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The drawing on the cover is from Kjell Aukrust's *«Guttene på broen»*.

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GROWTH CHAMBERS WITH CONTROL OF LIGHT QUALITY

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Mortensen L.M., E. Strømme, Z. Sebasta & D. Wenner 1987. Growth chambers with control of light quality. Norwegian Journal of Agriccultural Sciences 1: 1-5. ISSN 0801-5341.

As a means of studying the effect of light quality on plant growth and development, growth chambers were made of double acrylic sheets which allowed the passage of coloured solutions. Four differently coloured solutions (blue, green, yellow and red), composed of different water soluble dyes, were used to fill the top and the walls of the chambers. The temperature was controlled by heating and cooling devices to within ± 0.7 °C of the set point. At times of maximum solar radiation (800 W/m^2) the temperature rose 2-3 °C above the set point. The interception of photosynthetic active radiation (PAR) was measured daily in all chambers. The chambers set-up proved very efficient for studying light quality in plant development.

Key words: Growth chamber, light quality control.

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The traditional way of controlling air temperature in the greenhouse during periods of high solar radiation is by means of ventilation, but a disadvantage of this practise is the loss of CO_2 control at the time of day when high CO_2 levels are needed. A so-called «fluid-roof» system in which heat is absorbed and removed via double-layer greenhouse cladding has been described (Chiapale et al. 1977, 1983, Weichman 1981, Nilsen et al. 1983). Although a greenhouse in which the fluid roof consists of double acrylic sheets filled with a copper sulphate solution has been in use in Norway for three years (Pettersen 1984), no attempt has been made to store the heat absorbed by the solution. The system has functioned primarly for shading. If this will be the main use of the system in practise, attention should be paid to the colour of the solution, since light quality might affect plant growth and development quite significantly.

This paper describes growth chambers developed for studying the effect of light quality and other climatic factors on the growth and development of various greenhouse plants.

DESIGN AND FUNCTION OF THE GROWTH CHAMBERS

The growth chambers consist of 16 mm double acrylic sheets and are similar to those described previously by Mortensen (1982). Each chamber (870 mm wide, 1 200 mm long and 1 000 mm in height by inside measurements) has a volume capacity of 1 040 litres (Fig. 1). Air is forced by two fans through a channel and a perforated

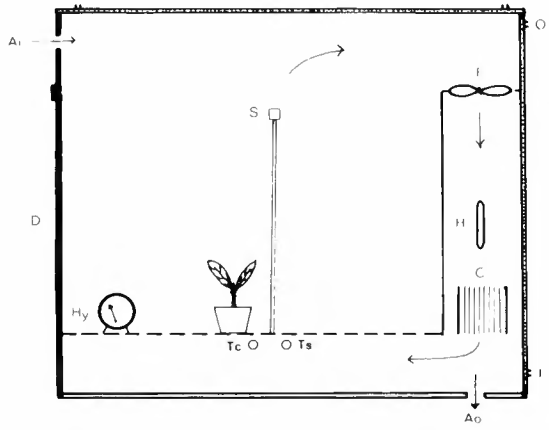

Fig 1. Growth chamber for light quality studies. A_1, air inlet; A_0, air outlet; C, cooling coil; D, door; F, fan; H, electric heater; Hy, hygrometer; I, liquid inlet; O, liquid outlet; S, quantum sensor; Tc, termocouple; Ts, thermostat sensor.

polyethylene plate which functions as a plant table. The perforation holes are 10 mm in diameter and make up about 5% of the total table area (0.87 m^2). Air flow above the table is 0.25 m s^{-1} The temperature is controlled thermostatically. Heat is supplied by a 1 000 W electric heater placed in the channel. To obtain precise temperature control, cooled water is continously circulated through a copper coil placed below the heater in the channel. The chambers are cooled constantly. Heat is turned on thermostatically. The air temperature is controlled within ± 0.7 °C. Air changes are controlled up to 30 h^{-1} by varying the opening of a 50 mm hole in the chamber bottom under the channel. CO_2 concentration is controlled by introducing a certain flow rate of pure CO_2 gas into the chamber from a bottle with compressed liquid CO_2. The flow rate is controlled by a reduction valve and capillaries. The CO_2 concentration at normal CO_2 concentration (340 µl l^{-1}) may be controlled within ± 20 µl l^{-1} and at 900 µl l^{-1}within ± 50 µl l^{-1}. The CO_2 concentration is measured by an infrared gas analyzer (Beckman Model 864).

The 16 mm acrylic sheets used for the growth chamber construction were 900 or 1 200 mm wide, and have 20 mm wide channels sealed at both ends. A homogeneous water flow through the sheets was made possible by a technique developed by the company Hamax-Vefi A/S (Larvik, Norway).

A test was carried out to determinate any chamber effect on plant growth. The chambers were placed in a greenhouse compartment and ten seedlings of *Lactuca* 'Salina' were placed in each of the ten chambers and grown for four weeks in January. The temperature was 18.0 °C and 100 µmolm $^{-2}s^{-1}$ (PAR) supplementary light was given by means of fluorescent light (Philips TL 33 RS) for 24 h day^{-1}. The lamps were placed above the chambers. The photon flux density was measured with a Lambda LI-185 B Instrument with a quantum sensor (400-700 nm).

The plant dry weight was not significantly different at the P=0.05 level, which means that all chambers give similar growth conditions.

EXPERIMENTAL PROCEDURE

Ten chambers were placed in an open field (Figure 2), the wall in which the door was situated was oriented north. Cooled water from a central reservoir was led to the chambers through insulated tubes, and two thermocouples for temperature measurement were put in each chamber. A sensor for measuring light (Lambda In-

Fig. 2. Experimental set-up in the open field. The picture is taken towards the East.

strument, quantum sensor, 400-700 nm) was also placed in each chamber. The photon flux density and the temperature were recorded every ten minutes using a Hewlett Packard 32521A dato logger. The daily PAR (photosynthetic active radiation) was accumulated and recorded. Average temperatures in the light and dark periods were recorded. The dark period was programmed to start when the PAR level fell below 5 μmol $m^{-2}s^{-1}$.

LIGHT QUALITY CONTROL

Different dyes were tested with respect to stability, and the four chosen as the basis for the control of light quality were: Green (1358), yellow (1409 Tartrazine), red (14123 Red 2G), all from D.F. Anstead Limited, and blue made by coppersulphate ($CuSO_4$). The dyes were dissolved in water. To obtain the same photon flux density (400-700 nm), the concentration of each dye was adjusted to give the same light transmission as 2.5 % $CuSO_4$. The spectral distribution curves in photons in the range 350-900 nm were made by a Kontron Instrument, Uvikon 860 spectrophotometer (Figure 3). The different colour solutions were poured into a quartz cuvette and light transmissoin at the different wave lengths was compared to transmission through an air-filled cuvette. The transmission in 100 nm sectors is given in Table 1. Pure water increased the transmission compared to air.

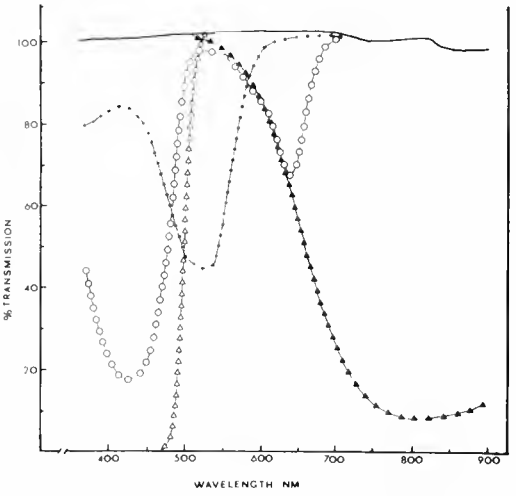

Fig. 3. Light transmission of water with different dyes in the range 350 to 900 nm wavelength, pure water; ▲, blue; ○, green; △, yellow; *, red.

Table 1. Light transmission (%) through water and differently coloured solutions compared to air, as measured in a spectrophotometer.

Dyes	Wave length (nm)				
	400-500	500-600	600-700	700-800	800-900
Water	101	105	103	103	99
Blue	101	99	55	14	10
Green	36	96	84	102	98
Yellow	5	103	106	106	102
Red	73	66	101	102	102

Table 2. Average temperature, relative air humidity and PAR level throughout a period of 30 days in June-July in ten «fluid roof» growth chambers with five different light qualities

Control (water)	Chamber no.										
	Blue		Green		Yellow		Red				
	1	2	3	4	5	6	7	8	9	10	Aver
Temp.											
light	18.8	18.3	19.7	19.3	18.7	19.0	19.1	18.9	19.0	19.2	19.0
dark	17.8	17.8	18.6	18.1	17.7	17.7	17.8	17.8	17.2	17.7	17.8
Relative humid. (%)	65	65	64	66	61	64	68	68	65	66	65
PAR (mol m^{-2} day^{-1})	21.5	21.5	22.2	20.8	19.8	21.6	21.5	22.2	21.9	21.1	21.4

The different solutions filled the walls and the top of the chambers. Light level inside the chambers compared to outside was approximately 50 % lower. The northern wall, where the door was situated was covered with alumina foil and was not filled with solution. Two chambers were filled with each of the four coloured solutions. The two chambers left were shaded with a white cloth in order to give the same PAR level as the others.

GROWTH CHAMBER CONTROL

The climate in all chambers was recorded throughout the summer of 1985. Temperatures in the light and dark periods, relative air humidity and PAR level were recorded daily. The average values for a of 30 days period are given in Table 2. The difference in climatic conditions between the chambers was relatively small. The thermostat was set at 18.0 °C day and night, but because of the many sunny days in the period, the day temperature was 1 °C higher on average.

A series of plants were grown during summer. The CO_2 concentration varied between 310 and 335 µl l^{-1} during the light period, depending on the light level and the quantity of biomass in the chambers. The air exchange number was 30 h^{-1}.

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A PAPER CHROMATOGRAPHIC SURVEY OF THE PHENOL CONTENT OF APPLE PEEL FROM SCAB RESISTANT AND SCAB SUSCEPTIBLE GENOTYPES

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Øydvin, J. 1987. A paper chromatographic survey of the phenol content of apple peel from scab resistant and scab susceptible genotypes. Norwegian Journal of Agricultural Sciences 1: 7-13. ISSN 0801-5341.

Two apple species and 37 cultivars and selections screened for fruit peel phenol content by two-way paper chromatographic separation revealed a total of 40 spots. The apple peel phenolic pattern was shown to be of taxonomic importance. A study of the relationship between apple peel phenol content and scab resistance, powdery mildew and fresh fruit taste indicated no clear connection. However, the group of genotypes possessing the V_f-gene for strong scab resistance had on average a more pronounced spot no. 6, which may denote isochlorogenetic acid, and also a higher total phenol content.

Key words: Apple peel, Apple scab, Fruit quality, *Malus florentina, Malus floribunda,* Paper chromatography, Phenols, Powdery mildew.

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There have been many studies into the relationship between phenolic compounds and disease and pest resistance in plants, but because of the inconsistency of the results, this area remains controversial (Challice & Westwood 1972).

On the one hand, there are som investigators who regard phenolic compounds as part of the defence system, for example Kiekiewics & van de Vrie in their recent report (1983) on histological studies of strawberry leaves damaged by the two-spotted spider mite *Tetranychus urticae* (Koch). These authors found higher levels of phenols in leaves resistant to mite than in those susceptible to it, while the content of phenols in uninfested leaves was the same.

On the other hand, while stating that the presence of certain phenols in the genus *Pyrus* sometimes coincides with disease resistance, Catlin & Olsson (1966) and Challice & Westwood (1972) concluded that there is no actual connection between them.

Resistance to scab *Venturia inaequalis* (Cke.) Wint. in the genus *Malus* has been attributed to phytoalexin-like oxidation products of phloridzin or its aglucone, phloretin (Noveroske et al. 1964a, 1964b, Raa 1968, Williams & Luc 1969, Kuc 1972). Nicholson et al. 1977 concluded, however, that neither phloridzin, phloretin, nor their oxodation products represent the primary means of host resistance to apple scab in the hypersensitive response.

The importance of leaf phenols as taxonomic characteristics in *Pyrus* and *Malus* is discussed in a number of reports (Williams 1969, Catlin

& Olsson 1966, 1968, Challice & Williams 1968a, 1968b, 1970, Challice 1972a, 1972b, 1973, Challice & Westwood 1972, 1973, Williams 1982).

The main aim of this study was to compare the content of phenol in apple peel from scab susceptible cultivars and from newer scab resistant genotypes possessing the V_f-gene derived from *Malus floribunda* 821.

MATERIALS AND METHODS

Apple material

Most apple specimens were obtained from Oregon State University (Lewis/Brown Farm and Botany Farm), Corvallis. The following were received from Southern Oregon Exp. Stn. Medford: 'Red Gravenstein' and 'Tydeman's Red'; from Western Washington Res. and Ext. Unit. Mt. Vernon: 'Akane', 'Discovery', 'Oriole', and 'Tydeman's Early'; from the University of Illinois: COOP 13, COOP 14, COOP 20, 'Paulared' and 'Prima'. 'Prima' was also sampled at Corvallis.

Extraction of apple peel

Fresh apples in two replicates were dipped in chloroform for 15-20 sec to remove surface waxes. Immediately afterwards apple peel (5 g) was immersed in boiling alcohol containing 1% v/v HCl in methylalcohol (MeOH) and boiled for 1 min. The peel was then stored at -1 °C for 5-8 days completely covered in a sealed beaker before beeing rapidly cut into small pieces, squeezed in a mortar and filtered through glass fibre into test tubes. The samples were made up to 10 ml (evaporating in nitrogen flow or by adding alcohol) before use.

Paper chromatography

200 microlitres, corresponding to 100 mg apple peel, was put in one corner of each paper sheet (Watman No. 3MM paper) and run in two directions. Solvent used for the 1st run (ca. 45 cm) was SBA (secondary buthylalcohol, glacial acetic acid and water (sec. BuOH: glac. HAc: H_2O, 70:2:28)) and for the second run (ca. 37 cm) 2% HAc (glacial acetic acid and water (2% v/v glac, HAc in H_2O)) (see Challice & Williams 1968a, 1968b for the suitability of these solvents for separating the range of phenols in the related genus *Pyrus*.

Detection of spots on paper chromatograms

All chromatograms were examined and stored in a dark room under u.v. light (366 w.l.) both with and without ammonia-fuming. Detected spots were numbered, recorded for colour and R_f-values, and scored on a 1-9 scale for spot size and intensity.

Record of diseases

Available records of scab and mildew infestation from the Western Washington Res. and Ext. Unit Mt. Vernon (Norton 1981) were used to study relationships between diseases and phenol contents within the group of cultivars lacking the V_f-gene (cv. nos. 1-14 in Fig. 2). For genotypes possessing the V_f-gene (nos. 16-37), unsprayed trees at Corvallis were scored (in 1980) on a 1-5 scale (1 most severe, 5 no visible symptoms) for scab infestation of fruits at harvest and for powdery mildew infestation on shoot tips in early September. Thus, the presence and amounts of phenol detected relate to fruits and shoots which have been heavily attacked by the scab and mildew fungi throughout a wet spring and early summer and a dry summer and autumn.

For selected genotypes of nos. 16-37, fruit quality, including the trait taste, was sensorially assessed at Corvallis in 1980.

RESULTS AND DISCUSSION

Separation of phenols and R_f-values

Fig. 1 summerizes the results of the apple peel phenol in a survey of 37 apple cultivars and selections and 2 apple species, while Table 1 gives the R_f-values obtained in two solvents. Results of colour reactions and of attempts to identify some of these spots have been omitted from this report. Each spot is denoted simply by

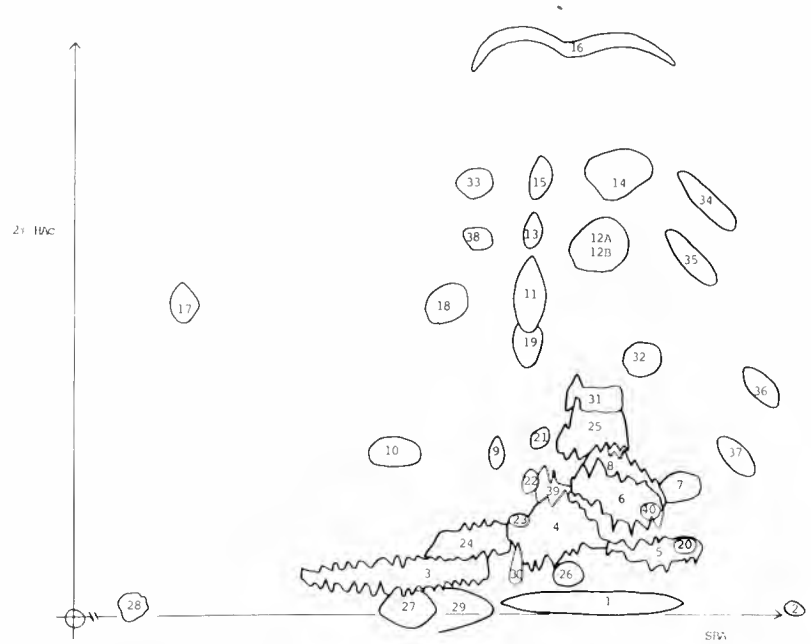

Fig. 1. Two-dimensional composite map of apple peel phenols (SBA - 2% HAc solvent-pair)

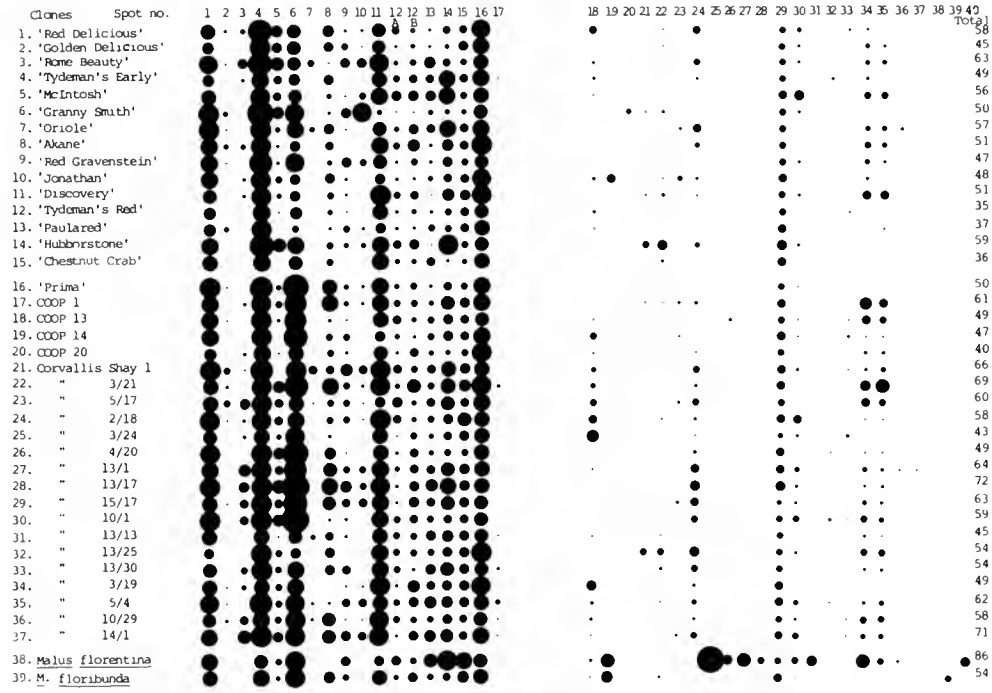

Fig. 2. Map of apple peel phenols (spot nos. 1-40) for 39 clones

Table 1. R_f-values of apple peel phenols on two-dimensional paper chromatograms.

Spot no.	SBA[1]	2% HAc[1]	Spot no.	SBA[1]	2% HAc[1]
1.	.77	.02	21	.72	.30
2.	.97	.01	22	.71	.22
3.	.60	.07	23	.70	.16
4.	.72	.15	24	.64	.12
5.	.84	.10	25	.77	.31
6.	.79	.22	26	.75	.07
7.	.86	.22	27	.60	.01
8.	.80	.26	28	.32	.01
9.	.68	.27	29	.67	.01
10.	.58	.28	30	.70	.08
11.	.71	.55	31	.77	.37
12 A, B.	.78	.63	32	.82	.44
13.	.71	.65	33	.66	.74
14.	.79	.76	34	.88	.71
15.	.72	.74	35	.87	.61
16.	.75	.96	36	.94	.39
17.	.37	.53	37	.91	.31
18.	.63	.53	38	.67	.64
19.	.71	.47	39	.74	.20
20.	.86	.12	40	.83	.18

[1] For explanation, see Materials and Methods, Paper Chronomatography

a number, with no attempt beeing made at classification.

The separations gave a total of 40 spots. This reveals a much higher number of phenols in the apple genus than has hitherto been detected. Spot No. 12 may refer to two components since its colour is blue in u.v. light without ammonia-fuming, but green and sometimes much larger in the presence of ammonia.

Apple peel phenols as taxonomic characteristics

The similarity between replicates was striking, and suggests that apple peel phenolic pattern may often be of taxonomic importance, as leaf phenols have been shown to be in previous reports. The pattern of apple peel phenols for single genotypes are shown in Fig. 2 with figures for total amount on the right hand side.

Malus florentina, the only species with spot nos 25 (very high level), 27, 28, 31 and 40, is also unique for its very high total phenol content.

Only *Malus floribunda* revealed spot no. 39 otherwise this species produced a pattern similar to many cultivars and selections. Unfortunately, fruits of the *M. floribunda* 821 clone, which possesses the V_f-gene and which was the original source for strong scab resistance in newer selections (Dayton 1980), were not obtainable for this investigation.

In named cultivars, large differences in the occurence and size of different spots were found. Cv. 'Red Delicious', for instance, possessed a wider range of apple peel phenol than did 'Golden Delicious', 'Red Gravenstein', Tydeman's Red' or 'Paulared'. 'Rome Beauty' and 'Oriole' both showed the rarer spot no. 7. 'Granny Smith' was characterized by its major spots nos. 1, 4, 6, particularly the well-pronounced spot no. 10 and the abcense of spot nos. 34 and 35. 'Jonathan' had a pronounced

spot no. 19. 'Tydeman's Red', 'Paulared' and 'Chestnut Crab' possessed the lowest in total phenolic content.

Contents of apple peel phenols in relation to scab and mildew resistance

Spot nos. 1, 4, 6, 11, 12A, 12B, 13, 14, 15, 16 and 29 were present in all investigated genotypes. These spots accounted for most of the phenol in both resistant and disease susceptible cultivars and selections.

Fig. 2 shows that there was no sharp differentiation in apple peel phenol content and pattern between scab susceptible or tolerant cultivars (nos. 1-14) and those bearing the V_f-gene (nos. 16-37). Strong scab resistance could not be attributed to any single spot or group of spots. It seems, however, that the occurence of spot no. 6, which may be isochlorogenic acid (Challice & Williams 1968b), was on average higher for the scab resistant group:

	Scab suscept. cv. nos. 1-14 Mean (max. - min.)	Scab resistant cv. nos 16-37 Mean (max. - min.)
Spot no. 6	3.9 (6 - 2)	6.0 (8 - 3)
Total all spots	45.7 (63 - 35)	56.8 (72 - 40.5)

The maximun record for spot no. 6 in the first group, however, was twice the minimum level in the scab resistant group. There was also a pronounced difference in total phenol content between the two groups; again the maximum level of the first group exceeded the minimum record of the second group by ca. 50%. The results indicate that differences in apple peel phenol content alone cannot be used for distinguishing between the two groups, nor for predicting groups of untested individuals.

Although the *M. floribunda* derivatives (nos. 16-37) had been selected at the seedling stage for strong scab resistance (Dayton et al. 1977a), Corvallis 10/29 and a few others became infested with scab pits on the fruit surface. Within this group, records of scab pits on the fruits covered a range from 2 to 5; for powdery mildew on shoot tips a range from 2 to 4-5, where 5 indicates no visible disease symptoms.

Regression analysis performed separately within each group of genotypes (with and without the V_f-gene) suggested no clear connection between apple peel phenols or total phenol content and scab or mildew resistance/tolerance/susceptibility. Within the scab susceptible group the highly scab susceptible 'Rome Beauty' (Dayton 1980) followed by 'Hubborstone', 'Red Delicious', 'Oriole' and 'McIntosh' were the highest in phenol content, whereas Corvallis 13/17, 14/1 and 3/21 were the highest within the *M. floribunda* derivatives group. The high level of total phenols in many of the latter group is not surprising. All these selections can be traced back to sibcrosses of F_1 seedling plants from 'Rome Beauty' x *M. floribunda* 821 (Dayton et al. 1970, Williams et al. 1972, 1975a, 1975b, Dayton et al. 1977b, 1979). The higher phenol content in this group is more likely a result of their origin than of continued selection for scab resistance.

Results of single genotypes did not suggest any negative association between phenol content and corresponding scores of eating qualities of the fruit. For instance, Corvallis 3/21, a highly scab and mildew resistant selection which also revealed most spots and a high total phenol content, was considered very pleasant in taste, with no astringent flavour at harvest or off-flavour after storage. It is unlikely that the lower scores for eating quality of the newer scab resistant cultivars, compared to some widely grown older varieties (Aeppli et al. 1983), are connected with the presence of the V_f-gene, or to changed phenolic pattern in general. The eating quality of a fruit depends of cource on a range of factors and ingredients.

SUMMARY

Two apple species and 37 cultivars and selections have been screened for fruit peel phenol content. The results have been studied in relation to records of scab and mildew attack, and

also eating quality assessments of some selected genotypes.

The two-way paper chronographic separation revealed a total of 40 spots. Eleven of these were always present and accounted for most of the phenols in all genotypes.

The apple peel phenolic pattern, which was shown to be of taxonomic importance, can best be compared for single genotypes by careful examination of Fig. 2. *Malus florentina* stands in a class of its own. *M. floribunda* could also be separated, although the phenolic pattern of the latter is closer to the pattern of many cultivars and selections.

No sharp differentiation in phenolic pattern was found between scab susceptible genotypes and those possessing the V_f-gene for strong scab resistance. However, the latter group had on average a more pronounced spot no. 6 (which may denote isochlorogenitic acid), and also a higher total phenol content.

These results indicate that there is no clear connection between apple peel phenol content and scab resistance (on the fruit), powdery meldew (on shoot tips) or any clear relationship with fresh fruit taste.

ACKNOWLEDGEMENTS

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A STUDY OF 'VICTORIA' SEEDLINGS

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A study of 190 seedlings from open pollinated 'Victoria' was carried out during the period 1983-86. Vegetative traits such as tree size and frost hardiness were recorded. Time of flowering and amount of flowers are tabulated, along with time of maturation and productivity. Fruit samples were used for estimating colour, fruit size, stone adherence to fruit flesh, and chemical analysis of soluble solids and titratable acidity. 'Victoria' proved to be highly heterozygous for all registered traits, and distributions of the progenies indicate that most traits are polygenically inherited. An exeption is the one gene model for stone adherence to fruit flesh.

Key words: Chemical analysis, fruit morphology, inheritance, maturation, *Prunus domestica,* 'Victoria' seedlings, variation.

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According to Crane and Lawrence (1956), *Prunus domestica* is an allohexaploid species. The assumed parents are the tetraploid species *P. spinosa* (blackthorn) and the diploid species *P. cerasifera* (myrobalan). The hexaploid nature of *P. domestica,* gives a complex way of inheritance, which is only partly known (Wilson, 1971).

As is all plants, the juvenile phase in plums is determined by genotype and environment, some parents transmitting shorter juvenile periods than others (Paunović et al. 1968) The length of the juvenile period is highly dependent on tree size (e.g. Zimmermann 1972), and in plums the juvenile period is 4-5 years or more (Paunović et al. 1968).

Three size and tree habit vary greatly. Crane (1921) classified tree habit as spreading-erect, spreading-drooping, erect or drooping. Classification of habit and vigour have been given little attention.

Ripening dates usually reflect the parents, and crossing of two early varieties will produce mainly early maturing offspring (Yenikeyev 1978).

Fruit size, fruit form, and stone size show little variation between years (Cociu 1968). Paunović et al. (1968) found 41,5% of the progenies to be transgressive for fruit size, and small ones the most abundant. Crane (1921) noticed high variability in fruit shape, and expressed this character as the ratio between excess of length over mean diameter to mean diameter. Stone size reflects the parents in that the crossing of two varieties with small stones will usually result in smallstoned progenies (Yenikeyev 1978). Cociu (1968) found that stone size varied from 2.67% to 6.07% of fruit weight in the plum varieties used in his breeding programme.

With regard to store adherence to fruit flesh, varieties are described as clingstone, semifree or freestone (Crane & Lawrence 1956). Yenikeyev's (1978) experiments indicating that freestone is dominant to clingstone are in accordance with Crane (1921). By contrast, Crane & Lawr-

ence (1956) and Paunovic et al. (1968) found clingstone to be dominant over semifree and freestone. This discrepancy between these authors indicates that the genetical control for this trait is insufficiently known.

Fruit colour is a very complex trait in plums (e.g. Crane & Lawrence 1956). Ground colour and bloom will influence the appearance, and anthocyanins may be absent or present either fleckwise or evenly distributed. In addition to these genetical differences, Johansen & Oldén (1962) found that colour flecks on plums have a very high environmental component.

Crane & Lawrence (1956) report that *P. cerasifera* has yellow ground colour, and the anthocyanin is red. In *P. spinosa* the ground colour is green, and the anthocyanin blue. The variation within these two species is limited. The origin of *P. domestica* from these two species indicates that red/green and blue/yellow are at different chromosomes. All recombinants of these four colours occur in *P. domestica,* and a wide variation is observed.

The anthocyanins in plums have been analysed by several authors. Van Buren (1974) found 'Victoria' peel to be richest in Cyanidin-3-Glucoside, but Cy-3-Rutinoside, Peon-3-G and Pa-3-R are also present. Johansson & Oldén (1962) found that 'Victoria' was heterozygous for anthocyanins, as progenies without anthocyanins appear after selfing the variety.

Quality components such as dry matter and acidity show great variation in plum varieties (e.g. Cociu 1968). Vangdal (1982) studied sugar content in Norwegian grown plums, and found considerable variation. Total sugar content from 11 varieties evaluated, varied from 8.48 to 12.87%. Total sugar content averaged 77% of soluble solids.

MATERIALS AND METHODS

Stones from open pollinated 'Victoria' germinated in 1979 and 190 seedlings were planted in the field in 1980. A neighbouring field contained the varieties 'Reine Claude d'Oullins', Reine Claude d'Althanns' and 'Bonne de Bry'. 'Victoria' is self-fertile, but the seedlings were probably a mixture of full-sibs and half-sibs.

Time of flowering was estimated as early, medium or late. Amount of flowers was scored from 0-5. These two characters were registered in each year from 1983 to 1986.

Time of maturation and amount of fruit were estimated by the same scale as the corresponding flowering properties. Fruit size was scored on a scale of 1-5, 1 being very small.

Tree size was registered as tree height in 1983 and 1985. In 1985 crown width and trunk circumference were measured in addition.

Winter damage was registered in spring 1985 applying a scale from 0-9, 0 being without damage. The same scale was used for estimating amount of leaf-fall at a fixed date in autumn 1985.

Fruits were sampled at weekly intervals in the autumn of 1985. Maturity was determined by degree of fruit softening, and the sample size was 10 fruits if as many could be found. The fruits were classified for fruit colour: yellow, yellow with flecks, red and purple. Fruit size was determined by weight; fruit length and fruit breadths were also measured. Fruits were split and evaluated for stone adherence to fruit flesh on a scale of 1-5, where 1 was clingstone. Stone length, width and thickness were measured, too.

Fruit samples containing 5 fruits or more were pulped in a plastic bag by hand, and then filtered. The juice obtained was analysed for soluble solids by using an Atago DBX50 refractometer. Furthermore, pH and titratable acid were analysed in a Radiometer system 822.

RESULTS AND DISCUSSION

Tree size and juvenility

A close relationship between tree height and year of first flowering was observed. The first trees flowered in 1983 when the mean height of flowering trees was 215 cm compared with 171 cm for juvenile trees. This difference was highly significant ($P<0.001$). All trees higher than

290 cm flowered while no trees lower than 100 cm flowered.

When trees pass the juvenile period, they tend to grow more slowly. By handling in a one-way analysis of variance for juvenile status, a highly significant ($P<0.001$) difference was detected. Table 1 shows the mean growth increments for different physiological groups.

Vigour may be estimated by trunk circumference, while tree height and width give additional information concerning crown form. The correlations between trunk circumference and tree height and crown width were r = 0.609 and r = 0,713 respectively.

A plot of tree height to crown width is shown in figure 1. Generally the progenies show a tendency to be higher than wide, thus the upright tree-form is predominant.

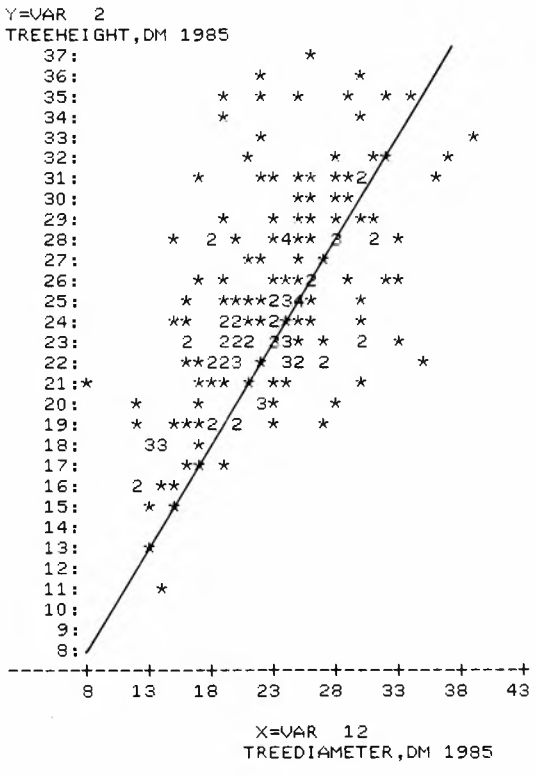


Figure 1. Plot of crown width to tree height 1985.

Leaf fall and winter hardiness

Winter damage was registered after the severe winter of 1984/85. About 80% of the trees scored 0 or 1 on a scale from 0-9, where 0 was no damage. The 1985-86 winter, however, was also rather cold, and 24% of the trees had a moderate condition in 1986.

The distribution for leaf fall showed no skewness, so there seems to have been no directional selection operating in the winter 1984/85. However, tree condition in the spring 1986 had a slightly positive correlation to leaf fall (r = 0.207).

FLOWER REGISTRATIONS

The time of flowering varied from year to year. The percentages of trees in the different classes are presented in Table 2.

Table 2 shows that there was a very high proportion of early flowering trees the first year of flowering. In successive years 31% of the genotypes were scored both early and late. This possible inaccuracy of scoring might be due to the fact that scoring was carried out once a year, and at a different time each year. However, early flowering genotypes tend to have a shorter juvenile phase.

The amount of flowers increased in relation to stage of adulthood as shown in Table 3.

The differences in amount of flowers for trees with first flowering in 1983-86 were highly significant in all years ($P<0.001$). Table 3 also shows that scores were very high in 1984 compared with other years.

Time of maturation

Since the time of maturation differs considerably within each tree, an exact date of maturation is quite difficult to estimate.

The present material showed great variability in time of maturation. As for time of flowering, scoring in early, medium or late once a year was divergent for the same genotypes in successive years. Estimating maturity at weekly intervals gave a more accurate expression of the

Table 1. Mean growth increase from 1983 to 1985 (cm)

	cm	No. of trees
Juvenile in 1985	83.2	37
Juvenile in 1983, adult in 1985	61.4	90
Adult in 1983	52.5	53
Mean	63.3	

Table 2. Percentage of trees in different flowering classes.

	1983	1984	1985	1986	Mean
Early	60	23	35	31	25
Medium	25	21	46	23	44
Late	15	56	19	46	31
No. of trees	55	127	146	156	

Table 3. Mean amount of flowers. Score 0-5.

Year of first flowering	Mean amount of flowers 1983	1984	1985	1986
1983	1.76	3.52	3.36	3.85
1984	0	2.26	2.41	3.44
1985	0	0	1.36	2.05
1986	0	0	0	1.44

trait; the distribution for trees carrying 5 or more fruits in 1985 is presented in Figure 2.

Figure 2 shows that most progenies had earlier or similar maturity to 'Victoria' (arrowed in the figure). In the analysis no consideration has been given to whether the trees could be half-sibs or full-sibs.

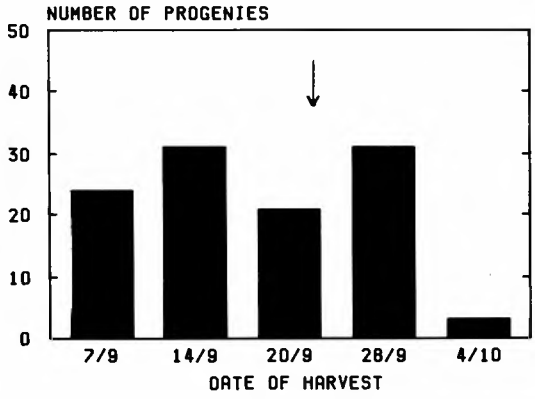
Productivity and fruit size

Productivity was highly correlated with the length of the juvenile period. The rise in productivity by increasing age from the first year of flowering is presented in Table 4.

The amount of fruit in the different adult classes were significantly ($P<0.001$) different in all years.

Registrations from the trees which flowered in 1983 indicate that yield score stabilizes 3

Figure 2.
VARIATION IN TIME OF MATURATION



years after the first year of flowering. Data of amount of fruit in 1983 were not taken, but the tendency seems to be identical for the first three groups. The trees flowering for the first time in 1985 had a somewhat low yield score in 1986. Trees flowering for the first time in 1986 had a rather high yield score in 1986. These tendencies do not correspond with the amount of flowers presented in Table 3.

Productivity was positively correlated with fruit size. This result was unexpected. No thinning was carried out, and therefore one would have expected the amount of fruit to be negatively correlated with fruit size. When this is not the case, the selection for high yield and fruit size should be a fairly good criterion for selecting good genotypes. This might be done by independent culling for these two characters, and culling levels for productivity could be set to 4, and for fruit size to 30 g. The number of genotypes in different groups is presented in Table 5. The culling level for yield in the table was established by means of two registrations in 1986 and one registration in 1985.

Table 5 shows that the fruit size follows a fairly normal distribution, while the scores for yield were much more equally distributed. The culling levels give 10 selected genotypes.

Scores on the 1-5 scale for fruit size and 0-5 for yield were taken by two judges in 1986. Culling levels at 4 for both characters gave 12 and 9 selected progenies, respectively. One genotype was selected by both judges in 1986 that was not selected by the «advanced» method, us-

Table 4. Mean amount of fruit. Score 0-5.

Year of first flowering	Yielding year 1984	1985	1986
1983	1.91	3.16	3.27
1984	.82	2.05	3.19
1985	0	.72	1.67
1986	0	0	1.13

Table 5. Number of trees in different classes of productivity and fruit weight.

Yield score	Fruit size in g 10-14	15-19	20-24	25-29	30-34	35-39	>40	Total
1.0	2	2	2	1	0	0	0	7
1.3	0	2	3	2	0	0	0	7
1.7	0	2	3	3	0	0	0	8
2.0	1	1	2	3	1	0	0	8
2.3	0	3	1	1	0	1	0	6
2.7	0	4	0	3	1	2	0	10
3.0	0	2	2	2	2	2	0	10
3.3	0	0	2	3	1	0	0	6
3.7	0	0	2	4	1	0	0	7
4.0	0	2	2	3	3	0	0	10
4.3	0	1	0	5	1	0	0	7
4.7	0	0	4	3	3	2	0	12
5.0	0	1	1	2	0	0	1	5
Total	3	20	24	35	13	7	1	103

ing fruit weight and mean yield score of three registrations. One genotype selected by all three methods was retained for further trials.

Crane & Lawrence (1956) state that fruits tend to be larger the second year after flowering. In this study rather the opposite was the case.

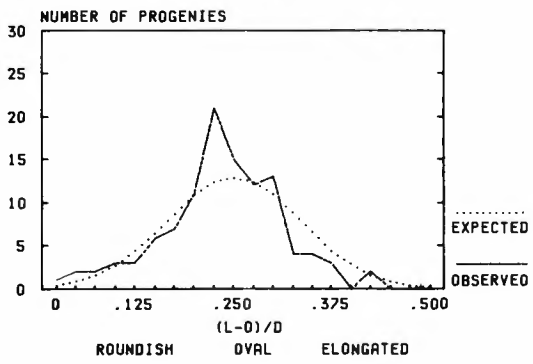
Fruit form

According to Cociu (1968) fruit form shows little variation between years. Crane (1921) grouped fruits in three classes: round, oval and elongated. However, the shape follows a continous distribution. No classes can be found, and this indicates polygenic inheritance. For illustrating fruit form, excess of lenght over mean diameter to mean diameter (L-D)/D, is suggested by Crane (1921). This distribution is presented in Figure 3.

The distribution in Figure 3 has no skewness, but has significant ($P<0.05$) positive kurtosis, indicating heavy tails. The roundest fruits are found to the left on the curve, the most elongated to the right. In one genotype the mean diameter exceeded length by 0.5 mm, but this was only one fruit, and the result is uncertain. The extremes based on mean of 10 fruits were 38 x 37 mm and 48 x 30 mm.

Crane's (1921) measurements of 'Victoria' were around 0.225, and the mean of the population in figure 3 is 0.226, which is very close to 'Victoria'. The heavy tails in the distribution indicate linked gene complexes for round and elongated form.

Figure 3.
VARIATION IN FRUIT FORM



Fruit colour

Scoring for colour is fairly difficult and subject to many sources of error if account is not taken of all components of appearance. In the present study, the colour was divided into 4 classes: yellow, yellow with flecks, red and purple. The results are presented in Table 6.

Table 6 shows that red and purple genotypes predominate in the present material. The discrepancies in ragard to Crane's experiment are significant $X^2=35.6$, and the main reason is found in classes 2 and 4. The present results support Johansson & Oldén's (1962) statement that 'Victoria' is highly heterozygous for anthocyanins. The excess for progenies in classes 3 and 4 indicates that alleles for red and blue predominate in 'Victoria'. According to Johansson & Oldén (1962), flecks are often due to a favourable environment. However, genes for these colours must be present. Probably genes controlling colour expression are different for class 2 compared with classes 3 and 4.

Stone properties

Stone size was characterized by length, breadth and thickness. Stone volume was estimated by calculating the product of these three factors. The variations in these characters are presented in Table 7.

As the table illustrates, all stone characters are highly variable from 17.7 x 10.3 x 6.5 mm to 24.4 x 17.7 x 9.9 mm.

Stone percent is more important than stone size alone. In the present study, stone percent was calculated as the volume ratio 10 x (stone length x breadth x thickness)/(fruit length x breadth 1 x breadth 2), Segregation of the different genotypes for this ratio is presented in figure 4.

The distribution shows a significant ($P<0.05$) positive skewness, which indicates that many values below the mean are very close to it, while high values are more distant (Snedecor & Cochran 1967). About 25% of the progenies are below 5% stone volume, which is suggested as culling level by Cociu (1976). The distribution function seems to have too high values on the abscissa, if compared with Cociu's (1968)

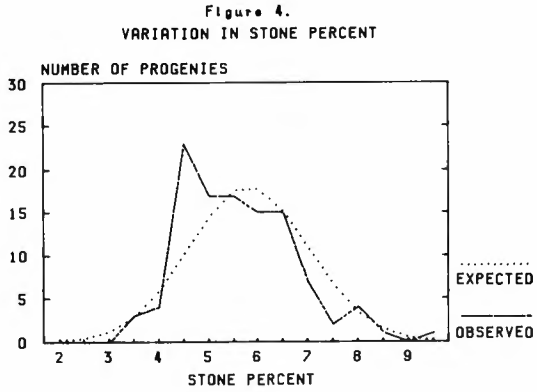
Table 6. Progenies in different colour classes.

	1	2	3	4	Total
Percentage	6	5	54	35	100
Crane (1921)	3	17	64	16	100

1 = Yellow, 2 = Yellow with flecks, 3 = Red (Victoria), 4 = Purple.

Table 7. Measures of stone size, mm.

	Min.	Max.	Mean
Stone length	16.7	28.5	22.4
Stone breadth	10.3	18.4	14.8
Stone thickness	6.0	9.9	7.9
Volume (mm^3/10)	119	428	264



registrations. However, stone and fruit weight may give a lower stone percent than calculating the percentage as volume ratio.

A significant negative correlation found between fruit size and stone percentage (r = -0.549) indicates that large fruits tend to have relatively smaller stones.

Stone adherence to fruit flesh

This trait is highly dependent on the environment, and fruits within a sample from one genotype may vary considerably. Grouping is usually characterized as clingstone, semifreestone and freestone (e.g. Crane and Lawrence 1956). The present study, however, indicates that the trait may have a more continous distribution. The genetical background is quite uncertain due to the large environmental component of variation. Table 8 gives the distribution of genotypes in different adherence classes.

The table shows that there are most genotypes with freestone or partly freestone genotypes. This indicates dominance for the freestone character (as found by Crane 1921). The frequencies of clingstone : semifreestone : freestone were 19:77:33. If the present material fits a one gene model, class 2 must be graded as clingstone. The ratio would then be 28:68:33 and this is in accordance with the expected 1:2:1 ratio for a one gene model with partial dominance.

Table 8. No. of genotypes in different store adherence classes.

	Class	Number
Clingstone	1	19
Semifree	2	9
Semifree	3	30
Semifree	4	38
Freestone	5	33

Chemical content

The soluble solid contents varied from 9.5 to

19.1 ° Brix, and the distribution fitted a normal curve N (14.0, 20.63²), as presented in Figure 5. Distribution with respect to normality seems to be skew. The statistical analysis carried out by the MSTAT computer program did not find this skewness to be significant. It is noticeable that 'Victoria' had soluble solid contents for unthinned and thinned fruits of 11.9 and 15.9, respectively. These values correspond with the two peaks in the figure. The mean of unthinned and thinned 'Victoria' for two harvesting times was 13.9, which is very close to the progeny mean of 14.0.

The content of soluble solids was negatively correlated to fruit weight and yield, and could have been due to a thinning effect and competition for assimilates, respectively. The main reason for the variability, however, is probably of genetic origin.

pH and titratable acidity

The content of dissociated acids in fruit juice, as indicated by pH measurements, showed a degree of variability ranging from 2.59 to 3.34 (Figure 6). Titratable acidity was calculated as malic acid and varied from 0.71 to 2.07% (Figure 7).

Both figures show a tendency to skewness, but neither one is significant. The curves show skewness in opposite directions, which is what would have been expected. Mean titratable acidity of thinned and unthinned 'Victoria' at two harvesting times was 1.52, which is slightly higher than the progeny mean of 1.38.

Figure 5.
VARIATION IN SOLUBLE SOLIDS

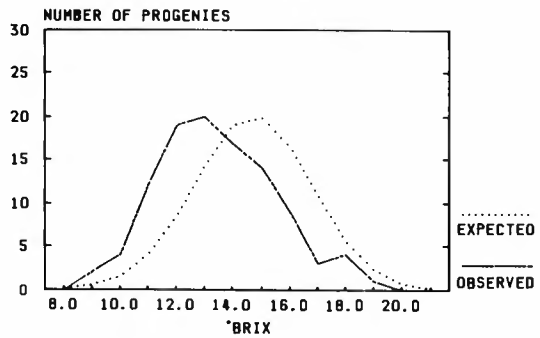


Figure 6.
VARIATION IN ACIDITY

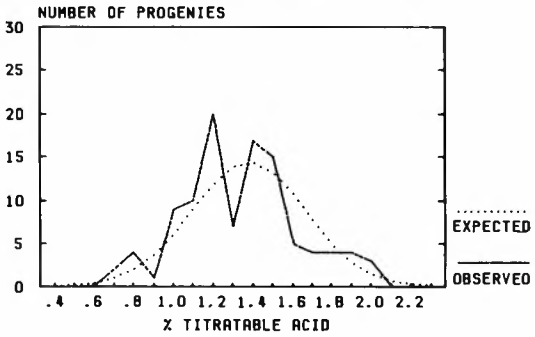
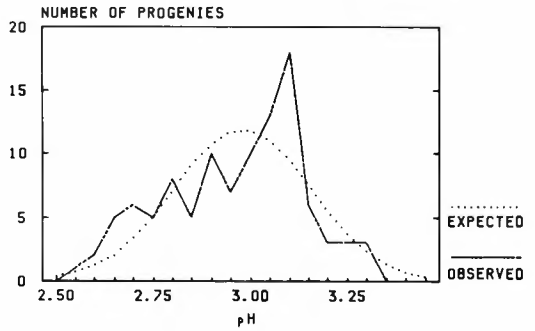


Figure 7.
VARIATION IN pH



CONCLUSIONS

The present study has shown that, in accordance with the study of Crane (1921), the variety 'Victoria' is highly heterozygous for all the registered traits. It was also observed that the hexaploid nature of *P. domestica* gives a complex way of inheritance. A very large number of the traits have a normal distribution, which indicates polygenic inheritance.

The origin of *P. domestica* indicates that colour could be explained by a simple gene model. However, this trait is extremely complex, and this study has not revealed such a model.

A one gene model for stone adherence to fruit flesh with dominance for the freestone character was sufficient for explaining the variation in the present material.

The correlation found between leaf fall and winter hardiness was too low to be used as indirect selection criteria for winter hardiness as could be suggested from Rudorf (1940).

ACKNOWLEDGEMENTS

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RESISTANCE OF STRAWBERRY TO PHOTOSYNTHETIC INHIBITORS AS A BASIS FOR RANKING OF CULTIVARS

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Fykse, H. 1987. Resistance of strawberry to photosynthetic inhibitors as a basis for ranking of cultivars. Norwegian Journal Agriculture Sciences 1: 25-34. ISSN 0801-5341.

The influence of lenacil, metamitron and simazine on the photosynthetic activity was measured in five cultivars of strawberry using an infrared gas analyser. Additionally, the effect of the same herbicides on growth was studied in a pot experiment. Measurement of the photosynthetic activity proved to offer a valuable apparatus for ranking the cultivars according to herbicide resistance. The following order of restiance was obtained:
Lenacil: Senga sengana > Bounty, Glima > Jonsok, Zephyr
Metamitron: Glima > Bounty, Senga sengana > Jonsok > Zephyr
Simazine: Bounty, Jonsok, Senga sengana > Glima Zephyr.

Key words: strawberry, cultivar-ranking, photosynthetic inhibitors.

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Field experiments aimed at evaluating herbicides for application to strawberry are labourious and expensive. Limited resources allow only a few experiments annually. Therefore, the evaluation process takes a long time and has to be concentrated on the main cultivars, while the new ones remain largely untested.

The purpose of this investigation was on the one hand to study different cultivars of strawberry with regard to their resistance to herbicides of currant interest, and on the other to try to find a reliable way of ranking the cultivars on the basis of resistance to the herbicides, without necessitating expensive field experiments.

MATERIALS AND METHODS

The investigation was carried out in two parts in which photosynthetic activity and growth rates were used as criteria of herbicidal effects.

Herbicides and plant material

The effects of lenacil, metamitron and simizine, which are well known inhibitors of photosynthesis (Anon. 1985, Schmidt et al. 1975), were studied in both parts of the investigation on the cultivars Bounty, Glima, Senga sengana and Zephyr. The latter two have long been the main cultivars of strawberries in Norway - Senga sengana as a late cultivar, Zephyr as an early. Compared with Zephyr, Senga sengana is regarded as being relatively resistant, especially to simazine (Erlandsen 1983). Resistance of the

remaining cultivars to herbicides is more or less unknown.

Multiplied vegetatively by A/S Gartnerhallen Nuclear Stock Plant Station, Sauherad, and Elite Plant Station, Reiersøl, the plants were collected in the autumn and stored until further use in darkness at ca. 5 °C.

Photosynthetic activity

Apparatus

The plants were put in separate chambers of clear polymethylmetacrylate (Fig. 1). Each chamber consisted of an upper and a lower vessel mounted on a sheet supported by four legs on a table. The herbicide was injected through the hose (a) by means of a pipette, while alteration to a herbicide-free nutrient solution was accomplished by changing the lower vessel.

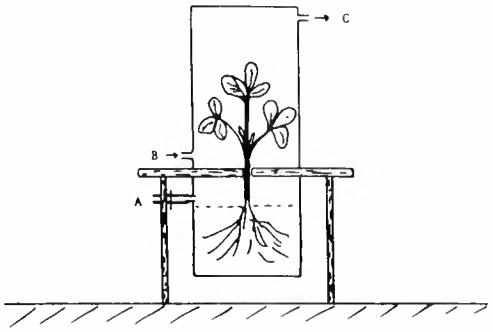

Fig. 1. Growth chamber. a = hose for injection of herbicide, b = air inlet, c = air outlet.

Having bubbled through a solution containing 20% KOH to remove all CO_2, the air was replenished with CO_2 to give a final CO_2-concentration of 0.04%. Under low pressure and at a speed of 35 l/h the air was blown into the chambers at the base of the upper vessel (b) and out at the top on the opposite side (c). The equipment consisted of six chambers; four were used for herbicide-treated plants, one for monitoring the CO_2 content of the air had no plants, while the last chamber contained an untreated plant for the observation of uninhibited growth.

The CO_2 content of the air leaving the chambers was determined by an infrared gas analyser. The differences in CO_2-concentrations between the reference chamber and each of the chambers containing plants were used as measures of the net photosynthesis or respiration of the plants.

The light source, strip lamps supplemented with sodium high pressure lamps, produced a photonflux of 550 $\mu E\ m^{-2}s^{-1}(400nm<\lambda 700nm)$ at plant level. Fig. 2 compares this light intensity with that on two successive days - one of which was sunny and clear, the other cloudy - in the middle of August.

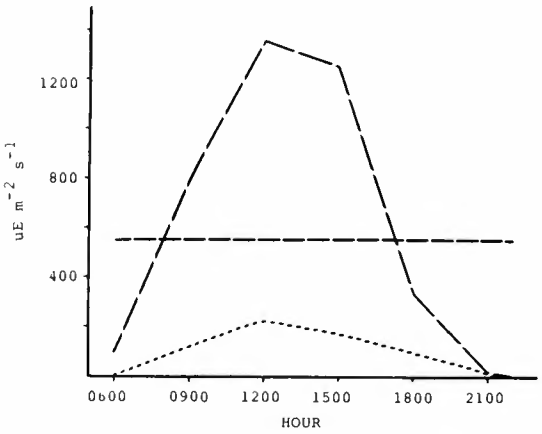

Fig. 2. Light intensity inside the growth chamber ----- compared with light intensity on a sunny day – – – – and a cloudy day ····· in the middle of August.

Raising the plants

The plants were removed from the cold storage and transplanted into pots with a mixed soil (Fykse 1982) at rates and amounts corresponding to the demand for plants in the experiments. The pots were set up in a growth cabinet at 20 °C, 16 h day and a photon flux density of 260 $\mu E\ m^{-2}\ s^{-1}$. After 21 days, leaves developed during the previous autumn were removed and the number of leaves reduced to three per plant. The roots were washed and the plants transferred to a nutrient solution for a period of eight

days under light conditions identical to those used in the experiments of photosynthesis. When plants were grown hydroponically, Hoaglands nutrient solution of ¼ strength was used.

Herbicide treatment and light programme

To calibrate the apparatus properly and to have a practical working routine the plants were put into the chambers one day before commencement of treatment. The herbicide was administered next morning at 0800 hours and removed at 1500 hours by changing to a herbicide-free nutrient solution. Recordings of the CO_2 content continued for three days after treatment. In a separate series of experiments, however, the herbicide was not removed and recording of the CO_2 content was terminated 48 hours after treatment.

In accordance with results of preliminary studies, a dose of 10^{-4}M ai formulated product was adopted as the standard. Because lenacil and metamitron in practical weed control are applied in doses of 2.2 and 5.6 times higher respectively than simazine, corresponding doses of these herbicides were also included.

The light period in most esperiments was from 0500 hours to 2100 hours, i.e. the dark period started 13 hours after administration of the herbicide or six hours after changing to the herbicide-free nutrient solution. To study the influence of light after treatment on the herbicidal effect, a light period from 0000 hours to 1600 was used, i.e. the same length of time as in the other experiments, but with commencement of darkness only one hour after removal of herbicide from the nutrient solution. All experiments on photosynthesis were performed with four replicas.

Absorption of herbicides

As the standard dose in the experiments described was higher than the solubility of both pure simazine and lenacil in water (5 mg/l and 6 mg/l respectively at 20-25 °C, compared with 18.2 g/l of metamitron), a separate experiment was undertaken to test whether the amount of herbicide absorbed by plants corresponded with the concentration of the herbicide in the nutrient solution. In this experiment only the Glima and Zephyr cultivars were used as test objects. The plants were cultivated and treated in the same way as those used in the experiments on photosynthesis.

Plants were selected at random for determination of the content of herbicide (a) at the time the herbicide-containing nutrient solution was removed, i.e. seven hours after administration of the herbicide, and (b) at the end of the three days' experimental period, the last 65 hours of which was without herbicide. The roots were thoroughly washed in tap water and the plants sectioned into 'roots' and 'leaves'. The sections were stored at -20 °C until further processing, and the analysis were performed at the Chemical Analytical Laboratory of the Agricultural University of Norway. The experiment was run with three replicas.

Growth rate

To compare the results of the experiments on photosynthesis with the phytotoxic effects of the herbicides when absorbed by roots via the soil or by the leaves as in practical situations, plants were transplanted on 14 May in plastic pots, 12 cm in diameter, in a soil with 4.6% organic matter and the following composition of the mineral fraction: 2 - 0.2 mm 34%, 0.2 - 0.02 mm 31%, 0.02 - 0.002 mm 23% and < 0.002 mm 12%.

The pots were placed outdoors under a roof of clear plastic and watered according to requirement. One week after transplanting all leaves from the previous year were removed, and after another week the plants were treated in accordance with the following scheme: (a) untreated, (b) simazine 0.5 and 1.0 kg ai/ha, (c) lenacil 1.2 and 2.4 kg ai/ha, (d) metamitron 3.0 and 6.0 kg ai/ha.

The smallest dose of each herbicide corresponded with the lower dose used under practical weed control, while the largest one was somewhat higher than the highest practical dose. To get the herbicide into the soil, the pots were watered soon after spraying.

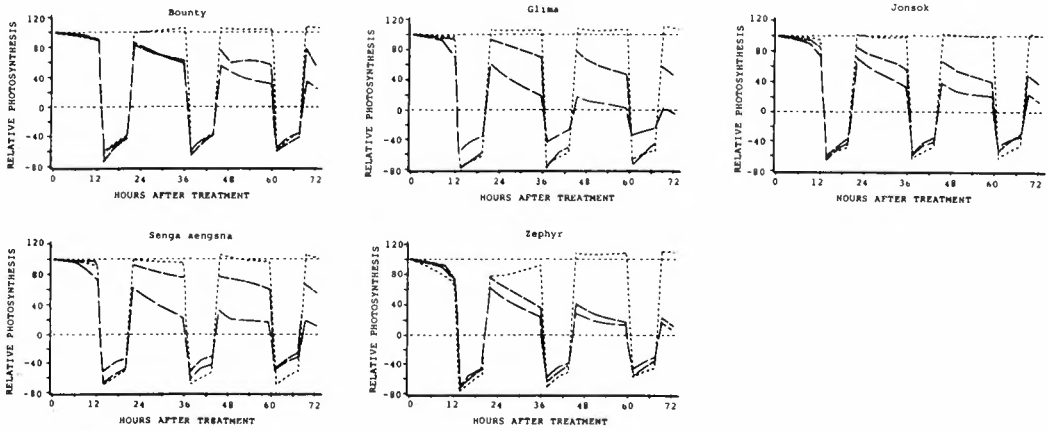

Fig. 3. Influence of 10^{-4}M of lenacil -----, metamitron ····· and simazine – – – – on the photosynthetic activity of different cultivars of strawberry. The herbicides were administered at time 0 and removed 7 hours later. Darkness 13-21, 37-45 and 61-69 hours after treatment.

One month after the treatment the plants were harvested. The number of flower buds were counted, the plants sectioned into 'leaves' and 'roots', dried at 90 °C for 48 hours and the weight of each section determined.

RESULTS

Photosynthetic activity

The effects of the three herbicides - lenacil, metamitron and simazine - on the different cultivars are shown in Fig. 3. During the seven hours of herbicidal exposure, alteration of the plant's photosynthetic activity was relatively limited, and the differences between herbicides and cultivars were negligible. During the following three days, however, marked differences between the herbicides emerged. Simazine proved to be the strongest photosynthetic inhibitor on all cultivars, and metamitron the weakest. The effect of simazine and lenacil increased with the time, even without further addition of herbicide. After a period of darkness (night), however, a slight tendency of recovery could be detected. By contrast, metamitron had significant effect only on Zephyr, and the effect decreased with time, even during the period of illumination.

When the light was switched off soon after the plants were given the herbicide-free nutrient solution (eight hours after administration), the herbicidal effect appeared slower, and remained at an appreciably lower level during the whole experimental period (72 hours). The main tendency, however, including the effect of the herbicides in relation to each other, was the same as above. The results for Senga sengana are shown in Fig. 4.

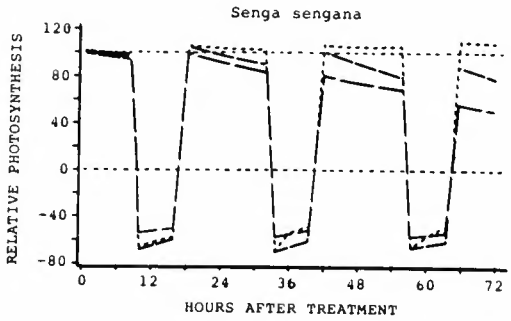

Fig. 4. Influence of 10^{-4}M lenacil -----, metamitron ····· and simazine – – – – on Senga sengana. The herbicides were administered at time 0 and removed 7 hours later. Darkness 8-16, 32-40 and 56-64 hours after trestment.

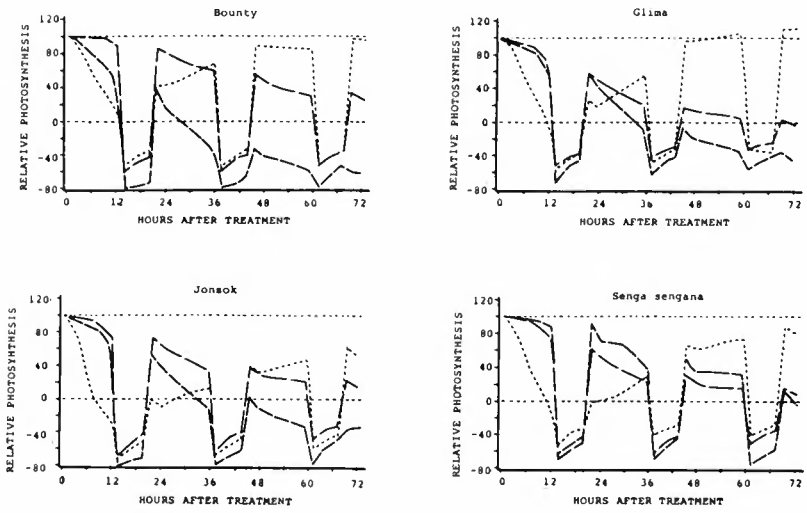

Fig. 5. Influence of $2.2x10^{-4}$M of lenacil -----, 5.6x10-4M of metamitron ····· and 10^{-4}M of simazine – – – – on different cultivars of strawberry. The herbicides were administered at time 0 and removed 7 hours later. Darkness 13-21, 37-45 and 61-69 hours after treatment.

The effect of the herbicides when administered in doses relating to each other, as in practical use, is illustrated in Fig. 5. Zephyr had to be excluded, however, because of a power failure. Under these conditions metamitron especially, and, to some extent, lenacil as well, acted more rapidly than simazine, and quicker than in the standard dose (cf. Fig. 3). Lenacil was very harmful to all cultivars, Senga sengana tolerating the herbicide better than the others - in fact more or less to the same extent as it tolerated the standard dose of simazine. At the beginning, metamitron strongly inhibited the photosynthetic activity of all the cultivars. At the end of the first day, only Bounty had a photosynthesis high enough to compensate for respiratory loss. During the following days, however, photosynthesis increased steadily. This was particularly obvious in Glima, which after only two days had regained the starting level of photosynthetic activity. Bounty was not far behind.

The results of the experiment with the herbicides applied at standard dose, but not removed as in the experiments mentioned above, are shown in Fig. 6. The effect of the herbicides increased appreciably at the end of the day of treatment. They were more powerful when continuously present in the nutrient solution compared to removal seven hours after administration (cf. Fig. 3). The most striking feature, however, was that metamitron, though strongly inhibiting photosynthesis of all cultivars at the end of the first day, lost very much of its effect during the next two days, especially in Glima, Jonsok and Senga sengana. Zephyr again turned out to be the most susceptible cultivar.

Absorption of herbicides from the nutrient solution

The amounts of active ingredient detected in leaves and roots are shown in Table 1. The concentration of simazine in the roots at the time of changing to herbicide-free nutrient solution (seven hours after administration of 10^{-4}M simazine) was significantly lower in Zephyr than in Glima. For the other two herbicides, no significant differences between the cultivars were observed. In the leaves, significantly higher

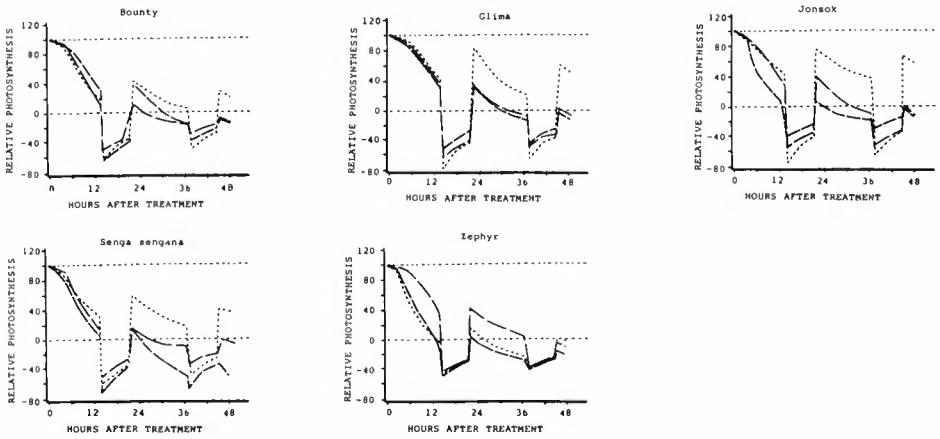

Fig. 6. Influence of 10^{-4}M of lenacil -----, metamitron ····· and simazine – – – – on different cultivars of strawberry. The herbicides were administered at time 0 and left in the nutrient solution for the duration of the experiment. Darkness 13-21 and 37-45 hours after treatment.

Table 1. Influence of dose and experimental conditions on the concentration (mg/kg) of different herbicides in leaves and roots of the strawberry cultivars Glima and Zephyr

Herbicide	Dose applied (M)	Exposure	Glima		Zephyr	
			Roots	Leaves	Roots	Leaves
Metamitron	10^{-4}	7 h of herbicidal	16.4	8.2	18.7	14.0
Lenacil	10^{-4}	exposure, then	8.8	1.1	10.2	1.0
Simazine	10^{-4}	harvest	16.0	1.4	3.7	3.0
Metamitron	10^{-4}	7 h of herbicidal	2.8	3.9	0.5	4.9
Lenacil	10^{-4}	exposure, followed by	9.2	2.7	5.3	2.0
Simazine	10^{-4}	65 h without herbicide, then harvest	2.9	5.6	0.3	6.1
Metamitron	5.6×10^{-4}	7 h of herbicidal	102.2	34.8	105.6	40.6
Lenacil	2.2×10^{-4}	exposure, then harvest	25.6	1.3	14.3	1.5

concentrations of metamitron and simazine were detected in Zephyr than in Glima, For lenacil, no differences were observed between the cultivars. Regardless of cultivar, metamitron was detected in leaves and roots in significantly larger amounts than lenacil and simazine.

By the end of the experiment (7 + 65 hours after administration) the concentration of metamitron had decreased appreciably in leaves as well as roots. For lenacil, however, the changes during the final 65 hours were very small, while the concentration of simazine decreased in the roots and increased in the leaves. During the latter part of the experimental period lenacil, therefore, proved to be less mobile in the plants than simazine.

When the applied doses corresponded to each other, as the herbicide rates in practical weed control, the concentration of metamitron increased significantly in roots as well as leaves

of both cultivars compared with the standard dose. In relative terms, the uptake of metamitron into the roots increased at least as much as the increase in the dosage. No significant differences were observed between the cultivars regarding the concentration of metamitron in leaves or roots. Subjected to the greater dose of lenacil, significantly higher amounts were taken up by the roots of Glima. The increased herbicide uptake corresponded well with the increased dose applied. In contrast, the roots of Zephyr did not show any enhanced uptake of lenacil. Neither did the increased dose of the herbicide lead to higher concentrations in the leaves of any of the cultivars, thus emphasizing the limited mobility of lenacil in the plants mentioned above.

Growth rate

The effect of the herbicides on the flower development was divergent and unsystematic (Table 2). In Glima, simazine significantly reduced the number of flower buds compared with untreated plants and plants sprayed with metamitron. In Jonsok the greater doses of lenacil and metamitron significantly reduced the number of flower buds compared with untreated plants, and in Zephyr the lower dose of all herbicides caused the plants to produce significantly more buds than the larger dose or in the non-treated plants. This apparantly stimulating effect on the induction of flowers in Zephyr, however, had nothing to do with vigorous growth. Quite the contrary, the plants were severely damaged, the induction og flowers appearing to be the last convulsion before the end.

The dry weight of the leaves, therefore, proved to be a better parameter of the herbicidal effect (cf. Table 3). Generally. simazine damaged the plants more severely than the other two herbicides, even though the effects were not always significantly different. It is also apparent from Table 3 that the Bounty, Glima and Senga sengana cultivars had a higher degree of resistance to the herbicides than Jonsok and Zephyr.

The dry weight of the roots (Table 4) largely reflected the dry weight of the leaves. The correlation between root weight (Y) and leaf weight (X), based on absolute figures, can be expressed by the equation: $Y = 1.02X + 0.318$ ($r = 0.91$).

DISCUSSION

When the herbicides were applied in equal amounts, simazine appeared to be the strongest inhibitor of photosynthesis and metamitron the weakest. Taking the more rapid translocation of metamitron into consideration (Table 1), this difference is further emphasized. Lenacil showed a weaker effect than simazine in all cultivars, a result which correlated well with the herbicide concentration in the leaves.

In the dark, the plants recovered slightly. This was particularly evident when the light was switched off soon after the change to herbicide-free nutrient solution. As regards lenacil

Table 2. Influence of different doses of various herbicides on the development of flower buds. Relative figures.

	Untreated	Simazine		Lenacil		Metamitron	
Dose, kg/ha	-	0.5	1.0	1.2	2.4	3.0	6.0
Cultivar:							
Bounty	100	89	81	105	100	84	86
Glima.....	100	74	76	102	83	95	106
Jonsok.....	100	81	93	85	63	81	70
Senga sengana.....	100	-	86	122	92	89	78
Zephyr.....	100	147	100	156	134	119	97

Table 3. Influence of different doses of various herbicides on the weight of dry matter of leaves. Relative figures.*)

	Untreated	Simazine		Lenacil		Metamitron	
Dose, kg/ha	-	0.5	1.0	1.2	2.4	3.0	6.0
Cultivar:							
Bounty	100a)	90ab)	72b)	102a)	88ab)	101a)	70b)
Glima	100a)	85a)	50b)	100a)	86a)	101a)	92a)
Jonsok	100a)	68ab)	76ab)	79ab)	54b)	73ab)	69ab)
Senga sengana	100a)	-	87b)	109ab)	86b)	124a)	83b)
Zephyr	100a)	77ab)	52cd)	89ab)	79ab)	63bc)	35d)

*)Different letters on the same line = significantly different figures ($p \leq 0.05$).

Table 4. Influence of different doses of various herbicides on the weight of dry matter of roots. Relative figures.*)

	Untreated	Simazine		Lenacil		Metamitron	
Dose, kg/ha	-	0.5	1.0	1.2	2.4	3.0	6.0
Cultivar:							
Bounty	100a)	86ab)	62b)	84ab)	72ab)	93ab)	60b)
Glima	100a)	86a)	39b)	98a)	91a)	96a)	76a)
Jonsok	100a)	65ab)	51ab)	73ab)	41b)	61ab)	72ab)
Senga sengana	100a)	-	96a)	100a)	80a)	127a)	95a)
Zephyr	100a)	70ab)	57b)	102a)	83ab)	57b)	49b)

*)Different letters on the same line = significantly different figures ($p - \leq 0.05$).

and simazine the inhibiting effect was not only delayed, but reduced as well (Figs. 3 and 4). The fact that the concentration of lenacil and simazine in the leaves did not decrease with time (Table 1) indicates that during the dark period the herbicides were inactivated in some way or another.

The effect of metamitron and of lenacil on photosynthesis was much more rapid in doses relating to each other as under practical conditions than in the standard doses. Furthermore, lenacil showed a stronger end-effect than simazine on all cultivars except Senga sengana. By contrast, metamitron lost much of its effect during the experimental period - virtually all for Glima and Bounty. The herbicide was obviously inactivated, a result that corresponded very well with those presented in Table 1, which shows a fairly rapid degradation of metamitron in strawberry plants. Degradation of lenacil and simazine, if any, was much slower.

Under continous exposure, photosynthesis of all cultivars was severely inhibited by all the herbicides. Nevertheless, with the exeption of Zephyr, the cultivars were more resistant to metamitron than to the other herbicides.

In the pot experiment, the great variations between the plants (Tables 2, 3 and 4) may be explained to some extent by the development of small cracks between the soil and the wall of the pots, allowing the herbicides to be washed down along the wall. This may have caused excessive absorption by the plant roots in some pots, and loss of herbicide in others. In spite of these variations, of the two standard cultivars Senga sengana again tolerated the herbicides

better than Zephyr. Among the new cultivars the tolerance of Bounty and Glima equalled that of Senga sengana, except for Glima against simazine. This is fully in accordance with the results of the experiments on photosynthesis, where Glima also appeared to be relatively susceptible to simazine (Fig. 3). In contrast, Glima showed a high degree of resistance to metamitron both in the experiments on photosynthesis and in the pot experiment. Glima in fact was the only cultivar that during the whole experimental period did not show any symptoms of injury from metamitron, even from the highest dose.

In conclusion, the experiments on photosynthesis provided fairly good evidence of the reaction of the different cultivars to the three herbicides tested. In evaluating new herbicides or in testing new cultivars of strawberry for resistance to photosynthetic inhibitors, measurements of the influence on photosynthesis may be a valuable supplement to, partly even a substitute for, expensive field experiments. A cultivar with known resistance is required as a reference. In this way it becomes possible to rank the cultivars on the basis of their resistance to the specific herbicide. According to the present experiments, the cultivars can be ranked as follows regarding resistance to:

Lenacil: Senga sengana > Bounty, Glima > Jonsok, Zephyr

Metamitron: Glima > Bounty, Senga sengana > Jonsok > Zephyr

Simazine: Bounty, Jonsok, Senga sengana > Glima Zephyr.

Measurements of photosynthesis, however, are not suitable for adjusting the herbicidal dosages to be used in practical situations. Properties of the soil, for example, which are of crucial importance for the effect of soil-acting herbicides, can not be tested using this type of experiment. The dosage, therefore, must be deduced from tha amounts of the herbicide that would have been applied had the standard cultivar been grown in that particular type of soil.

SUMMARY

The influence of lenacil, metamitron and simazine on photosynthetic activity was measured in five cultivars of strawberry. The herbicides were mixed with the nutrient solution in which the plants were growing. Seven hours after administration the plants were supplied with the nutrient solution free of herbicide, or alternatively left in the solution with herbicide for the duration of the experiment. Additionally, the effect of the same herbicides on growth was studied in a pot experiment, where the herbicides were sprayed on plants and soil.

The experiments on photosynthesis showed that in equal doses (10^{-4}M) the herbicides could be ranked according to decreasing inhibiting effect as follows: Simazine > lenacil > metamitron. When the light was switched off one hour after removing the herbicides, the effect appeared later and was weaker. When the herbicides were not removed, they all had a strong inhibitory effect, metamitron the least.

Applied in amounts relating to each other, as in practical situations, metamitron showed the fastest action and lenacil the strongest.

Metamitron was translocated more rapidly than lenacil and simazine, and, in contrast to the latter two, was degraded in the plants.

Altogether, Bounty and Senga sengana showed the greatest resistance to the herbicides tested. Glima was especially resistant to metamitron. Generally, Zephyr was the most susceptible cultivar, with Jonsok only slightly better.

The results of the pot experiment coincided to a large extent with those of the experiments on photosynthesis. Regarding herbicides acting mainly through the photosynthetic process, it was concluded that measurement of the photosynthetic activity may offer a valuable apparatus for ranking cultivars of strawberry according to herbicide resistance.

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WEATHER AND YIELD OF WESTERWOLTH RYEGRASS

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The effect of insolation and temperature on yields of first, second and third harvests was analysed by means of a regression model that takes the rate of phenological development into account. A low rate of development and delayed heading increased yields. Estimates of daily contributions to yield potential increased with increasing isolation, and decreased with rising temperature. Though, with low insolation, contributions to the first harvest decreased with decreasing temperature, which was ascribed to poorly established plant stands. The revealed relationships between weather and dry matter yield are statistical, and interpretation had to be partly based on plant stand characteristics.

Key words: Air temperature, development stages, insolation, Lolium multiflorum, yields.

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It is well-known that grass yields are influenced by weather in the growing season. The relationship can be described by modelling. A statistical analyses model that takes phenological development into account, revealed different effects of weather at separate stages of development on the final seed and haulm yields of field bean (Skjelvåg 1981b). In its vegetative growth ryegrass does not go through all the different developmental phases of a seed crop, and a simpler model could be expected. Baier et al. (1980) have analysed timothy yields by a similar model. They too assumed the response functions of timothy to be simpler than those of wheat, but they found several questions still not clarified.

MATERIALS AND METHODS

Westerwolth ryegrass *(Lolium multiflorum* Lam. var. *westerwoldicum)*, cv. Tewera, was in 1969-71 grown at 11 locations from the coast to 300 m a.s.l., and on one field 500 m a.s.l. in Aust-Agder, Norway. There were meteorological recordings on all fields (Skjelvåg 1981a, Skaar 1982 1983).

On each field three 4.2 m^2 plots were laid out among plots with other crops. Each plot consisted of ten rows with a distance of 13.3 cm between them. There were 1.1 m border strips without plant cover between the plots. Two m^2 were harvested from the six inner rows of each plot. Cutting height was 5 cm. The herbage was placed in a plastic bag and brought to the research station for weighing and dry matter determination. Dry matter was determined by chopping and mixing the whole plot yield and drying of three samples of about 20 g each at 80 °C for 20 hours. The first, the second and sometimes the third cuts were taken as soon as possible after heading, usually about one week

afterwards. The last cut, mostly the third one, was always taken late in the month of October without respect to the stage of development.

The basic fertilization with phosphorus and potassium was adjusted to soil analyses. It varied from 52 to 91 kg P and from 100 to 200 kg K per hectare. Nitrogen was given at rates of 114 kg N per hectare before sowing and 10 kg N per hectare as topdressing every fortnight after emergence. All fields were irrigated when tensiometer readings exceeded 0.5 bar at 15 cm depth in any of plots with oats, potatoes, field beans or peas.

The relationship between recorded yields (Y_p) and weather was analysed by the model:

$$Y_p = [f_0(t) + f_1(t)\,GR + f_2(t)GR^2 + f_3(t)TD + f_4(t)TD^2 + f_5(t)GR\,TD + f_6(t)Y_{p-1}]$$

where:

$$f_j(t) = u_{j1}t + u_{j2}t^2 \quad j = 0, 1, \ldots.6$$

List of symbols

Y_p Recorded yield at harvest p, gm^{-1}.

dY_p Estimated, daily contribution to yield of harvest p, $gm^{-2}d^{-1}$.

TD Diurnal mean temperature, °C at 2 m height.

GR Global radiation, $MJm^{-2}d^{-1}$, estimated on the basis of sunshine hours recordings (Skjelvåg 1981a).

t Biometeorological time scale, $t = \Sigma\, dP$, where the daily advance in phenological development $dP = f(TD)$ as described by Skjelvåg (1986) and summation, was done from sowing or cutting to the day in question. Fixed values were: $t = 0.0$ on the day of sowing and immediately after cutting, $t = 1.0$ on the day of emergence and on the heading days of regrowths, $t = 2.0$ on the heading day of first growth.

u Coefficients to be determined by the least square technique (Skjelvåg 1981a).

Daily values of t, GR and TD were used and summation done over all days of the growing period of each harvest. Thus, daily contributions (dY_p) to Y_p can be estimated by the model, which has been discussed by Skjelvåg (1981a). Both forward and backward stepwise selection of the terms in the regression model were used to eliminate nonsignificant terms and to find a model with the highest possible coefficient of determination, and with as few terms as possible.

RESULTS

The model explained 80% of dry matter yield variation in the first harvest, 68% in the second harvest and 44% in the third one (table 1). In the first and the third harvest the biometeorological time scale, as the first variable, accounted for about one half of the explained variation, and in the second cut, for about nine tenths.

Increasing global radiation always increased dY, but the GR term was significant only in the model of the first growth. The temperature term TD contributed significantly only in the models of the first and the third cut. In the latter, it was dependent on the GR term, which by removal reduced the coefficient of determination of the model significantly. Thus, GR and TD together contributed much to the model, and each of them alone little. Increasing temperature usually led to lower dY, but not in the first growth when insolation was lower than 14 $MJm^{-2}d^{-1}$. Then the temperature effect was positive. There was a weak tendency to higher dY_2 when yield of the first cut was high ($P<0.1$).

In the initial analyses a soil water term was included, according to the method described by Skjelvåg (1981a). This term was always removed early in the selection procedure, and was not considered in the final analyses.

The lowest yields of the first harvest were all taken in 1969 and the highest ones in 1971 (fig. 1). In both years field numbers 7, 8 and 10 on the coast had extremely high or low yields. At field 10 the period from sowing to the first harvest was long in 1969, dY_1 often negative or

Table 1. Contributions to the coefficient of determination (CD) and F-values from the analysis of variance by stepwise inclusion of terms in regression models showing daily contributions to dry matter yields at 1st, 2nd and 3rd cut. Explaining variables are: stage of development (t), global radiation (GR), diurnal mean temperature (TD) and yield of first harvest (Y_1).

Source of variance	CD	F
1st harvest: n=27, R^2=0.80		
- 59.564·t	0.43	46.2**
+ 4.492·GR·t	0.12	12.6*
+ 3.643·TD·t	0.08	8.1*
- 0.257·GR·TD·t	0.17	19.0*
2nd harvest: n=27, R^2=0.68		
24.953·t - 18.452·t^2	0.63	21.0**
+ 0.431·GR·t	<0.00	0.03
- 0.345·TD·t	<0.00	0.04
+ 0.00002 Y_1·t^2	0.05	3.6
3rd harvest: n=26, R^2=0.44		
11.330·t - 2.840·t^2	0.25	4.6(*)
+ 1.319·GR·t	0.02	0.7
- 1.372·TD·t	0.17	6.4*

(*): 0.05<P<0.1, *:P<0.05, **: P<0.01.

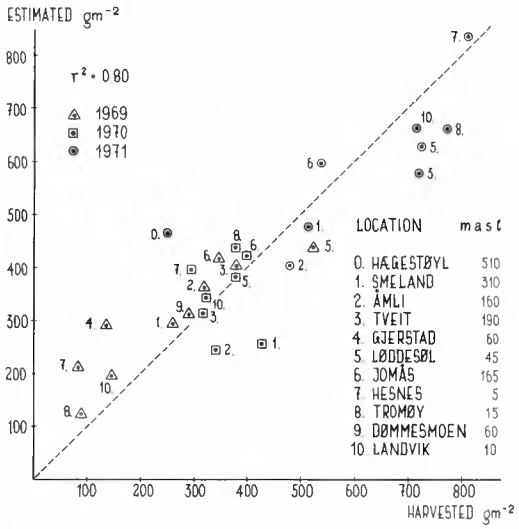

Figure 1. Observed and estimated dry matter yields of first harvest of Westerwolth ryegrass during three years at some locations in Aust-Agder.

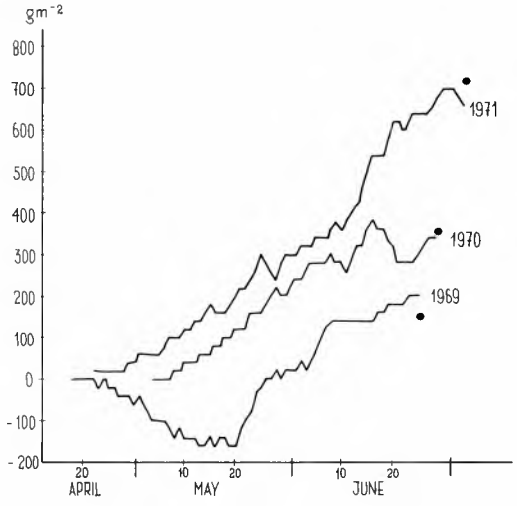

Figure 2. Accumulated daily contributions to the first harvest of Westerwolth ryegrass at location 10 Landvik during the period from sowing to harvest in three years. Harvested yields are indicated by points.

small, and the yield low (fig. 2). In 1970 dY_1 was usually positive, but the growing period shorter and the yield a little lower than the average. In 1971 the growing period was as long as in 1969, but dY_1 usually positive and yields the highest.

Figure 3 shows that insolation was higher in 1971 than in 1969. Temperature from sowing to early June was usually higher in 1971, whereas 1971 had the lower temperature from early June to cutting. In 1970, not shown, both radiation and temperature were on average the highest of all years.

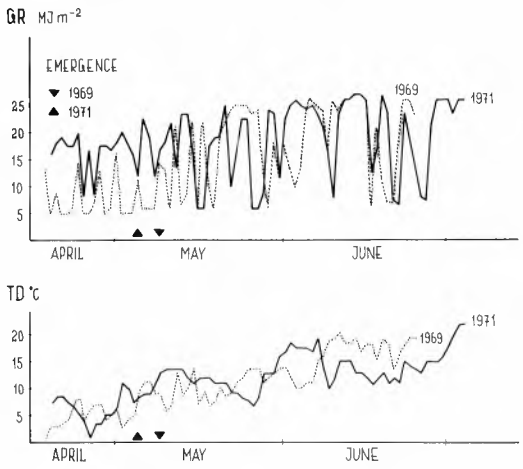

Figure 3. Insolation (GR) and diurnal mean temperature (TD) from sowing to first harvest of Westerwolth ryegrass at location 10 Landvik in 1969 and 1971.

DISCUSSION

In most cases the models were reduced to linear terms of the biometeorological time scale t. Thus, this simpler models can be interpreted as reflecting a homogenous response to weather throughout the vegetative growth of ryegrass, as compared with the more differential responses described by the complex model of a seed crop yield (Skjelvåg 1981b).

It is physiologically reasonable that the combination of high insolation and low temperature gave the highest dY to the second and the third harvest, and also to the first harvest, when insolation was higher than 14 $MJm^{-2}d^{-1}$. Consequently, the combination of high temperature and low insolation was disadvantageous for the yields of the second and the third cuts.

The negative dY_1 associated with low temperature when insolation was low, can not be explained by the effect of temperature on photosynthesis or on the rate of phenological development. In this case, establishment of plant stand was the most probable reason. According to recordings, plant stands of field 7, 8 and 10 in 1969 were extremely sparse. Cool, wet, overcast weather after sowing led to bad establishment. Thus, negative dY is associated not only with the light and temperature effects on CO_2 assimilation, but also with plant stand chacteristics. There is a parallel to this in height growth of field bean (Skjelvåg 1981a). Negative, daily contributions to the final plant height could be ascribed to sunny weather promoting flowering, which again promoted cessation of the height growth. This led to lower plants as compared with those experiencing overcast weather during the same phase. As to forage crop modelling, this only emphasizes the necessity of including plant population submodels (Torssell 1984).

The daily contributions are estimated by coefficients derived from summation terms, and the harvested yield as the dependent variable. This implies the possibility of negative dY on days with weather that resembles the weather regime of fields with low yields. By giving negative dY the interpretation above, it may not be necessary to eliminate negative dY from the model, as attempted by Baier et al. (1980) by fitting of the coefficients.

The effect of the biometeorological time scale t was dominating in the second harvest, and strong in the third one. Also in the first harvest there was a positive relationship between Σt and yield, but intercorrelation with Σ GR and Σ TD has made the sign negative for the t term in the complete equation. This positive relationship

shows that a slow rate of phenological development generally gives many production days and a higher yield.

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COLD HARDINESS IN THE BLACK VINE WEEVIL *(OTIORHYNCHUS SULCATUS* (F.)) *(COLEOPTERA: CURCULIONIDAE)*

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Stenseth, Chr. 1987. Cold Hardiness in the Black Vine Weevil *(Otiorhynchus sulcatus* (F.)) *(Coleoptera: Curculionidae)*. Norwegian Journal of Agricultural Sciences 1: 41-43. ISSN 0801-5341.

Investigating the capacity of adults and larval stages of *Otiorhynchus sulcatus* (F.) to survive at +2 °C, -3 °C and -6 °C revealed that the larvae were better able to survive sub-zero temperatures than the adults. The LT_{90} at -3 °C for adults and larvae was 30 days and 90 days, respectively. At -6 °C, both adults and larvae had an LT_{90} of about 9 days. The significanse of winter temperature for the distribution of *O. sulcatus* in Norway is discussed.

Key words: Otiorhyncus sulcatus, cold hardiness.

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Outdoor infestation of the black vine weevil *Otiorhyncus sulcatus* (F.) in Norway occurs particularly in the southern coastal districts (Hesjedal 1982). The species hibernates usually in the larval stages. A new generation of adults emerges from the end of June and eggs are deposited in August-September. Very few adults hibernate and lay eggs the following year (Stenseth 1976). During winter the larvae live in the soil, while the adults remain on the soil surface or in plant material. Under laboratoty conditions the adults live for between 90 and 250 days and have several oviposition periods (Stenseth 1979).

Smith (1932) suggested that high moisture content on the living sites reduces the life-span of adults. This paper presents data on the effect of low temperature on the survival of the black vine weevil.

MATERIALS AND METHODS

The experimental animals were from the Grimstad area and were cultivated om strawberry plants at 18-20 °C. The cold hardiness of fertile females and larval stages 3-6 was investigated at constand temperatures of -3 °C (±0.5) and -6 °C (±1 °C). The experimental animals were stored for 4 days at + 2 °C (± 1 °C) before exposure to sub-zero temperatures. Survival rate was also measured at + 2 °C. Larvae exposed to low temperature were placed in a moist peat-rich soil, 25 larvae per sample. Four samples were removed from each temperature treatment after different time periods and examined for larval mortality. Dead larvae were grey-black in colour, in contrast to the white living larvae.

The experimental samples of adults consisted of 10 specimens in a petri dish containing 3 strawberry leaflets. Five samples from each

temperature treatment were used to measure mortality at different time periods. After 24 hours at room temperature, adults were recorded as dead if they were immobile.

RESULTS AND DISCUSSION

Figure 1 shows that the larvae were more sensitive to -6 °C than to -3 °C. LT90 at -3 °C and -6 °C was approximately 90 days and 9 days, respectively. The mortality curve for -3 °C has a horizontal component at about 25% mortality, indicating that a minority of the population has a lower temperature tolerance than the majority. The fact that this pattern was not observed at -6 °C may be due to the very short survival period at that temperature, but might perhaps be revealed by the use of several different exposure times. At +2 °C, 93% of the larvae survived for 150 days.

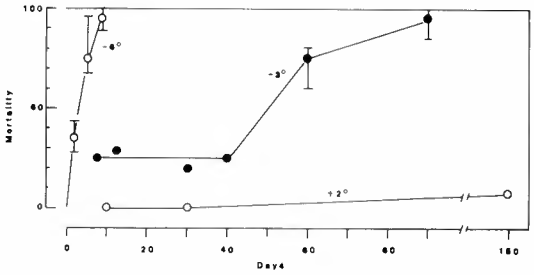

Figure 1. Percentage mortality of larvae of *Otiorchynchus sulcatus* stored at different temperatures.

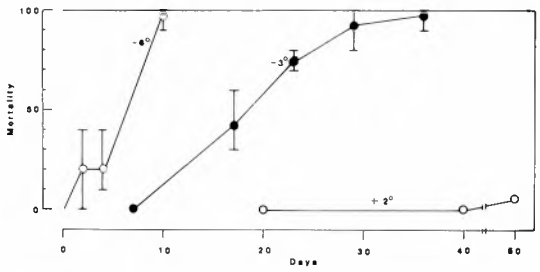

Figure 2. Percentage mortality of adult females of *Otiorhynchus sulcatus* stored at different temperatures.

The LT_{90} for adults at -3 °C and -6 °C was about 30 days and 9 days, respectively (Fig. 2), whereas there was a mortality of only 6% after 90 days storage at +2 °C. These results demonstrate that larvae have a considerably gerater ability to endure low temperatures. In addition to this inherent ability, larvae also find more sheltered overwintering sites than adults. Experiments have shown (Baadshaug 1973) that fields without a cover of snow at sub-zero air temperatures have lower temperatures on the soil surface than at a depth of 15 cm. On the other hand should a mild winter or a winter with snow-cover in periods with sub-zero temperatures occur, then high survival of adults and a corresponding stronger larval attack the following summer can result.

Comparing a temperature map (Det Norske Meteorologiske Institutt 1972) with the distribution of black vine weevil in Norway (Hesjedal 1982) shows that the species is concentrated in districts with a monthly average air temperature above -4 °C in January and in a few districts with an average temperature -4 °C to -6 °C. There are no records in districts with a monthly average temperature lower than -6 °C in January. Based on the cold hardiness in the larvae it is reasonable to assume that distribution of the black vine weevil is limited by winter climate.

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ACTIVATOR PROTEINS FOR LIPOPROTEIN LIPASE FROM BOVINE PLASMA.
A partial amino acid sequence analyses

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Astrup, H.N. 1987. Activator proteins for lipoprotein lipase from bovine plasma. *A partial amino acid sequence analyses.* Norwegian Journal of Agricultural Sciences 1: 45-48. ISSN 0801-5341.

Determination of a sequence of 44 amino acids of the major activator protein from bovine plasma is reported. A similarity is seen with human activator protein. The isolation of activator proteins from bovine plasma has been described previously (Comp. Biochem. Physiol. 1982, 72B, 487-91).

Key words: Activator protein, amino acids, bovine plasma, isolation.

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The ability to activate lipoprotein lipase in blood is found in most species. It has been shown to reside in low molecular weight apolipoproteins present in triglyceride rich lipoproteins, which are the natural substrate for the enzyme. The human activator protein, apolipoprotein C II, has been isolated and sequenced (Jackson et al. 1977a, Hospattankar et al. 1984). An activator protein has also been isolated from pig plasma (Jackson et al. 1977b). Bovine lipoprotein contains at least two activator proteins (Lim & Scanu 1976, Clegg 1978, Astrup & Bengtsson 1982, Sundheim et al. 1983) and there seems to be complete cross-reactivity between them. All species activators stimulate activity in all others (Bengtsson & Olivecrona 1977).

Consequently immuno responses are faint and activator immuno assays are hard to make. However, the compositions of the different species are not completely identical, the differences possibly being due to slight deviations in amino acid sequences or to a structure which couples extended sequences or groups to the mother compound.

METHODS

The activator proteins investigated were isolated from cows' blood. Apolipoproteins in plasma were isolated by means of a soybean oil emulsion stabilized with egg lecithin (Intralipid). After centrifugation and washings, the lipoproteins were treated in chloroform - methanol, 2:1, vol:vol. The proteins separated and were filtered off. After a gel choromatography step, the included fraction was then subjected to further separation by ionic exchange chromatography on diethyldiaminoethyl (DEAE) cellulose column (Astrup & Bengtsson 1982).

Of more than 10 proteins emerging from column, only 3 carried lipolytic activity. The isolation procedure and method of lipolytic activity determination have been described in a previous paper (Astrup & Bengtsson 1982).

RESULTS AND DISCUSSION

The active proteins appeared after those of the void volume, but ahead of the main protein of the apoproteins (Fig. 1). The first one was interesting because of its greater activity, judged to be about twice that of the second protein. The third protein appeared impure by gel electrophoresis, and was not analysed further. The electrophoretic columns gave a single protein disc with the other two activators. The overlapping fraction between the peaks produced a double disc, which means that the two isolated proteins were pure and distinct from each other. The amino acid compositions of the two proteins were similar.

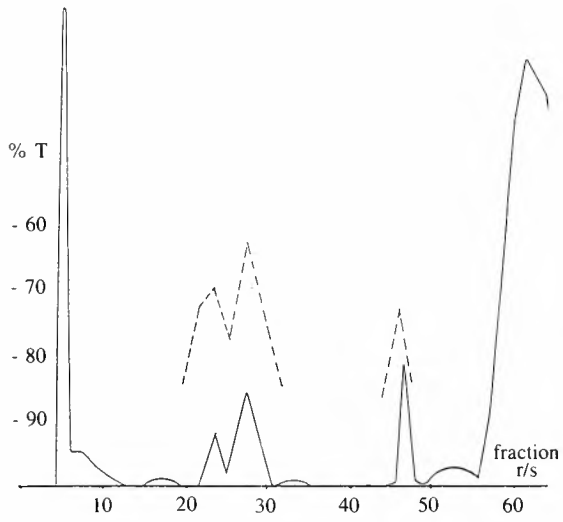

Fig. 1. Optical transmission (———) and the activating power (-----) of fractions eluated from DEAE column.

The dose response curve of lipolytic activity is reported in Fig.2, with the first protein. The second protein was available in amounts sufficient to approach a sequence analysis of the amino acids. The result of the Edman degradation (courtesy of Protein Structure Laboratory, Oslo) is given in Fig. 3. The sequence is seen from the amino terminal group and 44 amino

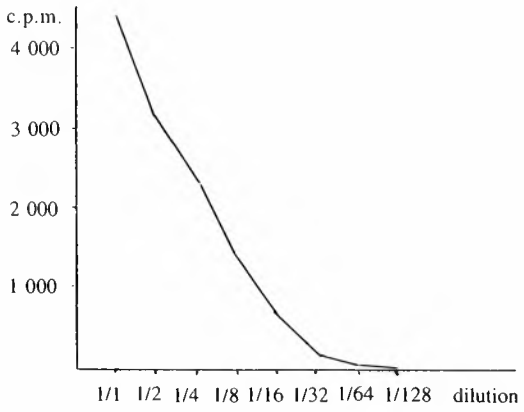

Fig. 2. Dose response curve of activator protein emerging first from the DEAE column. Initial solution 300 μg in 30 μl 6 M urea, 50 mM NaAcO pH 4.5. Each dilution was diluted a further 100 times in the reaction mixture.

acids towards the carboxyl end of the protein. It is similar to the sequence of the human protein between acids 7 and 50, reported by Hospattankar et al. (1984). Similarity is greatest towards the middle of the molecule, or the end of the sequence investigated. Certain amino acids, altogether 13, are exchanged: glutamine (13) against lysine, aspartic acid (21) against glutamic acid, lysine (29) against aspargine, and lysine (32) against glutamic acid.

The last nine exchanges are within the neutral amino acids. The introduction to the sequence in the human activator is missing in the cow activator. The cow protein lacking six of the first amino acids suggests a total of 73 amino acids in bovine activator versus 79 in the human activator. The sequence is strikingly similar, and if we take away the first six acids from the human structure, the amino acid composition is similar, too (Table 1).

There are two properties of the activator proteins which may be explained by the structure - their ability to attach themselves to phospholipids and the fat particle, and the coenzyme property in the lipolysis.

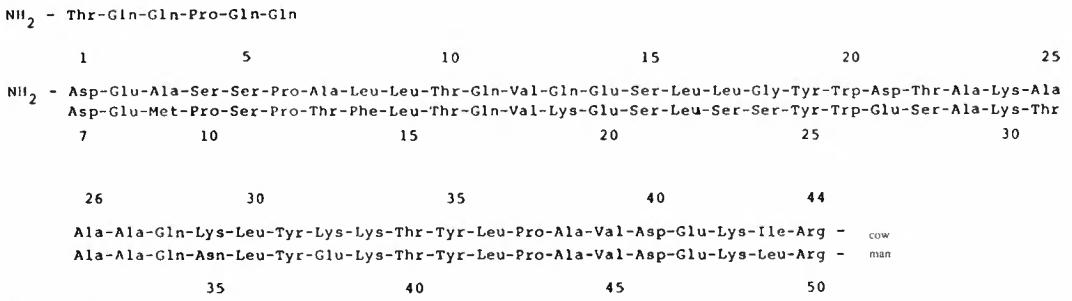

Fig. 3. Partial amino acids sequence of the second and major activator protein emerging from DEAE column compared with that of the activator protein isolated from human blood by Hospattankar et al. 1984.

Table 1. Amino acids in human[1]) and bovine lipoprotein lipase activators.

	Activator's number of amino acids		
	Human 1-79	Human 7-79	Bovine 1-73
Asp	5	5	6
Thr.....	9	8	6
Ser.....	9	9	8
Glu.....	14	10	9
Pro.....	4	3	2
Gly.....	2	2	3
Ala.....	6	6	11
Val.....	4	4	4
Met	2	2	0
Ile.....	1	1	3
Leu	8	8	7
Tyr.....	5	5	5
Phe.....	2	2	1
Trp.....	1	1	1
Lys.....	6	6	6
Arg	1	1	1

[1]) Hospattankar et al. 1984

The lipolytic activity has been ascribed to the sequences between the 55 unit and the carboxyl end of the human activator molecule (Smith et al. 1978). Although outside the lipolytic area proposed by Smith et al., the arginine (in position 50) in the bovine activator (this corresponds to position 44) seems important for the lipolytic function (Holdsworth et al. 1984). The various coenzyme functions may be integrated throughout the molecule. The N-terminal sequences, however, apparantly deviate between the species, as the comparison between man and bovine activator shows.

The proteins accompanied in the C-proteins may exhibit quite different structures, as seen for the so-called C III protein in human plasma (Sparrow et al. 1977). Because of their anti-lipolytic properties, they may be involved more indirectly in lipolysis.

The lipase in milk is a lipoprotein lipase, present in very high concentrations, and may be activated through the lipoprotein lipase activator, also present in the blood.

The lipolysis in milk is a quality defect in milk production, and is affected by the kind and amount of feed given (Astrup et al. 1980). The activator regulation may be a regulator also of cows' milk quality.

SUMMARY

A partial sequence analysis of the amino acids of bovine lipoprotein lipase activator shows great structural similarity with human apo C II, except for six amino acids, missing in the bovine activator, initiating an N-terminal sequence of the human protein. The full structure of this and the other two activating bovine proteins remains to be worked out.

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THE DISTRIBUTION OF ORALLY ADMINISTERED MAGNESIUM-28 IN TWO DAIRY COWS

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Astrup, H.N., H. Hvidsten & K. Stenberg 1987. Observations on the distribution of orally administered 28 Mg in two dairy cows. Norwegian Journal of Agricultural Sciences 1: 49-55. ISSN 0801-5341.

Magnesium absorption and distribution was followed in identical twin cows on a mixed winter ration and spring grass rations. The animals consumed less feed and less labelled magnesium from rumen in the spring ration. One animal kept on potassium rich pasture between experiments became more hypomagnesaemic than the other during the spring grass experiment.

Key words: Absorption, bovine, distribution, magnesium.

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Knowledge of the utilization and metabolism of magnesium is lacking in studies of hypomagnesaemic conditions in ruminants (Rook et al. 1958, Field et al. 1958, Hvidsten et al. 1959). Recent reviews have been given by Martens & Rayssiguier (1979), and Pehrson (1985).

Because of the availability of a radioactive magnesium isotope 28 Mg, with half-life of 21.4 hours, we used this isotope in studies of the magnesium metabolism in dairy cattle. Despite the short half-life, the isotope is still able to supply information on the behaviour of magnesium in animals. The difficulties caused by the short half-life of the radioisotopes and their high cost were partly compensated for by large samples (100 ml or 100 g) for the countings.

Our preliminary aim was to examine distribution of orally added 28 Mg in the rumen contents, serum, milk, urine and faeces, in winter stall and spring grass rations.

MATERIALS AND METHOD

A pair of identical cattle twins were selected as experimental animals. Their live weight in experiment I was about 470 kg and in experiment II 450 kg. They were 5 1/2 years old and calved their 4th calf on 20 February (No. 79) and 18 March (No. 80) 1959. The experiments were conducted at the Department of Animal Nutrition.

Quantitative collection of urine and faeces was made for a period of 58 h after dosing. A modified version of the harness described by Balch et al. (1951) was used for the separate collection of urine. Modification comprised enlarging the urine tube and its metal bridge, and adding spongy rubber to the contact surface between the urine tube and the skin around the vulva. The elastic thongs were replaced with non-elastic ones to which elastic straps of 5/16″ x 3/64″ rubber tubing were connected to the D-rings of the hindmost flank strap. With these

modifications no urine was spoiled during the experiments. Faeces were collected in iron containers in the gutter. The cows were accustomed to the harness some days before the first experimental period. They were milked at 0830 and at 2030.

The radioactive magnesium was added as an aqueous solution of 28 Mg SO_4 delivered from A.E.R.E., Harwell, England (cyclotron-produced). Each cow was given ca. 50 μCi by means of a rubber tube into the rumen. The specific activity was about 20 μCi/g Mg. Activity from radioactive contaminations was found to be less than 0,5% of 28 Mg activity at the time of administration to the cows.

At fixed intervals samples (100 g or 100 ml) for determination of 28 Mg activity were taken of the rumen content (by stomach tube), blood serum from the jugular vein, and milk, urine and faeces. The samples were dried and ashed and the 28 Mg content determined using a Geiger-counter. Large dishes were used to minimize selfabsorption in counting. When the 28 Mg activity died out, the samples were counted again to allow corrections for background activities. Activity after the complete decay of 28 Mg in urine, faeces, and rumen content was higher in experiment II than in experiment I. This was commensurate with a recorded increase in the content of radioactive fall-out (from nuclear weapon tests) in the fodder in experiment II at the time when fresh grass was given.

Minerals in the blood serum were determined by methods adapted at the department: The Mg assay was after Denis et al. from Hawk et al. (1947) and the phosphorus method was according to Raabe by Zeiss-Opton (1951). Ca was titrated at pH 10 with edta and ammonium purpurate as indicator. Volatile fatty acids in rumen liquor were determined after isolation in a Parnass-Wagner still.

Ammonia-N diffusion assays were carried out in Conway dishes on filtered samples. Minerals in feed, milk and faeces were determined on dried and ashed material in accordance with official methods.

Experiment I was carried out from 13 to 16 May 1959 under winter feeding conditions, Daily rations to both cows were identical from 28 March to the end of the experimental period and consisted of 4 kg hay, 7 kg treated straw (Bechmann method), 15 kg root tops silage, 7 kg concentrates, and 50 g mineral mixture. The radioactive magnesium was given at 1030 hrs, the collection of urine and faeces starting at the same time.

In experiment II, the same animals were used after they had been on pasture from 19 May to 2 June 1959. During this time, cow No. 79 grazed on plots fertilized in the spring with 113 kg of N per hectare (in ammonium nitrate/limestone), 36 kg of P (in superphosphate), and 174 kg of K (in muriate of potash). Cow No. 80 grazed on plots fertilized with the same amount of N and P, but not K. Three analyses carried out during this grazing period showed that no. 79 had an average of 1.6 mg per 100 ml magnesium in the serum while no. 80 had 2.2 mg per 100 ml.

The cows were brought into the stall on 2 June at 0900, harnessed, and fed cut grass from the same plots they had been grazing on. No. 79 was in heat and took smaller quantities of grass after being brought indoors. It went entirely off feed in the afternoon of the next day. It had stiffness in the legs and difficulty standing. Collection of urine and faeces had to be suspended and no milk was obtained on 4 June. On being given a subcutaneous injection of 30 g $MgSO_4$ and 35 g Ca-borogluconate after completion of the experimental period, it recovered some days afterwards. No. 80, also, refused to take as much grass as she was offered. The average 24 h consumption of grass during the experimental period for Nos. 79 and 80 was 10 kg and 42 kg respectively.

RESULTS AND DISCUSSION

Table 1 gives the amount of dry matter and minerals taken in the feed and of the minerals excreted with faeces and in the milk over 24 h within the experimental period. The period of feeding was shorter than usual for an assay of

Table 1. Average daily content of Ca, P and Mg in feeds, milk, faeces and urine in the experiments I and II for cow no. 79 and 80.

Experiment no.		I		II	
Cow no.		79	80	79	80
Feeds:					
Dry matter	kg	13.4	13.4	2.1	9.9
Crude protein	g	2 026	2 026	510	2 110
Ca	g	88	88	11	59
P	g	56	56	9.2	42
Mg	g	31	31	4.2	23
Milk:	kg	16.8	17.1	4.8	14.0
Ca	g	14.7	15.0	9.2	18.8
P	g	16.9	17.1	5.1	16.0
Mg	g	1.5	1.5	0.6	1.5
Faeces:	kg	20.1	16.9	3.5	11.1
Ca	g	62.3	40.8	6.3	19.9
P	g	30.2	27.2	6.0	15.5
Urine:	kg	9.3	8.0	7.0	12.9
Feed - Faeces:					
Ca	g	25.7	47.2	4.7	39.1
P	g	25.8	28.8	3.2	26.5
Mg	g	8.9	18.8	1.0	11.9

Table 2. Mean analytical results for the experimental period.

Experiment no./cow no.	Serum			Rumen liquor			Urine		Milk
	Mg (g/l)	Ca (g/l)	P (g/l)	pH	NH_3-N (g/l)	Volatale acids (M)	pH	NH_3-N (g/l)	fat (%)
I/79	0.024	0.093	0.035	7.6	0.048	0.039	8.3	0.026	4.3
I/80	0.027	0.091	0.042	7.6	0.045	0.040	8.2	0.015	4.6
II/79	0.008	0.064	0.033	8.0	0.086	0.049	7.5	0.218	13.8
II/80	0.019	0.091	0.035	7.4	0.104	0.074	7.2	0.172	6.1

digestibility. In experiment II, cow no. 79 had been fed potassium fertilized grass and was particularly low in serum and milk magnesium. She turned sick and finally went off during the experimental period. Phosphorus and calcium levels in serum were also depressed (Table 2). Cow no. 80 secreted more of its calcium into the milk in the second period on fresh grass than on the winter feed of experiment I. The serum values of calcium and phosphorus in cow No. 80 remained unchanged on the grass feeding, but magnesium in serum dropped in this cow when it was turned onto grass.

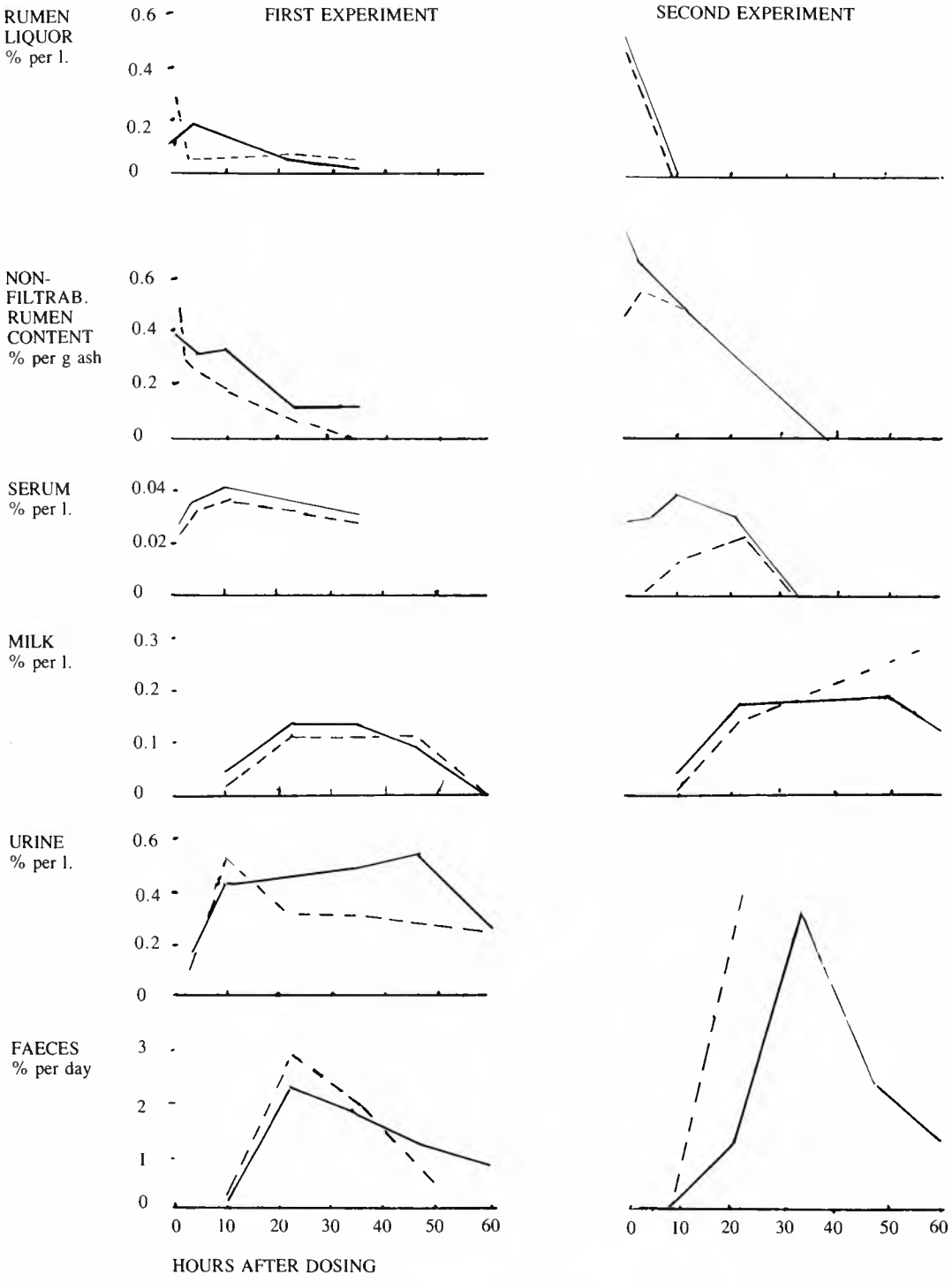

Fig. 1. Time distribution of labelled magnesium of two experiments in both cows.

Figur 1 shows the content of radioactive magnesium in rumen solids and liquor, in blood serum, milk urine and faeces obtained after rumen infusion. All the values were corrected for decay of the isotope.

In the rumen liquor the variable activity of each sample taken after 1 h was probably due to technical difficulties, with resulting saliva contamination. In experiment I activities in rumen liquor of the two cows were plotted semilogarithmically to make a straight line relationship with time. The exponential function of activity in the rumen calculated by the least squares method was $Y_t = Y_o \cdot e^{-0.0046t}$ where t is the time in h. This gives a half-life of magnesium in the rumen of 17 h, a value not far from the 24 h reported by Phillipson & Cuthbertson (1956) for the passage of hay through the rumen.

Figure 1 shows that 28 Mg disappeared more rapidly from the rumen in experiment II than in experiment I. The non-filterable solids of the rumen liquor reflected the changes seen in the total liquid. The rate of labelled Mg disappearance in experiment II was twice that of experiment I. Some residual activity might have resulted from reentrance of 28 Mg from saliva.

Serum activity appearing after only 2 h, and reaching its maximum levels within 10 h, strongly suggests an absorption of magnesium from the rumen. On the grass diet, both cows lost their serum magnesium activity more quickly than on an indoor diet. In cow no. 79, with the severe deficiency, also serum level took longer to peak, indicating an inferior transfer of magnesium from rumen into blood in experiment II. This fits with the rumen events referred to above, which showed a quicker turnover in rumen activity in experiment II, and thus must have made magnesium less available for transfer to the blood.

Since these experiments were carried out, several authors have confirmed that blood uptake of magnesium from the rumen takes place (Harrison et al. 1963, Wilcox & Hoff 1974, Tomas & Potter 1976). Recent results further claim that the rumen is the main route of absorption of magnesium, and that uptake from the intestines is insufficient to maintain serum magnesium levels (Tomas & Potter 1976).

Although the slowly soluble magnesium oxide alleviates magnesium defiency in ruminants, its absorption is probably more efficient after solubilization at lower pH, or in the gut. Furthermore, the non-ruminants satisfy their magnesium requirements without the rumen. The question needs further elucidation.

It is known that apart from absolute magnesium levels in the feed, the supply of nitrogen and potassium to the fields and the change from winterfeeding to pasture is likely to precipitate magnesium defiency (Breirem et al. 1949, Hvidsten & al. 1959, Hvidsten & Langebrekke 1972). Since absorption in the rumen is significant, the state of the rumen liquor may interfere with magnesium solubility and absorption from the rumen. A large number of such factors have been suggested.

In both experiments, activity in the milk was seen as early as the first milking, 10 h after infusion. Highest activity was obtained after 22 h in both animals in experiment I. In experiment II activity in milk lasted longer.

The first activity in urine appeared in urine sampled within 4 h. From 10 h on, activity remained constant. In experiment II activity in the urine was too low to give a reliable counting, and much lower than in the preceding. Low excretion of magnesium through the urine is evidently a measure of poor magnesium uptake from grass feeding.

Radioactivity in the faeces appeared between 10 and 22 h after infusion, when maximum activity occured in both experiments. Peak activity in cow no. 80, however, appeared in the 34 h sample. More activity appeared in experiment II, where the amount of faeces was less (Table 1).

Table 3 gives the integrated part of radioactive magnesium separated into milk, urine and faeces with time after infusion. In experiment I, 3% has gone into the milk after 46 h, and 7-8% into the urine after 58 h. In faeces, 25% is found after 22 h.

In experiment II, very small amounts of activity were excreted with urine. For milk and faeces there were no great differences between

Table 3. Percentage of 28 Mg dose recovered in milk, faeces, and urine of dairy cows within hours (h) after dosing.

Experiment no./cow no.		Period of time after dosing[1])				
		10 h	22 h	34 h	46 h	58 h
In milk	I/79	0.2	0.9	1.9	2.7	a
	I/80	0.5	1.3	2.6	3.3	a
	II/79	0.2	0.4	b	b	1.7
	II/80	0.3	1.2	2.6	3.6	4.6
In faeces	1/79	0.9	26.8	41.6	50.2	a
	I/80	0.3	24.7	41.0	47.9	53.9
	II/79	0.8	28.4	b	b	b
	II/80	0.6	4.0	31.9	48.8	62.0
In urine	I/79	1.6	3.5	4.5	5.4	6.6
	I/80	1.6	3.7	5.3	7.5	8.2

[1]) Within 4 h after dosing, activity (0.4%) was measureable only in urine in expt. I.
a) Activity too low to give a reliable counting this sp. concerns urine in expt. II.
b) The cow was sick no milk, urine or faeces.

the experiments. The quicker rumen bypass seen is not reflected in the more rapid faecation on the grass diet. Possibly, inhibition of rumen absorption was compensated for, after all, in the intestines.

Is magnesium uptake which occurs from the rumen only, or in the gut only, important for elucidating the mechanism? In the present experiments it was recognized that magnesium intake is reduced when the feed is changed, and also when the rumen passage rate increased, thereby reducing the magnesium transferral into the blood from the rumen.

SUMMARY

1. Identical twins received a ruminal infusion of 28 Mg with a ration of winter feed and then a ration of cut grass. Radioactivity was measured in the rumen liquor, blood, milk, urine and faeces at times following the infusion. Reduced intake of feed and magnesium was seen on the grass diet. Serum magnesium was low on this diet, and the cow fed on grass from highly potassium fertilized plots developed severe hypomagnesaemia.

2. Half-life for 28 Mg in the rumen was 17 h on winter feed. With grass feeding, it disappeared more quickly from the rumen.

3. 28 Mg activity was seen in serum after only 2 h, with the maximum between 10 and 22 h. Afterwards, peak values decrease was stronger on grass than on winter feed.

4. Activity in the milk only 8 h after infusion reached its maximum in 22 h milk.

5. Winter feeding produced activity in urine within 4 h after infusion. From 10 h on, activity was constant. When grass was fed, activity in the urine was too low to estimate.

6. There was activity in samples of faeces produced 10-22 h after dosing. After 22 h, 25 % was recovered, and after 46 h 50 % was accounted for.

7. The low serum and urine magnesium in experiment II reflected a deficiency in cows being fed potassium fertilized grass (initial in stage in cow No. 80 and manifest in cow No. 79). The low difference in chemically assayed digestibility between both experiments and a non-existant variance between 28 Mg excretion, suggest that inferior uptake of magnesium in experiment II was the main reason for deficiencies, rather than conditioned unavailability of the magnesium.

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INTERFERENCE BETWEEN SOLID FATS AND MAGNESIUM
Biochemical observations in veal calf feeding

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Feeding solid fat reduces serum magnesium in animals. A general interference between fat and magnesium absorptions is suggested, Two experiments on the feeding of calves reveal that hydrogenated marine and coconut-oil based milk substitutes reduce serum magnesium compared with milk reconstituted from butter oil.

Key words: Fats, interference, serum, magnesium.

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Magnesium is chemically related to the other earth alkali elements calcium and strontium. Chemically similar elements substitute one another in their bondings and compete for reactions with other elements. They interfere with each other in the earth and in living tissues. Since the level of magnesium is generally lower than that of the other macro elements it hardly affects these other elements and their reactions. In the earth's crust the carbonates of magnesium are abundant (dolomite, magnesite). Minerals used to supplement animal feeds include calcinated carbonates, carbonate, oxides and to some extent phosphates and sulphates.

Magnesium in the body

Whereas calcium is present in the bones, magnesium is more abundant in soft tissue. In mature animals the ability to mobilize the bone stores is less efficient (Georgievskii et al. 1979). On liquid diet, the net requirement of a calf per kilo per day is 3 mg of magnesium (Blaxter 1956). Magnesium has structural, electrolytical and, most importantly, catalytical functions. It is an activator of a variety of kinases, phosphorylases, phosphatases and estereases in the body.

Magnesium deficiency, feed level and dietary availability

Deficiencies are known in calves (Blaxter & McGill 1956; Georgievskii et al. 1979), and in Norway in sheep (Hvidsten 1967; Nygaard & Nedkvitne 1977) and cows (Breirem et al. 1949; Breirem 1954 Breirem, Hvidsten et al. 1966 Hvidsten et al. 1959; Hvidsten & Langebrekke 1972). Calves fed on liquid feed are apt to become deficient, according to Georgievskii et al. (1979).

Low serum magnesium levels used to occur in cows on winter feeds in particular, before it was common practice to incorporate magnesium in the mineral supplement. Typically, de-

ficiency is encountered when animals are let on to green and lush grass in the spring. The digestibility in the young grass is low (Kemp 1968) when dressings are applied in excess, availability becomes even lower. The magnesium content of grass is also reduced by the treatment.

Silages usually have their minerals available because of the acid conditions (Astrup et al. 1973 Astrup 1986), but the loss from drainage can be severe. While some silage feedings appear to reduce serum magnesium (Breirem, 1954, Nygaard, Nedkvitne 1977), high intakes tend to compensate for low levels. Serum values of Ca, P and Mg correlate positively and significantly with intake of silage in appetite fed animals (Astrup 1985).

The total level of magnesium is very important, but its absorption by the animal may be affected as well. The uptake of magnesium directly from the rumen site of the gut has been established (Hvidsten 1960; Harrison et al. 1963; Rogers & Vantklooster 1969; Wilcox & Hoff 1974; Tomas & Potter 1976). Consequently conditions prevailing in the rumen can affect magnesium absorption. Rumen contents rate of passage of the grass feeding has been raised(Astrup et al. 1987), and may reduce uptake of magnesium. If rumen content pH remains too high, magnesium is not released from its bondings, and is not available.

The precipitation of insoluble magnesium - ammonium phosphate also takes place under rumen conditions.

Further, high levels of potassium in the rumen interfere with active uptake of magnesium. This has recently been discussed by Martens & Rayssiguier (1979).

The final possibility to be considered is the formation of insoluble soaps of magnesium, which might be expected to interfere with the rumen absorption of the element and with the uptake in the lower gut. A recent review on the availability of magnesium in cows has been given by Aaes (1985).

Availability of magnesium affected by fat in the diet

The question we raise is whether soap formation inhibits the utilization of magnesium. Pasture grass contains fat and fatty acids whose hydrogenation products are solid. Their magnesium soaps may be unavailable to the animal (Kemp et al. 1966). Young grass contains fat and is a poor source of magnesium. Late stages of development improve the situation. Hay has more magnesium and less fat. Silage also have more fat than hay. With an increase in serum-cholesterol, serum magnesium went down while feeding silage to lambs (Astrup & Nedkvitne 1967). Digestibility of brassic and erucic acids is much better than its trans-isomeres in the presence of minerals (Astorg 1980). These and the related isomers appear in the rumen.

In several investigations at the Institute, the supplement feeding of fat has been seen to affect serum magnesium levels. Goats given palmitic acid (Astrup 1985a) and cows given hydrogenated soybeanoil (Astrup et al. 1976) came out with low serum levels, as did cows given hydrogenated marine oils (Sundstøl 1974). Experiments with calves are reported in this paper.

Improvements in milk fat by magnesium oxide supplements to cow

Several hypotheses have been proposed explaining how milk fat may be raised when magnesium oxide is fed. In a low pH situation in the rumen, the neutralizing effect is of value (Emery 1983; Chalupa, Kronfeld 1983). Beede & O'Connor (1985) point out that the relationship between the effect of neutralization and the increase in milk fat is not good. Emery (1983) believes that magnesium per se stimulates uptake of endogenous acetate and triglyceride from the blood.

Another factor that may be considered is the interference between magnesium and solid fats in the feed. Since solid fats inhibit magnesium uptake, the reverse might also happen. The formation of long chain fatty acid magnesium soaps will reduce solid fat digestibility if feed magnesium level is raised.

Since the feeding of hydrogenated commercial fats reduces milk fat synthesis (Astrup 1976; Selner, Schultz 1980; Banks et al. 1984) it is suggested that the inhibition will be eliminated by feeding magnesium. Such solid isomeric fatty acids are produced by hydrogenation of unsaturated fat in the rumen. Any biological effect of these products may be counteracted by an extra magnesium supply, in making them unavailable.

Calcium and phosphorus in magnesium fat interference

Calcium and magnesium are related in their behaviour towards phosphates and the solid fatty acids. The primary phosphates are soluble, while the secondary phosphates are almost insoluble. Both minerals give insoluble soaps with solid fatty acids. There are some differences, however. Calcium is more abundant, it gives stronger alkalinity to the solution, and its secondary phosphate is less soluble. Calcium competes with magnesium for the phosphorus.If fatty acids are present, magnesium will favour soap formation.

It has been demonstrated that phosphorous supplements improve magnesium absorption if phosphorus is low in the feed. This has been reviewed recently by Hemingway (1985). The lowering of serum phosphorus together with induction of magnesium deficiency in a previous experiment with cattle may have enforced the deficiency syndrome (Hvidsten et al. 1959). Low phosphorus together with low magnesium separated when hydrogenated soybean oil was fed (Astrup 1976). Beede & O'Connor (1985) consider that the effect of magnesium oxide on cows' milk is different from that of primary-magnesium phosphate. Magnesium oxide increases milk fat content,while phosphate of magnesium increases the amount of milk.

The milk fat increases have been suggested in the previous section to be due to the removal of inhibiting fatty acid isomeres from the gut by means of the magnesium oxide supplement.

Improvement in milk production with magnesium phosphate is not only easily explained, but it may also possibly result from a stimulation of enzymes within the body cell.

Magnesium in human nutrition

Since hard water is found to be coincident with a reduced incidence of heart disease (Schroeder 1961), calcium and magnesium may be the factors involved, if the relation is causative. Magnesium is held by some to be the causative factor (Anderson et al. 1975) and has gained a position as a terapeuticum in some treatments of heart disease.

Since the supply of magnesium through human food is low, a direct interference of magnesium through the fat in the feed may be subject to consideration.

Experimental feeding of fat to calves

The price difference between margarine and butter raises the question of using margarine in liquid feeds for veal calves. A preliminary experiment conducted on a private farm in 1956 compared milk substitute with hydrogenated marine oil (mp 33 °C) with whole milk (Homb 1960). The three animals in each group took the milk substitute well, without digestive disturbance. Growth was about 8% less than with whole milk. The liver was enlarged and contained more cholesterol and more fat than in the control animals. Serum levels of Ca and P in the calves were normal. At the end of the experimental period (after 10 weeks), serum magnesium was analysed revealing that animals fed the substitute diet had hypomagnesaemia.

Two experiments were then designed to take place at the university experimental farm in 1959.

MATERIALS AND METHODS

Twelve male calves were purchased from the market. After one week of whole milk feeding the animals were divided into three groups and fed skimmed milk emulsions with three kinds of fat:

Group I: unsalted butter
Group II: hydrogenated marine oil
Group III: coconut fat.

Each animal, from 3 weeks on was given 5 g vitamin concentrate with 1400 units vitamin A per g. The gradual change from whole milk to artificial milk, that is, fat emulsion despersed in skimmed milk, took place as follows:

Days from purchase	Whole milk litre/day	Milk substitute litre/day
1-7	4	0
8-9	4	1
10-11	3	2
12-13	2	3
14-15	1	4
16-28	0	5-8
29-74	0	9-14

The aim was to give equal amounts of fat and milk to calves in all groups. However, group III animals lost some of their appetite and had a tendency to loose stools during the experiment, and consequently took slightly less feed. Groups I and II animals had good appetites. The calves were all slaughtered on the 74th day of the experiment.

Experiment 2 was planned as a repetition of experiment 1. By mistake, all animals received less fat than was scheduled in the transistant period (2 weeks). Appetite was slightly impaired with the coconut oil feeding this time in group III.

The fat emulsion was prepared in germ-free conditions by Sandar Factories. Monoglycerides were added to facilitate emulgation of the fat. The hydrogenated marine oil had a melting point of 33 °C,an iodine value of 78 and a saponification value of 188. The emulsions contained 30-35% analytically assayed fat.

The consumption of fat in the experimental groups appears in Table 1, where the figures derive from chemical determinations of fat in primary emulsions and in the substitute milks. The group I calves ended up with slightly lower intakes of fat than scheduled.

Analytical methods

Calcium in blood serum was titrated at pH 10-10.5 with ethylenediamino acetic acid (EDTA) and with ammonium purpurate as the indicator. Magnesium in serum was found by the method of Denis(described by Hawk et al. 1947). Inorganic phosphorus was determined according to Urbach, and presented by Zeiss-Opton (1951) for colorimetric micro assay in serum.

Fat from intestines was minced in a meat grinder and melted at 90-100 °C in screw lid sealed jars filled with CO_2. Liver fat was extracted by a solvent mixture developed by Mojonnier and used by Bixby at al. (1954). Control analyses on milk and milk substitutes were made using the Gerber method (Knudsen 1944). The emulsions were split by acids and then extracted with ethyl ether. Iodine values in fat were assayed with Hawks and Wijs solutions, as described by Hawk et al. (1947). The saponification value procedure is described in the same place. The sample size, however, was only 2 grams. Cholesterol of blood serum and of liver was assayed according to Tschugaeff, as used by Hanel & Dam (1955).

Table 1. Average consumption of feed of each calf in the two experiments.

	Experiment I			Experiment II		
	Gr 1	Gr 2	Gr 3	Gr 1	Gr 2	Gr 3
Whole milk, l	48	48	48	43	44	44
Whole milk fat, kg	1.92	1.92	1.92	1.55	1.58	1.58
Skimmed milk, l	650	643	602	658	663	617
Emulsion fat, kg	22.1	23.1	21.7	22.0	23.7	21.9
Fat in substitute, %	3.40	3.59	3.60	3.34	3.57	3.55

RESULTS

Some of the important weight averages are given in Table 2. Growth rate was good on butter fat and on hydrogenated marine oil, but growth rate on coconut oil was reduced in both experiments. These latter animals took less food and also suffered from scourings. In the first experiment deposition of fat was seen from the weighings of intestinal fat deposits to be less on coconut oil feeding. Liver weights were enlarged in groups given fats other than butter.

Table 3 gives the results of the biochemical analyses of the bloodserum and fat in the animals.

Table 2. Average growth and slaughter weights.

	Experiment I			Experiment II		
	Gr 1	Gr 2	Gr 3	Gr 1	Gr 2	Gr 3
Live weight,						
at purchase, kg	40.0	40.3	40.5	38.2	37.0	37.5
at start, kg	43.3	43.5	43.5	41.2	41.2	41.2
at slaughter, kg	108.0	104.2	97.5	109.5	109.0	100.5
Growth, g/day	919	865	770	976	986	863
Growth, g/day corr	990	850	752	996	1 013	869
Carcass weight, kg	62.3	56.7	52.9	61.0	61.0	55.5
Dressing percentage[1])	57.6	54.4	54.2	55.7	56.0	55.1
Intestinal fat, kg	1.0	1.0	0.8			
Liver, kg	1.9	2.4	2.5	2.1	2.5	2.2

[1]) Corr for dressing percentage = 55.

Table 3. Average analytical results.

	Experiment I			Experiment II		
	Gr 1	Gr 2	Gr 3	Gr 1	Gr 2	Gr 3
Serum, mg %						
P	10.1	9.1	8.8	9.1	9.6	9.0
Ca	9.3	10.1	10.2	10.4	10.5	10.3
Mg, start	1.98	1.95	1.93	2.03	1.90	2.20
1. month	2.04	1.80	1.82	1.94	1.89	2.01
2. month	1.97	1.08	1.22	1.72	1.31	1.35
Cholesterol						
start	-	-	-	87	74	73
1. month	99	154	235	102	91	102
2. month	98	167	231	112	144	173
Liver fat						
Percent	4.6	4.6	15.0	3.5	4.2	11.0
Iodine value	74	79	33	94	91	50
Sapon. value	203	199	211	208	200	223
Cholesterol, mg %	233	250	329	263	323	353
Intestinal fat						
Iodine value	34.1	65.6	20.2	44.5	59.9	21.4
Sapon. value	194	190	204	204	203	202
Melting point, °C	43.5	36.5	40.3	41.1	37.8	41.1

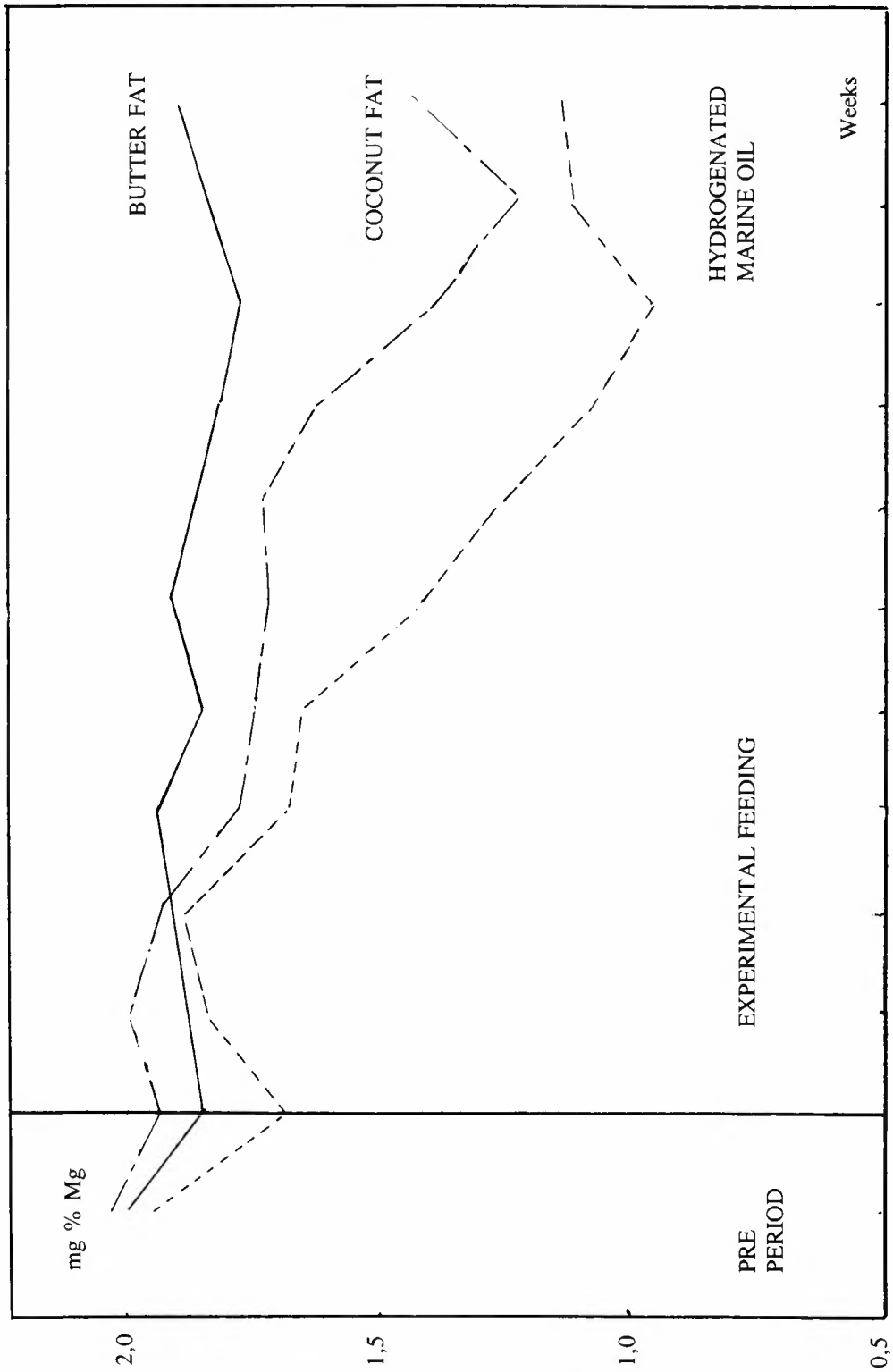

Fig. 1. Serum magnesium by feeding various fats in milk substitutes to calves. Butter fat -, coconut fat .-, hydrogenated marine oil--.

The influence of fat feedings on blood serum calcium and phosphorus is neglibible or inconsistent, but on magnesium it is remarkable (Fig. 1). This holds true also with serum cholesterol. Magnesium decreased with hydrogenated fat and coconut oil feeding, while serum cholesterol increased. Cholesterol in liver is also elevated compared with cholesterol in the livers of animals receiving butter fat in their feed.

The total liver fat of the animals on hydrogenated oil and coconut oil is increased. The liver fat characteristics are also changed in the direction of the fat in the milk substitutes. This is evident from the analytical results of fat from the intestines, too.

DISCUSSION

When the effect of fat feeding on magnesium serum levels was highest in the coconut oil group. However, when sample averages for magnesium and cholesterol in both experiments were correlated, significant coefficients of correlation were obtained ($R = -0.41$, $p<0.01$). An effect of magnesium deficient animals (Hellerstein et al. 1960; Breirem 1967). The increase in liver fat, cholesterol and serum cholesterol may be taken to be a lower turnover of the solid fats, perhaps more because of the low degree of unsaturation in the fats than the low magnesium level.

These experiments on calves demonstrate that interaction in animals does occur, fats reducing magnesium availability. The feeding of calves with milk substitutes may be a special situation, due to the large amounts of fat given. However, less fat was given, in goats,and in cows, where magnesium of the serum still seems to be depressed. The intake of fat on young pasture may be of the same order as in these experiments. When feeding high carbohydrate diets or young grass, the solid fats digested will consist mainly of trans and other isomeric acids.

To feed extra magnesium (50-100 g) is generous to enable soap formation of all the transacids. The removal of solid fat as soap has little noticeable effect on energy supply and production, but might have if the fatty acids removed have specific action on milk composition.

It is suggested above that magnesium oxide suppplements take the fatty acid isomers away from the rumen gut, and thereby restores milk fat synthesis.

SUMMARY

1. The effects of feeding butter, hydrogenated marine oil (mp 33 °C) and coconut oil in milk substitutes to calves were studied.

2. Compared with the serum of butter fed calves, feeding hydrogenated marine oil and coconut oil increased cholesterol and depressed magnesium content.

3. Compared with the liver values of butter fed calves, hydrogenated marine oil and coconut oil feeding raised total fat and cholesterol.

4. The fat iodine and saponification values in intestinal and liver fat were affected.

5. Growth tended to be best on butterfat milks, and inferior on coconut oil milks.

6. Since solid fat feeding interferes with magnesium uptake, it is concluded that the magnesium reduction effect of solid fat uptake may be beneficial in certain situations.

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EVALUATION OF COLOUR QUALITY IN BLACKCURRANT FRUITS GROWN FOR INDUSTRIAL JUICE AND SYRUP PRODUCTION

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Skrede, G. 1987. Evaluation of colour quality in blackcurrant fruits grown for industrial juice and syrup production. Norwegian Journal of Agriculture Sciences 1: 67-74. ISSN 0801-5341.

The colour quality of fruits of several commonly grown blackcurrant cultivars, harvested at different stages of ripeness, was evaluated by colour parameters Hunter L'a'b' and by absorbance in juices and syrups. The variation in instrumentally evaluated colour was determined by differences in total anthocyanin concentration in the fruits. The relative amounts of the four major anthocyanins of blackcurrants, as determined by HPLC analysis, were similar in all cultivars except one. The colour of fresh syrups could be estimated from the colour of juices. Colour evaluation of blackcurrant fruits for industrial syrup production is achieved by analysing absorbance or Hunter L'a'b' values directly in juice from homogenized fruits.

Key words: absorbance, anthocyanin, blackcurrant, colour, HPLC, industry, juice, syrup, tristimulus values, quality.

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The anthocyanins responsible for the colour quality of blackcurrant fruits are cyanidin-3-glucoside, delphinidin-3-glucoside, cyanidin-3-rutinoside and delphinidin-3-rutinoside (Chandler & Harper 1962). The presence of a further two acylated anthocyanins has been reported to occur in some cultivars (Jennings 1984). Each anthocyanin is characterized by special light-absorbing properties and contributes thereby to the typical colour of blackcurrants. The pigments can be separated and quantified by HPLC analysis (Williams et al. 1978, Strack et al. 1980, Blom 1983). Only small variations in the composition of anthocyanins with variety are reported (Nybom 1970). The aim of the present study was to screen the variation in colour characteristics and anthocyanin composition in fruit materials from several commonly utilized blackcurrant cultivars, grown at various places and harvested at various stages of ripeness.

In transparent dark fruit juices, an increase in pigment content causes decreased luminosity of the sample (Eagerman et al. 1973a, Francis 1977). By using colour scales originally developed for reflectance measurements, e.g. the CIE (1976) L*a*b* system, increases in pigment concentration beyond a certain limit give decreases in the chromaticity parameters a* and b*, i.e. in redness and yellowness respectively. To deal with this problem, special colour scales have been developed for transparent dark liquids where calculations are based on transmittance measurements (Eagerman et al. 1973b, Hunter 1975). With these scales, the chromaticity parameters are expanded in the region

where the luminosity (L) of the sample is low. After testing one of these colour scales, Johnson et al. (1976) concluded that the chromaticity parameters of the expanded system correlated well with the human observations, and that colour parameters derived from tristimulus values could be used for determining pigment concentration as well as for evaluating colour quality of transparent dark liquids. To obtain perfectly linear relationship between the chromaticity parameters and pigment concentrations, special colour scales would have to be developed for each product of interest.

In a previous study, we found that Hunter a' values or, better, a linear combination of Hunter L', a' and b' values, were well suited for predicting sensory scores of diluted (1:4) blackcurrant syrups (Skrede et al. 1983). The experiment comprised syrups with a wide range of colour qualities generated through a storage experiment. Hunter a' values have also been demonstrated to decrease according to first order reaction kinetics in aging blackcurrant syrups (Skrede 1985). Hunter a' values may therefore be used to predict storage life of syrups when the initial deterioration rate in known and acceptance limits have been established.

The present work was undertaken to study how colour quality of fresh blackcurrants intended for juice and syrup production could be evaluated using colour parameters derived from tristimulus values or absorbance measurements.

MATERIALS AND METHODS

Fruits from seven blackcurrant cultivars grown at the Department of Pomology, Agricultural University of Norway (NLH), were harvested at various stages of ripeness during a four week period. The cultivars were Amos Black, Lepaan Musta, Roodknop, Silvergieter, Svarteper, Wallace Seedling and Øjebyn. In addition, ripe fruits of nine cultivars were obtained from the Agricultural Research Stations at Kise (7 cultivars) and Holt (2 cultivars), Norway. The cultivars were Amos Black, Ben Lomond, Ben Nevis, Hedda, Roodknop, Silvergieter and Øjebyn, and Imandra and Sunderbyn, respectively. Samples of 0.5 kg were frozen soon after harvesting and stored at -20 °C until analysis.

Free anthocyanin composition of fruits (mg/100g) was determined using the pH-differential method described by Fuleki and Francis (1968). A molar extinction coefficient $E^{1\%}_{1\,cm}$ of 28 000 and a molecular weight of 595.2 g/mol were used for calculation (Wrolstad 1976). Absorbances at 515 nm were obtained from juice diluted 1:40 with water.

Blackcurrant juice was prepared by centrifugation of homogenized fruits at 12 000 x g for 20 min. For syrup preparation, homogenized fruits were treated with pectolytic enzymes (Panzym Super, 200 mg/kg) at 50 °C for 2 h. After centrifugation (3 000 x g, 20 min) the liquid was heated at 80 °C for 1 min in a water bath. Three parts of sucrose were added to two parts of liquid (w/w). Juices and syrups were stored frozen until analysis.

CIE (1976) L*a*b* and Hunter L'a'b' values (Hunter 1975) of diluted (1:10) juices and syrups were calculated from the transmittance spectra. The spectra were taken between 380 and 760 nm in 1 cm cuvettes using a Shimadzu UV-300 spectrophotometer connected to a Nord-10 computer, as described previously (Skrede et al. 1983). Calculations were based on measurements at every 10 nm. The equations for the Hunter L'a'b' system are: $L' = 10\,(Y)^{-1/2}$, $a' = 175\,(1.02\,X/Y - 1)$, $b' = 70\,(1\text{-}0.847\,Z/Y)$. X, Y and Z refer to the CIE tristimulus values. Light source C was used for the calculations. Hue angles, $h(^\circ)_{ab}$* and $h(^\circ)_{ab}$' were calculated as $\tan^{-1}b/a$ in both systems.

Separation of anthocyanins was performed by HPLC analysis (Blom 1983) modified by Thomassen (1987). The relative positions of the cyanidin-3-glucoside (Cy-3-gl) and cyanidin-3-rutinoside (Cy-3-rut) in the chromatograms were verified by co-chromatography with blackberry and raspberry extracts (Harborne & Hall 1964), while delphinidin-3-glucoside (Dl-3-gl) and delphinidine-3-rutinoside (Dl-3-rut) were verified by hydrolysis of the anthocyanins and identification of the reaction products. The aglycon was co-chromatograph-

ed by HPLC (Thomassen 1987) with delphinidin chloride (Carl Roth, Karlsruhe, Germany). Glucose and rhamnose were identified by co-chromatography with known sugars using an Aminex Ion Exclusion HPX-87H column (300 x 7.8 mm, Bio-Rad Laboratories, Richmond, CA) operated at 0.6 ml 0.01 N H_2SO_4 /min. Sugars were detected by Shodex-SE-51 refractive index detector (Showa Denko, K.K., Japan).

RESULTS

Colour parameters CIE (1976) L*a*b* from diluted blackcurrant syrups of the Øjebyn cultivar are shown in Fig. 1. With increasing syrup concentration, L* values decreased steadily corresponding to a darker colour in the less diluted samples. Chromaticity in red and yellow, expressed by a* and b* values, initially increased with increasing syrup concentration. Above a certain syrup concentration, however, a* and b* values decreased as syrup concentration increased. This corresponds to decreased redness and yellowness at the higher syrup concentrations. The changes in a* and b* values caused increased hue angle $h(°)_{ab}$*, and thereby a more yellowish hue with increasing syrup concentration (Fig. 2). At the highest syrup concentrations the hue remained nearly constant.

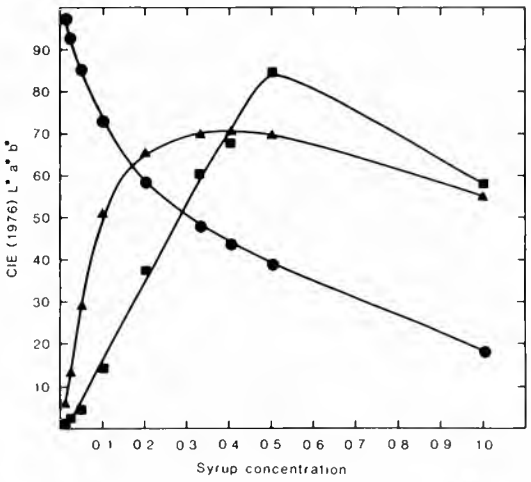

Fig. 1. CIE (1976) L* (●), a* (▲) and b*(■) values of diluted blackcurrant syrup of the Øjebyn cultivar.

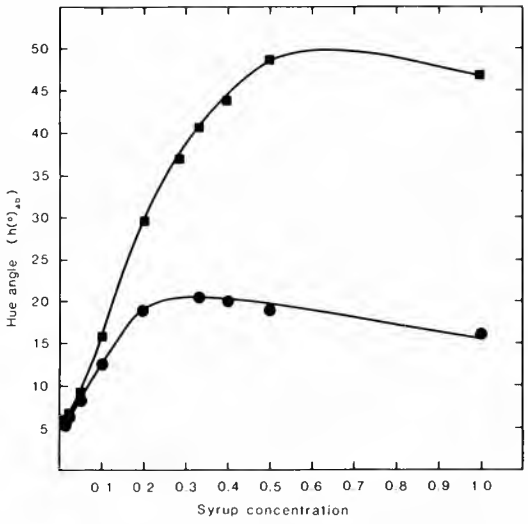

Fig. 2. Hue angles in the CIE (1976) L*a*b* (●) and in the Hunter L'a'b' (■) system of diluted blackcurrant syrup of the Øjebyn cultivar.

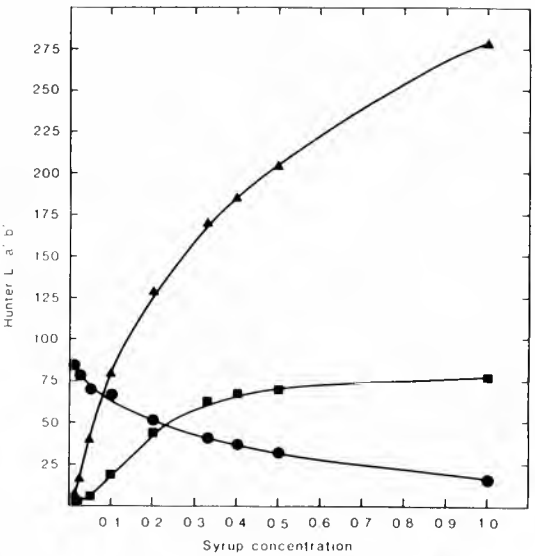

Fig. 3. Hunter L' (●), a' (▲) and b' (■) values of diluted blackcurrant syrup of the Øjebyn cultivar.

When the Hunter L'a'b' system was used for calculating colour parameters, lightness decreased and colour intensity increased with increasing syrup concentration throughout the entire range of dilution (Fig. 3). Hue angle $h(^{\circ})_{ab}$' increased initially, but also in this system decreased at the highest syrup concentrations mainly due to lack of sensibility in the b' value (Fig. 2). In the following, calculations were restricted to the Hunter L'a'b' system.

Colour analyses of juices and syrups from a total of 27 blackcurrant samples were performed. The experimental material included fruits from 7 blackcurrant cultivars harvested from the same field at various stages of ripeness. Colour parameters Hunter L'a'b' were calculated and plots of lightness (L') versus redness (a') (Fig. 4a) and of yellowness (b') versus redness (a') (Fig. 4b) are given. In the most highly pigmented juices a limited sensibility in b' was found. The addition of sucrose introduced a dilution effect in the syrups relative to the juices. This introduced more lightness and lower colour intensity to the syrups. When Hunter L', a' and b' values from a dilution series of syrup from the Øjebyn cultivar were included in the same plots, all juices and syrups examined were found to be aligned along the dilution lines of this chosen syrup. The resemblance in colour occurred regardless of blackcurrant cultivar and ripeness of fruits.

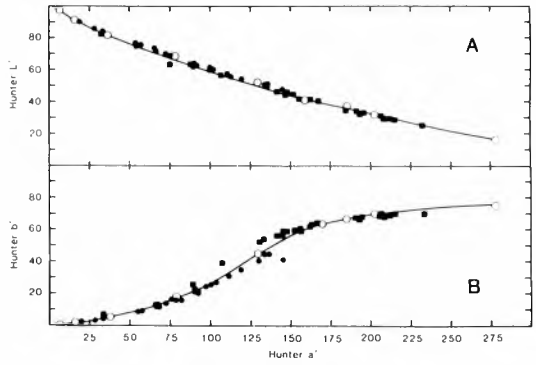

Fig. 4. Colour parameters of 27 diluted (1:10) blackcurrant syrups (●) and juices (■) originating from fruits of 7 cultivars harvested at various stages of ripeness. A: Hunter L' values versus Hunter a' values. B: Hunter b' values versus Hunter a' values. Lines and open rings represent colour parameters of syrup from the Øjebyn cultivar diluted up to eighty-fold with water.

Included in the experiments were blackcurrants with a more diverse origin regarding place of growth and year of harvest. Hunter L'a'b' colour parameters of juices and syrups from a total of 9 cultivars were nearly aligned along the dilution curve of the Øjebyn cultivar (Fig. 5), in the same way as those from fruits grown under more equal conditions.

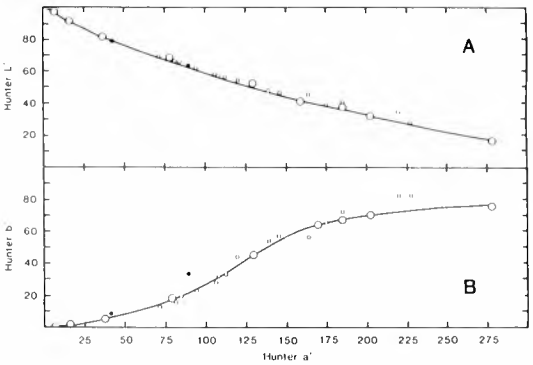

Fig. 5. Colour parameters of diluted (1:10) blackcurrant syrups (○) and juices (□) originating from fruits of 9 cultivars harvested in various years. A: Hunter L' values versus Hunter a' values. B: Hunter b' values versus Hunter a' values. Filled symbols represent the Sunderbyn cultivar while lines and large open rings represent colour parameters of syrup from the Øjebyn cultivar diluted up to eighty-fold with water.

The absorbances at 515 nm, which is the wavelength of maximum absorbance, ranged from 0.05 to 1.6 when juices from the present blackcurrant material were diluted 1:40. The correlations between the absorbances at 515 nm and the Hunter L', a' and b' values (Table 1) were significant ($P<0.001$).

Table 1. Correlation coefficients (r) and regressions for Hunter L', a' and b' values versus absorbances at 515 nm (A_{515}) in 27 blackcurrant juices. Colour parameters Hunter L'a'b' refer to 1:10 dilutions and absorbances to 1:40 dilutions of juices.

Parameter	r	Regressions
Hunter L'	-0.92	A_{515} = -0.0216 L' + 1.61
Hunter a'	0.94	A_{515} = 0.0061 a' – 0.30
Hunter b'	0.86	A_{515} = 0.0160 b' – 0.26

At the first date of harvest, about 10% of the harvested fruits of the Amos Black cultivar had the typical dark colour of ripe fruits, the remainder were green or reddish in colour. At the end of the harvesting period, most fruits were ripe and a few showed indications of overripeness. The relative amounts of anthocyanins in juice from these samples at various stages of ripening, as calculated from the HPLC chromatograms (Fig. 6), are shown in Fig. 7. The distribution among anthocyanins remained fairly constant throughout the ripening period. A similar picture of anthocyanin composition during ripening was obtained with all cultivars tested and the results are reported as the average through the ripening period (Table 2).

During the early part of the ripening period, the concentration of free anthocyanins, the absorbance at 515 nm and the chromaticity in red and yellow (Hunter a' and b') of the juices increased rapidly in the Amos Black cultivar (Fig. 8). The corresponding values during the late part of the ripening season were fairly stable.

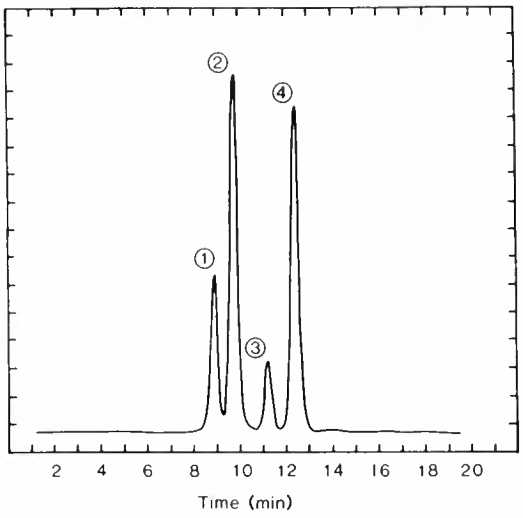

Fig. 6. HPLC-chromatogram of blackcurrant juice from the Amos Black cultivar. Peak 1: delphinidine-3-glucoside, peak 2: delphinidine-3-rutinoside, peak 3: cyanidin-3-glucoside, peak 4: cyanidine-3-rutinoside.

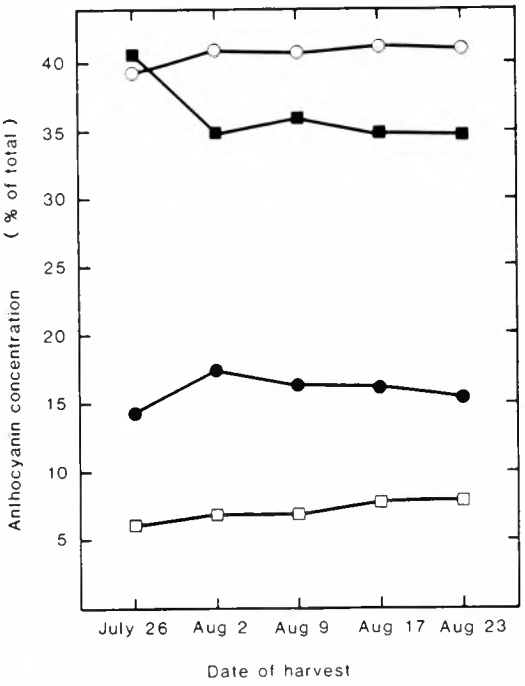

Fig. 7. Distribution of anthocyanins in juice from the Amos Black cultivar during the ripening period. Delphinidine-3-glucoside (●), delphinidine-3-rutinoside (○), cyanidine-3-glucoside (□), cyanidine-3-rutinoside (■).

Table 2. Anthocyanin concentration and relative amounts of anthocyanins in juices from several blackcurrant cultivars.

Cultivar	Place/year of harvest	Anthocyanins				
		Conc.	Relative amount, % of total			
		mg/100 g	Dl-3-gl	Dl-3-rut	Cy-3-gl	Cy-3-rut
Amos Black	NLH/82*	200	15.9	40.7	7.1	36.4
	Kise/82	250	13.6	43.1	6.5	38.8
Roodknop	NLH/82*	170	12.8	43.8	5.1	39.7
	Kise/82	190	11.4	47.4	3.9	37.4
Silvergieter	NLH/82*	330	14.5	38.6	7.2	39.8
	Kise 82	190	12.3	46.2	4.6	37.0
Øjebyn	NLH/82*	310	15.5	41.6	7.2	35.7
	Kise 82	210	14.5	47.4	4.6	37.0
Ben Lomond	Kise/80	150	10.2	52.7	3.6	34.2
	Kise/81	230	8.9	46.7	4.4	40.1
Ben Nevis	Kise/81	200	11.4	48.3	5.5	34.8
	Kise/82	240	14.6	47.4	5.8	32.2
Leepan Musta	NLH/82*	320	10.2	36.9	6.4	46.5
Svarteper	NLH/82*	430	10.9	49.1	4.3	35.7
Wallace Seedling	NLH/82*	250	17.7	37.8	7.1	37.4
Hedda	NLH/82	110	16.1	49.4	4.3	35.7
Imandra	Holt/84	130	11.3	48.0	6.2	34.6
Sunderbyn	Holt/84	150	5.2	10.3	19.9	64.6

*) Maximum pigment concentration during ripening and average relative amounts of pigments from different stages of ripening are given.

DISCUSSION

The results of the present experiments clearly demonstrate a decrease in chromaticity with increasing pigment concentration when the CIE (1976) L*a*b* colour scale is used for calculating the colour quality of blackcurrant syrups. This is in accordance with results described for anthocyanin solutions by Eagerman et al. (1973a). By using the expanded scale L'a'b' developed by Hunter (1975), chromaticity increases throughout the entire range of syrup concentration. Decreased sensibility was found at the higher syrup concentrations, especially in yellowness, however. If colour parameters are to be used for evaluating chromaticity or pigment concentration in undiluted blackcurrant juices or syrups, colour scales more expanded than the Hunter L'a'b' scale are needed (Eagerman et al. 1973b). In 1:10 dilutions of fresh blackcurrant juices and syrups, the Hunter L'a'b' scale is effective.

Previously, we found the Hunter L'a'b' parameters valuable in evaluating colour of stored blackcurrant syrups diluted four-fold with water (Skrede et al. 1983). To obtain similar results with juices, an adjustment for the higher pigment concentration of the juices has to be made through dilution prior to analysis. The resulting solutions correspond approximately with those recommended when these products are consumed. Thus, the analytical conditions are comparable to those under which the consumer evaluates the products.

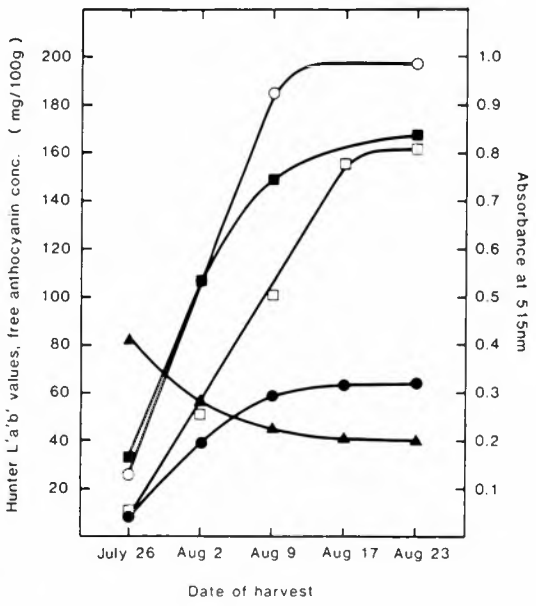

Fig. 8. Concentration of free anthocyanins (○), absorbance at 515 nm (□) and Hunter L' (▲), a' (■) and b'(●) values of blackcurrant juice from the Amos Black cultivar during the ripening period.

The colour quality of the blackcurrant juices and syrups proved to be very uniform. In all samples, the only variation found in colour quality could be ascribed to variation in pigment concentration. Variation in cultivar, in place of growth, year of harvest and in degree of ripeness of the berries, caused variations in colour quality which may be compensated for by dilution. Since the experimental fruit material represents a selection of commonly grown blackcurrant cultivars, this may apply to any blackcurrant juice and properly prepared syrup. One exception might be juice from the Sunderbyn cultivar, which seemed to have a more yellowish hue than the other juices.

The colour quality of syrups as determined by Hunter L'a'b' values was not, except for pigment concentration, significantly different from that of the juices. This implies that evalution of colour quality in blackcurrants for the syrup processing industry can be made directly in juice from fresh fruits.

The analytical conditions of the present study were not optimized for detecting the small amounts of acylated anthocyanins that have been reported to occur in some blackcurrant varieties (Jennings 1984), and calculations of anthocyanin composition were based on the four major anthocyanins (Chandler & Harper 1962). In all cultivars tested, about 60% of the anthocyanins in the ripe blackcurrants were found to be delphinidin derivatives while 40% were derivatives of cyanidin. One exception was found in the Sunderbyn cultivar, which contained about twice as much of the cyanidin derivatives as the other cultivars tested. The amount of anthocyanins derived from delphinidin was correspondingly smaller. Knowledge of this type may possibly be used in taxonomic studies of blackcurrants (Jennings 1984).

The variation in anthocyanin composition within cultivars, degree of ripeness and growing conditions in the present study, should be considered as small. This is in accordance with results reported by Nybom (1970). The variation in anthocyanin composition of the blackcurrant cultivars examined was not great enough to have any impact on the colour quality as described by the Hunter L'a'b' values or the absorbance measurements.

CONCLUSIONS

The relatively constant pigmentation found in the blackcurrants implies that fruit colour of the commonly grown blackcurrant cultivars can be standardized using instrumental colour analysis based on tristimulus values or by direct measurement of absorbance. Juices prepared by filtration or centrifugation of fruit homogenates should be diluted 1:10 for measurement of Hunter L'a'b' values or 1:40 for absorbance measurements. Hunter L' values (lightness) will then fall within the range 85 - 20, Hunter a' (redness) within 30 - 240, Hunter b' (yellowness) within 5 - 85 and the absorbance values within 0.05 - 1.6. A company utilizing blackcurrants

in its production should define its own colour standard within the range given for the parameter of choice and use this standard when evaluating fruits for processing. An even better solution would be to have values for colour quality, in accordance with the methods described, agreed upon by growers and by the industry using the fruits. The colour standards could further be worked into official standards for colour quality of blackcurrant fruits intended for industrial use.

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