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IV. ROOT GROWTH OF CUCUMBER IN WIDE NFT GULLIES

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Vlugt J.L.F. van der 1989. IV. Root growth of cucumber in wide NFT gullies. Norwegian Journal of Agricultural Sciences, 3, 301-308. ISSN 08001-5341

The growth of cucumber roots was studied in wide NFT gullies. Root growth rate was measured in cm per day and in number of lateral roots per day. The average length of the part of the root without laterals was also calculated. The periods before and after root death were compared.
Root growth was significantly reduced after root death, but a significant linear correlation found between growth rate before and after root death indicated that a fast-growing root mat may suffer less from root death than a more slowly growing mat. Also, significant differences between seasons were found.
Anatomical studies showed that the cortex had disintegrated when root death occurred. Thereby root surface area was reduced to 30-40%. No early indications of root death could be seen. Roots were still very young, physiologically, when they were affected by root death.

Key words: cucumber, *Cucumis sativus*, root anatomy, root growth

Abbreviations: l = increase in length (cm/day)
nl = increase in number of laterals/day
pwl = portion without laterals (cm)

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The degree of physiological root death may vary with the season, between plants in the same greenhouse and even between roots of the same plant. However, once in some roots root death occurs, then the other roots will follow suit (Hurd and Price, 1977; Van der Vlugt, 1986, part I).

The roots of the Cucurbitaceae can grow very fast,up to 2 1/2 inches (= 6.4 cm) per day in *Cucurbita* (Whitaker & Davis, 1962). The development of the vasculary system of the roots is much slower than the growth rate. Laterals can be initiated very early (Hayward, 1951).

Daughtrey & Schippers (1980) contended that the cortex decayed when root death occurred. Hayward (1951) mentioned that the cortex may persist for some time after secondary root growth has been initiated. Still, it would mean that roots are very young when they become affected with root death.

Therefore, it was considered necessary to study the growth and the anatomy of individual roots of the cucumber (*Cucumis sativus*) in connection with root death and if possible to find early indications of root death.

MATERIALS AND METHODS

Root growth measurements

An adaptation was made of the wide trays used by De Stigter (1969a). Instead of an individual system for each plant we

put 3 plants in a gully 4.5 m long and 0.6 m wide. Four of these gullies were supplied with the same nutrient solution, which was recirculated continuously. The nutrient solution was maintained at EC 2.0-2.5 mS and pH 5.5-6.5.

The best material for covering the gullies was found to be white polystyrene foam plates painted black on the under side. They were easy to move and the temperature in the gully did not become too high.

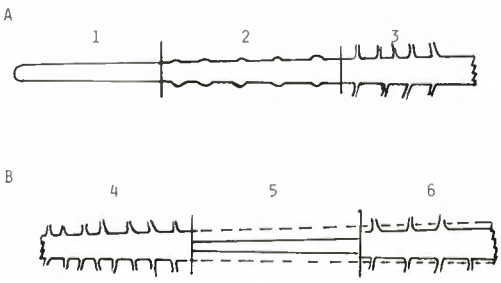
The gullies were lined with black plastic, and no other support for the roots was supplied. Different kinds of netting and non-woven polymer fibre tissue (Agryl) were unsatisfactory as lining because of toxic substances and problems with the roots growing through the holes.

Cucumber seeds of the cultivar Farbio were sown in small rockwool blocks (2.5 x 2.5 x 4.0 cm) and set out in the gullies 2 weeks after sowing. Natural light was supplemented with 5000 lux of fluorescent light in the period from October 1 to April 1 for 18 hours a day. At planting about 6 roots were growing out of the block; four of them were chosen at random for growth studies.

A wooden label was laid alongside the root, with a mark indicating the position of the root tip. The increases in length and in the number of laterals were measured two or three times per week. The portion of the root behind the root tip without laterals was also measured. (Parts 1 and 2, Fig. 1A).

The results were divided in the periods from planting to root death and from root death to the end of the experiment. Total length and total number of laterals were divided by the number of days in each period. In each period the average portion of root without laterals was calculated.

Once the system was functioning satisfactorily, five experiments were carried out (Table 1). The first experiment supplied material for microscopy. Experiment 2 was a repetition of an experiment carried out earlier using ordinary NFT-gullies (Van der Vlugt, 1986). In experiments 3 and 4 prevention of root death was attempted by adding auxin (see also Van der Vlugt, part V, in prep.). Also the effect on the whole root system was sought when only one root was treated (cf. De Stigter 1969a). In the last experiment the effect of earliness was studied, delayed fruit set would give a bigger plant which might be less susceptible to root death.



1. ROOT TIP, NO LATERAL ROOTS
2. LATERAL ROOTS VISIBLE AS KNOTS
3. COMPLETE LATERAL ROOT DEVELOPMENT
4. ZONE JUST DISTAL TO ROOT DEATH ZONE
5. ROOT DEATH ZONE
6. ZONE JUST PROXIMAL TO ROOT DEATH ZONE

Fig. 1A. Parts of the cucumber root used for microscopy before root death. B. Parts of the root with root death used for microscopy

Root anatomy

Roots excised for anatomical studies were divided into three parts (Fig. 1A). Part 1 consisted of the root apex, part 2 was taken where there were no laterals, only small knots on the roots. Part 3 was the most distal part in which the laterals were fully developed. The roots were embedded in paraffin and cut into 15μ slices using the microtome. The preparations were stained in safranine, picric acid and malachite green. The diameter of root and stele were measured under a light microscope. The percentage circumference was calculated by dividing the stele diameter by the root diameter.

Table 1. Important data in connection with the different experiments

Planting date	Flowering date	Root death	End of experiment	Treatment
October 26 1984 (1)	November 11	November 21	December 17	None, material for microscopy
February 18 1985 (2)	March 1	March 13	March 22	Root mass halved at planting or at flowering
May 29 1985 (3)	June 7	June 19	July 10	One root of each plant treated with 5 ppm IBA on June 19
July 17 1985 (4)	July 28	August 5	August 21	One root of each plant treated with 5 ppm IBA on August 5
October 8 1985 (5)	October 24	November 11	December 5	First fruit set in node 3, 4, 5, 6, 7 or 8

RESULTS

Root measurements

The data from all the experiments were analysed together with regard to correlation between characters and difference in growth rate before and after root death. The data were analysed by experiment for differences between treatments. The correlations between the studied characters were significant and positive:

$$pwl = 2.4 + 0.96 * l$$
$$nl = 2.4 + 1.82 * l$$
$$pwl = 1.8 + 0.39 * nl$$

This explained about 40-60% of the variation. Fast-growing roots grew more laterals per day but had also a larger portion without laterals than slow-growing roots.

For each character the growth after root death was significantly decreased (Table 2). Another interesting question was to know whether there was some correlation between growth before and growth after root death. Figs. 2, 3 and 4 show the correlation for l, nl and pwl before and after root death by experiment. A positive correlation was found; fastgrowing roots were less inhibited after root death than slow-growing roots. This explained about 50% of the variation. It seemed that the results from the same seasons were grouped together.

Linear models of the following type were tested:

Table 2. Increase per day of different root characters and a verage portion without laterals before and after root death. Mean of all experiments.

Character	Before root death	After root death
l (cm/day)	2.2	0.8
nl (nr/day)	7.2	3.0
pwl (cm)	4.5	3.2

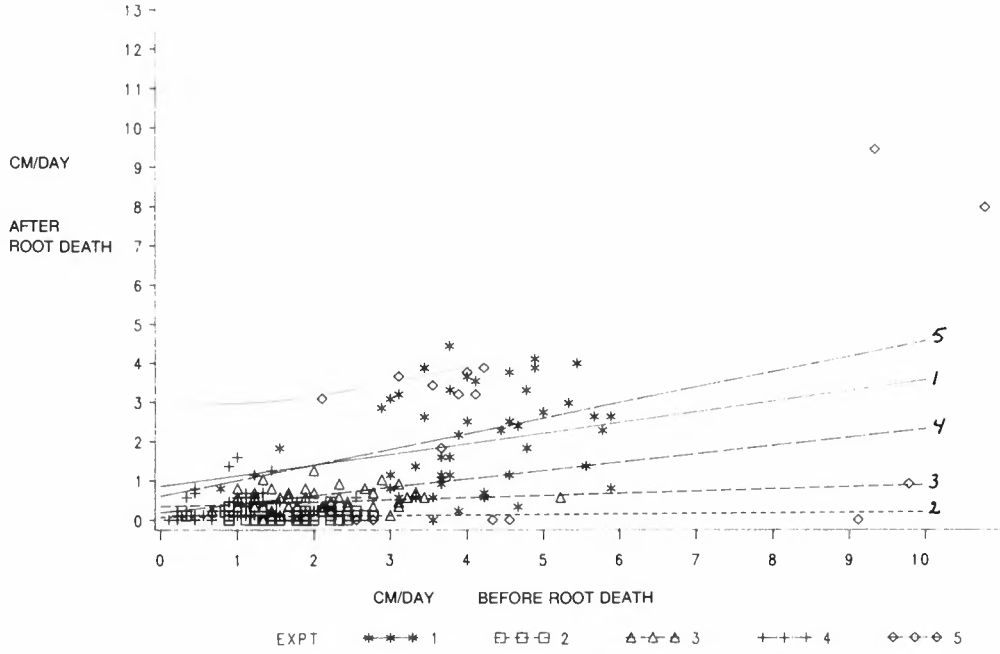


Fig. 2. Linear correlation between growth in cm per day of cucumber roots before and after root death. Data plotted separately for the experiments

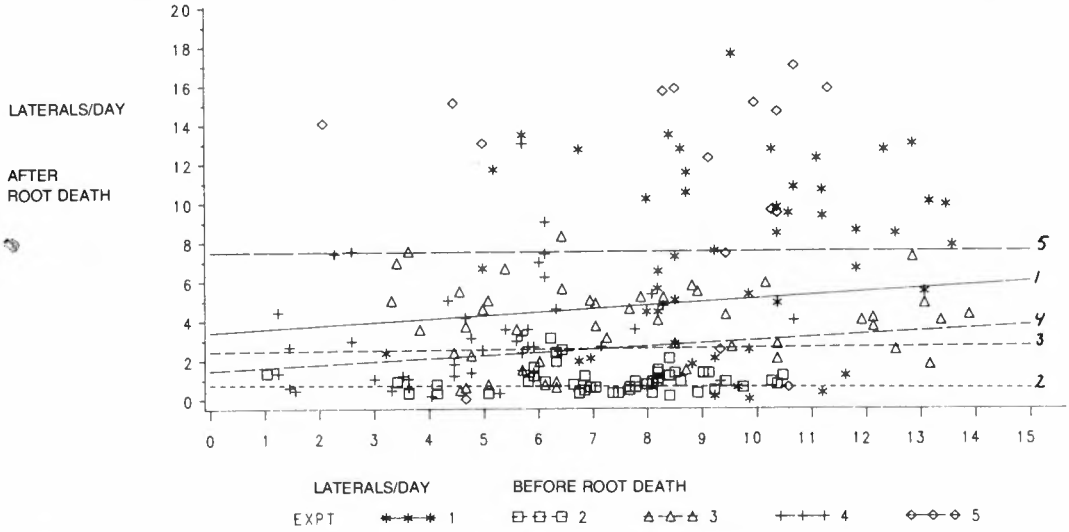


Fig. 3. Linear correlation between growth of cucumber roots in number of laterals per day before and after root death. Data plotted separately for the experiments

Character after root death = character before root death + season

This increased the explanation of variation considerably and gave very significant results for all characters. In particular the results from the autumn were significantly different from the other seasons.

When all the data were analysed together significant differences between

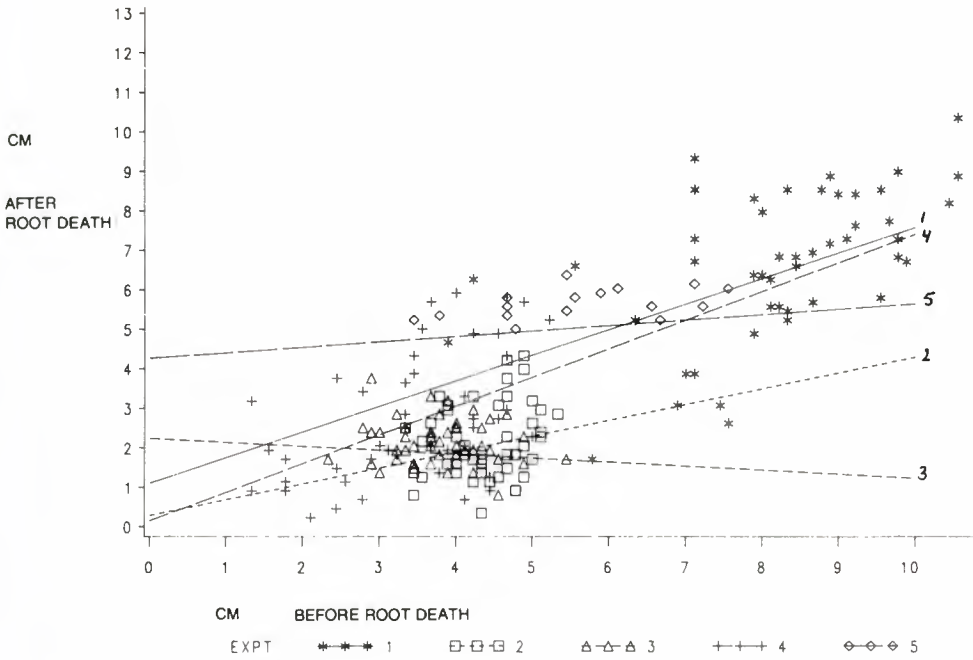


Fig. 4. Linear correlation between average portion of the cucumber root without laterals in cm before and after root death. Data plotted separately for the experiments

treatments were found which coincided with differences between experiments. Within experiments differences between treatments were not significant.

This showed that even though not all the roots were simultaneously affected to the same degree, the growth of all roots was reduced. Root growth might stop completely for some days and then resume. In the period with little root growth laterals might be developed to within 1 mm of the root apex. Sometimes the root tip would die and one of these laterals would take over as the leader root.

Root anatomy

The root measurements showed that the parts of the roots examined were not more than a few days old. Root preparations were made from a plant when it was 28-63 days old.

Fig. 5 shows a typical root in region 3. In some cases the metaxylem was fully developed with the characteristic central trachea. The cortex would consist of about seven layers of cells outside the endodermis. The epidermis might still be

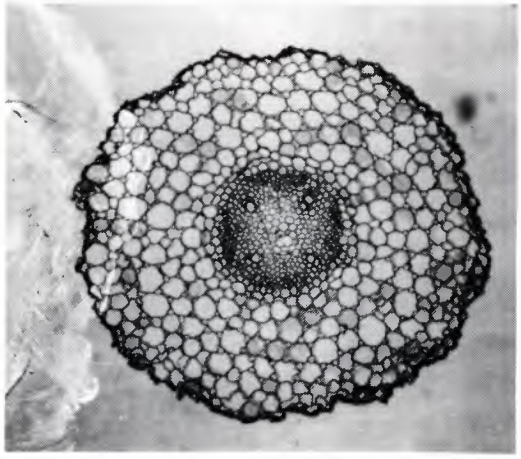


Fig. 5. Part 3 of a cucumber root on day 63. Metaxylem is under development, the cortex consists of 7 layers of cells outside the endodermis

intact. Very few root hairs were formed in NFT.

The development of lateral roots in region 2 could readily be observed under the microscope. Sometimes it would seem that part of the cortex was being dissolved (Fig. 6), but some days later this could not be observed in another root.

On day 63 root death was visible in part of the root (Fig. 1B). In regions 1, 2 and 3 no differences from earlier observations were observed. In region 4, just below the root death zone, the cortex seemed to have been destroyed from the outside (Fig. 7). Within the root death zone the cortex was almost completely dissolved (Fig. 8), but in an older region of the root at least some layers of cortex remained (Fig. 9). In these roots no secondary root growth was observed. The part of the root which was affected with root death was not more than 10 days old, being about 20 cm from the tip. The destruction of the cortex reduced the root surface area, which in turn reduced water and nutrient uptake. The reduction could be to 30-40% of the original root surface (Table 3).

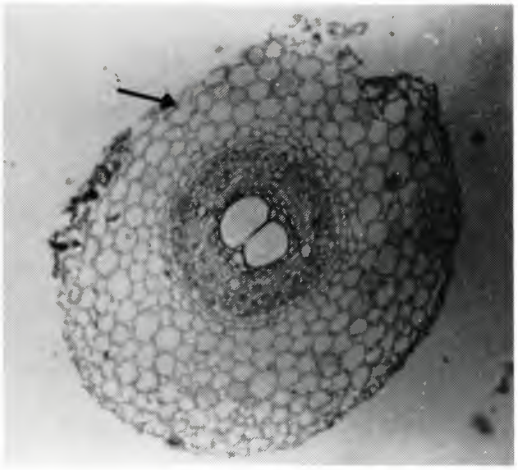


Fig. 7. Radial section of a cucumber root from the zone just distal to the root death zone on day 63. The outer layers of the cortex are being disrupted (arrow)

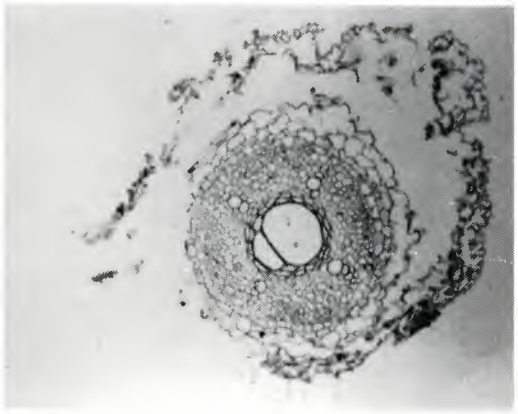


Fig. 8. Radial section of a cucumber root in the root death zone on day 63. The cortex is almost completely dissolved; the endodermis cells have collapsed

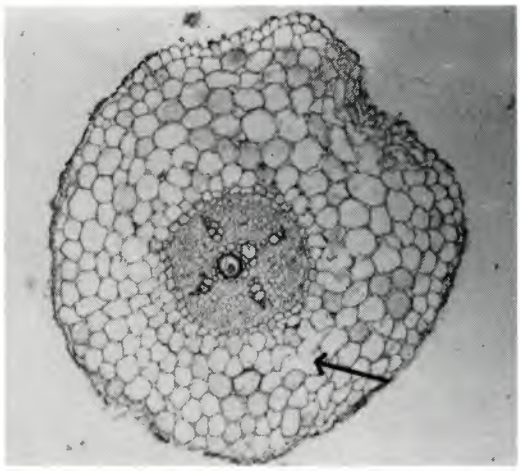


Fig. 6. Part 2 of a cucumber root on day 41. Some cells in the cortex seem to be dissolved (arrow).

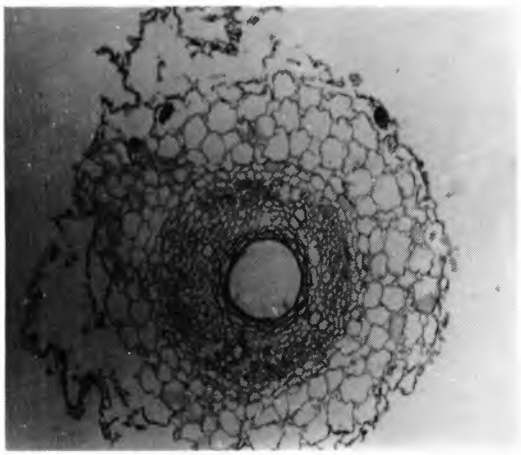


Fig. 9. Radial section of a cucumber root in the zone just proximal to the root death zone on day 63. Some layers of cortex still remain

DISCUSSION

The statistical analysis showed that there was a general reduction in root growth rate when some of the roots developed root death. Part of the variation could be explained by the growth rate before root death, which proved that a healthy and vigorous root system is preferable. Reduced growth rate and loss of material have been observed by many workers as explaining the decrease in root mass (De Stigter,1969b; Van der Post, 1968; Hall, 1977). Wilting and Fe-deficiency, which are sometimes observed in connection with root death, can be caused by the reduction in root surface area.

The roots were still in the primary stage of development when root death occurred, and they were also very young. Presumably destruction of the cortex was very rapid. The root measurements showed why renewing the root mass by root pruning had no effect on root death (Van der Vlugt, 1986).

The general reduction in root growth rate might be explained by root exudates poisoning the roots. Although not all the roots will be destroyed immediately, all will suffer from a high concentration of exudates. Exudates do not seem to be the only cause of root death, since hormone treatment with full fruit load did not prevent root death. (Exps. 3 and 4). On the other hand, reduction in fruit load did not prevent root death either (Exp. 5, Van der Vlugt 1986).

Table 3. The circumference of the stele of cucumber roots as percentage of the circumference of the whole root in different experiments.

Experiment	Stele % of root	Remarks
1	34.4	Regions 1 and 2 had a larger percentage in these roots than region 3.
2	24.5	Region 1 had a larger percentage than the other regions.
4	40.8	The cortex was disintegrated from 3 days after IBA application. About 4 layers of cells remained.
5	37.5	The oldest region, about 30 cm from the tip, had secondary root growth, the stele increased in size relative to the whole root. The younger regions had an average 31.8% stele.

ACKNOWLEDGEMENTS

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V. THE EFFECT OF PLANT GROWTH SUBSTANCES ON ROOT DEATH

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It has been suggested that root exudates have an influence on physiological root death. In a closed system like NFT the accumulation of exudates to toxic amounts may occur. Plant growth substances have been found among root exudates and some of these were investigated in the present experiments because of their considerable influence even when occurring in small amounts.
The effects of concentration, duration and time of application on root death in the cucumber were investigated for IBA, TIBA and silver ions. Auxins increased root death, irrespective of concentration, but time of application had a certain effect. TIBA did not counteract the effect of auxins.
After auxin treatment the roots became coiled as if after ethylene treatment. Auxin effects are often mediated through ethylene. Silver ions, inhibiting ethylene action, did not prevent root death. It is concluded that plant growth substances may play a part in root death.

Keywords: Cucumber, *Cucumis sativus*, root death, auxins, IBA, TIBA, $AgNO_3$

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Indications of root exudate involvement in physiological root death have been found (Van der Vlugt, in prep.). Exudates may accumulate in the nutrient solution because NFT is a closed system. These exudates could become poisonous to the roots above a certain concentration.

Vancura & Hanzlikova (1972) studied the exudates from cucumber seeds and seedlings, and found that they could be peptides, amino acids, organic acids or carbohydrates. They also observed that the composition of the exudate was different for seed and seedlings.

The timing of root death could be explained either by the total amount of exudates or by the occurrence of special substances in the exudate at a certain time and in a certain concentration. In the tomato it has been seen that also plant hormones occur in the root exudate (Cooper, 1979). A small change in the level of plant growth substances could have a considerable effect like root death.

In this study the effects of auxins and other substances involved in auxin transport and action on root death were investigated.

Exogenous auxins have been found to inhibit root growth, except at very low concentrations (Nissen, 1985), and to stimulate lateral root initiation. The pH ($\pm$ 6.0) of the nutrient solution renders the auxins metabolically active (Rao et al., 1976). Nissen (1985) states that the effect on root elongation may be caused by auxin-stimulated ethylene production. Endogenous auxins may have the

Table 1. Important cultural data from the experiments.

Experimental period	Growing medium	Treatment	Plant growth data
1982			
22 Feb.-7 April (1)	Containers	IBA 0, 1, 2, 5, 10 ppm added day 11	flowering day 11 first harvest day 30
13 April-20 May (2)	Containers	IBA 1 ppm added day 20, 27 or 35	flowering day 17 first harvest day 35
21 June-11 Aug. (3)	Containers	IBA 1 ppm added day 18 or 25 change/no change of solution after 1 week	flowering day 9 first harvest day 21
1983			
17 Jan.-11 March (4)	Containers	IBA 0, 1, 10, 20, 40 ppm added day 28	flowering day 16 first harvest day 35
16 May-28 June (5)	Containers	IBA 0, 10, 20, 40 ppm 1 week added day 28 'Farbio', 'Corona'	flowering day 17 first harvest day 32
1984			
2 July-10 Aug. (6)	Containers	TIBA 0.5 or 1% applied day 21 or 28	flowering day 14 first harvest day 23
13 Aug.-25 Sept. (7)	Containers	TIBA 1 or 2% applied day 16 or 23	flowering day 14 first harvest day 24
24 April-18 June (8)	Gullies	$AgNO_3$ 1 or 10 ppm for 24 hours added day 27 or 34	flowering day 15 first harvest day 29
2 May-25 June (9)	Containers	$AgNO_3$ 0.1 or 1 ppm on day 16 or 22 for 6 or 24 hours	flowering day 14 first harvest day 26
13 Aug.-10 Oct. (10)	Gullies	$AgNO_3$ 1 ppm added every $_4$th day from day 16	flowering day 13 first harvest day 22

same effect. Auxins from the shoot are transported to the roots. Batra et al. (1975) found that in the roots most of the auxin was found in the stele. When transport was inhibited, they observed that auxins were taken up by the cells and growth was inhibited. Both ethylene and TIBA affected auxin transport in

roots. TIBA inhibits auxin efflux from the cell (Depta et al., 1983). Lira & Freytag (1971) observed that TIBA and ethylene counteracted each other.

MATERIALS AND METHODS

The glasshouse cucumber cv. Farbio was used in all the experiments. In one experiment also cv. Corona was used (Table 1). Seeds were sown in rockwool blocks (7.5 x 7.5 x 7.5 cm) and propagated for 3 weeks, after which the plants were placed individually in 10 l containers. The containers were completely filled with nutrient solution (EC 2.0 - 2.5, pH 5.5 - 6.5). Air was bubbled through the solution continuously. The containers were refilled with nutrient solution every day. EC was measured every day, pH every other work-day. Plants were trained according to the umbrella system. In the period from October 1 to April 1 additional light was given for 18 hours a day. In some experiments 10

Table 2. Effect of different concentrations of IBA in the nutrient solution on root death symptoms in days after planting of the cucumber.
A. The effect of IBA connections of 0-10 ppm. Experiment 1, carried out with 8 containers per treatment, February-April 1982.

IBA concentration (ppm)	Root death (R)	Formation of new roots (N)	Recovery period N-R (days)
0	26.2	35.9	9.7
1	21.9	35.4	13.5
2	22.0	33.4	11.4
5	21.9	33.1	11.2
10	21.0	31.6	10.6

B. The effect of IBA concentrations of 0-40 ppm. Experiment 4 carried out with 8 containers per treatment, January-March 1983.

IBA concentration (ppm)	Browning	Root death R	Formation of new roots N	Recovery period N-R (days)
0	30.0	34.0	39.9	5.9
1	31.8	35.6	38.4	2.8
10	30.0	33.2	37.6	4.4
20	30.0	33.9	37.8	3.9
40	31.0	34.4	38.1	3.7

C. The effect of IBA concentrations of 0-40 ppm. Experiment 5 carried out with 10 containers per treatment, May-June 1983. Average for both cultivars.

IBA concentration (ppm)	Root Browning	Formation of death R	Recovery period N-R new roots N	(days)
0	30.4	33.4	35.6	2.2
10	30.9	34.2	37.1	2.9
20	30.9	34.7	39.9	5.2
40	30.6	35.0	41.5	6.5

plants were grown in a 4.5 m long gully, supplied by a 50 l catchment tank.

The potassium salt of indolebutyric acid (IBA) was dissolved in water, if necessary with a few drops of KOH, in a concentrated solution. Aliquots were syringed into the nutrient solution to the desired concentration. The same procedure was used with silver nitrate. 2,3,5-triiodobenzoic acid (TIBA) was mixed with lanolin in the desired proportion (w/w) and smeared around the hypocotyl, 20 mg of the mixture was used per plant. Data for the experiments are given in Table 1.

RESULTS

Three days after the addition of IBA lateral root development was observed. The effects of different auxin concentrations are shown in Table 2. Auxin treatment prolonged the recovery period (Table 2A, C). Root death often occurred before harvesting of the first fruit (Table 2B). Since there seemed to be no real differences between concentrations and no significant differences between cultivars, the results were pooled (Table 2C). With the highest concentrations (20 and 40 ppm) side-effects were noted: the lower part of the stem, especially the hypocotyl, burst. The lateral roots initiated by the auxin treatment very soon developed laterals themselves so that 2 or 3 roots seemed to come out of the root at the same place.

In the auxin treatments the root tips often became coiled. This was sometimes observed in untreated plants also (Fig. 1).

Since the timing of auxin addition seemed to be important, this was studied in some other experiments (Table 3). It seemed that root death was more severe with the later addition of auxins. However, the treatment was given so near the time of natural root death that no significant differences were observed.
If endogenous auxins are involved in root death in the same way as exogenous auxins, the stress might be relieved with TIBA treatment. Different concentrations of TIBA were tried and applied at different times (Table 4), but a negative correlation was found between concentration and root death symptoms. However, a positive correlation was found for the time of application. Root death always occurred, despite TIBA treatment.

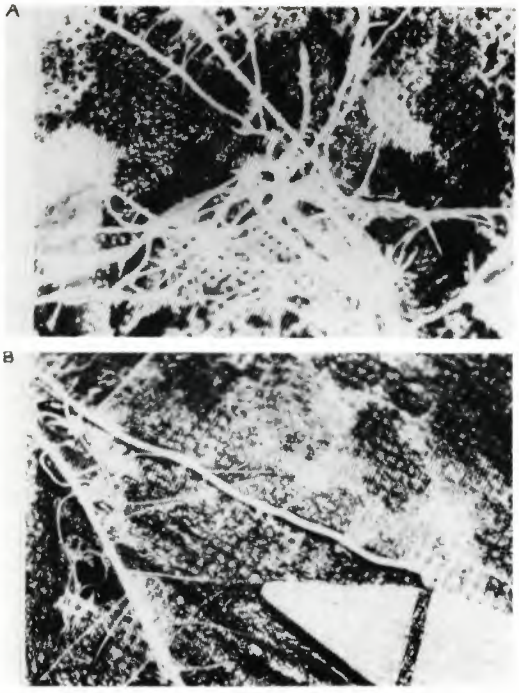

Figure 1 A. Part of a cucumber root mat with swollen and curved root tips, occurring naturally. B. Coiled root tip of cucumber, occurring naturally in NFT.

Ethylene could also influence auxin transport. Silver ions inhibit ethylene action. The results of the treatment with silver ions are given in Table 5. The $AgNO_3$ concentration of 10 ppm in the first experiment was too high and the plants wilted. No significant differences between concentrations or durations of treatment were found in the other experiments. The timing of the addition

Table 3. Effect of 1 ppm IBA added to the nutrient solution at different times on root death of the cucumber. Root death symptoms in days after planting.

A. Average of 10 containers per treatment. Experiment 2, carried out in April-May 1982.

Day of IBA addition	Browning	Root death R	Formation of new roots N	Recovery period N-R (days)
not added	28.3	29.8	42.7	12.9
20	28.6	32.0	40.1	8.1
27	29.4	33.0	40.0	7.0
35	29.6	33.0	41.8	8.8

B. IBA was added on day 18 or day 25 and the nutrient solution changed (c) or not changed (nc) after one week. Average of 10 containers per treatment. Experiment 3, carried out in June-August 1982.

IBA addition/ change of solution	Browning	Root death R	Formation of new roots N	Recovery period N-R (days)
not added c	24.6	29.0	32.6	3.6
18 nc	23.8	28.8	30.1	1.3
18 c	23.4	27.2	31.5	4.3
25 nc	24.4	27.9	32.0	4.1
25 c	25.8	29.5	34.4	4.9

seemed to have had some effect, especially when treatments were considered in groups which could have been influenced by the silver ions relative to root death of the control. In the earliest addition silver ions did not aggravate root death.

Table 4. Root death symptoms in days after planting, for cucumber plants treated with different 2,3,5-triiodobenzoic acid (TIBA) concentrations at different times. Experiments 6-7 carried out July-September 1984.

Experiment/ Day of application	Concentration % TIBA	Browning	Root death R	Formation of new roots N	Recovery period N-R (days)
6/	0 (control)	28.4	34.8	37.4	2.6
7/		19.2	26.4	29.8	3.4
7/16	1.0	17.6	25.4	31.0	5.6
	2.0	16.5	27.2	30.4	3.2
6/21	0.5	28.2	37.2	38.9	1.7
	1.0	27.2	34.4	39.2	4.8
7/23	1.0	20.6	27.1	31.6	4.5
	2.0	18.5	28.1	31.8	3.7
6/28	0.5	26.1	35.1	38.5	3.4
	1.0	29.0	33.2	37.1	3.9

Table 5. Root death symptoms in days after planting. Plants were treated with 1 ppm $AgNO_3$ in the nutrient solution at different times. The treatment 16/30 got 2 $AgNO_3$ applications on day 16 and 30. Data from all experiments, 8-10, control from exp. 10.

	Day of $AgNO_3$ addition to the nutrient solution										
	16	16/30	20	22	24	27	28	32	34	36	Control
Browning	17 19	17	17	23	22	23	28	20	26.5	24	20
Root death R	27 41	27	27	38.7	29	32.5	27	24	34	27	27
Formation of new roots N	31 42	31	34	39	38	42.5	34	29	35	34	31
Recovery period N-R (days)	4 1	4	7	0.3	9	10	7	5	1	7	4

DISCUSSION

Coiled roots were observed both after auxin treatment and in connection with naturally-occurring root death in NFT. Woods et al. (1984) observed coiling of tomato roots after ethylene treatment. Geneve & Heuser (1983) studied the interaction between auxin (IAA and IBA) and ethephon, and found that the roots were coiled because ethylene removed polarity.

In connection with root death it has been observed that growth decreases, and also the number of laterals (Van der Vlugt, in prep.). Ethylene, too, inhibits growth and decreases the number of laterals (Jackson, 1983).

The similarity between root death and ethylene effects seemed obvious, but finding out how the required level of ethylene is accumulated, is going to be more difficult.

The roots might become more sensitive to growth substances in the nutrient solution after flowering. Frimanslund (unpublished, 1980) grew cucumber seedlings in nutrient solution from plants with dead roots. The seedlings remained healthy. Daughtrey & Schippers (1980) obtained varying results by using old nutrient solution for tomatoes. A certain predisposition seemed to be necessary.

The trigger might be auxin-induced ethylene production or ethylene accumulation in the nutrient solution. Increased transport of auxins would have to occur just prior to root death. The solubility of ethylene is quite high, so large concentrations may occur in the nutrient solution (Jackson, 1980).

Ethylene is also involved in the formation of aerenchyma. Aerenchyma formation has been observed even in well-aerated hydroponics (Drew et al., 1981). The effect of aerenchyma formation is twofold. Large air spaces in the roots make an adequate 0_2-supply to the roots possible and the demand for oxygen is reduced because there are fewer cells.

In root death, also the number of cells is reduced, although it seems that destruction of the root bark starts from the outside (Van der Vlugt, in prep.). Possibly not the lack of oxygen but the lack of substrate for respiration leads to root bark destruction by ethylene in the case of root death.

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GENETIC ASPECTS OF FERTILITY OF BULLS

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NR-rates (non-return-rates) of 1158 A.I. bulls of the Norwegian Red breed used for test inseminations over a period of ten years, and the progeny test results of these same bulls for female fertility, growth rate and milk production were collected. The heritability of NR-rate was estimated using two statistical models, and the estimates obtained were 0.71 and 0.79, respectively. Heritability of the outcome of a single insemination was estimated at 0.0026 and 0.0029, while the estimates of repeatability were 0.0029 for both models. A highly significant correlation ($r = 0.13$) was found between the NR-rate for bulls and the NR-rate of their daughters, suggesting a positive genetic correlation of about 0.2 between male and female fertility. NR-rate of bulls was not significantly correlated with the carcass weight of their sons ($r = -0.03$) or with the milk yield of their daughters ($r = -0.02$). The small variation in NR-rate among bulls indicates that the response to selection would be slow in spite of the high heritability.

Key words: bull, cattle, fertility, heritability, NR-rate, repeatability.

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In almost all kinds of livestock, productivity is closely associated with reproduction. Fertility is therefore a trait of great economic importance. Moreover, the reproductive rate sets the upper limit for selection intensity and thus influences the potential for genetic improvement.

In cattle and other uniparous species, fertility is mainly a question of conception rate. Efforts to improve fertility by selection have been directed mainly towards the female side, as this is where the trait is manifested. It is, however, clear that the conception rate and associated traits are determined by the male and the female jointly.

Fertility of bulls used for A.I. is usually measured in the NR-rate (non-return-rate) of cows which have been inseminated with semen from the bull in question. The NR-rate is the proportion of these cows not inseminated a second time within a certain interval from first insemination. This proportion is slightly higher than the calving rate, but might be lower than the actual conception rate because some cows with early abortions might be inseminated again within the interval considered. The precision of NR-rate as an expression of the fertility of the bull depends on the number of cows inseminated.

Genetic aspects of bull fertility include its heritability and its genetic association with other traits. Previous studies in this field have led to ambiguous and partly conflicting results (see review by Syrstad 1981). The present article deals with heritability of annual NR-rates, heritability and repeatability of the outcome of single inseminations, and the as-

sociation of the bull's NR-rate with the fertility, growth rate and milk production of its progenies.

MATERIAL AND METHODS

The data used for this study were NR-rates of 1158 bulls entering A.I. from 1975 to 1984, and progeny tests of the same bulls for female fertility, growth rate and milk production. All the bulls belonged to the Norwegian Red breed (NRF). Before entering A.I. the bulls were tested for mating ability and semen production (volume and quality), and about 15% were culled at this stage (Graffer et al. 1988). Througout the period of semen collection, ejaculates of poor quality were discarded. The dilution rate was set according to the concentration of the ejaculate, the target being 15 million live sperms per dose after freezing and thawing.

The NR-rate of a bull was based on about 1400 test inseminations of a random sample of females with the purpose of producing a sufficient number of progenies for progeny testing. For bulls whose semen was released for use after the progeny test a separate NR-rate was computed for these inseminations.

The progeny test for female fertility was the NR-rate of 200 to 300 daughters (heifers), expressed as the estimated breeding value of their sire for this trait.

Growth rate of progenies was recorded in a field test in which information on age at slaughter and carcass weight of male progenies reared for beef production was collected. The carcass weight was adjusted to an age of 17 months (16 months for bulls entering A.I. in 1975). The number of progenies per sire ranged from ten upwards.

The progeny test for milk production included first lactation records of about 200 daughters. Relative breeding values were estimated by BLUP procedure.

The 1158 bulls were sired by 102 different sires. Most sires were represented in one or two years only. For the estimation of genetic variance and heritability two different statistical models were used:

1) Cross-classified model: Years and sires were cross-classified and the total variation split into fractions according to years, sires and residual.

2) Nested model: sires were nested within years, and the fractions of variation were for years, sires within years, and residual. With this model some of the sires will be repeated in consecutive years, so this might influence the sampling errors of the estimates, but the estimates themselves should be unbiased.

The additive genetic variance was estimated as four times the component of variance among sires. For estimation of the variation among bulls, the residual mean square was corrected for the variance due to the sampling error and then added to the sire component. The correction was calculated as $\bar{p}(1-\bar{p})/n$ where $\bar{p}$ is the overall NR-rate in the data set and n is the average number of inseminations per bull. The overall variance of the outcome of a single insemination is $\bar{p}(1-\bar{p})$.

Associations between NR-rate of a bull and his progeny tests for female fertility, growth rate and milk production were calculated on a "within year" basis.

RESULTS

Average NR-rate for test inseminations was 67.4%, with a standard deviation (within years) of 2.8%.

An extract of the results obtained from the two analyses of variance is presented in Table 1. A highly significant variation among years and among sires was found in both analyses ($P<0.01$). The additive genetic standard deviation was estimated at 2.38 percentage units using method 1 and 2.51 units using met-

Table 1. Analysis of variance of NR-rate of bulls

Model	Mean squares			k[1)]	σ_s^2	Herita-bility
	Year	Sire	Residual			
1 (cross-classified)	407.21	20.63	6.59	9.87	1.42	0.71
2 (nested)	187.97	15.07	6.45	5.45	1.58	0.79

[1)] k = effective number of bulls per sire (model 1) or per sire per year (model 2)
σ_s^2 = variance among sires

hod 2, and the two heritability estimates were 0.71 and 0.79, respectively. The sampling standard error of the estimates is approximately 0.13.

Estimates of heritability and repeatability of the outcome of a single insemination are given in Table 2. Heritability was estimated as the ratio between the additive genetic variance (obtained from Table 1) and the overall variance, while repeatability was the ratio between the component of variance among bulls and the overall variance. All estimates were very low (<0.003), and estimates of repeatability only slightly greater than those of heritability. Approximate standard errors were: heritability, 0.0005; repeatability, 0.00003.

For 220 bulls whose semen was also used after progeny testing, the coefficient of correlation between the two NR-rates was estimated at 0.38 ($P<0.001$). Bulls used in two consecutive years after progeny testing (≥1000 inseminations in each of the years) had a coefficient of correlation of 0.75 between the NR-rates of the two years (115 bulls).

Progeny test results for female fertility, growth rate and milk production were available for 1026 of the bulls. The coefficients of correlation between NR-rate of the bull and each of these traits are reported in Table 3.

The only significant correlation was that between the bull's own NR-rate and

Table 2. Heritability and repeatability of the outcome of a single insemination, considered as a trait of the bull

Model	Additive genetic variance	Variance among bulls	Herita-bility	Repeata-bility
1 (cross-classified)	5.69	6.44	0.0026	0.0029
2 (nested)	6.33	6.46	0.0029	0.0029

Table 3. Coefficients of correlation between NR-rate of bulls and their progeny tests for fertility, growth rate and milk production

Bulls' NR-rate and:	Coeff. of correlation	
NR-rate of daughters	0.13	*** ($P<0.001$)
Carcass weight of sons	-0.03	non.sign.
Milk production of daughters	-0.02	non-sign.

the NR-rate of his daughters. Even this correlation was low.

After correction for the accuracy of the progeny test and the heritability of the bulls' NR-rate this suggests a genetic correlation of around +0.2 between male and female fertility.

DISCUSSION

Previous estimates of the heritability of NR-rate of A.I. bulls (reviewed by Syrstad 1981) range from 0.10 to 0.59. Most of these estimates were based on limited sets of data, and sampling errors might account for a major part of the variation. Furthermore, the number of inseminations on which the NR-rate was based varied considerably. The high heritability estimates obtained in the present study are partly a concequence of the large number of inseminations per bull.

The most comprehensive study of the genetic variation of NR-rate as a trait in the bull is probably the one by Murray et al. (1977), whose data comprised records on more than six million inseminations. The heritability of a single insemination was estimated at 0.007. A similar estimate (0.006 ± 0.008) was reported by Stocker (1976). More recently, Karb (1986) reported estimates of 0.003 to 0.004 in three different breeds of cattle. This is only slightly higher than the one obtained in the present study.

How can it be that the heritability of the outcome of a single insemination is so low and yet the heritability of the NR-rate based on many inseminations is very high? This occurs because the repeatability (for a single insemination) is only slightly greater than the heritability. Estimates of repeatability and heritability were fairly similar (0.009 and 0.007, respectively) also in the study by Murray et al. (1977). With these estimates the heritability of NR-rates based on 1000 inseminations would be about 0.7, or similar to the present estimates. Hansen (1979) estimated the repeatability of NR for a single insemination at 0.011.

The phenotypic variation of NR-rate among bulls is closely related to the repeatability for a single insemination. A repeatability of 0.01 corresponds to a phenotypic standard deviation of about five percentage units if the number of inseminations per bull is reasonably high (say from 500 upwards). The low standard deviation of NR-rate in the present data (2.8%) shows immediately that the repeatability is also very low. The slight variation might be partly due to the culling of bulls with poor semen quality, and to the practice of standardizing the number of live sperms per dose.

The relatively low correlation between the NR-rate of a bull in test inseminations and the NR-rate of the same bull in subsequent inseminations (semen stored for four to five years) indicates that the two NR-rates to some extent reflect different properties of the semen.

The association between the NR-rate of bulls and the fertility of their daughters was studied by Maijala (1960), Gaillard (1974) and Hansen (1979). None of the estimates reported by these authors was significantly different from zero, but most of them (four out of five) were favourable. Skrettingland (1979) estimated the correlation between the NR-rate of A.I. bulls and the NR-rate of their daughters at 0.21, which was highly significant. His data were similar to those used in the present study.

A previous study based on a small fraction of the present data (Syrstad 1983) showed a significant negative genetic correlation ($r = -0.18$) between NR-rate of bulls and the carcass weight of their sons. This was not confirmed in the larger data set and was probably incidental (Type I error). The negative genetic correlation between NR-rate of bulls and the milk production of their daughters ($r_g = -0.27$) reported by Mur-

ray et al. (1977) was not confirmed either.

Because of the small phenotypic (and genetic) variation in NR-rate of bulls the response to selection for this trait would be slow in spite of the high heritability. Moreover, the degree of selection which could be applied to this trait would necessarily be small. However, the great importance of the trait and the positive association with female fertility seem to justify the inclusion of NR-rate among the traits considered in the final selection of bulls for use after progeny-testing.

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RESTRICTED FEEDING OF BROWN EGG LAYERS IN THE REARING PERIOD

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An experiment on restricted feeding of brown egg layers in the rearing period is described. By restricting feeding, the age at point of laying is delayed by 2-4 days. Restriction comprising a saving of 1.0-1.7 kg of rearing feed does not affect egg production or feed utilization in the laying period.

Key words: Brown egg pullets, brown egg layers, restricted feeding, rearing feeding.

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Brown egg layers were previously too heavy and consumed too much feed for competing with white egg layers in terms of economical egg production. Reduced body weight has therefore been a main target of modern breeding programmes for brown egg strains, and the resulting reduction in body size between the two types of layers has been significant.

However, high feed consumption is still a disadvantage of brown egg layers. Restricted or controlled feeding during the rearing period has therefore been recommended, and to investigate the effects on body weight, feed utilization and egg production, an experiment was carried out at the Department of Animal Science in the period 1985-86.

MATERIALS AND METHODS

The chicks used in the experiment originated from Hisex brown imported to Norway in 1981 and incorporated in the national breeding programme for brown egg layers. The experimental chicks were called Søve 43. From day-old to 4 weeks of age all chicks were fed ad libitum on a commercial chick starter with 11.7 MJ (2800 kcal) ME per kg and 20% crude protein. From 4 to 20 weeks, 576 chicks were divided across six feeding regimes as follows:

1. Commercial grower diet (CG), crumbles ad lib.
2. Low energy diet (LE) pelleted ad lib.
3. CG moderate restriction (MR) to 20 weeks
4. " " " " to 26 weeks
5. " strong " (SR) to 20 weeks
6. " " " " to 26 weeks

The commercial diet (Hønefôr 1) contained 11.3 MJ (2700 kcal) ME per kg and 15.4% crude protein. The LE diet, containing barley (30%), oats (45%), and wheat brand (15.5%) and pelleted on a laboratory press, was calculated to contain 10.4 MJ (2480 kcal) ME and 14.2% crude protein.

For the chicks on moderate restriction daily feed rations were changed once a week, so that a norm for weight gain given by Eurobreed for Hisex brown could be followed. In the treatments on strong restriction the target weight of

the chicks was considered to be around 10% lower than that of the chicks on moderate restriction.

The chicks were raised in cages with a floor area of 0.49 m^2. From 0 to 6 weeks there were 24 in each cage, but at 6 weeks the groups were divided into two, with 12 chicks in each cage. From 4 to 20 weeks the temperature varied between 20 and 22°C, and from the 1st to the 20th week they had a constant day length of 9 h. At 18 weeks of age the pullets were moved to individual laying cages and the number reduced from 96 per treatment at 6 weeks to 80 for the laying period. The reduction was carried out randomly so that selection according to body weight was avoided.

From 20 weeks all hens were fed a commercial laying mash with 11.1 MJ (2650 kcal) ME per kg and 15.8% crude protein. For treatments 4 and 6, restriction of the feed continued to 26 weeks of age. Day length was increased from 9 h to 11 h at 20 weeks, to 12 h at 21 weeks, and to 13 h at 22 weeks. From 22 weeks the day length was increased by ½ h per week until it reached 16 h at 28 weeks, at which time it was held constant.

During the laying period until the hens were 82 weeks, the number of eggs, egg weight and feed consumption were registered for every 4-week period. Shell quality (specific gravity) was registered for all eggs one day in each 4-week period.

RESULTS

The rearing period

The main results from the rearing period are set out in Table 1. Table 2 gives the figures for body weight and feed consumption from week to week.

Table 1 indicates that chicks on the LE-diet (treatment 2) consumed considerably more feed and gained more weight than chicks on the commercial diet (treatment 1). Also the intake of ME was higher in treatment 2 than in treatment 1. This fact might have been due to good pellet quality of the LE diet.

The chicks on quantitative restriction of feed had a growth rate as predicted. Compared with chicks in treatment 1 (on the same feed mixture), more than 1 kg feed was saved per pullet with the moderate restriction (treatments 3 + 4) and 1.7 kg feed with the strong restriction. From an individual weighing of the pullets at 18 weeks of age, it could be seen that the variation in body weight between individuals was greater for the treatment with the strong feed restriction (Table 1). A high mortality during the rearing period was caused mainly by cannibalism in a period after partition of

Table 1. The performance of chicks in the rearing period

	Treatment No.			
	1 CG diet ad lib.	2 LE diet ad lib.	3+4 CG MR	5+6 CG SK
No. of chicks 4 weeks	96	96	192	192
Mortality 1-18 weeks, %	7.3	8.3	4.2	10.5
Loss through cannibalism, %	5.2	3.1	0.2	2.5
Weight at 18 weeks x, g	1599b	1644a	1328c	1210d
» » 18 » s	112	122	125	142
Feed consumption per chick				
Starter 0-4 weeks, kg	0.69	0.70	0.68	0.68
Grower 4-18 weeks, kg	6.77	7.84	5.68	5.07
» 4-18 ME, MJ	76.5	81.5	64.2	57.3

Figures with dissimilar suffixed letters are significantly different ($P < 0.05$).

Table 2. Growth rate and feed consumption per day (FD) in the rearing period, g

Age weeks	Treatment No. 1 (CD) Ad lib. Weight	FD	2 (LE) Ad lib. Weight	FD	3+4 MR Weight	FD	5+6 SR Weight	FD
4	310	37	317	38	308	36	306	36
5	400	49	405	57	351	32*	342	30*
6	461	47	488	57	369	32	360	30
7	537	43	566	50	465	42	440	40
8	658	55	691	64	636	42	513	40
9	788	64	812	74	635	52	590	45
10	907	70	950	84	706	52	642	45
11	1015	78	1070	91	799	65	712	55
12	1123	81	1183	96	900	65	793	55
13	1213	81	1307	96	973	65	849	55
14	1307	81	1380	98	1034	65	910	55
15	1400	83	1470	97	1115	70	985	62
16	1475	80	1532	88	1192	70	1055	62
17	1539	79	1598	86	1274	75	1144	70
18	1599	77	1644	84	1328	85	1210	80

* Restriction started at 5 weeks.

the chick groups at 6 weeks of age. There was less cannibalism in the chicks given restricted feeding than in those given ad libitum feeding, however.

The laying period

Feed consumption

After the pullets were moved from the colony cages to individual cages at 18 weeks, feed restriction continued until 20 weeks for treatments 3 and 5 until 26 weeks for treatments 4 and 6. The daily rations of feed in grams for these periods were:

Age Weeks	Treatment 3	4	5	6
18-19	85	85	80	80
19-20	95	95	90	90
20-22	ad lib.	95	ad lib.	90
22-24	"	100	"	95
24-26	"	100	"	100

Total feed consumptions for the hens from 18 to 82 weeks are given in Table 3, which shows that at the end of the restricted feeding at 20 weeks (treatments 3 and 5) some compensation in feed intake occurred. Thus the hens in treatments 3 and 5 consumed about 0.5 kg

Table 3. Feed consumption per hen in the laying period, kg

Age Weeks	Treatment from the rearing period 1 (CG) ad lib.	2 (LE) ad lib.	3 MR20	4 MR26	5 SR20	6 SR26
18-26	5.2	5.3	5.8	5.3	5.8	5.1
26-34	6.5	6.6	6.5	6.6	6.6	6.5
34-58	19.8	20.0	19.4	19.4	19.7	19.2
58-82	19.6	19.8	19.3	19.4	19.5	19.0
18-82	51.1	51.7	51.0	50.7	51.6	49.8

more than those which had been fed ad libitum continuously. On ending the restriction at 26 weeks, however, very little compensation in feed intake followed.

After 34 weeks the hens which were on restricted feeding in the rearing period again had reduced feed intake. So for the laying period as a whole (18-82 weeks) there were small differences between treatments in feed consumption. However, the hens fed ad libitum on the low energy diet during rearing (treatment 2) continued with higher feed consumption in the laying period, too.

Egg production

The performances of the hens are shown in Table 4.

There were significant effects of feeding in the rearing period on age at maturity. Restricted feeding to 20 weeks delayed the age at start of laying by 2-3 days, and restricted feeding to 26 weeks to a further 1-2 days delay.

As a result of the delayed age at start of laying, there were also significant differences between the feeding regimes in number of eggs until 34 weeks. But for the whole laying period only hens in treatment 6 on the strong feed restriction to 26 weeks had reduced egg production and increased feed/egg ratio. The feeding in the rearing period had little influence on egg weight, but a small reduction occurred for the hens on restricted feeding to 26 weeks of age.

DISCUSSION

Restricted feeding in the rearing period is intended to save feed and to avoid fat and heavy pullets at the point of laying. A general result with restricted feeding is delayed age at the start of laying (Lee et al. 1971). Restricted feeding is not recommended for modern lightweight hybrids of White Leghorns. For the heavier meat type hens, however, strong restricted feeding in the rearing period is necessary for satisfactory egg production and feed utilization (Herstad 1980). For brown eggs layers which are somewhat heavier than white egg layers, controlled feeding for obtaining a target weight at point of laying may be favourable.

Diets low in energy content have been used to reduce the growth rate in chicks. That this is an uncertain method was clearly demonstrated in this experiment where the chicks on the diet with only 10.4 MJ ME per kg became heavier than the chicks on the diet with 11.3 MJ ME. Other factors associated with the

Table 4. Performance in the laying period, 18-82 weeks

	Treatment from the rearing period					
	1(CG) ad lib.	2(LF) ad lib.	3 MR20	4 MR26	5 MR20	6 MR26
No. of hens	80	80	80	80	80	80
No. of losses	2	2	2	2	1	3
Age at start of lay., d	158a	161b	161ab	162bc	163cd	165cd
No. of eggs at 34 w., g	72.5	69.6	70.8	69.7	67.5	64.8
Egg weight 22-34 w., g	57.3	58.1	57.0	56.3	57.8	56.7
No. of eggs at 82 w.	331a	329a	327a	328a	328a	314b
Egg weight 22-82 w., g	64.0ab	64.8a	63.8ab	63.5b	64.5ab	63.6a
Egg production, kg	21.2a	21.3a	20.9ab	20.8ab	21.2a	19.9b
Specific gravity of eggs	1.080b	1.081ab	1.082a	1.081	1.083a	1.081a
Feed/egg ratio	2.42a	2.43a	2.44a	2.44a	2.43a	2.50b
Body w. at 34 w., kg	2.09b	2.16a	2.04b	2.02bc	2.01a	1.96a
" " " 82 w., kg	2.22a	2.22a	2.20ab	2.16ab	2.13bc	2.08c

Figures with dissimilar suffixed letters are significantly different ($P < 0.05$).

feed such as physical structure and acceptability, might mean more for the energy intake than the energy consentration of the feed.

The growth curve for the chicks can be altered by restricted feeding as shown from this experiment in figure 1. The chicks on restricted feeding had a nearly linear growth curve from 6 to 20 weeks implying a stronger restriction until 16-18 weeks than later. Based on experiment results, Vells (1980) claimes that the greatest economical benefits arose from quantitative restriction of food intake which commenced at six weeks of age and markedly reduced growth rate in the middle of the rearing period and allowed compensatory growth to take place in later stages.

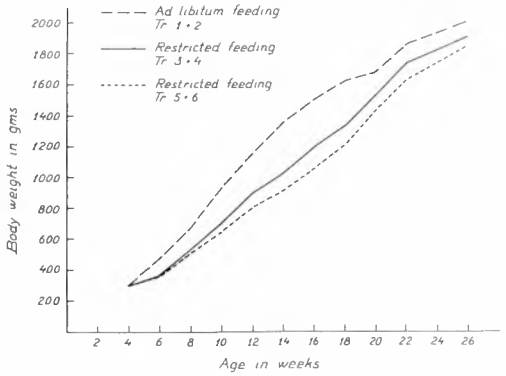

Figure 1. Growth curves of pullets during the rearing stage

Fig. 1 shows that the growth curve for the ad libitum fed chicks was decelerating from 14 to 20 weeks, followed by a new increase in weight. This breack in the growth curve may be a result of the move of the pullets to laying cages at 18 weeks but is more likely to reflect the development of ovary and oviduct at sexual maturity. The growth of the egg laying organs have been a direct continuation of the body weight for the restricted fed chicks.

A reduced performance during the laying period occorred for the hens in treatment 6 with the strong feed restriction lasting to 26 weeks of age. For the other treatment there were no significant difference in egg production and feed utilization in the laying period.

For eventually detecting any target weight of the pullets before point of laying, a regression analysis with individual body weight at 18 weeks as independent variable and egg mass per day as dependent variable was carried out. (Two hens with very low egg production were excluded from the analysis.) The equation found was:

$$ED = 34.6 + 8.1 \times LW \; (P < 0.01 \; R^2 = 0.05)$$

where ED = egg mass per day in grams and LW = body weight at 18 weeks in kg (Fig. 2).

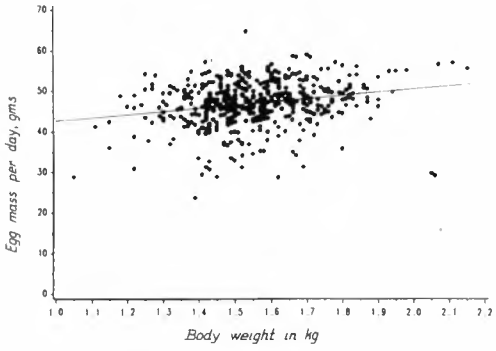

Figure 2. The relation between body weight at 18 weeks and succeeding eggproduction

The correlation between body weight and egg production was very small. However, the regression coefficient was positive and significantly different from zero. This means that with higher body weight at point of laying, a small increase in egg production might be expected. A similar analysis with the feed: egg ratio as dependent variable showed non-correlation with the body weight at 18 weeks.

From the data in this experiment it is difficult to define a target weight for pullets before sexual maturity. However, feed saving will mean an economic benefit from a moderately restricted feeding in the rearing period.

SUMMARY

An experiment was carried out to investigate the effects of restricted feeding of brown egg layers in the rearing period. A total of 576 chicks in colony cages were divided into six feeding regimes at 4 weeks. One treatment was feeding ad libitum on a diet with 11.3 MJ ME per kg. Another was feeding ad libitum on a low energy diet with 10.5 MJ ME per kg. Treatments 3 to 6 were feeding on two levels of restriction to 20 or 26 weeks.

The pullets were moved at 18 weeks to individual laying cages and all hens were fed a commercial laying mash in the laying period. Egg production and feed consumption were recorded until the hens were 82 weeks old. The data obtained in this experiment suggested that

- Feeding a low energy diet ad libitum does not reduce the live weight of the pullet compared with the weights of pullets on a higher energy diet.

- On the two levels of restricted feeding, reduced feed intake of about 1.0 and 1.7 kg are obtained to 20 weeks.

- When feed restriction terminates at 20 weeks, the hens increase their feed consumption, and in the period until 34 weeks about half of the saving of feed during restriction is caught up.

- After 34 weeks of age feed consumption by hens on restricted rearing feeding is again reduced compared with their ad libitum feed counterparts, and for the whole laying period differences in feed consumption are minimal.

-When restriction is prolonged to 26 weeks, no catching up in feed intake occurs when the hens obtain ad libitum feeding.

-By restricting feeding in the rearing period age at start of laying is delayed by 2-4 days.

-By moderate feed restriction in the rearing period egg production and feed utilization are equal with those of the hens on ad libitum feeding in the rearing period. Hens which have been fed on the strong restriction to 26 weeks show reduced performance in the laying period.

-There is a slight positive correlation between body weight at 18 weeks and succeeding egg production.

- The profit obtained by restricted feeding of brown egg layers seems to be limited to the saving of feed in the rearing period.

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UTILIZATION OF NH_3-TREATED STRAW BY SHEEP: EFFECTS OF SUPPLEMENTATION WITH DIFFERENT AMOUNTS OF GROUND BARLEY

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The inclusion of barley in a NH_3-treated straw diet depressed the rumen degradation and dry matter digestibility of the straw. The fibre digestibility of the diets was significantly reduced even with a 20% barley inclusion. The depression in fibre digestibility reduced the net energy value of the diets.

Key words: barley supplementation, fibre digestibility, energy utilization, NH_3-treated straw, rumen degradation, rumen pH, rumen VFA.

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Utilization of straw as an energy source is limited by its low feeding value and low voluntary intake by animals. However, the intake and digestibility of straw can be increased by various chemical pretreatments. Treatment with ammonia has been extensively studied and used during recent years (Sundstøl 1984), but the total effect when fed in mixed diets has not been fully investigated. In a previous paper (Bøe 1989a) it was reported that the degradation of NH_3-treated straw in the rumen of dairy cows was reduced to about the same level as that found for untreated straw when concentrates were added to a grass silage diet.

Similar results were obtained by Ternrud & Neergaard (1986) on cellulose digestibility when NaOH-treated straw was supplemented with barley.

The objective of this study was to examine the influence of barley on rumen degradation, digestibility and energy utilization of NH_3-treated straw fed to sheep.

MATERIALS AND METHODS

Animals, diets and experimental design
Four mature rams fitted with permanent rumen cannulas were used. The animals were those used in the previous experiment with diets of grass silage and barley (Bøe 1989b), and they were kept under the same conditions during this experiment. The animals were fed four different diets of NH_3-treated straw and ground barley near maintenance energy level. The proportions of NH_3-treated straw to barley were 100:0, 80:20, 60:40 and 40:60 on dry matter (DM) basis. The chemical composition of the dietary ingredients and composition of the diffe-

rent diets offered are given in Tables 1 and 2.

Table 1. Chemical composition of NH_3-treated straw and barley

	NH_3-straw	Barley
DM (g.kg^{-1})	843	886
Components of DM (kg):		
Gross energy (MJ)	18.0	18.3
Organic matter (g)	926	975
N total (g)	15	19
Ether extracts (g)	10	20
Crude fibre (g)	494	54
N-free extracts (g)	354	783
ADF (g)	523	49

Table 2. Daily intake of DM and crude fibre content of the diets offered

Diet straw: barley	DM intake g·k$^{-0.75}$)	Crude fibre g·kg DM^{-1}
100:0	38.3	494
80:20	34.9	406
60:40	31.0	317
40:60	25.4	230

The barley straw was treated with ammonia using the method of Sundstøl et al. (1978). The straw needed for the whole experimental period was mixed well, weighed and divided into dayly rations, then stored in plastic bags prior to use. A single batch of ground barley was used. To ensure that rumen degradable protein was not limiting, 10 g urea was added in the diets.

The animals were fed twice daily (at 0800 and 1500 hours) in two equal portions. Supplements of 10 g salt, 10 g mineral mixture and 5 g Vitol-granulat (A, D and E vitamins, Peter Møller A/S, Oslo) were given daily, and water was freely available.

Experimental design, calorimetric measurements, procedures for rumen degradation of feeds and rumen fluid samples and chemical and statistical analyses were as described in the previous experiment (Bøe 1989b).

Calorimetric calculations

Because of only one feeding level for the sheep in this study, fasting heat production (FHP) could not be calculated. The calculated FHP from the previous experiment (Bøe 1989b) was therefore used.

Efficiency of dietary metabolizable energy (ME), utilization for maintenance (k_m) and ME required for energy equilibrium were calculated after Blaxter & Wainman (1964).

Partial net energy of the diets for maintenance (NE_m) was determined after Vance et al. (1972).

RESULTS

Rumen fluid measurements

A depression in rumen pH was observed with increased proportion of barley in the diet (Figure 1). The minimum pH measured was above 6.3 for all diets ex-

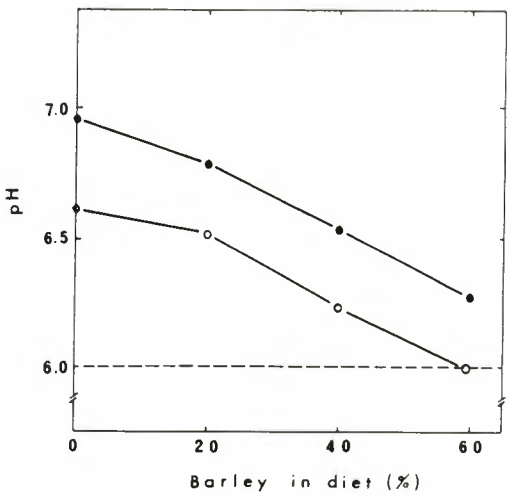

Figure 1. Average rumen pH (● ——— ●) and minimum pH values measured (o ——— o) as influenced by the level of barley

cept the one containing 60% barley, where pH was 5.8 four hours after feeding in two of the sheep. The other two sheep had pH values above 6.1.

The concentration of ammonia and VFA and the proportion of VFA in the rumen are presented i Table 3.

The average ammonia concentration was high for all diets, and varied between 16.8 and 22.5 mmol/l NH_3-N.

The total VFA-concentration increased with increasing amounts of barley consumed from 50.7 to 61.9 mmol/l for the all NH_3-straw diet and the diet including 60% barley respectively.

The molar proportion of acetic acid (C_2) was significantly depressed when 20% barley was included in the diet, but a further increase in barley supplementation had no effect on this proportion.

For propionic acid (C_3), the molar proportion decreased, whereas for butyric acid (C_4) it increased, with increasing amounts of barley in the diet.

Rumen degradation

There was little variation in the total potential degradable DM-fraction in the NH_3-treated straw when straw was supplemented with increasing amounts of barley (Table 4). Inclusion of 60% barley in the diet, however, depressed the potential degradation to a certain degree. The time needed to reach the potential degradability of the NH_3-straw increased with increasing inclusion of barley in the diet. Dry matter degradation of the NH_3-treated straw after 48 h incubation (DMD48) therefore decreased signifi-

Table 3. Average rumen fluid NH_3-N and VFA as influenced by the diet offered

Diet straw:barley	NH_3-N (mmol/l)	Total VFA (mmol/l)	Molar proportions C_2	C_3	C_4	IC_4	C_5	IC_5
100:0	17.5a	50.7a	72.0a	18.6a	6.4a	1.3	0.6a	1.2a
80:20	16.8a	57.8b	69.9b	17.8a	8.9bc	1.4	0.9b	1.3a
60:40	22.5b	59.1b	69.4b	17.1ab	9.5c	1.4	1.1c	1.6b
40:60	22.3b	61.9b	69.8b	15.6b	10.3d	1.3	1.3c	1.7b
SEM	4.3	8.8	1.6	1.3	0.8	0.3	0.2	0.4
Difference between diets	*	***	***	***	***	NS	***	***

a-c: means in each column with the same superscript are not significantly different ($p<0.05$).

Table 4. Rate and extent of disappearance of DM from NH3-treated straw incubated in the rumen

Diet straw:barley	potential degradation (a + b)	rate of degradability (c) [1]	RSD	DMD48
100:0	55.3	0.036	0.80	49.3a
80:20	57.7	0.032	1.15	47.3a
60:40	54.9	0.027	2.64	39.5b
40:60	49.1	0.017	1.25	37.4b
SEM				2.9
Difference between diets				***

a-b: means in column with the same superscript are not significantly different ($p<0.05$).

1) a, b and c are constants in the exponential equation $p = a + b\,(1\text{-}e^{-ct})$ where p is degradability at time t.
RSD = residual standard deviation.
DMD48 is dry matter degradability after 48 h incubation (p when t = 48).

cantly with increasing amounts of barley in the diet.

The loss of the ADF-fraction followed the same pattern as that of DM. Degradation of ADF after 48 h incubation is presented in Figure 2.

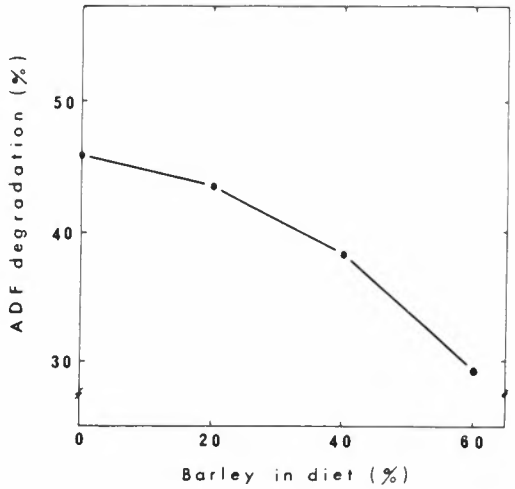

Figure 2. Degradation of acid detergent fibre (ADF) in NH_3-treated straw after 48 h incubation in nylon bags in the rumen, as influenced by he level of barley

Digestibilities

Apparent digestibilities of dry matter (DMD), organic matter (OMD), crude fibre (CFD) and acid detergent fibre (ADFD) of the diets are given in Table 5. The DMD and OMD of the diets increased as the level of barley consumed increased ($P<0.001$). However, the fibre digestibility declined and was at its lowest at highest level of barley inclusion, both for CFD ($p<0.05$) and ADFD ($p<0.01$). Regressions between CFD and ADFD and the percentage of barley in the diet had a significant quadratic component ($p<0.001$).

If the assumption can be made that barley is digested to the same extent regardless of the diet composition, as found in the nylon bag incubation for 48 h, where the barley DM degradation was 870 g kg^{-1} for all diets (no significant differences between diets), then the diet DMD can be predicted from the all-straw diet, assuming additivity. The predicted and observed DMD for the diets is shown in Figure 3. The depressions in straw digestibility, compared with the all-straw diet were 15.2%, 15.4% and 27.4% for the diets containing 20%, 40% and 60% barley, respectively.

Table 5. Digestibility of dry matter (DM), organic matter (OM), crude fibre (CF) and acid detergent fibre (ADF) as influenced by the diet offered

Diet straw: barley	Digestibility of the diet g·100g^{-1}				DM digestibility of the NH_3-treated straw[1)]	Depression in digestibility of straw (%)
	DM	OM	CF	ADF		
100:0	50.7^{a}	57.8^{a}	71.2^{a}	67.3^{a}	50.7^{a}	-
80:20	52.4^{a}	61.8^{a}	67.1^{ab}	61.7^{b}	43.0^{b}	15.2
60:40	59.9^{b}	67.5^{b}	65.7^{ab}	57.2^{bc}	42.9^{b}	15.4
40:60	66.2^{c}	72.9^{c}	61.7^{b}	53.2^{c}	36.8^{c}	27.4
SEM	3.0	3.1	4.3	2.9	3.9	
Difference between diets	***	***	*	**	**	

a-c: means in each column with the same superscript are not significantly different ($p<0.05$).

1) Calculated by differences from an assumed digestibility of barley (barley degradation in nylon bags after 48 h incubation).

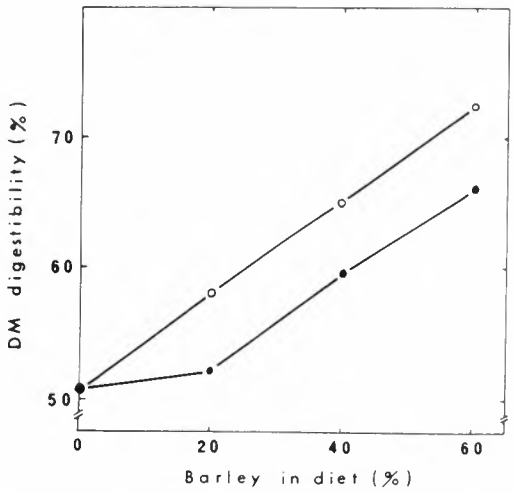

Figure 3. Mean dry matter digestbility of the diets as influenced by the level of barley, observed (● ——— ●) and predicted (o ——— o)

The digestion and utilization of dietry energy

The time the animals spent lying in the respiration chambers varied between 9.3 h and 15.6 h per 24 h period. There was no significant difference in activity between animals or diets.

Intake of gross energy (GE) and losses of energy in the faeces, urine and methane are given in Table 6. The proportion of GE lost in the faeces decreased and the loss in methane increased as the supplementation with barley in the diet increased. No significant difference was found between diets for urine loss.

Both the digestible (DE) and the metabolizable energy (ME) contents of the diets increased progressively with increased level of barley consumed (Table 7), with greater increases observed when the diet contained a high percentage of barley. This quadratic component of the

Table 6. Partitioning of dietry energy as influenced by the diet offered

Diet straw:barley	GE intake (MJ·day^{-1})	Proportion of GE intake in: Faeces	Urine	Methane
100:0	15.78	46.2^{a}	2.0	6.4^{a}
80:20	14.88	42.6^{a}	1.8	7.2^{ab}
60:40	12.59	34.9^{b}	2.0	8.2^{b}
40:60	10.53	26.6^{c}	2.8	9.6^{c}
SEM		2.1	0.6	0.9
Difference between diets		***	NS	**

a-c: means in each column with the same superscript are not significantly different ($p<0.05$).

Table 7. Digestible (DE) and metabolizable energy (ME) contents of the diets

Diet straw:barley	DE (% GE)	ME (% GE)	DE in dietary DM (MJ·kg^{-1})	ME in dietary DM (MJ·kg^{-1})
100:0	53.8^{a}	45.4^{a}	9.75^{a}	8.23^{a}
80:20	57.4^{a}	48.4^{a}	10.46^{b}	8.82^{a}
60:40	65.1^{b}	54.9^{b}	11.96^{c}	10.08^{b}
40:60	73.4^{c}	61.0^{c}	13.59^{d}	11.29^{c}
SEM	1.9	2.1	0.35	0.39
Difference between diets	***	***	***	***

a-d: means in each column with the same superscript are not significantly different ($p<0.05$).

relationship was highly significant ($p<0.001$).

Net availability of ME for maintenance (k_m) was low (50.8%) compared with the value calculated after ARC (1980), which was 66.2% for the diet containing NH_3-treated straw alone. There was a linear increase ($p<0.001$) in the k_m value with increasing amounts of barley in the diet, 56.1%, 64.3% and 73.9% for the diet including 20%, 40% and 60% barley, respectively.

Net energy content of the diets used for maintenance (NE_m) are given in Figure 4. Regression analysis of NE_m and the percentage of barley in the diet showed a significant ($p<0.001$) overall quadratic effect:

$NE_m = 4.39 + 0.0011\ B^2$
($r^2 = 0.99$, mean values)

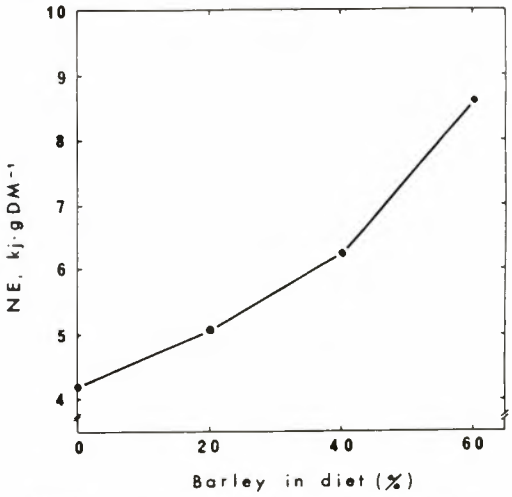

Figure 4. Net energy value of the diets as influenced by the level of barley

DISCUSSION

The effect of barley supplementation on fibre degradation

NH_3-treated straw supplemented with ground barley depressed the degradation of fibre in the diets, and the depression was highly significant even with a 20% barley inclusion.

The apparent DM digestibility of the NH_3-treated straw in the diets when straw was mixed with barley was determined by difference on the assumption that DMD of barley was unaffected. This approach seems valid, since in previous studies with dairy cows and sheep fed different amounts of grass silage and barley (Bøe 1989a, b), and in the present experiment, there was no difference between diet composition with regard to rumen degradation of barley after an incubation time of 24 h or longer. This has also been confirmed by other workers. Williams (1984) found that the starch in rolled barley was digested to the same extent regardless of the amount of NH_3-treated straw given along with the barley. Fahmy et al. (1984) found that the degradation of rolled barley was not greatly affected by the diet when given alone or together with NH_3-treated straw.

There was a good correlation between DM degradation of NH_3-treated straw incubated in nylon bags for 48 h (DMD48) and determined apparent digestibility of the straw.

For most diets based on agro-industrial by-products, such as straw, the primary limitation to the growth of rumen microorganisms is probably the concentration of ammonia in rumen fluid (Preston & Leng 1987). The level of ammonia that supports maximum digestibility in the rumen, and as such the largest population of microorganisms, will vary among diets. Following van Es and van der Meer (1980), rumen microbes need at least 1.6% total N in the diet DM. Since the NH_3-treated straw used in this study contained 1.5% N, the diets were supplemented with urea to exceed this threshold. Nitrogen limitations should therefore not contribute to the fibre depression.

The variation in fibre digestibility probably results from differences in the activity of the cellulolytic bacteria. As was found for grass silage supplemented with barley when fed at maintenance

level (Bøe 1989b), average rumen pH was depressed with increasing supplementation of barley, but did not fall below 6.0, which is supposed to be the "cellulolytic threshold" (Stewart 1977). A carbohydrate effect in addition to fall in pH therefore appears to be involved in the fibre depression, as discussed for grass silage and barley (Bøe 1989b).

Energy pratitioning

A curvilinear relationship was found between the net energy value and the level of barley in the diet, indicating negative associative effects between NH_3-treated straw and barley on the energy used for maintenance (NE_m), similar relationship was found by Byers et al for mixture of corn and corn silage (1976).

Other workers, however, have found that NE_m is linearly related to the proportions of concentrate in the diet (Lofgren & Garrett 1968, Vance et al. 1972, Garrett 1979). Garrett (1979) even concludes by saying that his "findings together with some observations made from the literature form the basis for the suggestion that experimental variability (technical and animal) together with the model chosen to present the results of a particular trial may be responsible for some reports of the associative effects of feeds".

In the present experiment, the curvilinear relationship between mean NE_m and percentage of barley in the diet reflects the same tendency as found for the diet fibre digestibility, DA and ME content. All of these regressions with barley content had significant curvilinear components.

In the work done by Garrett (1979), there was a strong linear relationship (no significant quadratic effect) between percentage concentrate in diet and digestible energy concentrations. The influence of concentrates on fibre digestibility was not measured.

Results from the present experiment, together with the findings of Byers et al. (1976), seem to indicate that a strongly reduced fibre digestibility in a mixed diet can make a substantial contribution to variation in energy values, even when the animals are fed near or below the level of nutrition required for maintenance.

The availability of ME for maintenance (k_m) was linearly related to the level of barley in the diet. The efficiency of ME utilization has therefore not contributed to the reduction in NE values.

In practical terms, these results show that NH_3-treated straw must represent the major part of the diet in order to utilize the available energy. The use of NH_3-treated straw should therefore be restricted to situations with low levels of production.

SUMMARY

1. The influence of barley on rumen degradation, digestibility and energy utilization of NH_3-treated straw was studied.

2. Four sheep fitted with permanent rumen cannulas were used. They were fed four different diets containing NH_3-straw and ground barley. The proportions of NH_3-straw to barley were 100:0, 80:20, 60:40 and 40:60 on DM basis.

3. Calorimetric experiments were performed using the open circuit type of respiration chamber.

4. Supplementation of NH_3-straw with barley depressed the fibre digestibility in the diet; the depression was highly significant even with a 20% barley inclusion.

5. The depressions in DM digestibility of NH_3-straw, calculated by difference from assumed digestibility of barley, were 15.2%, 15.4% and 27.4% when 20%, 40% or 60% barley was included in the diets, respectively.

6. A high correlation was found between DM degradation of NH_3-straw incubated in nylon bags for 48 h (DMD48) and the calculated DM digestibility of the straw.

7. A significant quadratic influence of the percentage of barley in the diet was found on the net energy content of the diets used for maintenance (NE_m). This curvilinear relationship indicates that the NE_m of each feed ingredient is not constant, but depends on the remaining diet composition.

8. The efficiency of ME utilization for maintenance (K_m) increased linearly with the levels of barley in the diet. Thus, the associative effects on the net energy values for maintenance are likely to be due to depressions in energy digestibility rather than to depressions in ME utilization.

9. It is concluded that NH_3-treated straw must represent the major proportion of the diet if the available energy is to be utilized. The use of NH_3-treated straw should therefore be restricted to situations in which moderate to low levels of production are desired.

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REPLACEMENT OF NH_3-TREATED STRAW BY CELLULOSE IN THE DIET OF SHEEP

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Bøe, U.B. 1989. Replacement of NH_3-treated straw by cellulose in the diet of sheep. Norwegian Journal of Agricultural Sciences 3: 337-341. ISSN 0801-5341.

1. The effect of cellulose given just prior to feeding NH_3-treated straw, alone or together with wheat starch, has been studied.
2. When some of the NH_3-treated straw was replaced with cellulose, the diet digestibility of dry matter and crude fibre increased. The extent and rate of ruminal degradation of NH_3-treated straw increased, too.
3. It seems reasonable that the provision of a readily digestible cellulose source just prior to feeding will increase the number of cellulolytic microbes in the rumen fluid, and hence increase the rate of fibre fermentation.

Key words: cellulose, fibre digestibility, NH3-treated straw, rumen degradation, starch inclusion.

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In a previous study (Bøe 1989a) the digestibility of fibre was depressed even when small amounts of ground barley were included in the diet of NH_3-treated straw.

Depressed fibre digestibility is often coupled with reduction in rumen pH when readly degradable carbohydrates are added to the roughage diet. This effect can be counteracted by the feeding of buffer salts to maintain pH above 6.0 - 6.1 (Mould et al. 1984), which appears to be the cellulolytic threshold.

In the previous study, where NH_3-treated straw was given along with up to 60% barley, the rumen pH did not fall below 6.0.

The depression in fibre digestion caused by moderate decreases in pH is not readily explained, and therefore difficult to counteract. It has been suggested that attachment problems of rumen cellulolytic bacteria to plant cell walls are involved in such a depression (Hoover 1986). A decline in the number of cellulolytic bacteria in the rumen, or a change in the proportion of species present may be involved, too (Henning et al. 1980). It has also been shown that some of the cellulolytic bacteria can grow on alternative substrates, and that synthesis of cellulases is inhibited by the presence of simple sugars (Fusee & Leatherwood 1972, Berg 1975).

Any manipulation which maintains a large pool of cellulolytic organisms in the rumen may contribute to counteracting the depression of fibre digestion. Addition of a readily fermentable cellulose source to the diet may possibly increase the microbial biomass after feeding and thus potentially increase the rate of colonization and degradation of the roughage particles (Chesson & Ørskov 1984, Nielsen 1981).

The objective of the present study was to examine the rumen degradation and digestibility of NH_3-treated straw, given alone or together with wheat starch, when readily digestible cellulose was given just prior to feeding.

MATERIALS AND METHODS

Four mature Dala x Texel rams fitted with permanent rumen cannulas (inner diameter 40 mm) were used. They were 2 - 4 years old, with an initial liveweight of 60 - 70 kg.

The animals were given four different diets according to a 4 x 4 latin square design. NH_3-treated straw given alone and NH_3-treated straw and wheat starch (50% of each on dry matter (DM) basis) were given as the two basal diets.

In the two other diets some of the straw was replaced by wood pulp cellulose (Table 1). The chemical composition of the feed is presented in Table 2.

The barley straw was treated with ammonia and handled as described in a previous study (Bøe 1989a).

Herring meal was added to give the NH_3-treated straw diet at least 13% digestible crude protein. In the other diets, urea was added to give iso-nitrogenous rations. Supplements of minerals and vitamins were as described earlier (Bøe 1989a).

The cellulose used was bleached wood pulp sulphite cellulose from Borregård, Norway, type SAI STRONG. The cellulose, which was available in rolled sheets, was torn into small pieces and soaked in water overnight before use. It was offered to the animals along with the herring meal half an hour before the rest of the diet.

The animals were held in metabolism cages during the whole experiment, each experimental period lasting 21 days. After a 10-day adjustment period, faeces were collected for 7 days. The last 4 days were used for nylon bag incubation of NH_3-treated straw and rumen fluid sampling.

At the end of the experimental period, two of the animals were given a diet of 800 g cellulose, 70 g herring meal and 15 g urea for 17 days to determine dry

Table 1. Composition of the diets in g·DM^{-1} day

Basal diet:	NH_3-straw		NH_3-straw + starch	
Cellulose replacement:	–	+	–	+
NH_3-straw	1000	800	500	300
Cellulose	-	200	-	200
Wheat starch	-	-	500	500
Herring meal	70	70	70	70
Urea	-	5	5	10
Salt, minerals, vitamins	+	+	+	+

Table 2. Chemical composition of the diet ingredients (g·100 g-1)

	DM	Ash	Crude protein	Ether-extr.	N-free extr.	Crude fibre
NH_3-straw	88.0	5.1	8.0	0.8	30.8	43.3
Cellulose	94.7	-	-	-	12.0	82.7
Wheat starch	89.4	-	0.3	0.03	89.1	-
Herring meal	92.8	11.4	73.4	7.5	0.5	-

matter and crude fibre digestibility of the cellulose. The DM digestibility was determined by difference method, assuming the DM digestibility of herring meal to be 87%.

Procedures for nylon bag degradation of the straw and rumen fluid samples and chemical and statistical analysis were as described by Bøe (1989b).

RESULTS

The dry matter and crude fibre digestibility of the cellulose was found to be 71.4 and 85.1%, respectively. When some of the NH_3-treated straw was replaced with cellulose, the diet digestibility of dry matter, organic matter, crude fibre and energy increased (Table 3). The differences were not statistically significant, except for the crude fibre digestibility for the diets containing starch. Here the digestibility increased from 64.6% in the diet without cellulose to 79.4% in the diet including cellulose ($p<0.05$).

The cellulose inclusion did not affect the pH values in the rumen content (Table 4), nor was there any effect of the cellulose on the concentrations of total VFA and NH_3-N (Table 5).

The molar proportion of acetate increased ($p<0.001$) and propionate decreased ($p<0.05$) when cellulose was included in the basal diet containing starch. For the diet containing NH_3-treated straw alone, the molar proportions of VFA were not affected by the cellulose. L-lactic acid was not accumulated in the rumen of the animals during this experiment.

The potential degradation of NH_3-treated straw and the degradation after 48 h incubation (DMD48) in the rumen increased slightly when cellulose was included in the basal diets (Table 6), as was also the case for rate of degradation.

DISCUSSION

The digestibility of dry matter and crude fibre of the cellulose was higher than the digestibility of the basal diets, thus contributing to increase in the diet digestibility when some of the NH_3-treated straw was replaced by cellulose. However, the

Table 3. Digestibility of ration dry matter (DM), organic matter (OM), crude fibre (CF) and energy (E), as influenced by the diet offered

Basal diet:	NH_3-straw		NH_3-straw + starch		
Cellulose replacement:	–	+	–	+	SEM
DM	61.1	65.5	71.9	77.0	4.5
OM	64.4	67.2	74.7	79.6	4.4
CF	77.1	83.0	64.6	79.4*	7.7
E	60.6	64.0	70.6	76.0	4.9

* Cellulose effect; significance of differences between diets: $p<0.05$

Table 4. Average rumen pH throughout the day and minimum pH value measured as influenced by the diet offered

Basal diet:	NH_3-straw		NH_3-straw + starch		
Cellulose replacement:	–	+	–	+	SEM
Average pH	6.69	6.65	6.17	6.17	0.3
Minimum pH	6.24	6.28	5.49	5.60	

Table 5. Rumen fluid volatile fatty acids (VFA) and NH_3-N as influenced by the various diets offered

Basal diet:	NH_3-straw		NH_3-straw + starch		
Cellulose replacement:	–	+	–	+	SEM
NH_3-N, mmol/l	15.1	14.8	9.6	10.5	4.2
Total VFA, mmol/l	53.9	58.9	71.6	66.9	27.1
Molar proportions					
Acetate (C_2)	69.7	70.9	61.6	66.6***	3.6
Propionate (C_3)	16.9	15.4	23.7	18.3*	2.0
Butyrate (C_4)	5.8	6.6	8.2	8.4	1.2
Iso-butyrate (IC_4)	6.6	5.7	5.2	5.2	1.9
Valerate (C_5)	0.1	0.3**	0.5	0.4	0.2
Iso-valerate (IC_5)	0.9	0.9	0.6	1.1*	0.3

*, **, *** Cellulose effect; significance of difference between diets: * = $p<0.05$; ** = $p<0.01$; *** $p<0.001$

Table 6. Rate and extent of dissappearance of DM from NH_3-treated straw incubated in nylon bags in the rumen

Basal diet:	NH_3-straw		NH_3-straw + starch	
Cellulose replacement:	–	+	–	+
Potential degradation (a + b)[1)]	55.8	60.6	34.8	41.5
Rate of degradability (c)	3.6	3.8	2.1	2.6
RSD	0.92	1.94	0.86	1.73
DMD48	45.6	47.9	32.3	35.2

1) a, b and c are constants in the exponential equation $p = a + b(1 - e^{-ct})$, where p is the degradability at time t. DMD48 is DM degradability after 48 h incubation (p when = 48) RSD = residual standard deviation.

quantities of cellulose added cannot account for the whole increase, especially with regard to improved crude fibre digestibility in the diet. This may indicate a better utilization of the NH_3-treated straw.

The rumen degradation of NH_3-treated straw confirms this indication, since replacement of NH_3-treated straw with cellulose in the diet increased the straw degradability. These observations are supported by studies with sheep fed diets based on sisal pulp, in which the provision of fresh grass significantly increased the degradation of pure cellulose in nylon bags suspended in the rumen (referred in Preston & Leng 1987).

It is difficult to compare the obtained results with other experiments including cellulose in the diet, because the digestibility of cellulose matter varies greatly depending on the wood used, the lignin content and the technology of the processing (Zelenák et al. 1985). The favourable effect of cellulose fibre on the digestibility of feed was, however, also found with sheep by Zelenák et al. (1985) and by Millet et al. (1973) in experiments with goats.

In conclusion, it seems reasonable that by providing a readily digestible cellulose source just prior to feeding there will be an increase in the number of cellulolytic microbes in the rumen fluid, and hence an increase in the rate of fibre fermentation.

However, further studies are needed to show whether this method of feeding could contribute towards neutralizing

the negative effect of starch inclusion on roughage fibre digestibility.

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INFLUENCE OF THE NITRIFICATION INHIBITOR DICYANDIAMIDE (DCD), ON THE NITROGEN EFFICIENCY OF CATTLE SLURRY

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DCD added to cattle slurry spread in the autumn increased grass yields the following year by 63% as compared with the increase from spring spreading of slurry without DCD. Barley yield also increased correspondingly by 63%. The application of 30 kg DCD per hectare was slightly less effective than 15 kg. Spring application of slurry without DCD was better than autumn application with DCD. Adding DCD to slurry spread in the spring had no effect.

Key words: Barley, cattle slurry, dicyandiamide, DCD, grass, herbage minerals, nitrification inhibitor, soil analyses, crop yield.

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In slurry from cattle and pigs 50-70% of the nitrogen is in ammonia form, while the rest is present in various organic compounds that are only slowly degraded. The effect of nitrogen in slurry is often less than that of fertilizer. Reasons of this are e.g. too much slurry per unit area, spreading without incorporation in the soil, uneven spreading and spreading during unfavourable seasons in the year.

When slurry is applied and spread correctly the total nitrogen effect may be about half that of fertilizer nitrogen. In less favourable conditions, like spreading slurry in the autumn or on the soil surface of grassland, the nitrogen effect may be far less.

Various additives are used in order to reduce loss of nitrogen as nitrate in water runoff from slurry and fertilizers.

In Norway, dicyandiamide (DCD) has been tried as a means of reducing the nitrogen loss from agricultural land where slurry has been spread. DCD is eventually degraded into nitrate and carbon dioxide. The soil temperature affects the time of degradation. With soil temperatures below 10°C the inhibitive effect may last for 8-10 weeks. Higher soil temperatures result in a shorter inhibition period.

MATERIAL AND METHODS

Field experiments were conducted at different sites in west and east Norway. Two Youden square designs, one with $t=11$, $k=r=5$, and the other with $t=7$, $k=r=4$ were used.

Table 1. DM yield of grass, kg ha^{-1}

	No. of trials	a	b	c	d	f	g	$LSD_{5\%}$
1. cut	16	4360	4890	4760	5220	5350	4460	340
2. cut	13	3850	4050	3890	4140	4180	3790	260
Ann. yield	16	7490	8180	7930	8590	8740	7540	450

Both designs comprise the following treatments: (Amounts in t or kg ha^{-1}, A = autumn, S = spring)

Design I
a. 50 t slurry (A)
b. 50 t slurry + 15 kg DCD (A)
c. 50 t slurry + 30 kg DCD (A)
d. 50 t slurry (S)
e. 50 t slurry + 15 kg DCD (S)
f. 600 kg NPK-fertilizer 14-6-16 (S)
g. 720 kg PK-fertilizer 5-16 (control)(S)

Design II comprises the same treatments as Design I except treatment e. In addition design II comprises the following treatments:

h. 100 t slurry (A)
i. 100 t slurry + 15 kg DCD (A)
j. 100 t slurry + 30 kg DCD (A)
k. 100 t slurry (S)
l. 1200 kg NPK-fertilizer 14-6-16 (S)

Cattle slurry was used in all trials. On average, 50 t slurry contains 180 kg Kjeldahl-N, 110 kg NH_4-N, 4 kg P and 17 kg K. In a few cases, NPK-fertilizers other than 14-6-16 were used. In trials with grass and ryegrass, 50 kg N per hectare was applied on all plots after the first cut. In the second experimental year, when residual effects were measured, artificial fertilizers in amounts a little less than normal in the respective areas were applied.

Altogether, results have been obtained from 19 trials following Design I, and 4 trials following Design II.

Statistical analysis of the material was carried out by means of the statistical programme MSTAT.

The ammonium-lactate (AL)-soluble P, K, Ca and Mg were analysed at the State Soil Investigation, Ås-NLH, Norway. Analysis of slurry and plant material was carried out partly by the Chemical Analytical Laboratory, Ås-NLH and partly by the Chemical Research Laboratory, Holt Agricultural Research Station, Tromsø.

RESULTS

Grass yields

The material consists of the results from 16 trials with perennial grasses and

Table 2. Yield (DM) of grass. Residual effect. Kg ha^{-1}

	No. of trials	a	b	c	d	f	g	$LSD_{5\%}$
1. cut	10	5860	5970	5800	6030	5760	5700	230
2. cut	8	3680	3780	3640	3610	3480	3580	220
Ann. yield	10	8800	8990	8710	8910	8540	8560	320

Table 3. Relative yield increase obtained by different slurry treatments as compared with the increase obtained by NPK- fertilizer (= 100). 16 trials with grasses

Treatment	a	b	c	d	f
Relative yield increase	16	91	46	119	100

Italian ryegrass (*Lolium multiflorum*), and 7 trials with barley. Out of the 23 trials, 19 were conducted according to Design I. A preliminary report was published by Tveitnes and Håland (1987).

In Table 1 results from trials with grass are shown.

In three of the 16 experiments the second cut was not harvested. DCD added to slurry at the time of spreading in October/November significantly increased the yield of grass DM, the response being 690 kg per hectare. Spring fertilization with slurry was significantly better than autumn application. Compared with this, the response to DCD applied in the autumn was 63%. Thirty kg DCD mixed into the slurry was less effective. Application of slurry in the autumn without inhibitor gave, on average, no increased yield response.

There was some residual effect of slurry the second year after application. The treatments with spring application of slurry without DCD and autumn application with 15 kg DCD gave the highest residual effects.

Yield response to slurry as compared with artificial fertilizer

In Table 3 yield responses to the different slurry treatments relative to the response to NPK-fertilizer are shown.

The yield response to NPK fertilizer is put at equal to 100 for the two-year experimental period. In comparison, spring application of slurry gave a 19% better response. However, slurry combined with DCD spread in the autumn responded quite well, while slurry without inhibitor responded poorly. As a percentage of the yield increase with a spring application of slurry compared with autumn application, an addition of 15 kg DCD to the slurry gave a yield response of 84%, while the effect of 30 kg DCD per hectare was less.

Yield of barley grain

In the trials with grain crops only the grain, not the straw, was weighed.

Spring application of manure was more effective than NPK- fertilizer. Application of DCD together with slurry increased the yield by 63% as compared with the difference in yield between spring and autumn applications of slurry.

In the second year the residual effect of the treatment rates was tested in two of the trials. Residual effects were also found on the NPK-fertilizer plots. However, the yield response to slurry applied in the spring and to slurry mixed with

Table 4. Yield (DM) of grain, kg ha^{-1}. Mean of 7 trials in the first experimental year

trials	No. of	a	b	c	d	e	f	g	LSD5
1. year	7	3020	3300	3340	3730	3740	3740	3070	550
2. year	2	3310	3670	3720	3880	-	3480	2770	700

Table 5. Apparent nitrogen recovery of applied Kjeldahl-N and NH_4-N (Percent)

	No. of trials	a	b	c	d	e	f
Kjeldahl-N							
Grass	11	-4	8	12	21	-	38
Barley (grain)	7	-1	3	4	9	9	14
NH_4-N							
Grass	11	-4	14	21	41	-	38
Barley (grain)	7	-3	5	7	13	14	13

DCD and applied in the autumn was higher, as shown in Table 4.

Summarized for the two-year experimental period, DCD added to the slurry in the autumn increased the crop yield response by 63-65% of the difference between autumn and spring applications of slurry.

Apparent nitrogen recovery and efficiency

Apparent nitrogen recovery is the increase in the amount of nitrogen contained in the harvested herbage expressed as a percentage of that applied in slurry or fertilizer.

Apparent efficiency of nitrogen is the increase in harvested herbage DM per kg N applied in slurry or fertilizer.

These values are calculated from the respective differences in N- uptake and DM yield between the slurry or fertilizer-treated plots and the control plots (g).

Compared with the artificial fertilizer treatment, the total apparent nitrogen recovery of slurry was a little more than half on the plots which received slurry in the spring. The recovery of ammonia N in slurry applied in the spring was better than that of N in fertilizer. For slurry spread in the autumn without DCD, no recovery of applied nitrogen was obtained. Addition of DCD improved the situation substantially, especially in the grass trials.

For every kg of nitrogen applied in fertilizer grass DM yield increase was almost 110 kg, while for barley grain it was 74 kg per hectare. Addition of DCD to slurry spread in the autumn increased the apparent nitrogen efficiency considerably.

Table 6. Apparent nitrogen efficiency. DM, kg ha ^{1}

	No. of trials	a	b	c	d	e	f
Kjeldahl-N							
Grass	11	6	36	28	71	-	109
Barley (grain)	7	-9	7	10	34	34	74
NH_4-N							
Grass	11	9	61	47	134	-	109
Barley	7	-26	15	18	51	52	74

Table 7. The effect of DCD added to slurry spread in the autumn and in the spring. DM, kg ha^{-1}

	No. of trials	5 t ha^{-1} slurry				$LSD_{5\%}$
		Autumn		Spring		
		Without DCD	With DCD	Without DCD	With DCD	
Grass or ryegrass						
1. cut	12	4310	4760	5120	5320	300
2. cut	9	3520	3770	3710	3740	170
Ann. yield	12	6950	7590	7900	8120	350
Barley (grain)	7	3020	3300	3730	3740	400

Crop yield response to DCD applied in the autumn and in the spring

This comparison comprises treatments with slurry with and without addition of 15 kg DCD per hectare, and was carried out in 12 trials with grass and in 7 trials with barley. The results are shown in Table 7.

Addition of DCD to slurry in the autumn increased the yield of grass DM significantly, and especially in the first cut. Although there was a tendency to a yield increase because of the addition of DCD in the spring, this effect was not significant. In the 7 trials with barley, a clear tendency to yield increase was found owing to DCD in the autumn, while there was no effect of DCD applied in the spring. DCD did not influence the residual effect of slurry on the crop yield.

Increasing amounts of slurry per unit area

In 4 trials 50 and 100 tons of slurry per hectare spread in the autumn and in the spring were compared.

Compared with autumn spreading, spring spreading significantly increased the yield in the first year, but not in the second year. Both in the first and second year there were negligible yield differences between the two application rates of slurry.

There was no indication that DCD had different effects whether 50 or 100 tons of slurry per hectare was applied.

Chemical analyses of slurry

Composite samples of slurry used on the plots of each trial were analysed and the results presented in Table 9.

The average DM of the slurry was 8.2-8.3%. The maximum value was 13.4% and the minimum 2.1%. The content of the various nutrient elements was about the same in samples collected in the autumn and in the spring.

Table 8. The effect of different amounts of slurry spread in the autumn and in the spring. Annual grass DM yield. Kg ha^{-1}.

	No. of trials	Slurry, t ha^{-1}				$LSD_{5\%}$
		Autumn		Spring		
		50	100	50	100	
1. year	4	910	957	1065	1091	122
2. year (residual effect)	3	1013	1050	1062	1070	NS

Table 9. Chemical analysis of slurry used in the trials

	No. of samples	DM	Percent Kjeldahl-N	NH_4-N	P	K	Ca	Mg
Autumn	18	8.2	0.37	0.20	0.07	0.36	0.11	0.04
Spring	18	8.3	0.37	0.22	0.07	0.33	0.10	0.05

Soils and soil sample analyses

All trials were conducted on fertile soils that had been cultivated and utilized for either grassland or arable crops for a number of years. Soil samples were collected from some of the trials, partly composite samples from each site at the start of the experiment, and partly samples from each treatment at the end of the experimental period. No significant differences were found between treatments in the content of various nutrient elements after the short experimental period. In Table 10 average soil analysis data are presented.

The analysis indicated a high to very high level of phosphorus, magnesium and calcium both in the topsoil (0-20 cm) layer and in the subsoil (20-40 cm) layer. The content of potassium was medium in the topsoil and low in the subsoil.

Chemical content of the grass crop

Samples of the crop were collected at each harvesting from most of the trials.

At the first cut, grass from plots with slurry applied in the autumn had a lower content of nitrogen, phosphorus and potassium than grass and ryegrass from plots which received other treatments (Table 11). Addition of DCD to the slurry in the autumn increased the content of

Table 10. Average soil analysis data

	No. of trials	Soil depth cm	pH	P-AL	K-AL	mg/100 g dry soil Ca-AL	Mg-AL	K-HNO_3
East Norway	9	0-20	6.1	13.6	13.9	146	11.2	73
	4	20-40	6.1	10.1	4.3	103	9.7	59
West Norway	6	0-20	5.7	20.6	10.4	141	8.2	104
	1	20-40	5.6	20.5	4.1	110	5.5	-

Table 11. Plant analysis data from the first cut. Percent of DM. Mean of 11 trials

	a	b	c	d	f	g	$LSD_{5\%}$
Kjeldahl-N	1.72	1.86	2.00	1.98	2.04	1.88	0.23
P	0.26	0.26	0.28	0.28	0.28	0.26	0.02
K	2.57	2.63	2.81	2.98	2.79	2.79	0.24
Ca	0.39	0.39	0.41	0.38	0.42	0.47	0.05
Mg	0.12	0.12	0.13	0.12	0.13	0.13	n.s.
Na	0.08	0.09	0.10	0.12	0.12	0.09	0.02

these elements in the crop almost to the same level as the slurry or fertilizer applied in the spring.e 9

Application of nitrogen in slurry or fertilizer decreased the content of calcium in the plant DM. There were no significant differences at the second cut.

Table 12. Content of plant nutrients in barley grains.
g/100 g DM. Mean of 7 trials

Kjeldahl-N	P	K	Ca	Mg	Na
1.71	0.43	0.48	0.05	0.13	0.01

In the barley trials there were no significant differences in nutrient content of the grains. In Table 12, the mean content of nutrient elements in grains is shown.

DISCUSSION

Spreading on agricultural land is the only reasonable way of dealing with livestock manure. It is also conclusive that slurry, as well as fertilizers, should be spread in the spring or early in the growing season in order to make satisfactory use of the nutrient elements and to reduce the risk of water pollution.

For various reasons, however, a substantial amount of manure is spread in the autumn. Main reasons are lack of adequate storing capacity for slurry during the winter, delayed seedbed preparation and sowing in the spring, shortage of labour capacity and machinery. Moreover, risk of soil compaction damage owing to traffic with heavy slurry tankers is sometimes the reason why farmers hesitate to spread slurry in early spring.

Although spring application of manure is preferable and should be encouraged, any means of improving the effect of manure spread in the autumn should also be appraised.

The effect of nitrification inhibitors depends on conditions that favour leaching of nitrates, and such conditions may sometimes be difficult to predict. A number of field experiments have shown that the inhibitive effect of DCD varies noticeably. On average, for 20 trials with perennial grasses, Italian ryegrass and barley, the effects obtained from the use of inhibitors in slurry applied in the autumn turned out to be positive and of significance. Of the two amounts of DCD tried, 15 kg per hectare was better than 30 kg. This treatment increased the yield of grass by 65% of the yield difference between autumn and spring applications of slurry without the use of inhibitors.

In several reports, it is concluded that after spreading slurry mixed with DCD in the autumn or winter, the utilization of N by the subsequent crop is improved, e.g. Amberger et al. (1982), Kjellerup (1985), Nilsson (1987), Scheffer et al. (1983).

The use of nitrification inhibitors in slurry spread in the spring improved the fertilizer effect negligibly in these experiments.

SUMMARY

The report deals with altogether, 23 field trials with slurry and the nitrification inhibitor DCD. Of these, twelve were situated in west Norway and eight in east Norway. On the trials sites in west Norway grass or ryegrass were grown, while barley was grown in all but one trial site in east Norway.

It can be concluded from the results that:

a. DCD, 15 kg ha-1 added to slurry spread in the autumn, significantly increased yields of grass by 63% as compared with the difference in yield between slurry spread in the autumn without the addition of inhibitors and slurry spread in the spring. The corre-

sponding increase in barley (grains) was also 63%.

b. There was a tendency that 30 kg DCD per hectare was less effective than 15 kg.

c. Spring application of slurry was the better alternative.

When spreading of manure has to be carried out in the autumn for other than agronomic reasons, application of a nitrification inhibitor DCD may be a useful tool in increasing the nitrogen efficiency in plant production and thereby reducing the water pollution risk involved caused by leaching of nitrate.

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PROPAGATING APPLE ROOTSTOCKS BY SEMI-HARDWOOD CUTTINGS

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Semi-hardwood cuttings of the apple rootstock 'MM106' root more readily than 'M26'. Shading the stock plants increase the rooting percentage in both cultivars but is most effective in 'M26' and in the latter part of the growing season. Three- and 4-node stem cuttings root more readily than 2-node stem cuttings and terminal cuttings, especially when the lowermost leaf is removed from each cutting. Distal stem cuttings root more easily than proximal stem cuttings. Wounding the base of each cutting improves rooting only slightly. Auxin application increases rooting; 1% IBA is the optimum talc concentration, but a 24-hour soak in 200 ppm K-IBA is the most successful treatment. Three- and 4-node stem cuttings treated with 1% IBA-talc grow most vigorously and produce the largest rootstocks in the first growing season.

Key words: Apples, auxins, mist propagation, propagation by cuttings, rooting, rootstocks, shading.

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Apple (*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.)) rootstocks are most commonly propagated by mound layering (stooling) or by hardwood cuttings (Howard 1971, Hartmann & Kester 1983). Traditionally, these have been the only methods used for commercial rootstock production in Norway. However, less than 20% of the rootstocks produced by these methods will be of saleable quality after one growing season. Micropropagation, by which small shoot tips are grown under aseptic conditions, are being used for the production of disease-free stock plants. This method, however, may be too expensive for the large-scale production of rootstocks.

The serious bacterial disease *fireblight* has spread to most European countries, and since the predisposed species in *Rosaceae*, including apples, cannot be imported to Norway from infected countries, there is a growing interest in Norway for producing the 200.000 apple rootstocks required annually by Norwegian growers.

Although softwood and semi-hardwood cuttings root fairly easily, these have been used to a very limited extent for the production of apple rootstocks (Tukey 1964, Howard 1971). In fruit species like pear (Hartmann et al. 1963), cherry (Hartmann & Brooks 1958, Roberts 1963), and peach (Coston et al. 1983, Gur et al. 1986), softwood or semi-hardwood cuttings have been used very successfully.

The stock plant has a profound influence on propagation success whether used for traditional cutting propagation or for propagation by micro-cuttings (Hartmann & Kester 1983, Read

et al. 1985). In many trees and shrubs, including apples, low light levels during stock plant growth are shown to improve cutting rootability (Harrison-Murray 1981, Bassuk et al. 1984). British practice now calls for extreme reduction of light; allowing about 2% of the day light (Harrison-Murray & Howard 1982). Shading, allowing 5-50% of ambient light, has improved rooting in several species (Johnson & Roberts 1971, Knox & Hamilton 1982). In this paper an 80% reduction of light, achieved by covering the rootstocks with shade cloth, is compared with an ambient light level during stock plant growth in a glasshouse.

The rooting response of semi-hardwood cuttings is affected by position within the shoot (Hartmann & Kester 1983). Tip cuttings often root more readily than basal cuttings. However, this may differ from species to species, the middle and lower part of the shoot sometimes exhibiting the greatest potential for rooting (Assaf 1966). Terminal cuttings within semi-hardwood shoots are often succulent and may be difficult to keep turgid througout the rooting period. Consequently, the environmental regime during the rooting process may be very important for successful propagation.

Exogenously applied auxins have made possible the propagation by cuttings of many hard-to-root species. Not only does the percentage rooting increase but the number of roots in each cutting as well as the quality of the roots are usually enhanced. Many synthetic auxins have root promoting effects, but only a few have become well established in commercial plant propagation, indole butyric acid (IBA) being the most commonly used. Several ways of applying the auxins have developed, and among the most important are: Auxin acids dispersed in talc or dissolved in alcohol, and auxin potassium salts dissolved in water. The response to formulation and concentration may be highly species-specific and may even differ from cultivar to cultivar within the same species (Chong 1981, Berry 1984). Interactions between type of cutting (maturity of stem) and auxin concentration have been observed (Dirr 1981).

In 1983 a programme was initiated with the ultimate goal of developing a quick and reliable method for the production of saleable apple rootstocks in one growing season. As part of this programme the potential effects of stock plant shading and treatments with auxins on the rooting of different categories of semi-hardwood cuttings were studied. Two different propagation systems, intermittent mist and fog, were available to study effects of the rooting environment.

MATERIALS AND METHODS

During 1983-87 ten experiments were carried out to examine the effects of stock plant shading, cutting material, auxin treatments, and rooting environment on the rooting of semi-hardwood apple cuttings. General information about the experiments is given in Table 1. All the experiments included the two rootstock cultivars 'MM106' and 'M26'. Stock plant shading was included in experiments 3, 4, and 5. The auxin treatments and the main categories of cuttings are listed in Table 2. In addition, some other factors were included in single experiments: Wounding the base of each cutting (Exp. 1), position of stem cuttings within the shoot (Exp. 6), and removal of the lowermost leaf in each cutting (Exp. 8).

Two series of one-year-old caliper 8-10 mm stock plants were potted in 5 dm^3 containers in a medium consisting of a fertilized peat (Floralux) and granules of expanded clay (Leca) in a 3:1 (v/v) ratio. The stock plants in the first series were rooted hardwood cuttings ('MM106') and micro-propagated rootstocks ('M26'). They were potted on 12 April 1983. Cuttings were obtained from the first

Table 1. General information about the 10 rooting experiments with semi-hardwood cuttings of the apple rootstock cultivars MM106 and M26. * = Stock plant shading is included; I.M. = intermittent mist; PP = peat/perlite (1v:1v); J4,J5 = Jiffy peat pots 4 cm and 5 cm; + = the growth of the rooted cuttings was studied.

Exp. no.	Sticking date	Stock plant series	Rooting period (weeks)	Rooting environ-ment	Average air temp. (°C) High	Low	Rooting medium	Growth
1	1/6-83	1	6	I.M.	31	18	PP and J5	+
2	2/6-83	1	6	I.M.	31	18	J5	+
3	15/7-83	1*	6	I.M.	32	18	J5	
4	7/9-83	1*	7	I.M.	29	18	J5	
5	25/5-84	1*	6	I.M./Fog	25	15	J4	+
6	24/7-84	2	5	Fog	25	15	J5	
7	23/5-85	1	7	I.M./Fog	26	16	J4	+
8	30/5-85	2	7	I.M./Fog	26	16	J4	+
9	15/5-86	2	5	I.M.	25	15	PP	
10	1/7-87	3	6	Fog	25	12	PP	

Table 2. Auxin applications and cutting categories included in the rooting experiments with semi-hardwood cuttings of the apple rootstock cultivars MM106 and M26.

Exp. no.	Total no. of cut-tings	No. of reps. x no. of cut-tings	Auxin												Cutting category			
			Con-trol	IBA-talc(%)			K-IBA(ppm) quick dip			24-h soak			Termi-nal	Stem (no. of nodes)		
				0.5	1.0	2.0	500	1000	2000	50	100	200		2	3	4
1	320	2x5	x		x								x			x
2	200	5x5		x									x		x	
3	1440	2x5		x	x								x		x	x
4	960	2x5			x								x	x	x	x
5	480	2x4			x								x			x
6	468	2x6			x			x				x	x	x	x	x
7	1008	3x7			x								x			x
8	1152	3x6		x	x								x	x	x	x
9	600	2x10	x	x	x	x			x				x	x	x	
10	480	2x6	x	x	x	x	x	x	x	x	x	x	x		x	

series during three seasons, 1983-85. The second series consisted of one-year-old rootstocks propagated by softwood and semi-hardwood cuttings the previous year and potted on 8 June 1984. A third series of stock plants included in 1987 consisted of one- and two-year-old stock plants originating from rooted softwood cuttings. They were planted in 15 cm deep flats. All the stock plants were fertilized regularly with a complete fertilizer.

Each year the stock plants were pruned to leave 3 to 5 buds of the previous year's growth and forced in a heated greenhouse starting in late March or early April. No supplementary light was given, resulting in a day length of about 13 hours at the start of forcing and increasing to about 19 hours at mid-

summer. The average daily maximum air temperature in the greenhouse during the growth period ranged from 22°C in April to 31°C in June-July. The average minimum night temperature was 12-15°C througout the season. Very similar temperature regimes existed in the glasshouse during stock plant growth each year.

Shading was achieved by constructing a burlap covered frame over the potted rootstocks. The shade cloth reduced the irradiance to about 20% of ambient light; the light level underneath the shade being about 600 lux (11 $\mu Em^{-2}s^{-1}$ PAR) on cloudy days and about 18.000 lux (330 $\mu Em^{-2}s^{-1}$ PAR) on sunny days. Shading was carried out during 8 June to 19 September 1983 and 24 April to 24 September 1984.

The cuttings were obtained from 20-50 cm long shoots. In Experiments 1 and 2 the shoots were only about 20 cm when obtaining the cuttings. Each of these shoots yielded one 7-10 cm terminal cutting and one 4-node stem cutting. In the remaining experiments the shoots were 25-50 cm long, allowing one terminal cutting and two to four stem cuttings from each shoot. The stem cuttings were of three categories; 2-node, 3-node, and 4-node cuttings. The distal cut was made just above a node. A part of the proximal internode was included in most cuttings. In the main part of the experiments the lower leaf was removed so that 1, 2, or 3 leaves, respectively, were left in the three categories of stem cuttings. A comparison between intact cuttings and cuttings with the lowermost leaf removed was carried out, as well as a study of the rootability of cuttings from the distal part of the stem as compared with the rootability of cuttings from the proximal part.

The effects of root-promoting substances were studied in several experiments (Table 2). Different concentrations of indole butyric acid (IBA) dispersed in talc (Rhizopon AA) were applied to the basal 0.5-1.0 cm while the bases of the cuttings were still wet, thus securing adherence of the rooting powder. The treated cuttings were inserted in dibbled holes to avoid loss of rooting powder. A potassium salt of indole butyric acid, K-IBA (supplied by ICN, Cleveland, Ohio), was dissolved in water in several different concentrations and the 0.5-1.0 cm basal part of each cutting was dipped in these solutions. This was done either by a 5 to 10 second «quick dip» in the concentrated solutions, or by a 24-hour soak in the dilute solutions.

The cuttings were inserted in a mixture consisting of 50% perlite and 50% percent fertilized peat (v/v). In a majority of the experiments, designated «J4» or «J5» in Table 1, the rooting medium was filled in 4 or 5 cm pots made of compressed peat (Jiffy-Strips). In a few experiments, designated «PP», the cuttings were inserted directly in flats containing the peat:perlite rooting mixture. The two potting systems were compared in Experiment 1 but no significant difference was observed.

Each combination of treatments consisted of two or three replications of 4 to 10 cuttings each (Table 2). The inconsistency of the experimental designs was caused by a restricted number of shoots available at the start of each experiment.

Two different environmental regimes were used in the rooting experiments; intermittent mist (I.M.) and fog. The intermittent mist supplied water for three to four seconds each 5 minutes in sunny weather. The frequency of misting was reduced to 10-30 minutes in accordance with the weather conditions. Misting was discontinued at night. The average daily maximum air temperature exceeded 30°C in 1983, but was about 25°C in subsequent years (Table 1). The fog system was based on an ultrasonic humidifier nozzle (Sonicore). Fog was supplied when the relative humidity fell below $90 \pm 10\%$ as controlled by a humidistat. The average daily maximum air temperature was about 25°C. Bottom

heat was maintained at 22±1°C, except when the air temperature exceeded the temperature of the medium on hot summer days. The average minimum air temperatures were 18°C in 1983 and 12-16°C in the subsequent years (Table 1).

Rooting was scored after 5 to 7 weeks in the propagation chamber according to a scale ranging from 0 to 5:

0 = wilted and dead
1 = callused but without roots
2 = poorly rooted
3 = rooted
4 = well rooted
5 = very well rooted

The rooting percentage was calculated by counting the cuttings obtaining root scores of 2, 3, 4, or 5 and dividing by the total number of cuttings behind the treatment at hand. In Experiment 10 the number of roots in each rooted cutting was counted and the average number of roots was calculated.

In five of the May and June experiments, designated «growing-on» in Table 1, the after-effects of shading, cutting category and auxin treatments on the growth of rooted cuttings were studied. Non-rooted cuttings were omitted from this part of the experiments. The cuttings were hardened off for 10-15 days under greenhouse conditions and subsequently planted 10 by 10 cm apart in a plastic greenhouse in a fertilized peat between 3 and 20 July. Fertilizer was added routinely throughout summer. At the end of the growing season the height and caliper of the rootstocks were recorded.

All the results are analysed statistically by using a standard F-test procedure for unbalanced designs. The models include all main effects and all two-way interactions. Three-way and higher level interactions become part of the error term. All the models are tested using an F-test and found to be significant at the 0.1% level. Differences between levels of each main effect are tested according to a multiple comparison procedure; REGWQ (SAS Institute 1982). In the tables, means that are not significantly different are followed by the same letter. All the presented results are significant at least at the 5% level.

RESULTS

Cultivar, stock plant shading, cutting category, treatment with the root promoting substance, and rooting environment all affected rooting in the semi-hardwood cuttings of the apple rootstocks. The most important interactions were observed between the rootstock cultivar and the stock plant shading, the type of cutting, and auxin application, respectively. A summary of the results of the statistical analyses is presented in Table 3.

Cultivar

'MM106' generally rooted better than 'M26'. The overall average rooting percentages for the two cultivars were 59 and 44, respectively. The corresponding average root scores were 2.1 and 1.5.

Stock plant shading

Shading was a highly successful stock plant treatment in all of the three experiments where this treatment was included (Table 3). The overall average rooting percentage was 60 for shaded rootstocks and 35 for non-shaded controls. The corresponding root scores were 2.0 and 1.3; reflecting a 57% increase in the shaded rootstocks.

Rooting environment

The cuttings rooted under two different environmental regimes; intermittent mist and fog. These regimes were compared in three May experiments. In two of the experiments a significant effect of the rooting environment on rooting was observed (Table 4). The fog regime produced the greatest overall rooting

Table 3. Results from the statistical analyses on the effects of cultivar (CV), stock plant shading (SS), auxin application (AA), cutting category (CC), rooting environment (RE), and their two-way interactions on the rooting percentage in semi-hardwood cuttings of the apple cultivars MM106 and M26. Levels of significance; ***: α = 0.1%, **: α = 1%, *: α = 5%, N.S. = not significant, - = not included in experiment.

Exp. no.	CV	SS	AA	CC	RE	CV xSS	CV xAA	CV xCC	CV xRE	SS xAA	SS xCC	SS xRE	AA xCC	AA xRE	CC xRE
1	N.S.	-	***	***	-	-	N.S.	N.S.	-	-	-	-	N.S.	-	-
2	***	-	N.S.	***	-	-	*	***	-	-	-	-	N.S.	-	-
3	***	***	***	***	-	***	***	N.S.	-	N.S.	N.S.	-	N.S.	-	-
4	***	***	-	***	-	***	-	*	-	-	N.S.	-	-	-	-
5	***	***	-	***	***	N.S.	-	***	N.S.	-	N.S.	N.S.	-	-	*
6	***	-	***	**	-	-	*	*	-	-	-	-	N.S.	-	-
7	***	-	-	***	N.S.	-	-	N.S.	N.S.	-	-	-	-	-	N.S.
8	N.S.	-	***	***	***	-	***	***	N.S.	-	-	-	**	N.S.	N.S.
9	***	-	***	***	-	-	N.S.	N.S.	-	-	-	-	**	-	-
10	**	-	***	***	-	-	***	N.S.	-	-	-	-	N.S.	-	-

percentage 50, and the highest root score, 1.9, as compared with the intermittent mist regime, 43% rooting and a root score of 1.7. However, the significant interaction between the rooting environment and the type of cutting which was observed for root score in Experiment 7, indicated that the terminal cuttings suffered under the intermittent mist regime. The root scores were 1.5 for terminal cuttings under mist and 1.7 under fog, while the root scores for 4-node stem cuttings were higher under mist, 2.6, than under fog, 2.3.

Table 4. Effect of rooting environment on the rooting (%) of semi-hardwood cuttings of the apple rootstock cultivars MM106 and M26. Comparisons are tested within experi-ment.

Exp. no.	Fog	Intermittent mist
5	41a	23b
7	57a	59a
8	48a	38b
Balanced mean	50	43

Wounding

In Experiment 1, 50% of the cuttings were wounded by removing a 1.5 cm slice of the bark on the opposite side of the lowermost bud in each cutting. Wounding improved the rooting percentage slightly in both cultivars, but the effect of this treatment was barely significant when compared with non-wounded controls. The rooting percentage increased from 85 to 90% in 'MM106' and from 76 to 90% in 'M26'. There was a significant interaction between wounding and auxin treatment. The wounded cuttings rooted equally well with or without the addition of auxins. In the non-wounded cuttings, however, the rooting percentage was only 69 without auxins, but 93% with a basal dip in 1.0% IBA-talc.

Cutting type

Terminal cuttings rooted less readily than 4-node stem cuttings in 7 out of the 8 experiments were the two cutting categories were included. An average of 40% of the terminal cuttings rooted, with a high of 78 and a low of 19, while 64% of the stem cuttings rooted, with a high of 96 and a low of 29. The average root scores were 1.3 for terminal cuttings and 2.4 for 4-node stem cuttings.

The rooting potential of terminal cuttings was compared with the rooting potential of different sizes of stem cuttings (Table 5). Only 19% of the terminal cuttings rooted in this experiment. An average of 44% of the 2-node stem cuttings rooted, while the rooting of 3- and 4-node cuttings could not be separated statistically; the rooting percentages being 57 and 50, respectively.

The proximal leaf should not be removed from small cuttings (Table 5). Rooting was halved in terminal cuttings and reduced by almost 30% in 2-node stem cuttings when the lowermost leaf was removed. However, in 3-node and 4-node cuttings rooting was not significantly affected by removing the proximal leaf.

Stem cuttings from the distal part of the mid-section of the shoot rooted better than cuttings from the proximal part.

The average rooting percentages were 26 and 14, respectively. The greatest difference was observed in 4-node cuttings, 29% and 11%, percent, while the least difference was observed in the 3-node cuttings, 32 and 22%. Two-node stem cuttings rooted poorly but rooting was twice as high in distal cuttings, 17%, as in proximal cuttings, 8%.

Root promoting substances

A positive effect of auxin applications on the rooting of semi- hardwood cuttings was observed in all three of the experiments where an untreated control was compared with auxin treatments. For instance, in Experiment 1 the rooting percentage increased from 78 to 91 when the bases of the cuttings were treated with 1.0% IBA-talc. The average root score increased by more than 50% from 2.4 to 3.7.

The effects of treating semi-hardwood cuttings with either a 0.5% or a 1.0% IBA-talc were studied in four experiments. Semi-hardwood cuttings of the apple rootstocks rooted consistently better when treated with 1.0% IBA-talc. The average rooting percentages were 61 for 1.0% IBA-talc and 45 for 0.5% IBA-talc. The corresponding average root scores were 2.1 and 1.6.

Cuttings were treated with IBA in several ways in Experiment 10; by dipping in IBA-talc, by a quick dip in 500-2000 ppm solutions, or by a 24-hour soak in 50-200 ppm solutions. The results are presented in Table 6 and in Figure 1. When averaging over cultivar, the percentage rooting increased subsequent to all the IBA treatments as compared with the untreated control, although many treatments did not differ significantly from the control. The quick dip and the 24-hour soak very often produced higher rooting percentages than treatments with comparable concentrations of IBA-talc. Within each kind of treatment

Table 5. Effect of removal of the proximal leaf on the percentage rooting in different categories of semi-hardwood cuttings of the apple rootstock cultivars 'MM106' and 'M26'.

Type of cutting	State of proximal leaf		Balanced mean
	Removed	Not removed	
Terminal	13c	26b	19c
Stem			
2-node	37b	51a	44b
3-node	62a	53a	57a
4-node	58a	42a	50ab
Balanced mean	43NS	43NS	

Table 6. Effects of different treatment procedures and concentrations of indole butyric acid (IBA) on percentage rooting in semi-hardwood cuttings of the apple root stock cultivars'MM106' and'M26'.

	'MM106'	'M26'	Bal. mean
Control	54bc	38b	46d
IBA-talc			
0.5 %	46c	67ab	56cd
1.0 %	79abc	42b	60cd
2.0 %	71abc	63ab	67abcd
IBA-5 sec. dip			
500 ppm	54bc	63ab	58cd
1000 ppm	71abc	67ab	69abcd
2000 ppm	83ab	92a	88ab
IBA-24 h. soak			
50 ppm	92a	33b	63bcd
100 ppm	88ab	63ab	75abc
200 ppm	96a	83a	90a
Bal. mean	73a	61b	

there was a concentration-dependent effect on the rooting percentage. The 2000 ppm quick dip and the 200 ppm 24-hour soak produced the highest rooting percentages.

The 24-hour soak as well as the 1.0% IBA-talc treatment produced the greatest number of roots (Fig. 1). On average, 'MM106' cuttings produced about twice as many roots as 'M26' cuttings; about 15 as opposed to about 8. The optimum treatment when both percentage rooting and number of roots were taken into account was a 24-hour soak in 200 ppm K-IBA.

Interactions

Shading affected the two rootstock cultivars in significantly different ways. However, this interaction was observed only in Experiments 3 and 4, which were carried out in July and September 1983 (Table 7). On average, the rooting percentage increased from 64 to 81% in 'MM106' and from 21 to 60% in 'M26' when the stock plants had been shaded as compared with non-shaded controls.

The effect of shading was also affected by the time of year (Table 7). The July rooting percentage increased by only 11% in 'MM106', from 78 to 89%, while the September rooting was improved by 24%, from 44 to 68%. In 'M26', rooting was also mostly improved in the latter part of the season. The rooting percen-

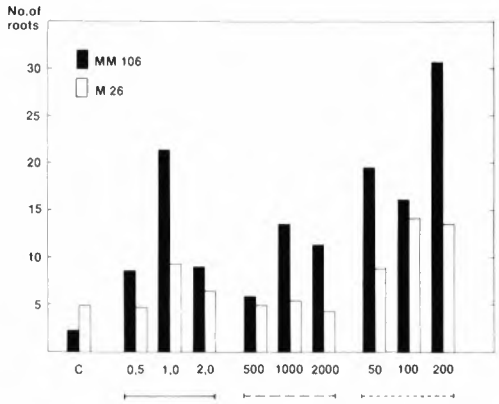

Figure 1. Effects of different indole butyric acid (IBA) treatment procedures and concentrations (C = control; ——talc, % conc.; - - - - "quick dip", ppm; ------ 24-hour soak, ppm) on the number of roots produced in each rooted semi-hardwood cutting of the apple rootstock cultivars MM106 an M26.

Table 7. Interaction between time of year, rootstock cultivar and shading on the rooting percentage in semi-hardwood cuttings of the apple rootstock cultivars 'MM106' and 'M26'.

		July	September	Bal. mean
'MM106'	Control	78	44	64
	Shade	89	68	81
'M26'	Control	24	15	21
	Shade	58	62	60

tage more than doubled, from 24 to 58% in July, but quadrupled, from 15 to 62% in September.

The auxin concentrations affected the rooting of 'MM106' and 'M26' cuttings somewhat differently (Table 6 and Fig. 1). In 'MM106' the concentration dependent effect was hardly noticeable, except for the quick dip treatments. The 0.5% IBA-talc and the 500 ppm quick dip produced a significantly lower number of roots than the 24-hour soak treatments. In 'M26' the concentration dependent effect was more obvious, especially for the 24-hour soak treatments.

The number of roots in 'MM106' cuttings was greatest subsequent to the 200 ppm 24-hour soak. In 'M26' the increase in root number was not so clearly demonstrated, although the 100 ppm and the 200 ppm 24-hour soaks were significantly better than most of the other treatments.

Terminal cuttings rooted less readily in 'M26' than in 'MM106' (Table 8). While an average of 37% of the terminal cuttings rooted in 'MM106', the average rooting percentage in 'M26' was only 15. In 4-node stem cuttings there was no noticeable difference between the two cultivars. While 4-node 'MM106' stem cuttings rooted only 60% better than terminal cuttings, the rooting percentage in 'M26' almost quadrupled when comparing 4-node stem cuttings with terminal cuttings.

There was no consistent interaction between applied auxins and type of cutting.

Growth of the rooted cuttings

In 4 of the 5 experiments in which the rooted cuttings were growing on to study the after-effects of shading, auxin application and cutting type, no difference was observed between the two rootstock cultivars. However, in Experiment 2 caliper and height were significantly smaller in 'M26' than in 'MM106' rootstocks. This was especially the case for terminal cuttings as witnessed by an average caliper of only 2.0 mm as compared to calipers of 4.5-5.5 for 4-node 'M26' stem cuttings and for both terminal and stem cuttings of 'MM106'.

Terminal cuttings generally grew less vigorously than 4-node stem cuttings. This was true both for caliper growth and for height. The average difference in the five experiments was 1 mm in caliper, 4.5 mm as opposed to 3.5 mm, and 8 cm in height, 30 cm as opposed to 22 cm.

In Experiment 1 IBA-treated cuttings grew more vigorously than non-treated controls (Table 9). The positive effect of IBA-treatment was most pronounced for the stem cuttings. Application of different auxin concentrations generally did not affect the growth of the rooted cuttings. Nor did stock plant shading affect the growth of rooted cuttings.

There was a close relationship between the average root scores and the average scores for caliper and height. Well-rooted cuttings grew more vigourously than poorly rooted cuttings.

Table 8. Interaction between cultivar and type of cutting on the percentage rooting in semi-hardwood cuttings of the apple rootstock cultivars 'MM106' and 'M26'.

Exp. no.	'MM106'		'M26'	
	Terminal	4-node stem	Terminal	4-node stem
2	93	99	40	90
5	22	40	8	60
8	24	56	15	45
Bal. mean	37	58	15	55

DISCUSSION

Rooting experiments often produce inconsistent results, as observed in some of these apple rootstock experiments. Nursery trials with softwood and semi-hardwood cuttings may succeed in many instances but often prove unsuccessful. The inconsistent results can often be traced to one of two factors: rooting environment or vigour of stock plants.

The rooting environment is essential for leafy cuttings. When the cutting is severed from the stock plant, it will need immediate transfer to an environment in which the cutting is allowed to keep turgid until new roots are formed. A relative air humidity close to 100% and a controlled air temperature are the most important factors for keeping the cuttings turgid over prolonged periods of time (Moss & Dalgleish 1985). The intermittent mist will keep a thin layer of water on the leaf surface. Evaporation of surface water will lower the leaf temperature and thereby reduce transpiration and respiration as well as increase the humidity around the leaves (Hartmann & Kester 1983). A fog system based on an ultrasonic humidifier nozzle (Sonicore) (MacDonald 1985) resulted in improved rooting in two out of three May experiments (Table 4). In early summer high air temperatures are less common than later in the growing season, making the fog system more advantageous at this time of year. This agrees with the statement by Moss & Dalgleish (1985) that fog is most suited for rapid rooting species and species that are not affected by high leaf temperatures during rooting. Apple rootstocks are not especially rapid rooting, nor do the big leaves tolerate high temperatures well.

Increasing age or loss of juvenility is one of the most important single factors

Table 9. Interaction between type of cutting and auxin application on the root score and caliper growth of rooted semi-hardwood cuttings of the apple rootstock cultivars 'MM106' and 'M26'.

	Type of cutting				Balanced mean Caliper
	Terminal		4-node stem		
	Root score	Caliper	Root score	Caliper	
Control	2.1	3.8	2.8	4.9	4.5b
IBA-talc (1%)	2.9	4.2	4.5	6.4	5.6a
Balanced mean		4.0b		5.8a	

limiting the rooting ability of many difficult-to-propagate species (Nelson 1977, Hartmann & Kester 1983). The most effective way of keeping stock plants juvenile is by heading hard back, thus securing new vigorous growth. In these experiments the stock plants at the start of the experiments were clearly juvenile. Furthermore, they were headed back at the start of each growing season. Stock plant growth, however, was affected by the growing conditions. Ageing undoubtedly progressed, as witnessed by some of the stock plants producing flowers in their fourth year. A decreasing rootability of cuttings obtained from stock plant series 1 during the three years 1983-85 may, at least in part, result from loss of juvenility of the stock plants.

Environmental factors during stock plant growth may influence the rootability. A reduced light level to the stock plants improves rooting of semi-hardwood cuttings of apple rootstocks. This result agrees with earlier observations (Doud & Carlson 1977, Delargy & Wright 1978, Harrison-Murray & Howard 1982, Hartmann & Kester 1983). An extreme reduction of the irradiance, excluding as much as 95-98% of normal daylight seems to be just as effective as complete exclusion of light. According to the results presented in this paper, even an 80% reduction of the light level will improve rooting in apple cuttings. A practical consequence of these findings is that stock plants may benefit from growing under low light, for example underneath greenhouse benches or in artificially lit storage rooms.

However, the low light level will normally reduce photosynthesis and biomass production in the stock plants and thereby also the number of cuttings produced. In hard-to-root species one will have to consider the combined effect of increased rootability and reduced number of cuttings. A larger number of poorly rooting cuttings obtained under full light may be less advantageous than a smaller number of easily rooted cuttings obtained from shaded stock plants.

The relative effect of shading was greater in the latter part of the growing season. Shading keeps the developing shoots green and less lignified than sun-grown shoots. This condition favours rooting. However, other factors, like air temperature, nutrition, and pathogen attacks, may also be important. In stock plants grown in controlled environment chambers the level of irradiance did not affect the rooting percentage or the number of roots per cutting in 'M26' rootstocks (Christensen et al. 1980). Cuttings obtained from the low irradiance stock plants, however, produced the greatest amount of roots as a response to auxin application.

'M26' and 'MM106' apple rootstocks are both considered easy to propagate (Hartmann & Kester 1983). However, 'M26' is often found to be harder to root than 'MM106' (Howard 1971, Gorecki 1979), a result in agreement with the findings in our experiments (cf. Tables 6, 7, and 8).

The rootability is affected by the time of year. In our experiments, May cuttings of 'M26' and May and July cuttings of 'MM106' rooted excellently, 97%. Cutting rootability was dramatically reduced on the later dates of sticking; the rooting percentage decreasing to 50-70. Cuttings obtained in spring and early summer when the shoot is growing vigorously often root more readily than cuttings taken later in the growing season (Fischer 1969, Hartmann & Kester 1983, Caldwell & Coston 1986, Hansen 1988). As the shoots mature the cuttings will become more lignified and harder to root. Also, the greenhouse environment is not the same in July as in May, temperatures being higher in July, relative air humidity lower. Such environmental factors may affect stem structure and the physiological condition of the shoot and thereby cause differences in rooting of cuttings obtained from the shoots.

Terminal cuttings within semi-hardwood shoots of the apple rootstocks rooted less readily than stem cuttings (Table 5). Terminal cuttings are difficult to keep turgid when severed from the stock plant, especially during hot periods in summer. Moisture stress caused by high temperatures and low relative humidity did occur during the rooting process in some of the experiments. This caused leaf scorching, and thus reduced the potential for rooting.

Terminal cuttings and 2-node stem cuttings are small and will contain less carbohydrates and other compounds (e.g. auxin) than larger cuttings. This may be a reason for poor rooting (Grange & Loach 1984, Caldwell & Coston 1986). On the other hand, when using small cuttings, stock plant material is utilized well, and even small cuttings root readily in some species (Orton 1978, Smalley & Dirr 1987).

Stem caliper has been associated with the ease of cuttings to root. In kiwi-fruit, cuttings of 2.0 to 4.0 mm diameter rooted readily while cuttings greater than 8.0 mm rooted very poorly (Avery & Beyl 1986). The stem caliper is related to position within the shoot. The proximal part of the stem will have a greater diameter than the distal part. In our experiments distal cuttings obtained from apple summer shoots rooted more readily than proximal cuttings. This agrees with observations in many other species (Fischer 1969, Hartmann & Kester 1983, Marini 1983, Caldwell & Coston 1986).

Cuttings should be allowed to keep as large a leaf area as they can support without wilting (Zimmermann 1925). Leaf removal is time consuming and did not improve rooting in 17 of 18 genera examined by Cumming (1968). Reuveni & Raviv (1981) found that the rooting percentage was correlated with the number of leaves retained on the cuttings. The speed of rooting, too, was determined by the number of leaves. In the apple cuttings studied in these experiments leaf removal was detrimental to rooting in the smaller cuttings, but did not affect rooting in the larger cuttings.

Wounding the base of each cutting is a common practice in many hard-to-root species, e.g. in rhododendrons (Hartmann & Kester 1983). Wounding *per se* has little effect on the ultimate rooting percentage, but has a definite effect on the quality and quantity of roots produced and the speed of rooting (Wells 1962, Orton 1978). However, when rooting is scored before it is completed, a higher percentage may be observed in wounded cuttings, because these cuttings root more rapidly than non-wounded cuttings. Wounding improved rooting percentage and root score in both apple rootstock cultivars, although only slightly. This corresponds with the observations by Gorecki (1979) that wounding increased the rooting percentage, as well as the number of roots and root length in each cutting in several apple rootstock cultivars, including 'M26' and 'MM106'. The positive interaction between auxin treatment and wounding is commonly observed (Nahlawi 1970). Auxins are made more readily available for the cuttings when the surface for uptake in each cutting is increased.

Auxin application enhances rooting in cuttings of most species. But it is not a precondition for rooting in semi- hardwood cuttings of the apple rootstocks. However, both the percentage rooting and the number of roots in each cutting increase subsequent to auxin treatments. In fact, an increase in the number of roots was observed in Experiment 10 (Fig. 1) and indirectly through a higher root score in most of the other experiments. Four-node stem cuttings, which were treated with 1.0% IBA-talc scored 4.5 as compared with scores of 2.1-2.9 in non-auxin-treated cuttings and in terminal cuttings (Table 9). These well-rooted cuttings grew substantially more vigorously than the poorer rooted cuttings (cf. Dirr 1981).

It is difficult to recommend one optimum concentration of auxins for each plant species (Howard 1986). The optimum concentration will depend on time of year, cutting material, auxin application method, and cultivar (Chong 1981, Berry 1984). However, semi-hardwood cuttings of apple rootstocks, if treated with IBA-talc, seem to benefit from concentrations of at least 1.0%. In Experiment 9 the rooting percentage dropped from 52 to 33 when the cuttings were treated with 2.0% IBA-talc instead of 1.0%. Conversely, rooting was not negatively affected by the higher concentration in Experiment 10 (Table 6). Further studies are obviously needed to examine the effects of IBA-talc concentrations on rooting of stem cuttings at different stages of maturity.

In the semi-hardwood apple cuttings auxin solutions generally improved rooting more than talc treatments did. The higher concentrations of quick dips and 24-hour soaks were more effective than the lower concentrations. A wide range of IBA concentrations are recommended for semi-hardwood cuttings of fruit species: 2.500-10.000 ppm for quick dips (Hartmann et al. 1963, Chong 1981, Dirr 1981, Lyons et al. 1985) and 20-200 ppm for 24-hour soaks (Hartmann & Kester 1983, Gur et al. 1986). The optimum concentration will depend on the cultivar and the maturity of the cutting stem; early-season cuttings requiring less auxins than late-season cuttings (Dirr 1981).

SUMMARY

When apple rootstocks are propagated by semi-hardwood cuttings one should use 3- or 4-node stem cuttings, preferably from the distal part of the shoots. Proximal stem cuttings as well as terminal cuttings root less readily. Wounding improves rooting only slightly. Auxin application is necessary for successful rooting. 1.0% IBA dispersed in talc is recommended, although the effects of higher talc concentrations need further studies. Solutions of IBA salts generally are more effective than IBA- talcs but these formulations are not available for Norwegian growers at the moment. A fog propagation system is likely to produce excellent results in spring, but a conventional intermittent mist may be more advantageous through the summer months. Shading the stock plants produces more easily rooted cuttings, but the total yield of cuttings is reduced.

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HOST PLANT PREFERENCES AND LARVAL PERFORMANCE OF *DELIA FABRICII* HOLMGREN (DIPTERA: ANTHOMYIIDAE) IN THE LABORATORY

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In two trials carried out on a laboratory population of *Delia fabricii* Holmgren it has been found that the flies prefer pots with *Poa pratensis* L. plants for egg deposition to any of the following species: *Phleum pratense* L., *Dactylis glomerata* L., *Agrostis capillaris* L., *Festuca rubra* L., *Festuca pratensis* Huds., *Phalaris arundinacea* L., *Bromus inermis* Leyss., *Elytrigia repens* Nevski, *Hordeum vulgare* L. and *Avena sativa* L. In a third trial with five species other than *P. pratensis* it has been found that significantly more eggs are deposited in pots with *A. capillaris* than with any other species. Larvae can infest shoots of *P. pratensis*, *P. pratense*, *D. glomerata*, *A. capillaris*, *F. rubra*, *F. pratensis*, *P. arundinacea* and *E. repens*. Pupae develop in all these species except *A. capillaris* and *F. rubra*, probably because of weak plant growth vigour.

Key words: Antibiosis, *Delia fabricii*, host preference, gramineae, larval performance, tolerance.

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Delia fabricii Holmgren has a circumpolar high latitude distribution (Hennig 1966-1976). Its natural habitats are meadows and grass fields (Lindroth 1931, Nielsen et al. 1954), birch woods and willow shrubs (Collin 1931, Nielsen et al. 1954) and boggy land (Lindroth 1931, Andersson 1967) based on catches of adult flies. Larval damage to agricultural plants has only occasionally been reported. Znamenskaya (1941) observed infestation by *D. fabricii* in young wheat, oats and barley in the Murmansk district (67.5°N) in the USSR. The larvae were assumed to have migrated from couch grass (*Elytrigia repens* Nevski). In Northern Norway, *D. fabricii* has infested *Poa pratensis* L. grown in sparse stands for seed production at Bardu (69°N) (Johansen 1990).

In the event of future high latitude seed production, it is important to assess whether species other than *P. pratensis* may act as host plants for *D. fabricii*. In this paper the oviposition preferences and larval performance on different gramineous species in the laboratory are reported. The paper is a revised part of a thesis on morphological and ecological descriptions of *D. fabricii* (Johansen 1988).

MATERIALS AND METHODS

The insect material was taken from a laboratory population of *D. fabricii*. The rearing methods are described elsewhere (Johansen 1988). In all experiments, the temperature was 18/12°C day/night with 16/8 hours light/dark. The lighting was provided by 400 W HPI/T lamps. The plant species and cultivars used were *Poa pratensis* L. 'Lavang', *Phleum pratense* L. 'Engmo', *Dactylis glomerata* L. 'Hattfjelldal', *Agrostis capillaris* L. 'Leikvin', *Festuca rubra* L. (breeding population no. 061098), *Festuca pratensis* Huds. 'Salten', *Phalaris arundinacea* L. (breeding population «Hansvoll»), *Bromus inermis* Leyss. 'Løfar', *Elytrigia repens* Nevski, *Hordeum vulgare* L. 'Agneta' and *Avena sativa* L. 'Gråkall'.

The host plant preference experiments were carried out with approximately 400 adult *D. fabricii* in a cage measuring 70 x 70 x 50 cm. The grass plants were grown in 8 cm pots in fertilized peat soil with an upper layer of sand. All the plants were 7 weeks old when the experiments started. Selected plants were arranged in three successive experiments in a latin square design within the cage. The exposure times covered six days. After exposure the eggs were separated from the soil by washing through meshes (Gemmill 1927).

The plants used in the larval performance experiments, except the cereal species, had been stored for 10 weeks at 6°C and 10/14 hours light/dark, followed by 18 weeks at 3°C in darkness. When the experiments started, the plants were transplanted to 8 cm pots with fertilized peat soil. The cereal plants had been sown directly in 8 cm pots two weeks earlier. Twenty post-diapausing eggs were deposited on the soil among the shoots in each of 20 pots per plant species. The total number of shoots varied from 5 in the cereal species to 24 in *P. pratensis* one week after egg deposition.

The rate of infested shoots was calculated after one week by dissecting all the shoots in two pots per plant species. After four weeks, the total number of attacked shoots was counted in two other plants. Also after four weeks, the vigour of the remaining plants was scored by comparison with an uninfested plant. The pupae were sieved from the soil and maintained at room temperature

Table 1. Percentages of eggs laid on different gramineae when exposed simultaneously to a laboratory population of *Delia fabricii*

Gramineae	Trial no.	% of eggs laid		
		1 (n=6)	2 (n=6)	3 (n=5)
Poa pratensis L.		48a[1)]	81a	-
Phleum pratense L.		17b	-	17b
Dactylis glomerata L.		12b	-	21b
Agrostis capillaris L.		9b	-	39a
Festuca rubra L.		8b	-	7b
Festuca pratensis Huds.		6b	-	-
Phalaris arundinacea L.		-	8b	16b
Bromus inermis Leyss.		-	1b	-
Elytrigia repens Nevski		-	6b	-
Hordeum vulgare L.		-	2b	-
Avena sativa L.		-	2b	-
	Egg totals	306	220	505

[1)] Entries with different letters in each column are significantly different (Newman-Keule, $P<0.05$).

until fly emergence. Weight and length estimates were calculated when more than 10 pupae per plant species were found.

RESULTS

P. pratensis was clearly the preferred host plant for egglaying in the first two trials (Table 1). No significant differences occurred among the other species. In the third trial, which was set up without *P. pratensis*, significantly more eggs were deposited in pots with *A. capillaris* than with any other species.

The newly hatched larvae were able to infest all the plant species included in the trials, except *B. inermis* and the cereal species (Table 2). *D. glomerata* and *P. arundinacea* seemed fairly unaffected in terms of vigour, despite being infested and fed on. No larvae developed into the pupal stage on *F. rubra* and *A. capillaris*, even though both were infested. These plants had impaired growth when the experiments started, however, and were severely injured after infestation.

The number of pupae differed from 0.3 to 3.8 per plant (Table 2). Regarding pupal size in most plant species, the total number of pupae was too small to give significant estimates. However, the pupae developed in *F. pratensis* were significantly larger than those developed in *P. pratensis* (Table 2). This also corresponded to a higher percentage of fly emergence in *F. pratensis*. The other fly emergence percentages were based on too few pupae to be reliable estimates.

DISCUSSION

These results do not allow ranking of all the plant species with regard to host plant preference of *D. fabricii* for egglaying. *P. pratensis*, however, was clearly preferred when more species were tested simultaneously. The preference for *A. capillaris* when *P. pratensis* was absent is somewhat striking and should be investigated further. Additional trials, with the plant species exposed individually to egglaying females in the laboratory, would probably give more information about the host attractive-

Table 2. Laboratory performance of *Delia fabricii* larvae in pots with different gramineae

Gramineae	% of shoots infested[1]	% of shoots attacked[2]	Growth vigour[3]	Mean no. of pupae (±S.E.) (n=12)	Average pupal weight (mg)	Pupal length (mm)[4]	% fly emergence
Poa pratensis L.	42	97	10	3.8 ± 0.9	6.8	4.8-5.0	61
Phleum pratense L.	13	100	50	1.2 ± 0.2	7.5	4.8-5.2	79
Dactylis glomerata L.	33	54	100	0.3 ± 0.2	-	-	33
Agrostis capillaris L.	25	86	30	0	-	-	-
Festuca rubra L.	18	95	10	0	-	-	-
F. pratensis Huds.	21	100	60	3.7 ± 0.8	8.5	5.2-5.4	89
Phalaris arundinacea L.	13	49	90	0.3 ± 0.2	-	-	67
Bromus inermis Leyss.	0	0	100	0	-	-	-
Elytrigia repens Nevski	20	18	50	0.7 ± 0.2	-	-	88
Hordeum vulgare L.	0	20	100	0	-	-	-
Avena sativa L.	0	0	100	0	-	-	-

[1] Average of two random plants one week after egg deposition.
[2] With feeding marks (average of two random plants four weeks after egg deposition).
[3] All (12) plants scored visually after four weeks (control plants = 100).
[4] 95% Confidence intervals.

ness of each species. Such experiments have been described by Vickerman (1978).

The larval performance experiments clearly show that several plant species are suitable for infestation by and the development of *D. fabricii* larvae. Obviously there are differences among the species, but, ranking the degree of antibiosis and/or tolerance (cf. Bardner & Fletcher 1974) is difficult based on these experiments. The difference in the number of shoots and the growth stage at infestation, the variable health status after storing, and some occasions of food deficit for the larvae, may also have an influence on the outcome of the infestation. However, the most suitable hosts for larval development seemed to be *P. pratensis*, *P. pratense* and *F. pratensis* (cf. Table 2). *F. pratensis* produced a similar number of pupae to *P. pratensis*, but was less affected with regard to vigour. This was probably due to a lower infestation rate and/or a higher tolerance. Food deficit for the larvae, caused by a high percentage of infestation, was probably the reason for smaller pupae and a lower percentage of fly emergence in *P. pratensis* than in *F. pratensis* (Table 2). Food deficit for the larvae was probably also the reason for unsuccessful pupal development in *F. rubra* and *A. capillaris*. Further experiments with healthy plants must be carried out before conclusions can be drawn about the host plant suitability of these species.

D. glomerata, *P. arundinacae* and *B. inermis* showed a high degree of antibiosis/tolerance, being little affected by infestation and by producing a small number of pupae. These species would probably not be damaged in a field situation. The larval performance in *E. repens* in these experiments supports Znamenskaya's (1941) assumption that *E. repens* is a natural host for *D. fabricii*. The cereal plants included in the present experiments seemed not to be subjected to egglaying and infestation by *D. fabricii* (Tables 1-2). This does not, however, contradict Znamenskaya (1941), who observed attacks on cereal plants. He probably observed third instar larvae that had migrated from *E. repens* in search of new shoots on which to feed (cf. Johansen 1988). Znamenskaya's (1941) observations indicate, however, a wider potential host range for large larvae than for newly hatched larvae, as do the present experiments.

The combined results from the egglaying and larval performance experiments strongly indicate that *P. pratensis* is the species most vulnerable to attack from *D. fabricii*. Only field observations can verify whether the other plant species used as larval hosts in the laboratory are also hosts for natural populations of *D. fabricii* in the field. Of special interest are «no choice» situations like monocultures.

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SAP-TRANSMISSIBLE VIRUSES IN LETTUCE (*Lactuca sativa* L.) IN NORWAY: IDENTIFICATION AND IMPORTANCE

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Blystad, D. 1989. Sap-transmissible viruses in lettuce (*Lactuca sativa* L) in Norway: indentification and importance. Norwegian Journal of Agricultural Sciences 3:373-384. ISSN 0801-5341.

Lettuce grown in the open or in glasshouses was surveyed for sap- transmissible virus diseases. The virus isolates obtained were identified by their reaction in test plants and by immunosorbent electron microscopy (ISEM). The viruses found were cucumber mosaic virus, dandelion yellow mosaic virus, lettuce mosaic virus and tobacco necrosis virus. Cucumber mosaic virus and dandelion yellow mosaic virus were found to occur in field crops in Asker, Akershus County, and in Lier, Buskerud County. Lettuce mosaic virus was found only in seed samples. Tobacco necrosis virus was found to be prevalent in roots of lettuce grown by the nutrient film technique. These viruses did not result in economic damage in any of the crops surveyed.

Key words: virus, sap-transmissible viruses, lettuce, immunosorbent electron microscopy.

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Several viruses infect lettuce and cause diseases (Campbell 1985) and because of the economic importance of this crop world-wide, considerable attention has been given to these virus diseases.

In Europe several sap-transmissible viruses of lettuce are reported, the most important of which are lettuce mosaic virus, LMV (Kassanis 1947, Broadbent et al. 1951, Lot 1988) and cucumber mosaic virus, CMV (Gippert 1973, Weidemann & Rohloff 1976). Also occurring, but more irregularly, are arabis mosaic virus, broad bean wilt virus, dandelion yellow mosaic virus, DYMV (lettuce necrosis virus), strawberry latent ringspot virus, tobacco necrosis virus, TNV, tobacco rattle virus, tomato black ring virus and tomato spotted wilt virus (Horváth 1980). Artichoke Italian latent virus (Martelli 1988) and alfalfa mosaic virus (Ragozzino et al. 1978) are reported to occur in lettuce in Italy. Carnation mottle virus has been isolated once from lettuce (Tomlinson & Faithfull 1976).

In Norway lettuce is grown to supply the home market. Some specialist growers grow it continuously in greenhouses all year round, but there is also lettuce grown in the open in summer. Because of the importance of lettuce as a vegetable crop and because of the many virus problems reported from abroad, it was of interest to find out which viruses were actually causing diseases in Norwegian lettuce production.

MATERIALS AND METHODS

Virus isolates, transmission and host range studies

Virus isolates were obtained from plant material with virus-like symptoms collected during surveys performed in 1982, 1983, 1985 and 1986 from both field- and greenhouse-grown lettuce. From lettuce grown in the open, leaf samples, or whole plants with symptoms, were taken for testing. From lettuce grown by the nutrient film technique (NFT) roots were collected for testing. Seed lots were examined for seed transmission of LMV by visual observation of seedlings or by testing of seed samples to *Chenopodium quinoa*.

The collected samples were stored at +4°C before inoculation to test plants. Mostly *C. quinoa* and lettuce seedlings of butterhead varieties were used in the first attempt to transmit virus to indicator plants. In the beginning this was done by grinding tissue in 0.02 or 0.03 M phosphate buffer, pH 7.0 without additives. Later it was found that the additives sodium disulfite (0.25%), sodium diethyl dithiocarbamate, DIECA (0.5%), and 7% activated charcoal (Sigma) were beneficial when inoculating leaf extracts (Bos et al. 1983).

Leaves of indicator plants were dusted with carborundum (400 mesh) and the inoculum was applied with a cotton swab. Excess inoculum was immediately rinsed away with water. Control plants were rubbed with buffer and carborundum only.

Test plants were placed in a heated greenhouse at a day temperature of ca. 20°C and a night temperature of ca. 16°C. In summer the greenhouse was shaded and in winter additional light was given. Test plants were observed daily and back inoculated to *C. quinoa* when necessary.

The isolates were periodically maintained in lettuce and *C. quinoa*, and they were stored as intact leaves in a deep-freezer (-20°C) or desiccated over $CaCl_2$ at +4°C.

After grouping the isolates by symptoms produced in *C. quinoa* and lettuce, the isolates of each virus group were compared in a limited number of test plants consisting of *C. quinoa*, *Cucumis sativus* cv. Farbio, *Lactuca sativa* cvs. Salina and Brasil 48, *Nicotiana tabacum* cv. White Burley and *Phaseolus vulgaris* cv. Stella. For LMV isolates *Chenopodium amaranticolor* was included and for the DYMV isolates *Gomphrena globosa* as test plants. The TNV isolates were compared in *C. quinoa* and *P. vulgaris* cvs. Stella and Prince only.

Among the isolates, one in each group was inoculated to a broader range of test plants. These selected isolates, called type isolates, were:

- CMV-isolate SaA85-1, originally isolated from a lettuce cv. Salina plant with severe leaf necrosis found in Asker, Akershus County.
- DYMV-isolate 704, obtained from a stunted, mottled lettuce plant found in Lier, Buskerud County.
- LMV-isolate SaF85-1 which was isolated from a seedling of lettuce cv. Prado with mosaic symptoms.
- TNV-isolate L1, obtained from roots of a lettuce plant from a commercial NFT culture in Østfold County.

Electron microscopy

Electron microscopy was performed using 400 mesh copper grids coated with pure carbon film. The grids were examined in a Jeol JEM 1200 EX electron microscope at the electron microscopy laboratory of the Agricultural University of Norway (AGREM). Negative staining was done according to the Brandes dip method as described by Milne (1984).

The LMV isolates were tested in a decoration test (Milne & Luisoni 1977). A piece of leaf tissue, 0.5 cm^2, was minced with six drops of distilled water. A grid was floated on a drop of the resultant extract for one minute, drained with filter paper and placed on a drop of LMV

antiserum diluted 1:100 in 0.1 M phosphate buffer, pH 6.5, for five minutes, stained with buffered (pH 7.0) 2% (w/v) phosphotungstic acid (PTA), drained and dried.

The identity of all isolates was further investigated by immunosorbent electron microscopy (ISEM) (Milne & Luisoni 1977). Antiserum to CMV (titer 1:258) was supplied by M. Christensen, Statens Planteværnscenter, Lyngby, Denmark, and antisera to LMV (titer 1:1024), DYMV (titer 1:64), «TNV-soil» (titer 1:1024) and «TNV-bean» (titer 1:1024) were supplied by D.Z. Maat, Instituut voor Plantenziektenkundig Onderzoek (IPO), Wageningen, The Netherlands. The «TNV-bean» antiserum has been produced against a stipple-streak strain of TNV. The antisera were diluted in 0.1 M phosphate buffer, pH 6.8. The ISEM procedure for DYMV is outlined in Fig. 1.

In the ISEM test of the isolates of CMV, the protein A step was omitted. The antiserum to CMV was diluted 1:10 only, and the tissue extracts were made by grinding 1 part leaf tissue in 2 parts of 0.5 M citrate buffer pH 6.5-7.0 containing 5 mM EDTA and 0.5% thioglycollic acid (Francki & Hatta 1980).

Incubation time	Incubation step	
5 min	PROTEIN A	10 µg/ml diluted in phosphate buffer[1])
	rinsing	30-50 drops phosphate buffer[2])
	draining	
5 min	ANTISERUM	diluted 1:100 in phosphate buffer[2])
	rinsing	30-50 drops phosphate buffer[2])
	draining	
20 min	LEAF EXTRACTS	diluted 1:3 in phosphate buffer with additives[3])
	rinsing	30-50 drops distilled water
	draining	
	STAINING	6 drops uranyl acetate (2%)
	draining	
	drying	
	EXAMINATION	

1) 0.03 M phosphate buffer, pH 7.0
2) 0.1 M phosphate buffer, pH 6.8
3) 0.03 M phosphate buffer, pH 7.0 with the additives sodium disulfite (0.25%), sodium diethyl dithiocarbamate (DIECA, 0.5%) and activated charcoal (7%)

Figure 1. The steps used when DYMV was subjected to immunosorbent electron microscopy (ISEM).

Table 1. The number of identified virus isolates obtained from lettuce plants in different counties

County	Number of lettuce cultures surveyed	Number of virus isolates		
		CMV	DYMV	TNV
Østfold	8	0	0	1
Akershus	8	5	3	0
Buskerud	13	0	1	0
Vestfold	5	0	0	5
Hordaland	7	0	0	4
	41	5	4	10

CMV = cucumber mosaic virus
DYMV = dandelion yellow mosaic virus
TNV = tobacco necrosis virus

Table 2. Results of host-range studies with the type isolates of four viruses isolated from lettuce plants

Plant species and cultivars tested		Viruses and type isolates			
		LMV SaF85-1	CMV SaA85-1	DYMV 704	TNV L1
Brassica campestris ssp. pekinensis cv. Nagaoka 50 days		--	-*-*		
Calendula officinalis cv. Dania				l-	L-*
Chenopodium amaranticolor		LS	L-*	L(S)	L-*
Chenopodium quinoa		LS	L-*	L(S)	L-
Cucumis sativus		--	LS	--	L-
Datura stramonium			LS	-*-	L-
Gomphrena globosa		L-		LS	L-*
Lactuca sativa cv.	Brasil 48	-*(s)	-*s	LS	
	Ostinata	-*S		LS	L-*
	Prado	-*S	-*s	LS	
	Salina	-*S	-*s	LS	
	Salmini		-*s		
Lycopersicon esculentum			-*S		
Nicotiana benthamiana -9		lS		ls	
Nicotiana clevelandii		-*-*	-*S	l-	L-*
Nicotiana glutinosa		--	LS		
Nicotiana tabacum cv. White Burley		--*	(L)S	l-	L-*
Ocimum basilicum				l-	L-*
Petunia hybrida		--	-*s		L-
Phaseolus vulgaris cv. Felibon			L-	-*-*	L-*
	Stella	--*	L-	--	L-
	Prince				L-*
Pisum sativum cv. Little Marvel		-*S			
Spinacia oleracea cv. Viking		-*s	-*S		L-*
Tetragonia expansa					L-*
Vicia faba cv. Pirhonen					L-

L = local symptoms; l = latent local infection; S = systemic symptoms; s = latent systemic infection; - = no infection; -* = no symptoms, but not tested by back inoculation
Symbol in parentheses = reaction erratic

LMV = lettuce mosaic virus; CMV = cucumber mosaic virus, DYMV = dandelion yellow mosaic virus; TNV = tobacco necrosis virus.

In the ISEM tests of TNV and LMV isolates, the protein A step was omitted and the antisera were diluted 1:1000. Tissue extracts were made and diluted to a final dilution of 1:10 in 0.1 M or 0.03 M phosphate buffer pH 6.8 and pH 7.0; the grids were stained with 2% (w/v) uranyl acetate or 2% PTA.

RESULTS

Viruses were isolated from 29 lettuce samples from growers' lettuce cultures. Nineteen of these virus isolates were further identified as CMV, DYMV and TNV with five, four and ten isolates respectively (Table 1). Three isolates of LMV were isolated from commercial seed samples of unknown origin. The results of the comparative test plant studies between the chosen type isolates of these viruses are given in Table 2.

Cucumber mosaic virus

CMV was obtained from severely stunted, field-grown lettuce plants (Fig. 2) found in Asker, Akershus County.

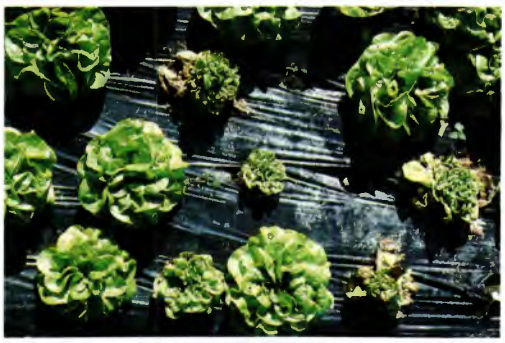


Figure 2. Lettuce plants of cv. Salina grown in the open by a commercial grower. CMV was isolated from stunted plants

All CMV isolates behaved similarly in the test plants used. In *C. quinoa* the local lesions were visible after 2-3 days, and they turned necrotic after a further 2 days. *C. sativus* cv. Farbio reacted with diffuse chlorotic local lesions after 4 days and a systemic yellow mosaic after 6 days. *P. vulgaris* cv. Stella developed small pin-point lesions in 4 days (Fig. 3). No systemic symptoms were observed and back inoculation to *C. quinoa* indicated no latent infection.

Figure 3. Phaseolus vulgaris cv. Stella with local lesions six days after inoculation with CMV-isolate SaS85-6.

The reaction in N. tabacum cv. White Burley consisted of diffuse chlorotic lesions after 4-6 days and after 5-7 days a transient mottle and vein yellowing occurred.

Lactuca sativa cvs. Salina and Brasil 48 were systemically infected, but they did not show any symptoms when grown as test plants in the greenhouse.

When using the ISEM procedure proposed by Francki & Hatta (1980), it was easy to trap a high number of CMV particles from extracts of infected tissue of lettuce or N. tabacum cv. White Burley (Fig. 4). It was important not to dilute the extract too much, the best results being obtained using a dilution of 1:3 to 1:4 (w/v). Sometimes unexpectedly high numbers of particles were trapped by the control normal serum, which was also diluted 1:10.

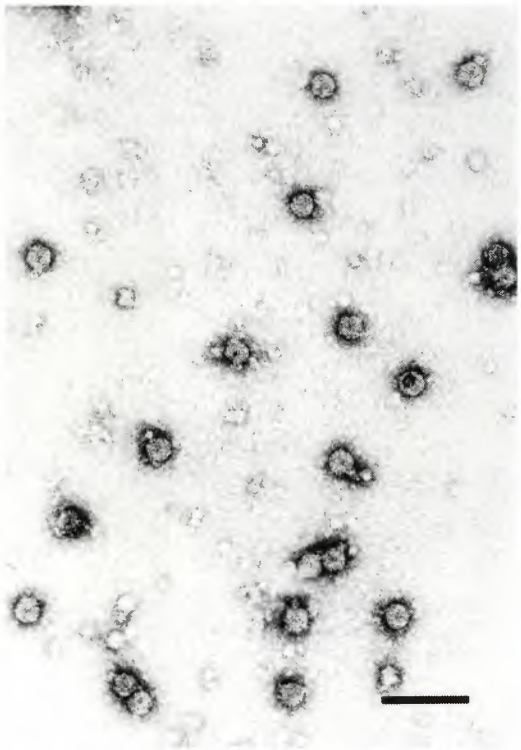


Figure 4. Particles of CMV-isolate SaA85-4 trapped by antiserum to CMV and negatively stained with uranyl acetate. Bar = 100 nm

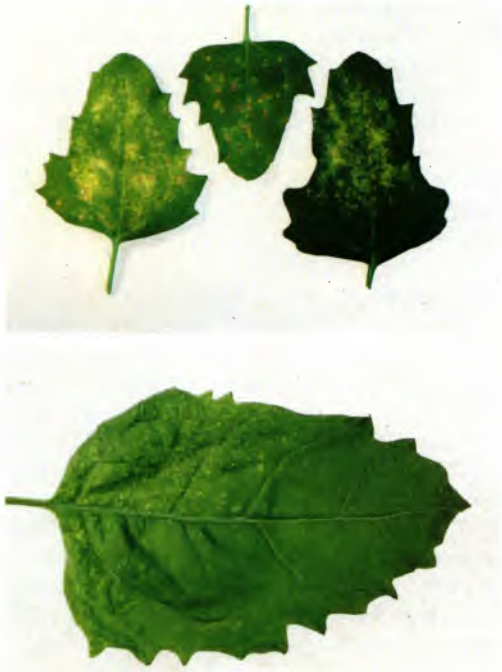


Figure 5. Chenopodium quinoa with local lesions (above) and systemic symptoms (below) 21 days after inoculation with DYMV-isolate 704

Dandelion yellow mosaic virus (DYMV)
DYMV was isolated from stunted, mottled, field-grown lettuce found in Asker, Akershus County, and in Lier, Buskerud County. This lettuce was not as stunted as that from which CMV was isolated, but the DYMV-infected lettuce was more mottled.

C. quinoa inoculated with DYMV developed local lesions after 4-5 days. These lesions were small, distinct and chlorotic (Fig. 5A). They did not expand or turn necrotic as did the lesions of CMV and LMV. Sometimes a systemic yellow stippling became visible after 10-14 days (Fig. 5B). *C. sativus* cv. Farbio developed no symptoms when inoculated, but had a latent, local infection. *N. tabacum* cv. White Burley and *P. vulgaris* cv. Stella were not infected. *L. sativa* cvs. Salina and Brasil 48 produced visible local lesions 4-5 days after inoculation. These were light brown to white, distinct spots or more diffuse, chlorotic and expanding depending on the isolate. The isolates 704 and 719 produced small, white local lesions in cv. Salina (Fig. 6A) and infected *G. globosa* both locally and systemically. The local lesions appeared in *G. globosa* after 7-10 days and after about 15 days a systemic mottle appeared. Isolates SaA85-5 and SaA85-6 induced rather big lesions in cv. Salina (Fig. 6B) and did not infect G. globosa. The systemically infected lettuce leaves became distorted and stiff, often with small necrotic spots. The lettuce plants did not recover. Although no virus particles were observed with ordinary negative staining, high numbers of particles

Figure 6. Local lesions in lettuce cv. Salina seven days after inoculation with the isolates 704 (above) and SaA85-5 (below)

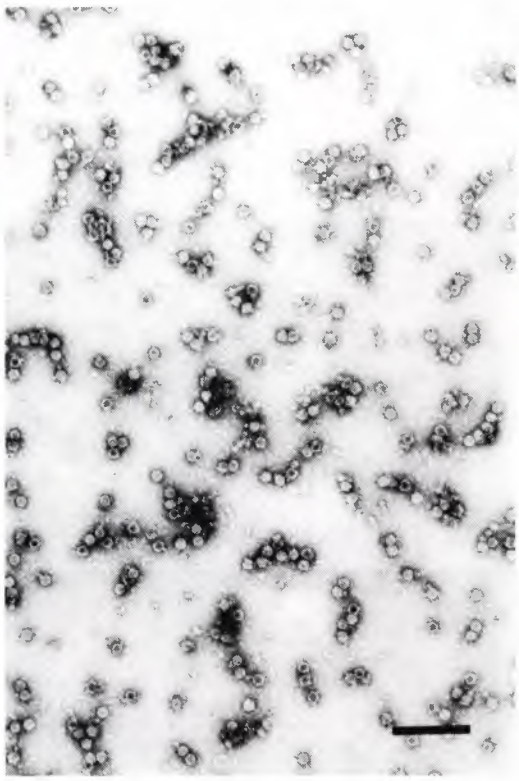


Figure 7. DYMV particles (isolate 719) trapped by antiserum to DYMV and stained with uranyl acetate. Bar = 200 nm

were trapped in ISEM. Grids coated with protein A and DYMV antiserum trapped 50- to 100-fold more particles (Fig. 7) than control grids coated with normal serum. Tubes of virus particles were also seen (Fig. 8).

Tobacco necrosis virus

TNV was readily isolated from roots of all tested lettuce plants grown by NFT, and the fungus *Olpidium brassicae* (Wor.) Dang. was found in the roots. The virus could also be isolated by inoculation of samples of the circulating nutrient solution directly on to leaves of *C. quinoa*. Isolates from lettuce roots from ten different growers were inoculated to *C. quinoa* and *P. vulgaris* cvs. Stella and Prince. The symptoms in *C. quinoa* consisted of numerous necrotic local lesions 2-4 days after inoculation. There were some differences in the symptoms in

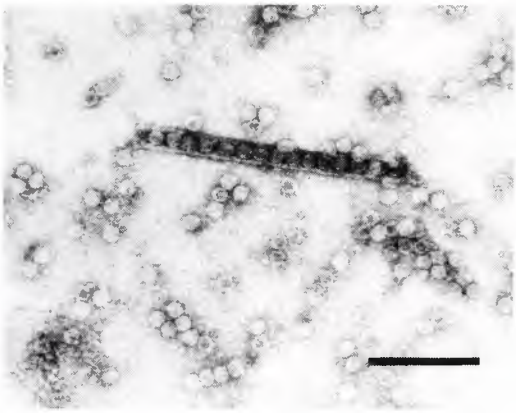


Figure 8. A tube of virus particles found in ISEM preparation using antiserum to DYMV and DYMV-isolate 704, stained with uranyl acetate. Bar = 200 nm

French bean produced by the ten TNV isolates. Some isolates gave mostly minute lesions (Fig. 9A), others produced necrotic rings about 2 mm in diameter extending evenly to all sides (Fig. 9B) and some had both types of lesions (Fig. 9C).

Figure 9. Three isolates of TNV giving different local lesions in French bean cv. Stella. Above: Isolate «Gjennestad» producing minute non-extending lesions; 15 days after inoculation. Middel: IsolateL1 producing rings 2mm in diameter extending evenly to all sides; 7 days after inoculation. Below: Isolate «Gutterød» giving irregular lesions

All isolates were trapped in great numbers in ISEM tests. Ten- to 100-fold more particles were trapped on grids coated with TNV- soil antiserum than on grids coated with normal serum (Fig. 10). The L1 isolate was also tested against TNV-bean antiserum. The number of particles seen in a field of view at a magnification of 100 000 was on average only 2 with TNV-bean antiserum compared with 156 when TNV-soil antiserum was used. Penetration of the particles by PTA stain varied for the different isolates.

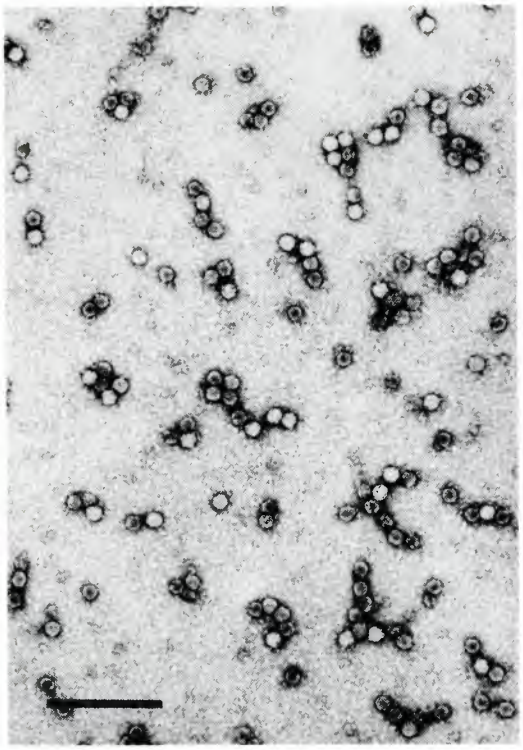


Figure 10. Particles of TNV-isolate L1 trapped by TNV-soil antiserum and stained with uranyl acetate. Bar = 200 nm

Lettuce mosaic virus

LMV was not found in commercial crops of lettuce, but three isolates were ob-

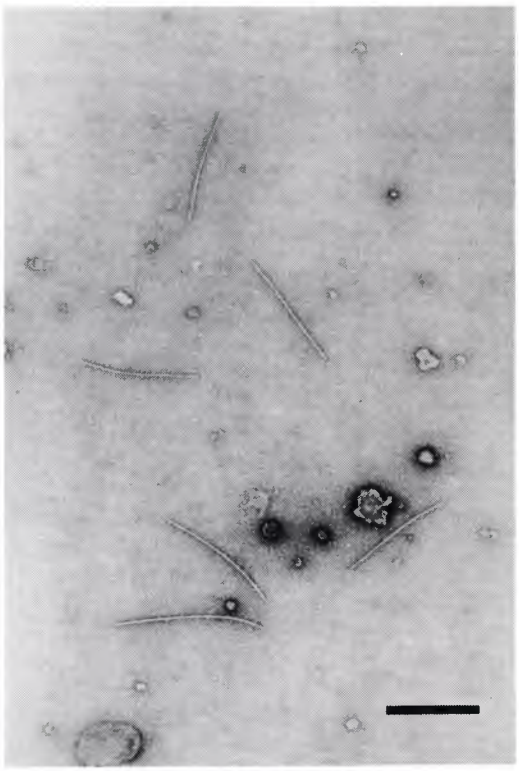
tained from commercially available seed lots of the cvs. Tom Thumb, Prado and Minetto. These three isolates could not be differentiated in the test plants used.

C. quinoa and *C. amaranticolor* reacted with local lesions 6-8 days after inoculation and systemic mosaic appeared 10-14 days after inoculation. *C. sativus* cv. Farbio, *N. tabacum* cv. White Burley and *P. vulgaris* cv. Stella were not infected by any of the isolates.

L. sativa cv. Salina developed vein-clearing 6-8 days after inoculation. Later more mosaic symptoms appeared and the lettuce plants became stunted. In cv. Brasil 48 no symptoms appeared and no stunting was observed, but virus could be found by back inoculation to *C. quinoa* two months after inoculation.

All three isolates were decorated by LMV antiserum (Fig. 11).

A



B

Figure 11. Particles of LMV-isolate SaF85-1. A. Decorated by antiserum to LMV and stained with phosphotungstic acid. Bar = 200 nm. B. Non-decorated particles stained with phosphotungstic acid. Bar = 500 nm

DISCUSSION

Sap-transmission of virus from lettuce to lettuce or other test plants was easy for all viruses when the additives mentioned were used. This is in contrast to the difficulties experienced by Kassanis (1947) when he tried to transmit DYMV mechanically without the use of these additives.

The symptoms produced in test plants by the CMV isolates were as described for the type strain (Gibbs & Harrison 1970, Francki et al. 1979). The DYMV isolates were more variable. The four isolates compared differed in the

local lesions they produced in lettuce cv. Salina and in their reaction in *G. globosa*. This could be used to differentiate the isolates. Bos et al. (1983) also mention that some lettuce cultivars reacted with local lesions but they did not use this to differentiate their isolates. *Calendula officinalis* is reported not to be infected by aphid-inoculation by Kassanis (1947), but in this study it was found to have a latent local infection, an infection type that may have been overlooked by Kassanis (1947) because he lacked an appropriate buffer for mechanical transmission.

Bos et al. (1983) and Brčák (1979) refer to several reports (Klinkowski 1960, Rønde Kristensen et al. 1965, Rønde Kristensen 1966, Rønde Kristensen et al. 1971) on DYMV (lettuce necrosis virus) occurring in Norway. Bos et al. (1983) question whether this virus is the same as the one which was characterized and identified as DYMV in the Netherlands, but the results from the ISEM tests, using the same antiserum as Bos et al. (1983), indicate that the Norwegian DYMV isolated from lettuce is identical or very closely related to the DYMV found in the Netherlands.

The results from the test plant studies with the TNV isolates indicate that these isolates are closely related to the D and E strains of TNV (Babos & Kassanis 1963). In French bean all the TNV isolates produced lesions that did not spread along the veins, a trait typical of strains D and E in the system of Babos & Kassanis (1963). The results from the serological tests corresponded to those from the test plant studies. The TNV identity of all the isolates was confirmed as they reacted positively with the TNV-soil antiserum, but the L1 isolate was not trapped by the TNV-bean antiserum, which has been produced against a stipple-streak strain of TNV, a strain belonging to a serological group other than strains D and E (Babos & Kassanis 1963). Thus it is likely that the L1 isolate is related to strains D and E of TNV. For accurate strain identification, more serological work is needed.

The use of IEM, and particularly the ISEM procedure, was found useful in a small-scale survey like this (Milne & Lesemann 1984). The technique is principally simple, but to obtain optimal stability of the virus particles it was important to know and to use the right buffers and stains.

Although LMV was found in commercial seed lots and was expected to be prevalent in the lettuce production, this was not the case. This could have been due to the production system used. Almost all growers start the culture by sowing in peat pots and later transplanting in the greenhouse or in the field. Probably smaller plants, and plants with abnormal shape or colour (as LMV-infected plants), would be discarded.

The only viruses found in the field were CMV and DYMV. But the occurrence varied between years, probably because of the number of aphids. These viruses were mostly found in Asker, an area with relatively warm summers, giving good temperatures for aphids to multiply. The lettuce is grown in small fields surrounded by rich natural vegetation or private gardens with perennials, giving good possibilities for these viruses to over-winter.

As is known from earlier reports (Tomlinson & Faithfull 1979, Bos et al. 1984, Tomlinson & Thomas 1986) outbreaks of *Olpidium*- transmitted virus diseases have followed the introduction of NFT and other soil-less culturing techniques. The occurrence of *O. brassica* and TNV in Norwegian NFT cultured lettuce shows how effectively *O. brassica* and the viruses it spreads are disseminated in such growing systems; this concurs with the results of Paludan (1985).

As mentioned by Teakle (1962) lettuce seems to be very tolerant to *O. brassica* and TNV infection. This is probably the reason why no direct symptoms or damage could be seen either on roots

or leaves despite the roots being heavily infected by *O. brassica* and TNV.

There seem to be no great economic importance attached to these four viruses in any of the fields surveyed. LMV will probably not create any problem with the present growing techniques, which include raising the seedlings under controlled, hygienic conditions for transplanting. This growing method will also reduce the risk of CMV and DYMV, because the time when infection can occur is greatly reduced as long as the seedling stage (3-4 weeks) takes place in protected greenhouses. However, in summers with large numbers of aphids, virus transmission from vegetation surrounding the fields into the lettuce crop will almost certainly take place, and damage caused by CMV and DYMV may then be of economic significance.

TNV has at the moment no economic importance but should cultivars with less tolerance to O. brassica and TNV be introduced, then the damage could be great.

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