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

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A laboratory soil incubation method to assess reactivity of liming materials for agriculture

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In order to establish a method for assessing reactivity of liming materials for agriculture, a soil incubation method is introduced, in which a loamy soil which should be raised from pH 5.5 to 6.0 is adopted as a standard. The lime-soil interaction consists of two processes: dissolution of the liming material particles, and diffusion into the soil. Liquid chemical methods only estimate the dissolution rate over a short period, whereas the soil itself is the most precise medium for testing the agricultural liming materials, and for the slowly reacting silicates in particular, this would be an appropriate method. To refine this laboratory incubation method four extensive experiments were carried out in Norway, using loamy and peaty soils, adding several levels of carbonate liming materials of different particle size groups. Some of the experiments were run for at least 2.5 years, and correcting for the Scandinavian winter season 2.5 years in the laboratory should correspond to 5 years in the field. The characteristics of the two soil groups were very different, but the relative differences between the liming materials remained principally the same, thereby presenting quite universal results. These investigations included limestones, Danish coral lime, dolomites and shell sand materials. The method was calibrated with Scandinavian field experiments, which also supplemented the look-up tables with data on chalks. These look-up tables have enabled STIL (The National Agriculture Inspection Service) to forward annual documents on the ENV (Effective Neutralizing Value) of liming materials on the Norwegian market. The ENV is calculated by multiplying the neutralizing value (NV, given in % CaO equivalents) by the reactivity (in per cent) during one year and five years. The ENV is stated with a fixed standard deviation of three units.

Key words: Fineness, liming materials, reactivity, rock carbonates, shell sand, soil incubation

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Bondorff (1974) described the process of dissolution of lime varieties and its velocity in mathematical terms.

The dissolution velocity ($\delta y/\delta t$) will depend upon surface area and characteristics of the lime particle, i.e. by integration

$$(K-y)^{1/3} = K^{1/3} - 1/3 \cdot k \cdot t$$

where K is a fixed mass of a liming material, y a dissolved mass after time t, and k a constant experimentally determined.

k is dependent upon k_4 (physical characteristics of a liming material) and k_1 (shape of surface). Testing different carbonates at the same small particle size, k_1 and its density will be equal, and an equation could be deduced

$$k_o = k \cdot (m \cdot c/K)^{1/3}$$

where m is the mass of the unisize particle, and c the purity of the actual liming material.

Similarly, Swartzendruber & Barber (1965) presented the equation

$(1-u)^{1/3} = 1 - c \cdot t$

writing $u = m/m_0$ for the fractional mass dissolution, and where t is time and c a constant. $(1-u)^{1/3}$ against t should be a straight line.

Besides ranking different liming materials, particle size groups within each product can also be experimentally tested and classified.

A number of scientists have been studying the effect of particle sizes upon reactivity (Albrecht 1946; Anderson 1969; Barber 1967; Bausch 1953; Beacher & Merkle 1949; Broughton et al. 1916; Bruin & Rowaan 1947; Davis 1951; DeTurk 1938; Elphick 1955; Hartwell & Damon 1919; Jokinen 1978; Kopeloff 1917; Love et al. 1960; Matzel & Ansorge 1971; Ohlsson & Torstensson 1955; Perkins 1961; Reith & Williams 1949; Salter & Schollenberger 1940; Smith et al. 1951; Stewart & Wyatt 1919; Ødelien 1961).

Morgan & Salter (1923) related solubility and dissolution rates of limestones to their other physical properties. Kriege (1929) found that the dissolution rates of limestones were inversely related to particle size, and crystallinity and harshness did not indicate the specific dissolution rate. Some dolomitic limestones (15-25% $MgCO_3$) were more unstable and dissolved more rapidly during the first half of their dissolution than pure calcites.

To quantify the importance of these characteristics chemical methods were tested. Morgan & Salter (1923) determined solubility in 2 N acetic acid, Thomas & Gross (1952) proposed to use a revised procedure of the method suggested by Barnes (1947). This revised method comprised a treatment of the liming materials with 0.1 N oxalic acid and titration with 1 N $KMnO_4$ after heating and cooling.

Gibaly & Axley (1955) developed a method for calcitic and dolomitic limestones, using boiling 0.07 N disodium ethylene-diaminetetraacetate, with a back titration with magnesium sulphate after adding an ammonia buffer solution.

Shaw & Robinson (1959) pointed out that dissolution in an ammonium chloride solution was superior to the acetic acid and oxalate methods. A 20-min digestion for calcites and dolomites was assumed to correspond with their soil reaction within a period of 3 to 12 months.

Kjær & Jensen (1974) tested the dissolution rate of 12 Danish calcium carbonate products using an acetate buffer. Jensen & Brink Pedersen (1977) introduced a pH-stat titration to rank liming materials. pH was kept constant at 2.00, 3.00, 4.00 and 5.00, combined with a fixed stirring of the sample, and a graph was drawn illustrating its reactivity profile R(t).

These liquid chemical methods would, however, only estimate the dissolution rate over a short period, whereas the lime-soil interaction also includes the slow diffusion process, and the agronomical perspective is covering months and years.

Therefore, many scientists have developed methods that include soil itself. Collins & Speer (1939) studied the decomposition of dolomite samples of different degrees of fineness, incubating them in five acid sandy soils (pH 4.50-5.60) for 65-75 days. Swartzendruber & Barber (1965) also built on incubation results to verify their mathematical approach.

Kac-Kacas (1966) developed a rapid method to determine the reactivity of limestones and dolomites. The liming materials were added to a soil suspension of an acid mineral soil and a KCl solution, and the elevated CO_2 production was measured volumetrically.

This paper is a compilation of Scandinavian research work concerning the effecti-

veness of different agricultural carbonate liming materials, as a function of particle size and incubation time. Most of the soil incubation experiments were run in laboratory, but some of the tests have also been verified and calibrated in field trials.

Luktvasslimo (1981) carried out his laboratory experiments with limestones, Jørgensen (Erstad) (1982) with limestones, dolomites and a few shell sands, Erstad (1986) with Danish coral lime, and Olsvik (1984) with shell sands of calcified seaweeds.

These results have been compared and complemented with field experiments by Jensen (1939) on Danish coral lime and chalk, Ohlsson & Torstensson (1955) on Swedish chalk and Precambrian and Silurian limestones, and, partly, Myhr (1980) on shell sand of bivalves, snails and worms.

Aarseth (1982) and Grøsfjeld (1990) have been mapping shell sand resources and testing their qualities in some areas on the west coast of Norway.

Sve (1989) and Sve et al. (1990) estimated the agricultural value of shell sand materials of bivalves, snails and worms from the west coast of Norway.

MATERIALS AND METHODS

Several soils were used in the incubation method, although simultaneously belonging to one of two main groups, loamy soils and ombrogenous peats.

The mineral soils used were mechanically analysed by the pipette method, after ignition of the organic material with H_2O_2, according to Elonen (1971). The results are presented in Table 1, together with a soil classification according to the Soil Survey of England and Wales (Hodgson 1974).

Table 1. Mechanical analyses (Elonen 1971) of the mineral material < 2 mm of the mineral soils (M) used in the laboratory incubation experiments, and their soil classification (Hodgson 1974)

Experiment - Soil		% sand	% silt	% clay	Soil class
Luktvasslimo (1981)	M	37	41	22	Clay loam
Jørgensen (1982)	M	37	41	22	Clay loam
Olsvik (1984)	M	47	39	14	Sandy silt loam

A set of parameters was chemically analysed.

The soil density values were obtained following the procedure described by Bondorff (1950). Total N was detected by the Kjeldahl method.

Loss of soil mass was decided by igniting the dried soil samples (378 K) at 823 K for 12 h, and organic matter found by subtracting the hygroscopic water kept in the clay minerals of the soils. Finally organic C was determined by dividing the value for organic matter by 1.724.

pH(H_2O) was achieved from the ratio soil:distilled water 1:2.5 v/v. P and K were extracted in ammonium lactate as described by Egner et al. (1960). The extracted P was analysed by means of the molybdenum blue method, as demonstrated by Murphy & Riley (1962), and the extracted K by means of flame photometer.

Exchangeable cations H, Ca, Mg, K and Na were extracted using the NH_4OAc method pH 7.00, as described by Thomas (1982). The acidity was found by back titration

to pH 7.00, and Ca, Mg, K and Na by atomic absorption spectrometry.

The results together with the calculated base saturation are listed in Table 2.

Table 2. Chemical analyses of the mineral (M) and peaty soils (O) used in the laboratory incubation experiments

Experiment - Soil		Soil density kg*dm^{-3}	Tot. N %	Org. C %	pH(H_2O)	P-AL mg*100g^{-1}	K-AL mg*100g^{-1}
Luktvasslimo (1981)	M	-	0.24	2.5	4.8	6.8	5.2
Luktvasslimo (1981)	O	-	2.25	47.7	3.8	5.7	12.0
Jørgensen (1982)	M	1.12	0.30	3.1	4.9	11.0	12.0
Jørgensen (1982)	O1	0.65	2.27	42.3	3.9	6.6	21.0
Jørgensen (1982)	O2	0.65	2.31	38.0	4.6	8.5	16.0
Olsvik (1984)	M	-	0.30	3.2	4.8	8.8	14.0
Olsvik (1984) & Erstad (1986)	O	-	0.92	48.6	3.7	3.6	93.0

Experiment-Soil		EXCHANGEABLE CATIONS Ca^{2+}	Mg^{2+}	K^+	Na^+	H^+ meq*100g^{-1}	Base saturation
Luktvasslimo (1981)	M	0.89	0.07	0.03	0.02	2.6	28.0
Luktvasslimo (1981)	O	9.18	0.65	0.33	0.13	81.0	11.3
Jørgensen (1982)	M	4.53	0.50	0.30	0.078	13.0	29.4
Jørgensen (1982)	O1	39.67	2.98	0.63	0.37	72.0	37.7
Jørgensen (1982)	O2	82.58	4.04	0.51	0.37	41.0	68.1
Olsvik (1984)	M	4.52	0.37	0.38	0.13	10.6	33.6
Olsvik (1984) & Erstad (1986)	O	7.32	4.16	3.12	2.95	120.5	12.7

To determine the reactivitiy of particle size groups of different carbonate liming materials, increments were supplied to each soil sample, each step at two replications. Soil samples and liming materials were thoroughly mixed in a tray.

Soils with a soil density of $\geq$1.0 g*cm^{-3} (mineral soils) were weighed into 1.5-l plastic pots at a mass of 1000 g dry matter, and at incubation water was added until 70% of field capacity at free drainage was reached. Soils with a lower density were packed into 1.0-l pots (soils rich in organic matter) in an attempt to achieve a normal soil compaction, and the adjusted water content represented 60% of field capacity at free drainage.

The pots were kept at room temperature, i.e. approximately 293-298 K.

The pots were covered with a parafilm during the incubation period of until 2.5 years. Samples were taken out 1, 3, 6, 12, 24 and 48 (or 52) weeks and 1.5, 2.0 and 2.5 years after liming, and the parafilm was removed approximately one week before sampling to make the soils easier to handle. From each pot 10 ml of soil was collected with a small auger, 8-10 pierce samples formed the final samples and these were transferred to small 50-ml cups and closed. Afterwards, the soils in the pots were mixed, again moistened and covered with the parafilm. The total masses of the pots were reduced each time by 10 g to compensate for the removal of soil samples.

To each soil sample in the cups was added 25 ml distilled water (soil:water ratio 1:2.5 v/v), the samples were thoroughly shaken for five minutes and then left to stand overnight. Next day the samples were briefly shaken again, and the pH measured after 10-15 min.

The equipment used was a METROHM E 632 digital pH meter with separate electrodes. The electrodes were always immersed in the supernatant of the cups, and after rapid stirring the highest pH value was read during sedimentation of coarse soil particles. This was done to avoid the suspension effect.

Concerning the dissolution and subsequent diffusion rate of lime into the soil, it is stated that the crystal size is a decisive factor within each mineral.

The rock carbonates were divided into seven categories relevant to Norwegian conditions:

Category I. Non crystalline and finely crystalline (< 1 mm) limestone
Category II. Medium crystalline (1-5 mm) limestone
Category III. Coarsely crystalline (5-30 mm) limestone
Category IV. Finely crystalline (< 1 mm) dolomite (marble dolomite)
Category V. Danish coral lime
Category VI. Swedish chalk (Skåne)
Category VII. Danish chalk

Bathurst (1975) gave a precise description of mineralogy and crystal structures of marine organisms.

We divided the shell sand materials into four classes according to origin and degree of weathering:

Class I. Type Fureneset:
Highly weathered, thin and fragile bivalves (*Ensis ensis*, *Mya arenaria*, *Chlamys islandica*, *Chlamys varia*, *Pecten maximus*), sea urchins, snails, cochleas and worms.
This type of shell sand is typical for the coastal area North Rogaland - Nordfjord, and is also found in smaller quantities at Sørlandet and north of Stad. This class could even include freshwater marl consisting of bivalves and snails (Hadeland, Mjøsa). Sometimes, however, this marl can consist of a foraminiferic mud (Senja in Troms) comparable with highly reactive chalk.

Class II. Type Fræna:
Highly weathered, dense bivalves (*Mytilus edulis*, *Modiolus modiolus*, *Arctica islandica*, *Ostrea edulis*, *Cerastoderma edule*), coarse species of snails (genera *Littorina*, *Neptunea*, *Buccinum*, *Aporrhais*, *Nassa*, *Sipho*) and worms.
This type is sturdy, but still greyish white in colour as a result of weathering. It is frequently found along most of the coast, particularly in waste banks at Møre and north of Andenes in Vesterålen. It can also be found outside Jæren, where the shell sand of class I is already exploited.

Class III. Type Nordfjord-Stad:
The same species as Class II, but sparsely weathered, with a bluish colour and a preserved nacreous structure (periostracum). This horny outer layer hampers the dissolution process.
This type is of relatively fresh depositions, and is frequently observed on the coast of Nordfjord and Møre.

Class IV. Type Troms:

Coral and seaweed (*Lithotamnion spp.*) shell sand.

This type is mainly related to high-boreal sea water in North Norway, well protected in smooth, shallow fjords, found particularly in Troms, but also further south to North Trøndelag.

This material is a high Mg calcite (2-4% Mg), exhibiting a very high porosity.

RESULTS

The replications in all experiments showed very similar results, and the methods were highly reproducible.

Almost any liming material would demonstrate a smooth sigmoid particle size distribution graph.

Olsvik (1984) fixed his regression analyses upon the mass of material coarser than 1 mm of his shell sand materials of class IV. He observed very close correlations between this parameter and pH of the soils, and the shell sands were slightly more reactive than rock carbonates of the same purity.

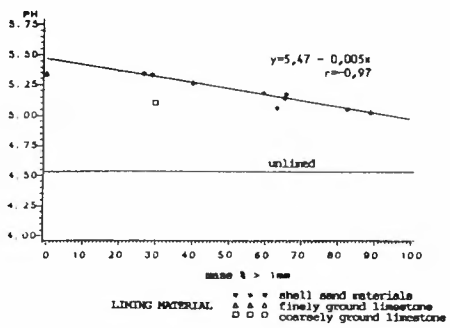

Fig. 1. Relationship between mass (%) of shell sand materials coarser than 1 mm and average pH values after 48 weeks of incubation in a peaty soil, including as a reference one finely ground and one coarsely ground limestone sample (Olsvik 1984)

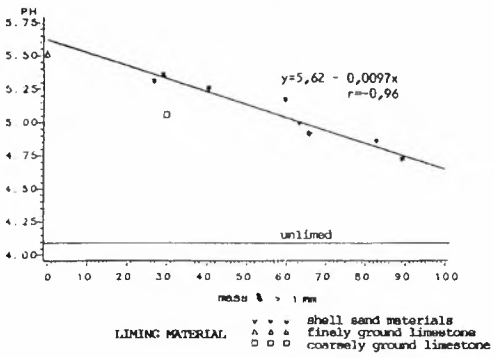

Fig. 2. Relationship between mass (%) of shell sand materials coarser than 1 mm and average pH values after 48 weeks of incubation in a sandy silt loam, including as a reference one finely ground and one coarsely ground limestone sample (Olsvik 1984)

Fig. 1. illustrates this relationship for a peaty soil, fig. 2. for the standard sandy silt loam.

The characteristics of the soils were very different, but the relative differences

between the liming materials remained the same.

Acid soils and soils with strong buffering systems over a wide pH range, i.e. high in organic matter, would increase the dissolution rates for dolomites and coarser particles of all liming materials, and vice versa.

Besides these reservations, the ranking of different liming materials is quite independent of soil type.

Choosing the loam (sandy silt loam / clay loam) as a standard reference soil which should be limed from pH(H_2O) 5.5 to 6.0, we were able to establish the look-up tables as presented in Table 3 (rock carbonates) and Table 4 (shell sand materials).

Table 3. Reactivity of increasing particle sizes of different rock carbonates during one year and five years, as a percentage of the completely dissolved particles <0.2 mm

Category I. Non-crystalline and finely crystalline (< 1 mm) limestone

	Particle size group (mm)							
Period of soil reaction	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	95	70	50	35	20	15	10	5
5 years	100	100	100	90	70	60	50	30

Category II. Medium crystalline (1-5 mm) limestone

	Particle size group (mm)							
Period of soil reaction	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	95	65	45	30	20	15	10	5
5 years	100	100	95	85	60	50	40	25

Category III. Coarsely crystalline (5-30 mm) limestone

	Particle size group (mm)							
Period of soil reaction	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	95	60	40	25	15	10	5	5
5 years	100	100	90	75	55	45	35	20

Category IV. Finely crystalline (< 1 mm) dolomite (marble dolomite)

	Particle size group (mm)							
Period of soil reaction	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	50	30	20	15	10	10	5	5
5 years	90	75	65	55	45	35	25	15

Category V. Danish coral lime

	Particle size group (mm)							
Period of soil reaction	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	100	100	85	75	60	40	20	10
5 years	100	100	100	100	100	80	50	30

Category VI. Swedish chalk (Skåne)

Period of soil reaction	Particle size group (mm) 0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	95	80	70	50	30	20	15	15
5 years	100	100	100	100	100	90	80	70

Category VII. Danish chalk

Period of soil reaction	Particle size group (mm) 0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	100	100	100	100	90	75	60	50
5 years	100	100	100	100	100	90	80	70

Table 4. Reactivity of particle sizes of shell sands of different qualities during one year and five years, as a percentage of the completely dissolved particles <0.2 mm

Class I. Highly weathered, thin and fragile bivalves (*Ensis ensis*, *Mya arenaria*, *Chlamys islandica*, *Chlamys varia*, *Pecten maximus*), sea urchins, snails, cochleas and worms (type Fureneset)

Period of soil reaction	Particle size group (mm) 0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	100	100	85	70	50	30	20	10
5 years	100	100	100	90	75	50	40	20

Class II. Highly weathered, dense bivalves, (*Mytilus edilus*, *Modiolus modiolus*, *Arctica islandica*, *Ostrea edulis*, *Cerastoderma edule*), coarse species of snails and worms (type Fræna)

Period of soil reaction	Particle size group (mm) 0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	100	95	80	60	40	20	15	5
5 years	100	100	100	90	70	45	35	15

Class III. The same species as Class II, but sparsely weathered, with a bluish colour and a preserved nacreous structure (periostracum) (type Nordfjord-Stad)

Period of soil reaction	Particle size group (mm) 0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	90	70	60	40	20	10	5	5
5 years	100	95	85	75	50	40	25	10

Class IV. Coral and seaweed (*Lithotamnion spp.*) shell sand (type Troms)

Period of soil reaction	Particle size group (mm) 0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.5	1.5-2.0	2.0-3.0	3.0-5.0
1 year	100	100	90	80	70	60	50	25
5 years	100	100	100	100	100	80	65	50

Keeping the pots at a constant room temperature during incubation was assumed to speed up the soil processes. Correcting for the Scandinavian winter season 2.5 years in the laboratory should correspond to five years in the field.

Any commercial product would obtain its calculated reactivity by summarizing the contribution of each particle size group.

DISCUSSION

Excluding the Scandinavian winter, the lime-soil reactions are presumed to run a little faster due to higher annual and seasonal temperatures.

Based on Norwegian Standard NS 2885 and these look-up tables, STIL (The National Agriculture Inspection Service) since 1987 has been certifying and annually revising a document of ENV (Effective Neutralizing Value) of liming materials on the Norwegian market.

The reactivity of any liming material is predicted by summarizing the contribution of each particle size group over a period of one year and five years. The ENV is calculated by multiplying the neutralizing value (NV, given in % CaO equivalents) by the two reactivity figures (in per cent). The ENV is stated with a fixed standard deviation of three units.

The types of liming materials estimated obviously reflect the demands of Norwegian agriculture.

Very coarsely crystalline (>30 mm) limestones and non crystalline (micritic) calcitic dolomites (50-90% dolomite) were actually not in commercial question, although geologically occurring in Norway.

Naturally quarried dolomitic limestones (10-50% dolomite) were calculated as a mixture of the two categories.

We were aware that chalk is even more diverse than presented in categories VI and VII, but these two look-up tables were adequate for the limited import of chalk from Denmark to Norway.

Danish studies were, however, much more detailed with respect to chalk products. Tind-Christensen (1951) observed that during a period of five years (1943-48) there was the same effectiveness of finely ground chalk, crude chalk as well as shell sand (class I) applied in the same amount of carbonate equivalents. The field experiments were run on mineral soils, varying from heavy clays to poor sandy soils.
In a similar research project (1948-52) Kofoed & Olesen (1952) confirmed most of these results, but for one type of chalk (Hillerslev) the dissolution rate of the crude material was reduced compared with that of the finely ground product.

There was a lack of data for British magnesian limestone (Permian), but to assess its ENV, STIL is temporarily adopting the table of Danish coral lime.

This laboratory soil incubation method could also be used for any other liming material, preferentially adopting a standard reference soil. Soil is the most precise medium for testing agricultural liming materials, and for the slowly reacting silicates in particular this would be an appropriate method.

Concerning the fragile, lumpy chalks highly exposed to weathering by rain, frost, drying and soil tillage, this method could, however, be supplemented by field experiments, as is already being done in Norwegian assessments.

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Evaluation of technique and DTPA and EDTA extraction methods for determining the availability of soil Zn, Mn, Fe and Cu

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In a study using 109 barley and soil samples collected from the fields in south-eastern Norway it was found that the inductively coupled plasma (ICP) spectrometry technique can be applied just as effectively as the atomic absorption spectrophotometry (AAS) method to soil and plant analysis for Zn, Mn, Fe and Cu. DTPA and ammonium acetate-EDTA extraction methods can be effectively used for determining the availability of soil Zn and Mn provided that the influence of soil alkalinity is properly taken into consideration. However, the results obtained using the methods have not proved to be encouraging for analysis of soil Fe and Cu.

Key words: Ammonium acetate-EDTA, atomic absorption, barley, calcareous soil, Cu, DTPA, Fe, inductively coupled plasma, Mn, Zn.

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The most usual means of determining metals is by the atomic absorption spectrophotometry method (AAS). Minuno (1987a,b) applied the inductively coupled plasma (ICP) spectrometry technique to soil analysis and obtained reasonable results for copper, manganese, zinc, magnesium, phosphorous and calcium. Use of the ICP technique has the advantages of high detectability, wide dynamic range, minimal interelement effects. good analytical precision and capability of simultaneous multielement determination. Although nowadays wherever the conditions are suitable the ICP technique has been widely applied in the analysis of various materials including plant and soil, results concerning the effectiveness of the ICP technique in comparison with that of AAS in soil and plant analysis are not often reported.

For simultaneous determination of the four metals, Zn, Mn, Fe and Cu, DTPA and EDTA have been generally accepted as effective extraction methods for various types of soils in many countries (Lindsay & Cox 1985). The DTPA extraction method was originally developed for calcareous soils by Lindsay et al. (1967) and Lindsay & Norvell (1969) and further modified by several investigators (Lindsay & Norvell 1978; Soltanpour & Schwab 1977; Baker & Amucher 1981). The use of EDTA in combination with other chemicals has been found to be comparable with that of DTPA (Haynes 1983; Lakanen &

Ervio 1971; Leclaire et al. 1984 Gupta & Mittal 1981; Ponnamperuma et al. 1981; Lindsay & Cox 1985; Levesque & Mathur 1988). The quantities extracted by the two chelating reagents are usually considered to represent the labile pool of the four micronutrients. However, results from previous reports concerning the two extraction methods for simultaneous analysis of the four metals are not completely satisfactory.

The aim of the present study was to compare the measurements obtained by ICP with those by AAS and to test the effectiveness of the DTPA and AA-EDTA extraction methods for estimating the availability of soil Zn, Mn, Fe and Cu.

MATERIALS AND METHODS

Plant and soil samples

Barley plants and corresponding soil samples were collected from 109 farm fields in different regions of southeastern Norway during the plant growing season between stages 10.0 and 10.1, in accordance with the Feekes' scale (Large, 1954). The sampling sites were chosen so that the samples would contain broad variations in soil pH, soil texture and in the level of concerned micronutrients.

Depending on the plant growth status, the sampling area of each barley sample varied from 0.094 to 0.25 m^2. The plants were cut to a height of about 4 cm above the ground, with precautions taken to avoid contamination. The soil samples vere taken from the 20 cm surface horizon.

The plant samples were dried at 70°C for 48h and milled to pass through a 0.8 mm sieve. The soil samples were air-dried and passed through a 2 mm sieve. For analysing the total element concentration and the soil organic cabon content, 10 g of each soil sample was further ground into fine particles to obtain homogeneity.

Determination of the element concentration

The method used for digesting the plant samples for the total element concentration analysis followed that by Wu et al. (1991). The soil samples were extracted with the DTPA-Tea-$CaCl_2$ and ammonium acetate-EDTA solutions in accordance with the procedures set out by Lindsay & Norvell (1978) and Levesque & Mathur (1988), respectively. The prepared solutions were measured by both ICP and AAS.

Determination of soil properties

The soil-water volume ratio used for soil pH determination is 1:2.5. The ammonium acetate (pH 7) method was applied for determining the soil potential cation exchange capacity. Soil organic carbon was determined by means of a carbon determinator, and soil mechanical analysis was carried out using the hydrometer method. Soil titratable alkalinity, defined as the milliequivalents of an acid required to acidify a soil to a specific pH (pH 5), was obtained using the method set out by Nelson et al. (1959).

Relevant terms and units

For convenience, relevant terms are defined as follows:

ZnPi, MnPi, FePi and CuPi = relevant element concentration in barley plants determined by ICP (mg/kg)

ZnPa, MnPi, FePi and CuPi = relevant element concentration in barley plants determinded by AAS (mg/kg)

ZnDi, MnDi, FeDi and CuDi = DTPA-extractable concentration by ICP (mg/l)

ZnDa, MnDa, FeDa and CuDa = DTPA-extractable concentration by AAS (mg/l)

ZnAi, MnAi, FeAi and CuAi = AA-EDTA-extractable concentration by ICP (mg/l)

ZnAa, MnAa, FeAa and CuAa = AA-EDTA-extractable concentration by AAS (mg/l)

TA = soil "titratable alkalinity" (m.e./100 ml)
CEC = soil cation exchange capacity determined using the ammonium acetate (pH 7.0) method (m.e./100 ml)
BC = soil exchangeable base cation concentration (m.e./100 ml)
C% = soil organic carbon content in percentage
Clay% = percentage of soil clay content

Statistical analyses
Aanlyses of correlation and regression and t-tests were carried out using the Statistical Analysis System SAS programs and the diagrams were drawn using Harvard Graphics HG on an IBM computer.

RESULTS AND DISCUSSIONS

Comparison between ICP and AAS
The means and range of variations in the measurements by the different methods are presented in Table 1. The t-test at the $p > 0.1$ level indicates that there is no significant difference between the results obtained by the ICP method and those obtained by the AAS technique.

The linear relationship of the results obtained from the two methods is illustrated in Figs. 1, 2 and 3 for the three types of solutions respectively. With the exception of Mn in the soil extracts, the element concentrations given by ICP are closely correlated with those given by AAS with correlation coefficient (r) values higher than 0.95.

Although the highest values for manganese concentration are excluded, the difference in values obtained by the two methods is still apparent in Figs. 2b and 3b for DTPA and AA-EDTA extracts. The deviation occurs particularly within the high concentration range and, in general ICP gives lower values than AAS. Repeated trials indicate that the Al ions interfere with Mn determination by depressing its absorbancy. It is important to use a clear solution when determining Mn values, as the occurrence of tiny soil colloids increases the Al level of the test solution and thus causes negative effects on the measurements. Proper

calibration is required when applying the ICP technique to soils with high levels of Mn.

Table 1. Descriptive statistic for soil properties and element concentrations in plant and soil samples (number of samples = 109)

Variable*	Mean	Std. Dev.	Minimum	Maximum	P value
ZnPa	23.65	8.78	7.00	55.50	
ZnPi	24.58	9.28	7.50	55.00	> 0.1
MnPa	44.28	48.72	8.50	519.95	
MnPi	44.87	58.58	7.50	627.00	> 0.1
FePa	99.68	63.13	55.00	445.00	
FePi	90.69	64.35	47.50	464.00	> 0.1
CuPa	10.46	3.11	6.50	27.00	
CuPi	9.69	3.31	5.00	28.50	> 0.1
ZnAa	1.69	2.09	0.18	17.31	
ZnAi	1.63	2.27	0.10	19.70	> 0.1
ZnDa	1.42	1.81	0.14	14.83	
ZnDi	1.35	1.91	0.10	15.80	> 0.1
MnAa	100.37	108.47	7.88	989.38#	
MnAi	117.06	102.86	7.60	553.40	> 0.1
MnDa	27.99	31.16	3.74	284.78	
MnDi	30.74	26.24	2.50	133.50	> 0.1
FeAa	130.01	64.14	37.00	320.00	
FeAi	130.12	62.83	36.40	311.00	> 0.1
FeDa	83.55	48.23	13.00	271.00	
FeDi	83.19	48.28	13.00	280.60	> 0.1
CuAa	2.52	1.49	0.70	8.40	
CuAi	2.50	1.47	0.70	8.20	> 0.1
CuDa	1.60	0.97	0.57	8.00	
CuDi	1.54	0.76	0.60	8.10	> 0.1
pH	6.35	0.59	4.55	7.89	
C%	2.89	0.88	1.21	6.01	
BC	18.49	8.59	3.13	50.61	
TA	2.05	1.59	0.02	11.13	
CEC	27.10	7.62	11.25	54.57	
Clay%	10.31	5.51	4.00	36.40	

* The definitions of the variables and their units are given in the section on Materials and Methods.

\# The highest values of Mn concentration in soil extracts are excluded in the t-test

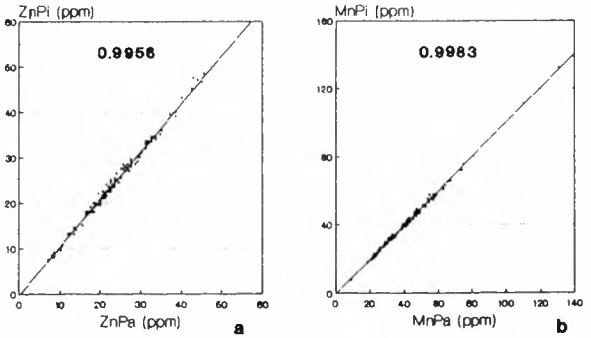
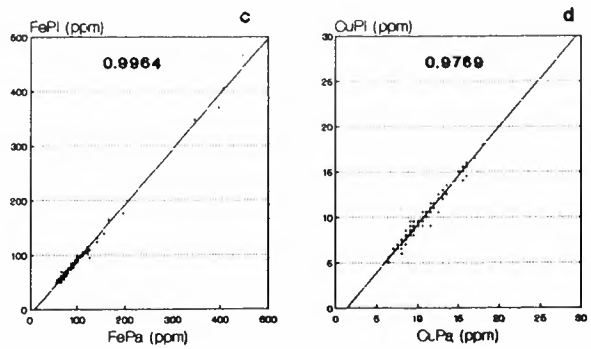


Fig. 1. Relationship between element concentrations in plants determined by ICP and by AAS: (a) Zn, (b) Mn, (c) Fe and (d) Cu



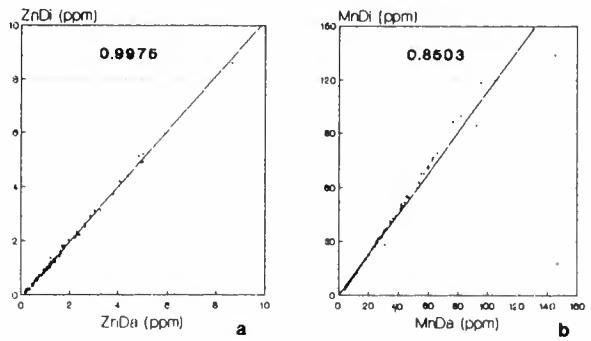
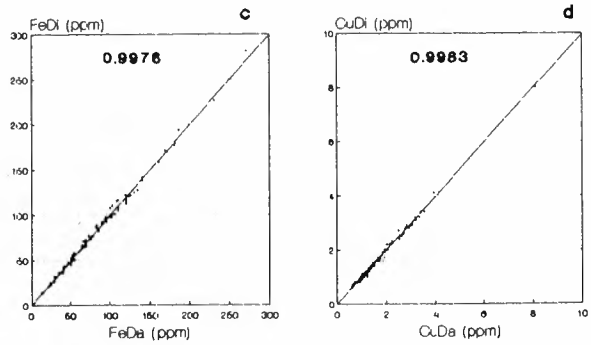


Fig. 2. Relationship between element concentrations in DTPA extracts determined by ICP and by AAS: (a) Zn, (b) Mn, (c) Fe and (d) Cu



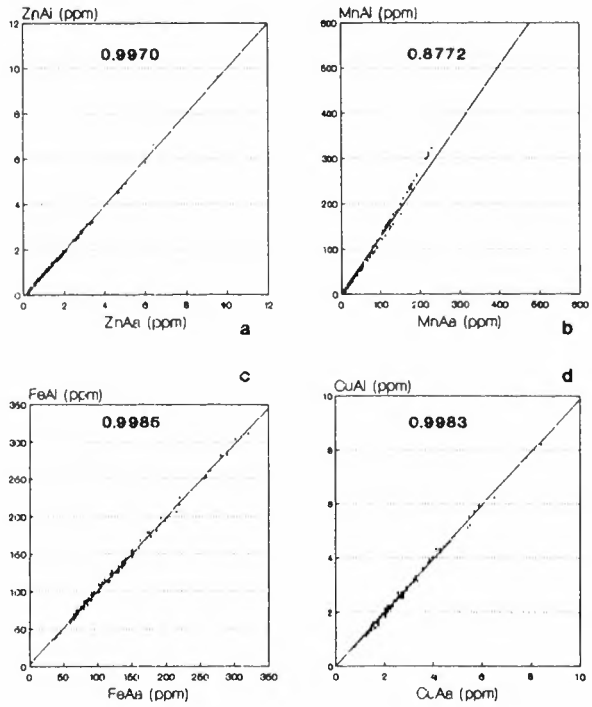


Fig. 3. Relationship between element concentrations in AA-EDTA extracts determined by ICP and by AAS: (a) Zn, (b) Mn, (c) Fe and (d) Cu

Correlation between element concentration in plant and soil

The results of the correlation analysis are given in Table 2. For a better comparison, two types of regression models are applied. Accounted for by the r values, the degree of suitability of the two extraction methods, DTPA and AA-EDTA, for relevant elements follows the order: Zn > Mn > > Fe/Cu. For Zn, and particularly for Mn, DTPA appears to be a better extractant than AA-EDTA. It can also be observed in Table 2 that the logarithmic model gives much higher r values than the commonly used linear model for Zn and Mn, regardless of the extraction method.

Table 2. Coefficients (r) of correlation between element concentration in plants and soils (number of samples = 109)

Extraction method	DTPA		AA-EDTA	
Regression model	Linear	Logarithmic	Linear	Logarithmic
Zn	0.4980	0.7690	0.4591	0.7441
Mn	0.4585	0.5296	0.2357	0.3040
Fe	-0.1906	-0.1832	-0.1999	-0.1899
Cu	0.0676	0.1020	0.0924	0.1613

The correlation levels are extremely low for Fe and Cu and the Fe concentration in the plants is even negatively related to Fe extracted from the soil by DTPA and AA-EDTA. Changes in the regression models do not make any significant differences in correlation levels.

Low levels of correlation between plant Fe and Cu concentrations and their soil DTPA- and AA-EDTA-extractable levels have been reported by many workers (Lindsay & Cox 1985). The present study definitely does not support the use of the two extraction methods for Fe and Cu.

Effects of soil properties

The coefficients of correlation between element concentrations in plants and some soil characteristics are listed in Table 3. The element concentrations in plants are virtually independent of most of the soil properties except for soil alkalinity factors. The relationship between the element status in plants and soil pH is further shown in Fig. 4.

Tabel 3. The coefficients (r) of correlation between element concentrations in plants and soil properties (number of samples = 109, by ICP)*

	pH	C%	BC	TA	CEC	Clay%
ZnP	-0.49	0.13	-0.05	-0.31	0.14	0.03
MnP	-0.45	-0.08	-0.25	-0.24	-0.12	-0.08
FeP	0.45	-0.08	0.17	0.43	-0.03	-0.01
CuP	0.13	0.12	0.10	0.14	0.09	0.06
Log (ZnP)	-0.54	0.16	0.06	-0.37	0.16	0.07
Log (MnP)	-0.72	0.02	-0.36	-0.48	-0.06	-0.01
Log (FeP)	0.42	-0.05	0.16	0.40	-0.02	0.04
Log (CuP)	0.07	0.11	0.07	0.0	0.08	0.10

* The definitions of the variables are given in the section on Materials and Methods

As seen from the linear plots and r values in Figs. 4a and 4b. there is a clear tendency toward a decrease in Zn and Mn concentrations in the plants with increasing soil pH. In comparison, the negtive effect of soil pH on the level of Mn in plants appears to be much stronger, suggesting that the uptake of Mn by barley plants is more subject to soil pH than that of Zn. If comparing the r values in Fig. 4b with those in Table 2 for Mn, it can be seen that Log(MnP) is much more closely correlated with soil pH than with Log(MnA) and Log(MnD). This further suggests that the soil Mn availability is more dependent on the soil alkalinity condition than on soil native Mn level.

The slightly positive effect of soil pH on Fe concentration in plants (Fig. 4c) is not easy to understand as, in general, the solubility of soil Fe decreases with increasing soil pH. This phenomenon, however, partly explains why the Fe concentration in plants is negatively related to soil DTPA- and AA-EDTA-extractable Fe in the present investigation.

The Cu concentration in plants does not appear to be affected by soil pH (Fig. 4d),

although it is generally believed that the solubility of Cu is also negatively related to soil pH as is that of the other three metals.

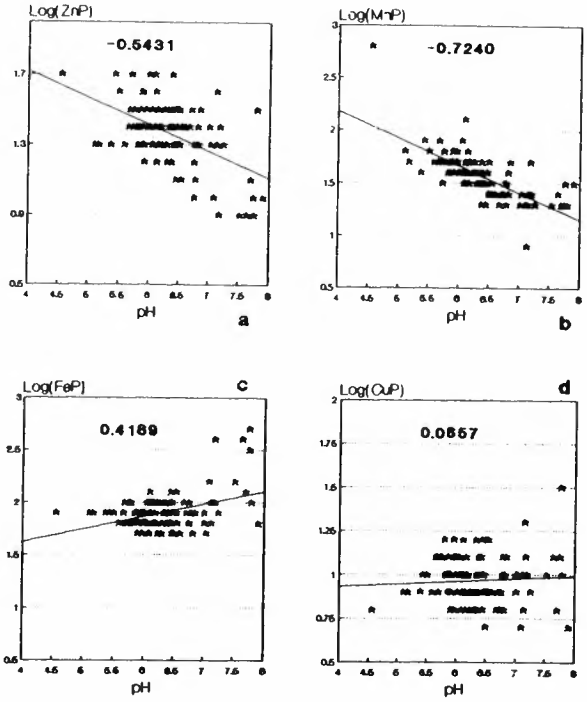


Fig. 4. Relationship between element concentrations in plants and soil pH: (a) Zn, (b) Mn, (c) Fe and (d) Cu

Multiple regression equations

The determined regression equations for prediction of Zn and Mn concentrations in barley plants are given in Table 4. The data used for regression analysis are those obtained by ICP. In comparison, the use of pH always results in the highest r values, equations including other soil properties are thus not presented. Attempts to establish proper regression models for prediction of Fe and Cu have also been made using the stepwise procedure. With respect to the correlation levels, however, no essential improvement has been made in all possible combinations of the extractable Fe and Cu with the investigated soil characteristics.

As can be seen in Table 4, high levels of correlation are obtained for Zn and Mn after the inclusion of soil pH in the logarithmic model. Based on the results from the present study, the conclusion can be drawn that the DTPA and AA-EDTA extraction methods can be effectively applied for prediction of the availability of soil Zn and Mn provided that the effect of soil alkalinity is properly taken into consideration.

Table 4. Regression equations for predicting the availability of soil Zn and Mn (number of samples = 109, by ICP)*

Log(ZnP)	=	1.9868	+	.2505Log(ZnD)	-	.0948(pH)	R	=	0.8267
Log(ZnP)	=	2.1104	+	.2817Log(ZnA)	-	.1190(pH)	R	=	0.8425
Log(MnP)	=	2.8964	+	.1127Log(MnD)	-	.2192(pH)	R	=	0.7497
Log(MnP)	=	3.0614	+	.0790Log(MnA)	-	.2473(pH)	R	=	0.7386

* The definitions of the variables and their units are given in the section on Materials and Methods

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Assessment of simple drought indices on the growth of timothy grass *(Phleum pratense)*

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Trials were carried out at Kise (60°47'N, 10°49'E, 135 m a.s.l.) in order to investigate the relationship between dry matter production of timothy *(Phleum pratense)* and values of simple drought indices (relative evapotranspiration and the number of stress days). Treatments which received either natural precipitation only or were sheltered from precipitation during various periods were compared with an irrigated control treatment. At a high level of N-input, there was a linear relationship between relative yield and calculated values of both relative evapotranspiration and the number of stress days. Poorer correlation was found with low nitrogen input, possibly because of confounding with leaching. Results were used in an extrapolation for three levels of available soil moisture capacity, using local weather data covering 28 years. These calculations suggested that non-irrigated grass yields are on average 22% lower than the irrigated potential on drought prone soil, but only 7% lower on drought resistant soil. The frequency distribution of relative yields is also presented.

Key words: Available moisture, DM production, drought stress, evapotranspiration, *Phleum pratense*, root zone capacity.

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Models which relate crop growth to weather conditions are increasingly being used in the extrapolation of research findings to new areas, in the mapping of production potential and in teaching. Such a model has been developed to describe timothy ley production in Sweden (Kornher & Torssell 1983; Torssell & Kornher 1983). This model estimates the effects of plant ageing, incoming radiation, air temperature and soil moisture on crop growth rate.

Trials have been carried out in various parts of Norway during the period 1989-91 in order to assess the overall applicability of the model under varying climatic conditions. This report presents the results of an investigation conducted at Kise to test the hypothesis that the effect of soil moisture shortage on relative grass growth rates may be described as a linear function of the relative evapotranspiration rate.

METHODS AND MATERIALS

Experimental design

Two varieties of timothy (cv. Grindstad from S.E. Norway and cv. Engmo from N. Norway) were undersown in barley in alternate 2 x 45 m strips in 1988. The resulting

sward was then used for a trial which, in 1989 and 1990, included the following treatments:

Main plots: (8 x 4 m)
A: Irrigated whenever soil moisture deficit exceeded 25 mm
B: Sheltered from rainfall for 4 weeks from start of growth
C: Sheltered from rainfall for 3-4 weeks prior to first cut
D: Sheltered from rainfall for 4 weeks after the first cut
E: Sheltered from rainfall for 4 weeks prior to the second cut

Subplots: (4 x 4 m)
N1: 140 kg N/ha
N2: 280 kg N/ha

There were four replicate blocks. One strip of each variety ran through each replicate, giving a smallest plot size of 4 x 2 m. Grass from 6 m^2 was weighed at each harvest.

Treatment A was irrigated using low pressure equipment mounted on 4 x 4 m shielded wagons. Treatments B to E were not irrigated, except in some cases under dry conditions when a small irrigation (5 mm) was given in order to dissolve fertilizer. Sheltering was achieved by means of 4 x 5 m mobile, open-sided, polythene tunnels mounted well above the growing crop. Access tubes for neutron probe measurements of soil moisture profiles were installed on two replicates of treatments B to E.

Fertilizer was applied at the start of each growth period, with 60% allocated to the first and 40% to the second period. The higher rate was chosen in order to be somewhat above the optimum rate for the region, whilst the lower rate was considerably below that normally used in practice. This meant that conditions could be both N-limited and non-limited.

Different main plot treatments were used in 1991 in an attempt to assess the effects of greater moisture deficits:

A: Irrigated whenever soil moisture deficit exceeded 25 mm
B: Non-irrigated, rainfed throughout
C: Sheltered from rainfall throughout the growing season

Fertilizer treatments were the same as before. Measurements of growth were also made on neighbouring plots at ten-day intervals during the first cut, during which time there was very little rainfall in that year.

The onset of growth was around 24-30 April each year. The first cut was taken around 20-24 June and the second cut around 21-23 August, corresponding to early heading in both cases.

Soil description

The soil is classified as an imperfectly drained brown earth (gleyed melanic brunisol, Canada Dept. of Agric.) derived from morainic till with a moderately high stone and boulder content. Topsoil depth is about 27-30 cm and drain depth is about 90-100 cm.

A soil profile pit was dug on each replicate. Three 100 cm^3 core samples were taken

in each pit at approximately 15 cm depth intervals down to 75 cm. Results of mechanical analyses and water-retaining and aeration properties are presented in Table 1. The soil texture is on the borderline between loam and clay loam, and both bulk density and aeration parameters suggest a relatively high state of compaction.

Table 1. Soil physical properties at the trial site

Depth	Texture (USDA)	Sand	Silt	Clay	Gravel	Ignition loss %	Density Mg m^{-3}
5 cm	Loam	40	35	25	3	7.6	1.42
20 cm	Loam	40	35	25	3	7.8	1.42
35 cm	Loam	44	35	21	4	3.0	1.79
50 cm	Loam	40	36	24	5	3.1	1.74
65 cm	Clay loam	30	41	29	5	3.2	1.74

Depth	Porosity %	Air cap. %	Air perm. μm^2	Water retention (vol. %) pF2-3	pF3-4.2	pF2-4.2	>pF4.2
5 cm	47.0	7.4	6.7	5.6	21.5	27.1	12.5
20 cm	46.6	4.0	1.2	6.0	23.6	29.6	13.0
35 cm	33.7	2.8	0.4	3.5	14.1	17.5	13.3
50 cm	34.7	4.0	0.9	3.6	13.3	16.9	13.9
65 cm	35.7	2.9	0.5	2.9	14.3	17.2	15.6

The topsoil has a high storage capacity for available water, whereas in the subsoil it is markedly lower. The available water storage capacity of the root zone (0-60 cm) is estimated at 110 mm using the method proposed by Riley (1989), in which the wilting point is set at pF 4.2 down to 40 cm, and at pF 3.0 for the lower horizon.

Weather conditions and amount of irrigation

The period was characterized by relatively warm and dry weather in May and early June, followed by wetter conditions in late June and July (Table 2). Irrigation of treatment A plots was carried out as follows (25 mm on each date):

1989	26.5	17.6	23.6	4.7	24.7		
1990	10.5	18.5	30.5	11.6	19.7	14.8	
1991	8.5	23.5	30.5	6.6	28.6	11.7	1.8

RESULTS

Dry matter (DM) yields

1989-90: (Table 3)

The effects of sheltering from rainfall, as compared to frequent irrigation, were broadly similar for both varieties at both nitrogen levels and in both years. The effects of sheltering were greater in the second growth period than in the first because soil moisture reserves were already partly depleted at the start of regrowth.

Table 2. Weather conditions in the trial years, compared with long-term mean values. Evaporation data obtained from an open water surface (Thorsrud 2500)

	Year	May	June	July	August
Air temp.	1989	9.1	13.7	15.6	13.2
(°C)	1990	7.1	13.7	14.8	14.8
	1991	8.9	10.5	16.3	15.2
	Normal[1]	8.2	13.5	15.2	14.1
Pot. evap.	1989	68	80	97	50
(mm)	1990	75	76	73	62
	1991	95	60	73	68
	Normal[2]	61	84	82	68
Rainfall	1989	46	49	111	74
(mm)	1990	10	136	52	29
	1991	10	136	52	29
	Normal[2]	45	65	63	67

[1]) 1951-1980 [2]) 1963-1987

Significant interactions were found in the second cut between sheltering treatment and both variety and nitrogen level. Engmo showed poorer ability to recover from sheltering prior to the first cut than Grindstad. The effect of late sheltering, prior to the second cut (treatment C), was greater at the higher nitrogen level than at the lower level.

Compared with the treatment with irrigation, total DM yields were on average 11% lower after sheltering in May, 15% lower after sheltering in June, 28% lower after sheltering in July and 14% lower after sheltering in August. There was little evidence to indicate compensatory growth during the second growth period on plots which had been sheltered during the first growth period.

1991: (Table 4)

First cut yields were reduced by 20% relative to the irrigated treatment in the treatment without sheltering and by 39% in the treatment which was sheltered from rainfall throughout the growth period. There was no interaction with either variety or nitrogen application level.

At the second cut Engmo again exhibited poorer regrowth ability than Grindstad, as might be expected with the arctic photoperiod adaptation of the former variety. There was also a significant interaction between sheltering treatment and nitrogen level. The effect of drought was most marked on plots with high nitrogen fertilization, where sheltering reduced yields by 69% compared with irrigation. The decline was much smaller at the lower nitrogen level. Some salt damage (dessication) was observed on heavily fertilized, sheltered plots, which may account for this interaction. Alternatively, leaching may have led to nitrogen deficiency on the irrigated treatment with low fertilization.

Table 3. Effects of irrigation and sheltering from rainfall during different periods on grass dry matter (DM) yields (t/ha) in 1989 and 1990

	Irrigation/sheltering treatment					Interaction
	A	B	C	D	E	s.e. of diff.
DM yield, 1st cut						
Grindstad	8.60	7.34	7.29	7.63	7.39	0.21
Engmo	8.46	7.30	6.95	7.57	7.56	
N1	8.04	7.06	6.40	6.86	6.96	0.19
N2	9.01	7.59	7.84	8.34	8.00	
1989	7.80	7.33	7.09	7.44	7.56	0.17
1990	9.26	7.31	7.15	7.76	7.39	
DM yield, 2nd cut						
Grindstad	5.89	5.51	5.52	2.84	5.20	0.18
Engmo	4.15	4.00	3.33	1.55	3.21	
N1	3.33	3.30	3.04	1.47	3.15	0.15
N2	6.71	6.21	5.81	2.92	5.25	
1989	5.17	3.99	3.54	2.16	3.51	0.16
1990	4.87	5.52	5.31	2.23	4.89	
Total DM yield						
Grindstad	14.48	12.85	12.81	10.47	12.59	0.29
Engmo	12.61	11.30	10.28	9.12	10.77	
N1	11.38	10.35	9.45	8.33	10.11	0.22
N2	15.72	13.80	13.64	11.25	13.25	
1989	12.96	11.32	10.64	9.59	11.07	0.25
1990	14.13	12.83	12.54	9.99	12.28	

Dry matter concentrations

Similar effects of sheltering and irrigation were found in both varieties, at both nitrogen levels and in all years (data not shown). Grindstad always had higher DM concentrations than Engmo, and high nitrogen fertilization always reduced DM concentrations, as might be expected.

Table 4. Effects of irrigation and sheltering from rainfall during different periods on grass dry matter (DM) yields (t/ha) in 1991

DM yield, 1st cut	Irrigated	Rainfed	Sheltered
Grindstad	7.84	6.53	5.25
Engmo	8.10	5.31	4.53
N1	7.48	5.93	4.39
N2	8.46	6.91	5.38
Mean	7.97	6.42	4.88
DM yield, 2nd cut			
Grindstad	6.14	5.73	3.11
Engmo	4.28	3.92	1.98
N1	4.39	3.71	3.18
N2	6.04	5.94	1.91
Mean	5.21	4.82	2.54

	1st cut	2nd cut
Variety	n.s.	$P < 0.01$
Sheltering	$P < 0.001$	$P < 0.001$
N-level	$P < 0.01$	$P < 0.01$

At the first cut DM concentrations declined in all years with increasing yield (Fig. 1), indicating that increasing the water supply prolonged the period with vegetative growth. Delayed heading was indeed observed on irrigated plots. Thus it is probable that increasing the water supply has a beneficial effect on both palatability and digestibility in this case, although these parameters were not measured.

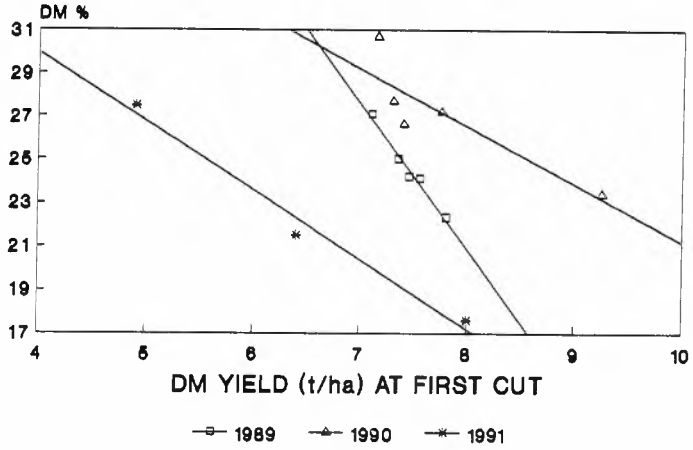
No consistent trend was found at the second cut, where somewhat higher DM concentrations were often found in the highest yielding plots. This was probably due to the more rapid start of regrowth, leading to earlier maturation, on plots which were well supplied with water early in the second growth period.

Nitrogen concentrations and crude protein yield (Table 5)

The effects of irrigation and sheltering treatments on nitrogen concentrations reflected their effect on DM yield. Higher nitrogen concentrations were found on plots which were sheltered, because of lower grass growth rates, than on plots which were irrigated.

The effect of sheltering on crude protein yield was thus less marked than that found in the case of DM yield. While there was little overall effect of sheltering in the first growth period, there was a compensatory uptake of nitrogen during the second growth period.

Fig. 1. The relationship between DM concentration and yield level at first cut



Soil moisture profiles

Moisture profiles measured in spring (26th April 1989) and at the end of each sheltering treatment in 1989 and 1990 are shown in Fig. 2, together with data for wilting point and field capacity obtained from the laboratory analysis of core samples. The latter indicated a soil moisture content about 20% higher than that measured in spring with the neutron probe. The reason for this may have been the presence of stones in the bulk soil, or it may have been due to hysteresis. In either case, a somewhat lower storage capacity for available water seems to be indicated than that based solely on the results of the laboratory analyses.

Soil moisture depletion at the end of each sheltering treatment was greater in 1990 than in 1989. Most of the water was extracted from the topsoil. Levels sank almost to wilting point in the uppermost layer, but remained above it at greater depths. There was little change in water content below the depth of 60 cm, suggesting that water uptake there is minimal.

The soil was somewhat drier in the spring of 1991 than in the earlier years (Fig. 3). Measurements made in late June (after the first cut) indicated that some rewetting of the profile occurred as a result of heavy rain while the plots were being harvested. This was taken into account in subsequent calculations of relative evapotranspiration for that period. The moisture deficit in the upper 60 cm of the soil amounted to 90 mm by the time of the second cut.

Table 5. Effects of irrigation and sheltering from rainfall during different periods on nitrogen concentrations and crude protein yield in 1989 and 1990

	Irrigation/sheltering treatment					Interaction
	A	B	C	D	E	s.e.of diff.
N%, 1st cut						
Grindstad	1.31	1.40	1.36	1.30	1.31	0.04
Engmo	1.30	1.53	1.47	1.28	1.27	
N1	1.12	1.23	1.25	1.09	1.13	0.04
N2	1.49	1.70	1.58	1.49	1.46	
1989	1.50	1.55	1.49	1.37	1.39	0.05
1990	1.10	1.38	1.34	1.21	1.20	
N%, 2nd cut						
Grindstad	1.16	1.31	1.40	1.98	1.46	0.09
Engmo	1.33	1.71	1.77	2.33	1.64	
N1	1.13	1.39	1.46	1.93	1.38	0.05
N2	1.35	1.63	1.71	2.38	1.72	
1989	1.44	1.81	1.96	2.36	1.88	0.05
1990	1.05	1.21	1.21	1.95	1.23	
Crude protein t/ha						
Grindstad	1.14	1.09	1.10	0.97	1.08	0.04
Engmo	1.03	1.12	0.99	0.83	0.94	
N1	0.79	0.80	0.75	0.63	0.75	0.02
N2	1.38	1.41	1.34	1.18	1.27	
1989	1.21	1.16	1.08	0.95	1.07	0.03
1990	0.96	1.06	1.01	0.85	0.94	

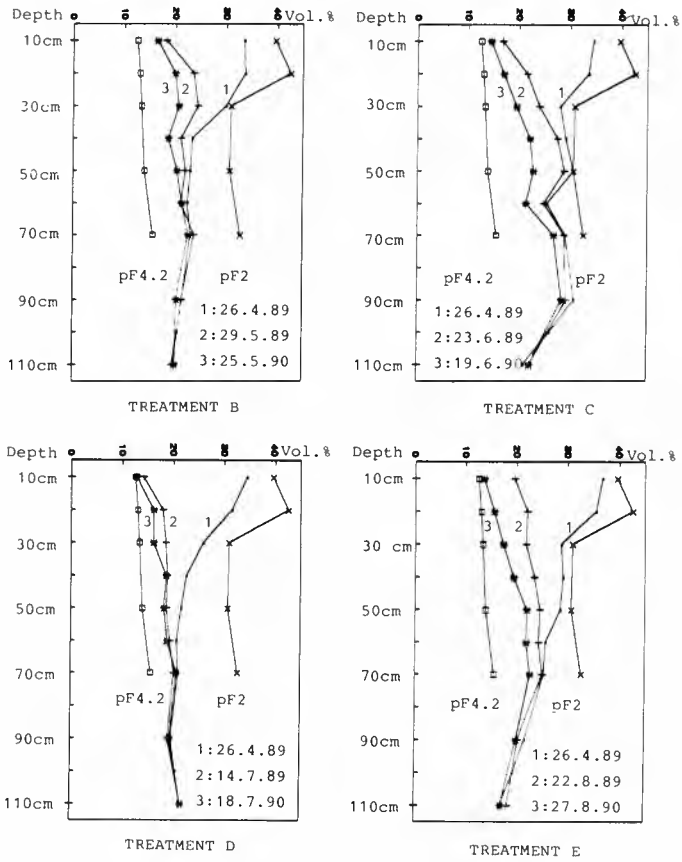


Fig. 2. Soil moisture profiles measured in the spring of 1989 and at the end of each sheltering treatment in 1989 and 1990 (means of two replicates). Laboratory data for wilting point (pF 4.2) and field capacity (pF 2) are also shown

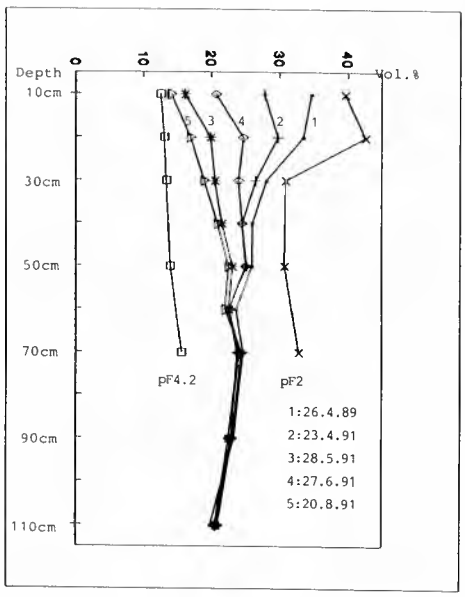


Fig. 3. Soil moisture profiles measured in the spring of 1989 and 1991 and at various stages during the growth season on sheltered plots in 1991 (means of eight replicates). Laboratory data for wilting point (pF 4.2) and field capacity (pF 2) are also shown

ASSESSMENT OF DROUGHT INDICES

Calculation method

Two simple indices of drought are the *relative evapotranspiration rate* (Ea/Et) and a related expression described as the *number of stress days* (Hiler & Clark 1971; Mogensen 1980). Both may be calculated using data for actual evaporation (Ea) derived from simple water balance models. Potential evapotranspiration (Et) is the evaporation calculated for a crop which is well supplied with water, in this case the irrigated control.

The relative evapotranspiration rate is calculated as the quotient of cumulative Ea and Et values, whilst the number of stress days is derived by summing the expression 1-Ea/Et for each day over a period. In the present case the growth periods for first and second cuts (nine and eight weeks respectively) were used in the calculations.

Actual evaporation was calculated from pan evaporation using the simple water balance model proposed by Kristensen & Jensen (1975), which takes into account the effects of variations in plant cover and soil moisture content, and which has previously been found to give satisfactory results with other crops under Norwegian conditions (Riley 1989).

The model is slightly more elaborate than that employed by Kornher & Torssell (1983), as it includes calculations of evaporation from both the soil surface layer and the root zone. This distinction is of importance especially during periods with incomplete crop cover. In the present case crop cover was assumed to be complete two weeks after the start of each growth period. A correction for incomplete cover was made in the calculation of Et during these periods.

Comparisons with measured results

Relative DM yields were calculated for individual growth periods, as percentages of the yields obtained on irrigated plots, using data for all three years, averaged over both varieties. Relative yields at the two nitrogen levels, as well as the mean values, were compared with values of the above-mentioned drought indices calculated for the relevant growth periods.

In view of the uncertainty about the level of the soil's field capacity, all calculations were made using two alternative values of root zone capacity, 110 and 90 mm. The former value is derived from the laboratory data as described above, whilst the latter makes allowances for the somewhat lower moisture content measured in the field. The choice of calculation method made little difference to the agreement between calculated and measured soil moisture deficits (Fig. 4).

Considerably closer relationships between relative yields and drought indices were found at the higher nitrogen level than at the lower level (Table 6). This is due to the fact that irrigated N1 plots yielded poorly at the second cut in 1990, possibly due to leaching. Marginally better correlations were obtained with a root zone capacity of 90 mm than with one of 110 mm.

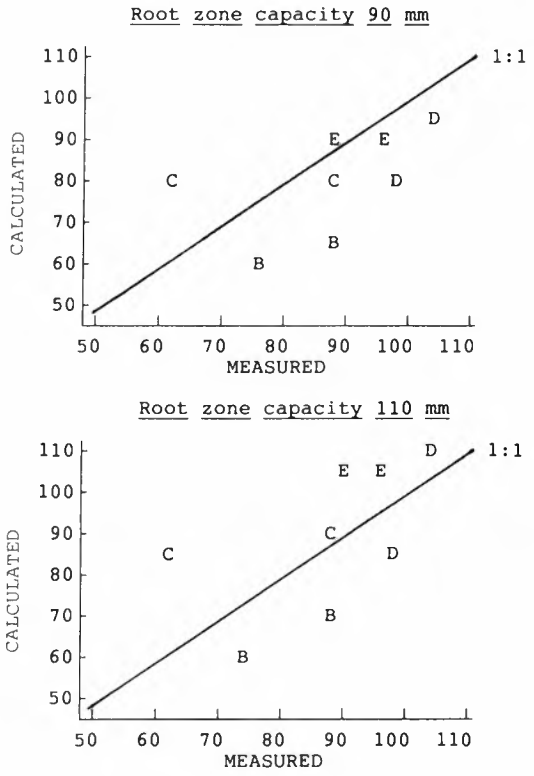


Fig. 4. Measured soil moisture deficits after sheltering at different times in 1989 and 1990 compared with values calculated using alternative values of root zone capacity (90 and 110 mm)
Letters denote sheltering period:
B = May C = June D = July E = August

Table 6. Correlation coefficients of relative yields in individual growth periods (% of fully irrigated) with alternatively calculated indices of drought. Data for 1989-91

Drought index:	Rel. evapotranspiration		No. of stress days	
Root zone capacity:	90 mm	110 mm	90 mm	110 mm
N1 yields	0.64	0.58	-0.56	-0.51
N2 yields	0.91	0.91	-0.88	-0.89
Mean yields	0.87	0.84	-0.82	-0.80

(Number of observations = 20)

The choice of root zone capacity is thus not critical for the evaluation of drought indices, but may be of importance in the extrapolation of results to soils with lower moisture-retaining capacity. In such a case, an overestimation of the root zone capacity of the original soil would lead to an overestimation of extrapolated drought effects. For this reason, and because a similar value was found in previous trials on a neighbouring site (Riley 1989), the value of 90 mm is used in the following calculations.

Both indices of drought appear to be physically meaningful (Fig. 5) when using a 90 mm root zone capacity, since they suggest that DM production declines to zero when the relative evapotranspiration rate reaches zero and when the number of stress days equals the

entire length of the growth period (approximately 60 days at each cut). Using 110 mm in the calculations would have suggested that DM production ceases at a lower level of drought (i.e. at a value of Ea/Et greater than zero), which seems unlikely.

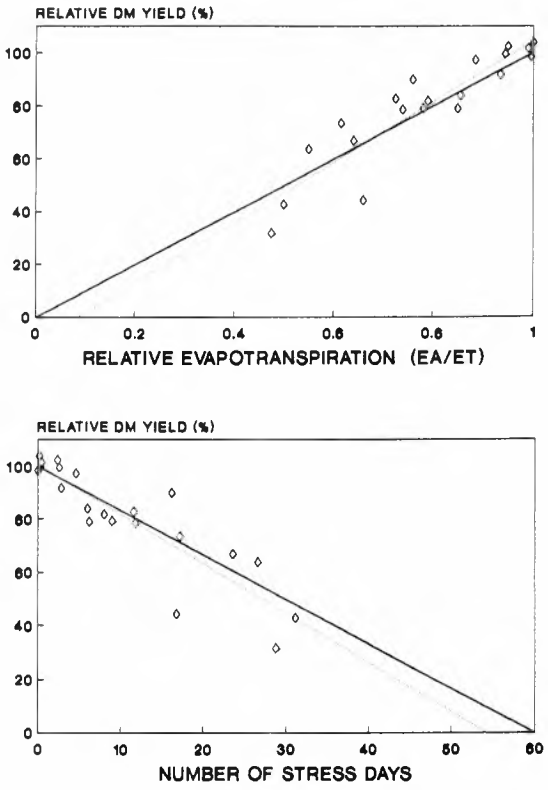


Fig. 5. The relationship between relative DM yields on unirrigated plots (Percentage of fully irrigated) and two indices of drought. Calculations made using a root zone capacity of 90 mm. Dotted lines indicate regression

The linear decline in DM production, from a maximum at Ea/Et = 1 to zero at Ea/Et = 0, is in agreement with the assumption made in the previously mentioned Swedish model. A test of its applicability over shorter time intervals was made using data from irrigated and rainfed plots harvested at ten-day intervals during the first growth period of 1991. Reasonable agreement was also found in this case (Fig. 6).

The use of data from individual growth periods, as performed here, would not be valid if drought effects during the first period were compensated for by enhanced growth in the later period. In order to examine this possibility, calculations were also made using data for total DM yield. This gave only a very slight decrease in the slope of yield decline with increasing drought, indicating that little compensation occurred. A linear, "one-to-one" relationship between relative yield and relative evapotranspiration is therefore considered suitable for use in extrapolation.

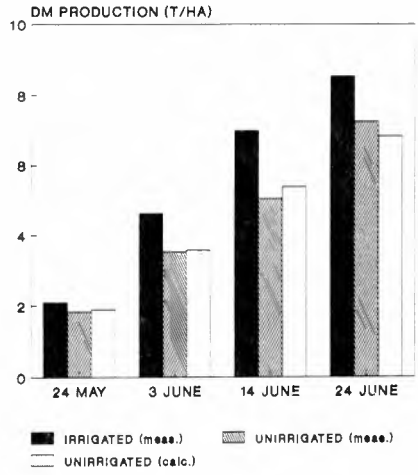


Fig. 6. Comparison of measured and calculated dry matter production during the first growth period of 1991. Calculations made by multiplying DM production of irrigated plots with relative evapotranspiration rate of unirrigated plots

Extrapolation of results

Calculations of relative DM yields were made on the basis of data covering 28 years of evaporation and rainfall measured at Kise Research Station for three contrasting levels of root zone capacity (50, 90 and 130 mm). These values are representative of drought prone, moderately drought resistant and drought resistant soils in the region.

Grass growth was assumed to start on 1 May each year, except in years with late snow cover, and two growth periods per year were simulated, with cuts taken in late June and late August. A weighting was made in the figures for total yields, since the contribution of the second cut is typically lower than that of the first. In the present trial the yield on irrigated plots with high N fertilization was approximately 25% lower at the second than at the first cut. This value was therefore used in the weighting.

The average results of these calculations are presented in Table 7. First cut yields appear to be less affected by drought than second cut yields. This is because the root zone is assumed to be refilled to field capacity after snow-melt at the start of each season. In the absence of irrigation, overall yields are on average 22% lower than the potential with irrigation on drought prone soil, whilst for drought resistant soil the reduction is only 7%.

Table 7. Long-term average yields in the absence of irrigation, relative to potential yields with irrigation, for soils with contrasting moisture-retaining capacity. Calculated from relative evapotranspiration rates using data from Kise 1963-90

Root zone capacity	First cut	Second cut	Weighted mean
130 mm	96	89	93
90 mm	91	83	88
50 mm	80	77	78

There is high variability between years in the need for irrigation. This is illustrated in Fig. 7, which shows the frequency of years in which non-irrigated yields exceed various percentage levels of the irrigated potential.

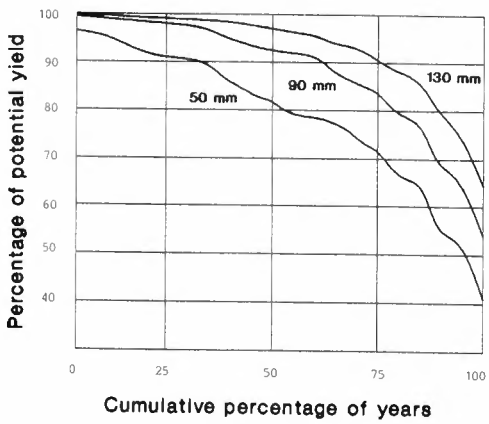


Fig. 7. Cumulative frequency of years when non-irrigated grass yields may be expected to exceed different proportions of the potential achievable with irrigation. Calculated using weather data for the period 1963-90 from Kise and for three levels of available soil moisture capacity in the root zone (50, 90 and 130 mm)

Further information obtained from these calculations includes the amount of irrigation water necessary on soil with different moisture-retaining capacity, and the amount of excess percolation that may be expected as a result of irrigation. A summary of such data is given in Table 8, assuming different levels of moisture deficit before the start of irrigation. A deficit equal to at least half the root zone capacity is normally acceptable without incurring any economic loss.

Table 8. Average water requirements for irrigation, assuming different acceptable moisture deficits (% of root zone capacity) and consequent excess percolation to depth (mm)

Deficit: Root zone capacity	Water requirements 25%	50%	75%	Excess percolation 25%	50%	75%
130 mm	116	82	42	61	33	13
90 mm	140	104	66	81	53	26
50 mm	160	131	99	85	63	42

DISCUSSION

The results of this investigation appear to confirm the hypothesis proposed in the Swedish ley model on the effect of soil moisture shortage, at least when there is no nitrogen deficiency. A smaller decline in the slope of yield versus relative evapotranspiration at lower levels of nitrogen input was also demonstrated by Brereton & Keane (1982). Extrapolation of such relationships suggests that DM production is maintained even at zero

evapotranspiration, which is clearly unlikely. Such low declines may possibly be caused by confoundation with leaching effects when nitrogen supply is limited.
At the other extreme, Danish studies have demonstrated a relationship which suggests that dry matter production ceases before relative evapotranspiration reaches zero (Gregersen & Olesen 1983). This may indicate the existence of a maintenance requirement which must be fulfilled before any new production takes place. Alternatively, carbohydrates may be translocated to roots under conditions of moisture stress, in which case one might expect plants to compensate for drought at a later date. However, recent Danish studies in ryegrass (Thomsen 1989) have suggested that the presence of green leaves in stubble is of greater importance for regrowth than such root and stubble carbohydrate reserves.

The adoption of a "one-to-one" linear relationship falls between the two extremes described above. It may be expected to give a reasonable description of the effects of drought both in short growth periods and over the whole season. The precise nature of the relationship at extremely low levels of relative evapotranspiration is probably of academic interest only under Norwegian conditions, since the extrapolatory calculations indicate that such levels occur only very rarely.

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Results of progeny testing and selection in Timothy (*Phleum pratense* L.)

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This article reports on population crosses, clone selection from local populations as well as from market varieties and cross populations, polycross (pc) fields with selected clones and testing of pc progenies at locations in southern and northern regions of Norway. The most important conclusions may be summarized as follows:

1. A large randomized duplicate block experiment with 100 clones from each of 10 populations gave broad sense heritability estimates of about 0.6 on average for heading time, leaf angle and relative yield, estimated on a plot basis by visual inspection. Highly significant direct and indirect responses were obtained after clone selection in the previous generation for heading time, plant height and general performance.
2. Highly significant interactions were found between pc progenies on the one hand, and locations and years on the other. Ecovalence as a stability parameter over locations and years indicated that alpine timothy, the marked variety Grindstad and mixed pc progenies from Grindstad were most unstable.
3. Large genetic variations were found for the quality traits protein content and *in vitro* digestability of the dry matter (DM). The most common and high-yielding market variety for the lowlands of South Norway, Grindstad, proved to be at the tailend of the distribution for protein content as well as for *in vitro* digestability.
4. At a location where there were no winter injuries protein content and *in vitro* digestability were highly genotypically correlated with each other in the positive direction, and they were both strongly genotypically correlated with DM yield in the negative direction. Our data do, however, indicate that it is easy to obtain combinations of high protein content, high *in vitro* digestability and high winter hardiness. Therefore, for regions where the yield depends to a large extent on the winter hardiness, the best possible results will probably be obtained by combinations of good winter hardiness with good quality.
5. Our results indicate that timothy breeding for northern regions and high altitudes should be based on populations derived from crosses between high quality and winter hardy populations from the North and high-yielding populations from the South.

Key words: Local populations, population crosses, progeny testing, timothy

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Wild growing types of the genus Phleum are distributed over most parts of the temperate regions of Europe, Asia and North Africa, and some *Phleum* species are also found in North and South America (Hegi 1936; Hulten 1950). Common timothy (*P. pratense* L.) has been found growing wild in Norway up to a latitude of about 67°N and in South Norway up to 600-700 m.a.s.l. (Schuebler 1886). In Norway, timothy has been grown as a fodder crop for about 200 years (Schuebler 1886; Witte 1915), and seems to have been seed propagated from as early as the beginning of the last century (Neumann 1809). From about 1850 timothy became increasingly important in cultivated grasslands until in this century it became completely dominant among the fodder grasses. For as long as hay-making has been the most usual method of storage, timothy has generally been sown in mixtures with red clover. Since increased cutting frequency and the use of silage in recent years have become increasingly more common, timothy has lost some of its dominance in Norwegian grasslands. However, it is still our most important fodder grass.

During the last century and at beginning of the present one, most farms produced their own timothy seed as well as that of clover. Repeated seed production at the same farm, or at least in the same region, led to adaptation by means of natural selection. From about 1880 trials were initiated by Larsen (1899) in order to investigate the practical value of local populations in comparison with populations imported from abroad. In the subsequent years several trial series of this type were conducted (Vik 1917; Elle 1930; Foss 1934; Jetne 1946; Rasmussen 1936; Fjærvoll 1935), the main findings of which were that relatively strong adaptation to the local growing conditions had taken place, and that the locally adapted populations were better than strains imported from abroad. Some of the local populations were better than others and were marketed as new varieties, with names indicating the farm from which they originated. The varieties Grindstad, Engmo and Bodin are still on the market, indeed, they dominate the market. Only one synthetic variety, namely Forus, has so far taken a significant share of the market. From the early 60s research and breeding of fodder grasses was taken up and gradually developed. This research started with basic work on cytogenetics and quantitative genetics in timothy (Opsahl 1964, Simonsen 1968, 1972). Later Schjelderup (1973, 1979, 1982) made extensive collections of timothy and other fodder grasses in North Norway. This work was later on extended to South Norway (Marum 1981) .

The present paper reports upon variations in local populations, population crosses, progeny testing and selection in materials derived from the local Nowegian populations, and, to some extent, populations from other countries.

MATERIALS AND METHODS

The materials reported upon in this article originate from the following varieties and local populations:

1.	Grindstad	Market variety originating from a local population in Southeast Norway
2.	Forus	Synthetic/Norwegian market variety

3.	Engmo	Market variety originating from a local population in North Norway
4.	Bodin	Market variety originating from mid-Norway, but selected in North Norway
5.	Mæresmyr	Local population from mid-Norway
6.	Øtofte	Danish-bred variety
7.	Pajbjerg	Danish-bred variety
8.	Omnia	Swedish-bred variety
9.	Climax	Canadian-bred variety
10.	Rjadovaja	Local population from the Kungur region in the USSR
11.	Alpine timothy (*P. alpinum*)	Population collected in the Alps in 1937 and since then grown and repeatedly seed propagated at the Løken Research Station in Norway
12.	Local population	No. 011035, from Karasjok, Finmark
13.	Local population	No. 011085, from Malangen, Troms
14.	Local population	No. 011043, from South-Varanger, Finmark
15.	Local population	No. 011012, from Svanvik, Finmark
16.	Local population	No. 011024, from South-Varanger, Finmark
17.	Local population	No. 011025, from Grense Jakobselv, Finmark

The local populations listed under nos. 12-17 are from collections made by Schjelderup in the 1970s. Populations 011035 and 011043 are the same as two of the populations studied for seed production by Rognli (1987).

Handling of our breeding material may be subdivided into various steps.

Step 1

The variety/population nos. 1-5, 7-10 and, in addition the British variety Aberystwyth S48, were space planted in an experiment laid out at the Research Station Saerheim in 1964 and observed for five quantitative characters over two years (1965-66). The results of this experiment were published by Simonsen (1968) in a dissertation thesis. Based on the observation means taken over two years, excluding Aberystwyth S48, and considering the rest of the plants as belonging to one population, the 72 (5%) most extreme plants in either direction of high and low were selected for the characters heading time, plant height and visually estimated general performance. The reason for excluding Aberystwyth S48 was that this variety was completely different from the others in heading time. In 1969 the selected plants were dug up, transferred to the Agricultural University in southeast Norway and planted in six separate groups. In the following year the plants in each group were isolated in small plastic houses and exposed to open pollination within each house. In this way the following six subpopulations were established:

EH	Selected for early heading
LH	Selected for late heading
LPLH	Selected for short plants and late heading
HPLH	Selected for tall plants and late heading
LGP	Selected for low general performance

HGP Selected for high general performance

Ste 2

In 1971 plants were raised in the glasshouse from seed of the six subpopulations mentioned under step 1 and, in addition, from seed of the varieties Grindstad, Engmo, Pajbjerg and Øtofte. The plants from these 10 populations were planted in the field as spaced plants the same year and left undisturbed until August 1973. Then 100 randomly selected plants from each population were cloned and the clones laid out in a randomized block experiment with two replications. The plots in this experiment were comprised of one row with five ramets set 30 cm apart within rows and with a row distance of 30 cm. In 1975 observations on a plot basis were taken on heading time, leaf angle at heading time and visually estimated size of the plants using a scale from 0 to 100. The first size estimate was taken before the first cut at heading time, and the regrowth was estimated on 30 July and 22 September. Average estimated yields were based on the means of the three estimates mentioned above. The quality of visual size estimates will, of course, to a high degree depend on the skill of the observer. In earlier experiments of the same type we have had genotypic correlations greater than 0.9 between weighted and visually estimated yields (cf. Aastveit & Aastveit 1989).

Step 3

Based on the results from an analysis of the data from the large clone experiment (step 2) the 10 highest and the 10 lowest clones for average relative yield within each of the 10 populations were selected for recloning and testing in a new clone experiment laid out in May 1976 (Garvik 1978). In addition to the 20 clones within each population selected for high and low relative yield, 10 clones with medium yields were included from each of the varieties Grindstad, Engmo, Pajbjerg and Øtofte. The new clone experiment had a total of 240 clones laid out in two randomized blocks. The plot size was the same as that mentioned for the first clone experiment (step 3)

The rates of fertilizer given each year in this clone experiment were 180, 42 and 72 kg per hectare of N, P and K, respectively. The experimental field was artificially irrigated twice in each of the years 1976 and 1977 with about 40 mm each time. The growing conditions were therefore quite optimal and the yield level high in 1977. In that same year, raw matter (RM) yield was determined by weighing after cuts at heading time and about two months later (end of August).

Step 4

In 1979, 1980 and 1984 a total of eight polycross (pc) fields were laid out at Ås in southeast Norway according to the design suggested by Olesen & Olesen (1973). A survey of these fields is presented in Table 1. All clones in pc's 21/79 and 23/79 were selected for high yield in the clone experiment mentioned under step 3. The clones of Engmo and Grindstad in pc 10/80 and pc 11/80, respectively, were raised from plants collected in a fourth year ley at Skagahøgdi, which is situated in Øystre Slidre in South Noway, about 1170 m.a.s.l. (Baadshaug et al. 1987). The material in pc 12/80 was quite different from that of the others, since it had clones of alpine timothy. The Engmo clones included in pc's 18/80, 19/80 and 20/80 were selected in a clone experiment at the Holt Research Station,

Alta section. The clones in pc 18/80 were selected for high yield, those in pc 19/80 for medium yield, while those in pc 20/80 were selected for low yield.

Table 1. The poiycross (pc) fields in step 4

Pc No.	No. of clones	Origin of clones	Types of pc prgenies
21/79	22	11 from the subpopulations mentioned under step 1. 1 from Grinstad, 2 from Øtofte 1 from Pajbjerg and 7 from Engmo	Mixed + 17 HS-families
23/79	10	3 from Grindstad + 7 from Engmo	Mixed
10/80	20	All from Engmo, Skagahøgdi (1170 m.a.s.l.), 4th year ley	Mixed + 10 HS-families
11/80	22	All from Grindstad, Skagahøgdi (1170 m.a.s.l.), 4th year ley	Mixed + 7 HS-families
12/80	22	All from the alpine population	Mixed
18/80	16	All from Engmo	Mixed + 16 HS families
19/80	15	All from Engmo	Mixed + 15 HS families
20/80	15	All from Engmo	Mixed + 15 HS families

In June 1983 a series of trials was laid out in which were included the mixed progenies and a number of HS-families listed in the right hand column of Table 1. The market varieties Engmo and Grindstad were also included. The experimental design was a special type of incomplete block (Aastveit 1977) where the "treatments" were laid out in groups of 20 progenies plus two control varieties (Engmo and Grindstad) in each group. Complete randomization was applied to groups and "treatments" within groups. Each individual trial had four replications, and the trials were laid out at the following locations:

1. Agricultural University, Ås in southeast Norway, 59°40'N, about 100 m.a.s.l.
2. Løken Research Station, South Norway, 61°07'N, about 550 m.a.s.l.
3. Vågønes Research Station, Bodø, Mid-Norway, 67°17'N, about 20 m.a.s.l.
4. Holt Research Station, Alta section, North-Norway, 77°57'N, about 10 m.a.s.l.

For the Ås trial, the rates of fertilizer were 120, 29 and 55 kg per hectare for N, P and K, respectively, at the time of sowing and repeated annually in the spring. Additional N, P and K at rates of 100, 29 and 46 kg per hectare, respectively, were applied after the first cut. Approximately the same rates of fertilizer were applied at the other locations in the spring. After the first cut the rates of fertilizer at the Vågønes and Holt Research Stations were about half those used at Ås and Løken.

The sizes of the machine-sown plots were 7.5, 3.24, 7.5 and 5.25 m^2 at Ås, Løken, Vågønes and Alta, respectively. At all locations sowing at a rate of 20 kg per hectare took place in June 1983. All trials were harvested at the beginning of heading (cut 1) and about two months later (cut 2). At harvest, samples of about 1 kg from each plot were weighed raw, dried at 60°C for 72 h and weighed again for determination of dry matter (DM) percentage. The same samples were used for determination of quality traits.

In vitro digestibility of the dry matter (IVDDM-%) from one of the trials was determined by the two-stage rumen fermentation technique of Tilley & Terry (1963) in the laboratory of the Vågønes Research Station, while NIRS analyses of this and the other quality traits were carried out at the Løken Research Station, as described by Marum (1990).

Step 5

As mentioned previously, a comprehensive collection of local populations of forage grasses was made by Schjelderup (1979, 1982) in the 1970s. The collected populations were first tested in dense stands on small (1.0 m^2) plots in two randomized blocks. In addition, 100 plants from each population were cloned and the ramets planted as spaced plants on small plots, usually five in each plot at a distance of 30 cm in both directions, and with two replications. The plots of dense stands and spaced plants were subjected to observations of many traits or characters of economic interest. Based on these observations some populations were chosen for progeny testing. Usually 22 clones were selected and planted in isolated pc fields. The layout was always in accordance with the design suggested by Olesen & Olesen (1973).

The pc's 1/83, 2/83, 1/85, 2/85, 1/86 and 2/86 were all conducted in the glass house (crossing house) at the Holt Research Station, near Tromsø, and the HS-progeny families were laid out in two series (A and B) of experiments, both series located in Tromsø and Alta.

Series A included 44 HS-families from the pc fields 1/83 and 2/83 mentioned above. The 22 HS-families from each polycross were laid out in separate experiments, each with three randomized blocks, but the individual experiments were sown in the same field at each of the two locations. The variety Engmo was included in all experiments for comparison over populations. In both series the plots had three drill-sown rows, 5m long and set 30 cm apart.

The two A-series experiments were sown on 15 August 1986 in Tromsø and on 17 June 1987 in Alta. All experiments in the B-series were sown on 24 June and on 10 June 1988 in Tromsø and Alta, respectively.

Yield was not measured in these experiments. To avoid border effects, only the rows in the middle were harvested and samples subjected to the NIRS analyses.

A total of 38 characters were recorded in these experiments. In this article we present data only for winter hardiness, earliness, general performance estimated by eye inspection and the quality characters digestibility of DM, crude protein content and NDF-%.

RESULTS

Experiments with clones

The population means in the first large clone experiment described under step 2 are given in Table 2. The table shows that selection in the previous generation for early and late heading time as well as selection for tall and short plants have led to significant differences in heading time in the progeny generation. The response to selection for plant height on earliness is, of course, an indirect effect as is the significant response to selection for high and low general performance on leaf angle. A large leaf angle seems to be associated with a high raw matter yield.

Table 2. Population means in the first clone experiment, 1971-75. Step 2

Population	Previous selection	Heading time (days in June)	Leaf angle (degrees)	Relative yield (0-100)
1. Grindstad	Unselected	11.4	82.1	22.8
2. Engmo	Unselected	11.9	78.2	20.1
3. Pajbjerg	Unselected	12.4	88.1	18.5
4. Øtofte	Unselected	12.0	85.8	20.7
5. EH	Sel. for early heading	10.1	77.5	22.3
6. LH	Sel. for late heading	14.3	78.2	19.5
7. LPLH	Sel. for short and late plants	10.9	78.5	24.3
8. HPLH	Sel. for tall and late plants	12.8	81.3	20.4
9. LGP	Sel. for low generalperformance	11.2	77.4	23.7
10. HGP	Sel. for high general performance	11.7	83.0	28.2
Average		11.9	81.0	22.1
LSD (0.05)		0.4	1.9	2.4

The estimates of broad sense heritability presented in Table 3 are all significant and on the same level for the three characters. The estimates indicate that great responses to selection can be expected in all characters.

The 10 highest and the 10 lowest clones for estimated raw matter yield within each population in the large clone experiment were selected, recloned and laid out in a new clone experiment as mentioned under step 3. In this experiment raw matter (RM) yield was determined by weighing after the first as well as after the second cut. Table 4 presents the differences between the means of clones selected for high an low yield. The table indicates that the responses to clone selection after one generation of vegetative propagation are significant for all four characters in question. High yielding clones in this experiment were recloned for a second time and included in pc fields 21/79 and 23/80 (cf. Table 1).

2. Trials with mixed polycross (pc) progenies and HS-families

The four individual trials in this series were observed over three seasons, 1984, 1985 and 1986. Because of severe winter damage, two blocks of the Vågønes trial had to be discarded after the first winter, and the remaining two blocks were observed in only two seasons. The precision of the observations as measured by the coefficient of variation, was

Table 3. Estimates of broad sense heritabilities from the large clone experiment. Step 2.

Population		Heading time	Leaf angle	Relative yield
1.	Grindstad	0.46	0.51	0.62
2.	Engmo	0.50	0.57	0.67
3.	Pajbjerg	0.42	0.47	0.60
4.	Øtofte	0.49	0.42	0.63
5.	EH	0.77	0.76	0.65
6.	LH	0.68	0.73	0.57
7.	LPLH	0.59	0.66	0.62
8.	HPLH	0.71	0.72	0.44
9.	LGP	0.58	0.55	0.61
10.	HGP	0.59	0.39	0.57
Average		0.58	0.58	0.60

Table 4. Response to clone selection for relative yield measured after recloning. Step 3

Population		Heading time Difference, late - early (days)	RM yield (g/plot) Difference, high - low		
			Cut 1	Cut 2	Total
1.	Grindstad	0.5	229	201	430
2.	Engmo	1.9	645	351	997
3.	Pajbjerg	1.1	278	290	589
4.	Øtofte	2.4	465	280	746
5.	EH	2.4	333	246	579
6.	LH	1.4	360	218	578
7.	LPLH	0.5	252	158	400
8.	HPLH	2.9	244	152	349
9.	LGP	0.1	310	205	515
10.	HGP	2.6	248	150	402
Mean difference		1.6±0.66	338±19	255±16	559±25
Population average		13.8	295	218	511

quite different in the four trials (Table 5). The location means presented in Table 6 show great variation between years, cuts and locations for dry matter yield (DM yield) as well as for the quality properties. In particular, the yearly location means for DM yield are related to winter survival. In the Ås trial the plant coverage in the spring was nearly 100% in all years, while at Vågønes the coverage was on average only 51% after the first winter. Table 7 presents winter survival for the Løken and Alta trials. Differences in winter survival may to some extent explain the great variation in the coefficients of variation (Table 5). From the data presented in Table 7 it can be seen that there was great genetic variation in winter hardiness. The pc fields 10/80, 18/80, 19/80 and 20/80 all had clones from the variety Engmo (Table 1). It can be seen from Table 7, although only slightly significant in some cases, that relatively small differences were found in winter survival between the mixed progenies from the pc-fields with Engmo clones, and between the same

mixed progenies and Engmo. However, highly significant genotypic variation was found between HS-families from the same pc fields, indicating that the variety Engmo is not homogeneous for winter hardiness.

Table 5. Coefficient of variation (CV) at each location over two years

Character	Cut	Ås	Løken	Vågønes	Alta
DM-yield	1	8.5	10.6	13 . 9	13.9
DM-yield	2	8.2	8.0	14.1	13.6
DM-yield	Total	6.5	7.5	12.0	10.1
IVDDM-%, NIRS	1	2.0	3.7	-	1.9
"	2	1.7	4.1	-	2.2
IVDDM-%, lab.	1	1.6	1.7*	-	-
"	2	2.0	1.4*	-	-
Crude prot. NIRS	1	5.9	7.4	18.1	
"	2	7.3	5.4	-	10.6
Stand in spring		0.9	4.9	27.1	21.5
Stand in autumn		0.9	5.1	25.0	19.9

*) Only one year

Table 6. Location means for DM yield (in kg per decare), digestibility and protein content (%)

Character	Ås			Løken			Vågønes			Alta		
	1984	1985	1986	1984	1985	1986	1984	1985	1986	1984	1985	1986
DM yield,cut 1	713	488	841	749	895	475	290	324	-	679	619	469
DM yield,cut 2	678	465	235	692	361	205	354	248	-	580	-	20
DM yield, Total	1391	953	1076	1441	1256	680	644	572	-	1259	-	675
IVDDM-%,cut 1 (NIRS)	74.9	75.2	-	71.4	68.7	-	-	71.1	-	69.9	68.9	-
IVDDM-%,cut 2 (NIRS)	70.0	69.7	-	74.1	78.7	-	-	76.9	-	77.4	-	
Crude protein (NIRS),cut 1	12.7	18.0	-	13.8	13.8	-	-	17.3	-	13.4	16.5	-
Crude protein (NIRS), cut 2	9.2	12.4	-	12.7	17.5	-	-	19.5	-	12.8	-	-

The pc field 11/80 had clones from plants collected in a fourth year ley of Grindstad grown at Skagahøgdi, 1150 m.a.s.l. (Table 1). There may be a tendency towards better winter survival of the mixed progeny from pc 11/80 as compared with Grindstad. The differences, however, are significant only in the first and second years at Løken. The significant genetic variation between HS-families from Engmo and the range of HS-families in Alta (Table 7) indicate that it should be possible to establish new synthetics from these materials with

better winter hardiness than Engmo.

Table 7. Winter survival at two locations. Percent coverage in the spring

Pc progeny or variety	Alta			Løken		
	1984	1985	1986	1984	1985	1986
21/79, mixed prog.	30	52	48	91	85	59
23/79, mixed prog.	71	60	70	82	87	64
10/80, mixed prog.	69	65	56	91	91	75
11/80, mixed prog.	42	36	46	93	89	61
12/80, mixed prog.	31	47	69	89	91	72
18/80, mixed prog.	76	74	65	87	90	70
19/80, mixed prog.	65	62	51	90	85	66
20/80, mixed prog.	67	65	67	90	85	63
HS-families, range from	18	27	28	83	76	53
to	83	84	78	92	94	75
Engmo	75	67	56	90	90	68
Grindstad	26	41	28	83	80	60
Average	60	61	55	88	87	66
LSD (0.05)	19	17	24	6	6	9

Table 8. Distribution of 88 pc progenies and to varieties for total DM yield averaged over blocks and 2 or 3 years (SD = standard deviation of entries)

Location, progenies or varieties	DM-yield (kg/decare)																			N	$\bar{x}$	SD
	550	600	650	700	750	800	850	900	950	1.000	1.050	1.100	1.150	1.200	1.250	1.300	1.350	1.400				
Ås, 3 years																						
Pc-progenies									5	10	15	18	15	13	10	1	1			88	1.142	
Engmo													1							1	1.078	28
Grindstad															1					1	1.300	
Alta, 2 years																						
Pc-progenies					1			3	23	38	18	5								88	971	
Engmo									1											1	944	40
Grindstad									1											1	912	
Løken, 3 years																						
Pc-progenies										2	16	19	24	20	7					88	1.162	
Engmo												1								1	1.124	44
Grindstad													1							1	1.165	
Vågønes, 2 years																						
Pc-progenies	6	30	35	15	2																609	
Engmo				1																	676	39
Grindstad	1																				587	

DM vield

For this character, highly significant two and three factor interactions were found between families/varieties, years and locations for the yields in the first and second cuts, and for

total yield. Table 8 presents the distribution of the 88 HS-families and mixed progenies in comparison with the two market varieties for total DM yield, averaged over blocks and years. The table indicates that only two progenies (HS-families) gave higher yields than the leading market variety, Grindstad, in the Ås trial. At Løken and in Alta many families gave higher yields than the highest of the market varieties. At Vågønes Engmo gave a much higher yield than Grindstad, and only two families gave a higher yield than Engmo.

Stability of the DM yields has been studied by estimating Wricke's (1962, 1965) ecovalence parameter from the data over years and three locations. Table 9 presents the estimates for the eight mixed pc progenies,. and in addition, for the commercial variety Grindstad. The table indicates that Grindstad, the alpine population and the mixed progeny from 11/80 had the lowest stability. The differences between the residual mixed pc progenies are relatively small. Pc 11/80 had only Grindstad clones. A comparison between the Grindstad commercial variety and the mixed progeny from 11/80 indicates that cultivation over a four-year period under the stressed winter conditions at Skagahøgdi has not altered the stability of Grindstad. The data also show that DM yield stability is not only a question of winter hardiness. In this connection, it should be noted that the alpine population included in the present series of trials has excellent winter hardiness (Table 7), but the lowest stability of all mixed pc progenies (Table 9)

Table 9. Estimates of the ecovalence parameter (divided by 1000) for the mixed pc progenies and the variety Grindstad. The estimates are based on means over blocks from three years at Ås three years at Løken and two years at Alta

Mixed progeny from polycross No.	Clones in the polycross from	Ecovalence for DM-yield	
		Cut1	Total
21/79	Grindstad, Øtofte Pajbjerg, Engmo	213	479
23/79	Grindstad, Engmo	169	474
10/80	Engmo, Skagahøgdi	274	526
11/80	Grindstad, Skagahøgdi	420	732
12/80	Aipine population	565	966
18/80	Engmo	266	348
19/80	Engmo	158	253
20/80	Engmo	183	366
Grindstad commercial		378	719

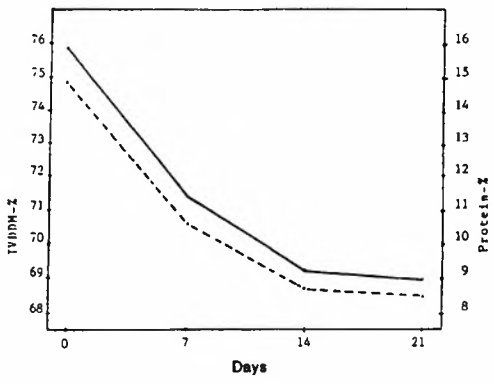
Quality

Complete data sets over two years from the NIRS analyses of IVDDM-% and crude protein-% are available from the trials at Løken and in Alta for both cuts, with the exception of the second cut in Alta 1985. Highly significant genetic variation was found for both characters after the first as well as after the second cut. The three factor interaction, family/variety x location x year, was highly significant for IVDDM-% in both cuts, but not significant for crude protein-%. The two factor interactions were not significant. Table 10 gives the distribution of IVDDM-% for the Ås and Løken trials, while Table 11 presents more detailed data from the NIRS-analyses at the three locations. It appears from these

Table 10. Distribution of 88 pc progenies and 2 market varieties for IVDDM-%, based on averages over blocks, 2 locations and 2 years

	IVDDM-%																					
	68		69		70		71		72		73		74	75		76		77		78		
Ås																						
Cut 1:																						
Pc-progenies			2	1	4	12	11	9	17	20	9	2	1									
Engmo									1													
Grindstad				1																		
Cut 2:																						
Pc-progenies	3	2	4	14	10	13	13	12	8	6	2		1									
Engmo								1														
Grindstad		1																				
Laken																						
Cut 1:																						
Pc-progenies	2	5	19	24	24	11	3															
Engmo					1																	
Grindstad		5																				
Cut 2:																						
Pc-progenies														1	6	4	13	12	16	18	12	5	1
Engmo																				1		
Grindstad															1							

Fig. 1. IVDDM-% (——) and protein-% (- - - -) in relation to time after beginning of heading



tables that the quality of Grindstad measured by IVDDM-% and crude protein-% is low compared with that of Engmo and most of the pc progenies. Since all the pc progenies and varieties were always harvested on the same day, the differences in quality should be discussed in relation to earliness. On average, Grindstad began heading 1.1 days earlier than Engmo (Table 14), and with the exception of the alpine timothy (12/80), Grindstad was also earlier in heading than most of the pc progenies. It is very well known that digestibility as well as protein content decrease rapidly after the beginning of heading. An example can be seen in Figure 1, which shows the results of an experiment carried out at the Research Station at Apelsvoll (Marum, unpublished), in which a number of varieties were harvested and analysed individually at different time intervals after the start of heading. Comparing the responses depicted in Figure 1 with the differences in Table 11, one comes to the

Tale 11. Quality means of 8 mixed pc-progenies, two varietis, and range of HS-families. Averages over the years 1984-85

	Ås				Løken				Alta			
	Prot. -%		IVDDM-%		Prot. -%		IVDDM-%		Prot. -%		IVDDM-%	
	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2
21/79 mixed	12.3	9.3	69.1	68.8	13.5	13.7	69.4	74.9	15.3	10.9	69.6	75.6
23/79 mixed	13.5	10.5	70.7	68.1	13.3	15.1	69.0	76.8	15.4	14.3	69.4	77.4
10/80 mixed	14.2	11.9	72.0	71.1	14.0	15.3	69.3	77.2	15.4	13.0	72.5	78.5
11/80 mixed	12.7	9.8	69.8	68.6	13.2	14.1	67.7	74.9	14.5	11.5	68.5	77.6
12/80 mixed	12.9	10.8	69.5	69.1	14.5	15.3	70.3	75.9	16.4	13.4	71.1	78.1
18/80 mixed	13.8	11.2	71.4	70.6	13.8	15.0	69.2	78.0	15.6	13.0	69.5	76.9
19/80 mixed	13.3	10.7	71.9	71.5	14.1	15.3	69.2	76.9	13.1	13.9	69.5	79.5
20/80 mixed	12.6	10.1	70.6	69.5	14.0	15.4	69.0	76.5	15.2	12.3	70.5	76.5
HS-families, range from	11.8	9.6	69.3	67.1	11.8	12.9	67.3	74.5	13.0	11.1	62.3	74.1
to	14.8	12.3	72.7	72.1	15.3	16.8	70.1	78.5	17.0	14.9	70.9	80.2
Engmo	13.4	11.0	71.2	71.0	15.0	15.5	69.6	76.5	13.6	13.3	69.5	79.4
Grindstad	12.6	10.3	68.4	67.7	13.0	13.9	68.0	74.9	16.5	10.6	69.8	75.8
LSD (0.05)	0.9	0.8	2.5	1.4	1.3	1.4	0.9	2.8	3.2	1.8	3.5	2.4

conclusion that the differences in earliness can, to some extent, explain the differences in quality, but not completely.

From table 11 it can be seen that the mixed progeny of pc 12/80, which was alpine timothy, was earlier than the other pc mixed progenies as well as the two varieties. Despite the extreme earliness, the pc 12/80 mixed progeny was fairly high in IVDDM-% as well as in protein content.

Genotypic and phenatypic correlations

The data from the Ås trial are complete for all characters observed over the two first years of harvest. In the third harvest year quality observations were not taken. In this trial only small interactions were found between family/variety and years, therefore the genotypic

Table 12. Genotypic (above diagonal) and phenotypic correlations for the Ås trial, based on data over two years

	DM Yield			Prot. % (NIRS)		IVDDM-% (NIRS)		IVDDM-% (lab)		Heading time
	Cut 1	Cut 2	Total	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	time
DM-Yield										
Cut 1	**1.00**	0.71	0.94	-0.54	-0.75	-0.73	-0.40	-0.90	-0.30	-0.54
Cut 2	0.38	**1.00**	0.99	-1.00	-0.98	-0.62	-0.90	-0.69	-0.76	-0.42
Total	0.41	0.65	**1.00**	-1.00	-0.97	-0.27	-0.99	-0.70	-0.80	-0.26
Protein-%										
Cut 1	-0.47	-0.72	-0.50	**1.00**	1.00	0.59	1.00	1.00	1.00	0.84
Cut 2	-0.29	-0.89	-0.60	0.73	**1.00**	0.55	1.00	0.72	0.91	0.34
IVDDM-% (NIRS)										
Cut 1	-0.21	-0.51	-0.20	0.47	0.45	**1.00**	0.66	1.00	0.59	0.66
Cut 2	-0.14	-0.78	-0.58	0.60	0.83	0.52	**1.00**	0.83	1.00	0.34
IVDDM-% (lab)										
Cut 1	-0.38	-0.43	-0.28	0.45	0.40	0.63	0.47	**1.00**	0.76	0.67
Cut 2	-0.14	-0.70	-0.50	0.51	0.70	0.46	0.87	0.51	**1.00**	0.32
Heading-time	-0.26	-0.29	-0.12	0.34	0.21	0.36	0.25	0.34	0.20	**1.00**

correlations were fairly constant over years. The estimates given in Table 12 are based on the averages of the two years. Digestibility was determined both by NIRS analysis and the slightly modified Tilley & Terry (1963) method. In Table 12 it is shown that the genotypic correlations were very high between these methods in this case. High positive genotypic correlations were found between digestability and protein content, and these two characters were highly genotypically correlated with yield in the negative direction. As found in other grass experiments (cf. Aastveit & Aastveit 1989) high genotypic correlations were observed between different cuts, and between the different cuts and total yield. The relatively high

and constant negative genotypic correlations between yield and earliness were surprising. From Table 12 it can be seen that, without exception, the phenotypic correlations were lower than the genotypic ones.

In the Ås trial, winter survival was nearly 100% in all years. At Løken there was a severe loss of plants during the third winter, and in Alta the most severe loss of plants came in the first winter (Table 7). The genotypic correlations between the DM yield and plant stand (coverage) in the spring are presented in Table 13 for the Løken and Alta trials. The table indicates high positive genotypic correlations between winter survival and yield in the first cut in this year at Løken, and in the first year, in the Alta trial. The high negative genotypic correlations between DM yield in the second cut and plant stand in the spring in both trials are difficult to explain. Perhaps one reason might be that highly productive weeds took over the vacant space after the disappearance of the sown timothy

Table 13. Genotypic correlations between DM yield and winter survival measured by coverage in the spring

Location		1984	1985	1986
	DM yield			
	Cut 1 vs coverage	0.08	0.23	0.82
Løken	Cut 2 vs coverage	-0.68	-0.37	-0.82
	Total vs coverage	-0.57	-0.09	0.33
	DM yield			
	Cut 1 vs coverage	0.91	0.23	0.82
Alta	Cut 2 vs coverage	-0.76	-	-0.81
	Total vs coverage	0.17	-	0.32

Table 14. Days in June to panicle emergence and relative figures for DM yield (DM) and digestable yield (D), averaged over three or two years

Pc-prog./variety	Heading time (days in June)	Ås		Løken		Alta*	
		DM	D	DM	D	DM	D
21/79 mixed	19.2	117	114	108	106	97	95
23/79 mixed	19.5	110	108	103	103	112	110
10/80 mixed	19.4	96	97	101	101	108	109
11/80 mixed	18.2	115	112	107	105	102	100
12/80 mixed	15.9	90	88	103	103	78	78
18/80 mixed	19.5	100	100	104	105	106	104
19/80 mixed	19.9	101	102	103	103	101	101
20/80 mixed	18.7	105	104	107	108	97	96
Engmo	19.3	100	100	100	100	100	100
Grindstad	18.2	120	115	104	101	97	95

*) 2 years

plants. For the Alta trial, we have systematic observations on the percentage of weeds in all years. In the third year a highly significant variation between entries was found for weed percentage, and there was a negative phenotypic correlation between winter survival (coverage) and weed percentage of r = -0.68. We do, however, think that the negative correlation between winter survival and DM yield can best be explained by variation in growth rhythm.

Digestable yield

We have seen that a considerable genetic variation exists in the present material for DM yield and quality. Since digestability and protein content are so closely correlated, and since they are both correlated with DM yield in the negative direction, we found it reasonable to estimate and compare the mixed progenies and varieties for digestable yield. Table 14 presents the relative figures for digestable yields of the eight mixed pc progenies and the two market varieties at the three locations, averaged over two or three years. It can be seen from the table that Grindstad gave the highest DM yield as well as the highest digestable yield in the Ås trial. The superiority of Grindstad in this trial was, however, only 1% for digestable yield as compared to the mixed progeny of pc 21/79. At the other two locations at least the pc 23/72 mixed progeny seems to be superior to Grindstad in total DM yield as well as in digestable yield. It is noteworthy that pc 23/79 had clones from Engmo and Grindstad (Table 1), The results from Alta indicate that for locations where the winter hardiness is an important component of DM yield, it seems possible to combine quality and winter hardiness from northern types with high-yielding capacity from southern types.

3. Experiments with progenies from polycrosses at Holt

The HS-progenies from the six polycross fields harvested in Tromsø during the years 1983-86 (step 5) were laid out in two series of experiments, denoted here as A and B. Series A was comprised of four experiments, two in Tromsø and the other two in Alta. Each of the experiments at each location included the 22 HS-families from pc's 1/83 and 2/83, and, in addition, the variety Engmo. The two Alta experiments were sown in August 1986, while the two experiments in Tromsø were sown in June 1987. Series B comprised of eight experiments, i.e. four in Alta and four in Tromsø. Each experiment at each location had a set of 22 HS families from one of the pc fields 1/85, 2/85, 1/86 and 2/86 and, in addition, Engmo. The sets of HS-families in Alta and Tromsø were the same.

Winter hardiness and earliness

In series A no significant differences were found in coverage during the first or second year of ley, in neither Tromsø or Alta. In the B series, on the other hand, significant genetic variations within populations were found in both years of ley and at both locations. In Table 15 the population means and ranges within populations are presented. The table indicates that on average, all four populations were inferior compared with Engmo, but they did contain HS-families that ranged far above Engmo in winter hardiness.

Table 15. Winter hardiness measured by the plot coverage (%) in the spring. 22 HS-families within each population + Engmo (E) (ranges in parentheses)

Series	Location	Year of ley	Population 11043 HS	11043 E	11012 HS	11012 E	11024 HS	11024 E	11025 HS	11025 E
B	Holt	1.	68 (55-81)	71	79 (61-87)	81	78 (68-86)	85	80 (60-90)	82
"	"	2.	55 (47-87)	53	71 (47-87)	77	66 (45-88)	83	64 (27-83)	65
"	Alta	1.	92 (81-99)	97	89 (68-99)	98	96 (90-100)	91	97 (91-100)	96
"	"	2.	52 (8-82)	48	67 (52-83)	77	68 (56-73)	62	67 (48-80)	70
Average			66.8	67.3	76.5	83.3	77.0	80.3	77.0	773

Earliness measured by the number of days from an arbitrary date to the beginning of heading indicated a highly significant variation between HS-families within all populations, and in most of the cases also significant deviation of the population means from the mean of Engmo. Significant interactions were found between HS families and years, and between HS-families and locations. The means and ranges presented in Table 16 demonstrate different reactions between locations. It is noteworthy that, on average, all populations were earlier in Alta as compared with Engmo, and the ranges were much larger too.

Table 16. Earliness measured by days in June to the beginning of heading. E=Engmo

Location	Year of ley		Population 11043	11043 E	11012	11012 E	11024	11024 E	11025	11025 E
Holt	1	Mean	6.3	5.3	6.3	6.0	6.7	5.3	6.7	6.3
		Range from	5.6		5.0		6.0		5.3	
		to	7.6		7.3		7.6		7.3	
"	2	Mean	4.4	4.3	3.1	2.3	4.3	4.3	3.0	3.0
Alta	1	Mean	22.8	29.0	27.0	29.0	27.3	29.3	26.2	28.7
		Range from	1.0		10.3		10.7		10.3	
		to	30.0		29.7		30.0		30.0	

General performance

The yield of raw- or dry matter was not measured by weighing in these experiments. Instead the general performance was judged visually using a scale ranging from 0 to 9, where 9 stands for extremely good. The evaluation of general performance was done on a plot basis at the normal stages of harvest for the first and second cut, i.e. at the beginning of heading and about 50 days later.

Earlier experience has shown that general performance, which in this case is nearly the same as the total value index used by Aastveit & Aastveit (1989), is closely correlated with weight of raw matter yield. Significant genetic variations were found within the populations for this character, too. The population means did, however, deviate only

Table 17. General performance evaluated by eye inspection using a scale from 0 to 9 (9 = extremly good)

Series	Location and cut	Year of ley	Population means (HS) 011035 HS	011035 E	011085 HS	011085 E	011043 HS	011043 E	011012 HS	011012 E	011024 HS	011024 E	011025 HS	011025 E	Average HS	Average E
A	Holt															
	Cut 1	1	4.8	5.0	4.8	5.0										
	Cut 2	1	-	-	-	-										
	Alta															
	Cut 1	1	6.4	5.7	6.7	5.3										
	Cut 2	1	5.4	4.3	4.6	5.7										
B	Holt	(1+2)														
	Cut 1		-	-	-	-	4.9	5.2	5.7	6.0	4.3	5.2	5.2	4.3	5.0	5.2
	Cut 2		-	-	-	-	4.7	5.4	5.4	5.1	4.4	5.5	4.8	5.2	4.8	5.3
	Alta	(1+2)														
	Cut 1		-	-	-	-	6.2	5.9	6.5	6.7	6.4	6.0	6.6	6.4	6.4	6.3
	Cut 2		-	-	-	-	5.0	5.2	4.7	5.2	5.1	5.0	4.9	4.9	4.9	5.1
Average			5.5	5.0	5.3	5.3	5.2	5.4	5.6	5.8	5.1	5.4	5.4	5.2	5.3	5.5

Table 18. Means of Hs populations and Engmo (E) for quality characters

	Series	Location and cut	Year offer	Population											
				011035		011035		011043		011012		011024		011025	
				Hs	E	Hs	E	Hs	E	Hs	E	Hs	E	Hs	E
IVDDM (%)	A	Holt, cut 1	1	68,1	87,2	67,5	67,0								
		" 1	2	72,8	73,3	72,9	72,8								
		" 2	2	77,0	77,2	79,6	79,7								
		Alta, cut 1	2	72,2	71,0	71,9	72,9								
		" 2	2	77,7	77,3	77,3	77,2								
	B	Holt, cut 1	1					73,3	74,0	73,6	73,7	72,9	72,7	73,7	74,5
		" 2	1					79,8	79,5	78,2	77,8	77,6	78,5	79,3	79,7
		"1	2					68,1	67,6	71,3	70,5	68,9	68,7	67,4	68,0
		Alta, cut 1	1					71,4	71,7	70,9	70,1	70,8	71,1	70,3	70,6
		" 2	1					78,6	78,6	79,2	78,5	78,3	78,1	78,8	79,5
		" 1	2					68,5	69,4	68,9	69,1	68,3	68,1	68,4	69,1
Average				73,6	73,2	73,8	73,9	73,3	73,5	73,7	73,3	72,8	72,9	73,0	73,6
Prot. (%)	A	Holt, cut 1	1	11,1	12,6	11,9	11,8								
		" 1	2	14,8	15,5	14,5	14,1								
		" 2	2	11,7	11,9	12,8	11,3								
		Alta, cut 1	2	11,5	14,1	15,7	15,6								
		" 2	2	21,4	20,9	21,3	21,8								
	B	Holt, cut 1	1					12,3	13,4	12,4	13,0	10,6	12,2	11,9	11,0
		" 2	1					15,9	16,6	15,8	15,0	16,4	16,7	16,2	15,1
		"1	2					10,2	10,4	12,4	12,1	10,7	11,9	9,8	9,4
		Alta, cut 1	1					13,3	14,2	10,0	9,4	10,1	9,8	10,6	10,2
		" 2	1					18,3	20,3	17,7	16,9	18,3	19,4	18,8	18,6
		" 1	2					11,5	13,1	11,3	11,5	11,3	10,4	11,5	13,0
Average				14,9	15,0	15,2	14,9	13,8	14,8	13,4	13,2	13,1	13,6	13,3	13,1
NDF (%)	A	Holt, cut 1	1	72,0	71,5	72,0	73,4								
		" 1	2	62,4	61,5	62,0	62,2								
		" 2	2	49,4	50,8	49,9	48,7								
		Alta, cut 1	2	63,5	63,2	63,5	62,8								
		" 2	2	45,4	44,5	45,4	45,0								
	B	Holt, cut 1	1					60,6	60,5	61,0	60,9	62,4	62,6	61,0	59,4
		" 2	1					48,5	57,3	50,4	49,5	57,7	54,8	50,6	48,1
		"1	2					64,5	63,9	64,3	63,5	66,1	63,0	67,2	68,0
		Alta, cut 1	1					63,3	62,2	63,2	64,1	64,3	64,3	64,2	62,4
		" 2	1					49,6	51,9	47,3	48,5	48,5	48,6	48,4	47,0
		" 1	2					60,2	58,0	58,7	58,9	60,1	58,1	60,3	57,4
Average				58,5	58,3	58,6	58,4	57,8	57,8	57,5	57,6	59,9	58,6	58,6	57,2

slightly from the mean values of Engmo (Table 17).

Quality

Table 18 presents the population means for the available data on *in vitro* digestability, protein content and the content of neutral detergent fibers (NDF). Highly significant genetic variations were found within populations for all these quality measures in individual cuts. However, the results were not consistent over locations, years and cuts for any of these characters. The IVDDM-% was in all cases highest in the second cut, as was the content of protein, with the exception of the two populations of series A in the second year at Holt.

The NDF-% as a measure of partly digestable cell wall contents (CWC) was always lower in the second cut. The relationships between the various quality measures found here seem to be quite common (cf. Østgård 1962, Østrem 1990).

The differences between the population means were relatively small (Table 18), as were the differences between the population means and the means of the standard variety Engmo. The data do, however, indicate that sufficient genetic variation exists within populations for considerable progress by selection.

DISCUSSION

The origin of cultivated timothy is not fully known (Witte 1915; Vestad 1953). In Norway and in the other Scandinavian countries timothy seems to have been grown as a fodder crop for 200 - 250 years (Schuebler 1886; Witte 1915). Towards the end of the last century there seems to have been a growing interest in the Nordic countries for taking up breeding work in timothy as well as in other herbage crop plants (Wexelsen 1971). Usually the breeding work was initiated by collection of local land races which had been grown and seed propagated on private farms over many generations. The next step was to conduct series of trials in which the local populations or land races were compared with each other and with varieties or local populations from other countries. Such series were organized in South Norway by Bastian Larsen (1899) in the 1880s, and were reported upon by Vik (1917). Similar series were later organized in other parts of our country, the results of which have been published in a number of reports (Elle 1930; Fjærvoll 1935; Vestad 1953; Østgård 1959; Hillestad et al. 1964 and others). The main results from these series of trials are that the locally adapted populations have been the best in respect of winter hardiness, and they have therefore produced the most stable and, on average highest yields.

In Sweden Witte (1912) had shown that timothy from different locations was highly differentiated with respect to many morphological and physiological characters. Studies by Foss (1968) and, later, by Heide (1982) have shown that adaptation is strongly correlated with latitude of origin and thereby related to photoperiod. Types adapted to the growing conditions in the North start growth later in the spring than southern types. The northern types do, however, grow faster in the early summer, but cease growth earlier in the autumn. These differences in growth rhythm are related to the superior winter hardiness of the northern types.

Andersen (1971), working at Holt Research Station in Tromsø, has demonstrated very clearly that adaptation to northern growing and reproduction conditions as a result of natural selection is a reversible process. Andersen (1971) found that seed propagation of the variety Engmo in southeast Norway from one to six generations led to a reduction in winter survival in his field experiments in Tromsø, from 79.1 % for Engmo seed propagated continuously in Tromsø to 54.0% for the same variety of seed propagated over six generations in southeast Norway.

Witte (1912) demonstrated great genetic variation within local populations for many typical quantitative characters of economic interest. A fairly high proportion of the plants within his local populations were weak or off-types of various kinds. Wexelsen (1971) reported similar variations in his unpublished studies. The conclusions of our studies are

exactly the same on this point. The local populations are genetically very heterogeneous. From a theoretical point of view one would expect reduced variability or even fixation for a fitness character like winter hardiness in local populations from the most marginal areas. The variety Engmo has proved to be extremely winter hardy, not only in Norway but also in northern regions of Sweden and Finland (Isatalo 1966). The loss of adaption by seed multiplication in South Norway reported by Andersen (1971) and mentioned above as well as our variability experiments at Holt and in Alta indicate the same tendency. Engmo is not at all homogeneous for winter survival. The same is the case with the other local populations from Finnmark and Troms. In our trials the recently collected local populations have been nearly equal to or somewhat inferior to Engmo on average (Table 15), but displaying with considerable genetic variations within populations. Based on these results, one would expect that it should be possible to establish synthetics with better winter hardiness than Engmo.

The high genotypic correlations presented in Table 13 between winter survival and DM-yield in the first cut at Løken and in Alta clearly show that winter hardiness is an important yield component. At Løken as well as in Alta, high and consistent negative genotypic correlations were found between coverage in the spring and DM yield in the second cut. Although we have no data that can explain these negative correlations directly, we think that the main explanation is to be found in the growth rhythm. Good winter survival is probably associated with fast growth in the early summer, but also with early growth cessation.

The variation in winter hardiness could to a large extent explain why so many HS-families and mixed pc progenies surpassed the best variety (Engmo) for DM yield in Alta and Løken (Table 8). In the Ås trial winter hardiness was not at all important, since all families and varieties had coverages in the spring of nearly 100% in all years. Under these conditions only two HS-families gave higher DM yields than the best market variety (Grindstad, Table 8).

Tables 10 and 11 indicate that large genotypic variations exist in the material for the quality characters IVDDM-% and protein content. It appears from these tables that Grindstad, the most common and most high yielding market variety in the lowlands and along the west coast of South Norway (cf. Aastveit 1991), is rather inferior in these quality traits as compared with Engmo and most of the HS-families and mixed pc progenies tested in the present work. This result is in good agreement with the results from the most recent variety trials (Foss & Bö1991).

Genotypic correlations based on the data from the Ås trial (Table 12), where winter hardiness was unimportant, demonstrated strong negative relationships between DM yield on the one hand, and protein content and IVDDM-% on the other. These strong correlations in an undesired direction will make it difficult to combine high DM yield, high protein content and high digestability in regions where winter stress is low. The data do, however, show that it is easy to combine good winter hardiness with high protein content and high in vitro digestability, and thereby it should be possible to achieve the desired combinations in regions where winter hardiness is an important component of DM yield. In Table 11 it can be seen that the mixed progeny from pc 23/79 had protein- and IVDDM percentages comparable to Engmo, while Table 14 indicates that the mixed progeny of 23/79 surpassed Engmo in Alta with 12% and 10% for DM yield and digestable yield (D),

respectively. It should be remembered that pc field 23/79 had clones from Engmo and Grindstad (Table 1). The results obtained from this polycross indicate that in the breeding work for northern and high altitude regions it may be advisable to base the breeding population on crosses between northern winter hardy and high quality populations and high yielding populations from southern regions.

The results and materials reported upon in this article have been used to establish new synthetic populations. These synthetics have now been seed-multiplied and are ready for testing in a series of variety testing trials.

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Use of a modified rising-plate meter to assess changes in sward height and structure

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Two designs of rising-plate meter were used to assess changes in mean compressed sward height (CSH), sward structure and sward height grazed. Mean CSH decreased significantly over each grazing period ($P < 0.001$) and between days ($P < 0.05$) within periods. Significant regressions were obtained between CSH and pasture dry matter (DM) yield (kg ha^{-1}), with the overall equation: $335.2CSH-4.3CSH^2-1585.7$ ($P < 0.001$). Due to non-uniform defoliation, the frequency distribution of CSH measurements (sward structure) became skewed and DM accumulated at CSH values below 10 cm. No appreciable build-up of mature material occurred. The measurement of fixed points over time showed that the decrease (D) in CSH due to grazing was significantly correlated with the initial CSH height: $D = 0.277CSH^2-0.043CSH-0.1208$ ($r^2 = 0.923$, $P < 0.001$) and that little apparent grazing occurred below a CSH height of 6.5 cm. It was identified that 200 samples ha^{-1} were required in order to obtain accurate mean values and provide sufficient samples to describe changes in sward structure.

Key words: CSH determination, dry matter yield, grazing

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An accurate assessment of the herbage mass available to the grazing animal is vital if pasture is to be efficiently utilised. Attempts have been made to estimate this as either the undisturbed sward height (sward stick, e.g. Bircham 1981) or the compressed sward height (rising-plate meter, e.g. Castle 1976). The values obtained with the latter are used to produce regressions related to herbage mass. Where swards are uniform, an estimate of the mean CSH, standard error and herbage mass per unit area are sufficient but over a grazing season a mosaic of mature plant material and overgrazed areas develops and more detailed measurements are required (Gibb & Ridout 1986). This paper describes the use of a modified rising-plate meter to identify sward height grazed and also the change in sward structure (height distribution) due to grazing.

MATERIALS AND METHODS

The sward production studies presented here are taken from an experiment in which the effects of providing supplementary roughage to grazing dairy cattle were investigated (Mould 1992). Two designs of rising-plate meter (RPM) were used to obtain compressed sward height (CSH) values from 1.0 ha of rotationally grazed permanent pasture. These

were obtained over four days (weeks 31 and 34) and three days (week 37). The larger of the two meters (LM) had a base plate diameter of 300 mm and weighed 200 g. This was used to take CSH measurements at approximately 1.0 m intervals along three equally spaced lines running the length of the field (180 m). This technique produced approximately 580 values per estimate and represented 0.4% of the total area (9990 m^2) being measured directly. Measurements were taken twice daily (08.00 h and 17.00 h). The smaller meter (SM) constructed by A.M. Services, N-4340 Bryne, had a base plate diameter of 95 mm and exerted a similar force per unit area to that of the LM. This was used to obtain CSH values at 10 cm intervals from three 5.0-metre randomly placed transects (153 measurements per estimate). The position of each transect remained constant throughout the study. Values were obtained before the start of each observation week and daily (16.00 h) during the week. All CSH measurements were taken to the nearest 0.5 cm, with bare or poached ground, areas contaminated with faeces or where the grass had been flattened given a value of zero.

Dry matter (DM) yield was assessed by cutting 0.25 m^2 quadrants with known CSH values (mean value of four measurements within each quadrant) to a height of 1.0 cm and drying the harvested material to constant weight at 80°C. Yields from 68 quadrants, with a CSH range of 5.0 to 16.0 cm, were estimated during the trial. Regressions were then produced relating CSH to DM yield. Pasture DM production over each of the three observation periods was estimated by means of growth cages.

Pasture intake (equivalent to the decrease in DM) was estimated by comparing CSH values over the observed grazing period, and including a correction factor for grass production. Increased precision was obtained by grouping the CSH values at 2.0 cm intervals and calculating the proportion of measurements within each division. The area of pasture (proportion of observations x 9990 m^2) within each division was then multiplied by the derived DM yield and the values totalled to give an estimate of the total yield. In addition, by comparing the range of CSH values before and after grazing, the preferred sward height grazed could be identified.

RESULTS

A total of 21 CSH estimates, comprising 12,172 measurements, were made over the three observation periods with the LM. Not only were mean CSH values found to decrease significantly over each period (Table 1), but significant changes could also be detected after each daily grazing period. CSH values were not reduced uniformly, as the proportion of values within each horizon altered significantly over each observation period (Table 2). In all three periods the proportion of CSH values in the highest horizons decreased, while those in horizons below 9.0 cm increased. Fourteen CSH estimates (2142 individual measurements) were taken with the SM. Similar values to those of the LM were obtained, with mean CSH decreasing significantly over the grazing period and between days within observation periods (Tables 1 and 2). A significant change in the proportion of measurements within horizons over each period was also recorded.

DM yield (kg ha^{-1}) was highly correlated ($r = 0.888$) with LM CSH according to the regression equation: DM yield = $335.2CSH - 4.3CSH^2 - 1585.7$ ($r^2 = 0.789$, $P < 0.001$).

Table 1. Mean CSH values obtained using the 95 mm (S) and 300 mm (L) rising-plate meters

		Week					
		31		34		37	
Time [1]	CSH	L	S	L	S	L	S
1B	cm	10.79^{a}	10.47^{a}	11.53^{a}	10.76^{a}	12.88^{a}	11.29^{a}
	s.d.	2.85	2.63	2.87	2.32	3.12	2.94
1A	cm	(9.39)	8.8^{b}	10.85^{b}	10.23^{a}	11.17^{b}	10.28^{b}
	s.d.	.	1.76	1.96	2.71	3.16	3.21
2B	cm	9.10^{b}	.	9.68^{c}	.	10.54^{c}	.
	s.d.	2.60	.	3.08			
2A	cm	8.38^{c}	(8.70^{bc})	9.17^{d}	8.33^{b}	9.83^{d}	8.55^{c}
	s.d.	2.39	.	2.95	2.78	2.64	2.54
3B	cm	8.19^{cd}	.	8.81^{ef}	.	9.30^{e}	.
	s.d.	2.60	.	2.78	.	2.78	.
3A	cm	7.92^{e}	8.04^{cd}	8.97^{de}	7.99^{b}	8.80^{f}	8.07^{c}
	s.d.	2.45	2.60	2.81	2.72	2.50	2.70
4B	cm	7.79^{ef}	.	8.56	.	.	.
	s.d.	2.65	.	2.71	.	3.12	.
4A	cm	7.57^{f}	7.75^{d}	8.25^{g}	7.81^{b}	.	.
	s.d.	2.27	2.14	2.69	2.80	.	.

Estimated values are given in parentheses. Values in columns without common superscripts are significantly different ($P > 0.05$).

[1] 1, 2, 3 and 4 = observation day, B = before grazing, A = after grazing

Table 2. Sward structure and DM yield before (B) and after (A) grazing according to compressed sward height(CSH)

Week	CSH (cm)	Area (%)		Yield (kg DM ha^{-1})	
		B	A	B	A
31	0	0.9	2.1	0	0
	0.5 -5.0	0.7	9.8	0	0
	5.5 -9.0	28.1	69.7	204.7	407.3
	9.5 -13.0	54.9	16.8	784.4	208.4
	+ 13.0	15.4	1.6	360.2	34.3
	Total	-	-	1349.3	650.0
34	0	1.5	3.1	0	0
	0.5 - 5.0	0	5.4	0	0
	5.5 - 9.0	17.3	62.3	184.2	577.6
	9.5 - 13.0	56.7	25.6	1010.6	414.7
	+ 13.0	24.5	3.6	628.3	91.6
	Total	-	-	1823.1	1083.9
37	0	0.7	1.8	0	0
	0.5 - 5.0	0.2	3.2	0	0
	5.5 - 9.0	11.6	56.5	157.5	740.6
	9.5 - 13.0	41.3	35.0	788.7	627.9
	+ 13.0	46.2	3.6	1165.2	84.4
	Total	-	-	211.4	1425.9

The relationship between CSH and pasture DM yield was also estimated for each observation period:

$Y31 = 5.75CSH^2 + 108.3SCH - 577.9$ ($P < 0.001$, $r^2 = 0.832$)
$Y34 = -11.23CSH^2 + 483.6CSH - 2209.8$ ($P < 0.001$, $r^2 = 0.645$)
$Y37 = 7.84CSH^2 - 40.1CSH + 1301.9$ ($P < 0.01$, $r^2 = 0.477$),

where Y31, Y34 and Y37 represent DM yields in weeks 31, 34 and 37, respectively. Not only did the estimated pre-grazing DM yields increase over the trial period (Table 2), but sward structure also varied between periods with the proportion of material initially found in the 5.5 to 13.0 cm horizons decreasing and that above 13.0 cm increasing. Over the grazing period DM accumulated in the lower horizons, especially at 5-9 cm, but decreased in the two highest horizons. Pasture production over the first grazing period (week 31) was approximately 5 g DM m^{-2} d^{-1}, while no measureable production was observed during the later two periods. Using CSH values, and including DM production, mean individual pasture intakes were estimated to be 10.9, 11.1 and 10.1 kg DM d^{-1} for weeks 31, 34 and 37, respectively.

DISCUSSION

Despite the differences in design, the number of CSH values taken per estimate and the total area assessed, both meters gave similar results. CSH values were significantly correlated ($r = 0.951$) according to the equation: $LM = 1.18SM - 1.056$ ($r^2 = 0.905$, $P < 0.001$). While Gibb et al. (1989), Mayne et al. (1990), Prache et al. (1990) and Hoden et al (1991), took 125-250, 400, 256 and 150 measurements ha^{-1}, respectively, other researchers used relatively few (Leaver 1982; Phillips & Leaver 1985; Baker & Leaver 1986: 10-12 ha^{-1} and Arriaga-Jordan & Holmes 1986: 60 ha^{-1}). The LM data were therefore randomly sampled to investigate the accuracy of using between 25 and 500 values ha^{-1} to determine mean CSH. Little benefit was derived when more than 200 values ha^{-1} were taken (Table 3), but using fewer than 200 values ha^{-1} gave variable mean CSH values and decreased the accuracy with which these were determined.

Table 3. Effect of number of values on the accuracy in determining mean compressed sward height (CSH)

Week	CSH	Number of values						
		25	50	100	200	300	400	500
31	cm	10.5	11.4	11.1	10.9	10.8	10.8	10.8
	s.d.	3.1	3.5	3.1	2.8	2.6	2.8	2.8
34	cm	11.9	11.8	11.8	11.6	11.5	11.6	11.6
	s.d.	4.4	3.1	2.8	2.9	2.8	2.8	2.9
37	cm	12.9	13.4	13.2	13.1	13.0	13.2	12.9
	s.d.	3.3	3.4	3.2	3.1	3.1	3.0	3.0

Because of the relatively low compressive force (2.83 kg m^{-2}) exerted by both meters actual

CSH values should not be compared directly with those obtained with heavier plates such as the "MMB meter" (Mayne et al. 1990) or the "Massey meter" (Michell 1982), which exert forces of 4.8 and 4.5 kg m^{-2}, respectively. Bransby et al. (1977) found little effect of increasing the downward force from 5 to 15 kg m^{-2} on the accuracy with which DM yield could be determined, but it is possible that due to the high compressive effect exerted by 5 kg m^{-2} the use of a lighter, rather than heavier, plate may have increased the accuracy with which this relationship is determined. The regressions used to estimate DM yield differed between weeks and showed an increasing loss of precision over the trial period. This was partly due to fewer observations (30, 22 and 16 for weeks 31, 34 and 37, respectively) and partly to the increased variation within the sward structure, indicating that recalibration of the meter over the pasture season is required. This was also reported by Michell (1982) although he found, when using a perennial ryegrass/clover sward, that regression correlations were normally constant over an extended period of time (months).

Sward structure was found to change as a result of grazing (Figs. 1 and 2) with the frequency distribution of CSH measurements becoming skewed and material accumulating in the lower divisions. Using a chi-squared analysis, the shape of the curve was found to change significantly ($P < 0.05$) suggesting that all grass heights were not uniformly grazed. The highly significant correlation ($r = 0.961$) between initial CSH and the decrease due to grazing (D), where $D = 0.277CSH^2 - 0.043CSH - 0.1208$ ($r^2 = 0.923$, $P < 0.001$), indicated that little apparent grazing occurred below 6.0 cm (Fig. 3). This was comparable to the finding of Meijs & Hoekstra (1984) who, using a meter with a compressive force of 4.77 kg m^{-2}, observed that even at high stocking densities cattle did not consume herbage below 4.0 cm. Michell (1982) concluded that the accumulation of herbage at low CSH values may be of little value to the grazing animal. However, a grass plant grazed to 6.5 cm will be in a different morphological state to a young, ungrazed plant with a similar CSH value and therefore subject to a different degree of grazing. Gibb & Ridout (1986) argued that summarising CSH values using the sample mean and standard error could be misleading where a mosaic of frequently and infrequently grazed areas had developed.

Total DM yield is not equivalent to that available for grazing as a proportion of the herbage present will be rejected. In this study it was found that, due to the relative short grazing periods utilized, there was no appreciable build-up (rejection) of herbage at high CSH values, nor was grazing pressure so severe that grass with low CSH values had to be grazed. If material with a CSH value of 6 cm or less is considered as unavailable, this represented 2.3, 7.1, 12.3 and 17.0% of the total grazing area available (days 1 to 4, respectively) but had little effect on available pasture DM. Apparent daily pasture intake, as assessed by the change in CSH values decreased markedly within grazing periods and further work is required to examine the significance of this effect in conjunction with sward selection and the intake of supplementary feeds.

Although similar results were obtained with the two meters the LM is better suited for obtaining CSH values on a field scale, while the SM is ideal for more detailed work such as measuring changes in sward structure or height of fixed points over time. It will also be of more use in experimental plot work or in amenity grass studies where more detailed observations have to be taken. Further work is in progress to evaluate these possibilities and to examine the effect of range of plate weights on the accuracy of determining DM yield.

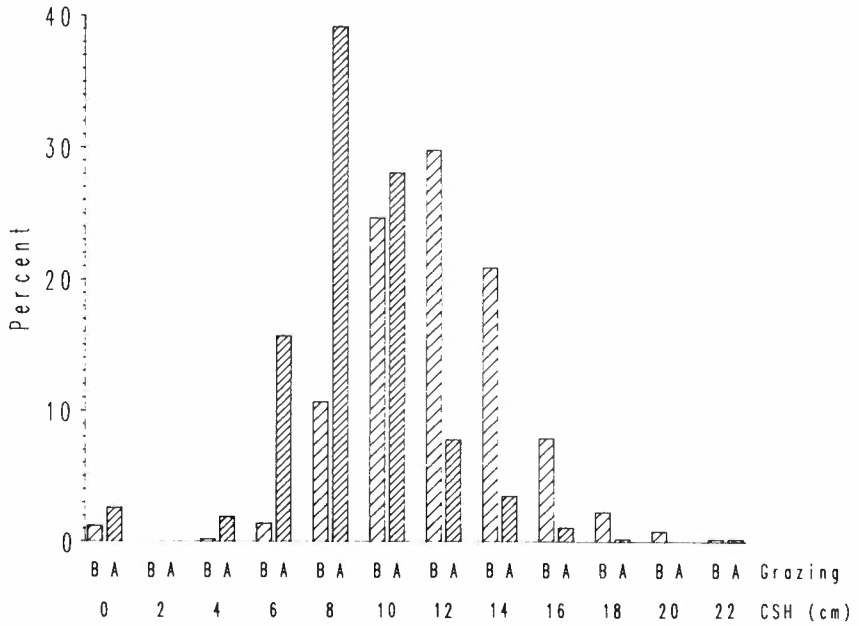

Fig. 1. Effect of grazing on the compressed sward height (CSH, cm) distribution frequency before (B) and after (A) grazing: 300 mm rising-plate meter

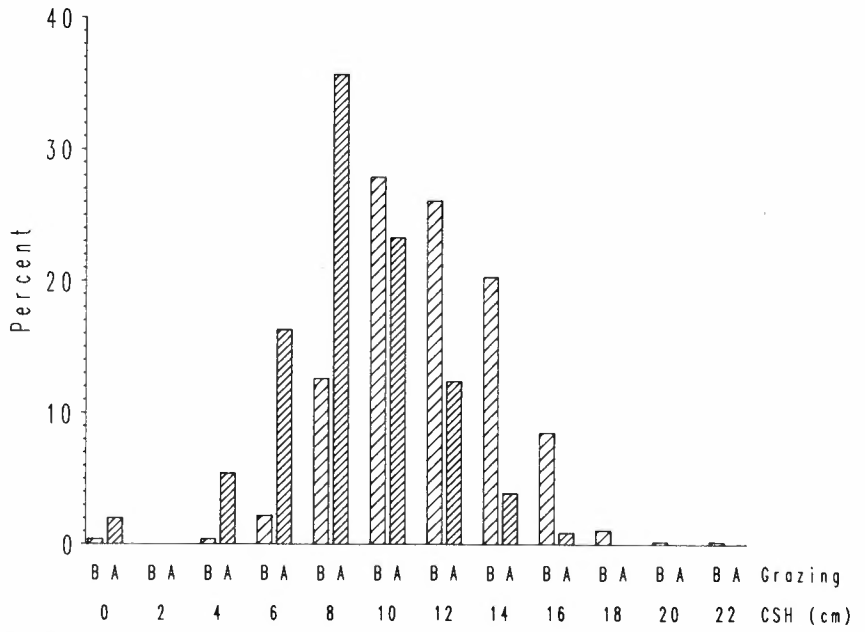

Fig. 2. Effect of grazing on the compressed sward height (CSH, cm) distribution frequency before (B) and after (A) grazing: 95 mm rising-plate meter

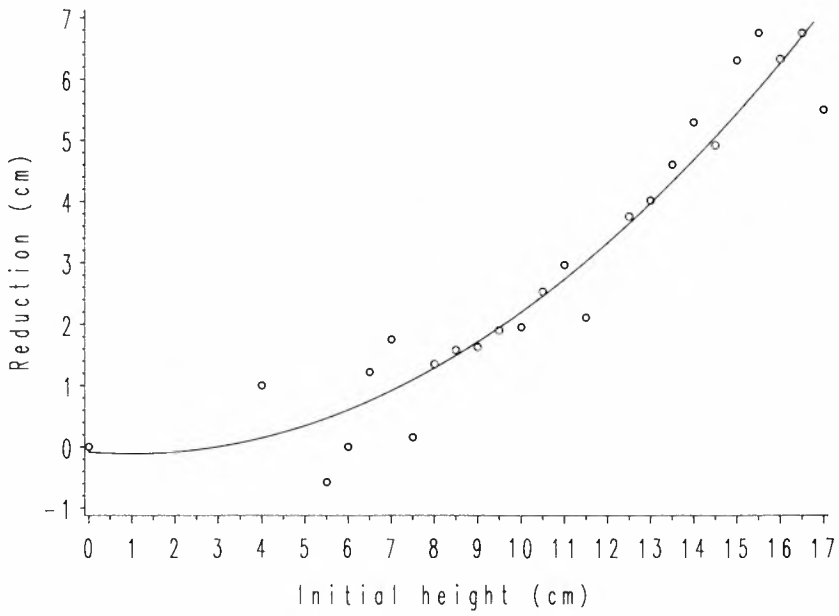

Fig. 3. The decrease in compressed sward height (CSH, cm) due to grazing as influenced by the initial CSH

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The influence of supplementary silage quality on milk production and performance of grazing dairy cattle

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The buffer-feeding system was used to examine the influence of supplementary silage quality on milk production, feed intake and grazing activity. High-yielding NRF cows (mean 27.1 kg d^{-1}) offered good quality silage were found to produce more milk, with a lower fat content, and more fat-corrected milk than those offered poor quality silage. This effect was not found with low- yielding cows (mean 20.1 kg d^{-1}). The cattle given poor silage consumed less than those given the better quality material, and while they compensated to some extent by increasing the time spent grazing, total intakes were insufficient to meet requirements and resulted in a decrease in either milk production (high-yielders) or liveweight (low-yielders). Grazing time was found to decrease, and supplementary feed consumption increase, as grass height was reduced under grazing.

Keywords: Buffer feeding system, feed intake, grass height, grazing activity

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The "buffer-feeding" system, which allows cattle to self-regulate supplementary feed intake has proven to be an effective method of offering additional feed to grazing dairy cows (Phillips & Leaver 1985). Ideally, a high quality roughage is used which is consumed only when grazing is insufficient to meet production requirements and not in preference to pasture. In a two-year study Mould (1992a) found that, using such a system, cattle offered baled-silage produced more milk and consumed more pasture dry matter than those offered ryegrass. Although the results suggested that baled-silage is a useful supplement for grazing cattle, many producers believe that it is unsuitable for high yielding cows. In this study the influence of silage quality on milk production, feed intake and grazing activity was examined.

MATERIALS AND METHODS

Twenty-four NRF spring-calving cows were blocked in groups of two on the basis of fat-corrected milk (FCM) yield (weeks 28-30), days since calving, liveweight and parity. One type of silage was then randomly allocated to each animal within a block. Two qualities of silage were produced, using the same primary growth of Italian ryegrass (*Lolium*

multiflorum, var. Meritra), by cutting either just prior to 50% shooting ("good") or after heading ("poor"). The grass was left to wilt overnight, an additive (FORAFORM, Norsk Fôrkonservering) was included at the rate of 4 l t^{-1} and the bales were wrapped with four layers of white plastic film. The silages were chopped (5-10 cm) prior to feeding and were made available *ad libitum* for 60 min daily following the a.m. milking, from week 31 until the end of the grazing season (week 41). The cattle grazed five paddocks (total area 5.6 ha) of permanent pasture on a rotational basis. In addition, all cattle received a basal ration of 3.0 kg concentrate daily and, from week 35, a small quantity of fresh ryegrass.

Records

Milk yields were measured on three consecutive days per week and an a.m./p.m. sample taken for composition and quality analysis. Intakes of concentrate and supplemental feeds were recorded individually five days per week and representative samples of all feeds offered, including pasture, were taken daily for dry matter and chemical analysis. Mature Dala rams were used to estimate the digestibility coefficients of samples of both silages taken at intervals during the study. Data from these studies and the chemical analyses were used to calculate feed energy values, expressed as feed units milk (FE_M) (Van Es 1975; Ekern et al. 1991). The cattle were weighed fortnightly. Herbage energy intakes were calculated as total FE_M requirements less the known FE_M intakes. A behaviour study was conducted to investigate the effect of silage quality on grazing activity. These observations were made on ten occasions (weeks 31 and 34 - four consecutive days and week 37 - three days). Individual activity was recorded at ten-min intervals during the period between milkings (ca. 09.00-15.30 h) according to the parameters listed in Table 3. For fuller details of this study, see Mould 1992c. Sward height was assessed as compressed sward height (CSH) using the method detailed by Mould (1992b).

Data handling and analysis

The results were analysed using SAS procedures (SAS 1987) to generate LSM and significances of difference. Covariates obtained in the pre-trial period, weeks 28-30, were used in the analyses of milk production data. Since calculated values (fat-corrected milk (FCM), fat, protein and lactose yields and effects of initial yield) are generated independently they may not necessarily be equal to the sum of their separately determined mean values.

RESULTS

In comparison with good silage, the poorer material had a higher fibre and lower nitrogen content and lower *in vitro* and *in vivo* digestibilities (Table 1). Energy contents (FE_M kg DM^{-1}) were calculated as 1.17 and 0.94 for the good and poor silages, respectively. Pasture quality varied only slightly over the grazing season, while the high fibre and low nitrogen content of the ryegrass was due to the relatively late harvesting date. Although no significant effects of silage quality on milk production or composition were observed (Table 2), the cattle offered the good silage produced slightly more milk and FCM than the other

Table 1. Chemical composition of feeds offered (g kg DM^{-1})

Type	n		OM[1]	CF	ADF	KjN	*In vitro*	*In vivo*
Pasture	28	mean	898	216	224	40	827	-
		s.d.	1.1	3.8	3.6	0.9	8.3	-
Silage								
- good	6	mean	896	229	231	36	887	816
		s.d.	1.6	3.7	3.9	0.5	2.3	-
- poor	6	mean	900	290	297	27	754	736
		s.d	1.6	3.5	1.9	0.8	5.5	-
Ryegrass	7	mean	894	240	247	26	779	-
		s.d.	1.8	-	4.4	1.3	8.9	-
Conc.[2]	-	-	939	71	73	29	-	-

[1] OM - organic matter, CF - crude fibre, ADF - acid digestible fibre, KjN - nitrogen, *in vitro* - *in vitro* digestibility (g kg^{-1}), *in vivo* - *in vivo* digestibility (g kg^{-1})
[2] Conc. - concentrate

Table 2. Production parameters as influenced by silage quality (good and poor) and inital yield (high and low)

Silage		Good	Poor	Good	Poor	Good	Poor
Yield		-	-	High		Low	
Milk	(kg d^{-1})	22.99	22.17	25.14	23.55	20.42	20.33
FCM[1]	(kg d^{-1})	21.90	21.57	23.76	22.98	19.52	19.75
Fat	(g kg^{-1})	37.1	37.8	36.6	38.6	37.5	38.6
	(kg d^{-1})	0.843	0.850	0.914	0.904	0.757	0.756
Protein	(g kg^{-1})	33.3	33.1	32.7	33.1	33.6	33.6
	(kg d^{-1})	0.757	0.734	0.816	0.778	0.778	0.679
Lactose	(g kg^{-1})	48.5	48.6	46.8	48.4	49.6	49.8
	(kg d^{-1})	1.112	1.076	1.176	1.139	1.014	1.012
DMI[2]	(kg d^{-1})	3.25^{a}	2.30^{b}	3.36^{a}	2.47^{b}	3.14^{c}	2.12^{d}
ADG[3]	(kg d^{-1})	-0.188	-0.212	-0.132	-0.129	-0.243	-0.296

[1] FCM - Fat-corrected milk
[2] DMI - Silage dry matter intake
[3] ADG - Average daily gain
Mean in rows with similar letters are not significantly different ($p < 0.001$)

group. When blocked by initial yields, high-yielding cattle (mean 27.1 kg FCM d^{-1}) offered the good quality silage produced more milk and FCM than those given poorer silage. Little difference, however, was observed between silages when offered to cattle with low initial yields (20.1 kg FCM d^{-1}). Intake of silage was significantly ($p < 0.001$) influenced by the type offered and level of milk production, with greatest intakes recorded with good quality material and high-yielding cattle (Table 2). During the hour that the supplements were made available, the cattle consumed the good quality silage for a longer period and at a faster rate than those given the poorer silage (40.6 and 37.1 min and 84 and 77 g min^{-1}, for the two silages, respectively). No significant effects on liveweight change were found, although those offered the poor quality silage had slightly greater daily liveweight losses and high-yielding cattle a reduced liveweight loss (-0.130 kg d^{-1}) compared to low yielders (-0.269 kg d^{-1}, $p < 0.06$).

The cattle given poor silage grazed for a slightly longer period and spent significantly less time resting ($p < 0.05$) than those offered good silage (Table 3). Within weeks as grass height significantly ($p < 0.001$) decreased as a result of grazing (Fig. 1), the time spent grazing was significantly reduced and supplementary feed consumption increased (Table 4). No effect on either biting or cudding rate was observed. Calculated pasture intakes (Fig. 2) showed that the intake of high-yielders was greater than that of low-yielders and those offered poor silage consumed more pasture than those given good silage.

Table 3. Effect of silage quality on grazing activity, expressed as a percentage of the observed time

Week	Silage	Grazing	Ruminating	Resting	Drinking/ other
31	Good	36.8	33.3	23.9	6.0
	Poor	39.5	35.1	19.1	6.3
34	Good	40.5	35.2	18.2	6.1
	Poor	46.4	33.6	15.5	4.5
37	Good	43.2	31.1	20.1	5.6
	Poor	44.6	30.5	20.0	4.9
Mean	Good	40.1	33.2	20.7	6.0
	Poor	43.7	33.2	17.9	5.2

DISCUSSION

No significant effects on either milk production or milk composition were obtained when different qualities of silage were offered using the buffer-feeding system. This was most probably due to the late, cold spring which delayed the first cut until week 27. As supplemental feeds had to be offered from week 31, limited time was available to allow grass quality to decline sufficiently to produce silage of a markedly poorer quality. Milk production and composition and intake values obtained with the good silage were similar to those found by Mould (1992a), when cattle were offered silage produced from

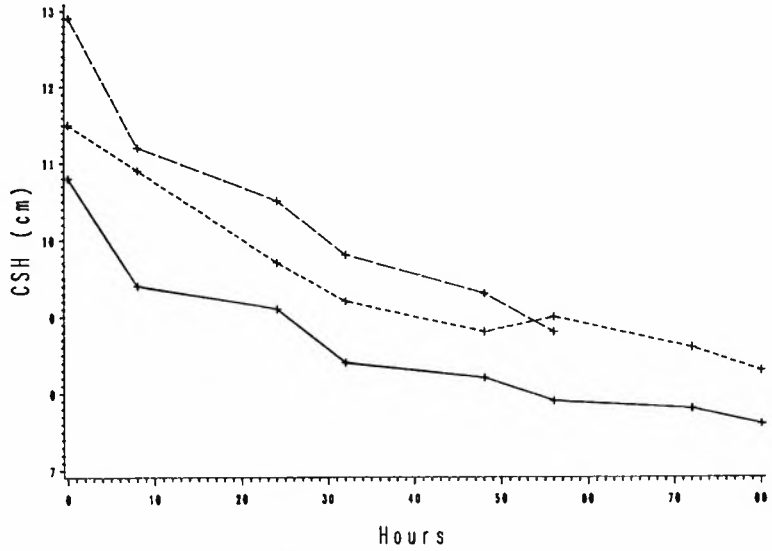

Fig. 1. Reduction in compressed sward height (CSH) due to grazing (days). Week 31———, week 34 - - - - - - and week 37 — — — —

ryegrass cut in week 25.

When analysed on the basis of initial yield, a response to silage quality was observed. High-yielding cattle offered good silage produced more milk, with a lower fat content and more FCM than those given poor quality silage. This trend was not observed with the low-

Table 4. Correlation coefficients relating compressed sward height (CSH), grazing activity and supplemental feed intake to duration spent grazing a given pasture

		Correlation coefficient (R)	Significance (p <)
CSH		-0.874	0.001
Grazing activity	- good silage	-0.895	0.001
	poor silage	-0.757	0.01
Silage intake	- good	0.465	n.s.
	- poor	0.094	n.s.

yielding cattle. The cattle offered the poorer quality silage were in part able to compensate for the lower level of supplementary energy by increasing pasture intake. This group grazed for a longer period of time and grazing activity was influenced less by CSH than those cattle offered good quality silage. Despite this, total intakes were insufficient to meet requirements and resulted in a decrease in either milk production (high-yielders) or liveweight (low-yielders).

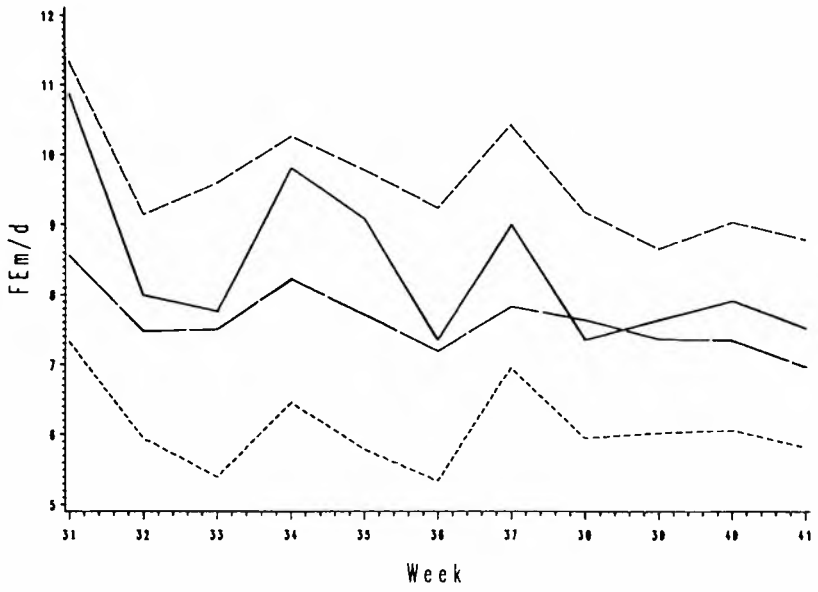

Fig. 2. Pasture intake, expressed as feed units milk (FE_M), as influenced by silage quality (good - G, poor P) and initial milk yield (high - H, low - L) GH ——— , GL -----, PH-- – – – and PL —— —— ——

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Restoring abandoned pasture by mowing - influences on frequency and cover of plant species

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Losvik, M.H. 1992. Restoring abandoned pasture by mowing - influences on frequency and cover of plant species. Norwegian Journal of Agricultural Sciences 6: 391-409. ISSN 0801-5341.

Abandoned pasture at Bergen, western Norway was mown once a year from 1983 till 1990. The ground layer, consisting mainly of *Rhytidiadelphus squarrosus* was removed before the experiment started. Three different mowing times were used. The cut sward was removed from the fields immediately after mowing. Out of a total of 39 species, 7 species were present both at the start and at the end of the experiment in all five plots. Six species disappeared during the experiment; 18 species appeared during the course of the experiment, but only one of these appeared in the control plot, while 9 of them appeared late in the experiment or persisted till 1990. Some grassland species, already present when the experiment started, increased in frequency and cover. Six species spread to other plots. Management by removal of the bottom layer every tenth year, cutting rather late once a year and spreading of the cuttings over the mown area, in order to avoid depletion of the soil are recommended.

Key words: Abandoned pasture, cutting times, plant species, turnover

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Mowing may be a useful alternative management regime in grasslands which have been traditionally grazed, but which are now difficult to fence, e.g. small or patchy nature reserves, recreation areas or parks. Management by mowing requires a light mowing-machine used for a short time once a year, and is an easy way to maintain open areas. It will prevent the regeneration of scrubs and trees and the resulting grassland will be suitable for recreation purposes, especially in spring and in autumn. During mid-summer, access should be restricted, but the hay meadow will by then offer a spectacular sight of wild flowers and grasses.

The time of cutting may be important to plant species composition of the sward, as may the effect on species composition of the removal of a luxuriant ground layer and of cutting and removal of the hay without applying any fertilizer.

The experiment described here aimed to test the following hypothesis:

1. Removal of most of the ground layer will increase the cover and frequency of hay meadow species at the expense of *Rhytidiadelphus squarrosus*, and create gaps where seeds may germinate.

2. Cutting in August will result in a greater species diversity than cutting at an earlier date (before 23 June).

The present experiment was set up to investigate the changes in occurrence, frequency and cover of species, when three plots were mown once a year, each consistently at different times in the summer, as compared to an uncut and an untreated plot, and to study the effect of the removal of the ground layer.

MATERIALS AND METHODS

Study area

The study area was located within The Norwegian Arboretum in Bergen, western Norway. It was situated in a former pasture, which had been abandoned for at least 10 years before the experiment was started. The pasture area was broken and comprised a mosaic of quite small patches which were either covered by a dense grass-sward or comprised rock outcrops dominated by cryptogams. The pasture had a scattered tree layer, mainly of oak (*Quercus robur*). The study site was situated about 30 m above sea level in a gently south-sloping area in one of the grass-dominated patches. It overlay a granitic bedrock (Kolderup & Kolderup 1940). The climate is oceanic, with a high humidity and low summer temperatures compared to the eastern parts of the country. Mean annual temperature is 7.8° C, no month having a mean temperature below 0° C (Bruun 1962). The precipitation is about 2300 mm a year (Det norske meteorologiske institutt 1981).

Sampling design and treatments

In 1982 five adjacent sites, each 1.7 m wide and 3.9 m long were set up, and numbered from 1 to 5 (Fig. 1). The sites bordered one another on the longer sides. A central 0.9 x 3.0 m^2 core (a plot) of each site was subdivided into 30 subplots of 0.09 m^2, for frequency analysis, and numbered from 1 to 30 (lower left to upper right subplot). Within each plot, two quadrats with sides 0.9 x 0.9 m^2 and containing subplots 4-12 and 19-27, respectively, were used to assess the cover of species. These quadrats were numbered from 1 to 10 (lower right to upper left quadrat). Tent pegs were used to mark the corners of sites and plots.

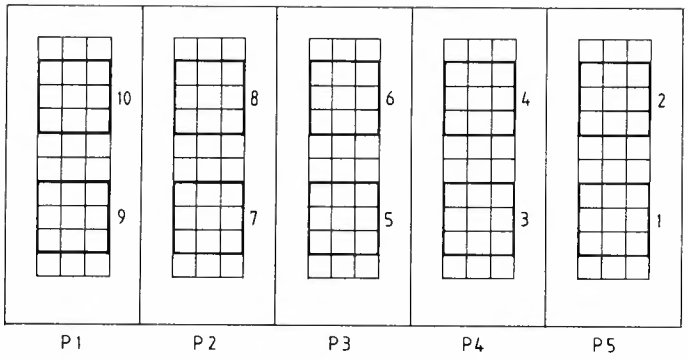

Fig. 1. Experimental design. Each site (P1-P5) has an inner plot divided into 30 subplots, used for frequency analysis. The quadrats 1-10 are situated inside the cores and are used for cover estimates

As a consequence of the layout of the experiment, each of the plots was surrounded by a buffer zone of at least 30 cm width which received the same treatment as the plot. Replicate plots would have added new variables, such as aspect and slope, to the experiment because of the broken topography of the area.

In November 1982 a ground layer of vegetation approximately 15 cm thick, consisting mainly of the moss *Rhytidiadelphus squarrosus*, which was quite evenly distributed all over the five sites, was removed from experimental plots 1 and 3-5 using a metal rake.

The ground layer from subplot 1 of each of the plots was collected as a sample, representing the ground layer in that plot, and dried at 105° C for 24 h. Each sample was then hand-sorted into cryptogams, dry leaves of trees, grasses and herbs, and twigs, which were then weighed (Table 1). Cryptogams consisted mostly of *Rhytidiadelphus squarrosus*, with a little *Scleropodium purum* and *Hypogymnia physodes*. Dry leaves of trees were mostly of *Quercus robur*, but also of *Betula pendula* and *Salix caprea*. Grasses and herbs included fresh material of *Agrostis capillaris*, *Veronica officinalis*, *Galium saxatilis*, *Rumex acetosa*, grasses, and seedlings of Rumex acetosa and grasses. Of the grasses and herbs category, 20-65 % was dead material, consisting of *Conopodium majus*, *Rumex acetosa*, other herbs and grasses. The sample subplot of plot 5 was atypical, with a greater proportion of grasses than the other ground layer samples.

Table 1. Total weight and weight of fractions of bottom layer (g) removed from one subsquare of each plot (P) 1-5 in 1982. Cryptogams = K, grasses and herbs = G/H, dry leaves of trees = L and twigs = T

	K	G/H	L	T	Total
P 1	44.7	11.7	14.2	3.3	73.9
P 2	52.8	9.1	3.9	3.9	69.7
P 3	46.3	12.1	12.3	7.6	78.2
P 4	35.6	12.8	6.9	3.0	58.4
P 5	0.6	24.6	5.6	0.7	31.5

In 1983-89 sites 1, 3 and 5 were mown once a year with a scythe. The cuttings were removed immediately after mowing. The stubble height was 6-8 cm. Site 1 was mown between 13 and 17 June. In the district, such an early cutting date implies no spring grazing and immediate removal of the grass for silage production. The farmers are therefore independent of weather conditions, and the date will be more or less the same each year. Site 3 was mown between 10 July and 5 August. The traditional time for cutting was after 10 July (Losvik 1988), but it varied according to the weather conditions as the grass from unmanured meadows had to be dried flat instead of on racks because of its stiffness. Site 5 was mown between 25 June and 5 July.

Site 2 received no treatment and was a main control. Site 4, where the moss layer had been removed (uncut experiment plot), was not mown at all and functioned as a control showing the sole effect of removal of mosses.

Recording of data

In 1983, 1984, 1987, 1988 and 1990 the occurrence of all flowering plants and bryophytes in each of the 150 subplots was recorded. Lichens were recorded, but not distinguished.

The species cover in the 10 quadrats was estimated by use of extended combined transformation values of the Hult-Sernander scale (van der Maarel 1979). In this scale, species represented in the quadrat by poorly developed individuals are in class 2. Classes 3, 4, 5, 6, 7 and 8 correspond to cover less than 1/16, 1/16-1/8, 1/8-1/4, 1/4-1/2, 1/2-3/4, 3/4-1 respectively. To facilitate the recording a net with 30 meshes, each 30 x 30 cm, was used to divide a plot into 30 subplots during analysis.

Variables

As the five sites were situated parallel to each other within a gently sloping area of approximately 4 x 9 m^2, the ecological conditions were considered to be fairly similar from one plot to another. Different management regimes were considered an important nominal factor, with the variables early (E), middle (M) and late (L) cutting time, uncut without mosses (C-) and control with the original ground layer (C+). The control with the ground layer intact was not presumed to be stable, but undergoing changes due to the previous abandonment. Time from 1983 until 1990 (1 - 8) is a semi-quantitative variable.

Data analysis

The overall change in species composition from 1983 till 1990 was assessed by correspondence analysis (CA) and canonical correspondence analysis (CCA) in CANOCO (Hill 1979; Hill & Gauch 1980; Jongman et al. 1987; ter Braak 1986, 1987a, 1987b), using (1) cover in 10 quadrats and (2) frequency in five plots, in five different years. Ordination of cover and of frequency of species showed only minor differences, and ordination of frequency is not further discussed. No covariables or product variables were defined, and equal weight was given to all species and samples. Sample scores are weighted mean species scores. An overall test using trace statistics and a test of significance of the first axis was performed using the Monte Carlo permutation test (Wichman & Hill 1982; ter Braak 1987b).

To compare the similarity of plots, the similarity index of Sørensen (1948) was used. Constancy classes I-V were used to denote that species occurred in less than 20, 21-40, 41-60, 61-80 and in more than 80% of the quadrats.

RESULTS AND DISCUSSION

Changes in species frequencies and cover (Table 2 & 3)

In 1983 the sward was quite scattered, with a luxuriant ground layer visible throughout. In the autumn dead leaves from nearby trees gathered in hollows in the sward. While the control showed no visible differences, the four experimental plots looked quite different in 1990 compared with in 1983. The sward was more even compared to the control, and the bottom layer was less well developed.

Table 2. Frequency of species (abbreviations, see Appendix) in plots 1-5, with different management (MAN), year, month and date of analysis and number of species (No.). MAN: medium cutting time (M), no cutting, moss layer removed (C-), late cutting (L), no cutting, with mosses (C+), early cutting time (E)

MAN	M	M	M	M	M	C-	C-	C-	C-	C-	L	L	L	L	L
Year	83	84	87	88	90	83	84	87	88	90	83	84	87	88	90
Month	06	06	07	07	09	06	07	07	08	09	07	07	07	08	09
Date	27	25	07	04	03	28	03	29	05	03	11	10	29	05	03
No.	12	16	13	13	15	11	16	14	14	14	11	13	12	14	14
Rhy squ	100	100	100	100	100	97	100	100	100	100	100	100	100	100	100
Agr cap	97	100	100	100	100	87	100	100	100	100	100	100	100	100	100
Fes rub	93	87	43	90	70	90	90	80	90	80	90	77	87	43	87
Rum ace	30	57	70	63	73	43	70	70	47	37	27	70	77	77	37
Ant odo	3	23	60	47	87	3	33	20	40	43	30	47	97	83	97
Con maj	67	83	67	60	77	53	80	73	53	77	40	67	63	43	80
Poa pra	97	97	40	33	40	90	67	23	60	93	97	97	47	73	57
Gal sax	17	27	40	53	50	17	53	70	80	57	40	60	100	100	53
Luz cam	27	37	13	20	40	23	20	7	.	7	30	27	60	30	27
Ver off	.	7	17	30	30	13	20	33	30	13	.	.	3	7	.
Ver cha	.	7	.	.	.	.	.	.	.	.	.	.	.	.	.
Scl pur	3	3	.	.	30	.	13	.	7	20	.	7	.	3	13
Pot ere	.	.	.	.	.	.	7	17	13	17	13	23	47	33	30
Hol lan	.	.	.	.	.	.	.	7	7	7	.	.	.	.	.
Que rob	.	.	.	.	.	.	.	.	.	.	3	3	3	3	3
Cam rot	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ane nem	3	3	3	3	.	.	.	.	.	.	.	.	.	.	.
Car pil	3	3	3	7	.	.	.	.	.	.	.	.	.	.	.
Car ova	.	.	.	.	.	13	10	.	.	.	.	.	.	.	.
Cen nig	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rub ide	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pol com	.	.	.	3	23	.	.	.	.	.	.	.	.	.	3
Pru vul	.	3	.	.	.	.	7	10	3	10	.	.	.	.	.
Cir pal	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ach mil	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Des ces	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ste gra	.	7	.	.	.	.	.	.	.	.	.	.	.	.	.
Dig pur	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.
Lic sp1	.	.	.	.	33	.	.	.	.	.	.	.	.	.	.
Lic sp2	.	.	.	.	23	.	.	.	.	.	.	.	.	.	.
Lic sp3	.	.	.	.	13	.	.	.	.	.	.	.	.	.	.
Nar str	.	.	.	.	.	.	3	3	.	.	.	.	.	.	.
Cer fon	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.
Rum lla	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.
Fes viv	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7
Fes ovi	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.
Eur sto	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.
Des fle	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hyl spl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 2. Continue

MAN	C+	C+	C+	C+	C+	E	E	E	E	E
YEAR	83	84	87	88	90	83	84	87	88	90
Month	07	06	06	06	08	06	06	06	06	08
Date	08	24	17	13	20	14	15	17	13	20
No	12	11	12	12	11	12	14	15	13	14
Rhy squ	100	100	100	100	100	93	100	100	100	100
Agr cap	90	100	100	100	100	97	100	100	100	100
Fes rub	50	80	50	53	77	97	97	87	97	87
Rum ace	40	33	30	33	33	37	53	73	83	50
Ant odo	57	37	80	50	60	13	23	77	73	93
Con maj	53	80	97	97	93	100	100	100	100	100
Poa pra	57	90	100	83	87	100	100	80	87	83
Gal sax	100	97	97	100	100	.	.	7	10	17
Luz cam	27	30	17	13	13	.	3	7	.	.
Ver off	10	.	3	7	.	.	.	.	.	.
Ver cha	87	93	100	100	13	70	27	13	3	.
Scl pur	.	.	.	.	.	7	30	3	13	40
Pot ere	.	.	.	.	.	.	.	.	.	.
Hol lan	.	.	.	.	.	3	20	40	40	37
Que rob	.	.	.	.	.	.	.	.	.	.
Cam rot	7	7	10	3	.	.	.	.	.	.
Ane nem	.	.	.	.	.	.	.	.	.	.
Car pil	.	.	.	.	.	.	.	.	.	.
Car ova	.	.	.	.	.	.	.	.	.	.
Cen nig	.	.	.	.	.	7	10	3	.	.
Rub ida	.	.	.	.	.	3	37	.	.	.
Pol com	.	.	.	.	.	.	.	.	.	.
Pru vul	.	.	.	.	.	.	.	.	.	.
Cir pal	.	.	.	.	.	.	10	7	7	10
Ach mil	.	.	.	.	.	.	.	10	20	3
Des ces	.	.	.	.	10	.	.	.	.	3
Ste gra	.	.	.	.	.	.	.	.	.	.
Dig pur	.	.	.	.	.	.	.	.	.	.
Lic sp1	.	.	.	.	.	.	.	.	.	.
Lic sp2	.	.	.	.	.	.	.	.	.	.
Lic sp3	.	.	.	.	.	.	.	.	.	.
Nar str	.	.	.	.	.	.	.	.	.	.
Cer fon	.	.	.	.	.	.	.	.	.	.
Rum lla	.	.	.	.	.	.	.	.	.	.
Fes viv	.	.	.	.	.	.	.	.	.	.
Fes ovi	.	.	.	.	.	.	.	.	.	.
Eur sto	.	.	.	.	.	.	.	.	.	.
Des fle	.	.	.	.	.	.	.	.	3	.
Hyl spl	.	.	.	.	.	.	.	.	.	20

A core of seven species was present in all five plots in every year of recording; in addition, *Galium saxatile* was very frequent in most plots during the experiment. These species are treated separately. *Luzula campestris* was seen to manage well in the middle and late mown

plots with their rather scattered field layer. As a rosette plant it may easily have been outshadowed in a dense sward. This is probably what happened in the control plot and in the uncut experimental plot, with their dense ground layer and sward, respectively. Much the same pattern was found for *Veronica officinalis*, which appeared anew in the middle mown plot in 1984 and established very well there. Like the above-mentioned species, *Veronica chamaedrys* was less frequent in the control plot after eight years. Early mowing seemed to be fatal for this species. To begin with, *Scleropodium purum* appeared to benefit from the removal of the dense ground layer, but also thrived best in the favourable light regime of the scattered field layer of the early mown plot. Also *Holcus lanatus* managed well under these conditions, probably by spreading vegetatively. *Potentilla erecta* was very tolerant to late mowing in the plot where it was established before the experiment started, and also made a successful establishment in the uncut experimental plot.

Galium saxatilis (Fig. 2)
When the experiment started, *Galium saxatilis* was growing at the top of, and in between the *Rhytidiadelphus squarrosus* carpet. As the moss carpet was removed from all plots except the control before the experiment started, individuals of *Galium* were removed together with the mosses. Consequently, the high frequency of the species in plot 2 and the rather low frequency values in the other plots in 1983 are easy to explain. The species seemed to recover well, even in mown plots, but then seemed to suffer a set-back sometime between 1987 and 1990. The fast-growing moss layer together with the rather dense grass layer may have outshadowed *Galium* individuals, as indicated from the increasing cover of *Rhytidiadelphus squarrosus* and grasses (Table 2). Grazing in spring and/or autumn is probably nescessary to keep this species in a hay meadow.

Anthoxanthum odoratum (Fig. 2)
Some rosettes of *Anthoxanthum* were removed together with the moss carpet in 1982 and the frequency of the species was higher in the control plot than in the other four plots in 1983. In the mown plots, *Anthoxanthum* recovered well to high frequencies in 1987 and onwards, and the frequencies were considerably higher than those in the control and the uncut experimental plot. Apart from the natural fluctuations which appeared from the curve of the control plot, the mown plots had about equal and high frequencies of *Anthoxanthum* in 1990 and the species obviously benefited from mowing, with late mowing time being the optimal alternative.

Poa pratensis (Fig. 2)
All the mown plots had high frequencies of *Poa* during the first couple of years, but from 1987 onwards the frequencies became lower in all three plots. This decrease may partly have been due to the removal of mosses in one way or another, as demonstrated by the curve of the uncut experimental plot, and compared to the control. But while the species had recovered to former levels in the uncut experimental plot by 1990, the mown plots had still much lower frequencies of *Poa*, especially the middle and late mown plots. This result is in agreement with records from old, middle and late mown meadows in Hordaland, which contain only very little or no *Poa pratensis* (Losvik 1988). A study by Lundekvam & Gauslaa (1986) showed that especially the biomass, but also the frequency of *Poa*

pratensis became lower with increasing age class. The species is, however, seen to persist for more than 20 years in abandoned hay meadows (Losvik 1985).

Table 3. Cover of species in 10 quadrats, two in each plot, estimated in 1983 (83), 1984 (84), 1987 (87), 1988 (88) and 1990 (90). 1-2: early mowing (E), 3-4: control (C+), 5-6: late mowing (L), 7-8: no mowing (C-), 9-10: middle mowing time (M). Abbreviated names of species, see Appendix

Plot	E	C+	L	C-	M
Quadrat	1212121212	3434343434	5656565656	7878787878	9090909090
Year	8384878890	8384878890	8384878890	8384878890	8384878890
Rhy squ	3355888888	8888888888	3354778888	3344766876	3343778887
Agr cap	4756674677	5543553376	6757655463	6545768788	5766766567
Poa pra	343533-3-3	5343333334	4434333333	3333333344	443333--33
Con maj	5366765733	3435568834	-3-4333334	4343333334	5354333333
Fes rub	4445333334	3443333434	4343333333	4444343335	3333333333
Rum ace	4342333333	42436-4-33	2333333333	3333333333	-333333344
Ant odo	33-3646454	3333653343	3333443365	3-4-343444	-333343343
Gal sax	-----33-33	35343343-4	--33445534	3-33333434	--3-335344
Luz cam	-----3----	--3-3---33	333333---3	3333-----3	-433-33-33
Ver cha	44-3---3--	35343444--	----------	----------	---2------
Cen nig	-3-3------	----------	----------	----------	----------
Rub ida	3333------	----------	----------	----------	----------
Que rob	----------	----------	2-2-2---3-	----------	----------
Hol lan	3-3-433333	----------	----------	----3-3-3-	----------
Ver off	----------	3---------	----------	-3-3-3-3-3	----333334
Pot ere	----------	----------	---4-3---3	-----2-333	----------
Cir pal	----3-3-3-	----------	----------	----------	----------
Ach mil	-----3-3-3	----------	----------	----------	----------
Des fle	------3---	----------	----------	----------	----------
Car ova	----------	----------	----------	3-3-------	----------
Pru vul	----------	----------	----------	---3---3-3	----------
Nar str	----------	----------	----------	---3------	----------
Cer fon	----------	----------	----------	---2------	----------
Dig pur	----------	----------	----------	----------	-----3----
Pol com	----------	----------	----------	----------	------3-33
Hyl spl	--------3-	----------	----------	----------	----------
Scl pur	---------5	----------	---------3	--------34	--------34
Des ces	---------3	--------33	----------	----------	----------
Lic sp1	----------	----------	----------	----------	--------33
Lic sp2	----------	----------	----------	----------	---------3
Lic sp3	----------	----------	----------	----------	--------3-

Agrostis capillaris

Some individuals of this species were removed together with the ground layer in 1982, but it soon recovered and the frequency was 100 % in all plots from 1984 onwards. The cover also increased, especially in the uncut experimental plot, but also in the control plot and in the early mown plot. The cover presented only small variations in middle and late mown plots, and this kind of management should be preferred if it is desirable to keep *Agrostis* from becoming dominant.

Conopodium majus (Fig. 2)
The variations within the uncut experimental plot, and the middle and late mown plots probably resulted from removal of mosses. In the two later mown plots the management allows seeds to ripen, on which the species is dependent for regeneration (Grime et al. 1988). In 1990 there was a tendency for frequency of the species in the control plot with mosses and the early mown plot to decrease. *Conopodium* seems to have an optimum phase some time after management has ceased, but is still quite persistent in earlier pastures (see also Losvik 1981). In the early mown plot, most of the biomass of *Conopodium* individuals is removed during flowering, and there is an obvious risk of the species dying out in the long run if this management practice is continued.

Rhytidiadelphus squarrosus
This species recovered most rapidly in the early mown plot (Table 2), but by 1988 was well developed with more than 75% cover in the middle and late mown plots too. However, there seemed to be some problem with recover in the uncut experimental plot where the species had been removed in 1982, probably because of the luxuriant grass layer in this plot.

Festuca rubra (Fig. 2)
The control plot indicated large fluctuations in the frequency of the species, as did the middle and late mown plots. Only in the early mown and in the uncut experimental plot did the frequencies of the species appear quite even and high all the way. The mean cover was also higher in these plots than in the others.

Rumex acetosa (Fig. 2)
The control plot showed little variation during the seven years of records. Probably the increase in frequency in the other plots from 1983 till 1987 was a result of removing the moss layer, as the seeds of *Rumex* could germinate in the gaps created. The frequency of the species decreased later, probably as the field layer became more dense. The species managed best in the plot with the middle mowing time, where the field layer never became very dense.

Species richness
In 1983 the number of species was low and quite even in all five plots (Fig. 3). In the control plot the number of species was lower in 1990 than in 1983. Earlier investigations have also shown that in abandoned meadows the sward quickly becomes so dense that new species have great difficulty in establishing, and such grasslands may remain as open grassland for decades after abandonment and the number of species usually declines (e.g. Ellenberg 1978; Persson 1984; Dierschke 1991). In the four plots where the ground layer had been removed, the species number increased by 2-4 more species after one year and in 1990 seemed to have stabilized with a greater number of species than in 1983. Undoubtedly the removal of mosses created gaps in the sward for new species to germinate and establish (see e.g. Wells 1971; Grubb 1976; 1977, Verkaar et al. 1983; Verkaar & Schenkeveld 1984; Hillier 1984). Successful invaders kept the species number high through to 1990.

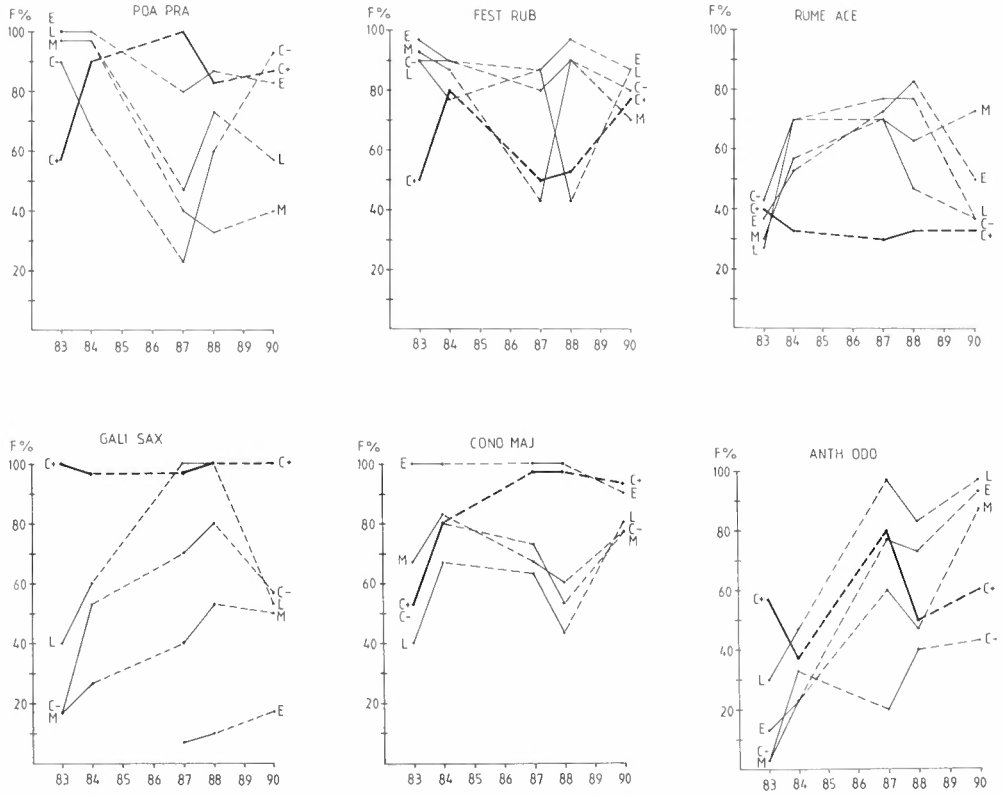

Fig. 2. Frequency (F %) of Galium saxatilis (GALI SAX), *Anthoxanthum odoratum* (ANTH ODO), *Poa pratensis* (POA PRA), *Conopodium majus* (CONO MAJ), *Festuca rubra* (FEST RUB) and *Rumex acetosa* (RUME ACE) in 1983-90 in control plot (C+), uncut experimental plot (C-), early (E), middle (M) and late (L) mown plot

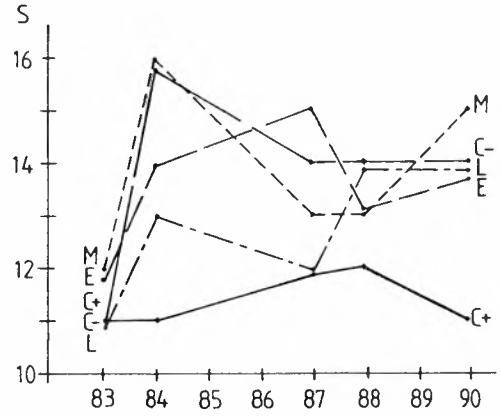

Fig. 3. Number of species (No sp.) in 1983-90 in control plot (C+), uncut experimental plot (C-), early (E), middle (M) and late (L) mown plot

Species richness is not greatly affected by the date of cutting (see also Parr & Way 1988), although other data connect high species numbers with a late mowing time (e.g. Losvik 1985, 1991; Bakker 1989; Smith & Jones 1991). A longer-term experiment is probably nescessary to reveal differences between cutting times where species richness is concerned.

Turnover

Out of a total of 39 species, 7 species were present both at the start and at the end of the experiment in all five plots. Six species, which were present at the start of the experiment, disappeared during the experiment: *Campanula rotundifolia* from the control plot, *Carex ovalis* from the uncut experiment plot, *Centaurea nigra* and *Rubus idaeus* from the early mown plot and *Carex pilulifera* and *Anemone nemorosa* from the middle mown plot. *Rubus idaeus* certainly would not be expected to survive mowing each year, and *Centaurea nigra* set seeds from July onwards (Grime et al. 1988) and thus would not survive in an early mown stand.

During the experiment *Campanula rotundifolia* and *Veronica officinalis* both disappeared from the control with mosses in 1988-90. Only *Deschampsia cespitosa* entered the plot anew. In the other plots, the number of species that entered the plots was larger than the number of species that disappeared from them in the period 1983-90 (Fig. 4). Species which entered the plots after the experiment started, and then disappeared, were in mown plots *Digitalis purpurea*, *Prunella vulgaris*, *Luzula campestris*, *Deschampsia flexuosa*, *Veronica officinalis*, *V. chamaedrys* and *Stellaria graminea*, and in the uncut experimental plot *Rumex acetosella*, *Cerastium fontanum* and *Nardus stricta*.

Successful new invaders were *Holcus lanatus*, *Potentilla erecta* and *Prunella vulgaris* in the uncut experimental plot, *Scleropodium purum* in all the experimental plots, *Veronica officinalis* in the middle cut plot and *Galium saxatile*, *Cirsium palustre* and *Achillea millefolium* in the early cut plot. *Holcus lanatus*, having good dispersal ability and high seed production (Peart & Foin 1985), is known to be an aggressive colonizer in several habitats (Brenchley & Warrington 1958) and probably has spread from the early mown plot, where it was present when the experiment started. *Potentilla erecta* and *Cirsium palustre* have persistent seeds (Grime et al. 1988) and may have germinated from the seedbank after the removal of the moss carpet.

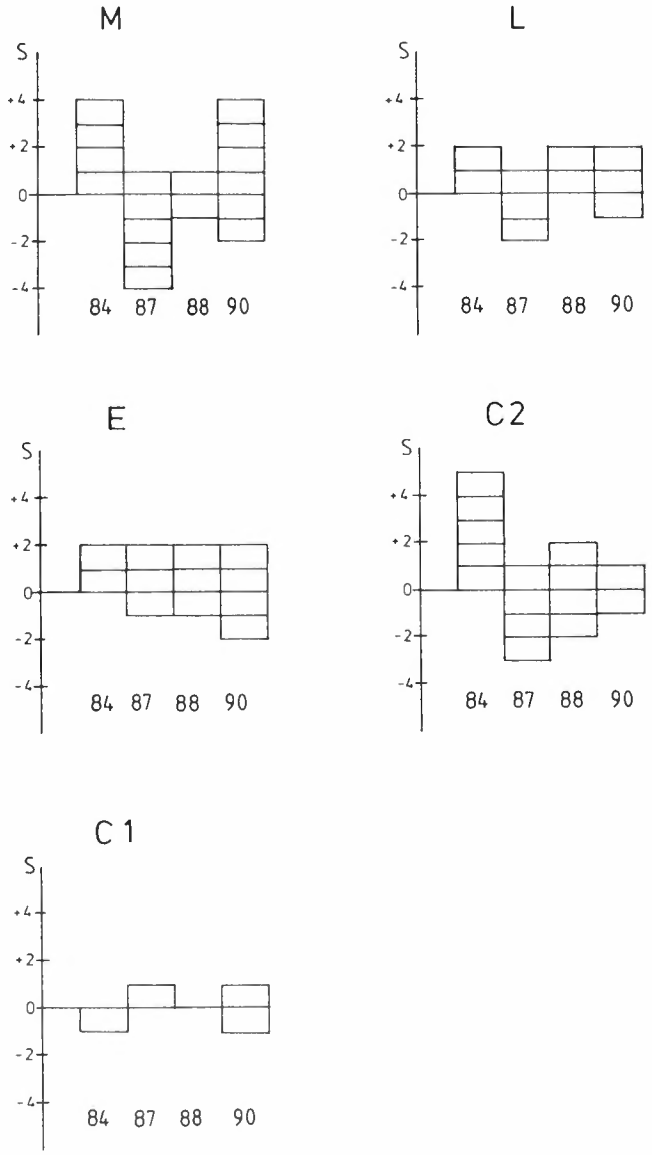

Fig. 4. Species turnover 1984-90 compared to species number in 1983 in control plot (C1), uncut experimental plot (C2), early (E), middle (M) and late (L) mown plot

After seven years of removal of cuttings, the plot seemed to become nutrient depleted, as indicated by the increasing number of mosses and lichens in the mown plots. High species diversity is, however, usually connected with unfertilized hay meadows (e.g. van der Maarel 1971; Zoller & Bischof 1980; Silvertown 1980; Collins & Barber 1985). Fine cutting and spreading of the grass over the plots is recommended to avoid further impoverishment of the soil.

Variation in species composition in relation to time and management

The vegetation in all the plots diverged from their original species composition but to a different extent. In 1983 the similarity between the vegetation in the control plot, the uncut experimental plot and the middle and late cut plots was high (75-87%), while similarity between the vegetation in the early mown plot and each of the other plots was 61-67%, probably as a result of lack of *Galium saxatile* and *Luzula campestris* and the occurrence of *Holcus lanatus*, *Rubus idaeus*, *Centaurea nigra* and *Veronica chamaedrys* in the early mown plot. By 1990, the vegetation in the uncut experimental plot, middle and late cut plots was still very similar (76-86%). Floristically, the vegetation in the main control plot diverged more from that of the other plots (69-72%), compared with in 1983. In 1990 the early mown plot was as far from the middle and late mown plots as in 1983, but a little closer to the uncut experiment plot and the control.

CA ordination with cover in 10 quadrats over five years demonstrated the subtle changes which occurred in the control site and in the late mown site (Fig. 5, see also Table 3). Thirthysix of the samples lie within the circle in the middle of the diagram. The vegetation in the early mown site was quite different from that in the other sites from the start, and it continued to be different as *Cirsium palustre*, *Achillea millefolium*, *Deschampsia cespitosa*, *Deschampsia flexuosa* and *Hylocomium splendens* entered the site. While one of the uncut experimental quadrats showed a larger difference early in the experiment, and then later returned to a position closer to the other quadrats, middle cut quadrats seemed to become even more divergent from the other quadrats at the end of the experiment. Axis 1 may be interpreted as a rich-poor gradient, with samples containing *Holcus lanatus* and *Rubus idaeus* on the left side, and lichens at the poor end of the gradient on the right side. It was assumed that the ecological conditions were fairly similar over the investigated area at the start of the experiment, as indicated also by the high number of constant species (constancy class III-V), namely half of the species occurring in 1983 (Table 4). The first axis has, however, a rather low eigenvalue (0.22) especially to be CA (Jongman et al. 1987, p. 139), so the amount of variance accounted for may be rather low. An obvious gradient is time, which runs from the upper left to the lower right in the diagram.

CCA ordination (Fig. 6) with cover of all species in 10 quadrats in five years, and the variables time and management, indicated that the early cutting time was correlated with the first axis (eigenvalue = 0.15, p = 0.01), associated with e. g. *Holcus lanatus* and *Achillea millefolium*. Along the second axis, middle cutting time was the more important factor (eigenvalue 0.10), while late cutting time was correlated to the third axis. The uncut experimental quadrats and the control quadrats were weakly positively correlated, and both medium and late cutting time were correlated with the uncut experimental quadrat. The control was strongly negatively correlated to late and to early cutting time. The time gradient runs from the lower right- to the higher left-hand side of the diagram. The *Nardus stricta* group was present early in the experiment in the uncut experiment quadrats, while *Prunella vulgaris* and *Potentilla erecta* came in later. In the same way, the order of species by the centroid of the early mown plot demonstrates the time gradient, with *Rubus idaeus* and *Centaurea nigra* being present from the start and *Hylocomium splendens* arriving late. As in the CA ordination, the uncut experimental quadrats, the control quadrats and the late mown quadrats are quite close in the diagram, while early and medium cut quadrats are

situated far from the others. As is seen from Figs. 3 and 5, the early and medium mown sites seem to be more unstable than the other sites

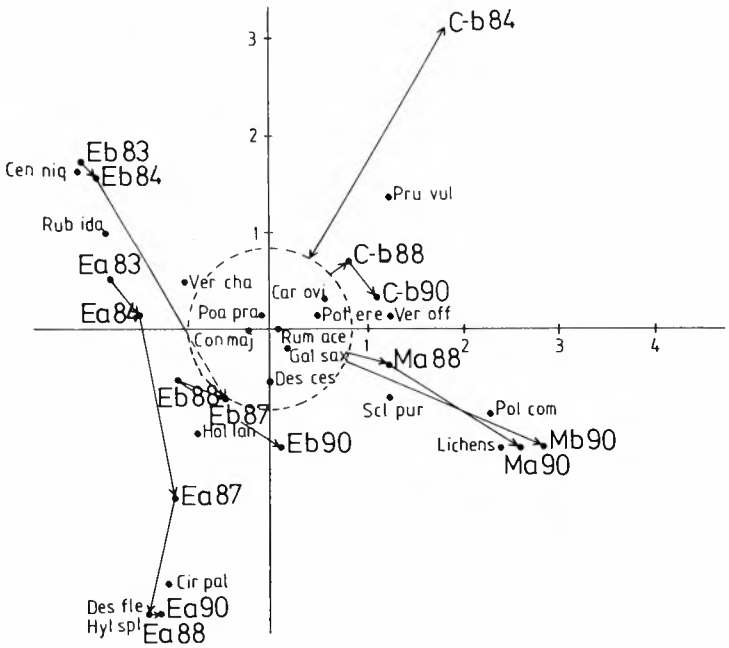

Fig. 5. CA ordination with cover of species in 10 quadrats in five years. Control with mosses (C+), the uncut experiment plot (C-), early (E), middle (M) and late (L) mown sites. 83-90 = 1983-1984-1987-1988-1990, a and b: quadrats a and b within the same plot. Samples within circle: C+a83-90, C+b83-90, C-a83-90, C-b83 & 87, La83-90, Lb83-90, Ma83-87, Mb83-88. Species abbreviations, see Appendix. Variation in species content in each quadrat through time is indicated by arrows

Table 4. Constancy (C) and mean cover (MC) of all species in 10 quadrats of the five sites in 1983

	CMC
Rhytidiadelphus squarrosus	V.4
Agrostis capillaris	V.5
Poa pratensis	V.4
Fesuca rubra	V.4
Conopodium majus	V.3
Rumex acetosa	V.3
Anthoxanthum odoratum	IV.3
Luzula campestris	III.3
Galium saxatile	II.4
Veronica chamaedrys	II.4
Centaurea nigra	I.3
Rubus idaeus	I.3
Quercus robur	I.2
Holcus lanatus	I.3
Veronica officinalis	I.3
Carex ovalis	I.3

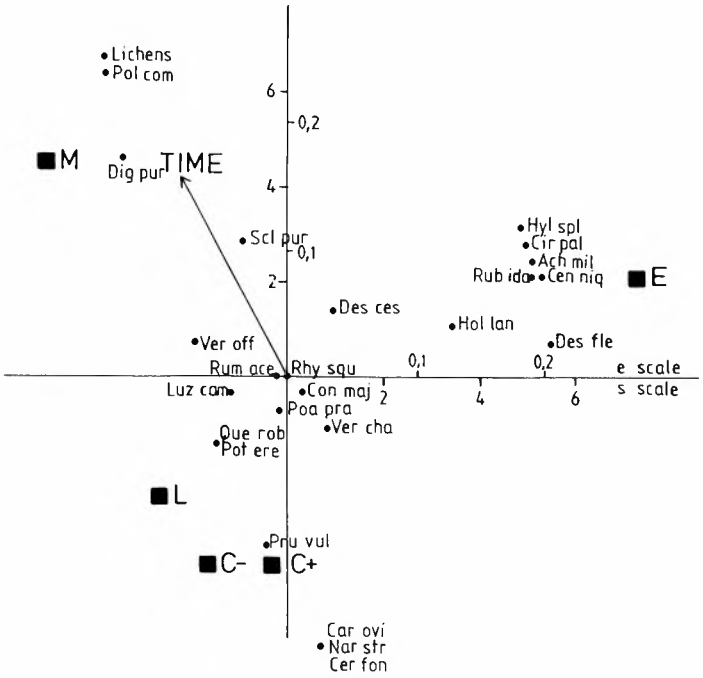

Fig. 6. Biplot diagram of CCA ordination with cover of species in 10 quadrats in five years and the environmental variables management regimes and time; s scale is species scale and e scale is environmental scale. Control site (C+), uncut experiment site (C-), early (E), middle (M) and late (L) mown sites. Species abbreviations, see Appendix

CONCLUSION

Removal of a dense ground layer is essential in order to increase species diversity in this grassland type. Although changes did occur in the control, too, mainly due to losses of species with time, the plots where the ground layer had been removed showed both higher species turnover and species richness at the end of the experiment. Removal of the ground layer facilitated germination of seeds resulting from the seed rain, vegetative spreading of grasses and herbs and the re-establishment of plants which formerly grew in the plots, presumably from the reserve of buried seeds (e.g. Hillier 1990). Even then, bryophytes and lichens may have a significant impact on seedling establishment (Keizer et al. 1985). The rapid recovery of the ground layer during the experiment showed that it may be advisable to remove it every 10 years to avoid loss of species. As a consequence, species such as *Agrostis capillaris, Galium saxatilis* and *Rumex acetosa* may be reduced in frequency (see Methods), but they will soon recover, and none of them disappeared from the plots during the present experiment.

Mowing did not result in a higher species richness compared to the uncut experimental plot in the time span of this study. Several grassland species, either occurring anew in the plots or increasing in frequency, are, however, known to grow mainly in grassland. Such species are e.g. *Prunella vulgaris*, *Holcus lanatus*, *Achillea millefolium* and *Conopodium majus*. A late mowing time is recommended. In this way the seeds of *Conopodium majus* will ripen before cutting, and *Agrostis capillaris* is kept from dominating the sward. A late mowing time seemed to be optimal for *Antoxanthum odoratum*, too. Several of the species

present, such as *Galium saxatile*, *Potentilla erecta*, *Veronica spp.* and *Luzula campestris*, are probably in the long run dependent on grazing to persist in the sward (e.g. Dahl 1957; Steen 1958; Kielland-Lund 1962; Skogen 1975). Spreading of the fine cut sward over the mown area is recommended to avoid depletion of the hay meadow soil. If the cuttings are removed, they should be left on the mown area for some days to dry, in order to ensure that ripened seeds are spread on the area.

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APPENDIX

Ach mil = Achillea millefolium
Agr cap = Agrostis capillaris
Ane nem = Anemone nemorosa
Ant odo = Anthoxanthum odoratum
Cam rot = Campanula rotundifolia
Car ova = Carex ovalis
Car pil = Carex pilulifera
Cen nig = Centaurea nigra
Cer fon = Cerastium fontanum
Cir pal = Cirsium palustre
Con maj = Conopodium majus
Des ces = Deschampsia cespitosa
Des fle = Deschampsia flexuosa
Dig pur = Digitalis purpurea
Eur sto = Eurhynchium stockesii
Fes ovi = Festuca ovina
Fes rub = Festuca rubra
Fes viv = Festuca vivipara
Gal sax = Galium saxatile
Hol lan = Holcus lanatus
Hyl spl = Hylocomium splendens
Lic sp1 = Lichen: species 1
Lic sp2 = Lichen: species 2
Lic sp3 = Lichen: species 3
Luz cam = Luzula campestris
Nar str = Nardus stricta
Poa pra = Poa pratensis
Pol com = Polytrichum commune
Pot ere = Potentilla erecta
Pru vul = Prunella vulgaris
Que rob = Quercus robur
Rhy squ = Rhytidiadelphus squarrosus
Rub ida = Rubus idaeus
Rum ace = Rumex acetosa
Rum lla = Rumex acetosella
Scl pur = Scleropodium purum
Ste gra = Stellaria graminea
Ver cha = Veronica chamaedrys
Ver off = Veronica officinalis

Effects of fenvalerate and esfenvalerate on carabid and staphylinid species in spring barley fields

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The effects of two pyrethroids were measured in pitfall traps in 16-70 ha plots in 1988-90. Because of reimmigration into treated plots, the effects were measured for only one to three weeks after treatment. Fenvalerate and esfenvalerate contain the same compound, but fenvalerate contains four isomers while esfenvalerate contains only the isomer with the greatest insecticidal activity. They were treated as comparable when fenvalerate had a concentration four times that of esfenvalerate. The activity abundance (= activity density) of the carabid genera *Bembidion* and *Trechus* was reduced to 20-35% after spraying with dosages recommended against cereal aphids (60 g a.i./ha for fenvalerate). No effect was observed on larger carabid species. Among the staphylinids, the subfamily Aleocharinae (excluding *Aloconota gregaria* and *Amischa* spp.) was reduced by 85%, while in *A. gregaria* there was increased activity by 200-900%, probably because of insecticide irritation, especially during the first week after spraying.

Key words: Carabidae, esfenvalerate, fenvalerate, insecticides, polyphagous predators, pyrethroids, Staphylinidae

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Most carabids, including the *Bembidion* and *Trechus* species, are polyphagous predators in agricultural fields. Although less is known about the diet of many staphylinids, most of these are probably also polyphagous predators (i.e. Sunderland et al. 1987). Among the staphylinid species specifically investigated here, Andersen (1992) demonstrated that *Aloconota gregaria* (Erichson) ate both dead and live aphids offered in laboratory and semifield experiments.

Motivated by their importance as polyphagous predators on many agricultural pest species, the effects of pyrethroids on carabids and staphylinids have been investigated during the past ten years. Compared to phosphorous compounds (i.e. dimethoate) and the less harmful, selective aphicides (i.e. pirimicarb), pyrethroids often have an intermediate effect (Poehling & Dehne 1984, Vickerman et al. 1987, Casteels & De Clercq 1990). Few investigations have compared different pyrethroids, but Heimbach (1991) found that lambda-cyhalothrin had a more negative effect on carabids than fenvalerate.

Different conclusions have often been drawn by different authors testing the same compound. For instance, Heimbach (1991) reported a reduced activity abundance (=

activity density) for carabids and staphylinids following a fenvalerate treatment, while Nilsson (1980) and Casteels & De Clercq (1990) found no effect of the same compound and Poehling et al. (1985) reported hyperactivity for several species.

These variations can sometimes be explained by different dosages tested, but more often they can be attributed to the methods used. Important differences in methods are, for instance, plot size, raised barriers around plots, use of pitfall traps (activity abundance) or quadrat samples (population density), how long the effects are measured and whether the beetles are identified to species or only to family. Still, it is not easy to draw firm conclusions from the existing literature as to the real effects of pyrethroids on different polyphagous predator species, although there certainly are effects.

The present investigation was carried out in order to evaluate the effects of the pyrethroids fenvalerate and esfenvalerate on the activity abundance of specific carabid and staphylinid species. The spraying was applied on rather large plots without barriers, and recolonization by the beetles therefore only allowed for evaluation of relatively short-term effects.

MATERIALS AND METHODS

The investigation was carried out in the barley cultivar "Bamse" on clay soil at Ås in southern Norway. In 1988 a 120 ha field was divided into two plots of 50 and 70 ha, respectively. Five pitfall traps were placed 40 m apart in one row in the middle of the 50 ha plot and seven traps in one row in the 70 ha plot. On 8 June (Zadoks growth stage 32) the 70 ha plot was sprayed with fenvalerate (Sumicidin FW, 50 g a.i./ha), while the 50 ha plot was left untreated.

In 1989 a 160 ha field was divided into four plots of 40 ha. In the middle of each plot, nine pairs of pitfall traps were placed in three rows 50 m apart, and with 50 m between traps in each row. On 6 June (Zadoks 22) three plots were sprayed with 60, 30 and 15 g a.i./ha of fenvalerate respectively, while one plot was left untreated.

In 1990 a 196 ha field was divided into 16 plots of approximately 16.3 ha, with four blocks of four treatments each. In the middle of each plot, four pairs of pitfall traps were placed in two rows 20 m apart, and with 20 m between traps in each row. On 6 June (Zadoks 37) three randomly chosen plots in each block were sprayed with 15, 7.5 and 3.8 g a.i./ha of esfenvalerate (Sumi-Alpha FW) respectively, while one plot was left untreated.

Fenvalerate and esfenvalerate contain the same active ingredient, but fenvalerate is a mixture of all four different isomers of the chemical, while esfenvalerate is a purification containing only the isomer with the greatest insecticidal activity. In this investigation, the two insecticides are treated as comparable when fenvalerate has a concentration four times that of esfenvalerate. The dosages used were those recommended for cereal aphid control in Norway and slightly reduced dosages.

Pitfall traps were set up shortly after sowing, and were emptied until at least one month after spraying. The cups used as traps were 38 mm deep and had an upper diameter of 92 mm. They were placed at least 40 m from the field edge, and contained water and some detergent. In 1988, the pitfall traps were placed singly, while in 1989 and 1990 "one trap" comprised of a pair of such pitfall traps placed 1 m apart, with a 10-cm-high plastic

barrier between them.

Among the carabids, *Bembidion quadrimaculatum* (L.) (66%), *B.lampros* (Herbst) (19%) and *B. guttula* (Fabricius) (14%) were the most numerous *Bembidion* species in the pitfall traps in all three years. In 1990, *Trechus secalis* (Paykull) (80%) and *T. quadristriatus* (Schrank) (18%) were the most numerous *Trechus species*.

Among the staphylinids, *Atheta fungi* (Gravenhorst) (48%), *Aleochara brevipennis* Gravenhorst (16%), *Dinaraea angustula* (Gyllenhal) (13%) and *Oxypoda exoleta* Erichson (12%) were the most numerous species in the subfamily Aleocharinae in 1990 (excluding *Aloconota gregaria* and *Amischa* spp.).

Pitfall traps are used for measuring activity abundance (= activity density). The catch is dependent not only on the population density of the species, but also on its activity on the ground (Thiele 1977). Since pitfall trapping is the only method used in this investigation, all results reflect the activity abundance of species.

For statistical analysis of the pitfall trap catches, the Wilcoxon paired-comparison test was used in 1988, and the Kruskal-Wallis test was used in 1989. In 1990 a one-way analysis of variance was used, and significant means were separated by Duncan's multiple range test. In all statistical calculations a significance level of 5% was used.

RESULTS

In 1988 and 1989 the differences in activity abundance between sprayed and unsprayed plots before spraying were tested separately, while in 1990 these were included in the analysis of variance. There were no significant differences in activity abundance between sprayed and unsprayed plots before spraying for *Bembidion* spp. in 1989 and for *Aloconota gregaria* in both years. In 1988 *Bembidion* spp. was caught in significantly higher numbers in the sprayed plot before spraying.

In all three years the activity abundance of *Bembidion* spp. was reduced for 4-19 days after application of the pyrethroid spray (Figures 1A, 2A and 3A). The activity abundance was only 20-35% compared with that of untreated plots, and the reduction was significant in 1988 and 1990. A tendency towards increased reduction with increased pyrethroid dosage was not statistically significant (Figures 2A and 3A).

In 1990 a significantly reduced activity abundance (20-25% compared with that for untreated plots) could also be observed for *Trechus* spp. and the Aleocharinae for a period of four days in the plots sprayed with the pyrethroid (Figures 3B and 3D), although there was no significant effect of dosage.

For *A. gregaria*, on the other hand, an increased activity abundance (200-900% compared with that in untreated plots) was indicated in the sprayed plots for 4-19 days after spraying in all three years (Figures 1B, 2B and 3C), although this was only statistically significant in 1988. There was a tendency towards increased activity abundance at increased pyrethroid dosage, but this was not statistically significant (Figures 2B and 3C). Figure 4 indicates that the activity abundance was especially increased during the first week after spraying.

No effect of the pyrethroids fenvalerate or esfenvalerate on the activity abundance of other carabids and staphylinids was observed during the three years.

Figure 1. Change (%) in number of beetles caught in pitfall traps 19 days after treatment, compared to 14 days before treatment, in unsprayed plot (U) and plot sprayed with 50 g a.i./ha of fenvalerate (F) in 1988. (A) *Bembidion* spp. (n=61), (B) *Aloconota gregaria* (n=42)

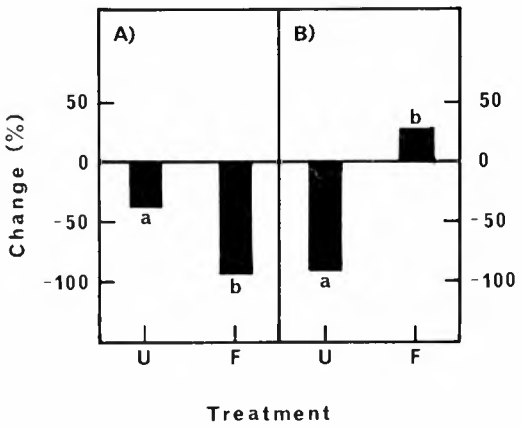
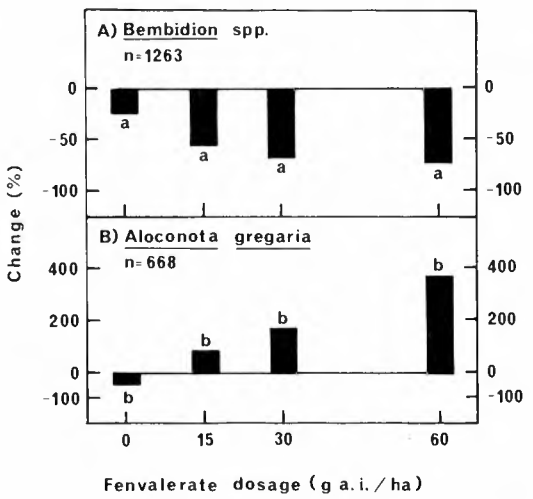


Figure 2. Change (%) in number of beetles caught in pitfall traps nine days after treatment, compared to 11 days before treatment, in plots sprayed with different dosages of fenvalerate in 1989. n= number of beetles caught during the 20 days. Bars with different letters are significantly different (p=0.05)



DISCUSSION

Among the carabids, reduced activity abundance after spraying pyrethroids at dosages recommended for cereal aphids (or lower) was only registered for the comparatively small genera *Bembidion* and *Trechus* (Figures 1-3). No effect was observed on the larger species. Insect size may partly explain the difference, as smaller species are more vulnerable than larger species to the insecticide spray. In addition, the most numerous *Bembidion* species were diurnal (Thiele 1977, Luff 1978) and thus more vulnerable because they are directly hit by the insecticide during spraying. Fenvalerate has previously been shown to cause some reduction in total carabid pitfall catch (Heimbach 1991), while the catch of larger carabids has been relatively unaffected (Poehling & Dehne 1986, Whitford & Showers 1987), or even increased because of starvation (Chiverton 1984).

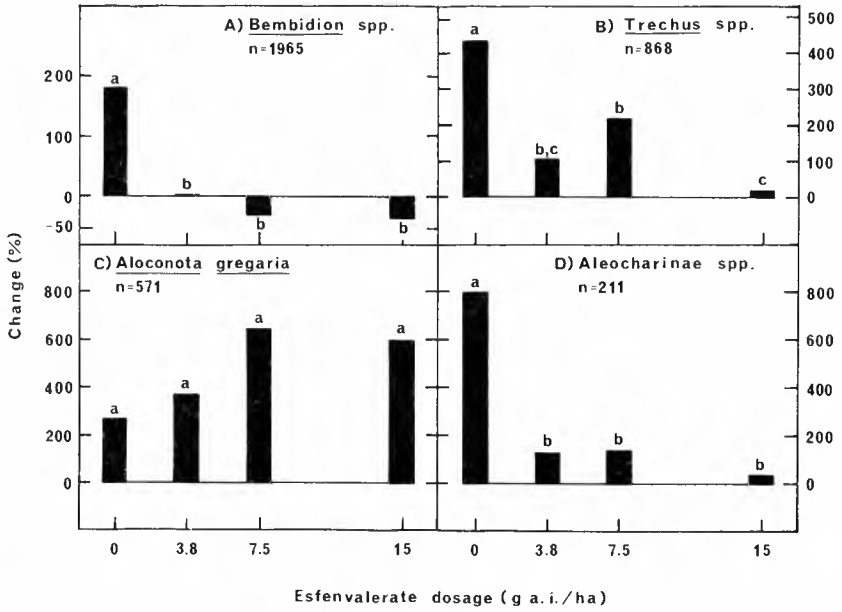


Figure 3. Change (%) in number of beetles caught in pitfall traps four days after treatment, compared to five days before treatment, in plots sprayed with different dosages of esfenvalerate in 1990. n = number of beetles caught during the nine days. Bars with different letters are significantly different (p=0.05)

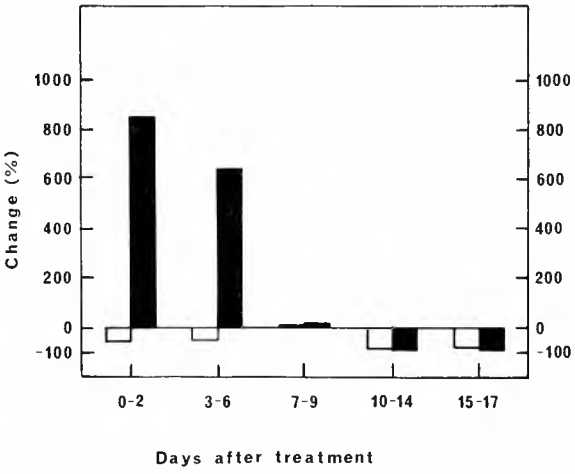


Figure 4. Change (%) in number of *Aloconota gregaria* caught in pitfall traps at different time intervals after treatment, compared to 11 days before treatment, in unsprayed plot (□) and plot sprayed with 60 g a.i./ha of fenvalerate (■) in 1989

Among the staphylinids, the pyrethroids tested induced increased activity abundance in *Aloconota gregaria*, especially during the first week after spraying (Figures 1-4). While reduced activity abundance in sprayed plots is thought to be caused by the insecticide killing

off a certain percentage of the beetle population, increased activity density could be due to increased activity as a result of starvation (Chiverton 1984) or hyperactivity due to irritation caused by the insecticide (Poehling et al. 1985). The measured reaction was immediate, strong and lasted for a short time (Figure 4) and was, therefore, most likely due to irritation by the insecticide.

The reduced activity abundance for Aleocharinae after spraying is thought to be caused by a part of the population being killed off by the insecticide. Previously, fenvalerate has been shown to lead to both reduced catch (Heimbach 1991) and hyperactivity (Poehling et al. 1985) in staphylinids.

If all the staphylinid species had been treated together as a family, the hyperactivity of some species and reduced activity abundance of other species would have been in opposition to one another, and thus the effects of the pyrethroids would have been masked. This clearly emphasizes the importance of identifying the material to species, as has previously been suggested by other authors (Poehling & Dehne 1986, Al Hussein et al. 1990).

The present investigation clearly shows that the effects of the pyrethroids tested were very varied on different carabid and staphylinid species. Further investigations are obviously needed to study the effects of pyrethroids in more detail on different polyphagous predator species in agriculture, both in cases of reduced and in increased activity abundance.

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Short term effects of different water content in feed on blue fox (*Alopex lagopus*) and silver fox (*Vulpes vulpes*)

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Twelve blue fox vixens and twelve silver fox vixens, both species supplied with two different watering systems, were given a pelleted commercial feed, either dry containing 94% dry matter (DM) or soaked, containing 45% DM. Total water intake was 3.0 g/g DM for silver fox and 4.0 g/g DM for blue fox. The difference between species was significant. The lower water content in the dry diet was fully compensated by a higher intake of drinking water in both species. Apparent protein digestibility and DM digestibility was 79 and 80% respectively for blue fox and 85 and 87% for silver fox. The difference between species was significant. There was no effect of water content in the diet. Water spillage from the drinking nipple system was significantly higher than that from the open drinking cup system. The volume of water spillage was 80% higher than the urine volume. The content of N, P and K in the manure DM was 13.6%, 2.5% and 2.3% for blue foxes and 18.2%, 3.1% and 2.9% for silver foxes. Differences between species were significant.

Key words: Digestibility, drinking water, dry diets, fur-bearing animals, manure, nutrient elements, water turnover.

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Several feeding experiments have been carried out on mink and other carnivorous animals like cats and dogs. Different scientific reports comprehend the subject areas as water turnover, feed intake, digestibility and excretion. Little information on these subjects concerning the blue fox and the silver fox is available in the literature. The blue fox (*Alopex lagopus*) and the silver fox (*Vulpes vulpes*) are two of the most important species in fur animal production.

The water requirement of foxes may depend on factors such as energy and protein consumption, digestibility, salt intake and ambient temperature, as reported for the mink (Tauson 1991; Møller 1991). Because of different physiolgical status, the water requirement may vary between individual animals. Thus it is recommended that fresh and clean drinking water should be available at all times (NRC 1982).

In accordance with the carnivorous nature of the blue fox and the silver fox, the conventional diet for farm foxes is primarily based on fish and animal byproducts. This type of wet diet contains approximately 65-70% water, and contributes greatly toward meeting

the total water requirement.

Pelleted, dry diets for foxes are designed to furnish all the nutrient requirements, except water. Thus it is of critical importance that pellet-fed animals are provided with an adequate water supply. In practice, this is often achieved by using different types of automatic watering systems. However, it may be questioned whether or not the animals can fully compensate for the lack of water in the diet by consuming more drinking water. Therefore, in order to reduce the dependence on drinking water, the dry pellets can be mixed with water to be fed in easily accessible trays or on feed boards. By using this technique, the total water consumption may be increased. Feed intake and nutrient digestibility may also be influenced.

Urine volume may depend on water intake, and water may be splashed or leaked out of drinking cups or nipples. Urine and water spillage may drain through the manure which is generally stored on the ground. Both the source of water and the watering system may affect the environment in the fox farm and accordingly also increase the risk of pollution problems.

Manure is a byproduct in all domestic animal production. Manure can be considered as a possible source of pollution, but, most importantly, it is a resource as a plant fertilizer. It is therefore of vital importance to know the content of plant nutrient elements in manure in order to reach optimal utilization of this resource.

The purpose of the present investigation was:

(1) To study the flow of water through a fur-production system for blue foxes and silver foxes, that is; the amount of water supplied to the animals in the feed and as drinking water, the total water intake of the foxes, spillage of water from the two drinking-water systems and excretion of water in urine and faeces.

(2) To study the effects of different water content in the feed on the digestibilities in the two species.

(3) To investigate the relation between the amount of nitrogen, phosphorous and potassium ingested by the foxes and that excreted in faeces and urine.

MATERIALS AND METHODS

The present experiment was carried out at the Agricultural University of Norway from 14 January to 8 February, 1991.

Experimental design

Twelve blue fox vixens and 12 silver fox vixens, aged 8-10 months, were used in the experiment, starting with the blue foxes. Within species, the animals were divided in two groups of six, with different drinking-water systems. A pelleted dry diet, fed dry or soaked, was used during two consecutive test periods. All experimental animals were fed dry pellets ad libitum four weeks prior to the test periods. After being moved to the experimental facilities, the animals were allowed an adaptation period of 3 days, followed by a collection period of 4 days. Then the diets were switched starting with another adaptation period of 3 days and terminating with a collection period of 4 days. The experimental design is shown in Fig. 1.

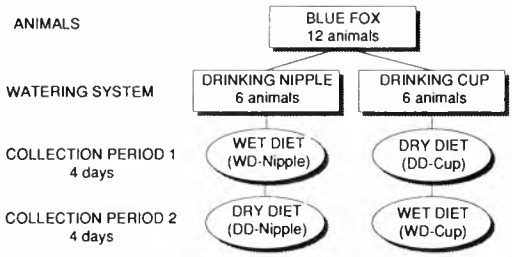

Fig. 1. Experimental design, trials with blue fox. An idenfical design was used for trials with silver fox

The animals were housed in individual cages (0.8 x 0.8 x 0.8 m), which were equipped for feeding both dry and wet feed and had separate systems for collection of urine and faeces.

Each cage had an individual drinking-water system either a pipe-nipple system or a drinking cup system. The watering systems are illustrated in Fig. 2. Water was supplied from a container above the top of the cages. The container was refilled every day and the water brought into the system was weighed to the nearest gram. At the end of the 4-day period, water was filled up to the starting level. The total consumption of water could then be calculated.

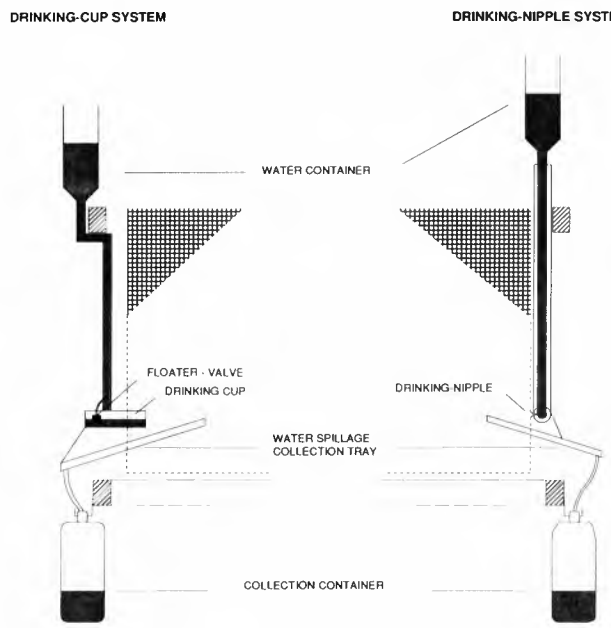

Fig. 2. Drinking water- and collection systems used in the experiment

Spilled drinking water was collected in a tray mounted under the drinking cup or the nipple and led to a container. Spillage was measured quantitatively after each collection period of four days.

The evaporative water losses were not measured, but were assumed to be minor and equal during every stage of the experiment.

Feeding and collection started at 12 a.m. following a strict daily routine.

The experimental room was temperature-controlled. Temperature during the experiment was 9.5 ± 0.5°C (mean ± SD), and relative humidity was 73.4 ± 4.4%.

Drinking water temperature was 9.1 ± 0.3°C for the nipple groups and 7.9 + 0.3°C for the drinking cup groups during the test periods.

Experimental diets

A commercial dry diet, "Skretting Revepellet", was used in this experiment. The composition of the diet is given in Table 1.

Table 1. Composition of the commercial diet. Skretting Revepellet[1)]

Ingredients (%)	
Herring meal (Norse-LT 94)	38.2
Extruded wheat	25.3
Soya-oil	11.8
Potato powder	11.0
Fish-oil	5.0
Potato fibre	4.0
Protamyl PF	3.0
Vitamin and mineral mixture	1.2
Limestone meal	0.5
	100.0

[1)] T. Skretting AS, Stavanger, Norway

The pellets were fed either dry (DD) or with added water (WD), i. e., with 120 g added water per 100 g dry pellets 21 h before feeding and stored in a refrigerator at +5°C.

The animals were fed restrictively in order to standardize feed intake. The daily allowance was 100 g dry pellets. This corresponded to 1.65 MJ ME per day (information from the manufacturer). Average daily energy consumption for blue fox and silver fox females in February is 1.88 and 1.84 MJ ME, respectively (Enggaard Hansen et al. 1991).

Sampling and analyses

The animals were weighed to the nearest 10 g before and after the test periods.

Feed offered, feed refusals, water and faeces were weighed to the nearest gram during the collection period.

Samples of feed were collected daily and frozen at -18°C. Pooled samples from the 4-day collection period were analysed for dry matter content (DM), ash, crude protein (N

X 6.25) and crude fat. In addition analyses for phosphorus (P), water-soluble P, potassium (K), sodium (Na) and chlorine (Cl) were carried out.

Faeces were collected daily in an air-tight plastic box and frozen. Urine was collected in a bottle for the whole 4-day period, weighed and then frozen until analysing. Pooled samples of urine and faeces were analysed for content of DM, nitrogen (Kjeldahl procedure) (N), NH_4 - N, P, water-soluble P and K.

All chemical analyses were carried out at The Chemical Analytical Laboratory, Agricultural University of Norway.

An analysis of variance on the data concerning water intake, water balance, digestibility and spillage was performed using the SYSTAT MGLH procedure (SYSTAT Inc. 1989) for split-plot designs after calculation of means for four days.

RESULTS

All the animals lost weight during the experiment. The blue foxes had an average body weight of 7.65 $\pm$ 1.25 kg at the start of the experiment and lost 0.63 $\pm$ 0.20 kg in the course of 14 days. The silver foxes weighed 5.57 $\pm$ 0.55 kg and lost 0.18 $\pm$ 0.12 kg body weight.

Feed consumption was acceptable during the two test periods for both blue fox and silver fox. There were some cases of minor feed refusals, but no observations were omitted. Chemical analyses of the two diets are presented in Table 2.

Table 2. Chemical analyses of the diets. n = 4. Values are means $\pm$ SD. n = 4

	Dry diet (DD)	Wet diet (WD)
Dry matter (DM), %	94.1±0.7	44.9±1.5
In % of DM		
-Ash	6.5±0.1	6.5±0.1
-Protein	32.7±0.7	33.4±0.4
-Fat	21.9±0.5	23.2±0.3
-Carbohydrate (calculated)	38.9±0.6	36.9±0.6
-P	0.94±0.01	0.94±0.02
-Water-soluble P	0.35±0.09	0.35±0.09
-K	0.89±0.02	0.88±0.05
-Na	0.46±0.01	0.44±0.02
-Cl	0.72±0.01	0.70±0.06

Feed consumption, water turnover parameters, total water intake in relation to body weight as well as apparent digestibility of protein and DM for the blue fox and the silver fox are presented in Tables 3 and 4 respectively. Apparent protein digestibility was calculated by deducting the faecal protein output from the protein of the ingested feed.

Drinking water intake and water output in urine were corrected for 3 out of 6 blue

foxes and 1 out of 6 silver foxes provided with the drinking cup system. These four animals splashed water out of the drinking cups using their front feet, and some of this water was not caught by the water collection system, thus leading to an unreasonably high urine volume. The urine volume was corrected for by using the average DM content in urine for the rest of the animals in the group as a correction factor. Water surplus in the urine was then added to the volume of water spillage. Both the original and the corrected values are listed in Tables 3 and 4. Only the corrected values are used in Tables 5 and 6.

Table 3. Feed comsumption, water turnover parameters and digestibility for the blue fox. Values are means for a 4-day period ± SD. Values in italics are corrected, using average DM content in urine as correction factor

	Blue fox				
	Nipple		Cup		
	Dry diet	Wet diet	Dry diet	Wet diet	Total
Number of observations	6	6	6	6	24
Feed consumption, g DM/day	90±8	99±8	89±12	84±20	91±13
Water intake, g					
-in feed	6±1	116±9	6±1	98±24	
-drinking	294±34	233±50	522±216	294±113	
-drinking, corrected values			*423±117*	*256±67*	
-total	300±34	349±55	528±217	392±122	
-total, corrected values			*429±117*	*354±80*	*358±87*
Drinking water intake g/g DM	3.3±0.3	2.4±0.4	*4.8±1.1*	*3.2±1.0*	
Total water intake g/g DM	3.3±0.3	3.5±0.4	*4.8±1.1*	*4.4±1.0*	*4.0±1.0*
Total water intake g/kg body weight	43±9	51±7	*60±21*	*47±17*	*50±15*
Water output, g					
-urine	66±20	100±54	186±112	107±54	
-urine, corrected values			*86±16*	*68±16*	*80±32*
-faeces	70±18	86±16	76±16	59±20	72±19
-total	136	186	262	166	
-total, corrected values			*162*	*127*	*152*
Water balance, g	164±19	163±34	267±105	226±62	205±75
Apparent digestibility of DM, %	77±3	76±4	79±3	83±5	79±4
Apparent protein digestibility, %	76±3	78±3	82±4	86±3	80±5

For the blue fox, there was a significant difference ($p < 0.001$) in total water intake as well as apparent protein digestibility between the two drinking-water systems.

In silver fox no differences between drinking-water systems were found. There was no significant difference in total water intake between the two diets. The lower water content in the dry diet was fully compensated by a higher intake of drinking water in both species.

Table 4. Feed comsumption, water turnover parameters and digestibility for the silver fox. Values are means for a 4-day period ± SD. Values in italics are corrected, using average DM content in urine as correction factor

	Silver fox				
	Nipple		Cup		
	Dry diet	Wet diet	Dry diet	Wet diet	Total
Number of observations	6	6	6	6	24
Feed consumption, g DM/day	91±10	87±21	94±0	96±2	92±11
Water intake, g					
-in feed	5±1	112±27	6±0	125±2	
-drinking	236±43	145±47	272±70	229±176	
-drinking, corrected values			*266±55*	*201±113*	
-total	241±43	257±43	278±70	354±174	
-total, corrected values			*272±55*	*326±111*	*274±72*
Drinking water intake g/g DM	2.6±0.5	1.9±1.2	*2.8±0.6*	*2.1±1.2*	
Total water intake g/g DM	2.7±0.5	3.2±1.2	*2.9±0.6*	*3.4±1.2*	*3.0±0.9*
Total water intake g/kg body weight	42±6	46±5	*53±17*	*63±27*	*51±17*
Water output, g					
-urine	86±45	89±57	134±29	121±66	
-urine, corrected values			*127±21*	*94±24*	*99±41*
-faeces	45±17	29±10	28±4	51±11	38±15
-total	131	118	162	172	
-total, corrected values			*155*	*145*	*137*
Water balance, g	110±32	139±36	116±41	182±116	137±68
Apparent digestibility of DM, %	83±2	85±2	86±1	84±2	85±2
Apparent protein digestibility, %	86±2	88±2	88±1	86±2	87±2

The analysis of variance indicated that the total water intake of blue fox was significantly higher than that of silver fox ($p < 0.001$). This difference was mainly caused by the higher drinking-water consumption by blue fox. Water output in urine was higher for silver fox ($p < 0.05$), but the fact that the urine volumes were corrected makes this assertion unreliable. Water output in faeces was significantly lower for silver fox ($p < 0.001$). During the collection it was observed that the silver fox had a firmer consistency of faeces, while some of the blue fox tended to have diarrhoea.

The silver fox had a higher apparent protein digestibility as well as a higher digestibility of DM than the blue fox ($p < 0.001$). There was no effect of diet on digestibility, however.

A comparison between the two drinking-water systems concerning the level of water spillage is presented in Table 5.

The average water spillage from blue fox and silver fox is 178 ml per day. This amounts to 41% of the water supplied to the foxes. Water spillage in relation to supplied water was significantly higher with the drinking nipple system ($p < 0.001$). Individual variation between the animals in the drinking cup groups was mainly due to the behavioural problems described earlier. If those observations are omitted, water spillage for the

drinking cup groups was 6.5 % of the water supplied. The average urine volume in all groups was 98 ml per day. Urine volumes were slightly higher in silver fox than in blue fox. No correlation between total water intake and urine volume ($r^2=0.185$) was found in this experiment.

Table 5. Water spillage from different drinking systems. Urine volumes from the different groups. Corrected values are used for cup system. Values are means

	Nipple system			Cup system		
	Blue fox	Silver fox	Total	Blue fox	Silver fox	Total
Number of observations	12	11 [1]	23	12	12	24
Water supplied, g±SD	433±80	390±155	412±121	613±413	311±240	462±365
Water spilt, g±SD	169±58	189±111	179±86	274±308	78±164	176±262
Water spillage, %	39	48	43	45	25	38
Urine volume, g±SD	91±44	100±49	95±46	86±20	119±29	102±29

1) One observation omitted due to leaking nipple

Data concerning content of dry matter and nutrient elements in faeces, urine and calculated content in manure (faeces + urine) are supplied in Table 6.

Table 6. Content of DM and some nutrient elements in fresh faeces and urine from blue fox and silver fox. Calculated values on manure (faeces + urine). Values in % of DM, means ± SD

	Number of samples	DM, %	N	NH_4 - N	P	Water-soluble P	K
BLUE FOX							
Faeces	24	22.1±1.9	4.99±0.64	1.33±0.54	2.36±0.32	0.67±0.19	0.75±0.13
Urine	24	8.2±2.9	34.79±3.19	2.12±0.27	2.82±0.27	2.38±0.24	6.12±0.48
Manure (calculated)		14.4±3.7	13.58±1.54	1.56±0.38	2.48±0.28	1.17±0.13	2.29±0.25
SILVER FOX							
Faeces	24	28.5±5.2	4.52±0.38	0.41±0.17	3.21±0.48	0.38±0.10	0.53±0.09
Urine	24	7.6±2.9	43.59±5.76	2.03±0.42	3.03±0.54	2.59±0.46	7.41±0.96
Manure (calculared)		14.0±3.7	18.16±3.26	0.98±0.17	3.13±0.31	1.16±0.18	2.92±0.51

Manure from silver fox had a higher content of N, P and K. The differences were significant ($p < 0.001$). On the other hand, content of NH_4-N was significantly higher for blue fox.

In Table 7 it can be seen that an average of 78% of ingested N, P and K is excreted in urine and faeces. The urine contributes strongly to the high content of N and K in the manure; 73 to 88% of N and K was excreted in urine.

Table 7. Total excretion of N, P and K in faeces and urine for blue fox and silver fox. Excretion of N, P and K in urine. Values are means ± SD

	N	P	K
BLUE FOX			
Total excreted, in % of ingested	74±12	76±11	78±13
Excreted in urine, in % of total	73±6	33±4	77±5
SILVER FOX			
Total excreted, in % of ingested	82±14	79±6	75±13
Excreted in urine, in % of total	83±5	34±8	88±5

DISCUSSION

Water turnover

Total water intake, that is; water in feed and drinking water, depends on the total consumption of dry matter (DM) and metabolizable energy (ME). The digestibilities of fat and carbohydrates were not measured in this experiment. The relation between total water intake and energy consumption could therefore not be calculated. For the same reason, calculation of metabolic water was not included in the present experiment.

The total water requirement includes metabolic water, in addition to water in feed and drinking water. Metabolic water is produced by oxidation of the energyyielding components of the feed. Oxidation of fat yields 107 g water per 100 g, while protein and carbohydrates yield 40 g and 55 g respectively (Blaza 1982). However, metabolic water contributes a considerable proportion of the required water when feeding dry rations. In experiments with cats by Thrall & Miller (1976), metabolic water was calculated to 16% of total water consumed when feeding rations containing 91-93% DM. Mäkelä & Valtonen (1982), working with mink, estimated the water of oxidation to 10% of the total water requirement. The normal variation in the content of energy-yielding components of the feed will only lead to a 1-3% variation in total water requirement.

The total water intake is usually quantified in relation to consumption of DM. In the present investigation the total water intake varied from 3.0 to 6.4 g/g DM with an average of 4.0 g/g DM for the blue fox. The average total water intake of the silver fox was 3.0 ± 1.0 g/g DM varying from 1.9 to 5.7 g/g DM.

In an experiment by Mäkelä (1971), male mink were given five diets with different contents of DM, varying from 27.0% to 92.0%. The total water intake varied from 3.3 g/g DM for the 27% diet, decreasing to 2.8 g/g DM for the 92% diet with an average of 3.0 g/g DM. Neil (1983) reports that mink fed commercial, dry diets had a total water intake amounting to 4.1 g/g DM. Cats fed dry rations had a total water intake of 2.0 - 2.4 g/g DM, as reported by Thrall & Miller (1976).

On average, total water intake was 50 g/kg body weight and 51 g/kg body weight for blue fox and silver fox respectively. A similar water intake/body weight ratio (51 g/kg) was found for cats fed dry rations (Thrall & Miller 1976). Seefeldt & Chapman (1979) measured total water intake by cats fed dry rations to 58 g/kg body weight. Burger et al. (1978) found that dogs fed dry rations had a total water intake of 65 g/kg body weight.

Farrell & Wood (1968) found a low correlation (r^2 = 0.278) between water intake and body weight during weight fluctations in their experiments with mink. In the present investigation all the animals lost weight. During weight balance, a high correlation (r^2= 0.906) between water intake and body weight was found. Water intake amounted to 133 g/kg for the average mink (Farrell & Wood 1968). They concluded that the mink presumably have a higher water requirement related to body weight than other species. This is possibly connected with the fact that the mink has a substantially higher energy requirement for maintenance (kJ/kg body weight) than the fox (Aarstrand 1989). It can be questioned whether comparisons between different carnivorous animals using body weight as a parameter are meaningful.

In the present study, the lower water content in the dry diet was fully compensated by a higher intake of drinking water. Compensation for low water content in feed, by drinking more, has been found in experiments with mink (Mäkelä 1971; Neil 1983), dogs (Anderson 1981) and cats (Seefeldt & Chapman 1979; Sauer et al. 1985). In contrast, Anderson (1981) found a significant decrease in total water intake by cats fed dry food (93% DM) compared with wet food (16% DM). This supports the findings of Burger et al. (1978).

The water balance figures in the present experiment include the water from oxidation of feed and oxidation of body fat as well as respiratory water loss and salivary losses. The sum of these water losses is usually considered as "insensible water loss". The insensible water loss amounted to 57% for blue fox and 50% for silver fox. Urinary water loss and water lost via faeces were 22 % and 20 % respectively for the blue fox, and 36% and 14% respectively for the silver fox. In their experiments with cats Thrall & Miller (1976) measured a 25% faecal water loss, and insensible water loss was only 39%.

Water turnover by carnivorous animals is affected by several factors, such as protein level, salt level and ambient temperature.

Protein level in the diet affects the water requirement. Excretion of byproducts from the protein metabolism, mainly urea, requires water. A rise in protein level from 20.8% to 44% of ME increases water consumption as well as urine concentration by the mink (Makela & Valtonen 1982). Berg et al. (1984) also found that total water intake by the mink was positively correlated with the protein content of the food. The water requirement increased with increasing protein level. With protein levels corresponding to 53, 35 and 19% of ME, the total water intake was 3.0, 2.7 and 2.2 g/g DM, respectively.

In the present experiment the protein level was approximately 33% of ME. This protein level corresponds to the medium protein level in the experiments by Berg et al. (1984). Thus, assuming that the protein level has a similar effect on water intake in blue fox and silver fox as in the mink, the present results may represent a medium level water intake. However, this could be influenced by the urinary concentration capability of foxes, which remains to be investigated.

Excretion of salt (NaCl) also requires water. In addition salt affects thirst regulation through the plasma concentration of sodium (Na) (Neil 1986). Raising the dietary NaCl content in the feed from 0.6% to 1.1% on a wet basis, leads to a minor, but not significant, increase in water consumption by the mink from 176 to 196 g/day. With a further increase in NaCl content to 1.6% and 2.6%, the water intake increased to 290 and 421 g/day, respectively, and urine concentration of the mink decreased (Eriksson et al. 1984). In the

present investigation the NaCl level was 0.5% and 1.1% for the wet diet and the dry diet respectively. In terms of DM, this corresponds to 1.2%. The effect of different salt levels in foxes has not been investigated, but compared with the findings in studies with mink, the salt level was moderate.

Ambient temperature affects the drinking-water intake. The respiratory water loss increases with higher ambient temperatures. In hot weather, evaporation in the lungs is an important temperature regulating mechanism because the body heat is used to vaporize the water. The respiratory water loss must be compensated by a higher water intake. A rise in temperature from 1-5°C to 20-30°C, increases the drinking-water intake by approximately 10 times according to Schicketanz (1981). Effects of ambient temperature on the water intake of the blue fox and the silver fox have not been reported so far.

The animals in the present study had a fully developed winter fur, and it can be questioned whether the environmental temperature of +10°C would lead to an increase in drinking-water intake due to heat regulation.

Digestibility

The energy supply of the animals is determined by feed intake and energy concentration in the feed. The availability of the feed energy depends on the capacity of the digestive system of different species. Factors determining this capacity are development of the digestive tract, time of feed passage and production of digestive enzymes.

Results from digestibility experiments with mink are often used to indicate digestibility in the foxes as well. However, Skrede & Ahlstrøm (1992) found that the protein digestibility of the blue fox was 7-8% higher than that of the mink, while the difference in digestibility of fat was 5-6%.

Furthermore, blue fox and silver fox are usually fed the same type of feed, even though they are of different species with different natural habitats. The present results show that the silver fox has a significantly higher apparent protein digestibility and dry matter digestibility than the blue fox when fed a commercial dry diet. These results might be of importance in order to optimalize the diets for the two fox species.

Water spillage

Water spillage from fur farms is a subject which has been given little attention. A growing awareness of water pollution problems has changed this. Manure stored on the ground should be sheltered from rain and water floating on the surface. If this is so, urine and water spillage will be the only remaining fluids that can penetrate through the manure, thereby causing infiltration of nutrient elements in ground water. The present investigation indicates that spillage of drinking water is the more important of these two factors. Average water spillage amounted to 178 ml per day while average urine volume was 98 ml. Leading the water spillage away from the cages would contribute to a decrease in the risk of pollution problems. Practical experience from the present trials has shown that it is technically easier to collect water spillage from drinking nipples than from drinking cups.

It is surprising that as many as one out of two blue foxes and one of six silver foxes are inclined to splash water out of their drinking cups. The question is whether the animals would act in the same way if the drinking cups were manually filled and the water supply was thereby limited. If so, this might lead to periodic water balance problems. The reason

for this behaviour is not easily explained, but a generally non-stimulating environment leading to stereotypies by the foxes may be one explanation. The cages used in this experiment were specially designed for experimental purposes. They were supplied with neither resting-shelves nor nest boxes. Furthermore, the animals were moved to an entirely new environment. The pre-experiment adaption period of three days, might be too short.

Foxes using drinking-cups with "normal" drinking behaviour seem to spill from 0% to 18% with an average of 6.5% of the supplied water. Water spillage varies from 8% to 62% with an average of 43% (Table 5) for the drinking-nipple groups. Drinking cups are more environmentally suitable, especially if the stress reactions which appeared in this experiment are avoided. The drinking-nipple may adapt rather poorly to the natural drinking behaviour of the foxes, thus causing extensive water spillage, but the animals displayed no signs of having problems with drinking enough water. Kangas (1973) reported that mink, both male and female, had a higher growth rate when they were provided with an open drinking cup instead of a drinking nipple. No such conclusions can be drawn in the present experiment. In addition to the level of water spillage, factors such as hygienic quality and possibilities of water temperature control, particularly during wintertime, are important in order to find the most favourable drinking-water system.

Content of nutrient elements in faeces and urine

The content of plant nutrient elements in manure depends on several factors, e.g. nutrient content in feed, digestibility, manure storage conditions and time of storage.

Little information concerning content of nutrient elements in manure from the blue fox and the silver fox is available in the literature. In most digestibility experiments little attention has been paid to nutrients other than nitrogen (protein), fat and carbohydrates. The possibility of comparing the present results with other studies is thereby limited.

A calculation of the difference between ingested and retained N and P in blue fox and silver fox made by Sundstøl & Mroz (1988) indicates that, theoretically, the excretion level of these nutrient elements reaches approximately 95% on a yearly basis. The figures for retained N and P in these calculations comprise growth of whelps produced by adult vixens in weight balance. In the present study, approximately 80% excretion of N and P was found. Some nitrogen from the urine and faeces might have become lost during the period from excretion to collection and freezing. Urine was collected for four days without adding any acids in order to reduce ammonia losses. The difference between the theoretical excretion of P and our actual findings is not easily explained.

The N content in stored manure from blue foxes and silver foxes was on average 6.74 and 4.63% of DM, repectively, in the studies by Tveitnes (1989), using samples from fur farms with different storage conditions. Compared with the results of the present study, this indicates that nitrogen loss during storage is substantial. Nitrogen losses as ammonia are likely to be high even with a short storage period and urine might have drained off. The samples of Tveitnes (1989) had a higher content of P, but a lower content of K for both species. This may have been caused by different diets.

A Danish analysis of fresh manure from mink (Kjellerup & Lindhard 1977) indicates a much lower content of N (5.56% of DM) and K (0.73% of DM) than the manure from the foxes in the present trials. The content of NH_4-N (2.05% of DM) and P (4.88 % of DM) was higher for the mink manure. It should be noted that these samples were taken

under farm conditions and from a substratum of sand, and no information on feeding and time of storage was given.

In practical fur-farming, wet diets are most commonly used for foxes. In this experiment the pelleted, dry food was preferred because it was of vital importance to ensure that the two diets had an equal content of nutrients except from water. Further investigations on the various subjects presented in the present study, using different wet diets, would be valuable.

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Growth regulation of pansy by chlormequat

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Pansy plants were raised under high summer temperature regimes and using chlormequat as a growth retardant spray. The treatments had no influence on days to marketing stage or flower size, but the application had an unstable and changeable effect on plant and flower peduncle height. Increased growth and flower peduncle stretching was found at low chlormequat concentrations. Plant quality was usually increased when chlormequat was used. A relatively high application rate (1500-2000 mgl^{-1}) and repeated treatments (3-4 times) are recommended for spraying with chlormequat.

Key words: Chlormequat (CCC), cycocel, pansy, *Viola* x *wittrockiana*

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Pansies are attractive bedding plants in Norway and those sold for planting in April and May are raised from seed sown in January and February. A low temperature programme is used throughout the cutivation period in order to produce plants with a compact growth. A more intensive cultivation with a higher temperature is of interest, but makes demands on growth regulation.

Pansies tend to have a slow growth rate to start with, but with some acceleration later. Unfortunately, stretching of the plants occurs at high temperatures and this is thought to take place in the last period before flowering (Pettersen & Litlere 1986). Alar (daminozide) and CCC (chlormequat) are both recommended as growth retardants for pansy (Oschek 1988; Evers 1989; Bjørnå 1990), but a too high concentration often results in leaf damage and therefore a low concentration and repeated treatments are recommended (Bjørnå 1990). An earlier experiment (Bævre unpubl.) was carried out using chlormequat (spray and soil drench), daminozide, ethephon, dikegulac and ancymidol. Chlormequat spray in concentrations of 2400 mgl^{-1} active matter and at higher concentrations resulted in considerable leaf damage, stunted leaves and flowers, while chlormequat soil drench delayed flowering. In the same experiment, dikegulac (1-10 mgl^{-1} active matter) led to the production of stunted plants only, while ethephon strongly delayed flowering and bleached the leaves. Daminozide (120-1000 mg^{-1}, 1-3 treatments) did not cause any reduction in growth and although ancymidol (1-10 mgl^{-1} active matter) damaged the leaves, it produced relatively compact plants when consentrations of 1-3 mgl^{-1} were used.

The purpose of this investigation was to give a recommendation which included

growth retardants in the cultural programme.

MATERIAL AND METHODS

This investigation, which was carried out in a glasshouse during the summer, included four experiments in May-July (Experiments 1 and 2) and July-September (Experiments 3 and 4). Experiments 1 and 2 were sown on 28 May and flowered in July and Experiments 3 and 4 were sown on 8 July and flowered at the beginning of September. The plants were potted 18 days after sowing, on 15 June and 26 July, respectively.

The plants were raised and grown in fertilized peat using a Vefi-System with about 100 plants per square metre.

Varieties in the experiments were:

Experiment 1	Experiment 2
Springtime White F_1	Spring Sun mørkeblå m/øje F_1
Coronation Gold	Europa White F_1
Favorite light red F_1	Europa Red F_1
Europa Blue w/ blotch F_1	Premiere Braunrote Töne F_2

Experiment 3	Experiment 4
Springtime White F_1	Premiere Weiss F_2
Favorite light red F_1	Premiere Braunrote Töne F2
Spring Sun mørkeblå m/ øje F_1	Premiere Dunkelblau F_2
Europa White F_1	
Europa Red F_1	
Europa Blue w/ blotch F_1	
Premiere Weiss F_2	
Premiere Braunrote Töne F_2	
Premiere Dunkelblau F_2	

Seeds were supplied by L. Dæhnfeldt a/s ('Spring Sun'), Sutton Seeds Ltd. ('Coronation Gold'), L. Clause ('Favorite'), KSA-Goldsmith a/s ('Europa'), Harris Seeds ('Springtime') and Ernst Benary Samenzucht GmbH ('Premiere').

The experiments were designed as a factorial combination between the concentration of the growth retardants and number of applications. Cycocel (chlormequat) growth retardant was sprayed in the following concentrations (active matter): 500, 1000, 1500 and 2000 mgl^{-1}. Each concentration of chlormequat was sprayed on the plants to drip point from one to four times. The growth retardant was used in the last period before sale and the first

application took place when the flower peduncle started to grow from the base of the plant. The plants were sprayed at intervals of two to four days. The treatments for Experiments 1 and 2 were - depending on variety - executed from 10 to 19 July, while plants in Experiments 3 and 4 were sprayed from 17 to 27 August. The experiments included each of three replications with nine (Experiments 1 and 2) or six (Experiments 3 and 4) plants per plot, making a total of 4086 plants.

The night temperature was maintained at 18°C, while the day temperature occasionally rose to 25°C on sunny days.

The marketing stage was taken as having been reached when the second flower on the plant had just opened. All data were recorded at this stage. The height and the width was measured on the first flower. Plant height was meaured from the pot rim to the top of the leaves and the flower peduncle was measured from the base up to the flower. The plant quality, i.e. firmness and compactness of plant, was estimated according to a scale from 0 to 7, where 7 indicated the best character. Leaf damage caused by the chlormequat spray treatment reduced the values.

Observations were subjected to a two-way analysis of variance, where $p < 0.001$ ***, $p < 0.01$ ** and $p < 0.05$ * indicate 0.1, 1 and 5% levels of significance, respectively.

RESULT

Cultivation period

The cultivation period from potting to flowering was 42 days in Experiments 1 and 2 and 50 days in 3 and 4. The plant variety had some influence ($p < 0.001$) on the cultivation period in all experiments, but not the different treatments with chlormequat. 'Europa' and especially 'Europa Red F_1' were early varieties. 'Favorite light red F_1' and 'Premiere Braunrote Töne F_2' were the two latest varieties, about two to five days later than 'Europa'. No significant effects on cultural period as a result of interactions between variety and chlormequat concentration, variety and number of treatments or between the chlormequat concentration and number of treatments were recorded.

Flower size

The flower size, measured as maximum height (h) and width (w) of the first flower, did not vary significantly with the concentration of chlormequat (no chlormequat included) or number of spray treatments. On the other hand, flower size varied ($p < 0.001$) among varieties. The varieties with the smallest and largest flowers in each experiment were:

Experiment 1. 'Favorite light red' 46 mm w/52 mm h,'Europa Blue with blotch' 52 mm w/56 mm h.
Experiments 2. 'Premiere Braunrote Töne' 41 mm w /46 mm h, 'Europa Red' 50 mm w/57 mm h.

Experiment 3. 'Premiere Braunrote Töne' 39 mm w/43 mm h, 'Europa Red' 48 mm w/54 mm h.
Experiment 4. 'Premiere Braunrote Töne' 38 mm w/42 mm h, 'Premiere Weiss' 40 mm w/45 mm h.

The coefficient of correlation between flower height and flower width was 0.90 *** based on single plants and 0.96*** on the basis of the average of each replicate .

Plant height

The height to the top of the leaves in single plants varied between variety and experiment from 7 to 11 cm. The plant height was ($p < 0.001$) different between varieties in all experiments. Treatments with growth retardant did not result in any interaction between variety and plant height. The highest and the lowest varieties in the control treatment were also the highest and lowest as an average of all treatments. The varieties with the highest and lowest plants, respectively, in each experiment were:

Experiment 1 'Europa Blue w/blotch', 'Favorite Red'
Experiment 2 'Premiere Braunrote Töne', 'Europa White'
Experiment 3 'Springtime White', 'Favorite Red'/'Premiere Dunkelblau'
Experiment 4 'Premiere Braunrote Töne', 'Premiere Dunkelblau'

A high concentration of chlormequat significantly reduced the plant height in two out of four experiments (Table 1) and this was ascribed to an increased plant growth at a low chlormequat concentration. The significant effect in Experiment 4 was an increase in growth at a low chlormequat application. Although there was a reduction in plant height at the highest concentration, the effect was variable.

Table 1. The plant height (cm) of pansy as affected by use of different concentrations (mgl^{-1} active matter) of chlormequat spray. 0 = untreated control plants

Chlormequat concentration	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	9.4	9.7	9.6	9.2
500	10.6	8.2		10.2
1000	9.4	8.2	9.2	9.9
1500	9.0	8.0	9.2	9.3
2000	8.7	7.9		9.1
Significance	***	n.s.	n.s.	***

The number of treatments with chlormequat had a poor effect on plant height (Table 2), with up to four treatments showing a significant reduction in only two out of four experiments.

There was no significant interaction between chlormequat concentration and the number of applications on plant height.

Table 2. The plant height (cm) of pansy affected by the number of treatments with chlormequat spray. 0 = untreated control plants

Number of treatments	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	9.4	9.7	9.6	9.7
1		8.5		9.6
2		8.2	9.8	9.4
3		7.7	9.3	9.5
4	9.4	7.8	8.6	9.9
Significance	n.s.	***	***	n.s.

Flower peduncle height

Flower peduncle height varied between 10 and 13 cm among varieties and the differences in length were significant ($p < 0.001$) between varieties in every experiment. There was no accordance between flower peduncle height on untreated plants and the flower peduncle height of all treatments. 'Coronation Gold' (Experiment 1), 'Premiere Braunrote Töne' (Experiment 2), and 'Premiere Dunkelblau' (Experiments 3 and 4) had the highest flower peduncles, while 'Europa Blue w/blotch' (Experiment 1), 'Spring Sun' (Experiment 2) and 'Premiere Braunrote Töne' (Experiments 3 and 4) had the shortest flower peduncles as an average of all treatments.

There was a tendency toward increased growth of the flower peduncle up to a low or a medium concentration of chlormequat (Table 3). The reduction in peduncle height at the highest concentration in two of the experiments was not significant compared with that in the untreated plants. The peduncle height increased significantly ($p < 0.001$) with number of applications in two experiments, while it was significantly ($p < 0.001$) reduced in the third experiment (Table 4).

Table 3. The height (cm) of the flower pedeuncle of pansy as affected by use of different concentrations (mhl^{-1} active matter) of chlormequat spray. 0 = untreated control plants

Chlormequat concentration	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	11.6	11.3	11.2	10.4
500	12.4	10.9		11.5
1000	11.1	10.8	11.8	11.5
1500	11.3	10.7	11.7	11.3
2000	10.7	10.4		11.3
Significance	***	n.s.	n.s.	n.s.

Table 4. The height (cm) of the flower peduncle of pansy as affected by different numbers of treatments with chlormequat spray. 0 = untreated control plants

Number of treatments	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	11.6	11.3	11.2	10.4
1		11.2		10.8
2		10.6	11.9	11.3
3		10.3	11.9	11.4
4	11.4	10.7	11.4	12.2
Significance	n.s.	***	***	***

Plant quality

The quality of treated plants increased compared with that of untreated plants, depending on a moderate or a high concentration of chlormequat and on the number of treatments in the experiments (Table 5). In Experiments 1-3, however, there was no significant interaction between the concentration of chlormequat and number of treatments on plant quality, and the highest point was reached with a high concentration (1500-2000 mgl^{-1}active matter) and repeated spraying (3 or 4 treatments). In Experiment 4, plant quality was highest at concentrations of 1000-2000 mgl^{-1} active matter and two treatments.

Table 5. Pansy plant quality 0-7) as affected by chlormequat spray in different concentrations (mgl^{-1} active matter) and different numbers of treatments. 0 = untreated control plants

Chlormequat concentration	Number of treatments	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0		4.3	4.0	4.3	4.3
500		4.3	5.1	4.6	4.0
1000		5.3	5.4	4.6	4.1
1500		5.2	5.5		4.6
2000		5.0	5.7		4.7
Significance		***	**	n.s.	***
	0	4.3	4.0	4.3	4.3
	1		4.8		4.4
	2		5.4	4.1	4.6
	3		5.9	4.5	4.3
	4	4.9	5.5	5.1	4.1
	Significance	***	***	***	**

There was no significant interaction between variety and the concentration of the growth retardant on plant quality in any experiment, but the interaction between variety and number of treatments was significant ($p<0.001$) in Experiments 2 and 3, where especially 'Premiere Braunrote Töne F_2' (Experiment 2) and 'Springtime White F_1', 'Spring Sun F_1', 'Europa Blue w/ blotch F_1' (Experiment 3) indicated a positive effect of increased number of treatments.

A correlation was found between plant quality, plant height and flower peduncle height. The coefficients of correlation between plant quality and plant height and plant quality and flower peduncle height, were -0.69 *** and -0.51***, respectively.

DISCUSSION

The elongation of the flower peduncles and plants with large leaves and lack of compactness constitute a problem when raising pansy plants under high temperature regimes. Different treatments with growth retardants in an earlier experiment revealed some negative effects on pansy plants (Bævre unpubl.). This investigation indicated a variable effect of chlormequat. A significant reduction in growth or stretching of the flower peduncles as a function of chlormequat concentration or number of treatments in one experiment changed to a non-significant difference in growth in another experiment. Even the use of low chlormequat concentrations significantly increased growth or peduncle stretching. Increased growth was observed by Halevy & Wittwer (1965) on snapdragon, by Daniel & Escher (1986) on seemannia and by Nørremark (1986) on verbena. Spraying with chormequat did not have a negative effect on cultivation period or the flower size. Plant quality was increased up to a medium or high chlormequat concentration and with repeated applications.

Chlormequat application in the late phase of plant development was not a satisfactory growth retardant for pansy raised under high temperature regimes. It is possible that an early application may have a more positive influence on plant growth, but there is a risk of delayed development and stunted plants. A better growth retardant for pansy was found by Lembeck & Siepker (1991). Increased concentrations of the fungicide Vigil reduced the plant height gradually and more effectively than daminozide. Growth regulation with chlormequat spray requires a relatively high concentration (1500-2000 mgl^{-1} active matter) and repeated treatments (3-4 times). Despite a weak and unstable effect on different parameters of the plants, spraying with chlormequat can affect the plant quality in a positive way. The better plant quality, in spite of the poor and variable effects on different plant parameters, must be attributed to a firmer plant and a stronger peduncle.

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Regulation of growth in *Begonia* x *cheimantha* by ancymidol and chlormequat treatments

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Leaf cuttings of *Begonia* x *cheimantha* cv. Nova were potted in the middle of September and grown according to a short culture programme with high air temperature and supplementary lighting described by Moe et al. (1992). The plants were treated with ancymidol or chlormequat applied as a soil drench (50 ml per 12-cm pot) or with chlormequat as a spray (two or four applications). The ancymidol and chlormequat treatments had no influence on days to marketing stage, but the elongation of the inflorescence (flower branches and flower peduncles) was strongly retarded and most pronounced at the highest application rate. The effect of growth retardants on the growth of the leaves (leaf height) was not as great. A high application rate reduced the flower size significantly. With chlormequat spray a concentration of 1000-2000 mgl^{-1} active matter seems to be appropriate when the treatments are applied twice. With four treatments a concentration of about 1000 mgl^{-1} seems optimal. With ancymidol or chlormequat applied as a soil drench the concentration should be 2 or 2000 mgl^{-1}, respectively, in order to reduce the elongation of the inflorescence and total plant height sufficiently. Starting the application of the growth retardants early at the beginning of short-day (SD) treatment, had the strongest effect on leaf growth (leaf height), while treatment at the end of SD or two and four weeks later had a similar effect on total plant height.

Key words: Ancymidol, *Begonia* x *cheimantha*, chlormequat

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In order to make the production of Christmas begonia (*Begonia* x *cheimantha* Everett) more economical, a short culture programme of about 12 weeks from potting until marketing stage has recently been reported (Moe et al. 1992). Such a growing programme requires higher growing temperatures and supplementary lighting, but this can create problems in that the growth of the plants is enhanched and there can be too great an elongation of the leaves, flower branches and flower peduncles. Application of growth retardants may therefore be required for production of compact plants. It has been reported that both chlormequat and ancymidol treatments reduced plant height of Christmas begonia, but the results of the treatments are not always adequate (Tangerås 1980, unpublished date).

The aim of the present study was to find the optimal application rate of growth retardants and how this works in a short culture programme of Christmas begonia.

MATERIAL AND METHODS

Two experiments were carried out in double-layer acrylic greenhouses at The Agricultural University of Norway, Ås and at Kvithamar Agricultural Research Station. Correspondingly results were attained, but only those from Kvithamar Agricultural Research Station are reported. Results from The Agricultural University are shown in two figures. Rooted leaf cuttings of the cv. Nova were planted in fertilized peat in 12-cm diameter plastic pots on 13 September. The plants were soft pinched on 25 September. Short-day (SD) treatment (10 h photoperiod) lasted from 3 October to 17 October. The plants were given supplementary lighting during the whole culture programme using high-pressure metal halide lamps (HPI/T) at an irradiance level of 7.5 Wm^{-2} PAR at the top of the plants. The irradiance was measured with a lux meter (conversion factor 2.8). The photoperiod was 10 h in the flower inductive phase, otherwise 24 h. The temperature was maintained at 20-21°C until the end of week no. 42 (the end of the SD period), then gradually decreased to 18°C. The plants were fertilized with a complete nutrition solution, as described earlier (Moe et al. 1992). A CO_2 concentration of about 600 $\mu l l^{-1}$ was established by enrichment of the greenhouse with pure liquid CO_2.

The growth retardants used were ancymidol (Reducymol) and chlormequat (Cycocel). Ancymidol was applied as a soil drench of 50 ml per pot at concentrations levels of 2,4 and 8 mgl^{-1} active matter. Chlormequat treatments were given as a soil drench of 50 ml per pot at concentrations of 2000, 3000, 4000 and 5000 mgl^{-1} active matter, or as a spray (500, 1000, 1500, 2000 and 3000 mgl^{-1} active matter) until drip point. The chlormequat spray was applied two or four times at intervals of one week. The first treatment was carried out at the end of the SD period (3 October). The treatments where growth retardants were applied as a soil drench were carried out at the end of the SD period, two weeks later or four weeks later, i.e. on 3 October, 17 October and 31 October, respectively. Treated plants were compared with untreated plants (control).

The marketing stage was reached when each plant had 15 completely open flowers. The experiment was terminated on 15 December and the following data were recorded:

- Number of open flowers per plant
- Leaf height measured from the pot rim to the top of the leaves
- Total plant height measured from the pot rim to the top of the inflorescences
- Plant diameter measured right-angled
- Different parts of the inflorescence were measured (Fig. 1):
  * The first flowering branch, from the leaf to the first branching (1)
  * Flower peduncle for the 1st and 2nd flowers on the first flowering branch, (1.1) and (1.2) respectively
- Flower diameter. The first (1.1) and the second (1.2) open flowers on the first flowering branch, and the first (2.1) and the second (2.2) open flowers on the second flowering branch were measured right-angled
- Plant quality was ranged according to a scale from 0 (poor quality) to 7 (very good quality)

Flower branch 1 (b. 1), the first flowering branch on the plant
Flower peduncle 1.1 (p. 1.1), the peduncle to the 1st flower on the first branch
Flower peduncle 1.2 (p. 1.2), the peduncle to the 2nd flower on the first branch
Flower 1.1 (f. 1.1), the 1st flower on the first branch
Flower 1.2 (f. 1.2), the 2nd flower on the first branch
Flower 2.1 (f. 2.1), the 1st flower on the second branch
Flower 2.2 (f. 2.2), the 2nd flower on the second branch

Fig. 1. Description of the inflorescences of *Begonia* x *cheimantha* associated with this investigation

Considerable importance was attached to a good proportion between leaves and inflorescence when estimating the plant quality.

The experiments were carried out with two replicates and with seven plants per plot. Observations were subjected to a two-way analysis of variance. The relationship between

any variables was determined by simple correlation analysis. $p < 0.001^{xxx}$, $p < 0.01^{xx}$ and $p < 0.05^{x}$ indicating a 0.1%, 1% and 5% level of significance, respectively.

RESULTS

Culture length

The average culture length from potting to marketing stage was 80.2 days, which varied from 79.6 days for plants treated with ancymidol to 81.2 days for the control plants. Use of different growth retardants and application rates had no significant influence on time to marketing stage. However, the growth retardant applications resulted in slightly greater flower number on 15 December. Plants treated with ancymidol had 24.4 open flowers at this registration, which was a significantly ($p < 0.01$) greater flower number than that for untreated control plants (20.2 flowers). The chlormequat treated plants had 23.5 flowers (chlormequat as soil drench), 22.8 flowers (sprayed twice) and 22.7 flowers (sprayed four times). There was no significant effect of time of treatment on the culture length or the number of flowers on 15 December, neither was there any significant interaction between the treatments and the time of treatments on culture length or number of flowers.

Length of flower branch and flower peduncles

Treatments of ancymidol and chlormequat resulted in a significant reduction in the length of both flower branch ($p < 0.001$) and flower peduncles of the inflorescences ($p < 0.001$) compared with those of untreated control plants (Table 1). Four applications of chlormequat spray resulted in significantly shorter flower branches and flower peduncles of first order (1.1) ($p < 0.01$) and second order (1.2) ($p < 0.05$) compared with the effects of two applications of chlormequat spray.

Chlormequat treatment sprayed twice or applied as a soil drench made no significant difference to the length of the flower branch or the flower peduncles (Table 1). Application of ancymidol or chlormequat as a soil drench had a similar effect on the length of the flower branch (Table 1).

Table 1. Main effects of growth retardant treatments (50 ml/pot as soil drench or spray) on the length (mm) of the first flower branch and the peduncles of the 1st and 2nd flowers of the first branch of *Begonia* x *cheimantha* cv. Nova. For inflorescence explanation see Fig. 1.

Part of the inflorescence	Control	Ancymidol as soil drench	Chlormequat as soil drench	Chlormequat spray Number of applications 2	Chlormequat spray Number of applications 4	P
Branch 1.1	63.9	52.7	51.5	52.9	46.8	***
Peduncle 1.1	31.4	25.8	27.4	27.3	25.0	***
Peduncle 1.2	18.4	15.6	15.7	16.4	15.0	***

Both ancymidol and chlormequat treatments applied as a soil drench at the lowest con-

centration significantly reduced the length of the flower branches compared with those in untreated control plants ($p < 0.05$ and $p < 0.001$ for ancymidol and chlormequat, respectively) (Fig. 2). The effect of the growth retardants applied as a soil drench was similar at low and high concentrations, but chlormequat spray at the lowest concentration (500 mgl^{-1}) had no influence on the flower branch. However, after a further increase in the chlormequat concentration from 1000 to 3000 mgl^{-1}, the growth of the flower branch was very significantly retarded ($p < 0.001$) compared with that of untreated control plants. The reducing effect was especially good after four applications (Fig. 2).

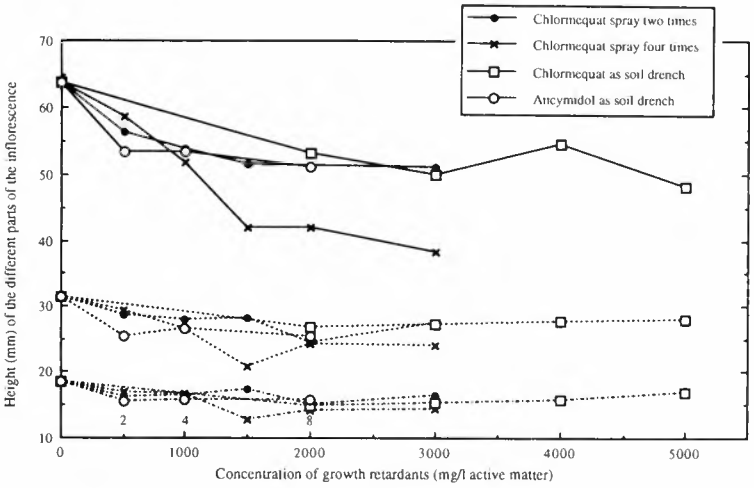


Fig. 2. The length (mm) of the first flower branch (1.2) ————, the first (1.1) ····· and the second (1.2) — · — · — · — · flower peduncle on the first flower branch of *Begonia* x *cheimantha* cv. Nova as affected by various treatments with growth retardants

The lowest concentration of ancymidol (2 mgl^{-1}) reduced flower peduncles 1.1 and 1.2 significantly ($p < 0.001$) to 25.3 and 15.4 mm compared with those of untreated control plants which were 31.4 and 18.4 mm, respectively. The same effect was obtained with chlormequat applied as a soil drench at a concentration of 2000 mgl^{-1}. Increasing the concentration of these two growth retardants applied as a soil drench did not result in a further reduction in length of the peduncles. Chlormequat spray has a slightly reducing effect on the length of the peduncles at the lowest concentration (500 mgl^{-1}) with both two ($p < 0.05$) and four (n.s.) applications. An increased concentration of chlormequat spray reduced the length of the peduncle considerably, especially when the plants were sprayed four times (Fig. 2).

When the application of ancymidol or chlormequat as a soil drench was delayed (0,2 or 4 weeks from the start of the SD treatment), the result was a non-significant increase in the first flowering branch from 49.1 mm to 51.3 mm and 54.6 mm. On the other hand, peduncle 1.1 was significantly ($p < 0.01$) reduced in length when the treatment was delayed.

Plant height and plant qualities

Both ancymidol and chlormequat treatments significantly reduced the total plant height ($p < 0.001$) and the leaf height ($p < 0.05$) compared with plant and leaf height of the untreated control plants (Table 2). Plants sprayed with chlormequat four times were significantly ($p < 0.01$) shorter than those sprayed twice. The leaf height was not significantly different for the various growth retardant treatments. The plant diameter was significantly reduced compared with that of untreated plants with four applications of chlormequat spray ($p < 0.001$) and with chlormequat applied as a soil drench.

Table 2. Main effects of growth retardants on total plant height (cm), leaf height (cm) and plant diameter (cm) of *Begonia* x *cheimantha* cv. Nova

			Chlormequat			
				spray		
		Ancymidol		Number of applications		
		as soil	as soil			
Plant growth	Control	drench	drench	2	4	P
Total plant height	21.5	18.9	19.4	19.6	18.1	***
Leaf height	11.7	9.8	10.0	9.9	9.8	ns
Plant diameter	16.4	15.5	15.2	4.3	14.9	**
Plant quality	3.3	4.1	3.6	4.3	3.7	*

Increasing the concentration of growth retardants had a great influence on the total plant height (Fig. 3). With two applications of chlormequat at 500 mgl^{-1} there was no significant reduction in the total plant height, but with four applications the total plant height was significantly ($p < 0.05$) shorter than that of the control plants. The lowest concentration (2 mgl^{-1}) of ancymidol reduced the plant height considerably ($p < 0.01$), but a further increase did not result in shorter plants. However, increasing the concentration of chlormequat resulted in a significant ($p < 0.001$) reduction in the total plant height compared with that of the untreated control plants.

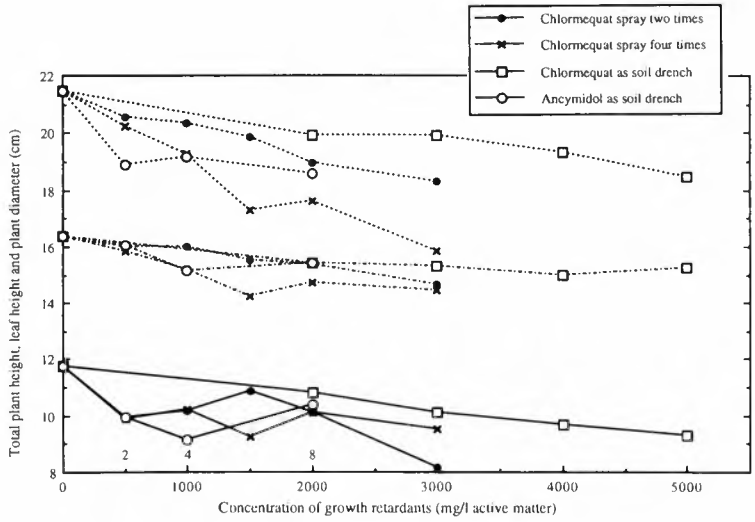


Fig. 3. The total plant height (·····), leaf height (– · – · – · –) and plant diameter (——) of *Begonia* x *cheimantha* cv. Nova as affected by various growth retardants

Plants sprayed with chlormequat at a concentration of 500 mgl^{-1} or applied as a soil drench of 2 mgl^{-1} ancymidol had a significantly ($p < 0.05$) shorter leaf height (9.9 cm) than that of untreated control plants (11.7 cm) (Fig. 3). Chlormequat supplied as a soil drench had the effect of gradually decreasing leaf height from 10.8 cm to 9.3 cm when the level of concentration was increased from 2000 to 5000 mgl^{-1}. The plants were significantly ($p < 0.05$) shorter than untreated control plants at a concentration of 4000 mgl^{-1}. There was no significant effect of two or four treatments with chlormequat spray on the leaf height, nor did time of application have any influence on total plant height, but early treatments (0 or 2 weeks after start of SD treatment) reduced leaf height and plant diameter significantly (Table 3).

Table 3. Total plant height (cm), leaf height (cm), plant diameter (cm) and plant quality (estimated by using a scale from 0 to 7) of *Begonia* x *cheimantha* cv. Nova, as affected by time of treatments with growth retardants (ancymidol and chlormequat as soil drench). The plants were treated 0, 2 or 4 weeks after the start of short-days

Plant growth and quality	Time of treatment (weeks) 0	2	4	P
Total plant height	19.0	18.8	19.7	ns
Leaf height	9.1	9.3	11.5	***
Plant diameter	15.2	15.2	15.7	*
Plant quality	4.7	3.6	3.4	***

Growth retardant treatments reduced the plant diameter (Table 2). Chlormequat supplied as a soil drench at the lowest concentration produced plants with a significantly ($p < 0.05$) smaller diameter than untreated control plants (Fig. 3). The plant diameter decreased markedly ($p < 0.01$) when the concentration was gradually increased to 3000 mgl^{-1}. The other treatments also produced plants with a significantly ($p < 0.05$ for ancymidol, $p < 0.001$ for chlormequat) smaller diameter than that of untreated control plants when the concentration was increased. Plants sprayed with chlormequat four times had a markedly ($p < 0.05$) smaller diameter than plants sprayed only twice.

The quality of the plants treated with growth retardants was improved and classified as better than that of the untreated control plants. Two applications with chlormequat produced a considerably ($p < 0.05$) better plant quality than four applications. A delay in time of treatment resulted in a gradual and significant reduction in plant quality (Table 3). There was no significant interaction between growth retardant treatments on the plant quality.

Flower size

The main effect of treatments with ancymidol and chlormequat on flower size is indicated in Table 4. The various growth retardant treatments reduced the flower size compared with that of the untreated control plants. The effect was most pronounced with the ancymidol treatment or with four applications of chlormequat as a spray (Table 4).

Ancymidol treatment at a concentration of 2 mgl^{-1} produced significantly ($p < 0.01$) smaller flowers than those of the untreated control plants (Fig. 4). Furthermore, chlo-

rmequat applied as a soil drench at the lowest concentration (2000 mgl^{-1}) had a significant influence on the flower size of flowers 1.1 ($p < 0.05$), 1.2 ($p < 0.01$) and 2.1 ($p < 0.001$). Chlormequat spray at the lowest concentration (500 mgl^{-1}) affected the flower size to a lesser extent with two applications than with four applications (Fig. 4). With a higher concentration (1500 mgl^{-1}) the flower size was markedly reduced and as the number of applications increased from two to four, the flower size became significantly ($p < 0.01$) smaller (Figure 4). Time of growth retardant treatments had no influence on flower size (data not shown).

Table 4. Flower diameter (mm) of *Begonia* x *cheimantha* as affected by various treatments with growth retardants. Explanation of flower number in Fig. 1

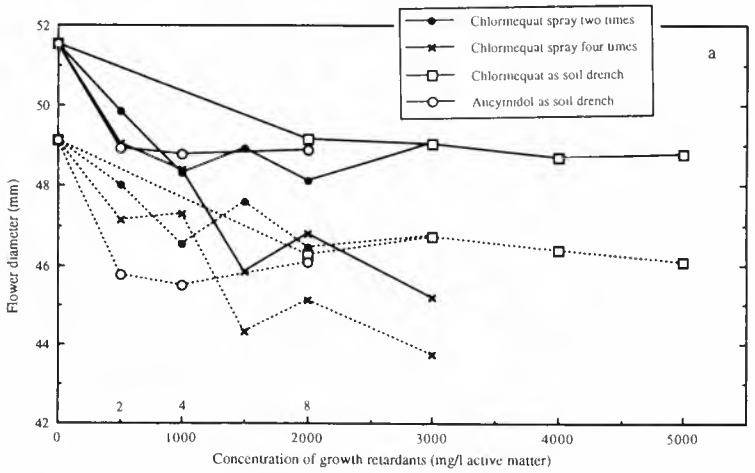
			Chlormequat			
		Ancymidol		spray		
		as soil	as soil	Number of applications		
Flower number	Control	drench	drench	2	4	P
Flower 1.1	51.5	48.9	48.9	48.9	47.1	***
Flower 1.2	49.1	45.8	46.3	47.1	45.6	***
Flower 2.1	50.9	47.0	47.1	47.9	46.6	***
Flower 2.2	48.4	45.5	46.5	46.5	45.1	***

DISCUSSION

Undesired internode (stem) and inflorescence elongation and large leaves constitute a serious problem in the cultivation of Christmas begonia (Heide 1962) and *Begonia* x *hiemalis* (Sandved 1971a). This is most critical when the plants are grown under high temperatures (above 18-20°C), particularly during the final period of flower development (Heide 1962; Sandved 1974). Sandved (1971b) reported that a rather short period of temperature increase from 15°C to 18 or 21°C promoted elongation of the inflorescences and the quality of the plants was therefore reduced. A temperature increase had a smaller effect on the leaf height. The problem of undesired elongation of stems and peduncles can be avoided by growing the Christmas begonia at lower temperatures (15-18°C) (Sandved 1968), but the production period from potting until marketing stage will be very long (more than 20 weeks) and not economical (Moe et al. 1992). Therefore, a short culture programme of about 12 weeks has been introduced. A high growing temperature and supplementary lighting are required in order to enhance flowering (Valsø 1987; Moe et al. 1992). However, such a growing programme tends to result in too great a total plant height and plant diamenter if no growth retardants are used (Table 2, Figs. 3, 5 and 6). As a result, the plant quality of the control plants is not acceptable for the customer.

Growth retardant applications with ancymidol and chlormequat reduced the total plant height significantly. The treatments reduced the elongation of the flower branches and flower peduncles to a greater extent than the leaves. This made a better balance between leaf height and total plant height in treated plants than that in untreated plants. (Figs. 5 and 6). If application rates are too high (high concentrations and many applications of

chlormequat), the result is too great a reduction in plant height. A concentration between 1-2 mgl^{-1} ancymidol and about 2000 mgl^{-1} chlormequat is therefore recommended.



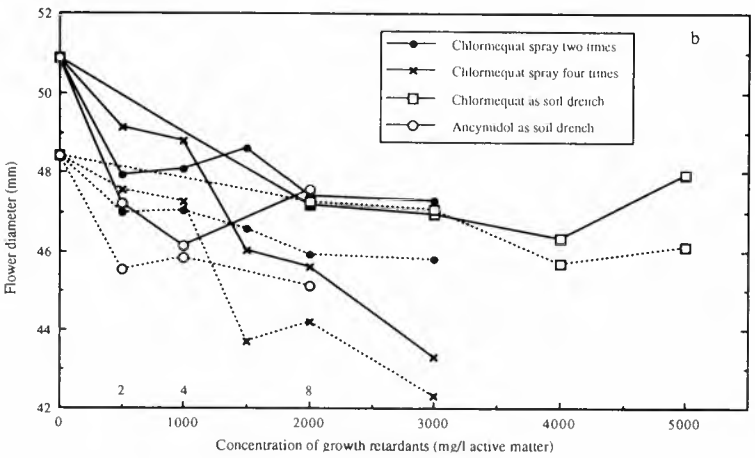


Fig. 4. The flower diameter (mm) of the first (1.1) —— and the second (1.2) ····· of the first flowering branch (a) and the first (2.1) —— and the second (2.2) ····· of the second flowering branch (b) of *Begonia* x *cheimantha* cv. Nova as affected by various treatments with growth retardants

Time of application is critical in Christmas begonia. Earlier experiments by Tangerås (1980, unpublished data) demonstrated that chlormequat spray had no influence on total plant height compared with that of control plants when the treatments were given late in the culture period. Our data show that early application of growth retardants at the beginning of SD treatment gave the strongest reduction in leaf height and plant diameter, while the reduction in total plant height was similar at all three times of application. It is reported by Sandved (1971a, 1974) that excessive stem and leaf growth in *Begonia* x *hiemalis* may be controlled by an additional week of SD treatment. It is expected that growth retardant

treatments have the strongest effect on leaf growth when applied early, before the leaf growth begins to slow down as a result of SD treatment.

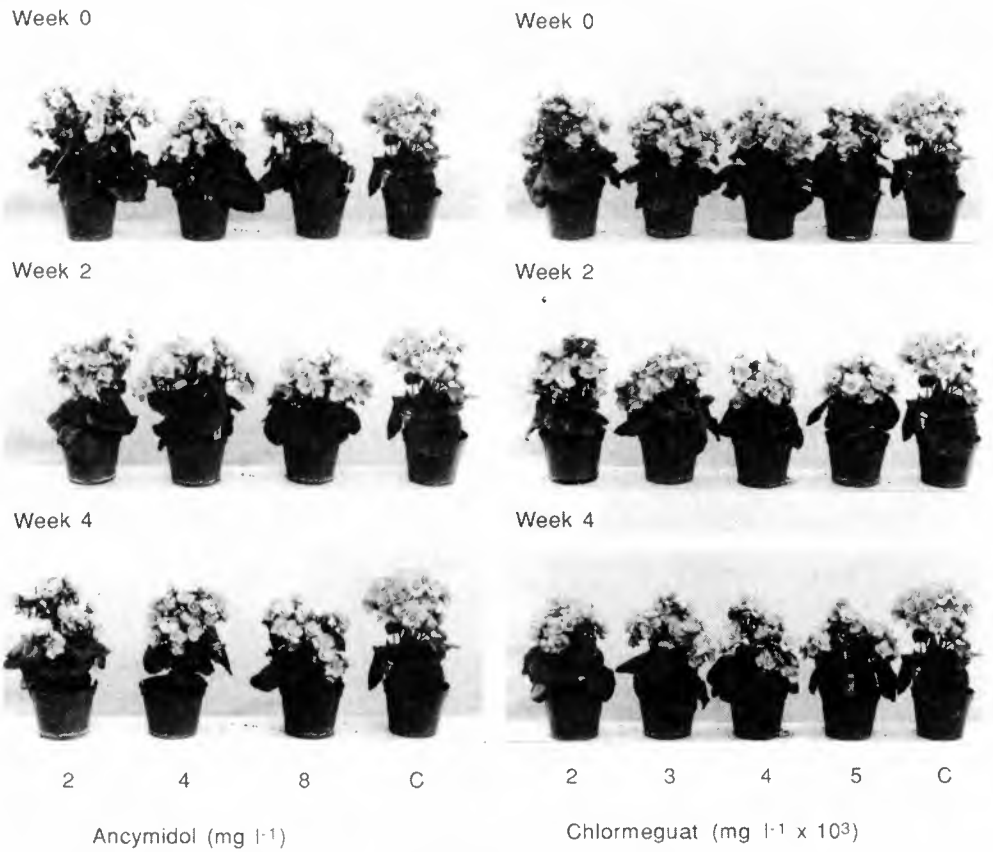


Fig. 5. Effect of time of applications (0, 2 and 4 weeks from start of short-day (SD) treatment) and different concentrations of active matter of ancymidol (left) and chlormequat (right) on growth and plant quality of *Begonia* x *cheimantha* cv. Nova. The treatments were given as a soil drench, 50 ml per pot at the following concentrations of ancymidol: 2, 4 and 8 mgl^{-1} and of chlormequat: 2000, 3000, 4000 and 5000 mgl^{-1}. C = untreated control plants. Potting date 13 September and start of SD 25 September. Photograph was taken on 10 December

Growth retardant treatments reduced the flower size (Table 4 and Fig. 4). This has also been reported in other crops such as *Begonia* x *hiemalis* (Hilding 1975). The reduction in flower size in Christmas begonia was most pronounced at higher application rates of growth retardants. It is therefore recommended that a rather low concentration of chlormequat and ancymidol be used, as discussed earlier. A decrease in the temperature to 15-18°C during the final two weeks before marketing stage is reached will improve the flower colour and size (Sandved 1968). Use of negative DIF or a short temperature drop to control plant height without the use of growth retardants, may not affect flower size (Moe 1991). This will be investigated in Christmas begonia in our next study.

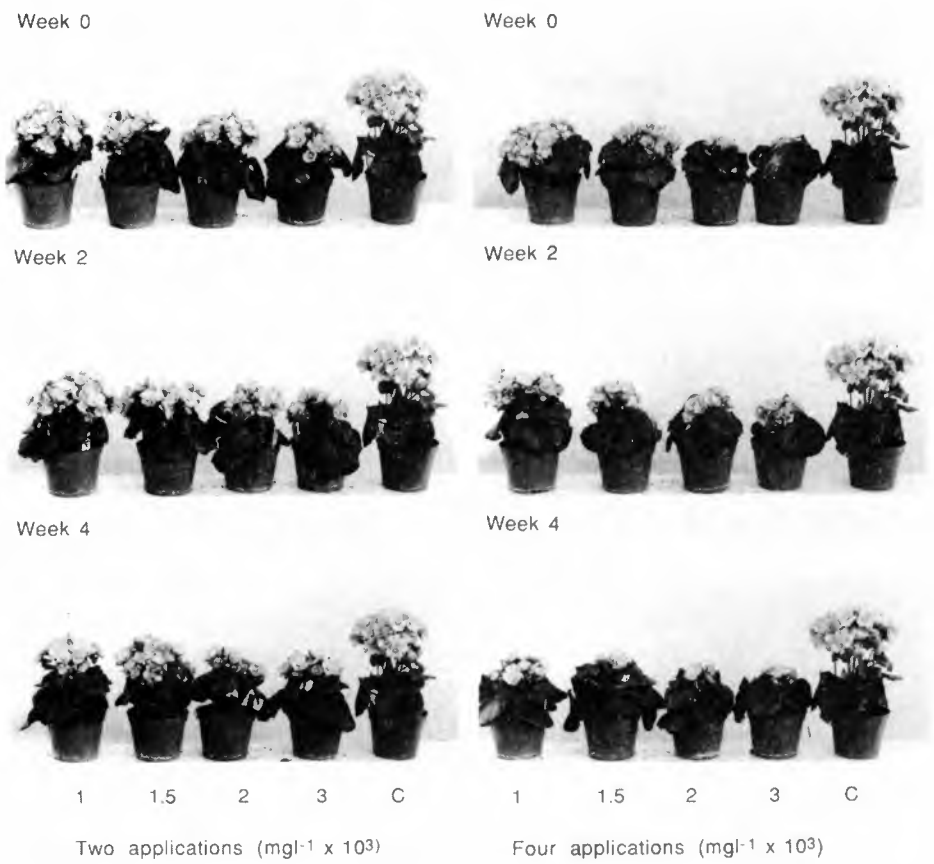


Fig. 6. Effect of time of applications (0, 2 and 4 weeks from start of SD treatment) and different concentrations of chlormequat spray applied two (left) or four (right) times on growth and plant quality of *Begonia* x *cheimantha* cv. Nova. The spray treatments were repeated at intervals of one week. The following concentrations were used: 1000, 1500, 2000 and 3000 mgl^{-1}. C = untreated control plants. Photograph was taken on 10 December

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Evaluation of the starch-iodine test for determination of optimum harvest dates of apples

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The degradation of starch in apples during ripening was studied by using the starch-iodine test on the apple cultivars 'Gravenstein', 'Summerred', 'Aroma' and 'Karin Schneider' at Ullensvang Research Station, western Norway over a period of three years (1986-88). Apples picked in five consecutive weeks (seven-day intervals), covering the actual harvest season for each cultivar, revealed a more or less uniform degradation of starch. Based on storage trials including quality evaluations of representative samples of fruit, recommendations were given for three of the four cultivars regarding optimal harvest dates determined by the starch-iodine test. A pronounced scattering of the degree of maturity within each fruit sample was revealed in the experiment. To meet the requirement of picking apples of equal maturity it was recommended that the harvesting of 'Gravenstein', 'Summerred' and 'Aroma' should be divided into several pickings. Number of pickings and a maximum duration of the actual harvest period were proposed. In 'Karin Schneider' the starch degradation starts very late in the actual harvest period, therefore other maturity indices have to be assessed in order to determine the optimal harvest period of this cultivar.

Key words: Apples, starch, starch-iodine test

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Conversion of starch to sugars occurs in most apple cultivars during the actual harvest period. This conversion can be followed by using the starch-iodine test; the iod in a potassium/iodide solution reacts chemically with the amylose of the starch, producing a very dark cut surface of apples where starch is present. This method is used in many districts to determine optimum harvest dates for apples and pears (Anon. 1987; Knee et al. 1989; Kvåle 1986; Nardin 1977; North 1971; Siegrist 1986, 1987; Truter et al. 1985; Österreicher 1989). Recently, recommendations regarding the use of this test were introduced to the guidelines for integrated fruit production in Südtirol (Mantinger et al. 1989).

Because of a short growing season and high labour costs Norwegian fruit growers usually picked the whole crop of a cultivar in one operation, although the benefits of dividing the harvest into two or more pickings were discussed by Thorsrud & Landfald (1956). In integrated fruit production a harvest divided into several pickings is included in the guidelines as a means of improving fruit quality (Mantinger et al. 1989).

It is known that fruits from the lower part of the tree and fruits growing inside the canopy develop different maturity indices compared with fruits growing in well-exposed

positions, as discussed by Farhoomand et al. (1977) and Krishnaprakash et al. (1983). With these reservations in mind, the starch-iodine test can be used to give some indication of the maturity of a sample of apples, as the developmental stage of each individual fruit is revealed.

The usefulness of the starch-iodine test in determining the optimal harvest date of four of the main commercially grown apple cultivars in Norway was evaluated in an experiment at Ullensvang Research Station, western Norway. In addition the data from the experiment were examined to obtain information about the scattering in degree of maturity among the individual fruits of each cultivar in a period around optimal harvest time, as this scattering most likely caused the quality inhomogeneity within each sample revealed in the quality evaluations of the same apples made during storage (Sekse 1990, 1992). This experiment also offered data for a discussion of the benefits of dividing the apple harvest into two or more pickings.

MATERIALS AND METHODS

Samples of apples of the cultivars 'Gravenstein', 'Summerred', 'Aroma' and 'Karin Schneider' were picked during five consecutive weeks, covering the commercial harvest season of each of these cultivars in the region (Table 1). In the experiment, which covered the years 1986-88, the apples were grown on free spindle trees in the experimental fields of Ullensvang Research Station, Lofthus, western Norway at latitude 60°N. During harvesting, fruits that did not meet the requirements of the official guidelines regarding size and quality for fresh consumption (Norges Standardiseringsforbund 1986) were deleted.

Table 1. The first out of five harvest dates (one week intervals) of four apple cultivars during three years

Cultivar	Year 1986	1987	1988
Gravenstein	09.04	09.02	08.22
Summerred	09.10	09.07	08.30
Aroma	09.23	09.22	09.14
Karin Schneider	09.29	10.01	09.22

Twelve randomly chosen apples from each picking were cut perpendicularly to their axis and the cut surfaces were immersed in an iodine solution (4 g KI plus 1 g iodine made up to 100 ml distilled water) and allowed to dry for 20-30 min. The amount of starch expressed as a dark-coloured surface was judged by five panellists using a percentage scale in 1986 and a scale rating from 1 (totally without starch) to 9 (completely dark-coloured surface) in 1987 and 1988.

RESULTS AND DISCUSSION

Optimum harvest dates

In the apple cv. 'Gravenstein' the starch-breakdown approximated a linear pattern which varied little from year to year (Fig. 1). Since the best quality apples were usually produced in the third week of harvest (Sekse 1990), it has been recommended that this cultivar be harvested when approximately 60-50 % of the surface is still black-coloured (Table 2). This corresponds well with the recommendations given for this cultivar by Mantinger et al. (1989).

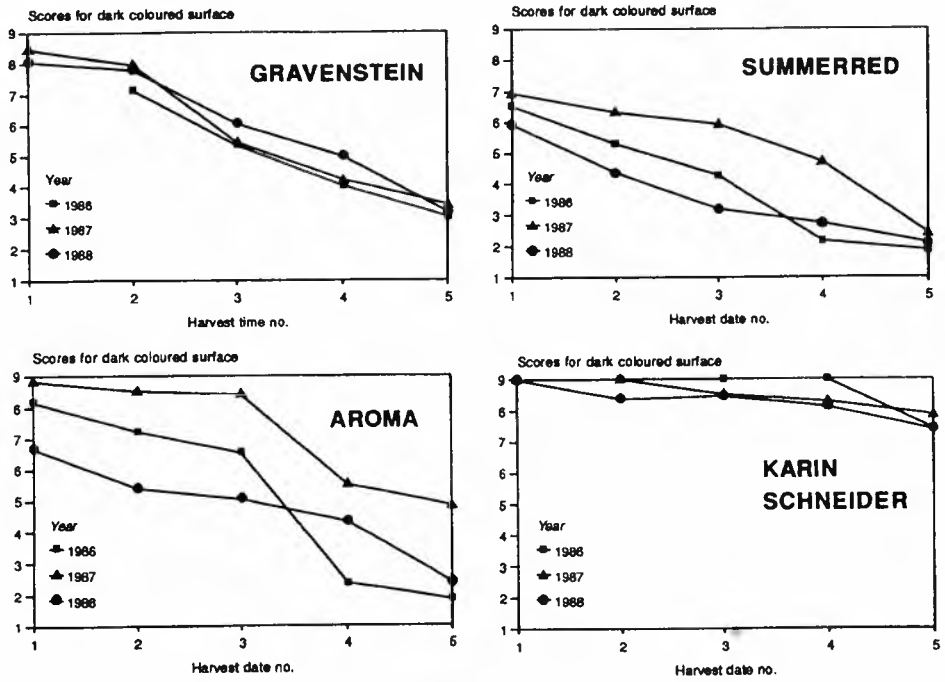

Figure 1. Changes in dark coloured cut surface as judged by a panel using a scale rating from 9 (completely dark coloured) to 1 (surface without dark colour) during a five-week harvest period of 4 apple cultivars and 3 years. The results from 1986 is converted from a percentage scale

In the cvs. 'Summerred' and 'Aroma' the degradation of starch did not follow the same linear pattern as that of 'Gravenstein', and both cultivars exhibited distinct differences regarding the starch degrading pattern between years (Fig. 1). Accordingly, recommendations for when to harvest apples of these cultivars based upon the starch-content observed by using the starch-iodine test were less accurate than those for 'Gravenstein'. However, as a general rule, 'Summerred' should not be harvested before

it has reached at least 40 % light-coloured surface, and harvesting should be complete when the apples reach a dark-coloured surface of 20-30 per cent (Table 2). Mantinger et al. (1989) recommend the harvesting of this cultivar when approximately 50 % of the dark-coloured surface has been reached. Likewise, 'Aroma' apples generally should not be harvested before they have reached a light-coloured surface of 30-40 %, and harvesting should be finished when they have reached 20-30 % dark-coloured surface (Table 2). The recommendations given for these three cultivars are well in accordance with those practised in our district (Anon. 1987).

Table 2. Recommended starch content measured as dark- coloured cut surface at optimum harvest date, number of pickings during a harvest season and maximum duration of the harvest season for three apple cultivars

Cultivar	Dark coloured surface (%)	No. of pickings	Maximum duration (weeks
Gravenstein	60 - 50	2 - 3	3
Summerred	60 - 20	2 - 3	3
Aroma	70 - 20	3 - 4	4

For the cv. 'Karin Schneider' most of the starch degradation occurs after actual harvest time (Fig. 1), and the starch-iodine test is therefore unsuitable for determining the optimun harvest time for this cultivar.

In addition to the starch-iodine test, well-known criteria such as the development of the ground colour, soluble solid content, the colouring of the seeds, taste, amount of fruit fall and size and shape of the fruit, should be taken into account when decisions about harvest dates are taken.

In apples from all the cultivars a decline in starch content occurred during the harvest season, but the patterns were different both for cultivars and for years. The effect of low temperatures should especially be mentioned. Cold nights with temperatures below +2°C occurred in late September 1986 which was just after the third picking of 'Summerred' that year, causing a sudden increase in starch degradation. In the same year there was a similar effect on the cv. 'Aroma' of one cold night early in October just before the third picking of this cultivar, manifest as an increase in the starch degradation in the apples of the fourth picking (Fig. 1). Comparatively, no cold nights occurred during the harvest season in 1988.

The influence of deleting from the experiment the apples that fell on the ground should also be mentioned. This occurred most frequently before the latest harvest dates due to overripening in a part of the crop. In many cases this produced a less steep fall in the starch degradation pattern than would have been obtained if these apples had been included.

Number of harvest times

The samples of apples of 'Gravenstein', 'Summerred' and 'Aroma' revealed a pronounced variation in maturity development (Table 3), especially in the middle of the harvesting periods, which in most cases were considered the optimum harvest dates during storage trials (Sekse 1990, 1992). This variation most likely caused the pronounced inhomogeneity within samples regarding the fruit quality revealed in the quality evaluations of

corresponding samples during storage. The variation was particularly pronounced in 'Aroma', but was evident also in 'Gravenstein' and 'Summerred'. Accordingly, harvesting the total crop in one picking will produce fruit of quite different maturity even although it is done at the optimal time of harvest. Even dividing the harvest into two pickings will usually cause a wide variation in stage of maturity among the fruits. Bearing in mind the recommendations of optimal developmental stage of the apples at harvest, information about the actual duration of the period in which apples are fit for harvest is also provided by the results presented in Table 3.

Table 3. The frequency (percentage) of apples with different degrees of dark-coloured cut surface as judged by a panel for three cultivars and five consecutive harvest dates (one week intervals) during three years

Year	Dark surface[1]	Gravenstein Harvest date no.					Summerred Harvest date no.					Aroma Harvest date no.				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1986	100	[2]	10									17	8	3		
	90		8	3			17	8	4			63	27	22		
	80		8	5	2		33	7	8			21	32	25		
	70		25	35	10	2	8	5	10				18	18		
	60		17	10	5		17	18	5				9	8		
	50		5	13	17	5	17	30	8				3	8		
	40		9	13	12	15	8	22	13				3	6	8	
	30		9	8	24	28		8	21	7	4		1	4	14	
	20		8	9	28	27		3	27	36	23		1	4	29	21
	10			3	3	22			5	54	48			1	39	65
	0					3				3	25				10	14
1987	9	57	33				10					82	57	52	5	
	8	33	37	13			20	18	18	5		18	37	42	8	12
	7	10	23	20	7		38	47	18	10			7	7	28	15
	6		7	15	12		28	9	28	20	2				17	23
	5			23	20	5	3	16	17	22	5				8	15
	4			13	30	47		2	12	15	13				12	3
	3			10	23	33		7	2	22	18				15	10
	2			5	8	15		2	5	7	37				3	13
	1										25				3	8
1988	9	44	35													
	8	47	37	22	13		2	3				42	28	3	8	
	7	2	18	22	5	5	32	5	2			24	10	30	18	
	6	2	2	25	20	3	38	10	5			15	13	12	12	2
	5	2	3	14	17	3	20	22	2			8	17	17	7	2
	4		3	8	25	15	3	30	27	15		5	7	12	10	5
	3	3		8	15	42	5	27	37	50	22	3	12	17	15	25
	2		2		5	30		3	25	30	67	2	5	10	22	60
	1					2			3	5	12		8		8	7

[1]): Judged as a percentage of the total surface in 1986, as scores on a scale rating from 9 (totally dark coloured) to 1 (completely without dark colour) in 1987 and 1988.

[2]): Data missing for the first harvest date of 'Gravenstein' 1986

Based on these findings, combined with the results from the storage experiments (Sekse 1990), it is recommended that the harvest of the actual cultivars be divided. Because of pore quality regarding sugar content and flavour when apples are picked too early and lack of storage quality when the apples are picked too late (Sekse 1990; Vestrheim 1970), it is recommended that the harvesting of 'Gravenstein' and 'Summerred' be divided into two to three pickings with a maximum duration of the total picking period of three weeks (Table 2). Mantinger et al. (1989) recommend that the harvesting of these cultivars be divided into three pickings. Likewise, the harvesting of 'Aroma' apples should be divided into three to four pickings with a maximum total picking period of four weeks (Table 2). The percentage of the total crop to be harvested at each time should be currently assessed.

The benefits of dividing the harvesting in this way will be fruits of better and more homogeneous quality and an improvement in the total grading result.

The occurrence of low temperatures during the harvest periods can accelerate the maturity development, which means that a narrowing of the total harvesting period will have to be assessed.

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Influence of harvest time and method on seed yield and quality in onion (*Allium cepa* L.)

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Vik, J. 1992. Influence of harvest time and method om seed yield and quality in onion. Norwegian Journal of Agricultural Sciences 6: 463-468. ISSN 0801-5341.

Experiments with different times of ripening stages and methods of harvesting in seed crop in *Allium Cepa* were carried out in 1988 and 1989. Seeds with a dry matter content ranging from 30% to 84% all gave a satisfactory germination of 89% or more. However, seeds with a dry matter content at around the 30% level were lighter and gave a lower yield than those with a dry matter content of 47% and 84%, which were equivalent in quality and yield. Harvesting of umbels with short or long stems had no influence either on the quality or on the quantity of the yield.

Key words: Harvest time, onion, seed-growing.

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Questions concerning harvest time and methods in order to obtain a better yield and seed of good quality are very important for practical seed production in onion. Factors involved are: development of the seeds and ripening indication; dry matter content and times of harvesting. Since our climate in Norway is worse than that in countries farther south, these questions are even more important.

Globerson et al. (1981) recommend the harvesting of onion seed when dry matter content is of the 60-70% level. Szalay (1983) recommends when the siliques start to open and Neal & Ellesbrock (1986) when 25% of the siliques are open. Jonassen (1985) demonstrated that maximum yield and satisfactory seed quality can be achieved when dry matter content reaches 46% or more, with corresponding open siliques of 6% or more. These recommendations and personal experience have promoted the carrying out of another experiment, adapted for ordinary seed production practice in onion in Norway.

MATERIALS AND METHODS

Three types of experiment were carried out in glasshouses:

1. Stages of ripening and different stem lengths at harvesting,1988-89
The umbels were harvested at three ripening stages and stem lengths. Ripening stages were as follow:
1. Green umbels, closed siliques and soft endosperm.
2. Grey/green umbels, closed siliques, endosperm medium firm.
3. Grey/green umbels, 30-60% open siliques, endosperm firm.

At all stages of ripening the seed had a dark colour. The umbles were harvested at stem lengths (a) 10 cm, (b) half stem length and (c) whole (1/1) stem length. Twenty umbels were harvested in each plot and three umbels for the dry matter test, divided into two parallels.

2. Harvesting the seed crop once, twice and three times, 1989
An attempt was made to begin each harvesting at exactly the same ripening stage, i.e. when a few siliques were open. The experimental plots were 2.0 m^2 with 50 motherbulbs per plot, transplanted 20 cm x 20 cm. For information on data of transplanting and harvesting, see the table below.

3. Harvesting once at different stages of ripening, 1989
The seed crop was harvested at four different ripening stages. These were estimated as being the time just before the first siliques were open, and when 10, 20 and 30% were open, referred to as the 1st, 2nd, 3rd and 4th ripening stages. The experimental plot measured 1.6 m^2, with 15 cm x 20 cm distances between the 40 motherbulbs included per plot.

The size of the motherbulbs in the experiments was about 150 g, and the crop was managed in the usual way throughout the season. All experiments had three replicates and seeds were tested according to the international seed testing design. Data taken were as follows: Percentage of normal germinated seeds after 7 (1988) or 10 (1989) and 14 days; percentage of abnormal germinated and ungerminated seeds; 1000-seed weight and yield (kg per 100 m^2). The table below gives a view of the dates of transplanting, start of flowering, period of harvesting and the germination tests performed.

Experiment number	Year	Date of transplanting	Flowering started	Period of harvesting	germ. test
1	1988	20/4	5/7	15-29/8	12-26/1
1	1989	20/4	3/7	15-23/8	5-18/1
2	1989	13/4	3/7	21-29/8	12/1- 4/2
3	1989	13/4	3/7	14-29/8	17-30/1

RESULTS

1. Stages of ripening and different stem lengths at harvesting
The growing periods for ripening stages nos. 1, 2 and 3 were 118, 122 and 132 days, respectively. These had a clear effect on the drymatter content of the seeds, which was 30, 47 and 84%, respectively. The germination percentage and the 1000-seeds weight had the same sequence, but the yield per umbel was partly another (Table 1).

Ripening stage 2 (siliques closed with green/grey umbels) gave the greatest yield, 2.1 g per umbel, and ripening stages 1 and 3 (siliques closed with green umbels and siliques two-thirds open with grey/green umbels) gave a lower and mutually significant yield at 1.6 and 1.7 g per umbel.

Table 1. Influence of ripening stages on the dry matter content of onion seeds, 1000-seed weight (g), percentage of normal seed after 7 (10) and 14 days, ungerminated seed and the yield (g) per umbel as a mean of two seasons 1988 and 1989

Ripening stages		Dry matter content, %	Weight of	Percentage of			*)
siliques	umbels	at harvest	1000-seeds	norm. 7(10) d	germ. 14 d	ungerm. 14 d	g seed per umbel
closed	green	30	3.1	64	89	4	1.7
closed	grey/green	47	3.6	71	92	3	2.1
open 2/3	grey/green	84	3.8	74	93	3	1.6
Mean		54	3.5	70	91	3	1.8
Sign. level		***	**	**	*	n.s.	**
LSD P=0.05		4	0.4	4	2	-	0.4

*) observed in 1988 only

The germination percentage in the laboratory was greatest at ripening stages 2 and 3, and smaller at ripening stage 1. But these differences were less clear-cut after a germination length of 14 days than after 7 (10) days. The germination percentages after 14 days of germination were 89, 92 and 93%, respectively for the 1st, 2nd and 3rd ripening stages and after 7 (10) days of germination, 64, 71 and 74%.

The 1000-seed weight follows the same sequence as the germination percentage, i.e. 3.1, 3.6 and 3.8 g, for the 1st, 2nd and 3rd ripening stages, respectively.

The number of ungerminated seeds was very low (mean 3%) and there was no difference in germination percentage among the ripening stages at the close of the germination trial (14 days).

The results differ somewhat between the experimental years 1988 and 1989, but despite this the trend of the results was the same as that illustrated by dry matter content in Fig. 1. The dry matter content was somewhat different in the 1st and 2nd ripening stages, but in the 3rd stage it was practically the same.

Harvesting the umbels at different lengths of stem had no significant effect on the characteristics observed, or on yield per umbel, 1000-seed weight, percentage of germination, or on dry matter content at harvesting, nor had it any no relationship to the ripening stages. The table which shows these results has therefore been omitted.

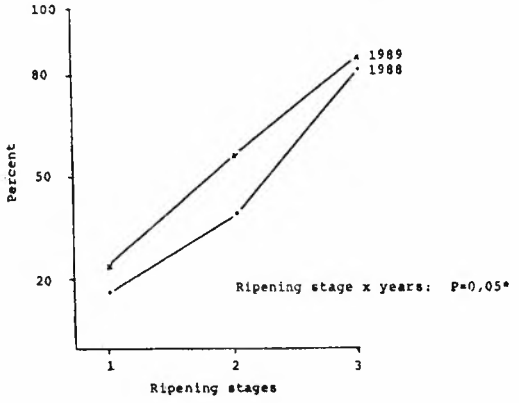

Fig. 1. Percentage of dry matter in onion seed at different ripening stages: 1st, 2nd and 3rd in 1988 and 1989

2. Harvesting once, twice or three times

The number of harvestings had no influence on germination after a 10 and 14 days' germination period, nor did it influence the 1000-seed weight

(Table 2). The germination percentages after 10 and 14 days were 74 and 93, and the 1000-seed weight was 4.2 g. However, the number of harvestings influenced the yield of seed crop tremendously. The yields at one, two or three harvestings were 12.2, 19.8 and 13.7 kg per 100 m^2.

Table 2. Seed yield (kg per 100 m^2), 1000-seed weight (g), percentage of normal germinated seed after 10 and 14 days and percentage of ungerminated onion seeds when harvested once, twice and three times

Number of harvestings	Kg seed per 100 m^2	Weight of 1000-seeds	Percentage of normal germ. 10 d	14 d	ungerm. 14
1	12.2	4.29	73	92	10
2	19.8	4.17	73	93	5
3	13.7	4.22	77	93	6
Mean	15.0	4.22	74	93	7
Sign. level *	n.s.	n.s.	n.s.	n.s.	
LSD P=0.05	4	-	-	-	-

3. Harvesting once, at different stages of ripening

The yield of seed increased from the 1st (no siliques open) to the 3rd ripening stages (20% siliques open), at the 4th ripening stage (30% siliques open) the yield was somewhat less. The yields were 12.2, 20.5, 22.0 and 17.3 kg per 100 m^2 respectively, from the 1st to the 4th ripening stage (Table 3).

Table 3. The influence of ripening stages on yield when harvested once, 1000-seed weight (g), percentage of normal germinated seed after 10 and 14 days and percentage of ungerminated seeds

Ripening stages	Kg seed per 100 m^2	Percentage of normal germ. 10 d	14 d	ungerm. 14 d	Weight of 1000-seeds
No open siliques	12.2	53	89	11	4.0
10% open siliques	20.5	62	92	7	4.2
20% " "	22.0	70	95	4	4.3
30% " "	17.3	72	95	5	4.5
Mean	18.	64	92	7	4.3
Sign. level		**	n.s.	n.s	**
LSD	6.3	7	-	-	0.2

The germination percentage in laboratory, however, rose according to more promoted ripening stage. These differences were highly significant after 10 days of germination, but at 14 days of germination these differences were very small, and insignificant. The germination percentages at 10 days were 53, 67, 70 and 72% for successive ripening stages, and at 14 days these percentages were 89, 92, 95 and 95% respectively. Concerning

ungerminated seeds, the 1st ripening stage had the biggest amount (11%) but did not differ significantly from the other ripening stages, which had 4 - 7% ungerminated seeds.

The 1000-seed weight increased according to successive ripening stages, as did the germination. The 1000-seed weight were 4.0, 4.2, 4.3 and 4.5 g.

DISCUSSION

In order to achieve a satisfactory germination percentage, the seed crop of onion needs to be harvested at a moderate ripening stage. Seeds from closed siliques with only 30% dry matter content gave a final germination of 89%. But the seeds were light, and as a result the lightest parts disappeared in the cleaning process. The experiments demonstrated that the seed quality (germination percentage and 1000-seed weight), with a dry matter content of around 50% and over, was an excellent one. This statement is also verified by others (Jonassen 1985, Neal & Ellesbrock 1986). Globerson et al. (1981) indicate a dry matter content of 60% and 70% with one harvesting.

Besides the dry matter content, the experiments also demonstrated that the colour of the umbels, together with more or less open siliques, was a satisfactory indication of ripening stage and harvesting time.

There are two factors in particular in the process of ripening that are decisive for the kind of yield one is likely to obtain: (1) feeding of the seed (weight of seeds); (2) the extent to which the ripening stage has proceeded (the number of open siliques).

When the umbels had turned grey/green and the siliques started to open, the feeding of seeds proceeded very rapidly. After reaching this ripening stage, the yield increased by 60 -70% in 5-7 days. Jonassen (1985) found that the yield increased by 41% over 14 days. Different growing conditions are the most reasonable cause of these differences in ripening length and increase in yield. In this experiment, the seed crop was grown in glasshouse, while Jonassen's experiment took place in a plastic greenhouse.

If ripening stage is too far advanced, it will easily lead to loss of seed crop, and consequently a reduced yield. When more than 20% of the siliques are open, one can expect an increasing loss of seed. Jonassen's experiment indicates similar figures. Neal & Ellesbrock (1986) refer to 25% open siliques as being the most favourable time, when harvesting once.

The experiments seem to indicate that one may chose either harvesting the crop once or several times without diminishing the yield and seed quality to any great extent. With one harvesting, the biggest crop yield was obtained when 10-20% of the siliques were open. In experiments with several harvest times, two harvestings (few siliques open) gave the biggest yield. In both cases the seed quality was good and the yield was around 20 kg per 100 m^2.

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Influence of top-dressing on autumn-drilled seed plants in early cabbage

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Seed crop experiments in early cabbage on autumn-drilled seed plants including top-dressing during winter were conducted in 1988/89. Two top-dressing applications (November-December) increased the plant growth to a considerable degree, with smaller increases occurring up to four times. The top-dressing had a similar influence on the quantity of the seed yield, but the increase was not significant, probably becaused of an attack of club-root during summer, which also accounted for the general low yield (5.3 kg per 100 m^2). The premier drilling, 5 August, gave a clear-cut higher growth and seed yield than that of 5 September, i.e. 7.54 and 3.07 kg per 100 m^2 respectively.

Key words: Top-dressing, early cabbage, seed plant.

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Autumn-drilled seed plants in early cabbage displayed a marked lower seed yield than seed plants started earlier or later in the year, as illustrated in a quotation of published data (Vik 1989).

Yield	Stem harvested May/June	Stem harvested October	Cuttings May/June	Transplants sown Autumn	Transplants sown Winter
g per plant	76	70	60	17	58
kg per 100 m^2	17.0	15.0	13.1	5.9	19.9

However, preliminary trials indicated an increasing yield when the vegetative growth was higher during winter. To obtain taller plants in better growing condition during winter, different drilling times and fertilizing practices (top-dressings) were implemented in a further trial.

MATERIALS AND METHODS

The seeds were drilled on 5 August and 5 September in 9 cm x 9 cm x 6 cm multipots filled with clay soil, half mixed with fertilized peat moss. During the raising period the seed plants received normal care, including sprinkling and fertilizing (1% solution of PKN 15-4-12) up to 20 October. Later, plants In treatment 1 were no longer given top-dressing (control), while those in treatment 2 received two applications, one in November and the

second in December, and those in treatment 3 were given four applications, in November, December, January and February. The plants were raised and overwintered in greenhouses (glasshouse) at a temperature fluctuating around the 5 °C level.

The seed plants were transplanted in plastic greenhouse (polyethylene 0.10 mm) with 15 seed plants per plot(4.5 m x 1.0 m) at a distance of 0.3 m x 1.0 m. The experimental design was a randomized block with three replicates. The pollinating insects were bees. The following data were registered: number of plants, times of harvesting, yield of cleaned seeds, 1000-seed weight and percentage of normal germination. The levels of signficance were indicated by P=0.001***, P=0.01** and P=0.5*, and LSD P=0.05. Growth of the seed plants, which also included branching, was estimated on 5 May, in a scale from 1 to 9, 9 being the highest. The climatic conditions in the year of seed production can be estimated as normally good. The mean temperature per month and the normal temperature (mean of 30 years) were:

Year	April	May	June	July	August	September
1989	5.3	11.1	14.1	16.6	14.5	11.8
Norm. temp.	5.2	10.6	16.0	16.6	14.0	10.1

RESULTS

Neither sowing dates nor fertilizing during winter had any effect on the 1000-seed weight or on the germination percentage. The mean 1000-seed weight was 5.48 g and the mean germination percentage 89 (Table 1). However, these two factors did have an effect on both the growth registered on 5 May and the seed yield in the autumn.

Growth was clearly greater in plants sown on 5 August than in those sown on 5 September, i.e. 7.8 and 5.3 respectively in the rating scale (Table 1). There was also a considerable effect of top-dressing on the plant growth, especially with two applications (November, December) in relation to none. Four applications (November, December, January and February) did not lead to any further significant increase (Table 2). The mean rates of growth from none to two and then to four applications were 4.2, 7.5 and 8.0 respectively (Table 2).

However, these factors (date of sowing and top-dressing during the winter) had only a partial effect on the seed yield in the same mode as the growth. The dates of sowing had a considerable effect on the seed yield. The date of sowing 5 August, gave a mean yield of 7.5 kg per 100 m^2, and 5 September 3.07 kg (Table 1). The yields per plant were 23g and 9 g, respectively. The effect of top-dressing was less noticeable. There was a slight increase (20%) in seed yield from none to two applications, and further a slight decrease to four applications (Table 2), although in both cases the decrease was not significant.

The fertilizing treatments did not affect the 1000-seed weight (data not presented).

Table 1. Different data in a seed growing experiment in early cabbage with two different sowing dates of seed plants in the autumn.

Date of sowing	Germ. %	g/1000-seeds	kg seeds per 100 m²	g seed per plant	Plant growth rated 1-9, 9 biggest
5 August	88	5.49	7.54	23	7.8
5 September	90	5.46	3.07	9	5.3
Mean	89	5.48	5.31	16	6.6
Sign. level	ns.	ns.	**	***	***

Table 2. Rating of the plant growth in a range from 1 to 9, 9 = biggest, and the yield of seed, kg per 100 m², as an effect of different numbers of top-dressings through the winter

Characters	Number of top-dress None	2	4	Mean	Sign. level	LSD
Plant growth	4.20	7.50	8.00	6.57	***	1.0
Kg seed/100 m²	4.86	5.89	5.18	7.31	ns.	

DISCUSSION

It is a common occurrence for larger seed plants with excellent growth to give a greater yield than smaller seed plants of inferior growth. In an earlier experiment, autumn-drilled seed plants received no fertilizing during winter, hence the plants turned blue and the new growth in the spring was minimal (Vik 1989). This experiment demonstrated that drilling time early August gave a greater growth and yield than a later drilling.

One could also expect that top-dressing (fertilizer application) would increase both plant growth and seed yield. This did occur, but it was more apparent in plant growth than in seed yield. The lower increase in yield was most probably caused by the increasing attacks of club-root in the course of the summer. The club-root attack, which was of some severity, also caused a general low yield level.

It should be borne in mind that it is important to maintain a low and steady temperature throughout the winter. In order to hit the right development of the seed plants at the time of transplantation (either in greenhouse or in the open), the growth has to be minimal. This is especially important where transplanting is carried out in the open. The flower cannot tolerate frost, and requires the right conditions for pollination. In this experiment the temperature during winter fluctuated around 5 °C, providing a suitable development for transplantation at the beginning of April, when flowering had just started.

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Effects of xylem cytokinin application on flower bud development in apple (*Malus x domestica* Borkh.)

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Continuous treatment (two or three weeks) of zeatin (Z) and benzyladenine (BA) directly to the xylem of small budded plants stimulated apical and lateral flowering on elongated shoots at concentrations 4 x 10^{-5} M and higher. Growth was reduced. No effects were found of isopentenyladenine (iP) at any concentrations. Single applications of a variety of cytokinins in amounts ranging from 10 to 1000 nmoles to spurs of established trees gave no effects. Simultaneous deleafing and defruiting of spurs reduced and enhanced flowering, respectively.

Key words: Apple, cytokinin, flowering, *Malus*, vegetative growth, xylem application.

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Since Luckwill's suggestion (1970) that cytokinins might play a role in flower bud formation in deciduous fruit trees, a few attempts have been made to verify this hypothesis by application of exogenous cytokinins. Ramirez-Rodrigues (1979; see also Ramirez & Hoad 1981) injected various cytokinin solutions into spurs of apple and found significant increase in return bloom. Later it has been found that spraying of benzyladenine (BA) during the first weeks after flowering resulted in an increase in return bloom (Greene & Autio 1989; McLaughlin & Greene 1984; Unrath 1989).

The effects of cytokinins on flower induction in other plants have been reported in a number of cases (see Bernier et al. 1981; Metzger 1987; Zeevaart 1978). The effects usually tend to be indirect (Ogawa & King 1979; Tse et al. 1974), but in some cases they can be synergistic, the cytokinins only increasing the effects of other flower-inducing factors (Abou-Haidar et al. 1985; Besnard-Wibaut 1981; Vince-Prue 1983). Only in a few cases did the flowering appear to have been triggered by the cytokinin application itself (see Bernier et al. 1981). Cytokinins have also been demonstrated to evoke partial reactions normally associated with the early stages of floral induction (Bernier et al. 1977; Havelange et al. 1986). In addition to the few cases mentioned in the introduction, cytokinins have also influenced flowering in other species of *Rosaceae* (Zieslin et al. 1985), apparently, however, by some releasing effect on correlative inhibition of lateral buds.

Hoad (1984) proposes in accordance with Luckwill (1970) that flower bud formation in apple is influenced by variations in the root-produced cytokinins transported to the bud by the xylem sap, and explains the effects of various flowerenhancing treatments by their

possible effects on root activity. If these assumptions are correct, an artificial enhancement of the xylem sap cytokinin level should increase the number of floral buds on an apple plant. In the present work a system for continuous elevation of the xylem cytokinin content was established in order to test the possible effects on lateral flower bud induction.

MATERIALS AND METHODS

Plant material and growth conditions

Regulated environment trials

The apple variety 'Aroma' was chip-budded onto small (3-4 mm shoot base diameter) M26 rootstocks during late winter. The budded plants were placed in plastic pots with limed and fertilized peat soil, and forced to break in a heated greenhouse. At scion shoot length the plants were selected for uniformity as far as was possible. However, some variation in shoot height had to be accepted, and considerable care was taken to distribute the variation as evenly as possible between the treatments.

Growth conditions varied somewhat from trial to trial, mostly according to season and available space. In the first trials temperature was maintained at 18°C. Later, ordinary greenhouse conditions with temperature varying between 18°C and 24°C had to be accepted. In one case a growth chamber with 18°C day/14-15°C night and 16 h daylenght at approximately 5000 lux was also used.

After the treatments ended, the plants remained at growth conditions for two or three additional months depending on the season (the longer the poorer the light conditions). Thereafter, the plants were transferred to cold storage for 2.5-3 months in order to break dormancy. The plants were then forced at 18-24°C for 4-6 weeks to allow recording of flower parameters.

When all the recordings had been carried out, the treated scion shoots were cut back to some few buds above the budding site, and one new shoot allowed to grow out to a length of 30 cm. This procedure was repeated three times to level out possible effects of the previous treatments before the plants were allowed to enter a new trial.

Orchard trials

Sixteen (1988) and ten (1989) 15-year-old trees of Aroma on MM106 in the experimental orchard of the Department of Horticulture, Norwegian University of Agriculture, were selected for uniformity. On each tree, spurs at a number high enough to include all combinations of treatments were selected for carrying at least one fruitlet and a non-elongated bourse bud. One week before treatment, the fruit number per spur was reduced to one.

Treated spurs were collected in October or November and stored in 70% ethanol until dissection. Spur diameter was measured at the point of cytokinin application, and the bourse bud dissected to determine bud status and number of flower initials.

Treatments

Direct xylem application of cytokinins was carried out either by microsyringe injection or by slight modifications of a method described by Iizuka et al. (1978). In the latter method

a thin non-stained cotton thread was pulled through the scion shoot base 3-5 cm above the budding site or through the spur just below the base of the bourse bud, and was used to feed the xylem with cytokinin solution from a small reservoir attached to the shoot or the spur (Fig. 1). In 1988, spurs on orchard trees received a single application of 1.5 ml solution containing various amounts of cytokinins, whereas in the regulated environment trials the reservoir was refilled once or twice daily over a period of two (or three) weeks.

Fig. 1. Application of cytokinin solution to the xylen

In 1989 cytokinins were applied to spurs of orchard-grown Aroma/MM106 by microsyringe injection, according to a method by Ramirez-Rodriguez (1979). A volume of 5 μl cytokinin solution was injected into the spur xylem through the base of the cut petiole of the leaf supporting the bourse bud.

Cytokinins were obtained from the Sigma Chemical Company. In the cases of zeatin (Z) and zeatin riboside (ZR) the trans-isomer grade 97% was used. The cytokinins were dissolved in a small volume of hot ethanol, diluted with distilled water, and the ethanol content adjusted to a final 5% in all solutions (including the controls); cytokinins for microsyringe injection were dissolved in 20% ethanol.

In one regulated environment trial the effect of a three- week continuous xylem application of benzyladenine (BA) was compared with a daily foliar spray at the same concentration and period as well as with a variety of other treatments known to enhance flowering of orchard trees: stem girdling, reduced water supply, root pruning and shoot bending.

In the orchard trials all cytokinin treatments were combined with simultaneous defruiting (+/-) and/or deleafing (+/-) of spurs, the first time at three weeks and then repeated at six weeks after full bloom.

The analysis of variance was carried out by means of the microcomputer program MSTAT (Nissen & Mosleth 1985).

RESULTS

Trials in regulated environment

A preliminary test using xylem application of the cytokinins isopentenyl (iP), t-zeatin (Z) and benzyladenine (BA) to 8-10 cm long, rooted shoots of the apple rootstock M26 revealed most of the effects on plant growth found for all experiments. In general, the appearance of plants treated with iP deviated little from that of control plants (Table 1). On the other

hand, Z and in particular BA produced a marked local swelling of the stem around the point of cytokinin application. The Z- and BA- treated plants also displayed less elongation in growth (Table 1), and their non-elongated lateral buds grew big and plump. These effects were more pronounced the higher the concentration applied, and more pronounced for BA than for Z at equal concentrations.

Table 1. Effects of xylem-applied cytokinins (100 mg/l; i.e. approx. 4.5×10^{4} M) to plants of M26. Averages of five plants

Cytokinin	Growth increment (cm)	Laterals per plant
Control	28.4	1.2
Zeatin	22.6	3.8
Isopentenyladenine	24.4	0.0
Benzyladenine	18.3	9.6

In the M26 plants application of Z and BA also increased the number of elongated laterals (Table 1); this was not found in any of the later experiments. Most laterals on Z and BA treated plants were found just above the point of application, where they extended horizontally a few centimetres out from the stem before they terminated growth. Laterals on control plants formed mainly on the upper half of the stem, and grew vigorously in an upward direction.

When a three-week xylem application of 100 mg/l BA was tested against a simultaneously applied daily foliar spray and other non-chemical treatments on budded Aroma/M26 plants, no increase in flower bud formation over the control was found (Table 2). However, many buds aborted before or during bud break, and several of these appeared to have contained flower parts. The distribution of the flower clusters was markedly affected by the xylem BA treatment. Whereas, without exception, flower clusters on non-treated plants appeared on the two or three upper nodes, flower clusters on the BA-treated plants were found along the entire stem, even below the point of application.

Daily foliar applications of 4.4×10^{-4} M BA over a period of three weeks produced quite another picture. Whereas xylem-applied BA caused some stunting of the plants, sprayed plants became elongated like the controls, but a marked swelling occurred along the entire shoot (Table 2). Instead of the big and plump buds of the xylem-treated plants, laterals grew out to form short (1-3 cm) and thick shoots. Flower formation remained almost absent (one flower on 18 plants).

None of the non-chemical treatments had a positivie influence on flowering, perhaps indicating that the flower-promoting effects of the system itself (possibly due to root restriction (Richards 1986)) dominated totally. Nevertheless, the number of flower clusters on girdled and bent plants was not significantly lower than that of the controls, despite their somewhat fewer nodes.

Xylem application of four different concentrations of zeatin to budded Aroma/M26 under low light conditions indicated that some effect might be expected after two weeks of xylem zeatin at approximately the 10^{-5} M level (Table 3).

Table 2. Effects of various treatments on growth and flowering of small Aroma/M26 plants in 10 cm plastic pots. Averages of 18 plants

Treatment	Shoot growth (cm)	Shoot diameter at half shoot height (mm)	Flowering terminals (%)	Flower buds per plant	% of buds
Control	24.1	4.1	72.2	1.67	12.4
BA, xylem appl.	17.8	3.9	61.1	1.67	13.9
BA, foliar spray	24.4	6.2	0.0	0.06	0.4
Girdled	21.1	3.6	66.7	1.45	12.1
Red. water supply	8.0	3.5	33.3	0.50	4.6
Root pruned 1x	15.6	2.8	22.2	0.34	2.8
" " 2x	17.9	3.0	16.7	0.17	1.5
Shoot bending	20.0	3.9	66.7	1.56	13.7
Levels of sign.	N.S.	0.01	0.01	0.01	0.01
LSD (0.05)	-	2.9	15.8	2.0	2.1

Table 3. Xylary t-zeatin application to chip-budded apple plants (Aroma/M26) under reduced light conditions (5000 lux)

t-Zeatin conc. (M)	No. of plants	Uptake per plant (μmol)	Shoot length (cm)	Flower buds per plant	Flowers per cluster
0.0	4	0.00	31.4	1.2	5.0
4.65×10^{-6}	4	0.07	33.9	1.2	5.0
4.65×10^{-5}	4	0.47	30.0	2.5	4.0
4.65×10^{-4}	4	6.05	28.1	0.5	4.5
2.33×10^{-3}	3	23.25	24.5	0.7	5.0

Treating 90 30-cm high decapitated Aroma/M26 plants with iP, Z and BA at three different concentrations produced the same variations in bud development as found for the rootstock M26. Within a week, increased growth of lateral buds was evident on plants treated with the two highest concentrations of Z and BA. The differences were most pronounced in the lower regions of the scion shoot, close to the point of cytokinin application. An unsuccessful attempt to determine bud status and primordia number by bud dissection resulted in the loss of almost half the material. However, in the three upper nodes development had proceeded far enough to be detected by dissection, and a pronounced effect of Z and BA on flower initiation was found, the effect of iP being more uncertain (Table 4).

An assessment of intact plants after dormancy confirmed the results obtained by dissection of dormant buds (Table 5). However, the most marked difference was actually found between the highest concentration of BA and the other treatments. This was particularly true for the distribution of the floral meristems along the scion shoot (Fig. 2). Whereas in other treatments 75-90% of the floral buds were found at the upper three nodes,

most of the floral buds on plants given 4 x 10^{-4} M BA were spread along the entire shoot below as well as above the point of cytokinin application. The buds at the upper node appeared to have a relatively high ability to form flower primordia irrespective of cytokinin treatment, and even low doses of both Z and BA were sufficient to bring the percentage of flower buds at this position to a high level.

Table 4. Percentage of generative buds at the three upper nodes of decapitated apple plants (Aroma/M26)

Treatment	Percent floral buds	
Water	13	13
iP 4 x 10^{-5} M	27	
iP 4 x 10^{-4} M	18	22
Z 4 x 10^{-6} M	44	
Z 4 x 10^{-5} M	37	
Z 4 x 10^{-4} M	62	48
BA 4 x 10^{-6} M	31	
BA 4 x 10^{-5} M	67	
BA 4 x 10^{-4} M	57	52
Average	39	39
LSD (0.05)	N.S.	29

Table 5. Effects of xylem application of three cytokinins on flowering of apple plants

Treatment	Flower buds per plant		Flower buds % of bud		Percentage of plants with flowering terminal	Flowers per flower bud
Water	0.8	0.8	5.5	5.5	40	5.0
iP 4 x 10^{-5} M	0.2		1.7		40	5.0
iP 4 x 10^{-4} M	0.2	0.2	1.3	1.5	10	6.0
Z 4 x 10^{-6} M	1.6		11.7		90	4.3
Z 4 x 10^{-5} M	0.4		3.2		60	4.0
Z 4 x 10^{-4} M	1.4	1.1	10.4	8.5	60	4.5
BA 4 x 10^{-6} M	0.8		6.4		50	5.3
BA 4 x 10^{-5} M	2.0		12.9		100	5.1
BA 4 x 10^{-4} M	6.0	2.9	40.8	20.1	80	4.0
Average	1.5	1.5	10.4	10.4	59	4.6
LSD(0.05)	1.5		11.4		N.S.	N.S.

However, no effects of a range of cytokinins (iP, iPA (isopentenyladenosine), Z, ZR (zeatin riboside), diHZ (dihydrozeatin), diHZR (dihydrozeatin riboside) and BA) at concentrations of 10^{-4} and 10^{-3} M were found in a similar experiment in the subsequent season using non-decapitated and slightly more vegetative plants, despite the fact that the appearance of BA- and Z- (including ZR-) treated plants with respect to stem swelling and lateral bud deve-lopment was identical to that in the preceeding experiments.

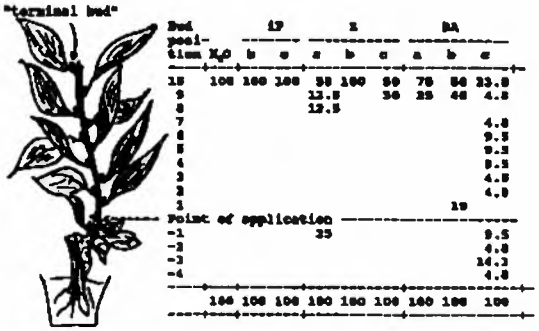

Fig. 2. Effects of xylem application of three cytokinins on distribution of the floral buds (%) at different positions along the scion shoot (a, b and c: $4 \cdot 10^{-6}$ M, $4 \cdot 10^{-5}$ M and $4 \cdot 10^{-4}$ M, respectively)

Orchard trials

Removal of spur leaves reduced the percentage of spur buds with flower initials in both years (Table 6), whereas removal of fruits had almost no effect on flowering. Irrespective of cytokinin type, application to the spur wood had no or a negative effect on the percentage of flowering spurs (Table 7). The number of flower initials was unaffected or slightly reduced, whereas in 1988 the spur diameter was considerably increased by most cytokinins.

Table 6. Effects of leaf and fruit removal on flower bud formation on single spurs (% floral spurs)

			Leaves	
1988		Intact	Removed	Average
	Intact	72.4	53.0	62.7
Fruit	Removed	79.9	47.9	63.0
	Average	76.2	50.5	63.3
1989				
	Intact	5.7	0.8	3.3
Fruit	Removed	5.6	1.1	3.4
	Average	5.7	1.0	3.4

DISCUSSION

The results indicate that flower formation in apple may be influenced by cytokinins, at least by the artificial compound benzyladenine, and are thus in accordance with results from previous studies (Ramirez-Rodrigues 1979; Unrath 1989). It was evident from the effects of both Z and BA on stem and buds that the presence of these compounds was registered by the tissues. The stem swelling, the reduced elongation and the almost Brussels sprouts-like appearance of the shoots treated with 10^{-4} to 10^{-3} M concentrations of BA and Z clearly indicated that the substances were taken up and transported along most of the shoot. The

tendency of the terminal buds to react by flower initiation may also be taken as an indication that the compounds even reached the shoot apex. The general lack of effects of iP on apple plant growth and development may be a parallel to its lack of effectivity in stimulating axillary bud development and shoot growth reported from tissue-cultured *Malus* (Hutchinson 1984a, b; Lundergan & Janick 1980).

Table 7. Effects of xylem cytokinin application to apple spurs

	Spur diameter (mm)	Percentage of spurs with flower buds		
	1988	1988	1989	Average
Control	4.7	80.0	5.4	42.7
Average of cytokinins	5.6	58.9	3.1	31.0
Average	5.2	69.5	4.2	36.9

A relatively unexpected observation was the flower-forming ability of the small plant system itself. If in a trial any flowers were formed at all, a substantial number of the control plants would also have a terminal flower bud. This was remarkable as budded scion/rootstock combinations do not normally form flowers until they reached a size considerably larger than the 20-30 cm-long shoots used in these trials, and more so as greenhouse grown apples are known to have poor flower-forming ability (Abbott 1984). The most likely explanation for the flower formation in untreated plants seems to be the small root volume. Root restriction has proved to increase the flower forming ability of several species (Ben-Tal 1986; Shinozake & Takimoto 1983) including peach (Richards 1986), in all cases accompanied by reduced vegetative growth.

However, in spite of the significant effects of Z and BA, their role in flower formation appeared to be supportive rather than decisive. Stimulation of flowering occurred only in plants with a certain immanent ability to flower even in the absence of these compounds. This effect could not be reproduced on plants lacking this ability, even though they clearly exhibited other responses to the cytokinins. Furthermore, flowering was only stimulated at relatively high concentrations, and more by the artificial than by the naturally occurring compound, possibly suggesting that the reponse was an abberration rather than reflecting inherent processes. In orchard trees cytokinins did not produce any enhancement of flowering, despite the fact that the spur buds responded to other treatments (deleafing) and the spur wood tissue itself responded to the applied cytokinins. As a wide range of cytokinin concentrations were tested (including those applied by Ramirez-Rodriguez (1979)), ruling out sub- or supraoptimality of concentration as an explanatory factor. In these trees, therefore, it seems that compared with the total impact of environmental factors, cytokinins were of little importance to flower bud formation.

Thus, the material does not justify any clear conclusions. However, most evidence indicates that cytokinin may not play the important role in flower induction of *Malus* ascribed to it by Hoad (1984). The most convincing evidence in this respect does not come from the planned trials, but from the already mentioned flower-inducing ability of the small plant system itself. Root cytokinin production appears to be associated with the number of

active root tips (Chen et al. 1985; Koda & Okazawa 1980); hence a reduced root volume or root activity would imply less export of cytokinins to the shoots. Actually, the vegetative growth potential of root-restricted peach seedlings could partly be restored by foliar applications of BA, indicating that reduced cytokinin export was at least part of the reason for the above ground effects (Richards & Rowe 1977). In *Pharbitis* root-applied BA and kinetin promoted flowering only to the extent that they also reduced root growth. When applied to the hypocotyl, kinetin had no effect on flowering (Shinozake & Takimoto 1983). Thus, if cytokinins play a role in flower induction they do so in concert with other equally vital elements.

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Xylem cytokinin content of apple (*Malus x domestica* Borkh.) as affected by season, soil management and root temperature

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Xylem sap of the apple variety 'Aroma' on M26 was tested for content of zeatin riboside (ZR) and isopentenyladenosine (iPA) by HPLC and enzyme immunoassay. The level of ZR dropped from approximately 80 pmoles/g sap in April to 10 pmoles/g in early June, and further to a 1-2 pmoles/g in August. ZR remained at this level until February, and attained a new maximum in late March. IPA constituted only some few percent of the total cytokinin, and showed no seasonal variation. Apart from a somewhat enhanced level of iPA in open soil, no effect of soil management was found on xylary cytokinin. The ZR level increased with increasing root temperature up to about 20°C, above which it declined.

Key words: Apple, cytokinin, endogenous, isopentenyladenosine, *Malus*, root temperature, season, soil management, xylem exudate, zeatin riboside.

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Cytokinins have been ascribed a role in flower induction of apple trees (Luckwill 1970), and the application of cytokinins to the spur tissue has been demonstrated to increase return bloom (Ramirez-Rodriguez 1979). Cytokinins are considered mainly root produced (Davies 1987), and various factors influencing flower bud formation in apple have been explained in terms of their effects on root activity and cytokinin production (Hoad 1984). In recent years the synthetic cytokinin benzyladenine applied as a foliar thinning agent has been shown to stimulate flower bud formation (Greene & Autio 1989; McLaughlin & Greene 1984). Benzyladenine also stimulated flowering in non-bearing trees (Unrath 1989).

Although Luckwill's hypothesis from 1970 has been maintained and further developed over the last two decades (Hoad 1984), few attempts to relate flower bud formation to endogenous cytokinin levels appear to have been made. Polish workers found that shoot cytokinin content in July (i.e. flower formation period) was twice as high in non-pruned (i.e. flower forming) trees as in pruned (vegetative) trees (Grochowska & Karaszewska 1978). Non-fruiting spur tissue was higher in cytokinin activity than fruiting tissue (Grochowska et al. 1984), and in the collar tissue a retarded decline in cytokinin content was observed after treatment with flower stimulating agents (Karaszewska et al. 1986).

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Apple plants receiving their nitrogen as ammonium, a treatment known to enhance flowering (Grasmanis & Leeper 1965, 1967; Grasmanis & Edwards 1973, 1974), displayed a sharp but transient increase in xylem sap cytokinin activity (Buban et al. 1978).

On the other hand, several workers (Hewett & Wareing 1973; Luckwill & Whyte 1968; Tromp & Ovaa 1990) found that natural cytokinin activity in xylem sap peaked in early spring, and fell abruptly towards the time of flower induction (i.e. full bloom + 3-6 weeks). In seeded and developing apple fruitlets, normally considered to affect flower bud formation adversely (Chan & Cain 1967), Letham and co-workers (Letham & Bollard 1961; Letham 1963; Letham & Williams 1969) repeatedly observed high levels of cytokinin-like activity. Bangerth et al. (1986) tested the effects of various growth regulators, and were not able to establish any correlation between endogenous cytokinin content and flower bud formation.

In a previously reported study on soil management systems, pronounced differences in flower bud formation occurred (Måge & Skogerbø 1992). To establish whether or not this could be ascribed to differences in xylem sap cytokinins, trees from four of the seven treatments were assayed for xylem sap cytokinin concentration.

MATERIALS AND METHODS

Plant material and treatments

Soil management trial

Plant material and experimental design and maintenance have been described elsewhere (Måge & Skogerbø 1992). Two adjacent replicates allocated in the middle of the experimental plot were selected and trees in bark mulch, grass ley, plastic film and herbicidal weed management were used for cytokinin assays. Twigs from two trees were sampled and pooled twice per month from April 1989 to April 1990. Soil temperatures were recorded at a depth of 25 cm for all eight blocks from early May to mid-July 1989. During the same period an indication of soil moisture content at a depth of 50 cm was obtained by two tensiometers in each of five plots (bark, grass, plastic, herbicide and mechanical cultivation) in one of the two replicates used for cytokinin analysis. Soil moisture and temperature were recorded twice per week between 1.00 p.m. and 3.00 p.m..

Root temperature trial

Rooted M26 *in vitro* produced shoots were allowed to grow to a 15-cm shoot length before entering the trial. At the start of the experiment the lower leaves of the plants were removed in order to facilitate instalment into the root temperature equipment, but the plants were otherwise else undisturbed. In addition, in one trial chip-budded Aroma/M26 plants were included in order to test possible effects on flower formation.

Different root zone temperatures were generated using a set-up illustrated in Fig. 1. An insulated three-chambered trolley was equipped with a cooled reservoir at one end and an aquarium-type electric heat bulb in the nutrient solution at the other end, thus erecting a temperature gradient over the three chambers. Each chamber contained a 10-l reservoir of a complete nutrient solution which was refreshed weekly without disturbing the roots. After instalment the plants were allowed to grow for three weeks. During this period the

root temperatures were recorded automatically at frequent intervals. Shoot temperatures were maintained at 21°C. At the end of each trial the shoots were cut at the upper insulation layer, and xylem sap collected from 30-cm-long pieces. In one case, exudates were also collected from the root part with a 5-cm piece of the stem base intact.

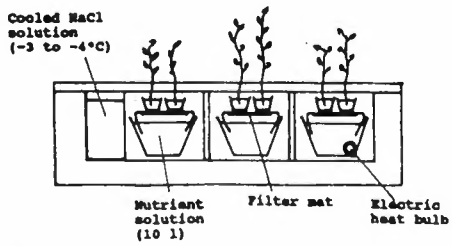

Fig. 1. Root temperature equipment

Xylem sap collection

Xylem sap was collected from 30-cm-long twigs of one or two years old wood using a technique described in Fig. 2. No more than 1-2 g sap was collected from each twig to prevent dilution by distilled water passing through the twig. The movement of pressurized water was studied adding potassium manganate, indicating that at least five grams of sap could be collected before any dye appeared at the upper end of the twig. When exudates were sampled from intact root systems the pressurized container (Fig. 2) was filled with tap water to cover the roots, only the stump of the stem protruding from the rubber seal.

Upon collection the xylem sap was mixed with 1 ml CH_3OH:Cl_3CH:H_2O 12:5:3 to denature possible proteins (enzymes) in the sap, and a small amount of di^{3}HZ (tritium-labelled dihydrozeatin, 1.37 TBq/mmol; Amersham) used as internal standard was added. The samples were then frozen at -20°C, thereafter lyophilized and stored further at -20°C until extraction.

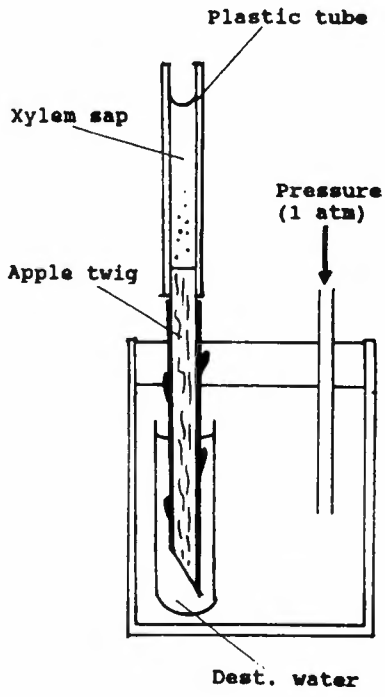

Fig. 2. Xylem sap collection

Xylem sap extraction

Lyophilized samples were dissolved in 2 ml distilled and deionized water, and pH adjusted above 9.0 by 0.1 N NaOH. The dissolved samples were then extracted three times with 2 ml water saturated n-butanol. The n-butanol phases were pooled, evaporated to dryness on a rotary evaporator (water bath 45°C) and dissolved in 500 μl water. The samples were stored at -20°C until fractioned on HPLC.

Fractioning on HPLC

The n-butanol fractions were purified further on a 250 x 4,6 mm column containing 5 μm particle size LC18 (Supelco). The column was originally eluted with a 25 min linear gradient of 10-40%

acetonitrile in 20 mM acetic acid adjusted to pH 5.6 with triethylamine according to a method by Hansen et al. (1984). However, interactions from the eluate causing overestimation of cytokinin values in the subsequent enzyme immunoassay (EIA) necessitated modifications. Thus, the method used for most samples was a 25 min system of two successive 10 min methanol gradients in mM H_3PO_4 pH 4.5 - the first running from 30 to 35% methanol, the second from 35 to 100% - followed by a 5 min sequence of 100% methanol. Flow rate was 1.0 ml per min, and fractions were collected at 1 min intervals, evaporated to dryness at 45°C, and stored (normally overnight) at -20°C until quantified on EIA.

Enzyme immunoassay of Z, ZR, iP and iPA

Assays of t-zeatin (Z), t-zeatinriboside (ZR), isopentenyladenine (iP) and isopentenyladenosine (iPA) were carried out in flatbottomed Immunolon M129 Micro ELISA plates (Dynatech Labs, South Windham, ME) or in flatbottomed Nunc MaxiSorb plates (InterMed, Denmark). The latter proved to have a higher binding capacity for the polyclonal antibody, allowing a lower concentration of iPA immunoglobulin (iPA-IgG) to be used. Anti-ZR and -iPA IgG were kindly provided by Carl Erik Hansen, and were obtained as described by Hansen et al. (1984) and Hansen (1986).

An EIA originally developed for tobacco callus and maize stem section analyses (Hansen et al. 1984; Hansen 1986) was somewhat modified to meet the requirements of this particular type of plant material and its often extremely low amounts of cytokinins.

Assay procedure

The procedure used was as follows: The wells were filled with 200 μl IgG in 50 mM $NaHCO_3$ pH 9.6. The IgG solutions were in all cases 10 μg per ml for ZR-IgG, but for iPA-IgG 20 μg per ml when Nunc Maxisorb plates were used and 40 μg per ml when Immunolon M129 plates were used. The plates were incubated overnight (> 12 h) at 4°C with gentle shaking. After rinsing twice with TBS (2.42 g Tris/l (tris(hydroxymethyl)aminomethane; US Biochemical), 29.24 g NaCl/l, pH to 7.5 with conc. HCl) containing 0.5 ml Tween 20 (polyoxyethylene sorbitane monolaurate; ICI) per litre, the wells were filled with 250 μl 1% BSA (bovine serum albumine, A-7638, Sigma) in TBS (Tween-free) and incubated at room temperature for another hour. After thorough rinsing (3x) with TBS with Tween, the coated wells were filled with 150 μl sample or standard dissolved in (normally 400 μl) TBS and incubated at 4°C with gentle shaking for a minimum of 30 min. Then 50 μl alkaline phosphatase conjugated to either ZR or iPA was added to each well (see below for procedure for preparing and diluting the respective conjugates). After an additional 30 min at 4°C with gentle shaking, the plates were rinsed three times with TBS with Tween, and then once with Tween-free TBS to avoid air bubbles in the final assay. Finally, 200 μl freshly prepared 4.24 mM (1mg/ml) p-nitrophenylphosphate in substrate buffer (800 ml water, 97 ml diethanolamine, 100 mg MgCl, 5 ml 2% sodium azide, pH to 9.8 with conc. HCl) was added to each well, and the plates incubated at room temperature for 15-40 min. The time required to reach measurable amounts of colouring was somewhat variable, and therefore absorbances was sought read when the well devoid of antigen gave absorbance values above 1.000 (normally between 1.000 and 1.500). If the plates could not be read immediately, the reaction was stopped with 50 μl 5 M KOH to each well.

Preparation of standard series

To account for the wide and unavoidable plate to plate variations, every single plate was furnished with a standard series of either t-ZR or iPA (Sigma) at 11 different concentrations. In addition, to account for the possible effects of the HPLC flow component, flow eluted from the rinsed column was mixed with the standard solution in amounts and composition corresponding to that of the samples assayed. The mixtures of standards and eluted flow were then evaporated to dryness at 45°C and otherwise handled as described for samples (above).

ZR and iPA conjugates of alkaline phosphatase

ZR and iPA conjugates of alkaline phosphatase previously prepared by C.E. Hansen (Hansen 1986; Hansen et al. 1984) were used for all the ZR and the first part of the iPA assays. However, as we ran out of iPA-conjugated alkaline phosphatase, this had to be prepared *de novo* from iPA and alkaline phosphatase (calf intestine; Boehringer Mannheim) following a procedure from Hansen et al. (1984). The alkaline phosphatase conjugates obtained from C.E. Hansen were diluted with TBS by a factor of 1:4000 just prior to use. Our iPA conjugate displayed a somewhat lower activity, and was diluted by a factor of 1:1500.

Calculations and statistics

The amount of cytokinin per well was calculated from a log-linear regression obtained from a standard dilution series applied to every plate:

$y = B/B_o = a + b\ln x$

x	=	amount of ZR or iPA in fmoles (10^{-15} mol)
B	=	absorbance obtained from sample or standard
B_o	=	absorbance obtained in absence of antigene (ZR or iPA)
a	=	constant; y-intercept of regression line
b	=	constant; slope of regression line

The obtained values per well were corrected for sample size, dilutions and recovery of di^{3}HZ after HPLC. As only about 70% of the radioactivity of the ^{3}H labelled diHZ solution co-eluted with non-labelled diHZ, the recovery was calculated on basis of a value equal to 70% of the reference tritium activity.

For the period April to September 1989 samples from both replicates and all four treatments were extracted with BuOH, and all extracts were fractioned on HPLC and assayed three times for both groups of cytokinins. For the most Z/ZR rich samples of replicate two, the HPLC fractions were dissolved in double volumes allowing two EIAs of each fraction. Because of limited time and scarcity of anti-iPA antibody, only Z and ZR of one of the replicates were analysed for the remaining period (October 1989 to March 1990). To avoid unnecessary use of the valuable anti-ZR antibody only samples from the open soil treatment were assayed first, and samples from the remaining three treatments were assayed from sampling date February and later, when a detectable increase in the ZR equivalent level was evident.

The effects of treatment and sampling date on cytokinin content were tested by

analysis of variance using the statistical analysis program Mstat (Nissen & Mosleth 1985). Possible influence of analytical factors (buffers, HPLC runs, columns, EIA plates etc.) was studied by the multivariate analysis Partial Least Square (PLS) regression applying the computer program Unscrambler (Martens & Næs 1989).

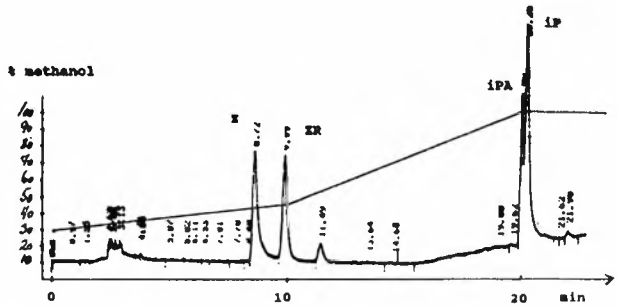

Fig. 3. Retention times of Z, ZR, iP and iPA on HPLC using a double gradient methanol/5 mM phosphate buffer pH 4.5 flow system

RESULTS

Analytical aspects

Retention times of the four cytokinins in question on the modified HPLC flow system were established by known standards (Fig. 3). Zeatin eluted between eight and nine minutes after injection, ZR around ten minutes, and iP and iPA in the twenty-first minute. However, the methanol/phosphate buffer flow system appears to have introduced an instability in retention times of zeatin and zeatin riboside that necessitated frequent resetting of the timing of the fraction sampling. Statistical calculations involving all available information on possible sources of variation carried out posteriorly on a larger part of the material, indicated the buffer component as a major source of variation. This instability prevented proper separation of zeatin and zeatin riboside, and the two compounds were taken together and presented as ZR equivalent in the following. As seen in Fig. 3, retention times for iP and iPA, although generally very stable, were too similar for separation, and these compounds are hence presented together as iPA equivalents.

Complete sensivity and specificity tests of the original assays had been carried out previously (Hansen 1986; Hansen et al. 1984). Final validation of the assay was obtained by adding ZR and iPA standards to xylem exudate lots at sampling, and comparing them with the values calculated from the EIA.

Seasonal variation in cytokinin contents

By far the most prominent effect observed was the seasonal variation in the concentration of ZR equivalents in the xylem sap (Fig. 4). The amount of ZR equivalents peaked at an average of approximately 80 pmoles/g sap in early April, dropping to an intermediate level of around 10 pmoles in mid-May, and then after a transient peak in June to the nearly undetectable level of 1-2 pmoles/g sap in mid-August. This situation was maintained until February, when the concentrations started to increase again to reach a new maximum in late March or early April.

The values obtained for iPA equivalents were only about 10% of those of ZR. The temporal changes were also less dramatic, showing a variation from 0.5 to 5 pmoles iPA

equivalents per gram of sap. Neither were there any signs of a seasonal pattern like that of ZR, with very high levels in the early spring and very low levels in late summer and autumn.

Effects of treatment

Plastic film and roofing felt increased soil temperatures by about 2°C over the open soil, whereas bark mulch and grass ley reduced soil temperature at a similar rate (Table 1). In this period bark mulch did not seem to influence soil moisture compared to the open soil treatments, whereas grass ley clearly reduced soil moisture. The data obtained on soil temperature and moisture were in accordance with those reported from more thorough investigations (Ashworth & Harrison 1989).

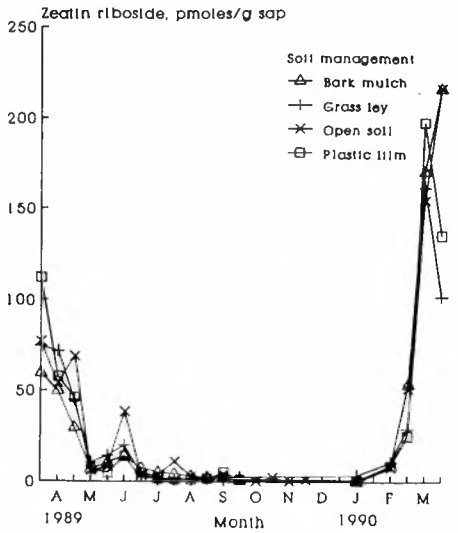

Fig. 4. Seasonal variation in xylem sap content of t-ZR equivalents. Pmoles per gram sap

Table 1. Soil temperature (25 cm) and moisture (50 cm). Average early May to medio July 1989

Treatment	Soil temperature (°C)	Soil water tension (bar)
Bark mulch	12.4	0.38
Grass lay	12.5	0.42
Open soil (herbicide)	13.9	0.38
Plastic film	16.4	0.21
Open soil (mechanical)	14.7	0.34
Plastic web	14.6	-
Roofing felt	17.4	-
Average	14.6	0.34
LSD (0.05)	1.0	0.25
Level of significance	0.01	0.01

The only significant effect of soil management on cytokinin content was found in the April-September average of iPA equivalents (Table 2). Analyses of spring (April-May) and induction period (June-July) contents revealed no significant differences in any of the cytokinins. However, trees in bark mulch and plastic film tended to have a lower and higher contents, respectively, of ZR equivalents than the other two treatments, whereas iPA levels in the same period appeared highest in bark mulch and grass ley trees. In the induction period, recorded ZR levels were on average only 20% of those in spring, and the order of treatments seemed to have changed, so that for both cytokinin groups open soil trees displayed the highest values, and plastic film the lowest. The relative amounts of

cytokinins in the four management systems were remarkably similar for the two cytokinins (Table 2). Flower bud formation, although not significantly different between assayed replicates, was highest in open soil and plastic film plots, and lowest in grass ley.

Table 2. Cytokinin levels in xylem sap of Aroma/MM106 trees under four different soil management system during different parts of the season 1989, and flower bud records the subsequent spring. Pmoles per gram sap, and flower clusters per tree, respectively

Cytokinin	Bark mulch	Grass ley	Open soil	Plastic film	Average	Lev. of signif.
Spring (April-May)						
t-ZR equiv.	35.8	55.3	49.5	63.3	51.0	N.S.
iPA equiv.	3.2	3.6	1.6	1.9	2.6	N.S.
Induction period (June-July)						
t-ZR eluiv.	9.8	10.7	14.5	6.1	10.3	N.S.
iPA equiv.	3.2	3.7	4.7	2.0	3.4	N.S.
Average (April-September)						
t-ZR equiv.	18.3	25.4	25.0	27.8	24.1	N.S.
iPA equiv.	2.7	3.5	2.7	1.6	2.6	0.05
Flower bud formation 1989	93.5	34.5	131.5	132.0	97.9	N.S.

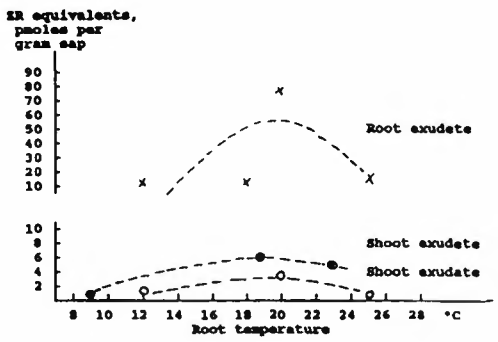

Fig. 5. Xylem sap ZR equivalent content as affected by root temperature

Effects of root temperature on cytokinin contents

Assays of the xylem cytokinin content of M26 plants maintained at different root temperatures displayed significant differences in the content of ZR equivalents (Fig. 5). The maximum levels were found at root temperatures of 18-20°C. The concentration of ZR in exudates from the shoot parts was in the range of 1-8 pmoles/g xylem sap, whereas exudates from intact root systems exposed values approximately one order of magnitude higher.

The level of iPA equivalents, although not significantly different, exhibited a tendency towards increase over the entire root temperature range applied (Fig. 6). Correspondingly to those of ZR, iPA levels were considerably higher in exudates from intact roots than in sap from the shoot.

DISCUSSION

The seasonal variation found in xylem cytokinin content is well in accordance with what was found by Luckwill & Whyte (1968) and Tromp & Ovaa (1990), indicating that in general the work carried out gives a reasonably correct picture of the cytokinin level of the samples analysed. Furthermore, the variation in xylem cytokinin content from sampling date to sampling date also exhibited in Tromp & Ovaa's work (1990), indicates that these are an inherent property of the cytokinin pattern of field-grown trees.

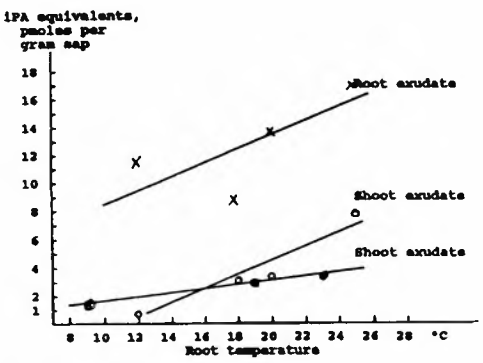

Fig. 6. Xylem sap iPA equivalent content as affected by root temperature

The findings of Tromp & Ovaa (1990) confirm the predominance of Z and ZR over their counterparts with un-oxidized sidechains. Interestingly, the only exception to this was found in late February, at the start of the spring elevation in cytokinin levels, when iP constituted 95% of the total cytokinin levels (Tromp & Ovaa 1990). Possibly, the rise in iP represents the biosynthetic precursor preceding the coming increase in Z and ZR levels. Belding & Young's analyses (1989) of apple xylem sap from dormancy to bud break likewise revealed an inclination in ZR content culminating at bud break. It is thus reasonable to believe that the variation in cytokinin activity in xylem sap of apple also found by Luckwill & Whyte (1968) mainly reflected the variation in Z and ZR.

The question as to what might be the physiological significance of the early spring rise in Z and ZR levels is intriguing, but still poorly understood. The elevation of xylem cytokinins most likely constitutes a part of the preparation for the coming growth season. However, the increase in xylem ZR content was not dependent on a fulfilled chilling requirement, and the maximum values were not significantly different between chilled and unchilled trees (Young 1989a). The fact that maximum ZR content in the xylem coincides with (the time of) bud break irrespective of root or shoot temperature regime (Belding & Young 1989), may indicate that the rapid decline observed in xylem content in May (see also Luckwill & Whyte 1968; Tromp & Ovaa 1990) may be a dilution effect due to increased transpiration rather than representing a reduction in the biosynthetic activity of the roots. The very high levels observed in March and April may merely be the result of an increase in root activity in the absence of transpiring leaves, raising the xylem cytokinin content to a high level. As shoot, and later fruit, growth proceeds, the depletion of stored assimilates may lead to an actual decrease in root cytokinin production during June and July. However, if root activity and transpiration were the only factors determining xylem cytokinin concentration, the consistently low level found in the autumn, when apple roots normally show a second peak in activity, remains to be explained.

As no significant differences in cytokinin levels were found during the first four months of the season, the results do not support the idea that the effects of soil management on growth and flowering are mediated by xylem cytokinins. This holds even if the non-

significant tendencies are taken into consideration. The trees of the two systems causing the most vigorous growth (bark and plastic film mulch) were also those displaying the greatest difference in early and average cytokinin content. Furthermore, trees in grass ley and plastic film with very dissimilar growth potentials were rather similar in cytokinin content. Growth kinetics were not studied, and possible relations between timing of bud break and early spring growth, and cytokinin levels, cannot totally be ruled out. Nevertheless, the results are well in accordance with the works of Belding & Young (1987, 1989) who found that bud break and shoot elongation were governed by shoot temperature, irrespective of xylem ZR content. If correlated at all, it appeared in their study as though commencement and intensity of shoot elongation were negatively correlated to xylem ZR content, as those temperature regimes leading to early and rapid growth were those that suppressed the cytokinin inclination most strongly. The only factor positively correlated with root temperature was the percentage of broken buds (Belding & Young 1987).

The same applies to flower formation. The (non-significant) differences in spring ZR content were not maintained till the time of flower induction, leaving no evidence of an effect signalled by cytokinins. Rather, the results indicate that flowering varies independently of xylem sap cytokinin content at this time. Neither were there any indications of an effect of temperature on xylem sap cytokinins. A possible positive effect of temperature, as indicated in Fig. 5, seems appears to have been absent in the outdoor trial, or at least masked by other, more dominating factors.

The relatively consistent patterns of cytokinin activity in the controlled environment trials are nevertheless intriguing; in particular the coincidence of an activity peak of ZR equivalents in the xylem sap and the peak in flower initiation (Tromp 1984) occurring at 15-20°C. However, more trivial explanations than stimulation of flower induction by cytokinins cannot be ruled out. As found by Tromp (1984) vegetative growth increases linearly with increasing root temperature. Assuming (1) that photosynthesis is independent of root temperature, and (2) that root cytokinin biosynthetic activity increases with temperature, a situation may occur in which the increased shoot growth may leave fewer assimilates available to the root, thus checking its biosynthetic potential. The same may apply to flower bud initiation dependent on the same assimilates. Increasing growth stimulated by higher root temperature increases the levels of available assimilates; however, if stimulated too much, the vegetative growth may call too intensively on the assimilates, thus leaving an insufficient level available for flower bud formation. In addition, potential flower buds may develop as vegetative shoots if growth is stimulated too far.

The absence of significant correlations between xylem cytokinin content and dormancy and growth parameters does suggest a more supplementary role for the cytokinins. The most popular among such is that of metabolite attraction (Young 1989b quoting Saure 1985). Alternatively, cytokinins (and other growth substances) may have a fundamental, but indirect role in all growth and development processes (Trewavas 1981; 1987), and the increase in xylem cytokinins may be accomplished in order to ensure ample supplies of the substances to prevent them from attaining a limiting and thereby controlling role in growth and development.

As pointed out in a previous report (Måge & Skogerbø 1992), the differences in growth and flower bud formation between the soil management systems could also well be attributable to different growth patterns. Although vital in cytogenesis and adventitious bud

regeneration, cytokinins have never been shown to have a general stimulating or controlling role on the rate of vegetative growth or flower induction in the intact plant. Rather, its function seems to be to maintain basic cell functions required for vegetative as well as for generative development. In the intact plant the level of cytokinin nearly always appears to be sufficiently high for maintenance of these functions, and cytokinins, although varying in content, seldom play a direct rate-controlling or triggering role.

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Effects of root pruning and trunk girdling on xylem cytokinin content of apple (*Malus x domestica* Borkh.)

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Root pruning and trunk girdling of apple trees at full bloom reduced growth, and enhanced flowering by 55% and 110%, respectively. Relative to control, xylem zeatin riboside (ZR) content as analysed by HPLC-EIA decreased in girdled trees, whereas root-pruned trees displayed a considerable increase in ZR levels after a transient decline. The levels of isopentenyladenosine (iPA) were unaffected by the treatments.

Key words: Apple, cytokinin, endogenous, flowering, isopentenyladenosine, *Malus*, ringing, root pruning, vegetative growth, zeatin riboside.

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Trunk or branch girdling is probably the most potent non-chemical measure available for enhancement of flower bud formation in apple trees. The effects have been known for centuries (Lawrence 1717; Davies 1957) and studied scientifically since early this century (Haller & Magness 1933; Kraybill 1923; Overholser & Claypool 1935). Girdling has proven superior to treatments with daminozide in increase and reliabilty of flower bud formation (Greene & Lord 1978; Veinbrants 1972), and appears to be effective in counteracting the negative effects of gibberellin applications (Dennis & Edgerton 1966). The increase in flower initiation found distal to a girdle is normally interpreted in terms of a build-up in assimilates above the broken phloem continuum (Kraybill 1923; Hansen 1972, 1975, 1985; Schumacher 1975a). However, the negative effects on vegetative growth (Dennis & Edgerton 1966; Greene & Lord 1978) do not appear to be as equally well accounted for. A possible contributory factor may be reduced cytokinin export as indicated in *Vitis* (Skene 1972) and *Salix* (Van Staden & Brown 1977,1978) due to reduced root activity (Hansen 1975) caused by lower availability of assimilates below the girdle (Hansen 1972).

Root pruning has in a number of cases been found to reduce vegetative growth and increase fertility in fruit trees (Richards & Rowe 1977a, b; Schupp & Ferree 1987b; Scibisz 1986), the effects on fertility partly being accounted for by enhanced flower forming ability (Schumacher 1975b; Schumacher et al. 1978). Schumacher et al. (1978) observed only small differences in flower formation of root pruning from December to April, whereas

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Abbott & Adam (1983; Abbott 1984) found that the highest number of flower buds were definitely on the trees root-pruned a month after full bloom. Noting that cytokinins were able to substitute for roots in stimulating flower development of induced bud of *Vitis* (Mullins 1967), Hoad (1984) suggests that the massive regrowth of roots occurring after root pruning (Abbott 1984) leads to higher cytokinin production and thereby enhanced flower induction in apple trees. Although Mullins' (1967) experiment concerned flower primordial development rather than flower induction, the fact that marcotted *Citrus* branches failed to form flowers unless roots were initiated above the girdle (Oslund & Davenport 1987) may lend some support to this view.

However, the effects of root pruning on root cytokinin production are far from clear. Whereas repeated foliar applications of benzyladenine (BA) could partly restore the vegetative growth of root-pruned peach seedlings (Richards & Rowe 1977a, b), trunk injections of BA or zeatin (Z) to young apple trees were not effective in overcoming the growth inhibition or other effects caused by the root pruning (Schupp & Ferree 1988), demonstrating perhaps more than anything else the contrasting effects of the application method (Skogerbø 1992). The effects of root pruning on endogenous cytokinin levels are just as confusing. Root pruning of MM106 apple rootstocks reduced the xylem sap content of cytokinin by 50% (Schupp & Ferree 1987a), and partial removal of the roots of decapitated *Phaseolus* plants reduced the accumulation of endogenous cytokinins in the leaves characterizing decapitated plants with their roots intact (Carmi & Van Staden 1983). Potato plants, on the other hand, responded to a 10-15% reduction in root volume by a transient depression in cytokinin activity followed by a marked increase in activity later (Bagautdinova 1983).

Given the very few and rather contradictory results concerning cytokinin involvement in the effects of girdling and root pruning on flower formation in apple, an attempt was made to assay xylem sap cytokinins of girdled and root-pruned apple trees during the first month after treatment at full bloom.

MATERIALS AND METHODS

Plant material and treatments

Experiment 1

Twenty-four four-year-old Lobo/MM106 trees arranged in one single row at the experimental orchard of the Department of Horticulture, Agricultural University of Norway were selected for uniformity and split into two replicates each comprising three plots of four trees. Within each plot three trees were used for sampling, while the fourth was left undisturbed for observation. All trees were deblossomed just prior to treatment.

Root pruning was carried out by forcing a broad-bladed spade to a depth of 25 cm in two 2-m-long lines parallel to the tree row, at a distance of 10 cm from the trunk. Girdling was done by removing two 1-cm half rings of bark 2 cm apart on opposite sides of the trunk. The lower ring was positioned 10 cm above the graft junction, and the two half rings had a 1-cm overlap at both ends. Both treatments were carried out at full bloom (13 June, 1988).

Xylem sap was collected from five 10-cm pieces of the previous year's wood taken randomly from all three trees of each plot. The xylem sap was collected according to the

procedure given by Skogerbø & Måge (1992) and the samples frozen at -20°C and subsequently lyophilized. Xylem sap was collected at 0, 1, 2, 4, 8, 18 and 32 days after treatment.

Experiment 2
To obtain material for testing the cytokinin analysis procedure, a simplified version of Experiment 1 was initiated three days later. Twelve of the remaining trees in the same row were selected for uniformity, and almost identical treatments to those described above were applied to four single trees distributed evenly among the twelve. Treatments were as described above, except that root pruning was carried out at a distance of 15 cm from the trunk base, and the trees were not deblossomed prior to treatment. Xylem sap was collected from one 25-cm twig of the previous year's wood from each tree at days 0, 1, 2, 4, 8, 17 and 32. The xylem sap from each treatment and day was in most cases collected as two volumes, which later were handled as separate samples.

Cytokinin analysis, calculations and statistical analysis

The content of ZR and iPA equivalents in the xylem sap was analysed by enzyme immunoassays (EIA) after fractioning on HPLC, and is described elsewhere (Skogerbø & Måge 1992). A minor modification was made as the internal standard (di^3HZ) was added just prior to extraction with n-butanol.

Calculation and statistical analyses were carried out as described by Skogerbø & Måge (1992). Considerations on assay validity made in that study also apply to this paper.

RESULTS AND DISCUSSION

Owing to the warm and dry weather in June, shoot growth more or less ceased within a month after full bloom on most trees. However, marked differences between the treatments were observed. Root-pruned trees of Experiment 1 suffered from afternoon wilting during the first few days after treatment, and growth ceased within one to two weeks. On control trees at least parts of the shoots were still growing after four weeks, whereas on girdled trees all shoots appeared to have stopped elongation between three and four weeks after treatment. The differences in the length of the shoot growth period were reflected in shoot length in the observation trees (Experiment 1) at the end of the sampling period (Table 1).

Leaves of girdled trees attained a light green to yellowish colour, and a stiff and somewhat crisp texture within a month after treatment. Root-pruned trees displayed no visible changes in leaf texture, and maintained a fresh green colour, although somewhat paler than that of control trees. Root pruning and girdling enhanced the number of flower buds per tree by 55% and 110%, respectively (Table 1).

Apart from some relatively high values of iPA equivalents for the first one to two days of Experiment 1, the cytokinin levels of the Lobo/MM106 trees were rather similar to those found in xylem sap of Aroma/MM106 trees at the same time in the subsequent season (Skogerbø & Måge 1992).

Table 1. Effects of girdling and root pruning on shoot growth and flower bud formation in Lobo/MM106 trees. Shoot length: average of ten randomly chosen shoots per tree (undisturbed), one month after treatment

Treatment	Shoot length (cm)	Flower buds per tree			
		Exp. 1	Exp. 2	Average	Relative
Control	25.3	98	34	66	100
Girdling	17.8	149	127	138	209
Root pruning	12.9	130	73	102	155

The two cytokinin groups reacted differently to the applied treatments. The level of iPA equivalents tended to fall during the first two days after full bloom irrespective of treatment (Experiment 1), and after day four there were hardly any differences found between the differently treated trees in any of the experiments (Fig. 1).

In contrast, the average level of ZR equivalents increased significantly as time passed. However, the increase was nearly entirely confined to the control and root-pruned trees, leaving the xylem ZR content of girdled trees far below that of the control trees by the last sampling date (Fig. 2). In Experiment 1 a significant difference was only found between control and root pruned trees, the final xylem ZR content of root-pruned trees in this experiment being nearly twice that of control trees. A fortnight earlier the situation was the opposite, with control trees exhibiting a higher xylem ZR content than root-pruned trees in both experiments, possibly indicating that root-pruned trees underwent a transient decrease in xylem ZR similar to that found for cytokinin activity in potato plants (Bagautdinova 1983). Since maintenance of photosynthetic activity in several cases has been found to rely on supplies of xylem cytokinin (Caers et al. 1985; Carmi & Koller 1979), the transient, though more persistent depression of net photosynthesis found in young, root-pruned apple trees (Geisler & Ferre 1984) may have reflected a reduced xylem cytokinin content. The failure of the root-pruned trees of Experiment 2 to exceed the controls in xylem ZR within the sampling period may be due to the fruit on these trees having first call on the assimilates, and thus delaying the development observed in deblossomed trees (Experiment 1).

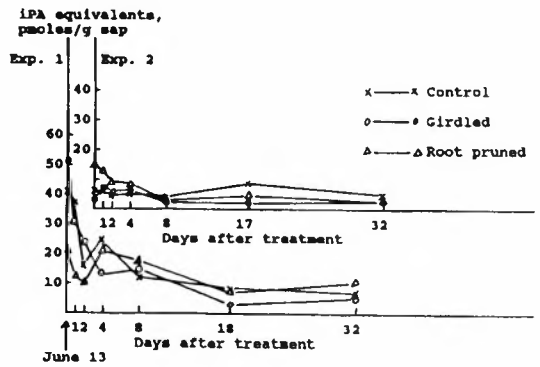

Fig. 1. Variation in xylem sap iPA equivalents after girdling and root pruning of 'Lobo/MM106' apple trees at full bloom

Reduced vegetative growth is frequently found upon root pruning (Richards & Rowe 1977a, b; Schupp & Ferree 1987b; Scibisz 1986), and among other factors it has been ascribed to

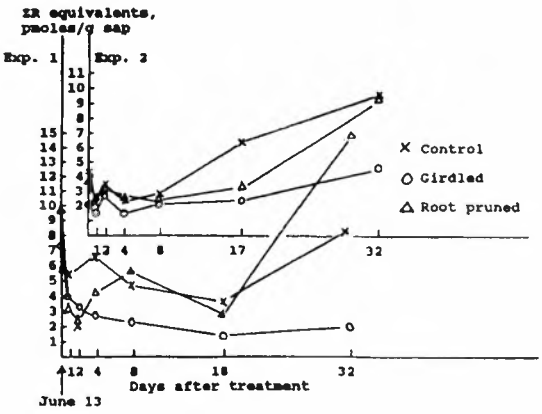

Fig. 2. Variation in xylem sap ZR equivalents after girdling and root pruning of 'Lobo/MM106' apple trees at full bloom

a reduced water supply. However, the reduction in leaf water potential of root-pruned apple trees was found to be temporary, and hardly detectable one day after treatment (Geisler & Ferree 1984). Hence, relocation of assimilate to root growth and reduced cytokinin supplies may be equally important (Richards & Rowe 1977a, b). Apart from the partial resumption of vegetative growth after foliar application of benzyladenine (Richards & Rowe 1977a, b), most attempts to relate the effects of exogenous (Miller 1982; Schupp & Ferree 1988) or endogenous (Belding & Young 1989) cytokinins to shoot elongation have failed. In most reported cases of root pruning, growth resumed after a while (Geisler & Ferree 1984; Richards & Rowe 1977a, b,). In our trials reduced water availability was aggravated by the subsequent warm and dry period, which also induced early growth cessation in the control trees, and it therefore seems likely that this was the cause of the rapid and complete cessation of shoot extension in root-pruned trees.

The reduced growth of girdled trees is equally well in accordance with the literature (Greene & Lord 1978; Veinbrants 1972), although less well explained. The assumption that reduced levels of xylem cytokinins (Skene 1972) will result in reduced shoot elongation is countered by the aforementioned lack of correlation between endogenous and exogenous cytokinins and growth. Furthermore, the fact that individual branches appear to react to girdling in the same manner as whole trees (Dennis & Edgerton 1966; Haller & Magness 1933) strongly indicates that the effects on growth are due to factors localized proximal to the girdle. Adverse effects of carbohydrate accumulation on the photosynthetic apparatus and photosynthetic activity have been described in a number of cases (Hansen 1972, 1975, 1985; Kraybill 1923). It is difficult to understand, however, how or why an abundant carbohydrate supply should reduce vegetative growth; the opposite trend would be more reasonable. An alternative explanation might be that the accumulation of auxin above the girdle imposes the apical dominance effect on the growing apices themselves. Furthermore, if the recent proposal by Bangerth (1989) on the role of IAA in correlative inhibition is valid, the IAA export rate may *per se* be an invigorationg factor of the dominating organ. Possibly also secondary effects (e.g. ethylene evolution) caused by the auxin accumulation may contribute to the loss of vigour of girdled trees or branches.

The observed variation in xylem ZR equivalents supports Hoad's expectations (1984) of an increase in root cytokinin export upon root pruning. However, assuming the increase in xylem ZR equivalents reflects *de novo* synthesis in an increased number of root apices (and possibly higher apex activity as a result of higher availability of assimilates after

growth cessation), a preceding inclination in the assumed biosynthetic precursors iP and iPA (Chen 1981; Maass & Klämbt 1981; McGaw 1987) would be expected. Although the results of this study do not justify a discussion on cytokinin biosynthesis and metabolism, the lack of such an enhanced level of iPA equivalents may indicate that explanations of the increased ZR levels other than *de novo* synthesis should be sought.

However, the variation in ZR equivalents does not accord with the view that xylem supplied cytokinins play an important role in apple flower induction (Hoad 1984), as by far the most effective flower-inducing treatment coincided with the definitely lowest xylem ZR values. The results rather indicate that flowering and xylem cytokinins vary independently. Nevertheless, this does not exclude the possibility that cytokinins may be necessary and, in certain situations, even controlling agents in flower induction in apple, but it certainly implies that other factors are equally important.

Furthermore, the view that growth cessation is a causal event in flower formation (Ben-Tal 1986; Davis 1957) is not supported either. The early and abrupt desistance of growth in root-pruned trees was not maintained in correspondance with the somewhat more vigorous growth of the girdled trees in flower induction. Not denying the fact that the elongation growth of the individual apex must be terminated before induction can occur, our results, supported by the literature (Delap 1967; Grasmanis & Edwards 1974; Grasmanis & Leeper 1965; Luckwill & Silva 1979; Tromp 1972), strongly suggest that the assumed negative correlation between vegetative growth and flower formation needs further consideration.

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