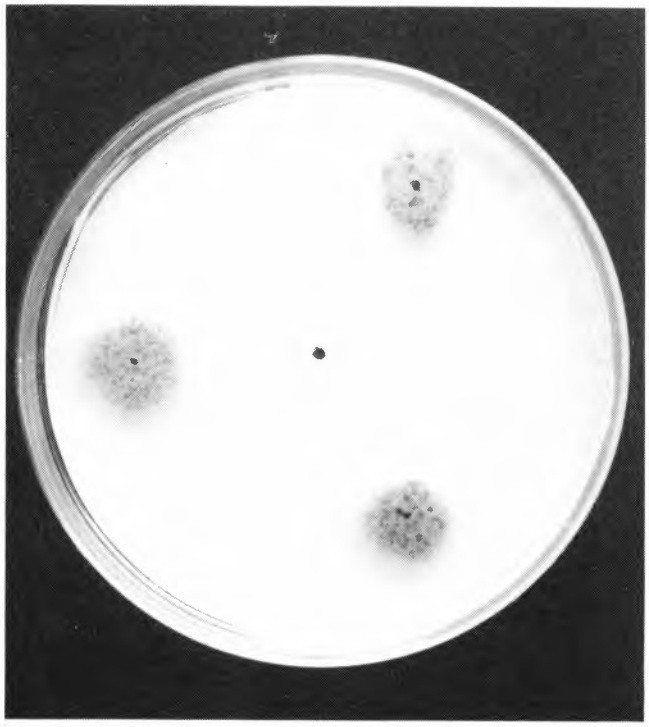

Fig. 2. Growth of transformants of *B. sorokiniana* on hygromycin-containing medium. Wild-type isolate in the middle

Two types of transformants are often recorded on a selective medium: those forming large colonies and those forming a large number of small colonies. Large colonies are stable transformants, while small colonies are an indication of transient expression of vector DNA and are Usually not mitotically or meiotically stable. Analyses of transformants by Southern hybridization have revealed that the integration of vector DNA into fungal genomes is often random (Oliver et al. 1987; Kistler & Benny 1988; Leung et al. 1990; Royer et al. 1991), but integraiton into single sites has also been observed (Tsuge et al. 1990), when vectors containing homology to fungal genomic DNA are used. The copy number of plasmid vectors integrated into fungal genomes varies widely, but there is no clear correlation between the number of copies and the resistance to hygromycin (Wang et al. 1988; Leung & al. 1990; Royer et al. 1991). Apparently the site of integration is more important than the copy number of vector DNA in determining the level of expression of a drug-resistant gene.

APPLICATION OF TRANSFORMATION TECHNOLOGY

Transformation technology offers an efficient strategy for cloning genes by complementation of mutants with a fungal genomic library. For example, Kronstadt & Leong (1989) isolated the mating type allele of the *b* locus of *U. maydis* by transforming a diploid strain homozygous for one *b* allele with a library of an isolate carrying a different *b* allele. Scultz et al. (1990) cloned four *b* mating type alleles by complementation strategy. So far, cloning by complementation is restricted to fungi where high frequency transformation is available. Cosmid rescue was used by Weltring et al. (1988) to clone

the pisatin dimethylating ability (PDA) gene from *Nectria haematococca*. This was cloned by detecting its expression in *A. nidulans*. However, although limited success in cloning fungal genes has been achieved, it appears that cloning relevant genes involved in host-pathogen interactions is a relatively difficult task. For example, the best way so far to clone genes encoding cell-wall degrading enzymes (e.g. cutinase, pectinases) involves cDNA cloning using antibody and oligonucleotide screening of expression libraries (Soliday et al. 1984; Dean & Timberlake 1989). It is anticipated that the novel strategies of PCR technology and pulsed-field electrophoresis will help plant pathologists to speed up the cloning of important genes from phytopathogenic fungi.

Transformation technology provides an effective way of testing the putative role of isolated genes in host-pathogen interactions. The PDA gene from *N. haematococca* was recently isolated by Weltring et al. (1988), and when this DNA sequence was transformed into non-pathogenic strains of *N. haematococca* unable to demethylate pisatin, it gave rise to transformants able to detoxify the phytoalexin and cause necrotic lesions on pea, thus providing direct evidence for the pathogenicity determinant of PDA in this fungus (Ciuffetti et al. 1988). The same PDA gene was also intriduced by transformation into *Cochliobolus heterostrophus*, which is a maize pathogen. Transformants that were obtained were able to attack pea leaves, a non-host for a maize pathogen, indicating that the host range of the pathogen can be altered by transformation of a single gene (Schäfer et al. 1988, 1989).

Recently the cutinase gene isolated from a pea pathogen, *Fusarium solani* f. sp. *pisi* (Soliday et al. 1984), was transferred into the genome of the phytopathogenic fungus of *Mycosphaerella* spp. that infects papaya fruits only if the fruit skin is mechanically breached before inoculation. Transformants of this wound-requiring fungus infected papaya fruits, and the specific role of the cutinase was verified when rabbit antibodies against the cutinase prevented infection. Van Kan et al. (1991) described the first piece of evidence for the isolation of fungal avirulence gene from the phytopathogenic fungus *Fulvia fulva*. Transformation of the avirulence gene into *Fulvia* races that lack the specific avirulence gene and testing host reactions of cultivars that carry corresponding resistance genes will be important steps towards the better understanding of the role of avirulence genes in plant-pathogen interactions.

The current technology allows the cloning of fungal genes, and it also allows the overexpression of cloned genes in fungal genomes because the gene can be used to a strong fungal promoter. In addition, specific alterations by gene disruption and gene replacement provide an effective way critically to test the role of cloned genes in host-pathogen interactions (Kronstadt & Leong 1989; Scultz 1990). Transformation technology may also have important applications for practical disease control. For example, biocontrol strains may be improved by introducing genes encoding antifungal compounds (chitinase, antibiotics, etc.) into antagonist strains. Modification of the target pathogen by altering hypervirulence traits or manipulation of fungal age may also offer effective strategies to control certain diseases.

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Studies on common root rot (*Aphanomyces euteiches*) of peas (*Pisum sativum*) in Sweden

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In Sweden common root rot of pea caused by the fungus *Aphanomyces euteiches* is one of the most damaging diseases associated with intensive cultivation of peas, *Pisum sativum* L. In this study 518 fields were found infected with the fungus. The fungus was found in 18 of 22 counties where samples were taken. In test studies 223 fields were randomly chosen from the important pea-growing areas of Sweden and 16 of these, that is 7%, were shown to have such a high degree of infection that it would be inadvisable to grow peas in them. As much as a 50% yield reduction was observed in seriously infected fields. Varietal differences in tolerance were observed. The fungus was found to cause a decrease in protein content, and a decrease in cookability. Varietal differences in degree of affection of protein content and cookability were observed. The fungus was further found to reduce the stalk length, thereby increasing the stalk stiffness.

Key words: *Aphanomyces euteiches*, interaction, *Pisum sativum*.

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In 1989 the area under pea cultivation in Sweden comprised about 47,000 hectares. This was divided among fodder peas (32,000 ha), cooking peas (5,000 ha), and freezing peas (10,000 ha).

Common root rot of pea caused by *Aphanomyces euteiches* is one of the most damaging diseases found on farms with intensive cultivation of peas in Sweden (Olofsson 1963; Engqvist 1986).

The fungus is soilborne. The characteristic symptom of the disease is a watery rotting of the root system together with the presence of oospores of the fungus which are found in the rootbark (Jones & Drechsler 1925; Stamps 1978).

This paper gives details of the incidence of common root rot infection of peas in soil samples of potential pea fields in Sweden. It also reveals the reaction of some varieties when grown on healthy v. Aphanomyces root-rot-infected fields for the characters yield, protein content, cookability, stalk length and stalk stiffness.

MATERIAL AND METHODS

Origin of soil samples

Soil samples were collected from two sources: the first being 223 fields from randomly selected locations of official pea trials, while the second source comprised 626 fields

mainly from pea-growers, who wanted to test their fields prior to sowing. All soil indexes reported in this paper are greenhouse indexes.

Soil testing

Svalöf AB operates a service in which soil from fields to be sown with peas can be tested for presence of *Aphanomyces euteiches* before sowing. A 6-l soil sample (general sample of 50 soil cores taken to ploughing depth representatively over the field or trial area) is thoroughly mixed and divided into six new pots. These are then sown with 10 seeds each of a pea variety. Two pots each of the varieties Timo, Vreta, and Petra were used in these investigations. (We are now using only Bodil.) The pots are placed on a glasshouse bench at an air temperature of 25°C with 16 h light per day. During a 14-day period, starting 10 days after sowing, heavy watering (double the normal) is carried out. For the rest of the period, lighting and watering is kept at a normal level. After 30 days the plants are assessed for *Aphanomyces euteiches* root rot severity. The plants are classified (Sherwood & Hagedorn 1958) into five disease classes: 0 = no disease, 1 = for water soaked, light brown areas on roots, 2 = water soaked, light brown areas confluent and more extensive but not involving the entire root system, tissue firm, 3 = water soaking and browning involving all roots and epicotyl (stem above seed piece), tissue soft but not collapsed, epicotyl not markedly shrivelled, 4 = water soaking, browning decay involving all roots and epicotyl, cortex easily sloughed off, epicotyl shrivelled, rotted. Microscopic identification of oospores is performed and only root rot symptoms attributable to *A. euteiches* are graded. A disease index indicating the number of plants that fall into each disease class is calculated for each pot. The mean for the six replicates is designated the greenhouse index of the soil sample. The index is calculated in the following way: Index = (p' x s') x 100/(4 x n) where p' = number of plants in respective class, s' = disease class (0-4), n = total number of plants. An index of 0 = no disease, 100 = very severe disease, and intermediate values indicate intermediate infection. At present the following advice is given when presenting the result: a field that shows a greenhouse index between

- 0-30 can relatively safely be cultivated with peas
- 31-50 can with questionable safety be cultivated with peas
- 51-100 is regarded as dangerous. These fields should not be cultivated with peas.

If peas have been grown within the past 5-6 years in a field with an index of 31-50, then they should no longer be grown in this field.

RESULTS

Occurrence of the fungus

The 1984-90 results of soil tests by Svalöf AB presented in Table 1 indicate the occurence of Aphanomyces root rot of peas. Samples were taken from fields which were intended to be sown with peas. Aphanomyces root rot was found in 37% of the soil samples originating from the official pea trial locations. The fungus was found in 18 of the counties where samples were taken.

Table 1. Results of soil tests for the presence of Aphanomyces root rot of peas. Samples were collected in fields to be used for pea cultivation, 1984-90

County of	Soil samples from official trial locations		Soil samples mainly from pea-growers		Total no. of samples	No. of samples with Aphanomyces root rot
	No. of tests	No. of positive	No. of tests	No. of positive		
B Stockholm	10	6	6	2	16	8
C Uppsala	15	4	35	32	50	36
D Södermanland	13	10	15	6	28	16
E Östergötland	12	2	85	62	97	64
F Jönköping	5	0	1	1	6	1
G Kronoberg	1	0	1	0	2	0
H Kalmar	7	2	28	12	35	14
I Gotland	10	3	-	-	10	3
K Blekinge	10	3	3	1	13	4
L Kristianstad	26	8	65	48	91	56
M Malmöhus	42	16	150	118	192	134
N Halland	9	3	56	44	65	47
O Göteborg & Bohus	8	1	5	3	13	4
P Älvsborg	9	1	47	34	56	35
R Skaraborg	7	3	96	46	103	49
S Värmland	4	0	7	6	11	6
T Örebro	14	6	7	5	21	11
U Västmanland	15	12	15	14	30	26
W Kopparberg	1	0	-	-	1	0
X Gävleborg	1	0	-	-	1	0
Y Västernorrland	2	2	3	2	5	4
AC Västerbotten	2	0	1	0	3	0
B-AC	223	82 (37%)	626	436 (70%)	849	518

The frequency distribution of soil samples for degree of infection by the fungus is given in Table 2. The degree of infection ranges from 0 to 100%. The 223 soil samples from the official trial locations had an average greenhouse index of 10, while seven % of the soil samples from the official trials had a greenhouse index which exceeded 50.

Table 2. Frequency distribution of soil samples for degree of infection by *Aphanomyces euteiches*, Sweden, 1984-90

Degree of Infection Index										
0	1-20	21-30	31-40	41-50	51-60	61-70	71-90	91-100	Total no.	Mean
Soil samples from official trial locations county B-AC										
141	44	2	11	9	3	7	6	0	223	10
Soil samples mainly from pea-growers' fields county A-AC										
190	177	43	26	43	29	40	63	15	626	26
Total no. of samples county B-AC										
331	221	45	37	52	32	47	69	15	849	22

Influence on yield

The yield levels of the pea variety Timo are presented in Table 3 for trial grounds with different degrees of infection by *Aphanomyces euteiches*. In fields with a high degree of infection the yield loss can amount to 50% of the mean yield of uninfected fields.

Table 3. Peas: yield of the variety Timo on trial grounds with different degrees of infection of *Aphanomyces euteiches*, 1985-89

Mean Aphanomyces root rot index	No. of trials	Seed yield kg/ha
0	65	3410
23	34	3200
77	16	1638

There are differences between varieties in level of tolerance to Aphanomyces root rot. Among the marketed pea varieties used for dry pea production, the variety Bodil suffers a considerably higher yield loss than Timo, Vreta and Capella on severely infected soils (Table 4). The relative yields of these varieties in the official trials of 1980-89 in the corresponding regions with a zero or low degree of infection were for Vreta 100 (3403 kg/ha), Timo 108, Bodil 103 and Capella 124 (number of years in trial: Vreta, Timo and Bodil 10, Capella 5 years) (Bengtsson et al. 1989).

Table 4. Peas: kernel yields for different varieties on highly Aphanomyces root-rot-infected locations in southern Sweden, 1985-89

Variety	No. of trials	kg/ha	Rel.	Aphanomyces root rot index
Vreta	16	1560	100	77
Timo	16	1638	105	77
Bodil	16	1014	65	77
Capella	16	1997	128	77

In Table 5 a yield comparison is made between the two most cultivated pea varieties in Sweden during the 1980s, variety Timo and the 1990-released variety Capella. In healthy fields with no occurence of the fungus, Capella shows a 13% higher yield than Timo. On fields with the fungus, Capella shows a 23% higher yield than Timo. Capella can therefore be said to have a 10% better tolerance level to root rot than Timo.

The better tolerance to root rot in Capella compared with Timo helps to reduce the loss on soils with a low or moderate degree of infection. On highly infected fields it is uneconomical to grow peas and another crop should be chosen instead.

Influence on protein content

The protein content in peas harvested from fields infected with the fungus shows a highly significant decrease compared with protein content of peas harvested from

Aphanomyces root rot index	No. of trials	Timo kg/ha	Capella rel. Timo = 100
0	65	3410	113
23	34	3200	123
77	16	1638	122

Table 5. Peas: yield comparison between Capella and Timo on trial locations at different levels of Aphanomyces root rot infection in southern Sweden, 1985-89

uninfected fields (Table 6). The average decrease was 1.6%. Since protein payment is applied on peas intended for fodder purposes in Sweden, this is likely to have economic consequences.

Protein content of peas grown in healthy fields without infection	Protein content of peas grown in Aphanomyces-infected fields	Diff.
24.7 ± 2.4	23.1 ± 2.3	-1.6 units
No. of observations		
222	308	
Test of significance		t = 7.5***

Table 6. Peas: protein content, percentage of DM mean ± standard error, in peas grown on fields without and with infection of Aphanomyces root rot, 1985-86

In Table 7 the protein content is given for different varieties grown on healthy and on Aphanomyces-infected soils. A comparision of the two most thoroughly tested varieties, Timo and Vreta, reveals that there are varietal differences in this respect. The decrease in protein content in the variety Timo is less than that in the variety Vreta.

Table 7. Peas: protein content of different varieties grown on healthy and Aphanomyces-infected soils respectively

A.e. index = 0	Non-infected fields		A.e.-infected fields		Diff.	t-value	Mean A.e. index
Variety	No. of obs.	Protein % of DM	No. of obs.	Protein % of DM			
Vreta	25	26.1 ± 1.6	33	24.2 ± 1.7	-1.8	9.16***	26
Timo	24	26.5 ± 1.8	33	25.7 ± 1.9	-0.8	1.63n.s.	26
Bodil	13	21.8 ± 2.4	8	20.5 ± 2.2	-1.2	1.20n.s.	26
Petra	25	23.6 ± 2.1	32	22.4 ± 1.7	-1.2	4.50***	25
Rigel	19	23.3 ± 2.5	27	21.1 ± 1.8	-2.2	4.90***	34
Svenne	13	24.5 ± 2.3	18	24.0 ± 2.0	-0.5	0.75n.s.	27
Bohatyr	10	24.5 ± 1.4	19	22.1 ± 1.6	-2.4	7.26***	24
Capella	11	25.4 ± 2.9	18	23.8 ± 2.1	-1.5	1.49n.s.	26
Solara	7	24.4 ± 1.6	16	22.1 ± 1.8	-2.3	4.05***	29

Influence on cookability

Cookability of peas harvested from Aphanomyces root-rot-infected fields is reduced compared to peas harvested from uninfected fields. The average decrease was 10% (Table 8). In Table 9 the cookability of different varieties is compared in trials with presence and absence respectively of *Aphanomyces euteiches*. The variety Capella is shown to have a decrease in cookability of only half that of the variety Rigel.

Table 8. Peas: cookability of peas grown on healthy and Aphanomyces root-rot-infected fields respectively, 1985-87. Percentage of cooked after 60 min boiling

	Non-infected fields	A.e.-infected fields	Diff.
Cookability (%)	89 ± 16	79 ± 23	-10
No. of observations	95	139	
Test of significance			t = 3.86***

Table 9. Peas: cookability of different pea varieties harvested from healthy and infected fields respectively, 1985-87

Variety	Non-infected fields A.e. index = 0		A.e.-infected fields		Diff.
	No. of obs.	Cookability %	No. of obs.	Cookability %	
Vreta	9	89	14	83	-4
Capella	9	96	14	90	-6
Solara	9	91	14	81	-10
Rigel	9	81	14	69	-12

Influence on stalk length

Root rot decreases the stalk length of the plants, see Table 10. On average over all varieties in the official 1985-1986 trials the decrease in stalk length was 23%, Table 11.

Table 10. Peas: stalk length (cm)

	Healthy fields A.e. index = 0	Root-rot-infected fields Average A.e. index = 0	Diff.
Plant length (cm)	78	60	-18
No of observations	103	161	
Test of significance			t = 6.3***

Influence on stalk stiffness

Stalk stiffness has increased in fields infected with root rot by an average of 9% units (see Table 12).

Table 11. Peas: stalk length of different varieties grown in fields with and without *Aphanomyces euteiches*. Official trials 1985-86

Variety	Non-infected fields		A.e.-infected fields		Diff. cm	Reduction %
	No. of obs.	Height cm	No. of obs.	Height cm		
Vreta	7	111	10	89	-22	20
Timo	7	114	10	88	-24	21
Petra	7	69	9	53	-16	23
Fjord	6	81	10	63	-18	22
Rigel	7	77	10	59	-18	23
Svenne	6	69	10	48	-21	30
Capella	6	81	10	64	-17	21
Bohatyr	6	86	10	67	-19	22
R 396	7	74	10	56	-18	24
CE 103	7	56	10	42	-14	25
Helka	6	70	10	55	-15	21
Solara	6	63	10	47	-16	25
Stehgolt	6	59	10	43	-16	27
Progreta	6	79	10	57	-22	28

	Healthy fields	A.e.-infected fields	Diff.
Stalk stiffness	21	30	+9
No. of observations	178	266	
Test of significance			t = 3.9***

Table 12. Peas: stalk stiffness in fields with and without *Aphanomyces euteiches*. Official trials 1985-86. 0-100, 100 = upright stand

CONCLUSION AND DISCUSSION

The results indicate the widespread incidence of *Aphanomyces euteiches* in field soils in the important pea-growing areas of Sweden.

The fungus can seriously decrease yield levels when the infection is severe.

Differences exist between varieties in their tolerance to the disease and it is highly worthwhile to consider these differences when choosing varieties. However, it has been observed that none of the varieties have a high resistance to attack in fields with a high degree of infection. Such fields should therefore not be planted with peas.

The observed reduction in protein content is likely to be due to the inhibiting role the fungus plays in the establishment of the nitrogen-fixing bacteria and due to the rotting away of nodules in severe root rot infections.

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