Norwegian Journal of Agricultural Sciences

Vol. 7 1993 No. 2

Norwegian Agricultural Advisory Service, Ås, Norway

NORWEGIAN JOURNAL OF AGRICULTURAL SCIENCES

Norwegian Journal of Agricultural Sciences fills a need created by the folding of Scientific Reports of the Agricultural University of Norway and Research in Norwegian Agriculture for a forum for publishing Norwegian research with international interest within the following areas: Aquaculture, Animal Science, Soil Science, Agricultural Engineering and Technology, Natural Resources and Environment, Food Technology, Crop Science, Forestry, Economics and Society Planning.

***Managing Editor,* Margrete Wiig**

Subject Editors

Even Bratberg, Rolf Enge, Ketil Gravir, Unni Dahl Grue, Knut Heie, Arne Hermansen, Rolf Horntvedt, Atle Kvåle, Trygve Skjevdal, Jon Stene, Steinar Tveitnes

Editorial Board

Ragnar Bærug, Agricultural University of Norway, Department of Soil Science
Sigmund Huse, Agricultural University of Norway, Department of Biology and Nature Conservation
Ådne Håland, Særheim Research Station
Åshild Krogdahl, Institute of Aquacultural Research
Karl Alf Løken, Agricultural University of Norway, Department of Agricultural Engineering
Toralv Matre, Agricultural University of Norway, Department of Animal Sciences
Einar Myhr, Agricultural University of Norway, Department of Agricultural Engineering
Nils K. Nesheim, Agricultural University of Norway, Department of Economics and Social Sciences
Kjell Bjarte Ringøy, Norwegian Agricultural Economics Research Institute
Ragnar Salte, Institute of Aquacultural Research
Martin Sandvik, Norwegian Forest Research Institute
Hans Sevatdal, Agricultural University of Norway, Department of Land Use Planning
Arne Oddvar Skjelvåg, Agricultural University of Norway, Department of Crop Science
Anders Skrede, Agricultural University of Norway, Department of Animal Sciences
Grete Skrede, Norwegian Food Research Institute
Kjell Steinsholt, Agricultural University of Norway, Department of Dairy and Food Industries
Arne H. Strand, Agricultural University of Norway, Department of Dairy and Food Industries
Hans Staaland, Agricultural University of Norway, Department of Biology and Nature Conservation
Asbjørn Svendsrud, Agricultural University of Norway, Department of Forestry
Geir Tutturen, Agricultural University of Norway, Department of Agricultural Engineering
Odd Vangen, Agricultural University of Norway, Department of Animal Sciences
Sigbjørn Vestrheim, Agricultural University of Norway, Department of Horticulture
Kåre Årsvoll, Norwegian Plant Protection Institute

PUBLISHER

The journal is published by Norwegian Agricultural Advisory Service, Moerveien 12, N-1432 Ås, Norway. Norwegian Journal of Agricultural Sciences (ISSN 0801-5341) is published four times a year, each issue containing approximately 100 pages. Four issues comprise one volume. Annual subscription NOK 500,–. Subscribers receive supplement issues free of charge, but these can also be ordered separately through the publisher.

CORRESPONDENCE

All Correspondence, editorial or otherwise, should be adressed to Norwegian Agricultural Advisory Service.

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

ISSN 0801-5341

The succession of seedlings of *Betula* spp. and *Sorbus aucuparia* after clear-felling of a forest area

KÅRE LUND-HØIE[1] & ROBERT ANDERSEN[2]
[1] Norwegian Plant Protection Institute, Department of Herbology, Ås, Norway
[2] Norwegian Institute of Forest Research, Ås, Norway

Lund-Høie, K. & R. Andersen 1993. The succession of seedlings of *Betula* spp. and *Sorbus aucuparia* after clear-felling of a forest area. Norwegian Journal of Agricultural Sciences 7: 111-119. ISSN 0801-5341.

An investigation was carried out in five experimental areas, two in Akershus and three in Vestfold, in which the invasion of seedlings of *Betula* spp. and *Sorbus aucuparia* was evaluated during the first 4-5 years after clear-felling. The evaluations took place in 1990-91. The results indicate that most of the *Sorbus* seedlings developed shortly after or possibly even before clear-felling. A general decline in the total number of seedlings and just slight differences in the distribution of seedlings on height classes between the two years of evaluation, indicate intense browsing by moose as the most likely reason for the suppression of the *Sorbus* seedlings regarding both number and height. The *Betula* picture is essentially similar to that of *Sorbus*. However, the *Betula* seedlings invaded the area over a longer period of time after clear-felling than those of *Sorbus*. A more pronouced variation in the height of the seedlings indicates that *Betula* seedlings are less attractive than those of *Sorbus* for browsing.

Key words: *Betula* spp., colonization, ecology, glyphosate, *Sorbus aucuparia*, succession.

Kåre Lund-Høie, Norwegian Plant Protection Institute, Department of Herbology, Fellesbygget, N-1432 Ås, Norway.

After clear-felling of a forest area it is often observed that deciduous species, usually *Betula* spp. and *Sorbus aucuparia*, colonize the area within a few years. Vegetative regeneration undoubtedly plays an important role in this respect. But the main source of the brushwood propagation is most likely seeds originally stored on the site or imported after clear-felling (Granstrøm 1986).

Since *Betula* spp. and to a lesser extent *Sorbus aucuparia* are serious competitors of the preferred coniferous species, mechanical or chemical brushwood control is common silvicultural practice. However, *Betula* spp. represent an increasing economic and environmental potential in Norwegian forestry and *Sorbus aucuparia* is very important as a moose browse (Hjeljord & Grønvold 1988). Consequently, any silvicultural practice in conifer plantations has to take these factors into consideration.

In brushwood management, chemical control still plays an important role. But such application, depending on the successional stage at the time of the application, may have a strong and

long-lasting effect on the two hardwoods mentioned (Lund-Høie & Grønvold 1987).

The purpose of this paper was to evaluate the seed-based colonization of *Betula* and *Sorbus* after clear-felling in order to decide whether chemical control is expedient and, if so, at which successional stage this control should occur.

MATERIALS AND METHODS

Experimental sites

Five sites located in the counties of Vestfold and Akershus were selected for the investigation. Each of the sites revealed a good habitat for *Betula* and *Sorbus* seedlings.

In Tallakstad, Vestfold, the experimental area covered 3 ha. The site was of high quality (index G20), and the main type of conifer before logging was Norway spruce. The area was clear-felled in 1987.

The Seljarsbekk, Vestfold site, was 3.7 ha in size. The area, which was of medium quality (G17), was clear-felled in 1987.

The Laenga, Vestfold experimental site, was of medium quality (G17) and 3.7 ha in size. The area was clear-felled in 1986.

Bjørknes, Akershus, (3.0 ha) was clear-felled in 1986/87, and the quality was relatively high (G20).

The Haakalia, Akershus site, covering 7.5 ha, was also of high quality, and the area was clear-felled in 1987/88.

Before the evaluations in 1990 and 1991, no vegetation management had taken place on any of the experimental sites.

Evaluation

The evaluations were based on 60 m² plots at 25 m intervals on lines 25 m apart. Table 1 shows the number of plots within each experimental site.

Table 1. No. of evaluation plots

Location				
Tallakstad	Seljarsbekk	Laenga	Bjørknes	Haakalia
No. of plots				
26	63	50	39	95

The evaluations were based on number of seedlings of *Betula* spp. and *Sorbus aucuparia* in the four height classes 0-10 cm; 11-25 cm; 26-50 cm and > 51 cm.

Vegetative suckers were not included.

RESULTS

Experimental sites

The experimental sites in Vestfold (Tallakstad, Seljarsbekk and Laenga) show almost the same

picture regarding frequencies of plots with *Sorbus* or *Betula* seedlings (Table 2) and the distribution of seedlings on height classes (Figs. 1, 2, 3).

Table 2 shows quite low frequencies of plots with both types of seedlings lower than 11 cm and very high frequencies for *Sorbus* seedlings higher than 11 cm. *Betula* seedlings, however, show a more unequal distribution.

Table 2. Frequency of plots with seedlings of *Sorbus aucuparia* and *Betula* spp

Location	Years after clear-felling	Height class (cm)	Species *Sorbus aucuparia* Frequency (%)	*Betula* spp.
Tallakstad, Vestfold	3	0-10	4.3	13.1
	4		2.2	1.3
	3	11-25	95.7	34.8
	4		95.9	56.5
	3	26-50	95.7	43.5
	4		95.8	63.2
	3	>51	95.8	56.6
	4		95.6	39.1
Seljars-bekk, Vestfold	3	0-10	8.6	5.2
	4		18.9	8.6
	3	11-25	91.3	44.8
	4		86.2	39.7
	3	26-50	96.6	56.9
	4		100.0	63.8
	3	>51	89.7	31.0
	4		87.9	50.0
Laenga, Vestfold	4	0-10	10.6	19.1
	5		23.4	23.4
	4	11-25	95.7	40.4
	5		89.4	48.9
	4	26-50	95.8	44.7
	5		91.5	47.8
	4	>51	63.8	27.7
	5		55.3	31.9
Bjørknes, Akershus	4	0-10	5.4	5.4
	5		13.5	16.2
	4	11-25	59.5	40.5
	5		62.2	40.5
	4	26-50	94.6	70.3
	5		91.9	78.4
	4	>51	97.3	81.1
	5		91.9	89.2
Haakalia, Akershus	3	0-10	4.4	21.9
	4		15.4	34.1
	3	11-25	95.6	64.8
	4		82.4	65.9
	3	26-50	96.7	75.8
	4		91.2	89.0
	3	>51	89.0	41.8
	4		74.7	62.6

Figs. 1, 2 and 3, illustrate an increasing number of *Betula* seedlings from the first to the second year of evaluation. For *Sorbus aucuparia*, except at Seljarsbekk, the opposite situation was recorded.

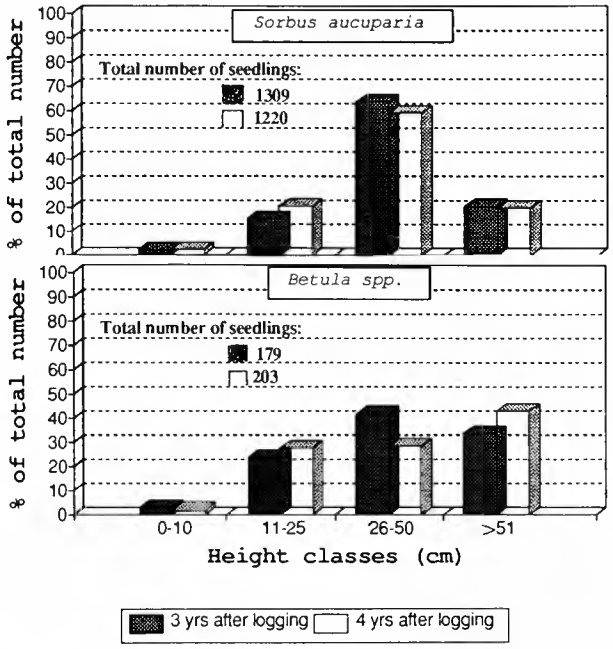


Fig. 1. Distribution on height classes of seedlings of *Sorbus aucuparia* and *Betula* spp., 3 and 4 years after clear-felling. Location: Tallakstad, Vestfold

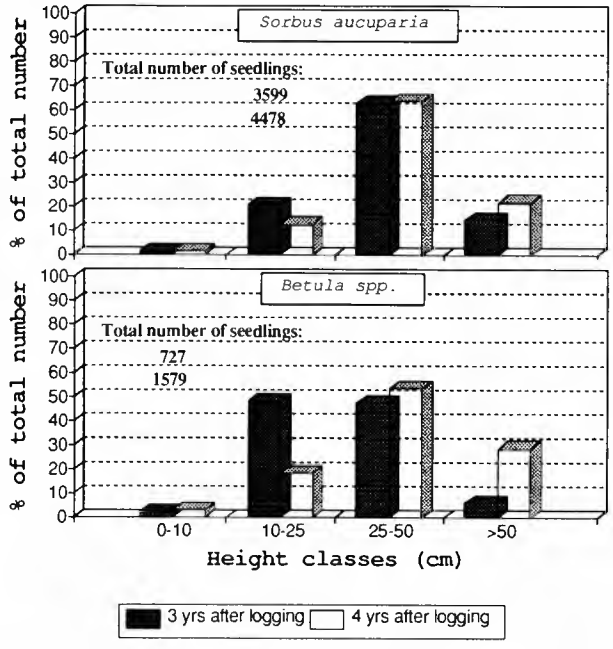


Fig. 2. As for Fig. 1. Location: Seljarsstad, Vestfold

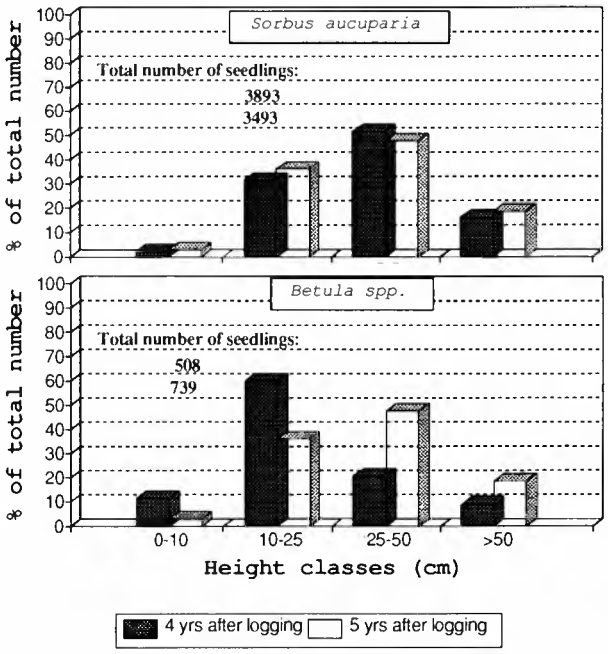


Fig. 3. As for Fig. 1, but 4 and 5 years after clear-felling. Location: Laenga, Vestfold

Most of the *Sorbus* seedlings were found concentrated in the 26-50 cm height class with approximately the same relative distribution on height classes in both evaluation years.

For *Betula*, the picture seems more complex. There was a more widespread distribution in height classes between 11 and 50 cm and a more well-defined shift of seedlings towards the >51 cm height class from the first to the second year of evaluation.

Fig. 4, illustrating the results from the *Bjørknes* site, shows that most of the *Sorbus* seedlings were concentrated in the >51 cm height class, with practically equal relative distribution on height classes over the two years of evaluation.

A decline in total number of *Sorbus* seedlings was observed between the two years of evaluation. The number of *Betula* seedlings, however, increased, with principally the same distribution pattern on height classes as explained for the Vestfold sites.

The experimental site at Haakalia (Fig. 5) shows a picture similar to that at the Vestfold sites: a majority of *Sorbus* seedlings in the 26-50 cm height class, almost equal distribution of the species in the various height classes in the two years of evaluation, and a more disparate distribution of the *Betula* seedlings. As for the other sites, the total number of *Betula* seedlings at Haakalia increased by about 57% in the experimental period.

DISCUSSION

According to Granstrøm (1986) *Sorbus aucuparia* belongs to a group of forestry species, the seeds of which exhibit a strong degree of innate dormancy in soil. Before germination *in situ*, for example on a clear-felled area, the seeds may have been stored for several years

in the soil. As stated by Roberts (1981), it is possible that seeds of many forest species may have been present in the area since the last coppice.

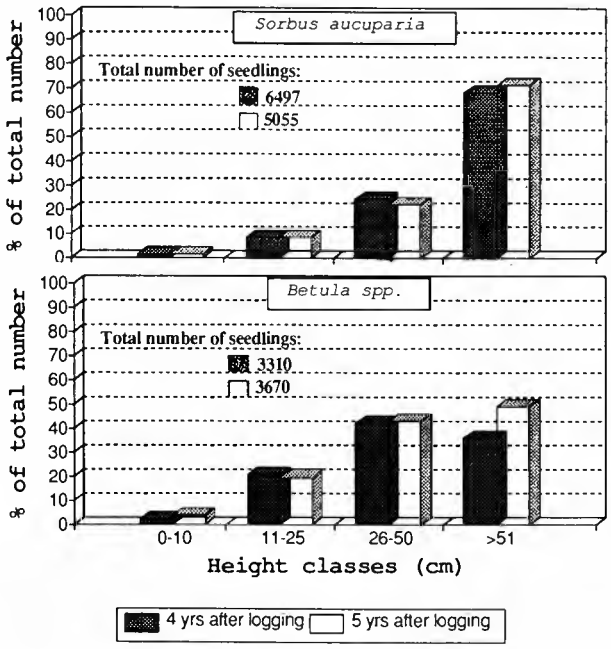


Fig. 4. As for Fig. 3. Location: Bjørknes, Akershus

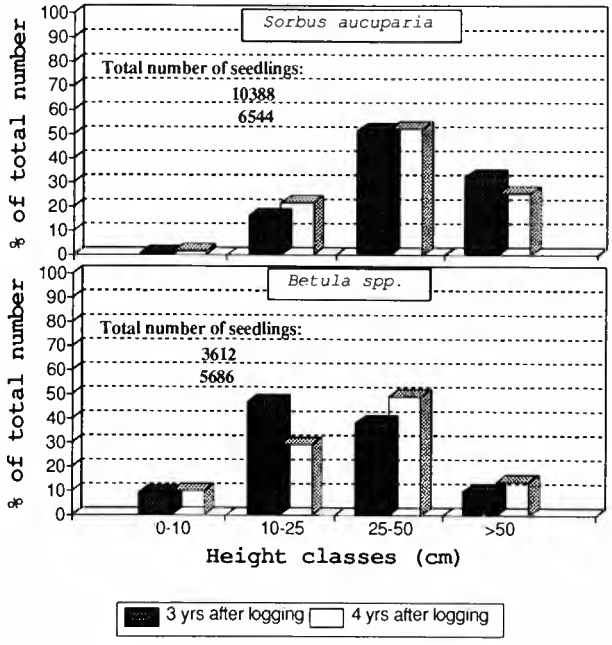


Fig. 5. As for Fig. 1. Location: Haakalia, Akershus

Granstrøm (1986) found viable seeds of *Sorbus aucuparia* as late as five years after sowing in the mor layer of a northern Swedish coniferous forest even when the seed coat was degraded.

With this in mind, it may be supposed that colonization of a clear-felled area with *Sorbus* seedlings takes place mainly from three to four years after felling. Furthermore, it is reasonable to assume that such colonization originates from seeds present in the area before the time of clear-felling.

The present study only partly supports such assumptions. Figs. 1,2,3,4 and 5 and Table 2 generally show a dominance of *Sorbus* seedlings within 26-50 cm with relatively no increase in the number of seedlings exceeding 51 cm between the two years of evaluation. In fact, the results show a decline in the total number of seedlings in the same period. Intense browsing by moose is most likely the reason for the suppression of the *Sorbus* seedlings.

The results also indicate a broad and, with one exception, general germination of *Sorbus* seeds before or shortly after clear-felling. The scant number of seedlings lower than 10 cm 3-4 years after clear-felling, indicates only sporadic germination after the felling.

At present, it is recommended practice that chemical brushwood control should take place no later than two to three years after clear-felling. A basic assumption for such a recommendation was knowledge of the strong innate dormancy of *Sorbus* seeds. Consequently, an application with, for example, glyphosate at the referred time was not expected to interfere with the seed-based *Sorbus* colonization. From an ecological point of view, the present study reveals the recommendation as not acceptable. On the other hand, the suppression of *Sorbus* in a coniferous plantation by moose makes chemical or mechanical control more or less superfluous.

As reported by Granstrøm (1986), *Sorbus* seedlings regenerate in established vegetation close to the mother tree. The high relative frequency of plots with seedlings higher than 11 cm in the present study indicates an import of seeds from outside the area.

Betula spp. generally have a high production of seeds almost every year (Børset 1985). Granstrøm & Fries (1985) found viable seeds of *Betula pubescens* and *Betula verrucosa* three years after sowing, but Fries (1984) states that the germination frequency is maximum during the year of most intense seed rain. Fries (1984) also found that *Betula* seeds could be air-borne up to 100 m from the mother tree. Consequently, it may be supposed that a clear-felled area can be colonized by *Betula* seedlings from a seed bank developing soon after clear-felling and, additionally, from seeds imported from outside the area after clear-felling. The latter type of colonization may proceed for several years after clear-felling, as long as the conditions for germination are optimal and mother trees are available.

The present investigation supports this assumption. Figs. 1-5 show a general shift of seedlings from lower to higher height classes between the first and second years of evaluation and imply at the same time a supply of new seedlings. The results indicate a low frequency of browsing by moose.

The height classes exceeding 11 cm show generally lower frequencies of plots with *Betula* seedlings than with *Sorbus* seedlings (Table 2). This indicates a more cluster-like growth habit of *Betula* spp. than of *Sorbus aucuparia*.

Betula spp. are known as serious competitors to coniferous species (Braathe 1992).

Consequently, in mixtures with preferred conifers, *Betula* colonization has to be controlled at an early successional stage. Fries (1984) recommends the removal of seed-producing mother trees at least one year before clear-felling. Provided *Sorbus aucuparia* is not a dominant species, glyphosate application at an early seedling stage may be a good substitute or alternative. Because of the high seeding and recolonization potential of *Betula* spp., such a chemical treatment would not interfere with the requirements for specified stocking of *Betula* spp. with Norway spruce. This has also been documented by Solbraa & Lund-Høie (1989). It is essential, however, that the application takes place no later than about two years after clear-felling.

CONCLUSIONS

The *Sorbus* seedlings colonizing the experimental areas probably originated from seeds present in the area at the time of clear-felling. The seedlings were most likely suppressed by moose browsing. Because of the colonization pattern and the high browsing potential of the species, *Sorbus aucuparia* should not generally be controlled chemically as a part of a brushwood management programme.

Seedlings of *Betula* spp. establish over a period of several years after clear-felling. A chemical control not carried out later than about two years after clear-felling, should consequently not interfere with the requirements of multiple use of the areas.

ACKNOWLEDGEMENT

This investigation is part of the project "NLVF-205020. Long-term effects of glyphosate application in forest", supported by The Norwegian Agricultural Council.

REFERENCES

Braathe, P. 1992. Forsøk med gran og bjørk i blanding. In: NISK 75 år. Jubileumsberetning 1992. Norsk Inst. for Skogforsk., 1432 Ås. Pp. 230-232.

Børset, O. 1985. Skogskjøtsel II. Skogøkologi. Landbruksforlaget, Oslo. Pp. 339-351.

Fries, C. 1984. Den frøsådda bjørkens invandring på hygget. In: Att behandla løv. Sveriges Skogvårdsførbunds Tidsskrift 3-4: 35-49.

Granstrøm, A. 1986. Seed viability of fourteen species during five years of storage in a forest soil. Swedish Univ. Agr. Sci., Dept. For. Site Res. Stencil No. 6: 1-20.

Granstrøm, A. & C. Fries 1985. Depletion of viable seeds of *Betula pubescens* and *Betula verrucosa* sown onto some north Swedish forest soils. Can. J. For. Res. 15: 1176-1180.

Hjeljord, O. & S. Grønvold 1988. Glyphosate application in forest - ecological aspects. VI. Browsing by moose (*Alces alces)* in relation to chemical and mechanical brush control. Scand. J. For. Res. 3: 115-121.

Lund-Høie, K. & S. Grønvold 1987. Glyphosate application in forest- ecological aspects. I. Successional vegetation changes. Scand. J. For. Res. 2: 455-468.

Roberts, H. A. 1981. Seed banks in soils. In: T. H. Coaker (ed.), Advances in Applied Biology, vol. VI. Academic Press, London, New York, Toronto, Sydney, San Francisco. Pp. 31-55.

Solbraa, K. & K. Lund-Høie 1989. Fortsatt mye løv på flatene etter sprøyting. Norsk Skogbruk 4: 14-16.

The impact of glyphosate application on seedling colonization

KÅRE LUND-HØIE[1] & ROBERT ANDERSEN[2]
[1]Norwegian Plant Protection Institute, Department of Herbology, Ås, Norway
[2]Norwegian Institute of Forest Research, Ås, Norway

Lund-Høie, K. & R. Andersen 1993. The impact of glyphosate application on seedling colonization in seed beds of various quality. Norwegian Journal of Agricultural Sciences 7:121-128. ISSN 0801-5341.

An investigation was carried out at three locations in Akershus. The experiments, were divided into two series, one with and one without glyphosate treated vegetation. Each of the series was based on plots with a) intact vegetation cover; b) aerial vegetation cover removed and c) mineral soil exposed. Glyphosate treatment the year before the start of the experiments, caused a temporary change in the recolonized flora, from the original perennial type towards flora dominated by annual species. The glyphosate-treated vegetation cover selected strongly between species as did the underlying seed beds inclusive exposed mineral soil. The glyphosate treatment stimulated the selected germinating species and inhibited others, regardless of the quality of the seed bed.

Key words: Allelopathy, colonization, germination, glyphosate, seedlings, seed-bed quality.

Kåre Lund-Høie, Norwegian Plant Protection Institute, Department of Herbology, Fellesbygget, N-1432 Ås, Norway.

It is generelly recognized that the dead vegetation cover following glyphosate application, especially when grasses constitute the major component, provides poor conditions for seed germination. On the other hand, some annual species, like *Galeopsis tetrahit* and *Senecio vulgaris*, are suited to a seed bed of this kind. However, the colonization of such species declines rapidly and reaches pre-spray status within 2-3 years after the chemical treatment (Lund-Høie & Grønvold 1987; Lund-Høie & Solbraa, in press). These referred species are generally replaced by perennials, mostly grasses to begin with.

The purpose of the present investigation was to clarify whether the inhibiting/promoting effect on recolonization of a chemically killed vegetation cover was restricted to the cover as such or whether sub-cover conditions were also involved.

MATERIALS AND METHODS

The investigation was carried out at three locations in Akershus, about 40 km south of Oslo. The experiments were divided into two series; one based on glyphosate application

the year before the start of the experiments and the other based on untreated plots. Each of the series was grouped into plots with a) intact vegetation cover; b) just the aerial vegetation cover removed and c) mineral soil exposed. Each plot was 1 x 1 m^2 in size.

Site 1 was clear-felled in 1985/86 and was of medium quality (index G17), with *Deschampsia flexuosa* as the dominant species. The area was glyphosate-treated in August 1988 and the plot preparations were carried out on 27 July, 1989.

Site 2 had a high production potential (G20) and was clear-felled in 1986. The dominant species at the start of the experiment were monocots like *Agrostis tenuis* and several dicots. The area was glyphosate sprayed in August 1989 and the site preparations were carried out on 9 May, 1990.

Site 3 was of high quality (G23) and the area was clear-felled in 1985. The glyphosate treatment took place in August 1989 and the site preparations on 3 May, 1990. Dominant vegetation consisted of dicots like *Rubus idaeus* and monocots like *Agrostis tenuis*.

The glyphosate applications, evaluated at the beginning of July 1990, caused complete death of the original vegetation.

The glyphosate used was formulated as "Roundup" (360 g a.i./L) and was applied at a rate of 1 kg per ha.

The germination and succession of common forest seedlings were evaluated in the first and second years after the glyphosate applications, with unsprayed plots as references. Plots of equivalent quality and dominant vegetation had eight replicates.

The evaluations were based on frequency of observations of individual species in the following ground cover classes: <10%; 11-20%; 21-30%; 31-40%; 41-50%; 51-60%; 61-70%; >71%.

RESULTS

The results are expressed as frequency indexes defined by the equation:

$$\text{Frequency index}_{\text{Species}} = \frac{(n_1 \times m_1) + (n_2 \times m_2) .. (n_8 \times m_8)}{N}$$

$n_1 \ldots n_8$: No. of observations of a given species in the various cover classes

$m_1 \ldots m_8$: Mean percentage coverage of a given species in the various cover classes

N : Total number of cover classes

The results are presented in Figs. 1, 2 and 3.

Location 1 (Fig.1)

Deschampsia flexuosa was the most dominant species on the unsprayed plots. The species established poorly on the b-bed, but showed a rapid succession on the c-bed. A similar situation was recorded on the glyphosate-treated plots on all the three seed-bed qualities, although mostly on the dead vegetation cover.

Rubus idaeus showed restricted establishment on unsprayed plots, regardless of bed quality. Glyphosate application, however, highly stimulated the germination, and the treatment caused a rapid succession on the a and b-beds.

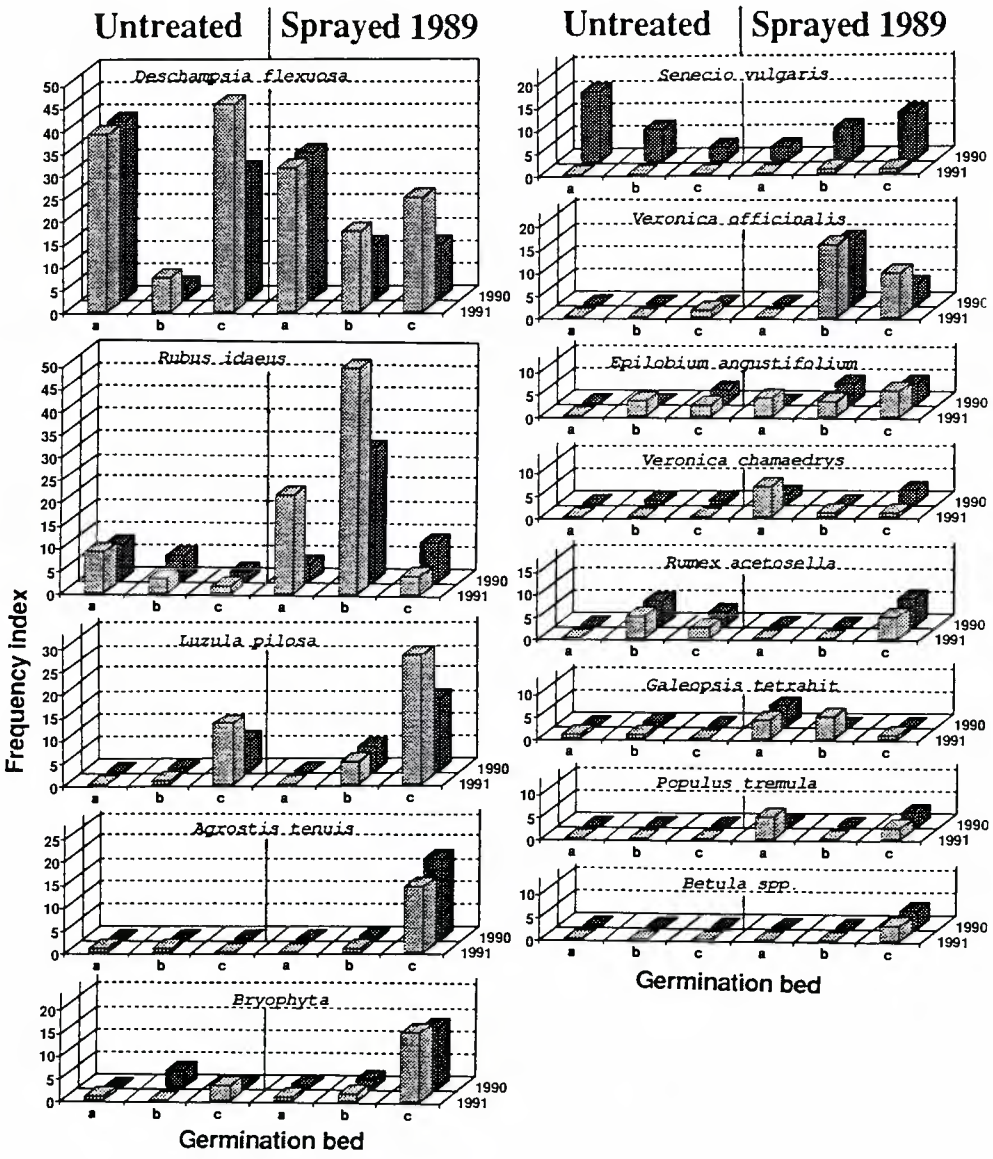


Fig. 1. Experimental location No. 1. Establishment and succession of seedlings of common annual/perennial forest species on seed beds of various qualities: a) Intact vegetation; b) aerial part of the vegetation removed and c) mineral soil exposed. The chemical vegetation control in 1989 was carried out as an overall application with glyphosate (1 kg a.i./ha)

Luzula pilosa germinated easily on exposed soil on both the sprayed and unsprayed plots, although glyphosate application promoted germination and succession for this species also.

Senecio vulgaris on unsprayed plots showed a declining establishment on the a, b and c-beds, but on the glyphosate-treated plots, the opposite situation was recorded. Regardless of chemical treatment, the succession was seen to maximize in the first growth season evaluated, but the species had practically disappeared by the second growth season.

Veronica officinalis was not found on the a and b-beds, and only a sparse occurrence was recorded on the c-bed on unsprayed plots. However, the germination and development of the seedlings on the b and c-beds seemed highly stimulated by the glyphosate treatment.

Agrostis tenuis showed a development similar to that of the *Veronica* species: a highly stimulated effect of the glyphosate treatment on germination and development on the c-bed.

Rumex acetosella was not found on the unsprayed a-bed, but germinated easily on the b and c-beds. Also, the glyphosate treatment seemed to have stimulated the establishment of this species on the c-bed .

Veronica chamaedrys, Epilobium angustifolium, Galeopsis tetrahit and to a lesser extent *Linaria vulgaris* all showed principally similar reactions to the glyphosate treatment, a stimulated germination especially on the a and c-beds, *Galeopsis* also on the b-bed. None of the species were found on unsprayed a-beds in the first year of evaluation.

Bryophyta species were also highly stimulated on glyphosate treated c-beds, as were the hardwoods *Betula* spp. and *Populus tremula*. They all germinated and developed freely on the glyphosate plots, in spite of the fact that none of them were found on unsprayed beds.

Location 2 (Fig. 2)

The site quality was slightly better than that of location 1, and the vegetation was dominated by *Sorbus aucuparia* in the brush layer and *Agrostis tenuis, Deschampsia flexuosa* and some dicots in the ground flora.

The results presented in Fig. 2, show a similar tendency to that indicated in Fig. 1, a stimulation by the glyphosate treatment of the establishment of the various species except for *Rubus idaeus* and *Sorbus aucuparia*. The *Sorbus* species was the only one which did not re-establish after the glyphosate application, regardless of the seed-bed quality.

Location 3 (Fig. 3)

This was a high quality site, with *Rubus idaeus, Maianthemum bifolium* and *Agrostis tenuis* as the dominant species. The *Rubus* and *Maianthemum* species were both highly suppressed by the glyphosate treatment in both evaluation years.

Agrostis tenuis showed a non-significant establishment on the glyphosate-killed vegetation cover. But there was almost an explosion of the species on the glyphosate-influenced b and c-beds in the second year after treatment. This was also true for species like *Galeopsis tetrahit, Anemone nemorosa* and *Epilobium angustifolium*, mainly on the dead vegetation cover. The *Epilobium* species also found good conditions for establishment on the glyphosate treated b and c-beds.

Bryophyta established rapidly on the glyphosate a-bed in both years after the application.

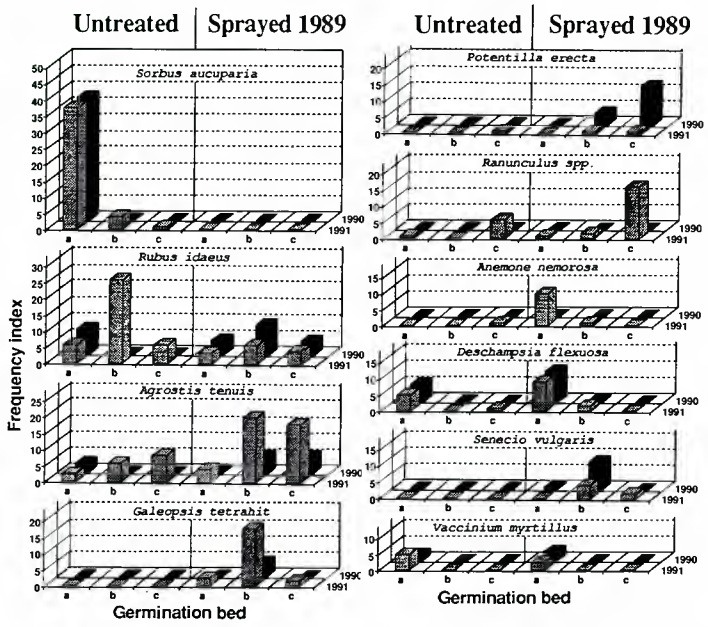


Fig. 2. Experimental location No. 2. Legend as in Fig. 1

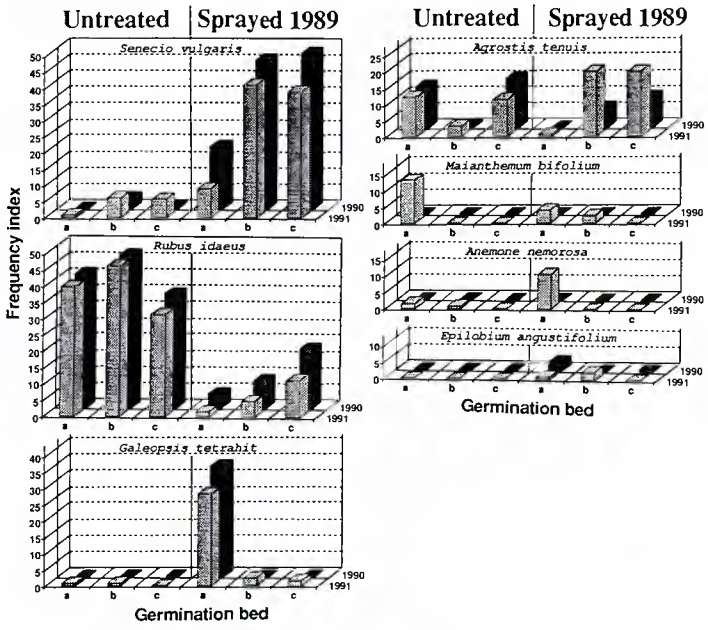


Fig. 3. Experimental location No. 3. Legend as in Fig. 1

DISCUSSION

Glyphosate treatment of an area with a dense ground vegetation will cause a shift in the dominance ratio between monocots and dicots. It has frequently been observed that during the first period after treatment, specific species, mostly annual dicots, tend to invade the area. But over time, perennials, especially monocots, begin to supersede the annuals, and the original balance is re-established (Lund-Høie & Rognstad 1990; Skuterud 1989).

A glyphosate-killed vegetation cover represents a barrier to germination. The reasons may be strictly physical, but allelopathic factors may also play an important role. It is well known that plants produce phytotoxins that interact among the plants (Putnam & Tang 1986; Rice 1984; Rizvi & Rizvi 1992; Waller 1987). Many of the allelochemicals have chemical structures and characteristics similar to those of the synthetic herbicides (Jobidone 1992).

The allelochemicals may be released from the plants during life and from plant residues after death, for example, after a glyphosate treatment. As reported by Purvis *et al.* (1985), such compounds exhibit selective effects that inhibit weed germination and growth under field conditions. It is also possible that such compounds may be synthesized by microorganisms utilizing plant residues as a nutrient source (Levesque *et al.* 1992, Lovett 1990). This is also reported by Torstensson (1992).

The present experiment supports only to a limited extent the above-mentioned statements about a germination-inhibiting effect of a dead vegetation cover. Grass species like *Agrostis tenuis* and to some extent *Luzula pilosa*, did not germinate at all on the dead vegetation cover (a-bed); this was also true of dicots like *Potentilla erecta, Rumex acetosella* and *Veronica officinalis*, though they were all very evident on the b and c-beds.

Limited germination was also recorded for *Ranunculus* spp. and *Rubus idaeus* in the first year after the glyphosate treatment. During the second growth season, however, the *Rubus species* in particular showed a more pronounced succession.

The hardwoods *Betula* spp. and *Populus tremula* were found to have different requirements regarding the seed-bed quality. The *Betula* species germinated easily on exposed mineral soil, but not on the dead vegetation cover, in either the first or the second year after the glyphosate application. *Populus*, however, appeared frequently on both the glyphosate-treated a and c- beds, but not on the corresponding untreated beds.

Sorbus aucuparia exhibited a dominant existence on the untreated vegetation beds but not on any of the glyphosate-treated ones even in the second year after the chemical treatment. The main reason for this may be the strong degree of innate dormancy of *Sorbus* seeds in the soil (Granstrøm 1986) and not an allelopathic inhibition of germination.

A few species germinated freely on the glyphosate-killed vegetation cover. The most typical in this respect were *Galeopsis tetrahit, Epilobium angustifolium, Veronica chamaedrys, Vaccinium myrtillus, Deschampsia flexuosa* and to a lesser extent *Senecio vulgaris*. However, the *Senecio* species evidently found the best conditions for germination on the b-bed.

The *Bryophyta* species became established on the dead vegetation bed, although apparently not on the one originally composed of *Deschampsia flexuosa*.

Taking all the seed beds into consideration, the results indicate an improvement in germination and succession of most of the species involved following the glyphosate treatment. Changes in the microflora/fauna and in the physical and chemical composition

of the seed bed following the glyphosate treatment, as well as reduced competition, may be the main contributory factors for this stimulation. That allelopathy includes such stimulating effects, is also reported by Rice (1986).

CONCLUSIONS

The glyphosate application created favourable seed-bed conditions for germination of specific dicots like *Galeopsis tetrahit, Senecio vulgaris* and *Epilobium angustifolium* and monocots like *Deschampsia flexuosa*, regardless of whether or not they were originally present. Hardwoods like *Betula* spp. and *Sorbus aucuparia* did not become established within the experimental period (two years). *Populus tremula*, however, found optimum conditions for germination even in the first year after the chemical treatment. Soil preparation which exposed the mineral soil had a similar but less obvious effect. The chemical treatment before soil exposure promoted germination even on bare soil.

Although the reasons for the selective effect of glyphosate application on the establishment of species from seeds are not known, physical and/or allelopathic parameters are probably involved.

ACKNOWLEDGEMENT

This investigation is part of the project "NLVF-205020. Long-term effects of glyphosate application in forest", supported by The Norwegian Agricultural Council.

REFERENCES

Granstrøm, A. 1986. Seed viability of fourteen species during five years of storage in a forest soil. Swedish Univ. of Agr. Sci., Dept. of For. Site Res. Stencil No. 6: 1-20.

Jobidon, R. 1992. Allelopathy in Quebec forestry-case studies in natural and managed ecosystems. In: S.J.H. Rizvi, V. Rizvi (eds.), Allelopathy. Basic and Applied Aspects. Chapman & Hall, London, New York. Pp. 341-356.

Levesque, C.A., J.E. Rahe & D.M. Eaves 1992. The effect of soil heat treatment and microflora on the efficacy of glyphosate in seedlings. Weed Res. 32 (5): 363-373.

Lovett, J.V. 1990. Chemicals in plant protection: Is there a natural alternative? In: C. Basset, L.J. Whitehouse, J. A. Zabkiewicz (eds.), Alternatives to the Chemical Control of Weeds. FRC Bulletin 155, Ministry of Forestry, For. Res. Inst., Priv. Bag 3020, Rotorua, New Zealand. Pp. 57-65.

Lund-Høie, K. & S. Grønvold 1987. Glyphosate application in forest - ecological aspects. Successional vegetation changes. Scand. J. For. Res. 2: 455-468.

Lund-Høie, K. & A. Rognstad 1990. Effect of foliage-applied imazapyr and glyphosate on common forest weed species and Norway spruce. Crop Prot. 9: 52-58.

Lund-Høie, K. & K. Solbraa 1993. Effects of vegetation management on succession and on hardwood competition with Norway spruce (*Picea abies* L.) Norw. J. of Agr. Sci. 7: 89-110.

Purvis, C.E., R.S. Jessop & J.V. Lovett 1985. Selective regulation of germination and growth of annual weeds by crop residues. Weed Res. 25 (6): 415-421.

Putnam, A.R. & C.-S. Tang 1986. Allelopathy: State of the science. In: A.R. Putnam, C.-S. Tang (eds.), The Science of Allelopathy. Wiley, New York. Pp. 1-19.

Rice, E.L. 1984. Allelopathy. Academic Press, Inc., New York, London, 424 pp.

Rice, E.L. 1986. Allelopathic growth stimulation. In: A.R. Putnam, C.-S. Tang (eds.), The Science of Allelopathy. Wiley, New York. Pp. 23-42.

Rizvi, S.J.H. & V. Rizvi 1992. Allelopathy. Basic and Applied Aspects. Chapman & Hall. London, New York, 480 pp.

Skuterud, R. 1989. Ugrasbekjempelse på åkerkanter - Fordeler og ulemper. In: Statens fagtj. for landbr. (ed.), Informasjonsmøte, Plantevern. Aktuelt fra Statens Fagtj. No. 3: 79-88.

Torstensson, L. 1992. Effekten av glyfosat på icke direkt behandlade växter. In: Ogräs och ogräsbekamping. 33:e Svenska Växtskyddkonf., Uppsala, 29. - 30. januar. Pp. 111-119.

Waller, C.R. 1987. Allelochemicals: Role in Agriculture and Forestry. ACS Symp. series 330. Am. Chem. Soc., Washington, DC, 589 pp.

Effects of ozone on growth of several subalpine plant species

LEIV M. MORTENSEN
The Norwegian State Agricultural Research stations, Særheim Research Station, Klepp station, Norway/Agricultural University of Norway, Department of Horticulture, Ås, Norway

Mortensen, L.M. 1993. Effects of ozone on growth of several subalpine plant species in Norway. Norwegian Journal of Agricultural Sciences 7: 129-138. ISSN 0801-5341.

The effects of three ozone (O_3) regimes (12-27, 40-53 and 86-96 nmol mol^{-1} during 8 h day^{-1}) on growth of 19 plant species from the Jotunheimen district (61.5°N, 8.5°E, 900-1350 m a.s.l.) were studied. Three subsequent experiments with seedlings were carried out in growth chambers supplied with a mixture of daylight and supplementary light. An increase from the low to the intermediate O_3 level resulted in a decrease in the dry weight of *Phleum commutatum*, and an increase in dry weight of *Saussurea alpina*, *Rumex acetosa* and *Silene vulgaris*. Raising the O_3 concentration from the lowest to the highest level decreased the dry weight of *Angelica archangelica* (28%), *Antennaria dioica* (58%), *Chamaenerion angustifolium* (73%), *Fragaria vesca* (32%), *Leontodon autumnalis* (14%), *Oxyria digyna* (14%), *Phleum commutatum* (99%), *Ranunculus acris* (21%), *Rumex acetosa* (17%), *Salix glauca* (42%), *S. lanata* (28%), *S. reticulata* (39%) and *Solidago virgaurea* (17%). No effect of O_3 was found on dry weights of *Circium palustre*, *Saussurea alpina*, *Salix herbacea*, *S. phylicifolia* and *Taraxacum croceum*. The leaf:stem fresh weight ratio was decreased by O_3 in the three *Salix* species where this parameter was measured. Number of shoots was decreased in three of 12 species, and number of leaves in two of seven species by the highest O_3 concentration. Number of shoots was enhanced by O_3 in two of the species and number of leaves in one species. Shoot length was decreased in four of 17 species by the highest O_3 level and was enhanced by the intermediate level in four species. Leaf injury caused by O_3 varied considerably between species but occured in all species at the highest O_3 concentration. Nine of 19 species showed distinct O_3 injury (yellow stipples) at the intermediate O_3 level, but this was accompanied by a reduction in dry weight in *Phleum commutatum* only.

Key words: Alpine plants, growth, ozone.

Leiv. M. Mortensen, Særheim Research Station, N-4062 Klepp station, Norway

The effect of ozone (O_3) pollution on plant growth has recently been reported for several wild species from a wide range of latitudes in Scandinavia (56-79°N) (Mortensen & Nilsen 1992). A large variation in O_3 sensitivity was found between the 24 species studied. The most important result so far with Norwegian wild plant species is that *Betula pubescens*, one of the most widespread species in Norway, is sensitive to O_3 (Mortensen & Skre 1990).

Relatively few studies have so far included wild plant species of relevance for Norwegian climatic conditions at realistic O_3 concentrations (Mortensen 1991). Ashmore (1984), however, categorized several native British plant species with respect to O_3 sensitivity by exposing the plants to a short-term, very high O_3 concentration. Wide variations in O_3 sensitivity were found between these species. The maximum O_3 concentration in Norway seldom exceeds 100 nmol mol^{-1}. A typical 7-h day mean concentration for the months of May and June is 40-50 nmol mol^{-1} (Pedersen & Semb 1990). Such a pollution level is known to cause growth reductions in different plant species (Guderian et al. 1985; Heagle 1989). In order to increase our knowledge about responses of subalpine vegetation to O_3 pollution, a range of plant species from a mountain region in Norway was studied. This work formed part of the national work on "Critical loads" in Norway.

MATERIAL AND METHODS

Seeds were harvested from plants growing at an elevation of 900-1350 m a.s.l. in the Jotunheimen region (61.5°N, 8.5°E) in August and September 1991. The seeds of five *Salix* species were sown soon after the harvest on 16 September since storage of these seeds would reduce their ability to germinate (Experiment 1, Table 1). The seeds of the species included in Experiments 2 and 3 were sown on 24 December (1991), and 18 February (1992), respectively (Table 1). The air temperature during the propagation of the seedlings was about 15°C and supplementary lighting by means of high pressure sodium vapour lamps (SON XL-T) was supplied 18 h day^{-1} at a level of about 100 $\mu molm^{-2}s^{-1}$ photosynthetic photon flux density (PPFD). The light was measured by means of a Lambda LI-185B instrument with a quantum sensor. The experiments started four, three and five weeks after sowing in Experiments 1, 2 and 3, respectively. A reference species, *Phleum pratense* cv. Forus, was included in the three experiments since from previous experiments this species is known to be very sensitive to O_3 (Mortensen 1992). The seedlings were planted in standard fertilized peat (Floralux) in 0.5 l pots, three seedlings per pot. In Experiments 2 and 3 the peat was mixed with 25% perlite. The peat contained 230 g N, 100 g P, 290 g K, 360 g Mg, 2.4 kg Ca, 150 g S, 18 g Fe, 4 g Mn, 4 g Cu, 4 g Zn, 1 g B and 1 g Mo per m^3 peat, and the pH was 5.5.

Four to eight pots (three seedlings per pot) of each species were placed in each one of six growth chambers previously described by Mortensen (1982). The chambers were placed in a greenhouse compartment. The length of the experimental period, number of days from sowing until start of the experiment, number of pots per chamber, plant dry weight per pot and shoot length at start of the experiment are recorded in Table 1 for each of the three experiments. Experiments 1, 2 and 3 commenced on 23 October 1991, 13 January and 26 March 1992, respectively. Three O_3 concentrations were given during an 8 h day^{-1} (10.00-18.00 h), two chambers at each concentration (Table 2). Ozone was generated from dry air using a high voltage O_3 generator (Nomizon, Nordmiljø ab, Sweden). The O_3 concentration was measured twice an hour by a scanner switching air flows from the chambers sequentially to an O_3 analyser (Monitor Labs Inc., Model 8810). The mean and maximum O_3 concentrations throughout the daily 8-h application period and the rest of the diurnal period were recorded separately by datalogger (Table 2). The O_3 concentration outside the exposure period (18.00 to 10.00 h) was < 15 nmol mol^{-1} in all

chambers, and these values are not presented. The CO_2 concentration was 350 ± 30 μmol mol^{-1} in all chambers as measured by an infra-red gas analyser (ADC, Model 225 MK3). Supplementary light was provided by the same light source as that used during the propagation of seedlings, at a PPFD level of 130 $\mu molm^{-2}s^{-1}$ during 18 h day^{-1}. This corresponded to a daily photon flux of 8.4 mol m^{-2}. The total photosynthetic active flux (PAP) is recorded in Table 2. PAP is the sum of supplementary and natural radiation. The natural radiation was measured at the Meteorological station at Særheim Research Station, and this value was reduced 50% by the shading caused by the greenhouse and growth chamber constructions. The temperature and the relative humidity were measured every five minutes by thermocouples and humidity sensors respectively, and datalogged. The mean values throughout the experimental periods are given in Table 2.

Table 1. Experimental period, days from sowing to start of the experiment, and initial dry weight and shoot length of different species in Experiments 1-3. *Phleum pratense* was included as a reference species (O_3 sensitive). Each pot included three seedlings

	Experimental period (days)	Days from sowing to exp.start	No. of pots per chamber	Initial dry weight ($g \cdot pot^{-1}$)	Initial shoot length (cm)
Experiment 1:					
Salix glauca L.	79	37	8	0.021	1.0
Salix herbacea L.	78	37	8	0.009	0.5
Salix lanata L.	79	37	8	0.020	1.0
Salix phylicifolia L.	79	37	4	0.026	1.0
Salix reticulata L.	78	37	8	0.004	0.5
Ref. species:					
Phleum pratense L.'Forus'	50	-	5	1.0	-
Experiment 2:					
Antennaria dioica (L.) Gaertn.	65	20	6	<0.01	0.5
Chamaenerion angustifolium (L.) Scop.	65	20	6	<0.01	0.5
Oxyria digyna (L.) Hill.	50	20	6	0.03	4.0
Phleum commutatum Gaud.	64	20	6	<0.01	6.0
Rumex acetosa subsp. lapponius Hiit.	32	20	6	0.03	2.0
Silene vulgaris (Moench.) Garcke	50	20	6	0.03	2.0
Solidago virgaurea L.	65	20	6	0.01	1.0
Taraxacum croceum Dt.	65	20	6	0.03	[1]5.0
Ref. species:					
Phleum pratense L. 'Forus'	60	14	5	<0.01	3.0
Experiment 3:					
Angelica archangelica L.	41	37		0.003	3.0
Cirsium palustre (L.) Scop.	42	37	6	0.069	5.0
Fragaria vesca L.	41	37	6	0.027	3.0
Leontodon autumnalis L.	40	37	6	0.210	6.5
Ranunculus acris L.	41	37	6	0.150	5.5
Saussurea alpina (L.) Dc.	42	37	6	0.039	3.5
Ref. species:					
Phleum pratense L. 'Forus'	38	14	5	<0.001	3.0

[1]Leaf length

Table 2. The 8-h (10.00-18.00h) mean and maximum O_3 concentration (±SD) as means for the whole experimental period in Experiments 1-3, and mean temperature, air humidity and photosynthetic active photons (PAP) are given. PAP is the total of supplementary and natural radiation which was the same for all treatments and replicates

	O_3 level					
	Low		Intermediate		High	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
Experiment 1:						
Mean O_3 conc. (nmol mol^{-1})	28±6	26±7	54±7	51±8	94±16	97±13
Mean max. O_3 conc. (nmol mol^{-1})	32±7	29±7	61±6	58±7	110±15	112±12
Mean temperature (°C)	12.7	13.2	12.9	12.9	13.0	13.0
Relative humidity (%)	77±8	74±8	72±5	74±7	71±10	75±7
PAP (mol $m^{-2}day^{-1}$)	9.8					
Mean outside O_3 conc.(nmol mol^{-1})	33±8					
Experiment 2:						
Mean O_3 conc. (nmol mol^{-1})	11±4	13±4	40±8	39±7	87±14	85±23
Mean max. O_3 conc. (nmol mol^{-1})	16±9	18±8	47±9	45±8	101±20	101±26
Mean temperature (°C)	13.8	13.5	13.7	13.6	13.8	13.9
Relative humidity (%)	68±2	73±3	68±3	71±2	71±2	77±3
PAP (nmol$m^{-2}day^{-1}$)	11.7					
Mean outside O_3 conc.(nmol mol^{-1})	17±5					
Experiment 3:						
Mean O_3 conc. (nmol mol^{-1})	20±6	19±3	45±7	44±9	89±18	90±20
Mean max. O_3 conc. (nmol mol^{-1})	26±7	22±3	52±9	51±10	104±23	105±22
Mean temperature (°C)	14.0	13.9	13.9	14.2	14.1	14.2
Relative humidity (%)	74±8	76±6	75±5	75±7	75±6	79±5
PAP (mol $m^{-2}day^{-1}$)	21.5					
Mean outside O_3 conc. (nmol mol^{-1})	26±3					

The plants were watered regularly. The electrical conductivity in the pots was kept between 1.5 and 2.0 mS cm^{-1}. A complete nutrient solution was supplied when needed, and this consisted of (mg l^{-1}): N, 188; P, 37; K, 242; Ca, 130; Mg, 41; S, 53; Fe, 2.0; Mn, 0.6; Zn, 0.14; Cu, 0.29; B, 0.34; Mo, 0.027; Co, 0.009 - giving an electrical conductivity of 1.7 mS cm^{-1}.

At the end of the experiments shoot fresh and dry weight, number of shoots, shoot length, leaf injury and pigmentation caused by O_3, leaf area of the three largest leaves per pot and root development were recorded. The last two parameters were not measured in Experiment 1. The O_3 injury (yellow stipples) was scaled from 0 (no injury) to 5, where 1 = 1-10%, 2 = 10-20%, 3 = 20-30%, 4 = 30-40% and 5 = 40-50% of the leaf area stippled. The root development was scaled from 0 (no roots observed on the bottom of the pot) to 5 (a dense root system covering the entire bottom of the pot). All data were subjected to an analysis of variance with pots as replicates, and Duncan's multiple range test was used to determine significant differences between treatment means.

RESULTS

The dry weight was decreased by 14-99% in 13 of 19 species when the O_3 concentration increased from a low level (12-27 nmol mol^{-1}) to a high level (86-95 nmol mol^{-1}) in Experiments 1-3 (Tables 3-5). The dry weight of the O_3 sensitive reference species, *Phleum pratense*, decreased by 77-96% by the same increase in O_3 concentration in the three experiments. The dry weight of *Phleum commutatum* was decreased (53%) and the dry weights of *Saussurea alpina*, *Silene vulgaris* and *Rumex acetosa* were increased when the O_3 concentration was raised from a low to an intermediate level (40-53 nmol mol^{-1}). The percentage dry weight was increased by a rise in the O_3 concentration in nine of the species, and was decreased in one species (*Rumex acetosa*) (Table 4).

Table 3. The effect of O_3 concentration on growth of different *Salix* species and *Phleum pratense* in Experiment 1. Each value represents three plants per pot with the exeption of leaf area, which represents the three largest leaves per pot. The leaf injury (yellow stipples) is scaled from 0 (none) to 5 (>50% of leaf area stippled). Significance level: ns, not significant; *, $p<0.5$; **, $p<0.01$; ***, $p<0.001$. Values followed by different letters are significantly different according to Duncan's multiple range test at $p<0.05$ level

Species	O_3 conc. (nmolmol^{-1})[1]	Dry weight (g)	% dry weight	Leaf: stem ratio	No. of shoots	No. of leaves	Shoot length (cm)	Leaf injury Index	Leaf injury Symptoms
S.glauca	27	3.82a	19.8b	1.9a	14.6	142a	26.7	0.0b	
	53	3.55a	21.0ab	1.6b	14.9	128a	27.5	0.3b	Yellow stipples
	96	2.23b	21.8a	1.5b	9.6	84b	24.5	2.3a	
Significance level		***	*	**	ns	***	ns	***	
S.herbacea	27	0.76	23.1b	-	26.0	-	5.0	0.0b	
	53	0.55	21.0b	-	21.4	-	5.0	0.0b	Yellow stipples
	96	0.76	26.6a	-	22.8	-	5.3	0.6a	
Significance level		ns	**	-	ns	-	ns	***	
S.lanata	27	4.90a	19.2b	1.6a	10.3	108	36.6b	0.0c	Yellow stipples/
	53	5.53a	20.1ab	1.3b	9.1	92	45.3a	0.8b	Red-brownish
	96	3.52b	21.4a	1.2b	8.3	84	35.0b	3.1a	pigmentation
Significance level		**	*	***	ns	ns	***	***	
S.phylicifolia	27	2.66	19.0	1.9a	13.3	-	23.3	0.0b	
	53	3.42	20.1	1.7ab	15.0	-	25.5	0.0b	Yellow stipples
	96	2.38	21.8	1.4b	11.4	-	26.6	0.4a	
Significance level		ns	ns	*	ns	-	ns	*	
S.reticulata	27	0.59a	18.4b	-	12.3	-	3.1	0.0c	Yellow stipples/
	53	0.64a	19.4b	-	12.5	-	3.6	0.9b	Red-brownish
	96	0.36b	24.6a	-	10.6	-	3.1	3.7a	pigmentation
Significance level		**	***	-	ns	-	ns	***	
Reference species:									
Phleum pratense	27	7.77a	9.7	-	22.7a	-	81.2a	[1]4b	Chlorosis/
	53	7.68a	19.3	-	26.8a	-	80.5a	7b	necrosis
	96	1.76b	28.8	-	14.7b	-	51.2b	59a	
Significance level		***	ns	-	***	-	***	***	

[1] Percentage dead leaf area

Table 4. The effects of O_3 concentration on growth of different species in Experiment 2. The root development was scaled from 0 to 5. SLA = specific leaf area. For more information see Table 3 text

Species	O_3 conc. (nmol mol^{-1})	Dry weight (g)	% dry weight	No. of shoot	Shoot length (cm)	Leaf area (cm^2)	SLA (cm^2 g^{-1})	Root develop-ment	Leaf injury Index	Leaf injury Symptoms
Antennaria dioica	12	1.70a	14.0b	31.1a	5.5	6.6a	246	2.2a	0.0b	Yellow stipples/ necrosis
	40	1.55a	14.0b	32.3a	5.3	6.3a	276	2.6a	0.0b	
	86	0.72b	16.0a	19.0b	4.9	3.6b	329	1.1b	2.8a	
Significance level		***	***	***	ns	***	ns	***	***	
Chamaenerion angustifolium	12	1.06a	8.7b	-	7.8b	109a	54	5.0a	0.0c	Yellow stipples
	40	1.26a	8.7b	-	10.9a	119a	53	5.0a	1.0b	
	86	0.29b	10.6a	-	3.7c	26b	47	1.8b	3.8a	
Significance level		***	***		***	***	ns	***	***	
Oxyria digyna	12	2.46a	7.8	57b	8.6	55a	240	2.8	0.0b	Yellow stipples
	40	2.55a	8.0	63ab	9.1	55a	252	2.3	0.2b	
	86	2.11b	8.2	68a	9.7	34b	254	2.8	2.6a	
Significance level		*	ns	*	ns	***	ns	ns	***	
Phleum commutatum	12	3.22a	23.1b	20.8a	30.3a	-	-	4.5a	[2]2c	Chlorosis/ necrosis
	40	1.52b	20.0b	18.7a	29.3a	-	-	3.2b	15b	
	86	0.03c	94.5a	2.2b	2.3b	-	-	0.0c	99a	
Significance level		***	***	***	***			***	***	
Rumex acetosa subsp. lapponicus	12	1.05b	8.9a	[1]26b	16.7	72b	362	4.6a	0.0b	Yellow stipples/ chlorosis
	40	1.19a	8.5ab	32a	17.9	83a	390	4.5a	0.0b	
	86	0.87c	8.3b	30a	17.1	60c	402	2.8b	2.8a	
Significance level		***	*	**	ns	***	ns	***	***	
Silene vulgaris	12	2.49b	11.4	16.3a	22.9b	25	249	3.7	0.0b	Yellow stipples/ necrosis/ red-brownish pigmentation
	40	3.09a	12.6	16.4a	39.6a	27	273	3.5	0.0b	
	86	2.16b	11.7	11.6b	32.8a	22	277	3.5	1.8a	
Significance level		***	ns	**	***	ns	ns	ns	***	
Solidago virgaurea	12	3.73a	17.1	[1]53	16.7	77	235b	4.1	0.0c	Yellow stipples/ red-brownish pigmentation
	40	3.98a	17.1	52	17.7	88	251ab	3.8	0.7b	
	86	3.11b	16.4	46	15.7	75	272a	4.0	4.4a	
Significance level		**	ns	ns	ns	ns	*	ns	***	
Taraxacum croceum	12	4.06	12.8	-	16.8	122	368	4.9	0.0b	Yellow stipples/ necrosis
	40	3.70	13.1	-	16.5	108	363	4.6	0.2b	
	86	3.76	13.3	-	17.5	106	363	4.5	3.7a	
Significance level		ns	ns	ns	ns	ns	ns	***		
Reference species: *Phleum pratens*	12	4.78a	16.8b	16.8b	63.3a	-	-	4.0a	[2]1b	Chlorosis/ necrosis
	40	4.62a	17.5b	21.3a	61.4a	-	-	3.7a	3b	
	86	0.34b	26.3a	5.6c	13.2b	-	-	0.9b	85a	
Significance level		***	***	***	***			***	***	

[1] No. of leaves [2] Percentage dead leaf area

The leaf:stem fresh weight ratio was decreased by increasing the O_3 concentration in the three *Salix* species where this parameter was measured (Table 3).

The number of shoots was unaffected by the O_3 concentration in the *Salix* species (Table 3), was decreased in three and increased in one (*Oxyria digyna*) of the species in Experiment 2 (Table 4), and increased in one species (*Circium palustre*) in Experiment 3 (Table 5). Number of leaves was counted in a few species and it was found that this had

decreased with high O_3 levels in one of two *Salix* species (Table 3), was enhanced in *Rumex acetosa*, but was unaffected in *Solidago virgaurea* (Table 4) and in the three species where the number was recorded in Experiment 3 (Table 5).

Table 5. The effects of O_3 concentration on growth of different species in Experiment 3. See Table 2 text

Species	O_3 conc. (nmol mol^{-1})	Dry weight (g)	% dry weight	No. of shoots	Shoot length (cm)	Leaf area (cm^2)	SLA (cm^2·g^{-1})	Root development	Leaf injury Index	Leaf injury Symptoms
Angelica archangelica	20	1.63a	12.8	14.3	11.0a	105a	283	4.4a	0.0c	Yellow stipples/ white spots
	45	1.70a	13.0	14.4	11.3a	106a	272	4.3a	1.3b	
	90	1.18b	12.4	15.1	9.3b	76b	285	3.0b	4.5a	
Significance level		***	ns	ns	*	**	ns	***	***	
Cirsium palustre	20	3.40	10.4	34.2b	-	92	198b	5.0a	0.0c	Yellow stipples/ necrosis
	45	3.52	10.4	35.3ab	-	81	208b	4.9a	2.2b	
	90	3.41	10.4	37.1a	-	92	248a	4.6b	4.6a	
Significance level		ns	ns	*	-	ns	***	***	***	
Fragaria vesca	20	1.99a	21.3	[1]31.6	8.8a	75a	272b	2.7a	0.0c	Yellow stipples
	45	1.82a	21.4	33.5	8.7a	74a	267b	2.4a	1.7b	
	90	1.36b	21.5	30.8	7.3b	59b	299a	1.8b	4.4a	
Significance level		***	ns	ns	**	***	*	***	***	
Leontodon autumnalis	20	2.77a	12.3b	11.3	-	-	-	4.6a	0.7b	Yellow stipples/ red-brownish pigmentation
	45	2.81a	12.3b	9.8	-	-	-	4.9a	1.8a	
	90	2.39b	14.2a	10.1	-	-	-	4.0b	2.3a	
Significance level		**	***	ns	-	-	-	***	***	
Ranunculus acris	20	1.65a	15.0b	[1]29.7	7.7	55a	239	4.7a	0.0b	Yellow stipples/ necrosis
	45	1.63a	14.9b	25.8	7.9	57a	240	4.3a	0.0b	
	90	1.30b	16.9a	29.4	7.1	44b	233	3.3b	4.6a	
Significance level		**	***	ns	ns	**	ns	***	***	
Saussurea alpina	20	1.65b	11.0	[1]23.5	14.3b	90b	251	3.4b	0.0b	Yellow stipples/ necrosis
	45	2.08a	11.3	24.3	17.3a	117a	236	3.3b	0.0b	
	90	1.81ab	11.7	26.8	16.3ab	94b	236	3.9a	1.7a	
Significance level		*	ns	ns	*	*	ns	***	***	
Reference: *Phleum pratense*	20	3.34a	16.0b	20.8a	46.1a	-	-	3.3a	[2]0b	Chlorosis/ necrosis
	45	3.28a	16.2b	20.8a	46.0a	-	-	2.9a	3b	
	90	0.12b	22.9a	5.8b	12.1b	-	-	0.6b	71a	
Significance level		***	*	***	***	.	.	***	***	

[1]Number of leaves. [2]Percentage dead leaf area.

The shoot length was increased by raising the O_3 concentration from the low to the intermediate level in *Salix lanata* (Table 3), *Chamaenerion angustifolium* and *Silene vulgaris* (Table 4), and *Saussurea alpina* (Table 5). Raising the concentration from the lowest to the highest level decreased the shoot length in *Chamaenerion angustifolium* and *Phleum commutatum* (Table 4), *Angelica archangelica* (Table 5) and *Fragaria vesca* (Table 5), but the shoot length of the other species was unaffected.

The leaf area of the three largest leaves per pot was decreased by the highest O_3 concentration in 6 of 12 species (Tables 4 and 5). Raising the O_3 level from the low to the intermediate level enhanced the leaf area (30%) in *Saussurea alpina* (Table 5). The specific leaf area was increased by high O_3 concentrations in 3 of 12 species (Tables 4 and 5).

The root development was negatively affected by the highest O_3 level in 8 of 14

species (Tables 4 and 5), positively affected in *Saussurrea alpina* (Table 5) and unaffected in 5 of the 14 species recorded.

All species developed leaf injury (yellow stipples) at the highest O_3 level, while 9 of the 19 species showed distinct stipples at the intermediate level (i.e. >5% of the leaf area stippled) (Tables 3-5). Chlorosis, necrosis, white spots and red-brown pigmentation developed to a variable extent in the different species (Tables 3-5).

The dry weight, shoot length and shoot number of the reference species, *Phleum pratense*, were decreased by increasing the O_3 level from low to high but not from low to intermediate in the three experiments (Tables 3-5). No effect on leaf injury was observed when the O_3 concentration was increased from the low to the intermediate level, but at a high O_3 level 59, 86 and 71% of the leaves became necrotic in Experiments 1, 2 and 3, respectively (Tables 3-5).

DISCUSSION

Large differences in O_3 sensitivity between the 19 alpine plant species were found in the present investigation. The growth rate of relatively few of these species, however, is likely to be affected by the 40-50 nmol mol^{-1} O_3 level (7-h day mean) prevailing in South Norway in summer (Pedersen & Semb 1990). One exception is *Phleum commutatum*, which appeared to be very sensitive to O_3. Another species of the same genus, *Phleum pratense*, has previously been shown to be very sensitive to O_3 (Mortensen 1992) and therefore it was also included as a reference species in the present investigations. Compared with this species, *Phleum commutatum* was even more sensitive to O_3. This alpine species may therefore be of great interest as a bioindicator for the effects of O_3 on alpine vegetation. Unfortunately, the visual injuries caused by O_3 (leaf wilting and necrosis) were not specific for O_3 injury, and can therefore not be distinguished from the symptoms of other stresses such as drought. However, the performance of this species in nature seems to be important as an indicator for the effects of O_3 in subalpine ecosystems.

The light conditions during the O_3 experiments were relatively low (10-20 mol m^{-2} day^{-1}) compared with the natural conditions during summer (May-July) in South Norway (40-50 mol m^{-2} day^{-1}). Previous experiments with tomato demonstrated that increasing the light level may in some cases decrease the O_3 effect (Mortensen 1992). However, the photosynthetic active flux in Experiment 3 was twice that in Experiment 2 without changing the O_3 effect on the reference species (*Phleum pratense*). Nevertheless, the effect of about 40 nmol mol^{-1} O_3 on *Phleum commutatum* in its natural habitat would probabely be less than the 50% dry weight reduction found in the controlled experimental conditions.

Occasionally, moderate O_3 levels may enhance plant dry weight (Rajput & Ormrod 1986), as was also found in three (*Rumex acetosa*, *Saussurea alpina* and *Silene vulgaris*) of the 19 species in the present study. This increase was correlated with an increased shoot elongation in two of the species. Often, the effect of O_3 on the root growth is greater than that on the shoot growth (Cooley & Manning 1988). Despite an enhancement of the shoot growth in the three species, the total plant dry weight might not have been enhanced. However, the visual observation of the root system in the three species indicated that raising the O_3 concentration to an intermediate level had no effect.

In a previous study with 24 wild plant species most species were tolerant to high (80 nmol mol^{-1}) O_3 concentrations (Mortensen & Nilsen 1992). In that investigation *Phleum pratense* was by far the most sensitive to O_3, followed by *Betula pubescens*, which is one of the most widespread tree species in the mountain regions in Norway. Three of the species were the same in the present study and in the previous study, namely *Oxyria digyna*, *Rumex acetosa* and *Solidago virgaurea*. No visual injuries at 40-50 nmol mol^{-1} O_3 could be observed in the last two species in the two investigations. *Oxyria digyna*, however, was unaffected by 80 nmol mol^{-1} in the previous investigation but developed injury symptoms in the present investigation at 86 nmol mol^{-1}. Only in *Phleum commutatum* were the visible O_3 injuries at 40-50 nmol mol^{-1} accompanied by a decrease in dry weight. In the other eight species showing symptoms of O_3 injury at this level the dry weight was unchanged. Tingey (1985) emphasized that growth reductions caused by O_3 are not necessarily concomitant with visible leaf injury, or vice versa. In the present experiments, however, dry weight reductions were always accompanied by visible O_3 injuries. In a previous study with *Betula pubescens*, however, growth reduction took place without visible injury symptoms (Mortensen & Skre 1990).

The genus *Salix* is important in the Norwegian mountain regions, and the present results indicate that species from this genus seem to be tolerant to O_3 pollution. At 40-50 nmol mol^{-1} O_3 only a slight visible O_3 injury could be observed in two of the five species, and in nature it does not seem likely that any O_3 effect can be identified.

So far, a total of 40 wild plant species have been studied for O_3 sensitivity in the present and previous investigations (Mortensen & Nilsen 1992). Of these, only *Phleum commutatum* can be categorized as very sensitive, which means that growth reductions and leaf necrosis (senescence process) at commonly occuring O_3 concentrations (40-50 nmol mol^{-1} over a period of weeks) take place. Other species such as *Angelica archangelica*, *Betula pubescens*, *Centaurea jacea*, *Chamaenerion angustifolium*, *Chrysanthemum leucanthemum*, *Cirsium palustre*, *Fragaria vesca*, *Hypericum perforatum*, *Leontodon autumnalis*, *Plantago lanceolata*, *Salix lanata*, *Salix reticulata*, *Solidago lanceolata* may develop some visible leaf injuries at about 50 nmol mol^{-1} (8-h day means) over a period of some weeks. On the basis of the results so far, however, it seems most probable that O_3 pollution has only a marginal effect on the subalpine vegetation in Norway. But if the O_3 concentration in nature continues to increase by about 1% per year (Hartmannsgruber et al. 1985), many plant species will most likely be negatively affected.

ACKNOWLEDGEMENTS

This work was carried out as a part of the national work on "Critical loads" supported by the Ministry of Environment.

REFERENCES

Ashmore, M.R. 1984. Effects of ozone on vegetation in the United Kingdom. In: A. Greenfeldt (ed.), The Evaluation and Assessment of the Effects of Photochemical Oxidants

on Human Health, Agricultural Crops, Forestry, Materials and Visibility. Gøteborg, IVL. Pp. 92-104.

Cooley, D.R. & J.W. Manning 1988. The impact of ozone on photoassimilate partitioning in plants. Persp. Environ. Bot., 2: 121-146.

Guderian, R., D.T. Tingey, & R. Rabe 1985. Effects of photochemical oxidants on plants. In: R. Guderian (ed.), Ecological Studies 52. Air Pollution by Photochenical Oxidants. Springer-Verlag, Berlin. Pp. 129-346.

Hartmannsgruber, R., W. Attmanspacher, & H. Claude 1985. Opposite behaviour of the ozone amount in the stratosphere and the lower troposphere during the last years. In: C. Zerefos & A. Ghazi (eds.), Quadriennal Atmospheric Ozone Symposium, 1984. Reidel, Dordrecht. Pp. 770-774.

Heagle, A.S. 1989. Ozone and crop yield. Annu. Rev. Phytopathol. 27: 397-423.

Mortensen, L.M. 1982. Growth responses of some greenhouse plants to environment. III. Design and function of a growth chamber prototype. Scientia Hortic. 16: 57-63.

Mortensen, L.M. 1991. Ozone pollution and effects on vegetation in Norway (in Norwegian). Norsk Landbr. Forsk. 5: 235-264.

Mortensen, L.M. 1992. Effects of low ozone concentrations on growth of seven grass and one clover species. Acta Agr. Scand. Sect. B, Soil and Plant Sci. 42:235-239.

Mortensen & Nilsen 1992. Effects of ozone and temperature on growth of several wild plant species. Norwegian Journal of Agricultural Sciences 6: 195-204.

Mortensen, L.M. & O. Skre 1990. Effects of low ozone concentrations on growth of *Betula pubescens* Ehrh., *Betula verrucosa* Ehrh. and *Alnus incana* (L.) Moench. New Phytol. 115: 165-170.

Pedersen, U. & A. Semb 1990. Ozone measurements in Norway. In: C. Baumann (ed.), Biochemical Stress Indicators. Seminar Report, Norw. Forest Res. Inst. Pp. 10-23.

Rajput, C.B.S. & D.P. Ormrod 1986. Stimulation of plant growth in pumpkin by ozone. HortScience 21: 498-499.

Tingey, D.T. 1985. The impact of ozone on vegetation. Persp. Environ. Bot. 1: 1-25.

Extractable heavy metals in newly cultivated and long-term cultivated soils

Q. B. HE[1] & B. R. SINGH[2]
[12]Agricultural University of Norway, Department of Soil Sciences, Ås, Norway
[1]Future address: The Office of Regional Planning Commission on Agriculture, Xing Hu Road 35, Nanning city, Guangxi province, China.

Q.B. He & Singh B.R. 1993. Extractable heavy metals in newly cultivated and long-term cultivated soils. Norwegian Journal of Agricultural Sciences 7:139-146. ISSN 0801-5341.

The effects of long-term use of commercial fertilizers and/or farmyard manure on the extractable heavy metals in agricultural soils, collected from newly cultivated and long-term cultivated fields in southwestern, southeastern, and central parts of Norway were investigated. The soil samples were analysed for Pb, Cr, Zn, Cu, and Mn by extraction with 1 M NH_4OAc (pH 4.8). Those samples were also analysized for pH, organic C, cation exchange capacity (CEC), clay content, easily soluble P, and dithionite-extractable Fe and Mn. The extractable Pb in the long-term cultivated soils from southeastern Norway was significantly lower than that in the newly cultivated soils. The long-term cultivated soils from both southeastern and central Norway contained significantly higher concentrations of Mn than the newly cultivated soils. No significant differences were observed for Cr, Zn, and Cu between the newly and long-term cultivated soils. Long-term use of commercial fertilizers and/or farmyard manure did not seem to have an appreciable effect on the NH_4OAc-extractable Pb, Cr, Zn, and Cu in the agricultural soils of Norway. These metals generally decreased with increasing soil pH, but were found to increase with increasing soil CEC, easily soluble P, and organic matter content.

Key words: Chromium, copper, cultivated soils, lead, manganese, soil properties, zinc

B.R. Singh, Agricultural University of Norway, Department of Soil Sciences, P.O. Box 5028, N-1432 Ås, Norway

Cultivated soils are continuously exposed to heavy metals through atmospheric deposition and application of phosphate fertilizers and/or farmyard manure. Removal of heavy metals from cultivated soils takes place through harvested crops and leaching. In general, phosphate fertilizers contain 0.1-170, 7-225, 66-245, 50-1450, 1-300, and 40-2000 mg kg^{-1}; and farmyard manure contains 0.3-0.8, 6.6-15, 5.2-55, 15-250, 2-60, and 30-550 mg kg^{-1} of Cd, Pb, Cr, Zn, Cu, and Mn, respectively (Kabata-Pendias and Pendias, 1989). Christensen and Tjell (1989) found that long-term use of NPK fertilizers had increased the 6 M HNO_3-soluble Pb, Zn, and Cu in Danish agricultural soils, and the increase of Pb and Zn was significant. In a field investigation, Erviö et al. (1990) reported that the NH_4OAc-EDTA-extractable Cr, Cu, and Mn in Finnish agricultural soils increased, but the extractable Zn and Pb decreased in a 13-year period.

In the previous studies, long-term use of phosphate fertilizers and/or farmyard manure was found to increase the total (Bærug and Singh, 1990) and extractable (He and Singh, 1993a) Cd in some regions of Norway. It was therefore thought desirable to assess whether such increases could also occur for other metals generally present in commercial fertilizers. This paper presents the results of NH4OAc(pH 4.8)-extractable Pb, Cr, Zn, Cu, and Mn in the newly cultivated and long-term cultivated soils of southwestern, southeastern, and central Norway. The relationships between the extractable metals and some important soil properties are also presented.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from different farms located in southwestern, southeastern, and central Norway to test the effects of long-term applications of P fertilizers and farmyard manure on heavy metal concentrations in soils (Fig. 1). The soil sampling was carried out in pairs from the surface layer (0 - 20 cm) of the newly cultivated (< 4 years) and long-term cultivated (> 30 years) fields located near each other in order to minimize the variations in soils and environments. An area of about 5 m^2 in each field was representatively selected for sampling. Six to eight sub-samples were randomly collected from this area and then combined into one representative sample for the whole field. Fertilization concentrations for the sampled fields ranged from 20 to 30 kg P ha^{-1} yr^{-1} using commercial P fertilizers, or by supplementing with farmyard manure in the grass-growing fields. There was no previous history of sludge application in the fields. In southeastern Norway the sampling was carried out during the cropping season of 1983, and in central and southwestern Norway it was done during the cropping season of 1986. A total of 146 (73 pairs) soil samples were collected, of which, 100 (50 pairs) samples were collected from southeastern Norway, 38 (19 pairs) from central Norway (Trøndelag county), and 8 (4 pairs) from southwestern Norway (Rogaland county). Soils were air-dried, crushed and passed through a 2 mm sieve prior to chemical determinations.

Soil analyses

Determinations of soil chemical properties

Soil pH was measured in a 1:2.5 soil to water ratio. The soil suspension was allowed to stand overnight prior to pH determination using a pH-meter. Soil organic carbon was measured by combustion in an EC-12 LECO-carbon analyzer. Available phosphorus of the soils was determined by the ammonium-lactate (AL) method (Egner et al. 1960). Cation exchange capacity was determined by extraction with 1 N NH_4OAc (pH 7.0) (Page et al. 1982). Dithionite-extractable Fe and Mn were determined by shaking two grams of soil and 100 ml of Na-dithionite solution for 12 hours. After filtration through a S&S blue ribbon filter paper, Fe and Mn concentrations in the extracts were determined in a 0.2% KCl solution by an atomic absorption spectrophotometer. The soil particle-size distribution was measured by the pipette method (Elonen, 1971).

Determination of soil cadmium

The extractable soil Pb, Cr, Zn, Cu, and Mn concentrations were determined by extraction with 1 M NH_4OAc (pH4.8) (Andersson, 1976). Soil samples were shaken with the extractant on an end-over-end shaker for one hour and then filtered through a S&S blue ribbon filter paper. The extract was analyzed for Pb, Cr, Zn, Cu, and Mn using a PERKIN-ELMER 3030 graphite furnace atomic absorption spectrophotometer with background correction (HGA-400 programme).

Fig. 1. Location of the sampling sites

RESULTS AND DISCUSSION

pH, easily soluble P, and organic C in soils

The soil pH was generally higher in southwestern Norway than in southeastern and central Norway. The soils from the long-term cultivated fields in southeastern Norway had significantly higher pH than those from the newly cultivated fields (Table 1). The soil pH in the other two regions also tended to be higher in the long-term cultivated soils. The ammonium lactate-soluble P (P-AL) was significantly higher in soils from the long-term cultivated fields than those from the newly cultivated fields, indicating that long-term use of phosphatic fertilizers and farmyard manure has increased the easily soluble P levels in the soils. The organic C content was lower in the long-term cultivated soils than in the newly cultivated soils in all three regions, being more pronounced in the soils from southeastern Norway (Table 1).

Extractable heavy metals in soils

The NH_4OAc-extractable Pb, Cr, Zn, Cu, and Mn are listed in Table 2. The extractable Pb was lower in the long-term cultivated soils than in the newly cultivated soils in all three regions. The difference between the long-term and newly cultivated soils was significant in southeastern Norway, but not in other two regions. The extractable Cr and Zn in soils from the long-term cultivated fields in southeastern Norway were slightly lower than those from the newly cultivated fields, but in southwestern Norway the long-term cultivated soils contained slightly higher levels of Cr and Zn than the newly cultivated soils. The extractable Cu in southeastern and central Norway was slightly higher in the long-term cultivated soils than in the newly cultivated soils. In both southeastern and central Norway, the extractable Mn was significantly higher in the long-term cultivated soils than in the

newly cultivated soils. No significant difference was found for NH_4OAc-extractable Mn between the newly cultivated and long-term cultivated soils of southwestern Norway.

Table 1. pH, easily soluble P (P-AL), and organic matter content in newly cultivated and long-term cultivated soils

Location	Soil group	pH	P-AL(mg kg-1)	Org.-C (%)
Southeastern	Long-term-cultiv.	5.95±0.42⇑	8.93±4.65	2.78±1.66
Norway	Newly cultiv.	5.48±0.57	4.74±3.73	4.78±3.78
n = 100	Long-term vs. newly	0.47***	4.19***	-2.00***
Southwestern	Long-term-cultiv.	6.38±0.97	10.12±7.31	5.36±3.57
Norway	Newly cultiv.	6.18±1.27	6.33±3.49	5.40±3.33
n = 8	Long-term vs. newly	0.20	3.79	-0.04
Central	Long-term-cultiv.	5.82±0.50	15.21±6.95	22.42±17.73
Norway	Newly cultiv.	5.74±0.76	8.51±5.85	23.48±18.30
n = 38	Long-term vs. newly	0.08	6.70**	-1.06

⇑Mean ± standard deviation
** and ***Significant at the 1 and 0.1% confidence level, respectively

Table 2. The NH_4OAc-extractable heavy metals in newly and long-term cultivated soils

Location	Soil group	Pb	Cr	Zn	Cu	Mn
		--mg kg^{-1}--				
Southeastern	Long-term cultiv.	0.39±0.27⇑	0.18±0.15	0.91±1.16	0.45±0.34	26.73±28.18
Norway	Newly cultiv.	1.01±1.48	0.25±0.25	1.36±1.48	0.40±0.36	16.91±17.67
n = 100	Long-term vs. newly	-0,62**	-0.07	-0.45	0.05	9.83*
Southwestern	Long-term cultiv.	0.75±0.42	0.42±0.28	3.39±0.47	0.39±0.47	17.59±12.79
Norway	Newly cultiv.	2.03±1.54	0.35±0.11	3.26±1.50	0.45±0.41	18.83±3.88
n = 8	Long-term vs. newly	-1.28	0.07	0.13	-0.06	-1.24
Central	Long-term cultiv.	0.30±0.20	0.43±0.23	3.16±2.34	0.60±0.34	61.48±42.08
Norway	Newly cultiv.	0.36±0.23	0.42±0.28	2.37±2.86	0.43±0.31	48.66±70.52
n = 38	Long-term vs. newly	-0.06	0.01	0.79	0.17	12.82*

⇑Mean ± standard deviation. * and ** Significant at the 5 and 1% confidence levels. respectively

With the exception of the three cases mentioned above, there were no significant differences in NH_4OAc-extractable Pb, Cr, Zn, Cu, and Mn between the newly cultivated and long-term cultivated soils. The lower concentration of Pb in the long-term cultivated soils than in the newly cultivated soils could be due partly to its removal by harvested crops and partly to cultivation practices over a longer period which might have mixed Pb in a greater volume of soil than in the newly cultivated soil. At the same time, the significantly higher organic matter content in the newly cultivated soils than in the long-term cultivated soils may also contribute to the higher Pb content in the former soils as Pb is known to form

complexes with organic matter. The higher concentration of Mn in the long-term cultivated soils than in the newly cultivated soils may be a result of the long-term use of commercial fertilizers that contain appreciable amounts of Mn (Kabata-Pendias and Pendias, 1989).

The extractable Pb in the soils from both southwestern and southeastern Norway was significantly higher than those from central Norway (Table 3), and it was found to be higher in southwestern Norway than in southeastern Norway. The soils from southwestern and central Norway contained significantly higher Cr and Zn than those from southeastern Norway. No such difference was found for the extractable Cu among the three regions. The highest Mn concentration was found in soils from central Norway and the lowest in soils from southwestern Norway. Besides the differences in parent materials and fertilizers applied, long-distance atmospheric transport of heavy metals from other European countries may be an important factor causing regional differences in heavy metal concentrations. Steinnes (1987) found significantly higher concentrations of many heavy metals in southern and southwestern Norway than in other parts of the country further north. The long-distance atmospheric transport is especially true for the more volatile metals such as Pb, Cd, and As which are preferentially concentrated on the small particle fraction available for transport over long distance.

Table 3. Regional differences in extractable heavy metals

Region	Pb	Cr	Zn	Cu	Mn
	mg kg-1				
Southwest.†	1.32 ± 1.20^a	0.39 ± 0.21^a	3.33 ± 1.70^a	0.42 ± 0.42^a	18.1 ± 9.4^a
Central	0.33 ± 0.21^b	0.43 ± 0.25^a	2.78 ± 2.61^a	0.52 ± 0.33^a	55.8 ± 55.9^b
Southeast.	0.69 ± 1.10^a	0.22 ± 0.21^b	1.13 ± 1.34^b	0.43 ± 0.35^a	21.7 ± 23.8^a

†Means ± standard deviation with the same letter in the same column are not significant at the 5% confidence level

Table 4. Correlations between the extractable heavy metals and soil properties from stepwise procedure

Variables in equation	R2 (n)†
Pb = 1.50 - 0.18pH	0.06* (84)
Cr = 0.218 + 0.016CEC (0.32) - 0.046pH	0.35*** (77)
Zn = 1.19 + 0.21organic C (0.34) + 0.04P-AL (0.42) - 0.23pH	0.45*** (81)
Cu = 0.275 + 0.02P-AL	0.07* (77)
Mn = -6.28 + 372Mn-dith. (0.75) + 0.87CEC	0.77*** (77)

†The number of samples (n).
* and ***Significant at the 5 and 0.1% confidence level, respectively.

Relationships between the extractable heavy metals and soil properties

Stepwise regression analysis was performed by relating the extractable heavy metals to various soil properties of 84 soil samples from southeastern Norway. The variables included

soil pH, cation exchange capacity (CEC), easily soluble P (P-AL), dithionite-extractable Fe and Mn, organic C, and clay content. The parameters significantly affecting the NH4OAc-extractable Pb, Cr, Zn, Cu, and Mn are presented in Table 4. The extractable Pb decreased with increasing pH in the soils, but the correlation coefficient was low. The Cr concentration increased with increasing values of soil CEC but it decreased with increasing soil pH, with CEC alone explaining 32% of the variations in extractable Cr (Fig. 2). The extractable Zn was significantly related to organic C, P-AL, and pH in the soils. It showed the highest correlation with organic C content (Fig. 3). The extractable Cu was significantly correlated only to the ammonium lactate-soluble P. The highest correlation of extractable Mn was found with the dithionite-extractable Mn which explained 75% of the variations (Fig. 4).

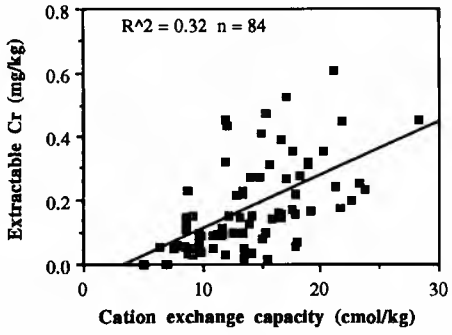


Fig. 2. The relationship between the NH_4OAc-extractable Cr and soil CEC

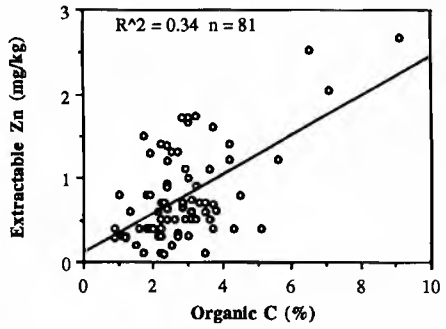


Fig. 3. The relationship between the NH_4OAc-extractable Zn and soil organic C content

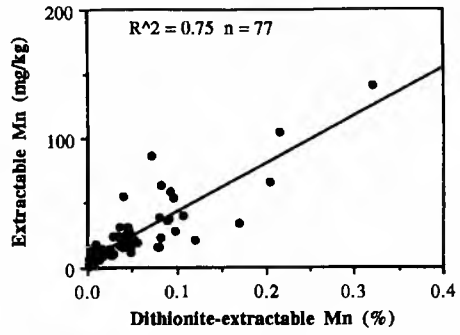


Fig. 4. The relationship between the NH_4OAc-extractable Mn and dithionite-extractable Mn

Soil pH is an important factor regulating the solubility of heavy metals. There is a general decrease in the solubility of Pb, Cr, Zn, Cu, and Mn when the soil pH is increased (Harter, 1983; Tyler and McBride, 1982; Andersson, 1975). The adsorption of these metals by clay minerals and organic matter also increases with increasing pH. The increases of extractable Cr, Zn, and Mn with increasing values of soil CEC and organic matter content imply that a larger fraction of these metals adsorbed by the soil minerals and organic matter was easily extracted by NH_4OAc (pH 4.8). Similar results have been found for Cd in these soils (He and Singh, 1993b). The positive correlation between the extractable Zn and Cu and the easily soluble P could be due to the long-term application of phosphate

fertilizers and/or farmyard manure as these are known to contain both Zn and Cu impurities (Kabata-Pendias and Pendias, 1989).

ACKNOWLEDGEMENT

The first author thanks the Agricultural Research Council, Norsk Hydro, and the Ministry of Agriculture for the fellowship grant which made it possible to carry out this research programme.

REFERENCES

Andersson, A. 1975. Relative efficiency of nine different soil extractants. Swedish J. Agric. Res. 5: 125-135.

Andersson, A. 1976. On the determination of ecologically significant fractions of some heavy metals in soils. Swedish J. Agric. Res. 6: 19-25.

Bærug, R. & B. R. Singh 1990. Cadmium levels in soils and crops after long-term use of commercial fertilizers. Norwegian J. Agric. Sci. 4: 251-260.

Christensen, T. H. & J. C. Tjell 1989. Long term trends in heavy metal contents of agricultural soils. Heavy Metals in the Environment. Geneva, CEP Consultants Ltd., Edinburgh, UK.

Egner, H., H. Riehm & W. R. Domingo 1960. Investigations of the chemical soil analysis as a basis for the evaluation of nutrient status in soil: II. chemical extraction methods for phosphorus and potassium determination (in German). K. Lantbruks Høgsk. Ann. 26: 199-215.

Elonen, P. 1971. Particle-Size Analysis of Soil. Ph. D. thesis. Department of Agricultural chemistry, University of Helsinki, Finland.

Erviö, R., Mäkelä-Kurtto & J. Sippola 1990. Chemical characteristics of Finnish agricultural soils in 1974 and 1987. In: Kauppi et al. (eds). Acidification in Finland. Springer-Verlag Berlin Heidelberg. Pp. 217-234.

Harter, R. D. 1983. Effect of soil pH on adsorption of lead, copper, zinc, and nickel. Soil Sci. Soc. Am. J. 47: 47-51.

He, Q. B. & B. R. Singh 1993a. Plant availability of Cd in soils: I. extractable Cd in newly- and long-term-cultivated soils. Acta Agric. Scand. (in press).

He, Q. B. & B. R. Singh 1993b. Plant availability of Cd in soils: II. factors affecting the extractability and plant uptake of Cd in cultivated soils. Acta Agric. Scand. (in press).

Kabata-Pendias, A. & H. Pendias (eds.) 1989. Trace Elements in Soils and Plants. CRC Press. Pp. 9-10.

Page, A. L., R. H. Miller & D. R. Keeney (eds.) 1982. Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. Second edition. Am. Soci. Agron., Inc. and Soil Sci. Soci. Am., Inc. Madison, Wisconsin USA.

Steinnes, E. 1987. Impact of long-range atmospheric transport of heavy metals to the terrestrial environment in Norway. In: Hutchinson, T. C. & K. M. Meema (eds.). Lead, Mercury, Cadmium and Arsenic in the Environment. John Wiley & Sons. Pp. 107-117.

Tyler, L. D. & M. B. McBride 1982. Mobility and extractability of cadmium, copper, nickel, and zinc in organic and mineral soil columns. Soil Sci. 134, 198-205.

Uptake of zinc, cadmium, mercury, lead, chromium and nickel by ryegrass grown in a sandy soil

BAL RAM SINGH & ALHAJI S. JENG
Agricultural University of Norway, Department of Soil Sciences, Ås, Norway

Singh, B.R. & A.S. Jeng 1993. Uptake of zinc, cadmium, mercury, lead, chromium and nickel by ryegrass grown in a sandy soil. Norwegian Journal of Agricultural Sciences 7:147-157. ISSN 0801-5341.

A greenhouse experiment on a sandy soil was conducted to investigate the effects of relatively high rates of zinc, cadmium, mercury, lead, nickel and chromium application on ryegrass yield, metal concentration and uptake. The metals were applied only in the first year and their residual effect on crop yield and metal concentration was studied in the subsequent years. All the metals were applied as salts. The results revealed that Cd and Hg at rates greater than 10 mg kg^{-1} soil, caused yield depressions in the first year due to their toxic effects. Very little or no residual effect of Hg was recorded in the second and third years. Some negative effect of Pb on yield was noticed in the second year. With the exception of Cr, the concentration of all the metals in ryegrass was found to increase with increasing application rates in the first year, then to decrease drastically in the subsequent years, thus showing reduced availability of these metals to plants over time. Total metal uptake by plants generally followed the same trend as metals concentration in the plants. The concentration of Cd and Ni in ryegrass at all levels of application was much higher than normally found in crop plants grown in non-contaminated soils, and this was also the case for Hg at the highest level of application. For other metals only a small deviation from the normal range was observed.

Key words: Cadmium, chromium, heavy metals, lead, mercury, nickel, ryegrass, sandy soil, toxicity

Bal Ram Singh, Agricultural University of Norway, Department of Soil Sciences, P.O. Box 5028, 1432 Ås, Norway

In view of the importance of plants in most food chains, a number of studies have been directed towards plant accumulation and effects of metals on plant growth and yield. There are wide differences in the uptake and translocation of metals in plants and therefore the total amounts of metals present in the soil may not always reflect their concentrations in the plant. Some metals like Cd and Ni in plants have shown good relationship to their soluble or exchangeable forms in soils (Lund et al. 1981; He & Singh 1993; Hutchinson 1981), whereas others such as Pb and Cr showed no relationship to their contents in soils (Tjell et al. 1979). Several of the metals, such as Hg, Pb, and Cd are normally regarded as biologically unfavourable. Others are detrimental in larger amounts but essential to the

organism in smaller concentrations. According to Adriano (1986), crops such as turnip and the beet family are very sensitive to metals, while grains and most grasses are more tolerant. For this reason, yield decreases in grasses or grain crops are seldom recorded even though the heavy metal concentrations are high. This is, however, a general statement, as crop sensitivity to individual elements differs considerably. The prospect of detrimental effects of heavy metals has become of greater interest because of use of sewage sludge or other industrial by-products which may enrich plants with heavy metals.

The objectives of this study were to investigate the effect of relatively high applications of various heavy metals on the yield and metal concentrations in ryegrass and to assess whether such rates of application could result in metal concentrations in plants considered toxic to plants or harmful in the food chain.

MATERIALS AND METHODS

Greenhouse pot experiments were carried out on a sandy soil (collected from Ås, southeastern Norway) with a pH of 5.6 and an organic carbon content of 0.4%. The concentrations of P, K, and Mg extracted by ammonium lactate (Egner et al. 1960) were 21, 26, and 3 mg kg^{-1} soil, respectively. The soil contained 0.6 and 0.1% Fe and Al, determined by shaking 2 g soil and 10 ml of Na-dithionate solution for 12 h.

The soil was limed at the rate of 10 g $CaCO_3$ pot^{-1} to raise the pH level to about 6.0. The metals were applied at the rates of 0, 2, 10 and 50 mg kg^{-1} soil, corresponding to 0, 0.4, 2.4 and 10 kg da^{-1}. Zinc, Pb, Hg, Cd, and Ni were applied as metal chlorides but Cr as Cr_2O_3. The metals were applied only in the first year and the residual effect was studied in the subsequent years. Apart from the control (nine replications), each treatment was replicated three times, giving a total of 63 pots.

Nitrogen as $Ca(NO_3)_2$, P as $Ca(H_2PO_4)_2$ and K as K_2SO_4 were applied to all treatments as basal dressings at the rates of 120, 45 and 120 mg kg^{-1} soil, respectively. A basal dose of 19.7 mg Mg kg^{-1} soil was given as $MgSO_4.7\ H_20$. The micronutrients Cu, Mn, B and Mo were applied to all pots at the rates of 6.4, 6.5, 0.28 and 0.27 mg kg^{-1}, respectively. These applications were repeated annually for the duration of the experiment.

The lime, fertilizers, micronutrients, and metals were thoroughly mixed with 6.67 l of soil (about 8 kg) which was filled in 7 l plastic pots. About 1.5 g ryegrass seed was sown in each pot and the moisture content in the pots was maintained at near field capacity by regular irrigation with deionized water. The ryegrass crop was harvested four times during the growing season, i.e. every three weeks ($\pm$ 3 days). The material was dried at 60°C for 48 hours and the dry weights recorded.

After harvest, the soil-filled pots were kept in the greenhouse until the next cropping season and used again in the subsequent years. The procedure employed in the first year was repeated in the second and third years.

Samples of dried plant material were ground, dry-ashed at 450°C and the ash dissolved in 5 ml concentrated H_2SO_4. The solution was then diluted to 100 ml with distilled water, and Zn, Pb, Cd, Hg, Ni and Cr were determined by atomic absorption spectrophotometry. The results are reported on oven-dry basis. Metal uptake was calculated as yield (kg) multiplied by concentration (mg kg^{-1} D.M). Weighted mean metal con-

centration was calculated as the concentration in each of four harvests per year (mg kg^{-1}), for three years, multiplied by the weight of the corresponding harvests (kg), and divided by the total harvest (kg). Statistical analysis of yield, concentration, and uptake data was carried out using the "StatView™" program, developed by Abascus Concepts Inc. (1988).

RESULTS AND DISCUSSION

Dry matter yields

The effects of increasing rates of heavy metal application on mean dry matter yield are presented in Table 1. As the metals were applied only in the first year, the effects on yields in the second and third year are residual.

Table 1. Average dry matter yields (g pot^{-1}) of ryegrass grown on a sandy soil as affected by increasing rates of metal application

	*Mean dry matter yield					
	Zn	Pb	Hg	Cd	Ni	Cr
Year 1						
Rates (mg kg^{-1})						
0	24.8a	24.8a	24.8a	24.8a	24.8a	24.8a
2	24.5a	25.6a	25.1a	24.6a	25.5a	25.0a
10	25.3a	24.4a	20.2a	21.4a	24.8a	25.2a
50	23.8a	25.5a	5.8b	16.3a	24.7a	22.3a
Year 2						
0	16.9a	16.9a	16.9a	16.9a	16.9a	16.9a
2	16.9a	14.4ab	16.4a	16.9a	17.5a	16.3a
10	18.7a	16.7a	18.3a	17.7a	18.2a	17.2a
50	19.1a	13.2b	20.3a	16.2a	19.4a	18.2a
Year 3						
0	16.5a	16.5a	16.5a	16.5a	16.5a	16.5a
2	19.0a	17.2a	16.3a	17.5a	16.5a	17.6a
10	17.2a	18.0a	17.0a	16.2ab	17.3a	17.5a
50	17.9a	19.2a	19.2a	9.6b	19.8a	17.3a
	** Weighted mean dry matter yields (1-3 years)					
Rates (mg kg^{-1})	Zn	Pb	Hg	Cd	Ni	Cr
0	19.4a	19.4a	19.4a	19.4a	19.4a	19.4a
2	20.1a	19.0a	19.3a	19.7a	19.8a	19.6a
10	20.4a	19.7a	18.5a	18.4a	20.1a	20.0a
50	20.2a	19.3a	15.3a	14.0a	21.3a	19.3a

* Means are based on 3 replicate treatments and 4 harvests per year, i.e. means of 12 figures. ** Mean values are based on 3 replicate treatments, 4 harvests per year for a 3-year period, i.e. means of 36 figures. Values followed by the same letter within a column are not significantly different at 5% level, according to Fischer PLSD

Only Hg reduced ryegrass yield significantly ($p < 0.05$) at the highest application rate in the first year. Cadmium also tended to decrease the yield at this level, although the differences among treatments were not statistically significant (Table 1). Visual observations during the growing season showed clear stunting of the plants at the highest Hg level, and reduced growth at the highest Cd level.

In the second and third years the detrimental effects of even Hg and Cd were generally not seen, and no significant effects of any other metal applied in the first year were observed on dry matter yield. The weighted dry matter yield for three years tended to be reduced at the highest level of Hg and Cd, most certainly due to drastic reduction in the first year, but no such effects were observed for other metals (Table 1).

Metal concentration and uptake

Weighted mean metal concentrations on a yearly basis are shown in Figs. 1 and 2, and for the three-year period, in Fig. 3.

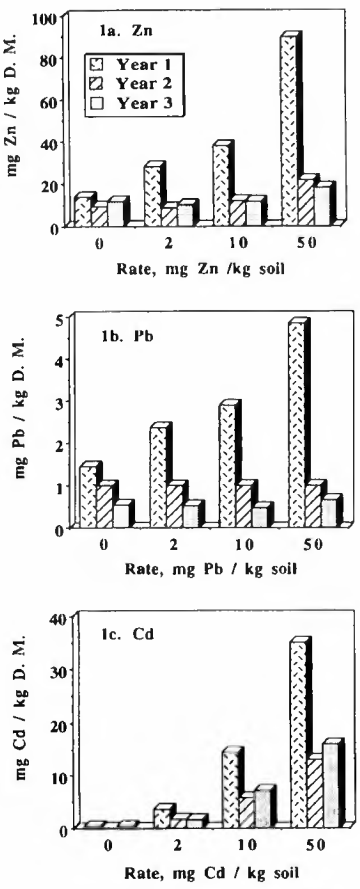


Fig. 1. Yeraly weighted mean concentrations of Zn, Pb, and Cd in ryegrass

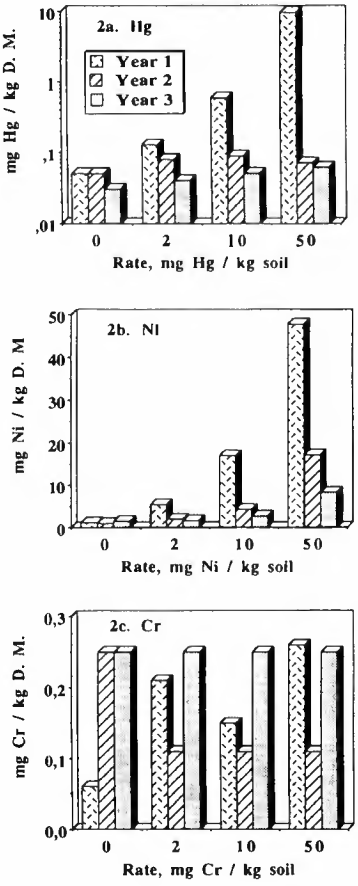


Fig. 2. Yearly weighted mean concentrations of Hg, Ni and Cr in ryegrass

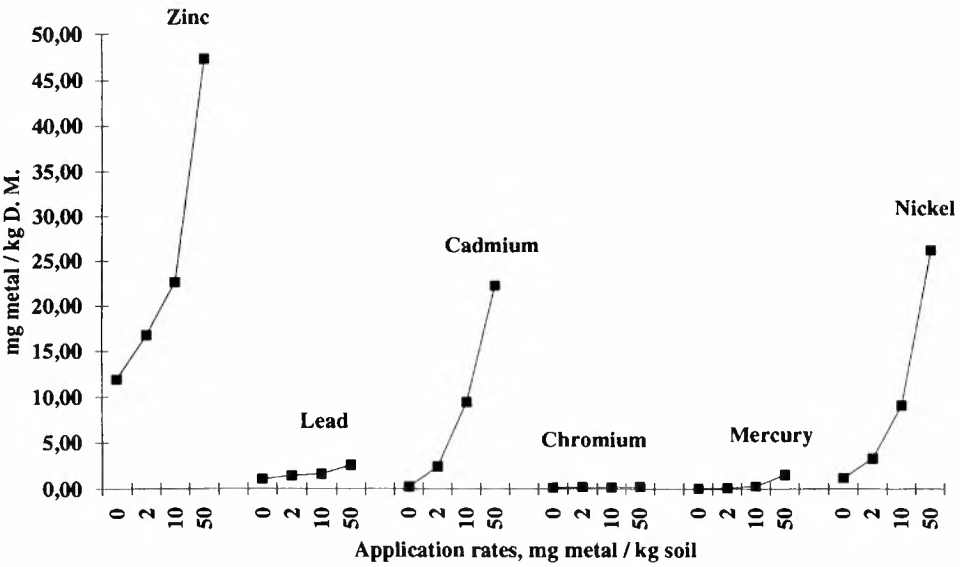


Fig. 3. Weighted mean metal concentrations in ryegrass as a function of increasing metal application rates - three years' data

Zinc

The concentration of Zn increased linearly with increasing zinc application rates in the first year (Fig. 1a). In the second and third years, however, this effect was reduced tremendously, indicating that the greater proportion of the element, added as easily soluble chloride salt, was taken up by the ryegrass crop, and that the residual amounts were either small or were made less available to the crop in the second and third years. It is known that the availability of metals in soils decreases with time (Boawn 1974; Follet & Lindsay 1971). Boawn (1974) found that DTPA extractability of Zn added as $ZnSO_4$ decreased for four years and appeared to reach an equilibrium availability thereafter. Follet & Lindsay (1971) found reversion of Zn, Cu, Fe, and Mn added as soluble salts or chelates in soils of varying textures and pH. The results of this study also indicate the same trend as observed by Boawn (1974). Fig. 3 shows that, compared with the other elements, the weighted mean concentration of zinc, for the three years, was the highest. This indicates that in this sandy growth medium, with low content of organic matter and low cation exchange capacity, zinc is highly mobile and is easily taken up by this plant species. Tissue concentration of about 48 mg Zn kg^{-1} DM found in this study was in the normal range and consequently no yield depressions could be expected at this level.

In the three years of the experiment, application of 2 mg Zn kg^{-1} soil did not seem to increase the Zn uptake by ryegrass substantially, relative to the control (Table 2). However, in the first and second years, metal uptake increased significantly ($p < 0.001$) after

application of 10 and 50 mg of the element kg^{-1} soil. In the third year only the highest rate resulted in significant uptake relative to the control (Table 2).

Table 2. Mean and total metal uptake by ryegrass as a function of metal application rates

Application rate (mg element kg^{-1} soil)	Year 1	Year 2	Year 3	Total uptake (3 years)
Zn		Uptake (mg pot^{-1})		
0	0.34a*	0.16ab	0.19a	0.69a
2	0.68ab	0.14a	0.19a	1.01ab
10	0.96b	0.23b	0.20a	1.39b
50	2.00c	0.42c	0.32b	2.74c
Pb				
0	0.036a	0.017b	0.009a	0.063a
2	0.060a	0.015ab	0.009a	0.084a
10	0.071a	0.016b	0.008a	0.096a
50	0.124b	0.013a	0.013a	0.150b
Cd				
0	0.008a	0.002a	0.006a	0.016a
2	0.080a	0.030a	0.027a	0.146a
10	0.307b	0.102b	0.115b	0.524b
50	0.570c	0.211c	0.153c	0.934c
Hg				
0	0.001a	0.001a	0.001a	0.003a
2	0.003a	0.001a	0.001a	0.005a
10	0.012a	0.002a	0.001a	0.015a
50	0.055b	0.015a	0.001a	0.071b
Ni				
0	0.03a	0.02a	0.03a	0.08a
2	0.14a	0.04ab	0.03a	0.21a
10	0.42b	0.06b	0.05a	0.53b
50	1.17c	0.33c	0.17b	1.67c
Cr				
0	0.002a	0.004a	0.004a	0.010a
2	0.005a	0.002a	0.005a	0.012a
10	0.004a	0.002a	0.005a	0.011a
50	0.006a	0.002a	0.004a	0.012a

* Values followed by the same letter within a column and treatment are not significantly different at the 5% level, according to Fischer PLSD

Lead

Like Zn, the concentration of Pb also increased with increased rate of Pb application in the first year. However, the magnitude of increase in Pb concentration was much lower (Fig.

1b). In the second and third years, no such increase was observed even at the highest level of its application. The yearly weighted mean concentration was very low relative to that of Zn, Cd, and Ni (Fig. 1a & c and Fig. 2b), lying below 5 mg Pb kg^{-1} DM, and being reduced to less than 1 mg Pb kg^{-1} DM in the subsequent years. Only the highest level of Pb applied increased the Pb uptake by ryegrass significantly in the first year (Table 2). As it was for concentration, Pb total uptake was also very low compared with the uptake of Zn, and Ni. There is a general agreement that only a small proportion of the Pb present in a soil is available to plants. Tjell et al. (1979) used ^{210}Pb as a tracer in rural Denmark and showed very low uptake of Pb into grass from the soil, and they deduced that 90-99% of Pb in the leaf material was due to foliar uptake. Marten & Hammond (1966) studied Pb uptake by bromegrass from a sandy loam soil with Pb content ranging from 12 to 680 ppm, and found that only plants growing in the soil with 680 ppm Pb accumulated any significant amounts of the element. Similarly, Singh & Steinnes (1976) found very low concentrations of Pb (about 1 mg kg^{-1}) in barley grown on metal-contaminated soil, with Pb concentrations ranging from 83 to 134 mg kg^{-1}. In crops grown on contaminated soil and acid-washed sand, Motto et al. (1970) found limited translocation of Pb to other parts of the plant. These results agree well with the Pb uptake in the present study. Despite the relatively high amounts added to the soil, low concentrations (< 5 mg kg^{-1} DM.) were found in the upper parts of the plant.

Cadmium

Cd followed almost the same trend as Zn but its concentrations in ryegrass at various levels of Cd application were about half to one-third as large as that of Zn (Fig. 1c). Unlike Zn, Cd concentration among various treatments, and especially at 10 and 50 mg Cd kg^{-1} soil levels, persisted at high levels also in the second and third years (Fig. 1c). The weighted mean concentration of Cd increased relatively rapidly, almost linearly, as the amounts added were increased. However, like Zn, tissue concentration dropped to less than one-third after the first year (Fig. 1). A regression on Cd applied versus weighted mean Cd concentration in the ryegrass tissue gave an r-value of 0.98. This is in good agreement with the general finding that there is a positive, almost linear correlation between levels of added Cd to the soil and the resulting Cd concentration in the plant tissues (Adriano, 1986). Allaway (1968) reported that although food plants normally contain less than 0.5 mg Cd kg^{-1}, plants may accumulate up to about 3 mg Cd kg^{-1} before severe plant growth depressions occur. Bærug & Singh (1990) found that most of the grain and grass crops in Norway contained Cd ranging from < 0.1 to 0.2 mg kg^{-1}. Cadmium concentrations observed in the ryegrass used in this greenhouse study (Fig. 1c) are much higher than those normally found in field crop plants, and this may explain the growth and yield depressions (Table 1). The Cd concentration in the ryegrass even at the lowest rate of Cd application (2 mg kg^{-1} soil) is in the range normally considered unsafe for food or fodder crops.

At the lowest application rate (2 mg kg^{-1}), no increase in plant uptake Cd relative to the control was found (Table 2), but as the rate was increased to 10 and 50 mg Cd kg^{-1} soil, a significant ($p < 0.05$) rise in plant Cd content resulted, and this persisted during the whole of the three-year period.

Mercury

Similar to the metals described above, the concentration of Hg in ryegrass also increased consistently with increasing rate of Hg (Fig. 2a) in the first year, but the differences in the concentration between treated and untreated plants disappeared in the second and third years. The weighted mean concentration of Hg did not differ considerably among treatments (Fig. 3). Significant differences in plant uptake of Hg were found only when the highest rate of application (50 mg Hg kg^{-1}) was compared with the lower rates, and this occurred only in the first year (Table 2). In the subsequent years differences in uptake, caused by the different levels, were not significant. Semu et al. (1985) also found that the concentration of Hg in wheat and bean straw increased significantly with increased levels of $HgCl_2$ application. Similar observations were also made by other investigators (Bache et al. 1973; John 1972; Sorteberg 1974). In general, there is a tendency for Hg to accumulate in the roots, i.e. the roots serving as a barrier to Hg uptake (Hogg et al. 1978; Beauford et al. 1977; Fang 1978), and may be an important reason for the low weighted mean concentrations of generally below 1 mg kg^{-1} found here (Fig. 2). Another may be that the mercury used was applied as $HgCl_2$, which has been reported to be less readily translocated in plants than the organic form (Adriano 1986).

Nickel

The concentration of Ni in ryegrass each year or the weighted mean over three years showed a trend similar to that observed for Zn and Cd, and all increases in Ni application caused a significant increase ($p < 0.001$) in plant content of the element relative to the control (Fig. 2b). Similarly, the total uptake of Ni followed a pattern similar to that of Cd and Zn (Table 2). Significant differences in Ni uptake by the plant were found at rates higher than 2 mg kg^{-1}. The element was applied as easily soluble $NiCl_2$. This, together with the low sorption capacity and relatively low pH (about 6.0) of the growth medium, makes it reasonable to assume that the element was presented as Ni^{2+} in the soil solution. The concentration of Ni in plants generally reflects the concentration of the element in the soil, although, clearly, the relationship is more directly linked with the presence of soluble and exchangeable forms of the element (Hutchinson 1981; Cataldo et al. 1978). Factors which increase solubility and exchangeability of Ni in soil also lead to an increased concentration of the element in plants. Concentrations of Ni in plants growing in non-contaminated and non-serpentine soils are generally in the range 0.1 - 5 mg kg^{-1} (Hutchinson 1981; Hutchinson et al. 1981). The concentration of Ni in ryegrass in this study, even at the lowest rate of Ni application, are on the higher side (Fig. 2b).

Chromium

The concentration of Cr in ryegrass on yearly basis (Fig. 2c) or the weighted mean concentration over three years (Fig. 3) is very low and there was no consistent effect of Cr application on its concentration in plants. In Table 2 it can be seen that there are significant differences in Cr uptake by plants between the control and any of the application rates. Concentrations in the foliar parts of the plants bear little relationship to the overall content of Cr in soil; non-contaminated or background concentrations are of the order of 0.23 mg kg^{-1} and, in general, concentrations are less than 1 mg kg^{-1} (Bowen 1979). Several workers have demonstrated that added Cr remains primarily in the roots and is poorly translocated

(Huffman & Allaway 1973; Cary et al. 1977; Wallace et al. 1976). The results indicate that even at a high rate of 50 mg kg^{-1} soil, Cr concentration in ryegrass was in the normal range.

ACKNOWLEDGEMENTS

The authors thank Norsk Hydro for providing the funds for this work through a cooperative research programme between them and the department of Soil Sciences. They also thank S. Ingebrigtsen and T. Trælhaug for conducting the greenhouse experiment.

REFERENCES

Abacus Concepts, Inc. 1988. The solution for Data Analysis and Presentation Graphics. StatView™ SE + Graphics.

Adriano, D.C. 1986. *Trace Elements in the Terrestrial Environment*. Springer-Verlag New York, Berlin, Heidelberg, Tokyo. 533 pp.

Allaway, W.H. 1968. Agronomic control over environmental cycling of trace elements. Adv. Agron. 20: 235-274.

Bache, C.A., W.H. Gutenmann, L. E. St. John Jr., R.D. Sweet, H.H. Hatfield & D.J. Lisk 1973. Mercury and methylmercury content of agricultural crops grown on soil treated with various mercury compounds. J. Agric. Food Chem. 21: 607-613.

Beauford, W.J. Barber & A.R. Barringer 1977. Uptake and distribution of mercury within higher plants. Physiologia Plantarum 39: 261-265.

Boawn, L.C. 1974. Residual availability of fertiliser zinc. Soil Sci. Soc. Am. Proc. 38: 800-803.

Bowen, H.J.M. 1979. *Environmental Chemistry of the Elements*. Academic Press, Troy, Mo. 333 pp.

Bærug, R. & B.R. Singh 1990. Cadmium levels in soils and crops after long-term use of commercial fertilisers. Norw. J. Agric. Res. 4: 251-260.

Cary, E.E., W.H. Allaway & O.E. Olson 1977. Control of chromium concentrations in food plants. I. Absorption and translocation of chromium by plants. J. Agric. Food Chem. 25: 300-304.

Cataldo, D.A., T.R. Garland & R.E. Wildung 1978. Nickel in plants. I. Uptake kinetics using intact soybean seedlings. Plant Physiol. 62: 563-565.

Egner, H.H., H. Reihm & W.R. Domingo 1960. Investigations of the chemical soil analysis as a basis for the evaluation of nutrient status of soil. II. Chemical extraction methods for P and K determination (in German). Kgl. Lantsbruks. Høgsk. Ann. 26: 199-215.

Fang, S.C. 1978. Sorption and transformation of mercury vapour by dry soil. Environ Sci. Technol. 12: 285-288.

Follet, R.H. & W.L. Lindsay 1971. Changes in DTPA-extractable Zn, Fe, Mn, Cu in soils following fertilisation. Soil Sci. Soc. Am. Proc. 35: 600-603.

He, Q.B. & B.R. Singh 1993. Plant availability of Cd in soils. I. Ex tractability of Cd in newly and long-term cultivated soils. Acta Agric Scand. Sect. B, Soil and Plant Sci. (in press).

Hogg, T.J., J.R. Bettany & J.W.B. Stewart 1978. The uptake of ^{203}Hg-labelled mercury compounds by bromegrass from irrigated undisturbed soil columns. J. Environ. Qual. 7: 445-45.

Huffman, E.W.D. Jr. & W.H. Allaway 1973. Chromium in plants: Distribution in tissues, organelles, and extracts and availability of bean leaf Cr to animals. J. Agric. Food Chem. 21: 982-986.

Hutchinson, T.C. 1981. In: Lepp, N.W. (ed). *Effect of Heavy Metal Pollution on Plants*. Applied Science Publishers, London, Ch. 6.

Hutchinson, T.C., B. Freedman & L. Whitby 1981. In *Effect of Ni in the Canadian Environment*, NRC, Canada, Ch. 5.

John, M.K. 1972. Mercury uptake from soil by various plant species. Bull. Environ. Contam. Toxocol. 8: 77-88.

Lund, L.J., E.E. Betty, A.L. Page & R.A. Elliott 1981. Occurrence of naturally high cadmium levels in soils and its accumulation by vegetation. J. Environ. Qual. 10: 551-556.

Marten, G.C. & P.B. Hammond 1966. Lead uptake by bromegrass from contaminated soils. Agron. J. 58: 553-554.

Motto, H.S., R.H. Daines, D.M. Chilko & C.K. Motto 1970. Lead in soils and plants: Its relationship to traffic volume and proximity to highways. Environ. Sci. Technol. 4: 231-237.

Semu, E., B.R. Singh, A.R. Selmer Olsen & K. Steenberg 1985. Uptake of Hg from ^{203}Hg-labelled mercury compounds by wheat and beans on an oxisol. Plant & Soil 87: 347-355.

Singh, B.R. & E. Steinnes 1976. Uptake of trace elements by barley in zinc-contaminated soils: 2. Lead, cadmium, mercury, selenium, arsenic, chromium, and vanadium in barley. Soil Sci. 121: 38-43.

Sorteberg A. 1974. The effect of some heavy metals on oats in a pot experiment with three different soil types. J. Scient. Agric. Soc. Finl. 46: 277-288.

Tjell, J.C., E.M.F. Hovmand & H. Moobæk 1979. Atmospheric lead pollution of grass grown in a background area in Denmark. Nature 280: 425-426.

Wallace, A., S.M. Soufi, J.W. Cha & E.M. Romney 1976. Some effects of chromium toxicity on bush bean plants grown in soil. Plant & Soil 44: 471-473.

Effect of Zn application and soil pH on yield and element nutritional status of wheat (*Triticum aestivum* L.)

XIAOFU WU & IVAR AASEN
Agricultural University of Norway, Department of Soil Sciences, Ås, Norway

Wu, Xiaofu & I. Aasen 1993. Effect of Zn application and soil pH on yield and element nutritional status of wheat (*Triticum aestivum* L.). Norwegian Journal of Agricultural Sciences 7: 159-174. ISSN 0801-5341.

A pot experiment in the greenhouse was conducted over a period of two years using a loamy sand to test the effects of Zn application rates, Zn sources and soil pH on the growth of wheat. Liming the soil to pH 7.5 induced both Zn deficiency and Mn deficiency. Plant yields increased with increasing Zn supply up to 5.6 kg ha^{-1} and decreased with increasing pH from 7.0 to 7.5. As a Zn source, zinc-containing NPK complex fertilizer was found to be at least as efficient as zinc sulphate in enhancing plant yields. The concentration of Zn was found to be two to three times higher in young plant shoots and grains than in mature straw. The percentage translocation of Zn to grain was enhanced by Zn application at lower rates, but was reduced at higher rates. Zinc deficiency induced apparent accumulations of N, P, K, Ca, Mg, S, Fe, Mn, Cu, and Mo in young plant shoots. Increasing the soil pH resulted in reduced plant concentrations of N, P, K, Mg, S, Fe, Mn, Zn, and B, and increased concentrations of Ca and Mo. Application of Zn had a positive effect on N and a negative effect on Fe and Mn concentrations in plants. Lower Zn application rates increased P concentration in plants, but higher Zn application rates at a lower pH level tended to reduce it. The concentrations of K, Ca, Mg, S, Cu, and Mo were not significantly affected by Zn supply.

Key words: Essential element, loamy sand, pot experiment, wheat, zinc-containing NPK complex fertilizer, zinc sulphate, Zn-pH interaction.

Xiaofu Wu, Central South Forestry University. Zhuzhou City, Hunan, China 412006.

The incidence of Zn deficiency is frequently associated with calcareous soils (Lindsay 1972; Liu et al. 1983; Jahiruddin & Hoque 1983) and coarse-textured soils low in native Zn content and organic matter, and high pH (Lindsay 1972; Sillanpää & Vlek 1985). Because of their high pH, saline and salt-affected soils are also prone to Zn deficiency (Chand et al. 1981; Mehrotra et al. 1986). Acid soils normally provide sufficient Zn for plant growth. With the intensification of agricultural production, however, Zn deficiency has often been induced in acid soils by overliming (Friesen et al. 1980; Iu et al. 1981; Martini & Mutters 1985), application of organic manure under flooding conditions (Amer et al. 1980; Gilmour & Kittrick 1979) and high rates of phosphorus fertilization (Elsokkary et al. 1981; Mandal & Haldar 1980).

Liming interacts with Zn mainly through increasing soil pH, thus reducing soil Zn

availability. Since the status of several other essential elements, e.g. Mn, B, and Mo, are also closely related to soil pH conditions, the effect of liming on Zn nutrition may also be a result of interactions between Zn and other elements.

Zinc fertilization has become a common practice for correcting Zn deficiency. Zinc sulphate is the most commonly used fertilizer largely because of its high solubility. Under different conditions, other types of fertilizers, such as ZnO, zincated superphosphate (Singh & Singh 1983) and Zn chelates (Rehm et al. 1980; Chand et al. 1981) are also recommended. However, there seems to be a paucity of more detailed information concerning the use of zinc-containing NPK fertilizers.

Zinc has been found to interact with several other elements both in soil reactions and in plant metabolism. Reports concerning such interactions, however, are often conflicting.

The present experiment was designed to test the effect of Zn application rates, Zn sources and soil pH on spring wheat yields and to compare the utilization efficiency of zinc-containing NPK complex fertilizer with that of zinc sulphate. The influence of treatments on the nutritional status of Zn and other essential elements was also investigated.

MATERIALS AND METHODS

A greenhouse experiment was conducted over a period of two years (1987 and 1988) using plastic pots filled with 6.7 l (7.02 kg) loamy sand, the characteristics of which are listed in Table 1. The treatments consisted of four levels of Zn (0, 2.8, 5.6, and 10 kg Zn ha^{-1}), three pH levels (6.5, 7.0, and 7.5) and two types of Zn fertilizer (zinc-containing NPK complex fertilizer (Zn-NPK) and zinc sulphate). Each treatment was replicated three times. The experimental design is presented in Table 2.

Table 1. Properties of the soil used in the experiment

pH	meq.(100g)$^{-1}$				mg kg^{-1}	%			
	TA[1)]	BC	EA	CEC	Total Zn	C	Clay	Silt	Sand
7.0	2.6	7.5	11.0	18.5	11.7	2.1	5.0	40	55

[1)] TA = titratable alkalinity; BC = exchangeable base cation content; EA = exchangeable acidity; CEC = cation exchange capacity; C= soil organic matter

Table 2. Experimental design

Rate of Zn application	Control	Zn fertilizers	
		Zn-NPK complex	Zinc sulphate
kg ha^{-1}	0(A)[1)]	2.8(B) 5.6(C)	2.8(b) 5.6(c) 10(D)
mg pot^{-1}	0	9.38 18.76	9.38 18.76 33.5
pH levels	6.5(1)	7.0(2) 7.5(3)	

[1)] Letters and numbers in parentheses correspond with those used in Fig. 1 and 2, and with the letters used in Table 7

The soil pH was adjusted by addition of either $CaCO_3$ or H_2SO_4. All pots received the same amount of basal fertilizers. The rates of N, P, and K applied were 670, 263, and 564 mg pot^{-1}, and those of Mg, Cu, Mn, Mo, and B were 66, 43, 54, 1.9, and 0.9 mg pot^{-1}, respectively. An NPK complex fertilizer was used for supplying N, P, and K. For Mg, Cu, and Mn, their sulphate salts were used, and for Mo and B, ammoniumheptamolybdate ($(NH_4)_6MO_7O_{24}.4H_2O$) and boric acid ($B(OH)_3$), respectively, were used. With the exception of the NPK complex fertilizer, all nutrients were applied in solution form. The Zn treatments, the soil pH adjustment and the basal supply were repeated in the second year.

Thirty seeds of wheat (*Triticum aestivum* L., the Norwegian cultivar Runar) were sown in each pot. After 10 days, 20 selected seedlings were retained in the pot for further growth. By using deionized water the water content of the soil was kept at approximately 50% of the soil water-holding capacity in the first two weeks and then increased to 60% in the latter part of the growing season.

For studying the element nutrition in the vegetative growth phase, three plants from each pot were harvested at the stage of ear emergence (Large 1954). At the end of the growing season all mature plants were harvested. The plant samples were dried at 70°C and milled to pass through a 0.8 mm sieve for further chemical analysis. A soil sample was taken from each pot after the final harvest.

Determinations of soil properties, DTPA-extractable soil Zn content and total Zn content in the plants were carried out following the methods by Wu et al. (1991). Total N content in plants was measured using the Kjeldahl digestion and the automated indophenol method (Selmer-Olsen 1971). Total content of P, K, Ca, Mg, S, Fe, Cu, Mn, B, and Mo in plants was determined by inductively-coupled plasma (ICP) emission spectrometry using the same solution as that prepared for Zn determination.

RESULTS AND DISCUSSION

Zinc deficiency

Symptoms of Zn deficiency did not appear in the pots without Zn addition at pH 6.5 and 7.0, indicating that the original soil contained sufficient zinc for normal growth. Liming the soil to pH 7.5 induced zinc deficiency in the control pots in both years. The deficiency symptoms, characterized as short internodes and chlorotic and necrotic areas on the older leaves, appeared about three to four weeks after sowing. Plant growth was apparently inhibited, followed by reduced tillering, restricted grain formation and delayed maturity, and a resultant considerable decrease in yield.

In comparison, Zn deficiency was much more severe in the second year. The deficiency symptoms appeared 10 days earlier, the growing period from sowing to ear emergence was 6 days shorter and the whole growth season was 9 days shorter in the second year than in the first year (Table 3). One possible reason for these differences in plant development could have been the higher temperature in the second year, especially in May and June, the first two months of the growing period. The mean air temperatures for May and June in the second year were 3.6°C and 6.4°C higher, respectively, than in the first year (Department of Physics and Meteorology, Agricultural University of Norway 1987, 1988).

Table 3. Length (days after sowing) of plant growth stages in different years

Year	Appearance of deficiency symptoms		Ear emergence	Final harvest	
	Mn	Zn		Normal plants	Deficient plants
1987	24	33	42	86	93
1988		23	36	77	84
Difference		10	6	9	9

The deficient plants were finally harvested 7 days later than the normal plants in both years. During this period in the first year, further growth of the deficient plants was observed and by the end, more than half of the ears became mature. In the second year during the last 7 days there were almost no significant changes in the growth status and further development of shoots and ears almost came to a stop. This was expected, however, since a large proportion of available Zn in the soil had been taken up by the plants growing in the first year. The depletion of native Zn in combination with further liming could have reduced the soil Zn availability from the first to the second year to such a low level that the deficient plants were unable to complete their life cycle.

Manganese deficiency

In the first year, Mn deficiency occurred at pH 7.5. Symptoms of Mn deficiency appeared 7 to 10 days before Zn deficiency symptoms became visible (Table 3). As further evidenced by the appearance of symptoms, the Mn deficiency was aggravated by Zn additions with more severe cases associated with higher Zn application levels. After spraying the deficient plants with a solution containing 2% $MnSO_4.H_2O$, the Mn deficiency symptoms disappeared in later developed leaves. The possibility of inducing Mn deficiency at marginal levels of soil Mn availability by Zn application does not seem to have been reported before. Although Mn deficiency symptoms were not observed in the second year, results from chemical analysis show that the Mn concentration in plant tissues is extremely low at high pH levels (see Fig. 2).

Plant yields

The average yield of grain (DG) and straw (DS) on a dry matter basis for each treatment is given in table 4. The influence of Zn application on plant yields obtained in the first year varied with soil pH levels, showing the importance of Zn-pH interactions in determining plant growth conditions. For the two lower pH levels, 6.5 and 7.0, the addition of 2.8 and 5.6 kg Zn ha^{-1} did not enhance the straw yields. At pH 7.5, however, the straw yields increased at the 2.8 kg Zn ha^{-1} level and then declined slightly with further increases in Zn supply.

The response of grain yields obtained in the first year also differed with pH changes (Table 4). Under slightly acidic conditions (pH 6.5) the grain yields remained nearly constant within the application level between 0 and 5.6 kg Zn ha^{-1} and then increased by about 4% at 10 kg Zn ha^{-1}. At pH 7.0, 2.8 kg Zn ha^{-1} increased the grain yield by 5%, but application levels at 5.6 and 10 kg Zn ha^{-1} did not further enhance the response. At pH 7.5,

the 2.8 kg Zn ha^{-1} treatment had a pronounced effect in enhancing the grain yield by correcting Zn deficiency, 5.6 kg Zn ha^{-1} did not further increase the yield, while 10 kg Zn ha^{-1} caused a 7% yield reduction.

Table 4. Dry matter yields of grain and straw (g pot^{-1}) in relation to soil pH, Zn fertilizers, and Zn application

Year	pH	Zn application, kg ha^{-1}						Mean
		Zn-NPK complex			Zinc sulphate			
		0	2.8	5.6	2.8	5.6	10.0	
1987				Grain				
	6.5	23.5	23.7	23.9	23.3	23.9	24.5	23.8
	7.0	24.8	26.4	26.9	25.6	25.3	26.5	26.0
	7.5	8.3	24.4	24.7	23.9	24.3	22.8	21.4
	Mean	18.9a[1)]	24.8b	25.2b	24.4b	24.5b	24.6b	23.7
				Straw				
	6.5	49.4	48.7	49.3	50.9	50.6	45.8	49.1
	7.0	44.0	43.0	41.5	43.2	42.8	43.8	43.5
	7.5	35.6	37.5	36.6	35.8	35.7	34.9	36.0
	Mean	43.0a	43.1a	42.5a	43.3a	43.0a	41.5a	42.8
1988				Grain				
	6.5	17.3	20.3	21.3	19.4	20.3	21.4	20.0
	7.0	17.5	19.7	20.0	19.5	19.7	21.4	19.6
	7.5	4.5	18.1	18.7	16.6	17.6	18.5	15.6
	Mean	13.1a	19.4c	20.3c	18.5b	19.2c	20.4c	18.4
				Straw				
	6.5	31.6	34.5	37.2	33.1	36.0	37.8	35.1
	7.0	30.6	32.4	36.7	31.6	34.8	37.6	34.0
	7.5	19.1	30.3	31.5	30.2	30.7	31.7	29.0
	Mean	27.1a	32.4b	35.3c	31.6b	33.8b	35.7c	32.7

[1)] Mean values within each line followed by different letters differ significantly ($p < 0.05$)

In contrast, the effect of Zn application on plant yields obtained in the second year is more consistent at different pH levels. In general, both grain and straw yields in the second year increased with increasing Zn supply up to 5.6 kg Zn ha^{-1} and then remained virtually unaffected, with the exception of pH 7.0, by the 10 kg Zn ha^{-1} treatment (Table 4). Negative effects of Zn application on plant yields were not observed in the second year as they were in the first year. A supply of 5.6 kg Zn ha^{-1} under the experimental conditions seems to be beneficial for obtaining an optimum yield.

The low grain and straw yields associated with the highest Zn application rate at pH 7.5 in the first year may be accounted for by the Zn-accentuated Mn deficiency. Although foliar spray of Mn was don to correct the deficiency, its negative effect can hardly be completely eliminated.

The effect of soil pH on straw yield was found to be negative in both years regardless

of Zn application levels. The response of grain yield to pH changes, however, indicates discrepancies in different years. In the first year, the highest grain yield was obtained at pH 7.0 while the lowest grain yield was found at pH 7.5 only in the control pots and in the 10 kg Zn ha^{-1} pots. In the second year, the grain yield was not significantly affected by increasing the soil pH from 6.5 to 7.0, but was apparently reduced when the pH level was increased to 7.5. Despite the fact that the highest Zn application rate did not further enhance plant growth in both years, the negative effect of liming the soil to pH 7.5 was not alleviated by Zn additions. This strongly suggests that the reduction in yield caused by increasing pH was not purely a result of the pH effect on the soil Zn availability. Presumably, other factors, e.g. Mn availability, must also have played a role in determining the observed plant growth pattern.

As noted in Table 4, much lower yields were obtained from all treatments in the second year than in the first year. One possible reason for this variation could have been the higher temperature in the first two months of the growing period in the second year, causing a shorter vegetative phase and also a shorter generative phase (Table 3). A shorter vegetative phase means a shorter time for nutrient uptake and vegetative development, while a shorter generative phase means a shorter time for translocation of assimilates to the grains.

The interaction between the soil native Zn supply capacity and the changes in soil acidity conditions during the plant growing season could be one of the causes for the inconsistent effect of Zn application on yield for different years. Table 5 gives the average pH values measured before sowing and after the final harvest for both years. The soil used was acidic in nature and low in organic matter and clay content (Table 1), and thus its pH dropped in the latter part of the growing season. In comparison, however, the decrease in pH was much lower in the second year than in the first year. Since soil Zn availability is negatively related to soil pH, it is very likely that in the first year when the soil native Zn level was somewhat high, the soil in acidic pots, including the control pots, contained sufficient Zn, and, thus, Zn application had virtually no effect on either the grain or straw yields. Because of the sharp decrease in soil pH during that year, the available soil Zn level may have increased with time as pH decreased. Consequently, it also reduced the differences between Zn treatments at higher pH levels.

Table 5. Average pH values measured before sowing and after final harvest

Year		pH treatment		
		1	2	3
1987	Before sowing	6.50	7.01	7.52
	After harvest	5.00	6.49	7.02
1988	Before sowing	6.46	7.05	7.55
	After harvest	6.18	6.72	7.20

Efficiency of Zn sources

The utilization efficiencies of the two types of Zn fertilizers are compared using contrast tests (Table 6). Zinc-containing NPK complex fertilizer (Zn-NPK) tended to give higher

values than zinc sulphate for grain yield in the first year and for both grain and straw in the second year. However, the yield differences between Zn sources are relatively small.

Table 6. Comparison of the average dry matter yields (g pot^{-1}) between Zn sources by contrast test

Year		Fertilizer type		
		Zn-NPK complex	$ZnSO_4$	Probability
1987				
	Grain	25.0	24.4	0.006
	Straw	42.8	43.2	0.427
1988				
	Grain	19.7	18.9	0.0001
	Straw	33.8	32.8	0.0001

Zinc nutrition

On average, the Zn concentrations in grains (ZnDG), young plants sampled at ear emergence (ZnPE), and mature straw (ZnDS) were found in the following order,

ZnDG > ZnPE > ZnDS

and the magnitudes of ZnDG and ZnPE were about two to three times higher than those of ZnDS (Table 7). Table 7 also shows that about 34 to 40% of Zn taken up by the above-ground part of the plant was retained in the straw, and about 60 to 66% was translocated to the grain. Because of Zn deficiency, the lowest percentage of Zn translocation to grains was found in the treatment without Zn addition. Compared to the control treatment, Zn application generally enhanced Zn translocation from straw to grain. However, the highest percentage ratio of Zn taken up by grain (ZnTG) to that by the above-ground plant part (ZnPT) was obtained at 2.8 kg Zn ha^{-1} for both Zn sources. Further increase in Zn supply reduced this ratio.

The ratio of Zn retention by grain to total uptake also varied at different soil pH levels. The lowest value is associated with pH 7.5 and the highest with pH 7.0 (Table 7). The pH effect on Zn uptake by different plant tissues is in principle consistent with the Zn application effect.

The results obtained from multiple regression analyses in which the plant dry matter yields and Zn concentration are taken as functions of soil DTPA-extractable Zn content (ZnDTPA) and soil pH are presented in Table 8. The magnitude of the multiple R values for the dependent variables followed the sequence:

plant Zn concentration > plant Zn uptake > plant yield

Table 7. Zinc levels in plants and soil relative to treatments

Treatment		mg (kg DM)$^{-1}$			µg pot^{-1}			mg l^{-1}
		ZnDS[1)]	ZnDG	ZnPE	ZnTS	ZnTG	ZnPT	ZnDTPA
Zn	A[2)]	5.5	15.5	16.3	160 (40.3)	237 (59.7)	397	0.47
	B	8.3	27.0	23.7	274 (34.1)	531 (66.0)	806	0.84
	C	11.3	31.3	27.7	405 (38.9)	635 (61.1)	1040	1.19
	b	8.0	26.2	22.4	256 (34.1)	495 (65.9)	750	0.89
	c	10.5	32.5	26.7	361 (36.2)	636 (63.8)	996	1.08
	D	12.8	37.7	32.0	465 (37.3)	782 (62.7)	1248	1.72
pH	6.5	12.8	39.3	32.5	457 (36.5)	796 (63.3)	1253	1.48
	7.0	8.9	28.8	26.1	308 (35.0)	573 (65.0)	881	0.85
	7.5	6.5	17.0	14.8	196 (40.4)	289 (59.6)	485	0.84
Mean		9.4	28.4	24.8	320 (36.9)	553 (63.1)	873	1.03

[1)] ZnDS = Zn concentration in mature straw
ZnDG = Zn concentration in grain
ZnPE = Zn concentration in young plant shoots
ZnTS = Zn retained by straw
ZnTG = Zn retained by grain
ZnPT = Zn retained by the above-ground plant part
ZnDTPA = soil DTPA-extractable Zn content

[2)] See Table 2 for Zn treatment A, B, C, b, c, and D. Values in parentheses are the percentage ratios of ZnTS, or ZnTG to ZnPT

Table 8. Relationship between soil Zn availability and plant yield, Zn concentration and Zn uptake

	Regression equation		R
Yield	log (DS)[1)]	= 1.84 + 0.21 log (ZnDTPA) - 0.05(pH)	0.64
	log (DG)	= 1.69 + 0.42 log (ZnDTPA) - 0.06(pH)	0.42
	log (DT)	= 2.06 + 0.26 log (ZnDTPA) - 0.05(pH)	0.55
Concentration	log (ZnDS)[2)]	= 2.16 + 0.65 log (ZnDTPA) - 0.17(pH)	0.91
	log (ZnDG)	= 3.18 + 0.65 log (ZnDTPA) - 0.25(pH)	0.85
	log (ZnPE)	= 3.30 + 0.51 log (ZnDTPA) - 0.28(pH)	0.81
Uptake	log (ZnTS)	= 4.00 + 0.86 log (ZnDTPA) - 0.22(pH)	0.87
	log (ZnTG)	= 4.88 + 1.06 log (ZnDTPA) - 0.31(pH)	0.73
	log (ZnPT)	= 4.82 + 0.95 log (ZnDTPA) - 0.27(pH)	0.80

[1)] DS = straw dry matter yield
DG = grain dry matter yield
DT = total above-ground plant dry matter yield

[2)] See notes below Table 7

The phenomenon that Zn concentration in plants is more closely related to soil Zn availability than are plant yields, has been observed by many investigators (Orabi et al. 1985; Singh & Shukla 1985; Kumar & Singh 1980; Rehm et al. 1983). Singh & Låg (1976)

demonstrated that the application of Zn could increase Zn concentration in barley plants up to as much as 489 ppm without causing a toxic effect. In other studies, supplying Zn at high rates was found to enhance plant growth only when P (Verma & Minhas 1987; Orabi et al. 1981), Fe (Nambiar and Motiramani 1981; Verma & Tripathi 1983), and K (Tiwari et al. 1982) were applied at adequate levels.

Zinc-deficiency induced element accumulation

The influence of treatments on element concentrations in young plants sampled at ear emergence is shown in Figs. 1a and 1b for macronutrients and Figs. 2a and 2b for micronutrients.

In the young plants, as illustrated in the respective figures, Zn deficiency caused an apparent accumulation of nearly all essential elements studied except Zn and B. According to their levels in Zn-deficient plants relative to those in non-deficient plants, the accumulated elements can be divided into three groups: (1) N, P, Ca, S, Fe, and Mo; (2) Cu; and (3) K, Mg, and Mn. The elements in the first group were concentrated in Zn-deficient plants at abnormally high levels with magnitude being the highest over all other treatments. The concentration of Cu in the second group was also found to be the highest in deficient plants, but the difference in the concentration of this element between deficient and non-deficient samples is relatively small. In the third group, the element concentrations are within the range of those in normal plants, and the accumulation of these elements caused by Zn deficiency is evidenced by their highest levels at either a given pH or a given zinc application level.

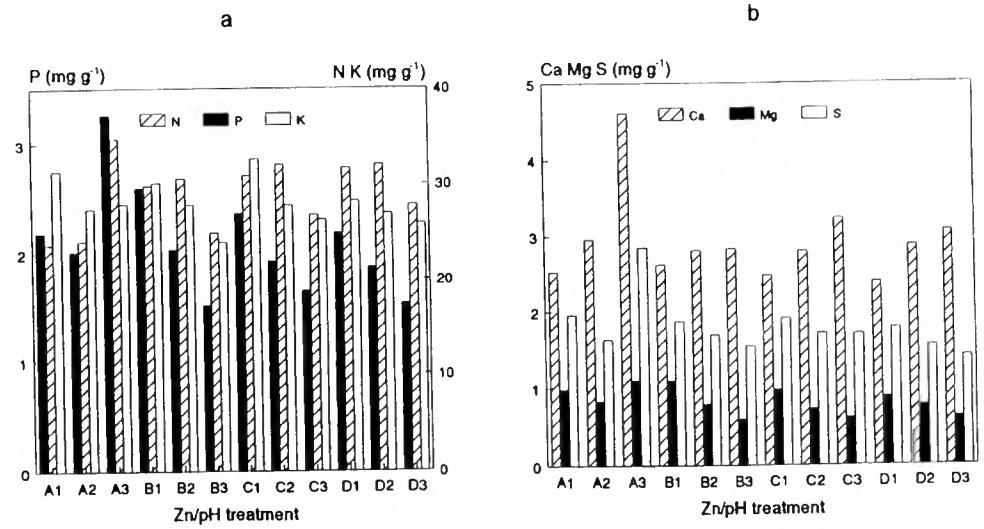


Fig. 1. Concentrations of macronutrients in wheat shoots sampled at the stage of ear emergence affected by zinc application and soil pH. Zinc application levels: A = 0, B = 2.8, C = 5.6, D = 10 kg ha^{-1}. pH levels: 1 = 6.5, 2 = 7.0, 3 = 7.5

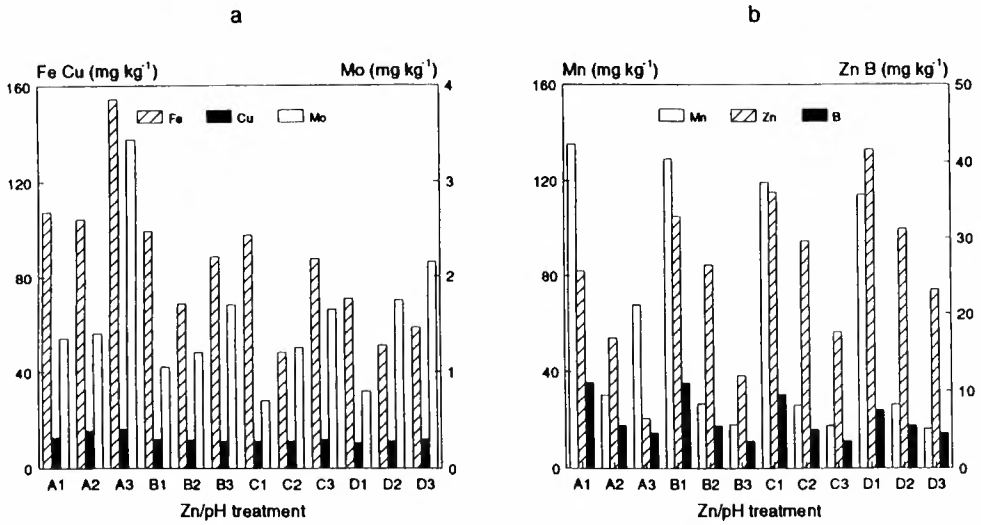


Fig. 2. Concentrations of micronutrients in wheat shoots sampled at the stage of ear emergence affected by zinc application and soil pH. Zinc application levels: A = 0, B = 2.8, C = 5.6, D = 10 kg ha^{-1}. pH levels: 1 = 6.5, 2 = 7.0, 3 = 7.5

Two types of Zn-deficiency-induced accumulations can be distinguished by changes in element concentrations in response to the treatments:

(1) Relative accumulation. When the treatments had a positive effect, such as the influence of increasing pH on the levels of Ca and Mo, the concentrations of these elements in Zn-deficient plants increased to higher levels compared with those in non-deficient plants. When the treatments had a negative effect, such as the influence of increasing pH on Mg, K, and Mn, the concentrations of these elements in Zn-deficient plants were less reduced than those in non-deficient plants.

(2) Absolute accumulation. Elements such as N, P, S, Fe, and Cu were concentrated in Zn-deficient plants at very high levels although the treatments reduced the concentrations of these elements in normal plants.

Cumbus (1985) observed the reduced shoot:root ratio of wheat plants grown in nutrient solution without Zn and attributed the accumulation of P, Fe, Mn, and N under such conditions to a possible enhanced ion transport caused by root morphological changes. Zhang et al. (1989) demonstrated that zinc deficiency enhanced the release of the phytosiderophore 2'-deoxymugineic acid from the roots of wheat. This compound was shown to be as effective in mobilizing Fe as in mobilizing Zn in the soil. In addition to the reduced plant shoot growth, the enhanced ion uptake under Zn deficiency conditions may also be responsible for the abnormal accumulation of those ionic species with levels above the

deficient range.

Among the elements analysed, B was the only one not found to have accumulated in the Zn-deficient plants. Gomez-Rodriguez et al. (1981) noted that B deficiency was associated with low Zn and Mn levels in cotton leaves. Graham et al. (1987), however, reported that the uptake of B by barley seedlings was negatively affected by Zn supply.

Accumulation of nutrient elements in plants suffering from deficiency of one single element may also be caused by larger yield reduction which may consequently result in increased concentrations of other elements.

Effect of treatments on element status in plants

With the exception of the Zn-deficiency-induced N accumulation, Zn application generally increased the N concentration in plants (Fig. 1a). The response of P concentration to Zn treatments varied with pH levels and Zn application rates. The 2.8 kg Zn ha^{-1} treatment increased P concentration at pH 6.5 while the 5.6 and 10 kg Zn ha^{-1} treatments reduced P concentration at both pH 6.5 and 7.0. The effect of Zn application on P concentration at pH 7.5 was not consistent (Fig. 1a). The concentration of Fe and Mn in plants was found to be negatively related to Zn application levels (Fig. 2). In comparison, Mn appears to be less affected by Zn treatment than Fe. The responses of K, Ca, Mg, S, B, Cu, and Mo in normal plants to Zn application are inconsistent and insignificant (Figs.1 and 2).

An increasing soil pH was found to have a negative effect on N, P, K, Mg, S, Zn, Mn, and B and a positive effect on Ca and Mo (Figs. 1 and 2). The influence of pH on Fe and Cu was not as consistent, however. At a given Zn application level, the lowest Fe concentration was always obtained at pH 7.0 and the next lowest, at pH 7.5. Contrary to what was expected, the Cu concentration increased slightly with increasing pH in pots with no added Zn and remained nearly constant when Zn was applied.

It may be worth mentioning that the negative effect of soil pH on Mn concentration is much more prominent than that of Zn application. The remarkable decrease in Mn concentration in all above-ground plant parts with increasing soil pH from 6.5 to 7.0 indicates that the effect of soil alkalinity on Mn uptake is more critical at pH levels higher than 6.5. The Mn concentrations in young plant shoots at both pH 7.0 and 7.5 are within the deficient range suggested for cereal crops (Aasen 1978, 1986; Finck 1987; Jones 1972).

The enhancement of N uptake due to Zn application observed in the present study is in agreement with reports from Singh & Singh (1980) and Hoyle (1979) for other plant species. Shukla & Yadav (1982) demonstrated that a balanced Zn and P nutrition was not only essential for promoting plant growth but also for enhancing the activity of Rhizobium for N fixation in legumes. Zinc is known to play an important role in N metabolism (Mengel & Kirkby 1987). Kitagishi et al. (1987) found that Zn deficiency depressed the content of 80S ribosomes in the meristematic tissue of rice plants and further reduced the plant protein content. The same phenomenon in cultured tobacco cells was also observed by Obata & Umebayashi (1988).

Reports concerning P-Zn interaction both in soils and in plants have mostly been conflicting. Pasricha et al. (1987) found that the application of P did not affect the Zn intensity in the soil, but Mandal & Haldar (1980) observed a negative correlation between soil extractable P and Zn. Orabi et al. (1981) and Basak et al. (1982a, 1982b) reported a positive relationship between P and Zn in corn and rice plants. In other studies, the effect

of Zn application on P uptake was found to be negative and vice versa (Shang & Bates 1987; Kumar et al. 1986; Verma & Neue 1984). The nature of the P-Zn interaction may be dependent on their relative levels and pH condition. The positive effect of Zn application at lower pH and Zn levels and the negative effect of Zn application at higher levels on P concentration in plants observed in the present study are in agreement with reports from Yadav & Shukla (1982), Elsokkary et al. (1981) and Wallace et al. (1978).

The concentration of Fe in the present plant samples is largely found to be subject to the interaction between pH and Zn treatments. Negative correlations between Fe and Zn in maize, rice, and wheat plants were reported by Nambiar & Motiramani (1981), Swarup (1981) and Kumar et al. (1981), respectively. Phosphorus was also found to play an important role in modifying the Zn-Fe interaction (Graham et al. 1987; Singh & Singh 1983). In general, increasing pH will decrease the availability of Fe in the soil, thus reducing Fe uptake by plants. On the other hand, however, the availability of Zn also decreases with increasing pH, which, possibly through Zn-Fe interaction in the plant metabolic processes, counteracts the negative effect of pH on Fe absorption. As can be seen in Fig. 2a, increasing the soil pH from 6.5 to 7.0 caused almost no changes in plant Fe levels when Zn was not applied but reduced it to very low levels when Zn was added. Further increasing pH from 7.0 to 7.5 perhaps had a greater influence on soil Zn availability than on soil Fe availability. Thus, the Fe concentration at pH 7.5 was not further reduced but, instead, increased to moderate levels. The present results suggest that the Fe concentration in wheat plants is more dependent on Zn nutrition, and perhaps also on Mn nutrition, than on its own availability in the soil.

The application of Zn was found to be beneficial to Mn uptake by barley (Singh & Steenberg 1975; Singh & Låg 1976) and rice plants (Verma & Neue 1984). Mandal & Haldar (1980) noted that soil DTPA-extractable Mn was not affected by Zn fertilization. Reports from Kumar et al. (1981), however, indicated that the Mn concentration decreased with increasing Zn levels in wheat shoots. In pearl millet plants, Kumar et al. (1986) found that low levels of Zn application enhanced the uptake of Mn, P, K, and Fe, but high levels of Zn application reduced it. In the present study, the observed negative effect of Zn application on the concentration of Mn is in accordance with the Zn-accentuated Mn deficiency symptoms that appeared in the first year.

In the present experiment, the responses of S, Zn, B, Ca, Mn, and Mo to changes in pH are in accordance with the influence of soil pH on the available levels of these elements in the soil. The negative effect of increasing levels of pH on N, P, K, and Mg may be a result of element interactions.

REFERENCES

Aasen, I. 1978. Mangan og sink i jord og planter. Norsk Landbruk 1978: (5) 12-13, 38. (6) 14-15, 33, 38.

Aasen, I. 1986. Mangelsjukdomar og andre ernæringsforstyrringar hos kulturplanter. Landbruksforlaget Oslo. Pp. 65-82.

Amer, F., A.I. Rezk & H.M. Khalid 1980. Fertilizer zinc efficiency in flooded calcareous soils. Soil Sci. Soc. Am. J. 44: 1025-1030.

Basak, A., I.N. Mandal & M. Haldar 1982a. Interaction of phosphorus and molybdenum in relation to uptake and utilization of molybdenum, phosphorus, zinc, copper, and manganese by rice. Plant and Soil 68: 261-269.

Basak, A., I.N. Mandal & M. Haldar 1982b. Interaction of phosphorus and molybdenum and the availablity of zinc, copper, manganese, molybdenum, and phosphorus in waterlogged rice soil. Plant and Soil 68: 271-278.

Chand, M., N.S. Randhawa & D.R. Bhumbla 1981. Effectiveness of zinc chelates in zinc nutrition of greenhouse rice crop in a saline-sodic soil. Plant and Soil 59: 217-225.

Cumbus, I.P. 1985. Development of wheat roots under zinc deficiency. Plant and Soil 83: 313-316.

Department of Physics and Meteorology, Agricultural University of Norway. 1987, 1988. Meteorological data for Ås 1987, 1988. Ås, Norway.

Elsokkary, I.H., H.A. El-Attar & M.A. Amer 1981. Influence of phosphorus and zinc fertilizers on the uptake of P and Zn by corn plants grown in highly calcareous soils. Plant and Soil 59: 227-236.

Finck, A. 1987. Improved manganese supply by fertilization in high-yielding crops in the northern regions of the Federal Republic of Germany. In: The Utilization of Secondary and Trace Elements in Agriculture. (Ed.: United Nations). Developments in Plant and Soil Sciences 29. Nijhoff, Dordrecht 1987. Pp. 85-95.

Friesen, D.K., M.H. Miller & A.S.R. Juo 1980. Liming and lime-phosphorus-zinc interactions in two Nigerian Ultisols: II. Effects on maize root and shoot growth. Soil Sci. Soc. Am. J. 44: 1227-1232.

Gilmour, J.T. & J.A. Kittrick 1979. Solubility and equilibria of zinc in a flooded soil. Soil Sci. Soc. Am. J. 43: 890-892.

Gomez-Rodriguez, M.V., M. Gomez-Ortega & M.C. Alvarez-Tinaut 1981. Boron, Copper, iron, manganeze, and zinc contents in leaves of flowering sunflower plants (*Helianthus annuus* L.) grown with different boron supplies. Plant and Soil 62: 461-464.

Graham, R.D., R.M. Welch, D.L. Grunes, E.E. Cary & W.A. Norvell 1987. Effect of zinc deficiency on the accumulation of boron and other mineral nutrients in barley. Soil Sci. Soc. Am. J. 51: 652-657.

Hoyle, M.C. 1979. Response of yellow birch (*Betula alleghaniensis* Britton) in acid subsoil to micronutrient additions. Plant and Soil 51: 453-455.

Iu, K.L., I.D. Pulford & H.J. Duncan 1981. Influence of waterlogging and lime or organic matter additions on the distribution of trace metals in an acid soil. Plant and Soil 59: 327-333.

Jahiruddin, M. & M.S. Hoque 1983. Factors affecting zinc availability to crops. A review. Bangladesh J. Agric. Sci. 10: 1-12.

Jones, J.B. Jr. 1972. Plant tissue analysis for micronutrients. In: J.J. Mortvedt, P.M. Giordano & W.L. Lindsay (eds.), Micronutrients in Agriculture. Soil Sci. Soc. Am. Madison, Wis. Pp. 319-346.

Kitagishi, K., H. Obata & T. Kondo 1987. Effect of zinc deficiency on 80S ribosome content of meristematic tissues of rice plant. Soil Sci. Plant Nutr. 33: 423-429.

Kumar, V. & M. Singh 1980. Sulfur and zinc interactions in relation to yield, uptake, and utilization of sulfur in soybean. Soil Sci. 130: 19-25.

Kumar, V., B.K. Bhatia & U.C. Shukla 1981. Manganese and zinc relationship in relation to dry matter yield and the concentration and uptake of nutrients in wheat. Soil Sci. 131: 151-155.

Kumar, V., V.S. Ahlawat, R.S. Antil & D.S. Yadav 1986. Interactions of nitrogen and zinc in pearl millet: 2. Effect on concentration and uptake of phosphorus, potassium, iron, manganese, and copper. Soil Sci. 142: 340-345.

Large, E.C. 1954. Growth stages in cereals: Illustration of the Feekes scale. Plant Pathol. 3: 128-129.

Lindsay, W.L. 1972. Zinc in soil and plant nutrition. Adv. Agron. 24: 147-186.

Liu, Z., Q. Zhu & L. Tang 1983. Microelements in the main soils of China. Soil Sci. 135: 40-46.

Mandal, L.N. & M. Haldar 1980. Influence of phosphorus and zinc application on the availability of zinc, copper, iron, manganese, and phosphorus in waterlogged rice soils. Soil Sci. 130: 251-257.

Martini, J.A. & R.G. Mutters 1985. Effect of lime rates on nutrient availability, mobility, and uptake during the soybean growing season: 2. Calcium, magnesium, potassium, iron, copper, and zinc. Soil Sci. 139: 333-343.

Mehrotra, N.K., V.K. Khanna & S.C. Agarwala 1986. Soil-sodicity-induced zinc deficien-

cy in maize. Plant and Soil 92: 63-71.

Mengel, K. & E. A. Kirkby 1987. Principles of Plant Nutrition. 4th Ed. International Potash Institute, Bern. Pp. 525-535.

Nambiar, K.K.M. & D.P. Motiramani 1981. Fe/Zn ratio as a diagnostic tool for prediction of Zn deficiency in crop plants. I. Critical Fe/Zn ratio in maize plants. Plant and Soil 60: 357-367.

Obata, H. & M. Umebayashi 1988. Effect of zinc deficiency on protein synthesis in cultured tobacco plant cells. Soil Sci. Plant Nutr. 34: 351-357.

Orabi, A.A., H. Mashadi, A. Abdallah & M. Morsy 1981. Effect of zinc and phosphorus on the grain yield of corn (*Zea mays* L.) grown on a calcareous soil. Plant and Soil 63: 291-294.

Orabi, A.A., T. El-Kobbia & A.I. Fathi 1985. Zinc-phosphorus relationship in the nutrition of corn plants (*Zea mays* L.) as affected by the total carbonate of the soil. Plant and Soil 83: 317-321.

Pasricha, N.S., H.S. Baddesha, M.S. Aulakh & V.K. Nayyar 1987. The zinc quantity-intensity relationships in four different soils as influenced by phosphorus. Soil Sci. 143: 1-4.

Rehm, G.W., R.A. Wiese & G.W. Hergert 1980. Response of corn to zinc sources and rate of zinc band applied with either orthophosphate or polyphosphate. Soil Sci. 129: 36-44.

Rehm, G.W., R.C. Sorensen & R.A. Wiese 1983. Application of phosphorus, potassium, and zinc to corn for grain or silage: Nutrient concentration and uptake. Soil Sci. Soc. Am. J. 47: 697-700.

Selmer-Olsen, A.R. 1971. Determination of ammonium in soil extracts by an automated indophenol method. Analyst 96: 565-568.

Shang, C. & T. Bates 1987. Comparison of zinc soil tests adjusted for soil and fertilizer phosphorus. Fertilizer Research 11: 209-220.

Shukla, U.C. & O.P. Yadav 1982. Effect of phosphorus and zinc on nodulation and nitrogen fixation in chickpea (*Cicer arietinum* L.). Plant and Soil 65: 239-248.

Sillanpää, M. and P.L.G. Vlek 1985. Micronutrients and the agroecology of tropical and Mediterranean regions. Fertilizer Research 7: 151-167.

Singh, B.R. & K. Steenberg 1975. Interaction of micronutrients in barley grown on Zn-polluted soils. Soil Sci. Soc. Am. Proc. 39: 674-679.

Singh, B.R. & J. Låg 1976. Uptake of trace elements by barley in zinc-polluted soils: 1. Availability of zinc to barley from indigenous and applied zinc and the effect of excessive zinc on the growth and chemical composition of barley. Soil Sci. 121: 32-37.

Singh, K. & U.C. Shukla 1985. Determination of critical level of zinc in non-calcareous soils for predicting response of wheat to applied zinc. Fertilizer Research 8: 97-100.

Singh, M. & S.P. Singh 1980. Yield of submerged paddy and uptake of Zn, P, and N as affected by liming and Zn fertilizers. Plant and Soil 56: 81-92.

Singh, M. & S.P. Singh 1983. Effects of zinc and phosphorus on absorption of iron and nitrogen by submerged paddy. Soil Sci. 135: 71-78.

Swarup, A. 1981. Effect of iron and manganese application on the availability of micronutrients to rice in sodic soil. Plant and Soil 60: 481-485.

Tiwari, K.N., V. Nigam & A.N. Pathak 1982. Effect of potassium and zinc applications on dry-matter production and nutrient uptake by potato variety `Kufri chandramukhi' (*Solanum tuberosum* L.) in an alluvial soil of Uttar Pradesh. Plant and Soil 65: 141-147.

Verma, T.S. & B.R. Tripathi 1983. Zinc and iron interaction in submerged paddy. Plant and Soil 72: 107-116.

Verma, T.S. & H.U. Neue 1984. Effect of soil salinity level and zinc application on growth, yield, and nutrient composition of rice. Plant and Soil 82: 3-14.

Verma, T.S. & R.S. Minhas 1987. Zinc and phosphorus interaction in a wheat-maize cropping system. Fertilizer Research 13: 77-86.

Wallace, A., R.T. Mueller & G.V. Alexander 1978. Influence of phosphorus on zinc, iron, manganese, and copper uptake by plants. Soil Sci. 126: 336-341.

Wu, X., I. Aasen & A.R. Selmer-Olsen 1991. A study of extraction methods for accessing soil zinc availability: I. Soil zinc extractability and soil zinc buffering capacity in relation to soil properties. Norw. J. Agric. Sci. 5: 89-107.

Yadav, O.P. & U.C. Shukla 1982. Effect of applied phosphorus and zinc on their absorption and distribution in chickpea plant. Soil Sci. 134: 239-243.

Zhang, F., V. Römheld & H. Marschner 1989. Effect of zinc deficiency in wheat on the release of zinc and iron mobilizing root exudates. Z. Pflanzenernähr. Bodenk. 152: 205-210.

Herring scrap as feed for silver foxes and mink in the growing-furring period

ØYSTEIN AHLSTRØM & ANDERS SKREDE
Agricultural University of Norway, Department of Animal Science, Ås, Norway

Ahlstrøm, Ø. & A. Skrede 1993. Herring scrap as feed for silver foxes and mink in the growing-furring period. Norwegian Journal of Agricultural Siences 7: 175-187. ISSN 0801-5341.

Up to 25% frozen-stored herring scrap was fed to silver foxes and mink in conventional wet diets during the period from 13 July (mink) or 8 August (silver foxes) to pelting time in November/December. Blood analyses revealed no effects of diet on vitamin E status or glutathione peroxidase activity. Body growth in silver foxes was improved by the herring scrap diet. Mink fed 25% herring scrap showed an initial growth impairment, but the differences in body weights vanished towards the final weighing. In silver foxes, the experiment revealed improvement in hair quality and general fur impression in animals fed herring scrap. The occurrence of brownish hairs in silver foxes was not affected. Fur quality or colour in mink was not influenced by the feeding of herring scrap.

Key words: Fish oils, foxes, fur-bearing animals, glutathione peroxidase, minks, vitamin E.

Øystein Ahlstrøm, Agricultural University of Norway, Department of Animal Science, P.O. Box 5025, N-1432 Ås, Norway

In Norway, herring (*Clupea harengus*) was extensively used in feed for fur animals before and during World War II. At that time herring was available as a cheap fat and protein source (Høie & Rimeslåtten 1951), but it was found that unbalanced diets containing high levels of dried herring often caused deficiency diseases, poor reproduction and fur quality, and occasionally death (Høie & Rimeslåtten 1951).

In the past few years, vast amounts of herring filleting scrap have been introduced to the market as feed for fur animals. The filleting scrap consists of the backbones with residual flesh, together with heads and viscera. Based on the protein content and the high but variable fat content, herring scrap may have a high potential as a feed ingredient for fur-bearing animals.

The aim of the present study was to investigate the effects of frozen-stored herring scrap in the diets of silver foxes and mink in the growing-furring period, particularly on the fur characteristics.

MATERIALS AND METHODS

Animals and diets

The experiment was carried out in 1989 at the Department of Animal Science, Agricultural University of Norway. The experiment began 13 July (mink) and 8 August (silver foxes) and ended at pelting time in November or early December. Mink of standard genotype were divided into three groups, which were balanced according to age, body weight, sex and genotype (Table 1). The silver foxes were allotted to two groups of males, of which the treatment group received 12.5% herring scrap until 13 September and 25% afterwards.

Table 1. Number of animals in the experimental groups

	Silver foxes	Mink
Diet 1. Control	16	92
Diet 2. 12.5% herring scrap		92
Diet 3. 25% herring scrap	16 *	92

* 12.5% herring scrap until 13 September.

Frozen herring scrap, stabilized with 200-300 mg ethoxyquin per kg, was obtained from R. Domstein & Co., Måløy. The storage temperature during the experiment was -15 to -20°C. The feed was prepared three times a week and stored in a refrigerator until feeding. Frozen feed ingredients were partly thawed and ground to pass through a 10 mm sieve. Feed and water were provided *ad libitum*. The feed was placed on feeding boards (foxes) or on top of the cage wire (mink). Rejected feed was collected daily. Feed consumption was calculated on a group basis. Samples of feed and feed ingredients were taken four times at regular intervals throughout the experiment.

Growth and fur quality

The animals were weighed at the start of the experiment and every four weeks thereafter. Final body weight was recorded at the beginning of the pelting season. Body length was measured as the distance from the tip of the nose to the base of the tail. Fur characteristics were evaluated on dried skins by the staff of the fur farm of the Department of Animal Science. The fur characteristics in mink skins were: density of guardfur and underfur, length of guardfur and underfur, fur colour, metallic, hair quality and general fur quality; and in silver fox skins: hair density, hair quality, texture, covering, colour of guardfur and underfur and general impression.

Analyses of feed

Feed samples were stored at -20°C pending analysis. Chemical composition, fat quality, total volatile nitrogen (TVN) and hygienic quality were determined at the laboratory of The Norwegian Fur Breeders' Association, Oslo. Nitrogen was analysed by the Kjeldahl method. Amino acids were determined according to standard procedures at the Biomedical Centre, University of Uppsala, Sweden. Fatty acid composition was determined at the Department of Animal Science, Ås. Free fatty acids (FFA) were dissolved in a neutral solution of

ethanol/carbon-chloride and titrated with sodium hydroxide to the neutral point (Welch 1976). The resultant fatty acid methyl-esters were separated and quantified by gas-liquid chromatography. The metabolizable energy (ME) content of the diets was calculated on the basis of proximate composition ME of each feed ingredient, estimated digestibility coefficients of the diets and these values of ME (kJ/g): protein (N x 6.25), 18.8 kJ; fat, 39.8 kJ; carbohydrate, 17.6 kJ (Enggaard Hansen et al. 1991).

Blood analyses

Blood samples were collected from six silver foxes and 10 mink in each group on 29 August and 10 October. The blood was collected from a vein in the front leg (silver fox), and by claw clipping (mink). Whole blood glutathione peroxidase concentrations were analysed at the Department of Animal Science in samples from both silver foxes and mink. Plasma vitamin E was analysed in samples from silver foxes. The samples were kept at -70°C until analysis. Glutathione peroxidase activity at 37°C was assayed following the method described by Paglia & Valentine (1967) with Cumene as ROOH substitute (Anderson et al. 1978). The method was modified for centrifugal analysis. Vitamin E was extracted from plasma by ethanol precipitation prior to extraction with hexane. After evaporation under nitrogen, the sample was added to ethanol and the solution injected into a high performance liquid chromatographer (Shimadzu). The columns were products of Brownlee Lab, precolumn: RP-18 Newguard 7μ 15x3.2 mm, the main column: Spheri-5 RP- 5μ 100x4.6 mm. The mobile phase was methanol/water (97/3). Detection was at 294 nm, and an external standard was used for quantification.

Autopsy

Autopsies were carried out on dead animals at the Norwegian College of Veterinary Medicine, Oslo.

Statistics

Differences between means were tested by analysis of variance (SAS Institute 1985).

RESULTS

Feed

The composition of the diets is presented in Table 2. The ME content and the distribution of ME were quite similar in the diets. Herring scrap was used as a protein and fat source, replacing cod scrap and lard in the control diet. The average proximate composition of the frozen herring scrap was (%): dry matter, 28.2; protein, 13.4; fat, 11.6; ash, 3.1.

The amino acid composition of the herring scrap resembled that found in the cod scrap (Table 3.), which meant that the amino acid composition in the diets was similar. However, the content of methionine and to a lesser extent lysine was lower in herring scrap than in cod scrap.

The fatty acid analysis of herring scrap revealed that saturated fatty acids accounted for about 28%, with palmitic acid (C16:0) as the most plentiful (Table 4). The monounsaturated fatty acids, especially the long-chained (C20:1, C22:1), accounted for a large proportion. The polyunsaturated fatty acids were dominated by the highly unsaturated C20:5 n3 and C22:6 n3, both common in fish oils.

Table 2. Composition of diets and metabolizable energy data

Ingredients	Control	Herring scrap	
		12.5%	25.0%
Herring scrap	-	12.5	25.0
Cod scrap	37.5	27.5	17.5
Fishmeal	3.0	3.0	3.0
Slaughterhouse offal	12.0	12.0	12.0
Blood	10.0	10.0	10.0
Lard	5.7	3.5	1.3
Soybean oil	1.0	1.0	1.0
Precooked wheat/oats (70/30)[1)]	10.0	10.0	10.0
Precooked corn	5.7	5.7	5.7
Vitamin mixture [2)]	1.0	1.0	1.0
Hemax [3)]	0.2	0.2	0.2
Water	13.9	13.6	13.3
Metabolizable energy:			
MJ/kg feed	7.1	7.0	6.9
From protein (%)	34.6	35.2	35.5
From fat (%)	43.2	42.3	41.7
From carbohydrates (%)	22.2	22.5	22.8

[1)] Containing 70% wheat and 30% oats.

[2)] Containing grass meal (50%), brewers yeast (50%) fortified with the following vitamins per 100 g: Vit.A 30000 I.U.; vit D_3 3000 I.U.; DL-α-tocopherol acetate, 300 mg; thiamine, 180 mg; riboflavin, 18 mg; niacin 15 mg; Ca-pantothenate, 15 mg; pyridoxine HCl, 30 mg; folic acid, 1.5 mg; biotin, 0.15 mg; B_{12}, 0.09 mg.

[3)] Product of Peter Møller A/S, Oslo, containing ferric glutamate at 20 mg Fe per g.

Table 3. Amino acid composition of herring scrap and cod scrap (g/16 g N). Average and standard deviation of four samples

	Herring scrap		Cod scrap	
Aspartic acid	6.9	(0.7)	8.4	(1.8)
Threonine	3.4	(0.7)	3.9	(0.8)
Serine	3.8	(0.2)	4.3	(0.5)
Glutamic acid	11.8	(2.4)	13.4	(3.1)
Proline	4.0	(0.3)	4.0	(0.3)
Glycine	5.0	(1.5)	5.2	(1.0)
Alanine	4.5	(1.2)	5.1	(0.5)
Half cystine	0.8	(0.1)	0.9	(0.2)
Valin	4.1	(0.6)	4.3	(0.9)
Methionine	2.1	(0.6)	3.1	(0.6)
Isoleucine	3.2	(0.5)	3.8	(1.0)
Leucine	5.7	(0.9)	6.6	(1.7)
Tyrosine	2.5	(0.4)	3.1	(0.7)
Phenylalanine	3.4	(0.3)	3.5	(0.6)
Histidine	1.8	(0.2)	2.0	(0.5)
Lysine	5.6	(0.8)	7.3	(2.0)
Arginine	6.7	(0.8)	5.9	(1.3)

Table 4. Fatty acid composition of herring scrap (%). Average and standard deviation of four samples

C14:0	8.0	(0.8)
C16:1 n7	4.8	(1.5)
C16:0	14.7	(1.1)
C18:4 n3	2.6	(1.0)
C18:3 n3	1.1	(O.3)
C18:2 n6	1.5	(0.2)
C18:1 n9/n11	10.8	(2.0)
C18:0	2.1	(0.7)
C20:5 n3	4.6	(0.7)
C20:4 n6	0.3	(0.1)
C20:1 n9	11.4	(2.3)
C22:6 n3	6.4	(2.2)
C22:1 n9/n11	15.9	(4.2)

The herring scrap that was used had a satisfactory microbiological status but a rather poor fat quality (Table 5). The fat quality of the herring scrap was reflected in diets 2 and 3 by a higher FFA percentage and higher peroxide values. The number of bacteria and fungi in the diets was within an acceptable range according to the standards set by The Norwegian Fur Breeders' Association (Kjos, personal information).

Table 5. Quality parameters of ingredients and diets. Average values of samples from 16 August and 1 October

				Bacteria/fungi, 10^3/g			
	Total volatile N (TVN) %	Free fatty acids (FFA) %	Peroxides mEq O_2/kg fat	Total bact. count	Coliforms	Fecale enterococcus	Fungi
Ingredients							
Herring scrap	0.8	4.1	20.6	87	<0.01	-	<0.01
Cod scrap	1.5	-	-	70	<0.01	-	<0.01
Slaughterhouse offal	1.1	2.4	3.0	8350	1.5	49	3.5
Feed mixtures							
Control	1.3	2.6	31.3	410	0.26	45	8.4
12.5% herring	1.2	2.9	46.5	426	0.44	39	0.9
25.0% herring	1.3	2.8	49.7	200	0.21	19	1.3

Growth and fur development

The growth of silver foxes fed herring scrap corresponded to that in the control group until the beginning of November (Figure 1). Later, the foxes in the herring scrap group had a greater weight gain, and at pelting the average body weights were significantly higher ($p < 0.05$). Daily average feed consumption was 3.5 MJ (ME) in the control group and 3.7 MJ (ME) in the herring scrap group.

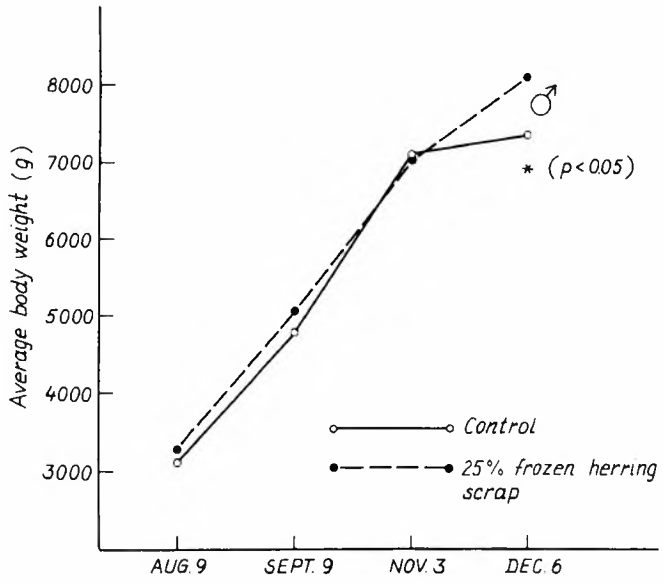


Figure 1. Body weight gain in silver foxes

The evaluation of silver fox pelts showed significantly improved hair quality ($p < 0.01$) and general impression ($p < 0.001$) in animals fed herring scrap (Table 6). Other parameters did not differ significantly. At the Oslo Fur Auctions the silver fox skins were also thoroughly evaluated for the occurrence of brownish hairs or discoloration. It was concluded that none of the silver fox skins had brownish hairs. The colour purity of the silver fox skins was graded from 1 (poorest) to 3 (best). The average results showed a minor insignificant difference between the control group (2.09) and the herring scrap group (1.92).

Table 6. Evaluation of silver fox pelts

	Control	Herring scrap	S.E.M.
Number of pelts	12	13	
Pelt length, cm	102.4	103.2	0.61
Pelt weight (dry), g	610.0	587.0	11.97
Density [1]	5.5	5.8	0.15
Hair quality [1]	4.6	5.4**	0.18
Texture [1]	4.9	5.5	0.17
Cover [1]	5.9	5.8	0.16
Colour of guardfur [1]	6.3	6.3	0.14
Colour of underfur [1]	4.8	5.1	0.20
General impression [1]	4.8	5.8***	0.18

[1] Subjectively graded from 1 (poorest) to 10 (best). S.E.M. = standard error of mean. Different from control group by analysis of variance; $p < 0.01$ = ** and $p < 0.001$ = ***

The body growth in mink was slightly affected by the experimental diets (Figure 2). On 7 September and 5 October it was found that the females in the group fed 25% herring scrap were significantly lighter than those in the control group ($p < 0.05$). The males revealed a similar tendency, but this was compensated for at the final weighing. The weight differences were probably due to temporary low feed intake, presumably because of changes in the taste of the feed. The average daily consumption of ME was 1.6, 1.6 and 1.5 MJ in Groups 1, 2 and 3, respectively.

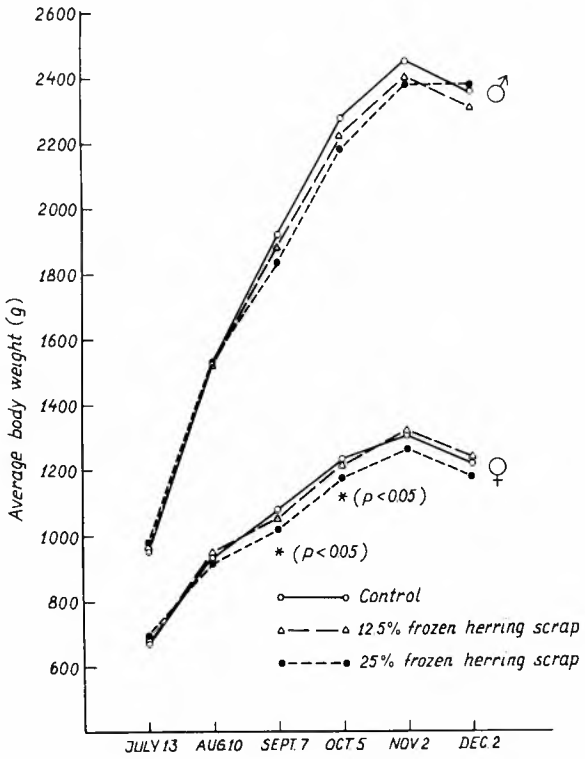


Figure 2. Body weight gain in mink

The evaluation of mink skins revealed minor differences among diets (Table 7). Group 3 (25% herring scrap) tended to have slightly reduced skin length, and the females in this group had a lower pelt dry weight ($p < 0.05$). The fur quality parameters and fur colour were not affected by the feeding of herring scrap.

Mortalities

One silver fox in the herring scrap group died on 13 August. It was considered unlikely that the death was connected with the treatment. In mink, one female in the control group, and two females and one male in the group fed 25% herring scrap died. The autopsy on all mink casualties revealed a *Streptococcus canis* infection.

Blood analyses

The vitamin E content of silver fox plasma was examined twice during the experiment. On 29 August, the averages for six silver foxes were 7.16 μg/ml in the control group and 7.04 μg/ml in the herring scrap group. On 10 October the average vitamin E levels in plasma had risen to 10.34 μg/ml in the control group and 7.55 μg/ml in the herring scrap group, but no significant differences were found. The glutathione peroxidase activity in whole blood revealed small differences between groups: 4.25 mkat/l (control) and 4.29 mkat/l (herring scrap) on 29 August, and 5.08 mkat/l and 5.04 mkat/l respectively on 10 October.

Table 7. Average pelt characteristics in mink

	Control	Herring scrap		S.E.M.
		12.5%	25.0%	
Number of pelts				
Males	34	33	37	
Females	28	30	33	
Skin length, cm				
Males	73.2	72.7	72.4	0.38
Females	59.4	59.5	58.8	0.28
Skin dry weight, g				
Males	151.0	154.6	152.1	2.29
Females	80.4	80.4	76.0*	1.41
Density guardfur [1]				
Males	5.6	5.4	5.6	0.09
Females	6.2	6.5	6.3	0.08
Density underfur [1]				
Males	5.3	5.2	5.5	0.11
Females	6.1	6.3	6.4	0.11
Length guardfur, mm				
Males	24.4	24.2	24.7	0.17
Females	22.1	21.8	21.6	0.15
Length underfur, mm				
Males	14.5	14.7	14.7	0.12
Females	13.6	13.9	13.4	0.11
Fur colour [2]				
Males	6.3	6.3	6.4	0.13
Females	6.3	6.4	6.5	0.14
Metallic [3]				
Males	3.6	3.6	3.7	0.08
Females	3.8	3.9	3.8	0.09
Hair quality [1]				
Males	5.1	5.2	5.3	0.11
Females	5.8	6.2	6.2	0.12
General fur quality [4]				
Males	2.4	2.4	2.6	0.08
Females	3.0	2.9	3.0	0.06

[1] Subjectively rated from 1 to 10 (best).
[2] Subjectively graded from 1 (black) to 7 (pale).
[3] Metallic is characterized by irregular light reflections because of abnormal guard hairs. Rated from 0 (no) to 5 (severe).
[4] Subjectively graded from 0 (poorest) to 4 (best).
S.E.M. = standard error of mean.
Different from control group by analysis of variance $p<0.05$ = *.

In mink, the whole blood glutathione peroxidase activity was 5.37, 6.00 and 5.70 mkat/l on 29 August, and 5.95, 6.31 and 6.17 mkat/l on 10 October, in Groups 1, 2 and 3, respectively. Thus, there were minor differences among groups, and no significant effect of herring scrap on selenium status in mink.

DISCUSSION

Whole herring and herring scrap usually have a high energy concentration because of high fat content. However, there are large seasonal variations. The fat levels of whole herring may vary from 5 to 30%, the peak level being reached just before spawning (Lambertsen 1973). Filleting scrap usually contains less fat than whole herring. The fat level in herring scrap used as feed for fur animals is usually 10-15% (Skrede 1986).

The amino acid composition of herring scrap in the present study was in good agreement with that observed in earlier studies on whole herring (Njaa & Utne 1982), and was close to that found in the present experiment in herring scrap. Also, the fatty acid composition of the herring scrap used in this experiment corresponds well with values for herring fillet (Lambertsen 1973) and herring oil (Ackman 1982). However, in a extensive analysis, including detection of about 40 fatty acids, Ackman (1982) reported that herring oil contains 6.7% of C16:3 n4. This fatty acid was not detected in the present study.

Diets high in unsaturated fat have been shown to reduce vitamin E absorption in mink (Eskeland & Rimeslåtten 1979, Brandt *et al.* 1990). Polyunsaturated fatty acids (PUFA) can cause vitamin E and selenium deficiency with symptoms such as yellow fat, muscular degeneration and sudden death in growing mink (Helgebostad 1971, Gorham & Hegreberg 1971). The same symptoms of vitamin E or seleninum deficiency have been induced experimentally in growing dogs (van Vleet 1975). Ender & Helgebostad (1945) observed spasms, swollen intestine, lesions in the abdominal wall, fatty liver and death in growing silver foxes fed diets containing large amounts of herring. Damgaard & Clausen (1990) found reduced plasma concentrations of glutathione peroxidase in mink fed a herring scrap diet. The cause of the depression in plasma glutathione peroxidase could have been an increased need for this enzyme induced by the polyunsaturated fat in herring scrap (Damgaard & Clausen 1990). The significant prophylactic effect of vitamin E supplementation to mink fed high levels of peroxidized fat has been described by Ender & Helgebostad (1975). The results of the present study showed that the vitamin E and selenium supply was sufficient to prevent deficiency symptoms.

Analyses of the herring scrap used in the present experiment disclosed higher peroxide values than those found by Clausen & Sandø Lund (1984). However, the storage period was only four days in the latter experiment. Rouvinen (1987), on the other hand, measured peroxide levels at 160 $mEqO_2/kg$ fat in herring scrap, which is well above the values obtained in the present experiment.

Herring is one of the fish species known to contain thiaminase (Høie & Rimeslåtten 1951). The herring scrap used in the present study did not contain enough thiaminase to destroy the supplied thiamine and to cause deficiency symptoms under the conditions of the experiment. It should be borne in mind, however, that the feed temperature was low during storage. Low temperature has been shown to reduce the thiaminase activity considerably (Helgebostad 1968).

The growth rate of the silver fox cubs fed the herring scrap diet was equal or superior to that of the control animals. This demonstrates the satisfactory palatability of diets with large amounts of frozen-stored herring scrap. Høie & Rimeslåtten (1951) reported similar results in experiments with silver foxes. The vitamin E status of the silver foxes in this

study tended to support the view that physiological stress induced by unsaturated fat from herring scrap could be efficiently prevented by providing supplementary vitamin E.

Some Norwegian fur breeders have suspected that herring scrap causes brownish hairs in silver foxes. In this study, herring scrap diets did not influence colour or general quality of silver fox fur. On the contrary, herring scrap seemed to improve fur quality. Høie & Rimeslåtten (1951) revealed minor differences in fur quality between a control group and groups fed herring products. Most likely, only unbalanced diets with large amount of poor quality herring and insufficient antioxidant protection, can be suspected of causing such fur defects in silver foxes as those described by Ender & Helgebostad (1944).

According to the present results, the growth rate of mink kits may be slightly retarded when the amount of frozen-stored herring scrap in the diet is as high as 25%. Similar results have been reported from Norwegian experiments when 20% frozen-stored herring scrap was used (Skrede 1986). Poulsen & Jørgensen (1986) discovered a reduced growth rate in mink fed 30% fresh and frozen stored-herring scrap. This diet was meant as a control, and allthough the reason for the growth impairment was not discussed further by the authors, it seems possible that the 30% herring scrap diet may have had some negative influence on growth performance. Ulmanen *et al.* (1991) found an increased mortality and a reduced growth rate in mink fed 40% partly rancid herring scrap. Because of the high mortality rate the amount of herring scrap was reduced to 20% in the last part of the experiment. In the present study, about 250 ppm ethoxyquin was added to the frozen-stored herring scrap, which gave approximately 30 ppm and 60 ppm in diets 2 and 3, respectively. In blue fox, Rouvinen (1991) found a marked reduction in appetite after adding 500-1000 ppm ethoxyquin to the diet. Pathological studies and blood samples of the animals revealed degenerative changes and malfunction of the liver. Lyngs (1991) observed reduced feed intake in mink when 23.1 ppm ethoxyquin was added to the diet. The ethoxyquin level, along with the fat quality of the herring scrap, is probably of crucial importance for feed acceptability in mink. It is likely that one or more of these factors may have caused the reduced feed consumption in mink fed high levels of herring scrap.

It has been observed that there is an increased frequency of fat metabolism disorders like fatty liver and liver necrosis in mink fed rancid herring scrap (Ulmanen *et al.* 1991). In this experiment, fatty liver or liver tissue damage was not discovered during the autopsy following mortalities.

Skrede (1986) found only marginal effects on mink skin quality parameters in experiments with herring scrap. Ulmanen et al. (1991) discovered a less dense underfur mass in mink fed 40% rancid herring scrap. In the present study, fur characteristics of mink fed on herring scrap did not differ from those of the control animals. Results of feeding experiments with different fat sources do not indicate any negative effect of capelin oil, which resembles herring oil with regard to fatty acid composition, on mink skin quality (Skrede 1984, Rouvinen *et al.* 1989). However, large amounts of rancid dietary fat may cause collagen damage in mink skins, indicated by a low breaking load of the leather (Rouvinen & Mäntysalo 1989).

CONCLUSIONS

In silver foxes, it was found that a diet containing 25% frozen-stored herring scrap had a positive effect on growth and no negative effect on fur quality. In mink, diets containing 12.5 % and 25 % herring scrap supported normal growth and fur quality. Mink seem to be more sensitive to the taste of feed than silver foxes, which suggests that herring scrap in diets for mink should be introduced gradually. The antioxidant system of the animals, as measured by vitamin E and glutathione peroxidase activity in whole blood, was not influenced when lard was replaced with herring fat.

The results of this study indicate that frozen-stored herring scrap is suitable as feed for silver foxes and mink in the growing-furring period.

REFERENCES

Ackman, R.G. 1982. Fatty acid composition of fish oils. Barlow, S.M. & M.E. Stansby, (eds.). Nutritional evaluation of long-chain fatty acids in fish oils. Academic Press, London: 26-88.

Anderson, P.H., S. Berret & P.S.P. Patterson 1978. Glutathione peroxidase activity in erythrocytes and muscle of cattle and sheep and its relationship to selenium. J. Comp. Path. 88: 181-189.

Brandt, A., C. Wolstrup & T. Krogh Nielsen 1990. The effect of dietary dl-α-tocopheryl acetate, sodium selenite and polyunsaturated fatty acids in mink (*Mustela vison*). J. Anim. Physiol. a. Anim. Nutr. 64: 280-288.

Clausen, T.N. & R. Sandø Lund 1984. Kvalitetsmessige undersøgelser af behandlet og ubehandlet sildeaffald, før og under en belastning. Faglig årsberetning. Dansk Pelsdyravlerforening, Glostrup, 1985: 111-129.

Damgaard, B.M. & T.N. Clausen 1990. Vitamin-E/selen status hos mink. Faglig årsberetning. Dansk Pelsdyravlerforening, Glostrup, 1991: 54-60.

Enggaard Hansen, N., L. Finne, A. Skrede & A.H. Tauson 1991. Energiforsyningen hos mink og ræv. NJF utredning/rapport nr. 63. DSR forlag, Landbohøjskolen, København. 59 s.

Ender, F. & A. Helgebostad 1944. Fôringens innflytelse på forskjellige pelsegenskaper hos sølvrev. Fiskefôring kontra kjøttfôring. Norsk Pelsdyrblad 18: 73-96.

Ender, F & A. Helgebostad 1945. Om påvisning av en vitamin B_1-destruerende faktor i prøver av sild og brisling. Antivitaminer som årsak til beri-beri hos rev. Norsk Vet. Tidskr. 55: 32-76.

Ender, F. & A. Helgebostad 1975. Unsaturated dietary fat and lipoperoxides as etiological factors in vitamin E deficiency in mink. Acta Vet. Scand. 16 Sup. 55, 25 s.

Eskeland, B. & H. Rimeslåtten 1979. Studies on the absorption of labelled and dietary α-tocopherol in mink as influenced by some dietary factors. Acta Agric. Scand. 29: 75-80.

Gorham, J.R. & G.A. Hegreberg 1971. Steatitis (yellow fat disease) in mink. Olympic report (Official Publication Olympic Fur Breeders Association Inc.), (249), Feb. 1971: 2,6,8.

Helgebostad, A. 1968. Vitamin B1-mangel hos mink og rev i forbindelse med fôring av sølvtorsk (*Gadiculus thori*). Norsk Pelsdyrblad 42: 619-622.

Helgebostad, A. 1971. Vitamin E mangel syndromet hos pelsdyr. Norsk Vet. Tidsskr. 83: 11-17.

Høie, J. & H. Rimeslåtten 1951. Forsøk med vårsild til pelsdyrfôr. Meld. Norg. LandbrHøgsk. 31: 53-90.

Lambertsen, G. 1973. Lipids in marine fish. Wissenschaftliche Veröffentlichungen der Deutschen Gesellschaft für Ernährung 24: 25-31.

Lyngs, B. 1991. Smagelighetsforsøg: Ethoxyquin i foderet til minkvalpe i pelssætnings-perioden. Faglig årsberetning. Dansk Pelsdyravlerforening, Glostrup, 1991: 92-96.

Njaa, L. R. & F. Utne 1982. A comparison of the amino acid composition of fifteen species of whole fish. Fisk. Dir. Skr., Ernæring, Vol II, 2: 25-33.

Paglia, D.E. & W.N. Valentine 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70: 158.

Poulsen, J.S.D. & G. Jørgensen 1986. Examination of mink (*Mustela vison*) fed a sulphuric acid preserved silage during lactation and growth period. II. Production and pathologic studies correlated to results of clinical-chemical studies. Nord. Vet.-Med. 38: 90-105.

Rouvinen, K. 1987. Olika fetter i pälsdjursfoder. NJF seminar nr. 128, Sept. 28-30, Tromsø, Norway, 22 s.

Rouvinen, K. 1991. Dietary effects of omega-3 polyunsaturated fatty acid on body fat composition and health status of farm-raised blue and silver foxes. Acta Agric. Scand. 41: 401-414.

Rouvinen, K. & E. Mäntysalo 1989. Influence of fatty acid composition in dried raw mink and blue fox skins on their storage aging and dressing properties. Acta Agric. Scand. 39: 289-300.

Rouvinen, K., P. Niemelä, & T. Kiiskinen 1989. Influence of dietary fat source on growth and fur quality of mink and blue fox. Acta Agric. Scand. 39: 269-278.

SAS Institute, 1985. Sas User's Guide: Basics. Sas Institute Inc., Box 8000, Cary, North Carolina, USA.

Skrede, A. 1984. Evaluation of capelin oil as an energy source in mink diets. Intern. Congress of fur animal production, Versailles, France, 9 s.

Skrede, A. 1986. Sildeavfall som fôr til rev og mink. Norsk Pelsdyrblad 60: 140-143.

Ulmanen, E., S. Turnunen & L. Blomstedt 1991. Effect of diet on skin fatty acid profile and fur condition in mink. Acta Agric. Scand. 41: 271-278.

van Vleet, J. 1975. Experimentally induced vitamin E/selenium deficiency in the growing dog. Am. Vet. Med. Ass. J. 166(8): 764-774.

Welch, R.W. 1976. A micro-method for the estimation of oil content and composition in seed crops. J. Sci. Fd. Agric. 28: 635-638.

Sexual steroid hormones in the reproductive cycle of silver fox

LUDMILA OSADCHUK
Institute of Cytology & Genetics, Siberian Branch of the Academy of Sciences of Russia, Novosibirsk, Russia

Osadchuk, L. 1993. Sexual steroid hormones in the reproductive cycle of silver fox. Norwegian Journal of Agricultural Sciences 7: 189-201. ISSN 0801-5341

A study has been carried out on sexual steroid hormone concentrations in the peripheral plasma of adult silver fox of both sexes and on the production of these hormones by gonads and adrenals *in vitro* using the radioimmunological method. In females a maximal oestradiol concentration was found during the pro-oestrus, just before onset of heat and ovulation and correlating with maximal production of oestradiol by ovaries *in vitro*. An increase in progesterone level was observed during the pro-oestrus and the maximum was reached before implantation. It is suggested that silver fox adrenals in females are an additional source of progesterone secreted into the systemic circulation and might play a role in the regulation of the reproductive function. In males, the maximal testosterone level coincided with the mating season. It was demonstrated that the testicles are the main source of testosterone and adrenals are insignificant in this respect.

Key words: Adrenals, oestradiol, oestrous cycle, ovaries, progesterone, silver fox, testosterone

Ludmila Osadchuk, Institute of Cytology & Genetics, Siberian Branch of the Academy of Sciences of Russia, Lavrentyev Ave. 10, 630090 Novosibirsk, Russia

The silver fox - a colour mutant of the red fox - is one of the many fur bearing species that are bred in captivity. Although the silver fox reproductive cycle has been described by several researchers in the past (Vasina 1937; Cler 1937; Starkov 1937; Johansson 1941; Pearson & Enders 1943), the endocrinological aspect of silver fox reproduction has hardly been studied at all. Most of the research studies were carried out on red fox from natural populations (Joffre 1977; Mondain-Monval et al. 1977; Bonnin et al. 1978; Maurel & Boissin 1981; Maurel et al. 1984) and the aim was to investigate the concentrations of sexual steroid hormones and gonadotrophins in peripheral plasma throughout the annual reproductive cycle. During the past few years a series of research studies on neuroendocrine and endocrine aspects of regulation of silver fox male reproduction have been carried out by Forsberg et al. (Forsberg et al. 1989; Forsberg & Madej 1990). Further information has been gathered on the reproductive endocrinology of the blue fox, taxonomically closely related to the silver fox (Moller et al. 1984; Mondain-Monval et al. 1985).

This work presents the results of many years of research on the hormonal steroid function of gonads in silver fox at different stages in the reproductive cycle. Adrenals can synthesize and secrete steroids into the general circulation and seem to be important for

facilitating ovulation and inducing female mating behaviour (Nequin & Schwartz 1971; Barfield & Lisk 1974; Wilson et al. 1978). Therefore, this work also introduces data on the contribution of adrenals to the formation of the peripheral pool of sexual steroid hormones in silver foxes.

MATERIALS AND METHODS

The animals used in this study were adult mature silver fox vixens and males (*Vulpes fulvus Desm.*) from a population maintained at the Experimental Animal Farm of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia). In order to investigate the seasonal dynamics of sexual steroid hormones in animals, preferentially of the same kind, blood samples were taken once or twice a month from the v. saphena during the anoestrus (12-15 vixens and 10-12 males). Samples were also taken during the pro-oestrus, oestrus and throughout the gestation period. Pro-oestrus was determined by the cytological condition of the smear sample and by vulval swelling. Following the method by Starkov (Starkov 1937), pro-oestrus was divided into three stages: the early, middle and late phases; and blood samples were taken at the beginning of each stage. Oestrus was determined according to vulval swelling, the smear composition and the readiness of the vixen to mate. The blood was taken on the day following the first mating. Throughout gestation blood samples were taken every 5-10 days. During the reproductive season (February) blood samples were taken from males after the vixen in oestros had been presented to them, whether coitus took place or not. Blood samples were collected in heparinized tubes and centrifuged. The plasma was frozen and stored at -20°C pending analysis.

In order to study the production of sex hormones by gonads and adrenals *in vitro*, mature vixens and males (in groups of 4-12 animals) were electrically sacrificed at different stages of the reproductive cycle (females twice in the anoestrus: at the end of November and in mid-December, in late pro-oestrus and in oestrus. Males - in November and in mid-March). The glands were removed, cleaned of adjoining tissues, weighed, minced and incubated in a shaker at 37°C in Crebs-Ringer bicarbonate buffer, glucose content of 200 mg%, in an atmosphere of 95% oxygen and 5% carbon dioxide gas mixture. Adrenals were incubated for 1.5 h in a 4 ml medium while ovaries and testicles (1 g portion of the latter was taken) were incubated for 3 h in a 10 ml medium. Ovaries with chorionic gonadotrophin (Antex Leo Company) were incubated for 2.5 h in 5 ml incubating medium in late anoestrus (the end of December). The minced tissue of ovaries of every animal under investigation was divided into three portions: the first was a control, the other two portions were treated with chorionic gonadotrophin - 50 and 100 IU, respectively. After the incubation the incubating medium was decanted, frozen and kept at -20°C.

Sexual steroid hormones in the plasma and the gland incubates were determined by radioimmunoassay, using a commercial kit by the Cea-Ire-Sorin Company. Before any determinations were made, steroids were extracted from plasma by freshly redistilled ethyl ether and then the ether was evaporated by dryness under nitrogen. The data obtained were statistically analysed using the Student's t-test.

RESULTS AND DISCUSSION

Females

The oestradiol concentrations in peripheral plasma of vixens during the oestrous cycle are presented in Fig. 1. Compared with other stages of the oestral cycle, during anoestrus it was found that the quantity of hormones is minimal and correlates with the quiescent period in the reproductive system. The lowest oestradiol concentration, i.e. 9-12 pg/ml, is reported as occurring in May-August. In autumn (September-October) an increase in plasma oestradiol was observed but with no visible alterations in vulval swelling. From the results obtained it was not possible to determine precisely the reason for the oestradiol surge during anoestrus. In our opinion, the oestradiol increase in the blood could be explained by alterations in oestrogen metabolism as a result of adaptation to the autumn-winter period rather than by enhancement of the hormonal function of gonads. It should be added that in a study of the red fox during anoestrus Mondain-Monval et al. (1977, 1979) reported (mostly in autumn) even a few peaks of oestradiol concentration in blood which coincided in the same vixens with an increase in electrical activity of the myometrium and the thickening of the vaginal epithelium, its proliferation and keratinization. However, they did not investigate either the sources of oestrogen in peaks or its regulation by hypophysial gonadotrophins during the anoestrus. It may be supposed that the simultaneous determination of gonadotrophins and oestrogens with the high frequency of blood sampling would offer deeper insight into the nature of episodic peaks of oestrogen throughout the anoestrus in the silver fox.

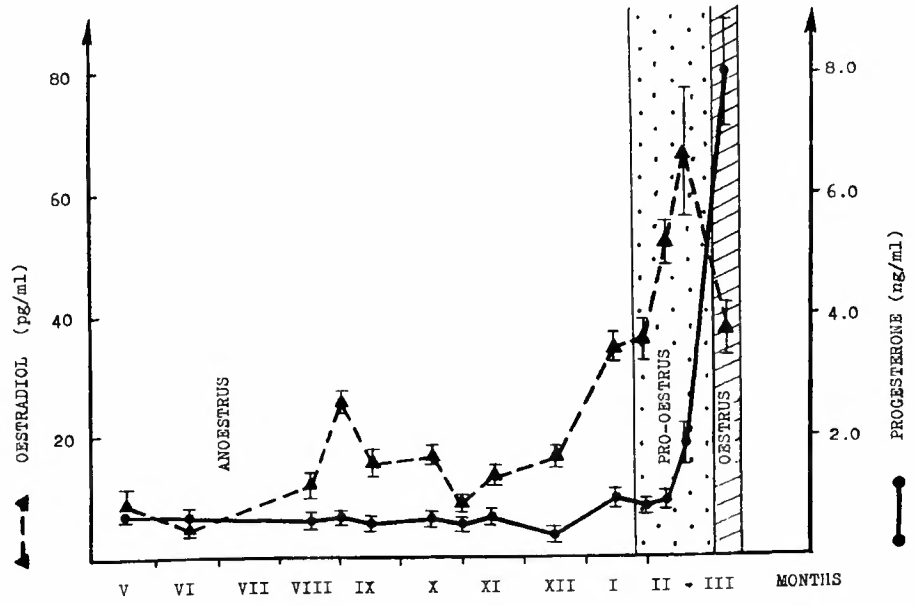


Figure 1. Plasma oestradiol and progesterone concentrations in silver fox females during the oestrus cycle. The shaded portion of the vertical bar indicates the period of oestrus

Fig. 1 also illustrates the dynamics of the progesterone level in silver fox vixens during the oestrous cycle. Contrary to oestradiol the concentration of this hormone does not significantly change throughout the anoestrus and the values range from 0.4 to 0.8 ng/ml. No elevations in the level of progesterone in the period of increased oestradiol concentration were observed. A low level of oestradiol and progesterone in peripheral blood in silver fox vixens in anoestrus agrees well with low production of these hormones by ovaries *in vitro* (Fig. 2). When compared with other stages of the oestrous cycle, during anoestrus the production of oestradiol and progesterone by ovaries *in vitro* is also minimal (Fig. 2).

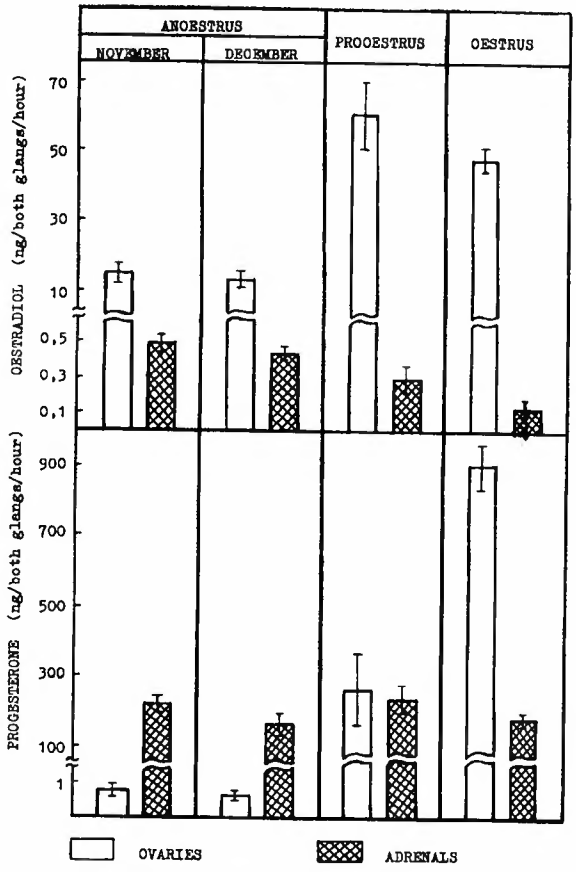


Figure 2. Oestradiol and progesterone production *in vitro* by ovaries and adrenals in silver fox females

Production of both hormones by ovaries *in vitro* in November and December was found to be the same. These data are in contrast with other observations which report that in December a growth and an atresia of follicles occur in silver fox ovaries (Cler 1937). However, during the period of active growth of follicles (December) no hormonal activity by the ovaries has been observed. It is possible that the low steroid activity of gonads is due to a low LH secretion by the hypophysis in this period. The ability of silver fox ovaries to respond to gonadotrophin stimulation in December was studied in a special experiment (see Table 1). It was demonstrated that chorionic gonadotrophin (HCG) in increased dosage does not stimulate the biosynthesis of progesterone and oestradiol by the ovaries. This may depend on a decreased activity of corresponding enzymes of the steroid biosynthesis or an insufficient amount of active receptors to gonadotrophin in structures of growing and atretic follicles in this period.

Table 1. Effects of chorionic gonadotrophin on the on the production of sexual hormones by ovaries *in vitro* in the female silver fox

Hormone	Control	50 units of HCG	100 units of HCG
Oestradiol (pg/100 mg/hr)	4.43±1.18 (6)	3.64±0.55 (10)	3.11±0.48 (10)
Progesterone (ng/100 mg/hr)	0.30±0.03 (10)	0.31±0.02 (10)	0.28±0.02 (10)

( ) - a number of animals in group

There is some interest in the problem of the sex hormone secretion by adrenals in the silver fox. In our experiment we compared the production of oestradiol and progesterone *in vitro* by the ovaries and adrenals of silver fox in anoestrus (Fig. 2) and obtained the following results. Oestradiol is produced by adrenals in November-December in quantities ranging from 0.4 to 0.5 ng/both glands/h while its production by ovaries varies between 13 and 15 ng/both glands/h. Progesterone production by ovaries and adrenals during this period makes up, respectively, 0.65-0.68 ng/both glands/h and 223-243 ng/both glands/h. Based on these data we can consider the ovaries as the main oestradiol-producing glands and the adrenals as the main glands for secreting progesterone in silver foxes in anoestrus.

Compared with the December results, it was found that in January the oestradiol and progesterone concentrations in peripheral plasma predictably increased ($p<0.05$) (Fig. 1). During this period no alterations were observed in the vixens' vaginal smear composition or in vulval swelling. The increase in oestradiol and progesterone levels before the onset of the reproductive season is undoubtedly connected with the increase in the secretory activity of growing follicles shaping up as graafian follicles (Cler 1937).

The concentrations of oestradiol in the peripheral plasma of silver fox females in pro-oestrus are reported in Fig. 1, where it can be seen that they increase to maximun in late pro-oestrus and are 5-7 times higher than during the anoestrus. In the oestrus the oestradiol level decreased significantly ($p<0.05$) (Fig. 1) and showed values typical of the onset of pro-oestrus. Similar alterations in the hormonal level are reported in the oestrous cycle of many mammals including canids - dog, silver and blue fox (Austad et al. 1976; Mondain-Monval et al. 1977; Moller et al. 1980; Moller et al. 1984).

Moller et al. (1984) studied the temporary correlation between concentrations of oestradiol and LH in blue fox in pro-oestrus and oestrus. They observed a few small peaks of LH in vixens in pro-oestrus that do not usually coincide in animals at that particular time. However, the main peak in all animals is reported as being 1-2 days before mating, lasting 1-3 days and coinciding with the time of peak blood oestradiol. An analogous study on dogs was carried out by Concannon et al. (1975). There are no grounds for doubting that in silver fox, as in other canids, the increased level of oestrogen in the follicular phase of the ovarian cycle is a factor stimulating the release of LH from the hypophysis through the positive feedback mechanism.

The progesterone level in the peripheral plasma in silver foxes increases even by the pro-oeatrus and its concentration in late pro-oestrus makes up 1.9±0.3 ng/ml, i.e. it is double the level recorded before the reproductive season (January) and is almost three times higher than that in the summer-autumn period (Fig. 1). During oestrus, the progesterone level progressively increases and its mean value is 8.0±0.1 ng/ml (Fig. 1). Progesterone

production by ovaries *in vitro* during the pro-oestrus also increases considerably compared with during the anoestrus (Fig. 2). During the anoestrus, progesterone production was 0.65 ± 0.12 ng/both glands/h, while in late pro-oestrus it increased to 267.3 ± 106.4 ng/both glands/h and in the oestrus to 906.5 ± 69.8 ng/both glands/h. Thus, the increase in progesterone concentrations throughout the pro-oestrus and the oestrus correlates with the enhancement of its production by ovaries *in vitro*. The obtained results assume progesterone biosynthesis by pre-ovulatory follicles, in other words, the formation of lutein structures in silver fox ovaries already in pro-oestrus. This assumption is compatible with previous histological observations that luteinization of the ripe follicles in the silver fox starts before ovulation (Vasina 1937; Pearson & Enders 1943). In blue fox and dog it was also found that there was a high peripheral plasma level of progesterone long before onset of oestrus and ovulation (Concannon et al. 1975; Moller et al. 1984).

A question arises concerning the physiological role of progesterone synthesized by silver fox follicles during the pro-oestrus, just before onset of ovulation and heat. Secretion of progesterone by pre-ovulatory follicles was reported in many animal species and humans. Moreover, the results of numerous experiments allowed the conclusion, generally adopted nowadays, that progesterone secreted by the ovaries before ovulation in synergism with oestradiol involves the pre-ovulatory release of LH and FSH from the hypophysis. It is not an overstatement that in silver fox in pro-oestrus, progesterone plays an important part in the signalling mechanism that initiates ovulation. Moreover, in silver fox vixens on heat the high progesterone concentration coincides with the animals' sexual receptivity and, along with oestrogen, is probably required to induce sexual behaviour. In this connection, it is interesting to note that these data do not correlate with results obtained on rat and sheep, in which a high progesterone concentration precedes the period of sexual receptivity (Neill & Smith 1974; Fink 1986).

A comparison of the production of progesterone and oestradiol by the ovaries and the adrenals during pro-oestrus and oestrus provides evidence for a quite insignificant biosynthesis of oestradiol and a sufficiently high biosynthesis of progesterone by the adrenals (Fig. 2). It was established that during pro-oestrus, ovaries and adrenals produce nearly the same amont of progesterone while during the oestrous the activity of the ovaries is only 4-5 times higher than that of the adrenals. Thus, the results of the present research project reveal that adrenals are a supplementary and significant source of progesterone in the silver fox. It can be said that the contribution of adrenals to the formation of the peripheral pool of progesterone at all stages of the oestrous cycle of silver fox vixens is quite important.

It is known that progesterone in adrenals was found to be one of the precursors in the corticosteroid biosynthesis. But there is a series of data that indicates a specific role of the adrenal progesterone in the regulation of some processes of the oestrous cycle in some rodents. It was shown that the adrenal progesterone as well as the ovarian progesterone during the pro-oestrus contribute to the ovulating release of LH in rat (Nequin & Schwartz 1971; Wilson et al. 1978) and the role of the adrenal progesterone in the development of sexual receptivity is also important (Barfield & Lisk 1974). Rats under stress increased secretion of adrenal progesterone and, depending on the stage of the oestrous cycle, ovulation and lordosal behaviour were either activated or inhibited (Roos et al. 1980; Plas-Roser & Aron 1982). The ability of adrenals in silver fox vixens to produce considerable quantities of progesterone and to change its peripheral level seems to be an important trait

in this species. Note that since the concentration of adrenal progesterone in silver fox vixens *in vitro* scarcely changes throughout the oestrous cycle (Fig. 2), it is quite difficult to imagine its participation in facilitating ovulation on the basis of a positive feedback. Still, if the progesterone level in blood increases as a result of stress or any other factors stimulating the activity of the hypophysial-adrenal system, it seems reasonable to suggest inhibiting effect of progesterone on the level of gonadotrophin hormones on the basis of a negative feedback with the subsequent inhibition of ovulation and heat. Certainly, determining the role of adrenal progesterone in the regulation of the reproductive function in silver fox would require further investigation.

In the present work estimates were also made on the concentration of progesterone and oestradiol in peripheral blood throughout gestation (Fig. 3). Although the oestradiol concentration scarcely changes, it remains 2-4 times higher than that found in anoestrus, thus evidencing a high secretion of oestradiol by corpora lutea. A slight increase in plasma oestradiol ($p<0.05$) was found on the 15th day of gestation. The embryo implantation in silver fox vixens occurs on the 15th-17th days (Cler 1937; Zybina et al. 1989). It is probable that the oestradiol increase during this period facilitates implantation of the blastocysts. During pregnancy the plasma progesterone level rises more steeply and reaches its maximum before implantation (Fig. 3). During the oestrus, it was found that when the progesterone level reached about 8 ng/ml, then on the 5th day of gestation it doubled and rose to 18.2 ng/ml, on the 10th day it was observed to be at the same level and after the 15th day it was found to decrease gradually until the end of gestation. The decrease in the progesterone level after implantation is typical of canine species (Moller 1973, 1974; Bonnin et al. 1978) and on this point they clearly differ from other species.

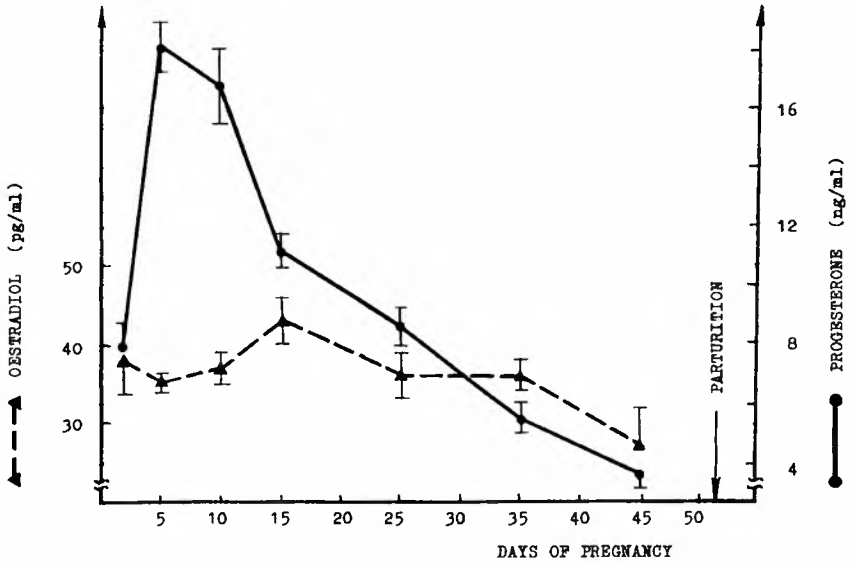


Figure 3. Plasma oestradiol and progesterone concentration in silver fox females during pregnancy

During the gestation period, the ovaries, the adrenals as well as the foeto-placental units may be the sources of sexual steroid hormones. The placenta does not produce enough progesterone to maintain pregnancy in the blue fox (Moller 1974). Since the placenta does not synthesize sex hormones, after implantation there is no increase in the progesterone level of dog, fox and blue fox compared with species with hormonally active placentas. Ovariectomy in blue fox at all stages of gestation resulted in a loss of embryos and an abrupt drop in the peripheral plasma progesterone (Moller 1974). Thus, corpora lutea seem to be the main sites of the secretions of sexual steroid hormornes during pregnancy in canine species. Since there appears to be no information to determine whether adrenals play any role in maintaining gestation in silver fox or not, this requires further investigation.

There have been very few studies on the mechanisms regulating the steroid activity of corpora lutea in fox gestation. When Moller (1973) compared the progesterone profiles in pregnant and non-pregnant vixens (not mated with males) of blue fox, he did not find any differences, i.e. the activity of corpora lutea does not really seem to depend on mating and the presence of embryos in the uterus.

The results from the study of dog indicate that LH and prolactin can be luteotropic factors in canids (Concannon 1980; Okkens et al. 1990). Moreover, when dogs were subjected to induced oestrus and ovulation by PMSG (pregnant mare serum gonadotrophin), HCG stimulated the production of progesterone by corpora lutea. However, neither HCG nor GnRH (gonadotrophin-releasing hormone) were able to prevent the regression of these induced corpora lutea (Barta et al. 1986). It is assumed that luteolysis is not related to the decrease in the gonadtrophic function of the hypophysis. Nevertheless, some authors assume that the hormonal activity of corpora lutea in foxes and dogs is maintained by the maternal hypophysis exclusively and the timing of corpora lutea regression is controlled by a biologioal clock of the maternal hypothalamus (Bonnin et al. 1978).

Males

In contrast to vixens, silver fox males have a longer period of mating. On average it lasts from mid-January until the end of March. Still, when confronted by a receptive female, some males can mate with females at the end of December and at the end of April. Fig. 4 presents the annual dynamics of the peripheral testosterone level in silver fox males. The lowest level was recorded from June until the end of October and varied from 0.1 to 0.2 ng/ml. From the beginning of November the level increased progressively, reaching the maximum values in January-February, i.e. during the reproductive season (2.4-3.0 ng/ml). In March it sharply declined, with a subsequent decrease in April (Fig. 4). Thus, the testosterone level in the blood of silver fox males is closely related to the sexual activity of the animal: the higher concentration coincides with the reproductive season, and the lower one with the period of sexual quiescence. Similar dynamics in the testosterone level were described in red fox males (Joffre 1977). Note the presence of testosterone "surges" in some animals during the period of minimal sex gland activity when they reach values registered in the reproductive season. Such "surges" do not correlate with spermatogenesis and sexual activity and, most probably, characterize only the pulsative release of the hormone from the testicles.

The hormonal activity of the testicles in red fox correlates with seasonal variations in testicle and epididymis mass (Maurel et al. 1984). The luteinizing hormone level in fox

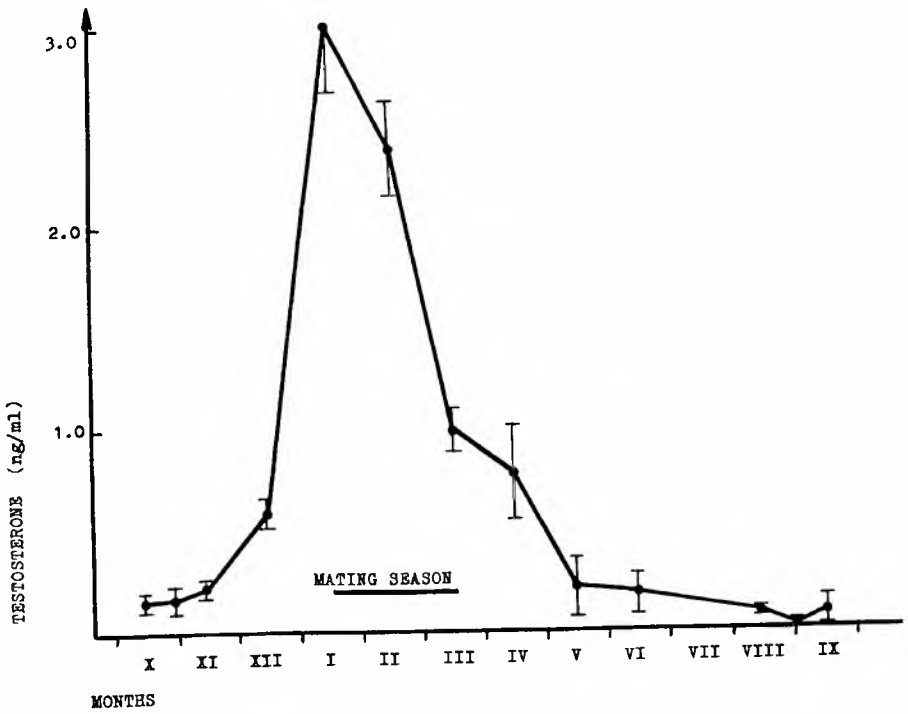


Figure 4. Plasma testosterone concentration in silver fox males during annual reproductive cycle

males also reaches peak values once a year. Yet, some other characteristics have been observed, such as a sharp increase in November that continued high until March, i.e. the significant elevation of LH in the blood occurs two months earlier than that of testosterone (Maurel et al. 1984). Seasonal alterations in testosterone were observed in the reproductive cycle of blue fox males (Smith et al. 1985). LH and FSH were estimated simultaneously with testosterone and an increase in FSH and LH from December to March was reported (Smith et al. 1987). Probably, both gonadotrophins are being controlled by the same negative feedback mechanism, and high concentrations of testosterone during the reproductive season may induce a decrease in FSH and LH. Although in this study LH and FSH estimates for silver fox males throughout the annual reproductive cycle were not assessed, it is thought that there may be a similarity between profiles of gonadotrophins in blue, red and silver foxes.

Interesting by enough, Smith et al. (1987) simultaneously estimated LH, FSH and testosterone concentrations in blue fox males administered gonadotrophin-releasing hormone (LH-RH) throughout the seasonal reproductive cycle. It was reported that there was virtually an immediate increase in the LH level and, in some animals, in the FSH level during all seasons, while the increase in testosterone in response to the LH-RH administration was observed only during the reproductive season. Thus, seasonal fluctuations in the hormonal activity of the testicles may depend not only on changes in hypothalamic

and hypophysial stimulation but also on alterations in the sensitivity of the testicles to gonadotrophins.

Testosterone in male fox can be secreted into the blood by both the testicles and the adrenals. The question then arises as to their relative contribution to the peripheral testosterone level. The data in Fig. 5 illustrate that in December (before the reproductive season) and in March (at the end of the reproductive season) the amont of testosterone produced by adrenals is in fact null compared with that produced by the testicles. In previous studies (Wassermann & Eik-Nes 1969) in venous blood in dog it was also noted that only "traces" of testosterone were produced by adrenals. From the given data it can be assested that in silver fox males as well as in dog, testosterone secreted by the adrenal glands into the blood is insignificant and the required testosterone level in the blood can only be maintained by the hormonal function of the testicles.

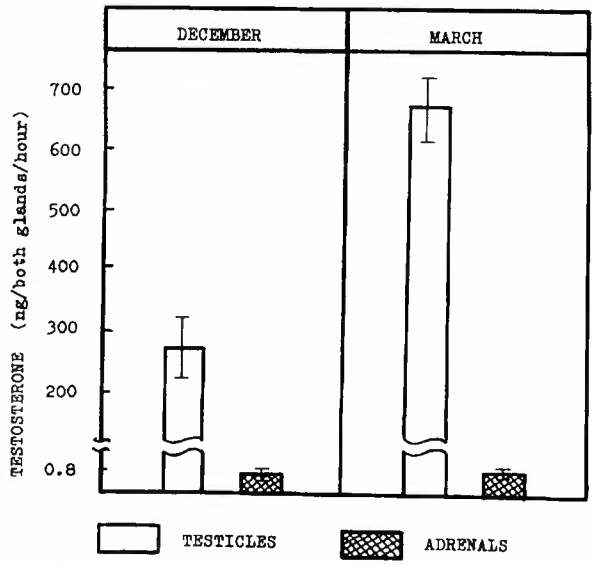


Figure 5. Testosterone production in vitro by testicles and adrenals in silver fox males

REFERENCES

Austad, R., A. Lunde & O.V. Sjaastad 1976. Peripheral plasma levels of oestradiol-17 and progesterone in the bitch during the oestrus cycle, in hormonal and after dexamethasone treatment. J. Reprod. Fert. 46: 129-136.

Barfield, M.A. & R.D. Lisk 1974. Relative contributions of ovarian and adrenal progesterone to the timing of heat in the 4-day cycle rat. Endocrinology 94: 571-579.

Barta, M., L.F. Archbald & R.A. Godke 1986. Progesterone secretion by induced corpora lutea (CL) of anestrous bitches after administration of gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (HCG). Theriogenology 25: 225-231.

Bonnin, M., M. Mondain-Monval & B. Dutourne 1978. Oestrogen and progesterone concentrations in peripheral blood in pregnant red foxes (*Vulpes vulpes*). J. Reprod. Fert. 54: 37-42.

Cler, R. 1931. Hystophysiology of silver fox reproduction. In: Sbornic rabot laboratorii histolgii po razmnogeniu lisi. Trest Sojusswerovod, Moscow: 34-49 (in Russian).

Concannon, P.W., W. Hansel & W.J. Visek 1975. The ovarian cycle of the Bitch: plasma estrogen, LH and progesterone. Biol. Reprod. 13:112-121.

Concannon, P.W. 1980. Effect of hypophysectomy and of LH administration on luteal phase progesterone levels in the Beagle bitch. J. Reprod. Fert. 588: 407-410.

Fink, G. 1986. The endocrine control of ovulation. Sci. Progr., Oxford. 70: 403-423.

Forsberg, M., J.A. Fougner, P.O. Holmo et al. 1989. Photoperiodic regulation of reproduction in the male silver fox (*Vulpes vulpes*). J. Reprod. Fert. 87: 115-123.

Forsberg, M. & A. Madej 1990. Effect of melatonin implants on plasma concentrations of testosterone, thyroxine and prolactin in the male silver fox (*Vulpes vulpes*). J. Reprod. Fert. 89: 351-358.

Joffre, M. 1977. Relationship between testicular blood flow: testosterone secretion and spermatogenic activity in young and adult wild red foxes (*Vulpes vulpes* L.). J. Reprod. Fert. 51: 35-40.

Johansson, I. 1941. Oestrus and mating in silver fox. Annals Agric. Coll. Sweden. 9: 239-263.

Maurel, D. & J. Boissin 1981. Plasma tyroxine and testosterone levels in the red fox (*Vulpes vulpes* L.) during the annual cycle. Gen. Comp. Endrocrinol. 43: 402-404.

Maurel, D., A. Lacroix & J. Boissin 1984. Seasonal reproductive endocrine profiles in two wild mammals: the red fox (*Vulpes vulpes* L.) and the European badger (*Meles meles* L.) considered as short-day mammals. Acta Endocrinol. 105: 130-138.

Moller, O.M. 1973. Progesterone concentration in the peripheral plasma of the blue fox (*Alopex lagopus*) during pregnancy and the oestrous cycle. J. Endocrinol. 59: 429-438.

Moller, O.M. 1974. Effect of ovariectomy on the plasma progesterone and gestation in the blue fox (*Alopex lagopus*). J. Reprod. Fert. 37: 141-143.

Moller, O.M., J.M. Aursjø, O.V. Sjaastad et al. 1980. Oestradiol-17 concentration in the peripheral plasma of the blue fox (*Alopex lagopus*) around oestrus. Acta Vet. Scand. 21: 140-142.

Moller, O.M., M. Mondain-Monval, A. Smith et al. 1984. Temporal relationships between hormonal concentration and the electrical resistance of the vaginal tract of blue fox (*Alopex lagopus*) at prooestrus and oestrus. J. Reprod. Fert. 70: 15-24.

Mondain-Monval, M., B. Dutourne, M. Bonnin-Laffargue et al. 1977. Ovarian activity during the anestrous and the reproduction season in the red fox (*Vulpes vulpes* L.). J. Steroid Biochem. 8: 761-769.

Mondain-Monval, M., M. Bonnin, R. Scholler & R. Canivenc: 1979. Androgens in peripheral blood of the red fox (*Vulpes vulpes* L.) during the reproductive season and the anoestrus. J. Steroid Biochem. 11: 1315-1322.

Mondain-Monval, M., O.M. Moller, A.J. Smith et al. 1985. Seasonal variations of plasma prolactin and LH concentrations in the female blue fox. J. Reprod. Fert. 74: 439-448.

Neill, J.D. & M.S. Smith 1974. Pituitary-ovarian interrelationships in the rat. In: "Current topics in experimental endocrinology". New-York; London: Acad. Press 2: 73-106.

Nequin, L.G. & N.B. Schwartz 1971. Adrenal participation in the timing of mating and LH release in the cyclic rat. Endocrinology 88: 325-331.

Okkens, A.C., M.M. Bevers, S.J. Dieleman & A.H. Willemse 1990. Evidence for prolactin as the main luteotropic factor in the cyclic dog. Vet. Quart. 12: 193-201.

Pearson, O.P. & R.K. Enders 1943. Ovulation, maturation and fertilizlation in the fox. Anat. Res. 85: 69-83.

Plas-Roser, S. & Cl. Aron 1981. Stress related effects in the control of sexual receptivity and in the secretion of progesterone by adrenals in cyclic female rats. Physiol. and Behav. 27: 261-264.

Roos, J., Ch. Lazarus & Cl Aron 1980. A hormonal and temporal analysis of the mechanisms involved in the control of ovulation induced by hemiovariectomy in the rat. Endokrinologie 75: 257-268.

Smith, A.J., M. Mondain-Monval, O.M. Moller et al. 1985. Seasonal variations of LH, prolactin, androstenedione, testosterone and testicular FSH binding in the male blue fox (*Alopex lagopus*). J. Reprod. Fert. 74: 449-459.

Smith, A.J., M. Mondain-Monval, K.A. Berg et al. 1987. Sexual development in the immature male blue fox (*Alopex lagopus*), investigated by testicular histology, DNA flow cytometry and measurement of plasma FSH, LH, testosterone, and soluble testicular Mn^{2+}-dependent adenylate cyclase activity. J. Reprod. and Fert. 81: 505-515.

Starkov, J.D. 1977. Sexual cycle of silver-black Vulpes Fulva. Uspechi zootecknical science 3: 385-401 (in Russian).

Vasina S.G. 1977. Development of silver fox corpus luteum. In: Sbornik rabot laboratorii histologii po razmnogeniu lisi. Trest Sojusswerovod, Moscow: 34-49 (in Russian).

Wassermann, G.F. & K.B. Eik-Nes 1969. Interrelation between adrenal function and formation of testosterone *in vitro* in the testis of the dog. Acta Endocrinol. 61: 33-47.

Wilson, C.A., J.C. Hadley, D. Gilbert & A.S McNeilly 1978. Steroidal control of the release of the preovulatory surge of luteinizing hormone in the rat. J. Endocrinol. 79: 223-234.

Zybina, E.V., T.G. Zybina, A.I. Zhelezova et al. 1989. Trophoblast cells in the placenta of the silver fox: their peculiarities and polyploidization. Cytology 31: 492-495 (in Russian).

Reduced phosphorus allowance in rearing and laying feed

OLAV HERSTAD
Agricultural University of Norway, Department of Animal Science, Ås, Norway

Herstad O. 1993. Reduced phosphorus allowance in rearing and laying feed. Norwegian Journal of Agricultural Sciences 7: 203-212. ISSN 0801-5341.

Two experiments were conducted to examine the allowances of phosphorus in feed for pullets and laying hens. For diets with around 0.5% total phosphorus (0.3% available P), there was a tendency to reduced ash content in the bones of both pullets and hens, but the shell quality of eggs tended to increase. No negative effects of the low phosphorus allowances were observed on mortality and egg production. There were indications that high calcium allowances may reduce egg production on diets with a reduced phosphorus content. An assumption that the availability of phosphorus should be higher in barley than in oats could not be confirmed. It is concluded that a phosphorus allowance of 0.50-0.55% (0.3-0.35% available P) in diets with a standard composition can be recommended for both pullets and laying hens. By reducing phosphorus allowances in the feed, reduced phosphorus content in the manure will be obtained.

Key words: Barley, bone quality, calcium, laying hens, oats, phosphorus, pullets, shell quality.

Olav Herstad, Agricultural University of Norway, Department of Animal Science, P.O. Box 5025, N-1432 Ås, Norway

In recent decades the allowance for phosphorus in feed for laying hens has been reduced whereas the allowance for calcium has been increased. In 1970 the standard Norwegian laying mashe contained 0.7-0.8% total phosphorus and 3.0-3.2% calcium. Today the feed for hens contains 0.55-0.65% phosphorus and 3.5-3.7% calcium. These changes are due to the fact that in many experiments high amounts of phosphorus have had a negative effect on shell quality. Furthermore, the increasing concern about pollution of the environment with phosphorus has promoted interests in reducing the phosphorus content of feeds. Lower phosphorus allowances will, of course, result in reduced excretion of phosphorus in animal manure.

Non-ruminants like pigs and poultry are not able to utilize the phytin phosphorus in vegetable feedstuffs. NRC (1984) and WPSA (1983, 1985) therefore recommend using available- or non-phytin phosphorus for adult birds and young chicks, respectively. Generally, 30% of the phosphorus in vegetable feeds is expected to be available. By supplementing the feeds with a bacterial enzyme - phytase - non-ruminants can also utilize phytin phosphorus (Simons et al. 1990).

Reducing the phosphorus allowance implies a reduction in the safety margins for

phosphorus in poultry feeds. In spite of the general assumption that only 30% of phosphorus in vegetable feeds is available, there is certainly a wide variation in availability among feedstuffs. Some values, summarized by Sauveur (1983), indicate that the availability of phosphorus in wheat and barley is around 50%, in oats about 25%, but in maize only 20%. Since barley and oats often constitute the major part of poultry feeds in Norway, such a difference in availability might be critical for laying hens on low phosphorus diets. Another area of uncertainty is that earlier experiments on low phosphorus allowances have often been of relatively short duration. Investigations on long term feeding with reduced dietary phosphorus allowance may therefore be needed.

This report deals with an experiment on low phosphorus allowances in laying diets based on barley or oats and a longer-term experiment in which the birds were fed low phosphorus diets in both the rearing- and the laying period, from 6 to 84 weeks of age.

MATERIAL AND METHODS

Experiment 1

The experiment 1, two basal diets with the following composition were made (figures in parentheses are for basal diet 2 if different from diet 1): herring meal 3.2%; meat and bone meal 2.0%; soybean meal 4% (5%); maize gluten meal 4%; barley 60% (0); oats 0% (60%); wheat 12%; wheat brand 3.7% (1.7%); animal fat 2% (3%); limestone meal 4%; sea shell meal 4.5%; salt 3.1%; micromineral mixture 0.10%; vitamin mixture 0.15%; and methionine 0.04%. The diets were calculated to contain 16% total protein and 10.8 MJ ME per kg.

From these two basal diets the phosphorus and calcium levels were adjusted by supplementing limestone meal and dicalcium phosphate (1%) to form the following experimental diets:

	Total P	Available P	Calcium
1. Barley-based diet (%)	0.46	0.28	3.40
2. Oat-based diet (%)	0.46	0.23	3.40
3. Barley-based diet (%)	0.51	0.33	3.37
4. Oat-based diet (%)	0.51	0.28	3.37

The level of total phosphorus in diets 1 and 2 (basal diets) were analysed to be 0.41% in each, somewhat lower than calculated.

Hens and housing

In the experiment, 480 brown egg layers (Søve 65) were used. The pullets were raised in colony cages and moved to individual laying cages (1050 cm^2) at 18 weeks of age and given the experimental feeds from 20 to 56 weeks of age (September 1987 to June 1988). Day length for hens was gradually increased from 9 h at 18 weeks until 15 h at 26 weeks. Thereafter the day length was kept constant. The room temperature was kept close to 20°C.

The experimental period was sub-divided in nine 4-week periods, with measurement of feed consumption and egg production. Shell quality as specific gravity was measured on

all eggs on one day of each 4-week period. When the experiment was completed, the phosphorus status in the skeleton of the hens was examined by analysing the ash content in the tibia of five hens from each feeding treatment.

Experiment 2

In experiment 2, the long-term feeding of low phosphorus diets was investigated by including both the rearing period, 6-18 weeks, and the full-time laying period, 18 to 84 weeks (November 1989-May 1991). The feeding treatments were as follows:

Rearing period, 6-18 weeks:

1. Diet with 0.5 % total P (0.26% aP) (aP=available P)
2. Diet with 0.6 % total P (0.36% aP)

Laying period, 18-84 weeks in a factorial design with the treatments in the rearing period:

1. Diet with 0.5% total P (0.30% aP) and 3.1% Ca
2. Diet with 0.6% total P (0.40% aP) and 3.1% Ca
3. Diet with 0.5% total P (0.30% aP) and 3.6% Ca
4. Diet with 0.6% total P (0.40% aP) and 3.6% Ca

The composition of the diets is presented in Table 1.

Table 1. Composition (in %) and calculated chemical content of the experimental diets in experiment 2

	Rearing diets		Laying diets			
	1	2	1	2	3	4
Herring meal	2.0	2.0	1.0	1.0	1.0	1.0
Meat and bone meal	2.5	2.5	4.0	4.0	4.0	4.0
Maize gluten meal	2.0	2.0	5.0	5.0	5.0	5.0
Soybean meal	4.0	4.0	3.0	3.0	3.0	3.0
Barley	40.0	40.0	36.0	36.0	36.0	36.0
Oats	40.0	40.0	36.0	36.0	36.0	36.0
Wheat bran	5.2	5.0	3.0	2.8	1.66	1.66
Grass meal	-	-	1.0	1.0	1.0	1.0
Animal fat	2.0	2.0	3.0	3.0	3.0	3.0
Micro minerals	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.25	0.25	0.25	0.25
Limestone meal	1.8	1.5	-	-	-	-
Sea shell meal	-	-	7.5	7.2	8.8	8.5
Monocal. phosphate	-	0.5	-	0.5	0.04	0.54
Vitamins	0.1	0.1	0.1	0.1	0.1	0.1
Lysine	-	-	0.05	0.05	0.05	0.05
Calculated contents:						
Total protein (%)	15.8	15.8	16.1	16.1	15.9	15.9
Digest. protein (%)	12.7	12.7	12.9	12.9	12.8	12.8
ME per kg, MJ	11.1	11.1	10.8	10.8	10.7	10.7
Calcium (%)	0.92	0.92	3.13	3.13	3.59	3.59
Total P (%)	0.51	0.62	0.51	0.62	0.50	0.60
Available P (%)	0.26	0.36	0.30	0.40	0.30	0.40

The experimental diets were produced at a commercial feed mill (Felleskjøpet Larvik) and delivered as pellets. The two rearing diets were produced in one batch and the four laying diets in three batches, each lasting for about one-third of the whole experimental period. The analysed levels of calcium and phosphorus in the different batches are presented in Table 2.

Table 2. Analysed levels of total phosphorus and calcium in the experimental feeds (%)

	Treatments							
	1		2		3		4	
	P	Ca	P	Ca	P	Ca	P	Ca
Rearing feed	0.57	1.17	0.61	0.91				
Laying feed:								
Batch 1	0.52	2.89	0.65	3.08	0.58	3.76	0.65	3.65
Batch 2	0.49	2.85	0.62	3.00	0.51	2.91	0.61	3.40
Batch 3	0.59	2.89	0.70	3.16	0.59	3.72	0.69	3.42

According to the analyses, the phosphorus content was somewhat higher than calculated in rearing diet 1, laying diet 3 - batch 1 and in the laying diets 1 and 3 - batch 3. The analysed value of calcium in batch 2 - diet 3 was lower than calculated.

Birds and housing

Day-old chicks of a white egg layer strain (Norbrid 41) were raised in colony cages and fed a standard starter ration up to 6 weeks of age. At 6 weeks, 672 chicks were allotted to 48 cages (14 chicks in each) and given the two rearing diets. The temperature and lighting programmes were the same as those for experiment 1, but the increase in day length started at 18 weeks instead of 20 weeks as in experiment 1. At 16 weeks, 288 pullets from each rearing diet were moved to individual laying cages and given the 4 laying diets from 18 weeks. At 16 weeks, ten pullets from each rearing diet were sacrificed and the femur and tibia removed and analysed for dry matter and ash content. After the laying period the same procedure and analysis were carried out on five hens on each of the four laying diets.

Statistics

The experimental data was analysed by the GLM procedure of the Statistical Analysing System (SAS 1990). For mortality data, a Chi-squar test was used.

RESULTS

Experiment 1

The main results of experiment 1 are given in Table 3.

Table 3. Performance of layers on barley- and oat-based diets with low phosphorus content (20-56 weeks)

	1 Barley	2 Oats	3 Barley	4 Oats
Total P, calculated (%)	0.46	0.46	0.51	0.51
Available P, calculated (%)	0.28	0.23	0.33	0.28
No. of hens at start	120	120	120	120
No. of deaths	3	0	2	2
Age at start of lay (d.)	152	152	152	153ns
Laying percentage	77.4ab	77.4ab	79.5a	76.1b
Egg weight (g)	62.9	62.7	63.2	63.7ns
Cracked eggs (%)	0.62a	0.26b	0.56ab	0.52ab
Specific gravity of eggs	1.0848a	855a	828b	849a
Feed per hen day (g)	108	106	110	107ns
Live weight, 56 weeks (kg)	2.21a	2.11b	2.21a	2.14b
Bone analysis (tibia)				
Dry matter (DM) (%)	70.1	68.6	70.8	70.3ns
Ash of DM (%)	40.8	42.5	44.9	42.8ns
P in ash (%)	17.1	17.2	16.7	16.5ns
Ca in ash (%)	38.4	38.9	37.0	37.4ns

Statistics: ns = non-significant differences. Values with dissimilar letters are significantly different ($p < 0.05$).

Egg production was significantly higher for the hens fed diet 3 (barley) than for those on diet 4 (oats), but the shell quality was poorer for the hens on diet 3. There were no differences in egg production and shell quality between the barley diet and the oats diet (diets 1 and 2) with the lowest phosphorus content. There was a tendency to higher ash content in the bones of the hens on diets 3 and 4 with the highest phosphorus levels than those on diets 1 and 2. No difference in bone ash were found between hens on the barley and oat diets.

The live weight of the hens on the barley diets was significantly higher than that of the hens on the oats diets. In addition, the feed consumption was significantly higher for hens on the barley diets up to 44 weeks of age, but equalised thereafter.

Experiment 2

The results from the rearing period are given in Table 4.

As shown in Table 4, the dry matter content in the femur and tibia was higher for pullets on the 0.6% phosphorus diet compared with the 0.5% phosphorus diet. There was also a tendency to an increased bone ash content on the higher phosphorus diet. However, performance in the succeeding laying period was not influenced by rearing diets containing different levels of phosphorus. The results of feeding different phosphorus diets in the laying period are presented in Table 5. As no interaction was detected by statistical analysis ($p > 0.05$), between feeding in the rearing period and feeding in the laying period, the table is arranged according to the feeding regimen in the laying period only.

Small differences between treatments occurred for different phosphorus allowances in the laying period. However, the porest shell quality (more cracked eggs and lowest specific gravity) was found in treatment 4 in laying feeds containing 0.6% phosphorus and

3.6% calcium. In contrast, hens on feed containing 0.5% phosphorus and 3.6% calcium seemed to produce eggs with good shell quality, but with reduced egg number (near significant level). No statistically significant differences between diets were observed for bone analysis. However, a tendency toward reduced bone dry matter and ash content in the bone dry matter was detected.

Table 4. Performances of pullets with different phosphorus contents in their feed (6-18 weeks)

	Diet 1 0.5% P	Diet 2 0.6% P
No. of chicks at 6 weeks	332	337
No. of deaths, 6-18 weeks	7	4ns
Live weight at 16 weeks (g)	1302	1307ns
Feed intake, 6-18 weeks (kg)	5.15	5.10ns
Bone analysis (femur + tibia)		
Dry matter (DM) (%)	69.1b	70.8a
Ash in bone DM (%)	35.5	36.0ns
Age at start of laying (d)	138	139ns
No. of deaths after 18 weeks	9	16ns
Number of eggs	364	359ns
Specific gravity of eggs, 30-48 weeks	1.0830	827ns

Table 5. Performances of layers on feeds with different levels of phosphorus and calcium (18-84 weeks)

	1 0.5% P 3.1% Ca	2 0.6% P 3.1% Ca	3 0.5% P 3.6% Ca	4 0.6% P 3.6% Ca
No. of hens at start	144	144	144	144
No. of deaths, 18-84 weeks	7	6	6	6ns
Age at start of laying (d)	139	140	139	139ns
Egg number per hen	365	363	354	365ns
Egg weight (g)	61.9ab	61.9ab	61.7b	62.6a
Cracked eggs (%)	1.40ab	1.65ab	1.24a	1.86b
Shell-less eggs (%)	0.22	0.30	0.31	0.30ns
Specific gravity of eggs				
30-48 weeks	1.0832a	831a	835a	818b
60-84 weeks	1.0797a	785ab	784ab	770b
Feed intake per hen (kg)	48.1	48.7	47.7	49.2ns
Feed/egg ratio (kg)	2.14	2.18	2.20	2.17na
Live weight, 84 weeks (kg)	1.87	1.92	1.89	1.92ns
Bone analysis at 84 weeks				
Dry matter (%)	74.8	72.1	73.1	74.4ns
Ash in DM (%)	47.5	51.1	45.1	49.7ns

DISCUSSION

In these two experiments there was a tendency to reduced ash content in the bones of hens fed diets with the lowest phosphorus content. This lack of significant effect may, however, be connected with the relatively small differences in phosphorus levels in the feeds. Significant increases in ash and dry matter content in bones of laying hens given feeds with 0.47, 0.57 and 0.67% phosphorus have ben reported in earlier experiments (Lund & Herstad 1990). In spite of the reduced ash content in bones, there were no negative effects on health and performance data, either in these or in the former experiments. Vogt (1992) found no significant effects on performance and mineralisation of the bones by reducing the phosphorus content in feed for laying hens to 0.42% (0.16% aP).

In experiment 1 the feed intake for the lower P diets were 108 and 103 g per day for the hens on the barley and oat diets, respectively. The corresponding intake of phosphorus was between 500 and 470 mg per day on the two diets. In experiment 2, the overall daily feed intake from 18 to 84 weeks was 103-104 g on the 0.5% P diets. The corresponding intake of phosphorus was then at least 520 mg per day, assuming the phosphorus content was somewhat higher than calculated. In a review, Roland (1986) referred to many authors reporting that an intake of around 400 mg total phosphorus per day is sufficient to maintain high egg production. Roland & Farmer (1986) found that phosphorus intakes of 384 mg and 307 mg per hen per day maintained egg production in two experiments but intakes of 288, 310, 322 and 329 mg per hen per day did not in two others. A working group under the European Federation of WPSA (WPSA 1984) has calculated the requirement for non-phytin phosphorus (NPP) of laying hens producing 50 or 60 g egg per day to be 280 and 320 mg per day, respectively.

In the mentioned experiments only insignificant increases in shell quality with reduced phosphorus allowances were found. With a higher phosphorus allowance (more than 700 mg per day) than that provided in the present experiments, a reduced shell quality was reported (Lund & Herstad 1990). According to Roland (1986), there are several reports, although inconsistent, showing that reduced phosphorus level in the feed can increase shell quality. On the other hand, reduced egg production has been observed on a phosphorus intake of around 300 mg per day (Roland & Farmer 1986), and on a diet with 0.15% available phosphorus (0.38% total P) (Rodriguez et al. 1984). Vandepopuliere and Lyons (1992) concluded that 0.4% total phosphorus was inadequate for satisfactory layer performance even though this phosphorus level improved egg specific gravity. Said et al. (1984) found that, depending on P sources, 0.5% total phosphorus gave the best response on egg production and other response criteria.

In experiment 2 the two phosphorus levels were provided in a factorial-type arrangement, with two calcium levels (3.1 and 3.6%). There was a tendency (near significant difference) to reduced egg number for the hens fed on a combination of 0.5% phosphorus and 3.6% calcium. Despite the uncertainty of the true level of calcium in feed from batch 2 in the experiment, this may be an indication that a high calcium allowance can cause phosphorus deficiencies on low phosphorus diets. Such an interaction between phosphorus and calcium has been demonstrated by Härtel (1989). He found reduced egg production on diets with 0.32% total phosphorus combined with 3% or more calcium. On diets with 0.52% total phosphorus, a reduction in egg production occurred in diets with 4%

calcium. In experiments with high calcium diets (3.0%) to pullets, Young et al. (1964) found reduced growth and increased mortality with 0.38% phosphorus compared with 0.6% phosphorus. Keshavarz (1990) demonstrated that a high dietary level of calcium in laying hens (6.5%), with a low dietary level of phosphorus (0.2% available P or 0.4% total P) decreased egg production performance and increased mortality. Roland & Farmer (1986) found no interaction in the percentage egg production or shell quality in experiments with 4.75%, 3.75% and 2.75% calcium and 0.7% and 0.31% total phosphorus. However, a significant interaction was found for egg weight (lowest egg weight from feed containing 4.75% calcium and 0.31% phosphorus). Although the results of experiment 2 demonstrate the importance of maintaining a proper Ca/P ratio in diets for laying hens, other experiments indicate that a wider Ca/P ratio than that used in experiment 2 is necessary before negative effects on egg production are likely to appear.

An adequate level of total phosphorus in poultry diets will, of course, be dependent on the bioavailability of phosphorus in the diets and the environmental conditions of the birds. The phosphorus content in barley and oats is around 3.25 g per kg. By using 60% of these cereals in the diets as in experiment 1, nearly 2 g of total phosphorus in the diets was provided from these cereals. As the data from Sauveur (1983) indicated an availability of phosphorus at 50% for barley and at 25% for oats, there should be a difference in available phosphorus of 0.5 g between the barley diets and the oats diets. Despite the low phosphorus content in the diets, none of the experimental results (bone analyses, egg production or shell quality) indicated any such difference in phosphorus availability between the two types of grain. However, the methods used for revealing such effects may have been too sketchy for any real conclusions to be drawn.

One incentive to reducing the phosphorus allowance in animal feeding is that there will be less phosphorus in the manure, and hence less pollution load on the environment. In the analyses of manure in the earlier experiments of Lund & Herstad (1990), such an effect was also noted. The amount of phosphorus that will be voided in the manure can be calculated from phosphorus intake minus phosphorus retained in the body (0.61%) and eggs (0.2%) (WPSA 1984). This calculation, based on the data in experiment 2, gives the following values for excreted phosphorus.

	P content in feed	
	0.5%	0.6%
Excreted P during rearing, 6-18 weeks (g)	20.1	24.9
Excreted P during laying, 18-84 weeks (g)	192.4	245.2

As indicated in the calculations, by reducing the phosphorus content in the feed from 0.6 to 0.5%, the excreted phosphorus in manure from replacement birds is reduced by 19-20% and from laying hens by 21-22%.

Experiments have revealed that the availability of phosphorus in vegetable feedstuffs can be increased by adding microbial phytase to the diets (Simons et al. 1990). By such enzyme treatments the phosphorus content can be reduced to 0.3% and even less in laying- and broiler feed. However, under practical feeding conditions with meat and bone meal as a relatively cheap feed ingredient, a phosphorus concentration of 0.5% and indeed, more will be obtained without adding other inorganic phosphorus sources. A reduction in the

phosphorus concentration from this level will therefore increase the feed cost through the addition of costly enzymes and also by replacing cheap feed ingredients such as meat and bone meal with more expensive ones.

CONCLUSION

The minimum allowance for phosphorus in poultry diets will be dependent on many factors, and a certain safety margin must be set before practical recommendations can be made. In diets with fish meal and meat and bone meal as the main phosphorus sources, and where the phosphorus content is reduced to around 0.5% (0.3% aP), the ash content in bones of the birds can be reduced. However, the shell quality can be increased and no negative effects has been observed on mortality and egg production. It should be stressed that with reduced phosphorus allowances, overfeeding calcium can reduce the egg production. There was no indication that the availability of phosphorus is different in barley or oats.

From these experiments, supported by other published data, dietary total phosphorus of 0.5-0.55% (0.3-0.35% aP) can be recommended for both rearing diets and laying diets. With reduced phosphorus allowances in the feed, less phosphorus will be excreted in the manure.

ACKNOWLEDGEMENTS

The experiments were partly financed by grants from the Norwegian Farmers Egg and Poultry Sales Association.

REFERENCES

Härtel, H. 1989. Evaluation of dietary interaction of calcium and phosphorus in the high producing laying hen. Br. Poult. Sci. 31: 471-494.

Keshavarz, K. 1987. Interaction between calcium and phosphorus in laying hens. Nutrition Reports International 36 (1): 9-19.

Lund, S. & O. Herstad 1990. Fosforinnholdet i fôret til verpehøner. Norsk Landbruksforsking 4: 213-220.

National Research Council (NRC) 1984. Nutrient Requirement of Poultry. National Academy Press, Washington DC.

Rodriguez, M., W.J.Owings & J.L. Sell 1984. Influence of phase feeding available phosphorus on egg production characteristics, carcass phosphorus content, and serum inorganic phosphorus levels of three commercial layer strains. Poult Sci. 63: 1553-1562.

Roland, D.A.Sr. 1986. Egg shell quality III: Calcium and phosphorus requirements of commercial Leghorns. Worlds. Poult. Sci. J. 42(2): 154-165.

Roland, D.A. SR. & M. Farmer 1986. Studies concerning possible explanations for the varying response of different phosphorus levels on eggshell quality. Poult. Sci. 65: 956-968.

Said, N.W., T.W. Sullivan, M.L. Sunde & H.R. Bird 1984. Effects of dietary phosphorus level and source on production performance and egg quality of two commercial strains of laying hens. Poult. Sci. 63: 2007-2019.

SAS, 1990. SAS User's Guide: Statistics. Version 6 ed. SAS Institute Inc., Cray, N.C.

Sauveur, B. 1983. Bio-availability to poultry of plant-origin phosphorus. Methodological criticisms and results. Proceedings of the 4th European Symposium on Poultry Nutrition, Tours: 103-113.

Simons, P.C.M., H.A.J. Versteegh, A.W. Jongbloed, P.A. Keme, P. Slump, K.D. Bos, M.G.E. Wolters, R.F. Beudeker & G.J. Verschoor 1990. Improvements of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64: 525-540.

Vandepopuliere, J.M. & J.J. Lyons 1992. Effect of inorganic phosphorus source and dietary phosphorus level on laying hen performance and eggshell quality. Poult. Sci. 71: 1022- 1031.

Vogt, H. 1992. Zum Phosphorgehalt im Futter von Kafighennen. Arch. Geflugelk. 56(9): 264-270.

WPSA, 1984. (Sub-committee on Mineral Requirements for Poultry of the Working Group No 2-Nutrition of the European Federation of Branches of W.P.S.A). Mineral requirements and recommendations for adult birds. World's Poult. Sci. J. 40: 183-187.

WPSA, 1985. (Sub-committee on Mineral Requirements for Poultry of the Working Group No 2-Nutrition of the European Federation of Branches of the W.P.S.A). Mineral requirements and recommendations for growing birds. World's Poult. Sci. J. 41: 252-258.

Young, R.J., M.C. Nesheim, I.D. Desai & M.L. Scott 1964. The effect of high dietary calcium on growing pullets and performance of laying hens. Proc. Cornell Nutr. Conf., Buffalo, N.Y., 45-49.

A comparison of diets for honeybee

KNUT PEDERSEN[1] & STIG W. OMHOLT[2]
[1]University of Bergen, Institute of Microbiology and Plant Physiology, Bergen, Norway
[2]Agricultural University of Norway, Department of Animal Science, Ås, Norway

Pedersen, K. & S.W. Omholt 1993. A comparison of diets for honeybee. Norwegian Journal of Agricultural Sciences 7: 213-219. ISSN 0801-5341.

A comparison between a commercially available diet, Soyapoll Extra, and a diet containing fishmeal and brewer's yeast was made on the basis of two outdoor flight cage experiments where each of two colonies had one of the diets as its sole protein source, and a field experiment where two colony groups were offered both diets simultaneously. The results indicate that the diet consisting fishmeal and brewer's yeast is superior to the Soyapoll Extra diet in palatability and effect on brood production.

Key words: *Apis mellifera*, fishmeal, pollen substitute, pollen supplement, protein diet, soybean flour.

Knut Pedersen, I M P, Jahnebakken 5, N-5007 Bergen, Norway

Protein diet formulations not fortified with natural pollen (potential disease carrier) have been of longstanding interest to the beekeeping industry. Consequently, formulations abound, but most of those currently in use are subject to high cost, deficiency of protein quality or other dietary requirements, presence of toxic agents, and low palatability (Chalmers 1980). Robinson & Nation (1966), defined pollen substitute as a protein diet that can completely replace pollen for the maintenance of a colony of bees for several brood generations. If the diet does not provide this, it should be considered as a pollen supplement.

However, from the point of view of a beekeeper the important issue is whether the use of a given protein diet will be profitable or not. The ultimate commercial test can only be tried by each beekeeper himself by weighing the increased profit derived from the application of the pollen supplement against the costs associated with its application. The outcome of such a test is dependent on so many different conditions that it is very difficult to generalize beyond limited geographical regions and different procedures of management. Yet, a good protein diet should at any rate meet certain general requirements (GR): Satisfying all or almost all honeybee requirements of amino acids, minerals and vitamins, and being highly digestible, i.e., having a high brood to diet ratio (GR1); being attractive to honeybees and maintaining palatability and consistency, so that it is actively stored and made use of even although some fresh pollen is available (GR2); being readily available (GR3); and being relatively cost effective (GR4).

These are the general requirements that the bee researchers focus on when developing new formulations. It is, however, very difficult to establish a strict testing protocol by

which different protein diets can be confidently evaluated. One way to reduce some of the experimental complexity is to compare the performance of existing commercially available protein diets with those of promising candidate formulations. In this paper such a comparison is reported between Soyapoll Extra, a commercially available protein diet that is commonly used in Europe, and a protein diet containing a blend of fishmeal and brewer's yeast according to the recommendations of Chalmers (1980). The comparison is based on field as well as flight cage feeding experiments.

MATERIALS AND METHODS

Three different experiments were conducted in the period 1983-85. All the experiments were carried out with honeybees from the apiary of the University of Bergen in Norway; the bees were of the Buckfast strain.

Flight cage experiments

Experiment 1 (1983)

Two different protein diets (PD1 and PD2) were prepared. The formulations of the two diets were PD1: Soyapoll Extra 22%, sucrose 33%, inverted sugar 45%; PD2: fishmeal 11%, brewer's yeast 11%, sucrose 45%, inverted sugar 33%. The brand of fishmeal used was Norse-lt 94 delivered by Norsildmel Bergen, Norway.

The meal contained 74% protein, 7% fat, 7.7% moisture and 13.7% ash. To increase digestibility it was ground to a particle size of 50-150 μm, as its mean particle size is usually much larger. The inverted sugar was prepared by mixing 3 l water, 6 kg sucrose and 5 ml lactic acid, and by boiling the solution lightly for 20 min. The ratio between sucrose and inverted sugar in PD1 and PD2 was adjusted so that the two diets had a similar consistency (rather like almond paste/marzipan).

An artificial swarm made from one colony on 21 July was split into two equal nuclei units, each containing 1.5 kg bees. Two one-year-old sister queens were introduced. The colonies the queens had been taken from were of similar strength, so both queens would presumably have similar egglaying capability. The two nuclei were placed in frameless swarm boxes in a dark room, and each was given 1 l 1:1 sucrose solution. After four days the nuclei were hived on five frames with comb foundations in one hive body and given 1.5 l 1:1 sucrose solution. The two hives were placed within an outdoor flight cage constructed of a rectangular iron frame (5 x 6 x 2 m) covered with a grey plastic mosquito net with a gauze size of 2.0 mm. Zippers in the corners allowed easy entrance.

The PD1 and PD2 diets were given *ad libitum* in plastic Petri dishes (15 mm x 90 mm) each containing 100 g. The dishes were inverted on the top of the frames beneath a cover board. In the first two feedings three dishes were given later, this was increased to six dishes. The experiment was run from 25 July to 10 October, and at intervals ranging from 7 to 11 days the colonies were given new Petri dishes with protein diets. Each time, the consumption was recorded and the old dishes were removed. In the period from 25 July to 22 August 1.0-1.5 l sucrose solution was given to each colony simultaneously with the protein diet. On 1 September and 8 September open and sealed brood cells was estimated by measuring the area of open and sealed brood. The brood production was not recorded

until any effect attributable to previously consumed natural pollen of the founder populations could be excluded. That is, only nurse bees that had been reared and fed solely on PD1 respectively PD2 protein diet were likely to be present at this time.

Experiment 2 (1985)
Two artificial swarms, each of 1.5 kg bees, were made on 12 June from a queenless cell builder that had been used for two weeks. Two one-year-old sister queens were introduced. The colonies the queens had been taken from were of similar strength, indicating similar egg-laying capability. The two nuclei were placed in frameless swarm boxes in a dark room, and each was given 0.7 l 1:1 sucrose solution with Fumidil (1 g Fumidil per kg sucrose). After two days the nuclei were hived on five frames with comb foundation and one drawn comb (with no honey or pollen) in one hive body, and were given 1.5 l 1:1 sucrose solution. The hives were placed within an outdoor flight cage of the same type as that described in Experiment 1. The PD1 and PD2 diets (see above) were given *ad libitum* as in Experiment 1. The experiment was run from 17 June to 19 August, and at intervals ranging from 2 to 7 days the colonies were given new Petri dishes with protein diets. The number of dishes (range 3-6) was adjusted depending on the consumption (the colonies never ran out of protein food). Each time, the consumption was recorded and the old dishes were removed. Sucrose solution was given to each colony approximately every third time the Petri dishes were exchanged. On 5 August and 19 August the brood frames were photographed and the number of sealed brood cells was counted.

Palatability test with field colonies
Experiment 3a, b (1984)
Two protein diets of the same formulation as those described above (PD1 and PD2) were given in the form of 200 g patties pressed out on a sheet of polyethylene plastic to 1 cm thickness. The patties were placed on top of the frames of the brood chamber so that the bees could choose the type of diet they preferred. The two types of diet were placed symmetrically with regard to the frames containing brood. The consumption for three days (20 July to 23 July) was recorded for 15 colonies (Experiment 3a), and for two days (24 July to 26 July) the consumption was recorded for another 13 colonies (Experiment 3b). To ensure equal availability of the two diets the experiments were terminated before two-thirds of the most palatable patty had been consumed. A Friedman two-way ANOVA test was used as the statistical method.

RESULTS

In the flight cage experiments the consumption of the diet containing fishmeal and brewer's yeast (PD2) exceeded that of the diet containing Soyapoll Extra (PD1). The difference in consumption increased sharply after the first new bees appeared (Figs 1 and 2). In addition, the brood production at the end of the flight cage experiments was much higher in the colonies given the PD2 diet than in those on the PD1 diet (Table 1).

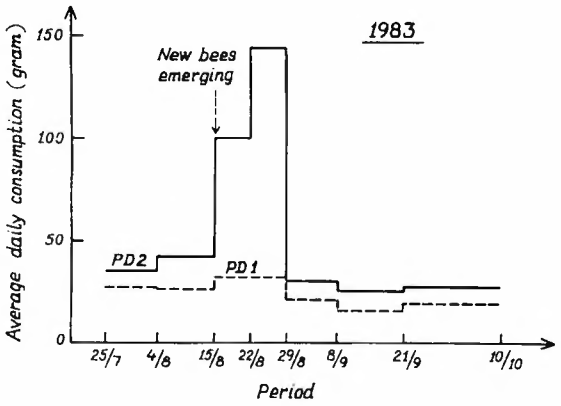


Fig. 1. Daily rate of consumption of the two colonies given PD1 and PD2 diets respectively in Exp. 1 (1983)

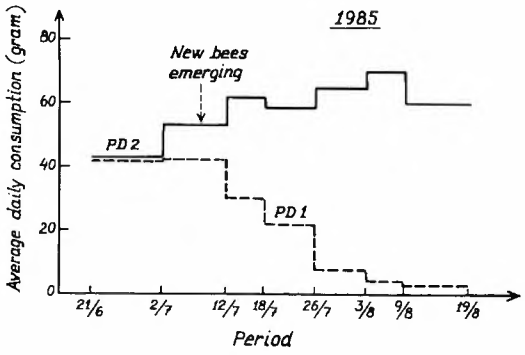


Fig. 2. Daily rate of consumption of the two colonies given the PD1 and PD2 diets respectively in Exp. 2 (1985)

In the palatability test the consumption of the diet containing fishmeal and brewer's yeast (PD2) exceeded significantly that of the diet containing Soyapoll Extra (PD1) (Table 2).

Table 1. Flight cage experiments

Exp. 1. 1983	Total consumption of protein diet 25/7-29/8	Total brood area 1/9	8/9
PD1	1005 g	400cm^2	350cm^2
PD2	2530 g	2500cm^2	3700cm^2

Exp. 2. 1985	Total consumption of protein diet 21/6-19/8	Number of sealed brood cells 5/8	19/9
PD1	1330 g	182	40
PD2	3542 g	2015	2500

Table 2. Palatability test

Exp. 3a 15 colonies Consumption in grams of PD1	PD2	
111.3	148.3	$t = 5.328$ $p = 0.001$

Exp. 3b 13 colonies Consumption in grams of PD1	PD2	
63.0	79.7	$t = 5.318$ $p = 0.001$

These results indicate that the PD2 diet is superior to the PD1 diet with regard to rate of consumption and stimulation of brood rearing. As fishmeal is also readily available at a moderate cost, this suggests that fishmeal deserves much more consideration as a major ingredient of protein diets.

DISCUSSION

One may object that too few colonies were used in the flight cage experiments to validate any strong conclusions.

In Experiments 1 and 2 the brood production was recorded two brood generations after the start of the experiment. The decrease in brood production in the colonies fed on PD1 and the increase in brood production in the colonies fed on PD2 indicate that the latter diet best meets the criteria for a pollen substitute (Robinson & Nation 1966), though the experimental period may have been too short for a definite conclusion to be drawn.

With regard to the general requirements (GR1-GR4) that have to be met by a good

protein diet as listed in the Introduction, it is evident that there are marked differences between PD1 and PD2 concerning GR1. The lack of information about the exact nutritional content of PD1 precludes a strict comparison of vitamin, mineral and amino acid profiles. But a blend of fishmeal and brewer's yeast has an amino acid and a vitamin profile that matches that of mixed pollen better than expeller processed soybean flour (Chalmers 1980). With the exception of manganese, the mineral content of PD2 as well as soybean flour seems to match that of natural pollen (Chalmers 1980).

In these experiments a 50:50% blend of fishmeal and brewer's yeast was used according to the recommendations made by Chalmers (1980). Because of the higher cost of brewer's yeast, it is desirable to reduce its proportion as much as possible. Only by carrying out further experiments can a decision on the minimum level required be made. In most practical situations, any pollen substitute will, to some degree, function as a pollen supplement because of the presence of stored natural pollen or nurse bees that have been reared or fed on natural pollen. Thus, one should not be too precise when comparing the nutritional content of pollen supplements with that of minimum optimal level requirements (de Groot 1953).

However, it should be noted that in Fig. 2 it is indicated that something is wrong with the nutritional content of Soyapoll Extra, as the consumption of PD1 dropped very rapidly after the first new bees emerged, while the consumption of PD2 stayed constant or even increased. A natural interpretation of this aberration is that nurse bees feeding solely on PD1 cannot rear brood successfully. In this way a feedback mechanism would have been established, causing a marked difference in rate of consumption between the two colonies due to the brood production in the colony given the PD2 diet. In Fig. 1 a similar pattern was found over a two-week period. After this period the consumption of PD2 and PD1 drops. The reason for the decreased consumption is presumably that the brood-rearing in the colony given PD2 started to decline at the beginning of September as a result of the adverse weather conditions that prevailed at that time.

Thus, it is difficult to decide to what extent the difference in brood production capability of PD1 and PD2 is attributable to the much higher consumption of PD2, and how much is attributable to different brood conversion ratios. In this context this is not so important. What really matters is the measurable difference in brood production, and only if this difference had been low would it have been worthwhile to investigate further the question of calculating the relative cost of pollen substitute per brood produced.

With respect to GR2, the PD2 diet is significantly more palatable to bees than PD1 when offered as patties on the top of the brood nest under field conditions. To minimize the effect of different number of new bees in the flight cage experiment, observation of consumption can be restricted to the first 20 days of that experiment. High palatability is a necessary criterion that must be met by any protein diet. Thus, the results confirm those of Winston et al. (1983) and refute those of Haydak (1936) with regard to the palatability of fishmeal. As Winston et al. (1983) pointed out, there has been a considerable improvement in the quality of fishmeal in the recent decades. Still, there are obvious differences between different types of fishmeal that may influence palatability to bees. For example, Norse-lt 94 is a low-temperature processed fishmeal made from fresh whole fish, while Canadian fishmeal is very often made from scraps from the fish industries, which causes a considerably higher ash content in the latter.

It would have been informative to have recorded the rate of consumption and brood production of colonies given fresh natural pollen *ad libitum* in the flight cage experiments. But as the main aim of the experiments was to compare the performance of the two supplements, such information is not of crucial importance in this context.

Concerning GR3 and GR4, fishmeal is readily available and relatively inexpensive. No exact comparison can be made concerning these requirements, but, clearly, fishmeal is competitive with soybean flour in this respect, and taking into the much better performance with GR2, it is concluded that fishmeal is a more suitable ingredient in protein diets than soybean flour. However, it should be stressed that the Soyapoll Extra produced nowadays may have quite a different new formulation from that produced in 1983 and 1985 (for further information see Herbert & Shimanuki (1980) and Ohe (1987)).

REFERENCES

Chalmers, W.T. 1980. Fish meal as pollen-protein substitutes of honeybees. Bee World 61: 89-96.

Groot, A.P. de 1953. Protein and amino acid requirements of the honeybee (*Apis mellifica* L.). Physiologia comp. Oecol. 3: 197-285.

Haydak, M.H. 1936. Value of foods other than pollen in nutrition of the honeybee. Journal of economic Entomology 29: 870-877.

Herbert, E.W. & H. Shimanuki 1980. An evaluation of seven potential pollen substitutes for honey bees. American Bee Journal May 1980: 349-350.

Ohe, W. von der 1987. Evaluation von diversen Protein-präparaten für den Einsatz als Bienengerechter Pollenersatz-stoff. Apidologie 18: 350-352.

Robinson, F.A. & J.L. Nation 1966. Artificial diets for honey bees, *Apis mellifera*. The Florida Entomologist 49: 176-184.

Winston, M.L., W.T. Chalmers & P.C. Lee 1983. Effects of two pollen substitutes on brood mortality and length of adult life in the honeybee. Journal of Apicultural Research 22: 49-52.

Glycoalkaloids, green discoloration and taste development during storage of some potato varieties (*Solanum tuberosum* L.)

LENE KAABER
MATFORSK Norwegian Food Research Institute, Ås, Norway

Kaaber, L. 1993. Glycoalkaloids, green discoloration and taste development during storage of some potato varieties (*Solanum tuberosum* L.). Norwegian Journal of Agricultural Sciences 7: 221-229. ISSN 0801-5341.

Total content of glycoalkaloids (TGA), greening and burning taste development were analysed in six varieties of potatoes (*Solanum tuberosum* L.) after exposure to either daylight or fluorescent light and/or high storage temperatures. TGA content was determined by an ELISA assay, greening by measuring L* values and taste by sensory evaluation. Illumination caused increased TGA content in the varieties Kerrs Pink, Troll, Beate and Peik but not in Bintje and Saturna, with the highest content found in Kerrs Pink potatoes (21.4 mg/100 g fresh potato). A bitter taste and burning sensation were recognized when the TGA content exceeded about 17 mg/100 g fresh potato. High storage temperature resulted in an increase in TGA content in Kerrs Pink and Troll, but not in Peik. Greening caused by illumination was less pronounced (Kerrs Pink, Peik, Troll) at 6 °C storage than at 18 and 24 °C. Indirect illumination did not induce increased levels of TGA (Peik, Troll). No transport of glycoalkaloids could be detected from the parts of the tuber with higher concentrations to parts with lower concentrations at low levels of TGA.

Key words: Direct/indirect light, glycoalkaloids, greening, high temperature, potato, taste.

Lena Kaaber, MATFORSK, Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway.

The total content of glycoalkaloids (TGA), which in potato (*Solanum tuberosum* L.) mostly consist of α-solanine and α-chakonine (total approx. 95%), has a recommended upper safety limit of 0.1% on a dry weight basis or 20 mg/100 g fresh weight (Bømer & Mattis 1924). This recommendation is generally accepted throughout the world. Glycoalkaloids are cholinesterase inhibitors (Orgell *et al.* 1958) and can cause serious illness if consumed in concentrations greater than 2.5 mg/kg body weight. Symptoms of poisoning are headache, vomiting, diarrhoea, mental confusion and visual disturbances (MacMillan & Thompson 1979).

The TGA content differs between varieties, but is generally between 2 and 10 mg/100 g fresh potato (Maga 1980, De Maine *et al.* 1988). The TGA content tends to be higher in immature than in mature tubers (Verbist & Monnet 1979), but various environmental conditions can also influence the level of TGA.

Sinden & Webb (1972) reported significant differences in TGA content between five commercial varieties grown at 39 locations in the United States. The effects of location were also significant. It was shown that a cool growing season accompanied by a high number of overcast days can result in an excessive glycoalkaloid concentration in a potato crop.

Exposure to daylight (Baerug 1962, Zitnak 1961, Patchett *et al.* 1976) and to artificial light (Zitnak 1955, Conner 1937) has been reported to increase the post- harvest levels of TGA in tubers.

Mechanical damage is also among factors that can increase the post-harvest levels of TGA (Ahmed & Mueller 1978). Two-stage harvesting has been studied (Statman & Cunnington 1990) because of a claimed positive effect in preventing mechanical injuries. However, this method may expose the tubers to daylight, thus inducing glycoalkaloid formation.

The reports mentioned are mostly based on experiments with varieties used in Central Europe and in the United States. These varieties are somewhat different from those usually grown in Norway.

The aim of the present work was to investigate how TGA content, greening and taste of some varieties grown in Norway are influenced by daylight and fluorescent light, and how storage temperatures (no light) affect the TGA level. Also, the effect of indirect fluorescent illumination on TGA content is investigated. Potato varieties for both the retail market and industry are included in the experiments.

MATERIALS AND METHODS

The potato varieties used were Beate (industry and retail), Bintje (industry), Kerrs Pink (retail), Peik (industry and retail), Saturna (industry) and Troll (retail). Bintje was imported from Sweden, Beate and Saturna were grown at Solør-Odal and Kerrs Pink, Peik and Troll at Vollebekk, Ås. The size of the tubers was in the interval 40-80 mm, with equal numbers of small, medium and large tubers. Prior to the experiments the potatoes were kept in dark storage at 6 °C.

Illumination

Artificial illumination was provided by means of fluorescence lamps (Tungsram 58 W F29 Warm White). The tubers were placed on white paper under a light intensity of 8000 lux. Time periods for illumination are indicated in the Resultssection.

Analysis of total glycoalkaloids

The total amount of α-solanine and α-chakonine was determined by an enzyme-linked immunosorbent assay (ELISA) (Morgan *et al.* 1983). The assay incorporates a competition step between α-solanine immobilized to the surface of each well and α-solanine and α-chakonine in solution for antibodies raised against glycoalkaloids in potatoes. The resulting distribution of antibodies between the immobilized and free phases is quantified by means of a second, enzyme-labelled antibody population active against the first. Recovery of the method is reported to be about 95%.

Anti-glycoalkaloid antiserum and micro-ELISA plates coated with α-solanine-bovine thyroglobulin conjugate were obtained from the AFRC Institute of Food Research, Norwich, UK. The plates were washed between each stage in the procedure using a Titertek Microplate Washer, and optical density at 414 nm was measured by a Titertek Multiscan Plus ELISA plate reader. Both instruments are produced by Flow Laboratories, Ayrshire, UK.

Green discoloration
Colour values (CIE(1976)L*a*b*) were obtained with a Minolta Chroma Meter CR-200 at three separate sites for each tuber after removing the cork layer. The L* value correlated to the green discoloration, and was used in the further study. L* values decreased with increasing green discoloration.

Corrections were made for the varietal differences in the potato flesh colour before greening by subtracting the initial L* values from those of the green samples.

Sensory evaluation
The tubers were cooked, peeled and analysed by Flavour profile test (ISO 6564 1985) using twelve trained assessors. The assessors evaluated the attributes of earthy flavour, grassy flavour, bitterness and burning sensation on a scale from 1 to 9, where 1 indicated no intensity and 9, high intensity.

Data analysis
Tukey's Studentized Range Test (HSD) was used to test differences in green discoloration between and within varieties. Differences were regarded as significant at p values ≤ 0.05.

RESULTS

Effect of daylight
Tubers of two varieties, Beate and Saturna, were left uncovered in the field until the next day to simulate two-stage harvesting with forced interruptions. TGA was determined after 1, 2, 3 and 27 h in samples of 2 x 6 tubers. The weather during the first day was hazy, and light intensity during daytime was about 25000 lux. The second day was partly cloudy with a light intensity of about 10000 lux.

The results revealed no increase in the TGA content for Beate and Saturna potatoes for the first 3 h of daylight exposure in the field (Figure 1). During the next 24 h the TGA content for Beate increased while the content for Saturna remained constant. None of the tubers developed green discoloration during the 27 h.

Effect of fluorescent light
Washed and unpeeled tubers of the varieties Kerrs Pink and Bintje were continuously illuminated for 12 days by fluorescence lamps at 24 °C.

Every second day samples of 2 x 6 tubers were analysed for TGA content and green discoloration, and 24 tubers for sensory evaluation. The tubers were turned over every day in order to expose the entire tuber to the same amount of light.

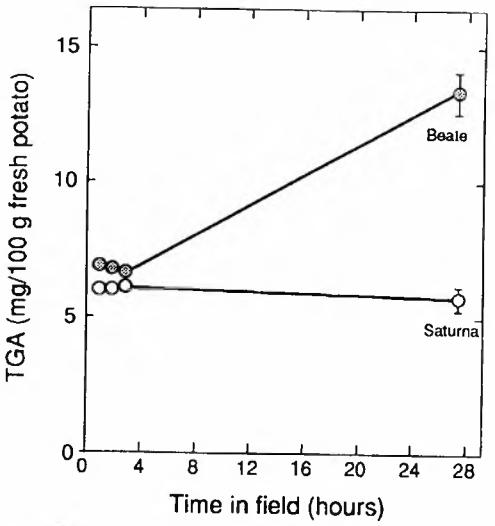


Figure 1. Effect of daylight on the TGA content of the varieties Saturna and Beate during field storage

From the results presented in Table 1, it can be seen that the content of TGA in Kerrs Pink tubers increased continuously for the first 6 days of illumination, and remained almost constant during the next 6 days. For Bintje tubers, there was no increase in TGA during the 12 days of illumination.

Green discoloration, described as a lowering of L* values, increased continuously for both varieties during the experiment, but was particularly pronounced in Kerrs Pink tubers. After six days the difference in green discoloration between the varieties was significant ($p = 0.017$).

Table 1. Development of TGA, burning sensation, bitterness and green discoloration (L*) during 12 days of illumination at 24 °C

Illumination (days)	KERRS PINK TGA (mg/100 g potato)	Burning sensation (scores)	Bitterness (scores)	L* values	BINTJE TGA (mg/100g potato)	Burning sensation (scores)	Bitterness (scores)	L* values
0	5.6	2.7	3.5	75.1	4.6	2.3	2.8	76.0
2	16.3	2.9	3.5	70.4	5.5	2.3	3.1	74.3
4	17.2	3.6	4.4	64.9	4.5	3.5	3.7	73.2
6	21.4	3.9	4.3	65.9	4.9	2.5	3.3	72.7
8	21.4	3.8	4.0	62.3	4.6	2.4	3.2	71.2
10	20.2	4.4	5.1	63.2	4.4	2.7	3.4	71.7
12	21.0	4.3	4.5	62.8	4.2	2.3	2.7	70.3

The sensory evaluation revealed that the correlation between TGA and burning sensation for Kerrs Pink was significant ($r = 0.81$, $p = 0.028$) while the correlation between TGA and bitterness was not significant ($r = 0.61$, $p = 0.143$). The increase in scores was also somewhat higher for burning sensation than for bitterness. There was no change in either burning sensation or bitterness in Bintje potatoes during the light exposure. Scores for grassy and earthy flavour were low and constant for both varieties during the experiment (results not shown).

Effect of temperature alone and in combination with fluorescent light

To estimate the effect of temperature on TGA content when illuminating tubers by

fluorescence lamps, the varieties Kerrs Pink, Peik and Troll were continuously illuminated for 1, 2, 6 and 12 days (lamps and light intensity as above, and the tubers were turned over every day). The storage temperatures were 6, 18 and 24 °C and relative humidity was 90%. TGA content and green discoloration were measured after each illumination for the 18 and 24 °C samples. The 6 °C samples were analysed only after 0 and 12 days. Similarly, samples were stored in the dark at the same temperatures and analysed as above, with the exception of the 6 °C sample, which was not analysed after 12 days.

For Troll, the content of TGA increased from 6.9 mg/100 g potato to 8.9 (18 °C) and 10.8 (24 °C) during the experimental period (Figure 2). The content of TGA in Kerrs Pink tubers decreased with increased temperatures compared with the content at the start of the experiment. However, there was a difference between the content of 18 °C samples and 24 °C samples after 48 h that remained constant until the end of the experiment. For Peik, no change in the level of glycoalkaloids as a result of increased temperatures was observed.

While exposed to fluorescent lighting in otherwise the same conditions as those above, the TGA content in Peik increased as a result of illumination in combination with high temperature (24 °C) (Table 2). The content remained constant at 6 and 18 °C. For Troll, storage at the lowest temperatures (6 and 18 °C) caused higher values of TGA after 12 days than with storage at 24 °C. The TGA content of Kerrs Pink increased to the highest level of all the varieties at all three storage temperatures.

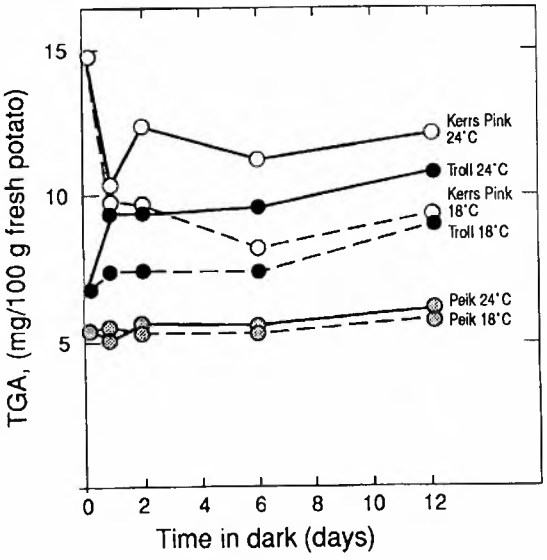


Figure 2. Effect of temperature on the TGA content of the varieties Kerrs Pink, Troll and Peik during dark storage

The development of green discoloration (L*) during storage is shown in Table 2 for the three varieties and temperatures. The degree of green discoloration was significantly different in the three varieties ($p \leq 0.05$) at each temperature level after 12 days.

Within varieties there was a significant difference in L* values at the three temperatures for all varieties except for Peik at 18 and 24 °C. The discoloration was lowest at 6 °C for the three varieties.

Effect of indirect illumination

To investigate whether exposure to light initiates production of TGA in the parts of the tubers that were not directly illuminated, tubers of Peik and Troll were exposed to light from fluorescence lamps, as above, for six days, but in this case the tubers were not turned over during the experiment.

When determining the TGA content, the tubers were cut longitudinally into two

halves, one of which had been exposed directly to light and the other one only to reflected light. Each sample consisted of halves from 2 x 6 tubers.

Table 2. Development of TGA content and green discoloration (L*) by fluorescent illumination at three different temperatures

Illumination time (days)	KERRS PINK 6 °C (TGA mg/100 g potato)	18 °C	24 °C	PEIK 6 °C (TGA mg/100 g potato)	18 °C	24 °C	TROLL 6 °C (TGA mg/100 g potato)	18 °C	24 C°
0	15.0	15.0	15.0	5.6	5.6	5.6	7.0	7.0	7.0
1	-	8.4	14.9	-	3.4	4.4	-	8.3	9.9
2	-	15.4	14.8	-	4.9	4.8	-	7.5	9.6
6	-	18.0	18.3	-	4.8	6.8	-	14.1	9.3
12	20.0	18.8	20.4	6.21	5.9	10.3	14.7	15.5	9.6
				Green discoloration (L*)					
0	72.3	72.3	72.3	73.5	73.5	73.5	70.3	70.3	70.3
1	-	72.2	71.7	-	73.9	74.8	-	67.4	67.0
2	-	71.1	68.7	-	70.0	72.9	-	64.5	62.9
6	-	61.1	62.4	-	61.4	64.5	-	51.1	59.4
12	64.2	49.0	59.3	68.0	53.5	53.4	59.3	41.6	52.2

Troll potatoes showed no differences in the TGA content whether the light was direct or indirect (Table 3). In both cases the TGA content increased compared to the sample stored in the dark at 6 °C. Peik potatoes displayed a somewhat increased TGA content with direct illumination compared with indirect illumination. With indirect illumination the content of TGA was the same as in the 6 °C sample.

Table 3.Effect of direct and indirect fluorescent light on TGA content

	TROLL TGA (mg/100 g potato)	SD[a)]	n[b)]	PEIK TGA (mg/100 g potato)	SD	n
Dark, 6 °C	7.0	0.39	4	5.4	0.26	7
Direct illumination, 24 °C	12.0	0.71	2	8.5	0.71	2
Indirect illumination, 24 °C	12.7	0.35	2	5.5	0.00	2

a) SD = Standard deviation
b) n = Sample size

DISCUSSION

The total content of glycoalkaloids has been reported to vary between 2 and 10 mg/100g fresh weight in different varieties of potatoes (*Solanum tuberosum* L) (Wolf & Duggar

1946). The present work confirmed these observations, but, in addition, it was demonstrated that potato varieties responded differently in the development of glycoalkaloids when exposed to light.

Beate and Saturna, used in the two-stage harvesting experiment, are among the most important potato varieties grown in Norway. They responded differently to daylight, despite having the same initial level of TGA. The TGA content in Saturna did not increase at all during 27 h in the field. Further experiments will have to be carried out to investigate the effects after a longer exposure to daylight. Beate, on the other hand, developed an increased TGA content after 3 h exposure to daylight. This is in agreement with Baerug (1962) who found a significant increase in TGA content in Kerrs Pink tubers after 6 h in bright sunlight. Leaving potatoes for up to 3 h in the field does not seem to affect the TGA content of any variety tested.

The TGA content was affected by fluorescent lighting in Kerrs Pink and to a lesser extent in Troll and Peik varieties, and seemed to reach a maximum level after six days of illumination. For Kerrs Pink this level was about 20 mg/100 g fresh weight. Further light exposure did not influence the TGA content. Baerug reported (1962) that the same level was reached for Kerrs Pink after only 6 h in bright sunlight, and still the TGA content increased for at least 48 h. These two experiments demonstrate that the light source is important in the formation of TGA. The formation of glycoalkaloids is faster and higher levels are reached in daylight compared with those reached in fluorescent light. This seems to be due to the difference in light quality and/or light intensity rather than the total amount of illumination.

All the tubers in the experiments turned green when illuminated with fluorescent light although the intensity of the colour differed, but not all of them increased in glycoalkaloid content. Green potatoes are usually associated with an increased level of glycoalkaloids. However, chlorophyll formation and glycoalkaloid formation have been shown to be independent processes (Jadhav & Salunkhe 1975). Bintje and Peik (6 and 18 °C) potatoes seem to verify this finding (Tables 1 and 2).

The rate of the chlorophyll synthesis was found to be lowest at 6 °C (Table 2), which means that potatoes for sale in shops are more vulnerable to greening than tubers stored in store houses where fluorescent lighting also has to be used at times.

Sinden & Deahl (1976) reported that a taste panel described several varieties with a glycoalkaloid content in excess of 14 mg/100g potato as having a bitter taste, and that a mild to severe burning sensation occurred in the mouth and throat when the glycoalkaloid level exceeded 22 mg/100g. This is more or less consistent with our observations of a burning sensation and bitterness recognized when the glycoalkaloid level exceeded about 17 mg/100 g potato (Kerrs Pink). However, the increase in scores for any of the characteristics is not great enough to guarantee that levels in excess of 20 mg/100 g fresh potato will be detected by untrained people, especially when potatoes are served as part of a meal.

In the experiments Kerrs Pink tubers from two different lots were used, one of which had an initial TGA content of 15 mg/100 g fresh weight (Table 2, Figure 2). When the storage temperature was changed to 18 and 24 °C (in the dark), the content of TGA decreased from 15 to about 10 mg/100g fresh weight during the first 24 h (Figure 2). In an earlier study Zitnak (1953) reports that some varieties obtain a higher concentration of

glycoalkaloids when stored at 4-8 °C than at 12-15 °C. Kerrs Pink may be such a variety, or perhaps our sample had been exposed to less than ideal conditions prior to storage, causing an increase in the level of TGA. If this is the case, the increased level is maintained during the cold storage and then falls to normal when the temperature increases to 18 and 24 °C. This suggests that potato lots with increased levels of glycoalkaloids caused by environmental conditions, would perhaps regain normal levels if conditioned at higher temperatures for some time. More attention ought to be paid to this question in the future.

The TGA content of some varieties, such as Peik, was unaffected by high storage temperatures (Figure 2), while temperature of especially 24 °C in Kerrs Pink and Troll tubers seemed to induce the production of more glycoalkaloids than normal. This is difficult to explain as anything other than varietal differences.

The increase in TGA content that was observed for Troll tubers at direct and indirect illumination (Table 3) is most probably a temperature effect, since the content is approximately the same as when Troll was stored in the dark at 24°C (Figure 2). Since Peik tubers where shown to be stable at raised temperatures in the same experiment, the increase at direct illumination can be due to light influence (Table 3). The results shown in Table 2 support this assumption, since the TGA content of Peik tubers became slightly raised when they were exposed to both light and a temperature of 24 °C. The indirect illumination is too weak to induce increased levels of TGA, and apparently glycoalkaloids do not diffuse from the exposed parts of the tubers to the unexposed parts, at least not at the low levels as described here.

ACKNOWLEDGEMENTS

The author thanks Ms Mona Ringstad for her technical assistance and Agricultural Adviser Rolf Krok, the Solør-Odal Agricultural Experimental Group and Professor Emeritus Lars Roer, Department of Crop Science, Agricultural University of Norway, for supporting the testing material.

REFERENCES

Ahmed, S.S. & K. Mueller 1978. Effect of wound-damage on the glycoalkaloid content in potato tubers and chips. Lebensm. wiss. Technol. 11: 144-146.

Baerug, R. 1962. Influence of different rates and intensity of light on solanine content and cooking quality of potato tubers. Eur. Potato J. 5 (3): 242-251.

Bømer, A. & H. Mattis 1924. High solanine content of potatoes. Z. Unters. Nahr. Genussm. Gebrauchsgegenstaende 47: 97-127.

Conner, H.W. 1937. The effect of light on solanine synthesis in potato tubers. Plant Physiol. 12: 79-98.

De Maine, M.J., H. Bain & J.A.L. Joyce 1988. Changes in total tuber glycoalkaloid content of potato cultivars on exposure to light. J. Agric. Sci. 111(1): 57-58.

Jadhav, S.J. & D.K. Salunkhe 1975. Formation and control of chlorophyll and glycoalkaloids in tubers of *Solanum tuberosum* L. and evaluation on glycoalkaloid toxicity. Adv. Food Res. 21: 307-354.

MacMillan, M. & J.G. Thompson 1979. Outbreak of suspected solanine poisoning in schoolboys - examination of criteria of solanine poisoning. Q. J.med. 48: 227-243.

Maga, J.A. 1980. Potato glycoalkaloid. CRC Critical Reviews in Food Science and nutrition 12: 371-404.

Morgan, M.R.A., R. McNerney, J.A. Mathew, D.T. Coxon & H.W.S. Chan 1983. An enzyme-linked immunosorbent assay for total glycoalkaloids in potato tubers. J. Sci. Food Agric. 34: 593-598.

Orgell, W.H., K.A. Vaidya & P.A. Dahm 1958. Cholinesterase inhibition *in vitro* by extracts of potato. Proc. Iowa Acad. Sci. 65: 160-162.

Patchett, B.J., P.S. Cunningham & R.E. Lill 1976. Glycoalkaloid levels in New Zealand potatoes. N.Z. J. Exper. Agric. 5: 55-57.

Sinden, S.L. & R.E. Webb 1972. Effect of variety and location on the glycoalkaloid content of potatoes. Am. Potato J. 49: 334-338.

Sinden, S.L. & K.L. Deahl 1976. Effect of glycoalkaloids and phenolics on potato flavour. J. Food Sci. 41: 520-523.

Statman, O.J.H. & A.C. Cunnington 1990. Two stage harvesting - An assessment of systems and their impact on lifting rate and tuber quality. EAPR Conf. Abstr. 388-389.

Verbist, J.F. & R. Monnet 1979. A propos de la teneur en solanine des petites tubercules nouveaux de pommes de terre (*Solanum tuberosum* L.) Potato Res. 22: 239-244.

Wolf, M.J. & B.M. Duggar 1946. Estimation and physiological role of solanine in the potato. J. Agric. Res. 73: 1-32.

Zitnak, A. 1953. The influence of certain treatments upon solanine synthesis in potatoes. M.S. thesis, University of Alberta, Edmonton.

Zitnak, A. 1955. Factors influencing the initiation and rate of solanine synthesis in tubers of *Solanum tuberosum* L. Ph.D. thesis, University of Alberta, Edmonton.

Zitnak, A. 1961. The occurrence and distribution of free alkaloid solanidine in Netted Gem potatoes. Can. J. Biochem. and Physiol. 39: 1257-1265.

Long-term effects of crop rotation, manure and straw on soil aggregation

SVEIN SKØIEN
Agricultural University of Norway, Department of Soil and Water Sciences, Ås, Norway

Skøien, S. 1993. Long-term effects of crop rotation, manure and straw on soil aggregation. Norwegian Journal of Agricultural Sciences 7: 231-247. ISSN 0801-5341.

Physical and chemical soil parameters were investigated in a crop rotation experiment which has been run since 1953 on a clay loam at the Agricultural University of Norway (AUN), south of Oslo. Four different crop rotations are used; 6 years of grain (I); 3 years grain + 3 years of row crops (II); 2 years of ley + 4 years of grain (III); 4 years of ley + 2 years of grain (IV). Three different fertilization treatments are compared: A. Low rate mineral fertilization; B. Medium rate mineral fertilization; C. Medium rate mineral fertilizers + manure. For rotation I and II the effect of straw incorporation vs. straw removal is also compared. The results indicate that for aggregate size distribution there is an increase in the percentage of aggregates in the 2-6 mm fraction, and a decrease in the fraction >20 mm for rotations with ley. For aggregate stability there are significant differences between all rotations and an increase in stability in the order II < I < III < IV. A significant increase in aggregate stability with use of manure was also observed. A and B did not differ significantly, but there was a slight tendency to increased aggregate stability with straw incorporation. Aggregate stability increases with increasing content of soil organic matter, but the correlations with Tot.-C and Tot.-N were weak. Consequences of crop rotation and previous soil use for soil erosion risk assessment are discussed.

Key words: Aggregate stability, aggregate site distribution, crop rotations, soil erodibility

Svein Skøien, Agricultural University of Norway, Department of Soil and Water Sciences, P.O. Box 5028, N-1432 Ås, Norway

There is reason to believe that soil erosion in agricultural areas in Norway has increased during recent decades due to an increased acreage of arable cropping, mainly for grain production. This increase has been greatest in regions with silt and clay soils which are known to be erodible. Erosion rates high above tolerance levels have been measured from soil erosion plots in these areas (Lundekvam 1992). The tolerance level in these areas is set to 0.1-0.2 kg/m^2 (Morgan 1986). However, examples of low soil erosion rates from other soils have also been reported (Haraldsen et al. 1992; Børresen & Uhlen 1991). There appear to be wide variations in soil erodibility as a result of differences in soil type, hydrology and previous land use. Soil loss from an old pasture which is recently brought into arable land is very low compared to a soil which has been levelled and is used for

continuous grain growing (Lundekvam 1992). The previous cropping and soil cultivation have to be taken into account when assessing an area for soil erosion risk.

MATERIAL AND METHODS

The results of analyses of soil samples from a crop rotation experiment are presented. The experiment, which has been run continuosly since 1953 is situated in an AUN experimental field at Ås on a clay loam (about 25% clay). The design includes the following crop rotations:

I. Continuous cereal cropping
II. Cereal crops (three years) and row crops (three years), from 1954 to 1977 five years of spring grain and one year of potatoes in a six year rotation.
III. Two years of rotational ley and four years of arable crops (spring grain)
IV. Four years of ley and two years of cereals

Rotation plan and crops 1992

Block Rotation	1	2	3	4	5	6
I	spring wheat	oats	barley	spring wheat	barley	oats
II	"	"	potatoes	swedes	"	beets
III	"	"	barley	spring wheat *)	1st year ley	2nd year ley
IV	3rd year ley	4th year ley	"	" *)	"	"

*) with grass+clover undersown

In the years 1972-77 the ley was left out of the rotations in order to assess the after-effects of grassland in spring grain. The described rotations were then established again.

Fertilizer treatments

A. Low rate NPK
B. Medium rate NPK
C. Medium rate NPK including FYM
D. Medium rate NPK + straw incorporation (rotations I and II)
High rate NPK + FYM (rotations III and IV)
Application of 60 tons/ha FYM once in the 6-year period.

The intention was to relate the 1992-yields, especially of spring grain, to soil structure properties. Unfortunately a severe drought caused very low and uneven yields. In order to

demonstrate the effects of rotational ley upon the following yields of spring grain, yield figures from 1970-1980 recalculated after Uhlen (1981), is presented.

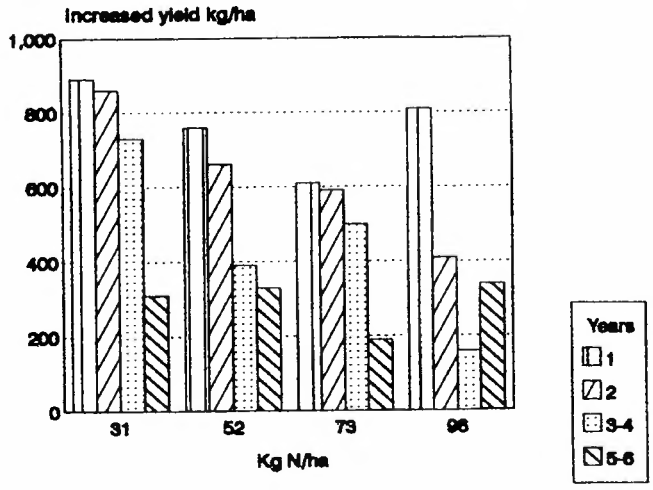


Figure 1. Increased grain yield (kg/ha) after 4 years ley compared to continuous grain. The columns express the effect on yields 1, 2, 3-4 (mean), 5-6 (mean) years after ley. Effects is also presented for the 4 different levels of N-fertilization

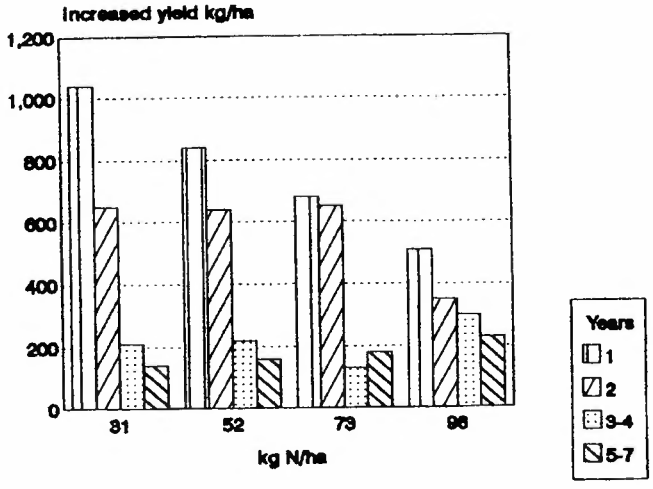


Figure 2. Increased grain yield (kg/ha) after 2 years ley compared to continuous grain. The columns express the effect on yields 1, 2, 3-4 (mean), 5-7 (mean) years after ley. Effects is also presented for the 4 different levels of N-fertilization

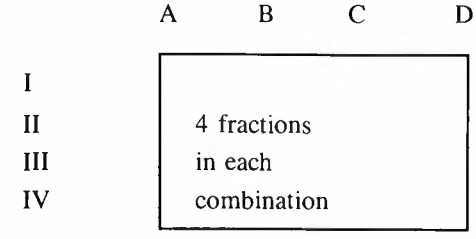
The effects of ley on the following grain yields are declining quite rapidly. It can also be seen that the effect is greatest at the lowest level of nitrogen fertilization, likely first of all due to a larger response on residual plant nutrients.

Two-litre soil samples from the 192 plots were taken in May 1992. On arable plots, soil from the upper 5 cm was taken. On ley plots, samples were taken just beneath the grass turf.

Samples were air dried and sieved into the fractions: >20 mm, 6-20 mm, 2-6 mm, 0.6-2 mm, and <0.6 mm. The aggregate size distribution is described as the percentage of weight of the total sample in each fraction. Stones were picked out of the total sample. Aggregate stability was measured for the two fractions 2-6 mm and 0.6-2 mm by means of a device used for rain simulation. A 20 g sample from an aggregate fraction is placed on a sieve with a mesh size of 0.5 mm. The sieves are placed on a rotating grid above which four spray nozzles provide the rain. The nozzles are of the type Tee-jet 8005E, with the distance of 31.5 cm between the nozzles and the sieve bottom and a water pressure of 1.5 kp/cm^2. Two parallels for each sample were tested. The samples were exposed to simulated rain for 3 min. During the rain some aggregates dissolved and were washed through the sieve. The remaining soil on the sieve was dried and weighed. In the 6-2 mm fraction some gravel remained after rain simulation, in addition to stable soil aggregates. After drying and weighing, the gravel was washed out of the remaining samples. The gravel content was roughly 5%. The weight of dry gravel was subtracted from both the initial and remaining sample weights before the aggregate stability was calculated.

$$\text{Aggregate stability (AS)} = \frac{\text{Dry weight of remaining soil}}{\text{Weight of initial sample}} \%$$

The soil samples were grouped into the 16 combinations of four rotations and four fertilization treatments. Within each combination, one mixed sample was prepared for chemical analysis for the fractions < 0.6, 0.6-2, 2-6, and > 6 mm.



RESULTS AND DISCUSSION

Aggregate size distribution

Effect of fertilizers, manure and straw

Table 1. Aggregate size distribution. Percentage of weight in different fractions. Mean values for treatments A, B and C.

Treatment	>20 mm	6-20 mm	2-6 mm	0,6-2 mm	<0,6 mm
A	5	20	31	28	16
B	5	19	31	29	16
C	5	19	32	28	16

No significant differences were found between the treatments A, B and C

Treatment D represents the incorporation of straw in rotations I and II, and for rotations III and IV, the use of manure. Results for treatment D compared with B are listed for each rotation.

Table 2. Aggregate size distribution. Percentage of weight in different fractions. Mean values for treatments D and B in rotations I-IV

Rotation	>20 mm		6-20 mm		2-6 mm		0.6-2 mm		<0.6 mm	
	D	B	D	B	D	B	D	B	D	B
I	6	5	20	21	29	28	28	28	17	17
II	6	7	21	21	28	28	28	27	17	17
III	3	3	17	19	34	32	30	30	16	17
IV	2	3	16	16	38	36	29	30	15	16

The aggregate size distribution in rotations I and II is quite similar and is not affected by straw incorporation. The distribution in III and IV is also similar and not affected by the somewhat different fertilizer applications.

Effects of crop rotation

Table 3. Percentage of weight in the different fractions. Mean values for rotations I-IV

Rotation	>20 mm	6-20 mm	2-6 mm	0.6-2 mm	<0.6 mm
I	6	20	29	28	17
II	7	21	28	28	17
III	4	19	33	29	16
IV	3	17	37	28	15
$LSD_{5\%}$ =	1.5	3.9	2.4	3.2	2.3

There are some significant differences between rotations. Rotations with ley have rendered the soil less coarse in structure with an increase of the 2-6 mm fraction.

A single characteristic parameter expressing the aggregate size distribution is the *mean weight diameter*, which is defined as:

$$X = (x_1w_1 + \ldots + x_nw_n)$$

In this calculation x_1 is the upper diameter in each interval of sieved fractions, and w_1 is the weight of the aggregates in that size range as a percentage of the weight of the total sample.

An analysis of variance of the mean weight diameter showed a significant difference for rotations, but not for fertilization treatments. Rotations with ley were shown to have a significantly less coarse aggregate structure than rotations without ley.

Effects of time in the rotation period

In this rotation experiment each phase, or rotational year, of each of the four crop rotations is represented every year. A grouping of results according to rotational years is presented below. The percentage of the aggregate 2-6 mm fraction is used.

Table 4. Aggregate size distribution. Percentage of the 2-6 mm fraction. Mean values for blocks in each rotation

Block Rotation	1	2	3	4	5	6
I	30	29	29	29	28	27
II	28	28	28	26	29	30
III	31	29	38	38	41	33
IV	39	29	30	39	41	44

$LSD_{5\%}$ = 2.4

A tendency to an increasing proportion of aggregates in the 2-6 mm fraction during the ley period might have been expected, but this is not confirmed by these results.

Earlier investigations

In 1964 Njøs (1967) investigated the aggregate size distribution from the same experiment. According to his results, the ley rotations resulted in a higher proportion of aggregates in the fraction >20 mm. There was no increase in the 2-6 mm fraction in the ley rotations compared to the arable rotations. In 1964 the soil samples were taken to a 10 cm depth, which might explain the contrasting results.

Uhlen (1974) has presented results from nine crop rotation experiments on farmers' fields in South-East Norway. The mean values of aggregate size distribution of the nine experiments are presented.

Table 5. Aggregate size distribution. Means of nine experiments. Samples taken in the spring after the ley period (Uhlen 1974).

Rotation	>6 mm	2-6 mm	0,6-2 mm	<0,6 mm
1. Cont. grain	21	29	26	24
2. After 3 years' ley	14	29	32	25
3. After 2 years' ley	11	27	33	29
4. After 1 year ley	15	27	30	27

In these experiments an increase was also found in the 2-6 mm fraction in some of the ley rotations.

Soil erodibility

Various parameters are suggested to explain differences in erodibility of soils. Soil strength constitutes the ability to withstand the erosive forces of water. Strength depends on cohesiveness and therefore clay content, but also the tendency to form aggregates. Poorly aggregated silt soils are highly erodible. Soils with a high clay content are the least erodible (Meyer 1985). Aggregate size distribution is not found to correlate well with erodibility (Skøien 1989), but it probably influences the strength properties. Formation of a high proportion of very small aggregates will also increase the tendency toward soil crust formation. Soil crusting will increase the erodibility to water (Alberts & Wendt 1985; Zobeck & Popham 1992). Soil erodibility is a dynamic factor. Shear strength is influenced by changes in soil physical properties such as moisture content and potential, pore size distribution, moisture distribution and bulk density. Freezing and thawing influence soil strength dramatically (Kok & McCool 1990). Detachment of soil into flowing water depends upon the soil shear strength and the hydraulic shear, as well as the tranport capacity of the flow. Process-oriented simulation models of soil erosion, such as the WEPP, use this function (Laflen et al. 1991). Soil erodibility by wind erosion is directly dependent on the aggregate size distribution. Aggregates larger than 0.85 mm in diameter are generally considered non-erodible by wind in the Wind Erosion Equation. A system of wind erosion prediction must also take into account the temporal variation in aggregate size

distribution throughout the year (Zobeck 1991). For peat-soils particles of about 1 mm in diameter are most likely to be blown.

Aggregate stability

Aggregates from the two fractions 6-2 mm and 0,6-2 mm were tested for water stability.

Effects of crop rotation

Table 6. Aggregate stability (AS). Means for each rotation

Rotation	AS % 6-2 mm	Rotation	AS % 6-2 mm
IV	81	IV	80
III	65	III	57
I	59	I	49
II	50	II	42

$LSD_{5\%}$ = 3.0 % $LSD_{5\%}$ = 3.2 %

For both fractions we find the highest stability for rotation IV and the lowest for rotation II. The effect of ley is evident.

Effects of time in the rotation period

Table 7. Aggregate stability for the fraction 0.6-2 mm. Means for blocks in each rotation.

Block Rotation	1	2	3	4	5	6
I	60	54	66	61	50	61
II	57	57	50	55	30	50
III	68	65	71	55	62	71
IV	83	81	78	81	83	82

$LSD_{5\%}$ = 7.5

Table 8. Aggregate stability for the fraction 6-2 mm. Means for blocks in each rotation

Block Rotation	1	2	3	4	5	6
I	51	42	57	55	44	46
II	49	47	39	44	30	42
III	63	55	62	39	56	64
IV	82	78	77	78	84	81

$LSD_{5\%}$ = 7.6

It is generally recognized that leys and good pastures increase the soil organic content and the number of water-stable aggregates. The addition of plant residues and root mass is greater than under arable crops, the decomposition of organic materials is decreased, and the activity of roots, hyphae and other forms of soil life that leads to aggregation is greater (Tisdall & Oades 1982). Several investigations indicate that a period of 3-4 years of ley increases the stability of soil aggregates. However, according to investigations in England by Low (1955) it takes a long time before the "mature" state of aggregation is reached; such as that found in old grassland. It would possibly take 50 years or more on some clay soils, but 5-10 years on coarse sandy soils. From this experiment it is quite clear that a high aggregate stability is maintained in rotation IV, whereas two years of ley, as in rotation III, does not seem to be sufficient for maintaining this level of stability.

Effects of fertilization

Table 9. Aggregate stability. Means for treatments A, B and C

Treatment	AS % 6-2 mm	Treatment	AS % 0.6-2 mm
C	61	C	67
A	54	B	62
B	54	A	61

$LSD_{5\%}$ = 3.2% LSD5% = 3.0%

A significantly higher stability is found where manure is used.

Effects of straw

Table 10. Aggregate stability for the 0.6-2 mm fraction. Comparison between treatments B and D

Block	Rotation	6	1	2	3	4	5
I	B	64	56	64	64	55	52
	D	54	62	58	64	64	49
II	B	39	61	52	42	49	28
	D	55	54	63	55	51	30

$LSD_{5\%}$ = 3

Table 11. Aggregate stability for the 6-2 mm fraction. Comparison between treatments B and D

Block Rotation		6	1	2	3	4	5
I	B	46	48	49	52	52	42
	D	46	53	42	55	57	47
II	B	34	51	44	31	39	28
	D	44	51	47	41	39	25

$LSD_{5\%} = 3.1$

The effect of straw incorporation is not evident, but appears to be most visible in rotation II. Here straw is only ploughed down in years with grain. Blocks 3 and 6 with beets and potatoes show the greatest differences, whereas no difference is shown in block 4 with swedes. Neither is there any great difference between treatments B and D in the blocks with grain. In another experiment in the same area, Njøs & Børresen (1991) found a small increase in aggregate stability with 19 years of straw incorporation compared with burning of straw. A significant effect of ley rotations on aggregate stability was also found by Njøs (1967), who also reported a tendency toward higher stability in treatments with animal manure and straw.

Soil erodibility

Aggregate stability is one of the main factors controlling erodibility (De Ploey & Poesen 1985). Aggregate stability of fractions >0.5 mm can be used in an index for soil erodibility (Bryan 1977). A high stability correlates with a low soil erosion. Soil erosion studies in the USA demonstrate that soil erosion is much higher on land used for intensive cultivation of row crops such as cotton, compared to soil which until recently has been used for permanent pasture. However, the favourable structure of these latter soils will decline over a period of two or three years, and the erosion thereafter will be about the same (Meyer 1985). The USLE considers these changes in the C-factor, but short-term changes are not considered in recent process-oriented models, such as CREAMS and WEPP, according to Meyer & Harmon (1992). A subfactor for prior land use will be incorporated in the revised USLE, which is presently under development in the USA (Renard et al. 1991). In the rotation experiment the lowest aggregate stability is found in rotation II, grain-rowcrops. Soil erosion occurring in potato fields may be partly due to compaction and a pulverized soil structure. The effect of a limited plant cover and rows placed up and down the slopes is of course of significance during the growing season. Chow et al. (1990) in Canada shows that soil erosion can be higher from potato cropping than from fallow, but that contour planting can reduce the soil erosion considerably on an 11% slope. After harvest, if the soil is left bare, the soil structure will have a greater effect on soil erosion than in the growing season with plant cover. Changes in aggregate stability during the season must therefore also be considered in a model for erosion risk (Lehrsh & Jolley 1992). The literature on soil aggregation is extensive. Aggregate formation and stabilization are influenced by mineral composition, organic content, and soil processes. A high content

of clay is generally favourable for aggregate stability. Both the clay content and the water content at ÷15 bars suction may be well correlated to aggregate stability. Skidmore & Layton (1992) showed that these parameters were good predictors for mean aggregate stability for Kansas soils. The relation between water-stable aggregates and organic carbon content is discussed in a review article by Tisdall & Oades (1982). For various reasons, the correlation between water-stable aggregates and organic carbon content in soils has not always been good; organic materials are not the only binding agents, the type of organic material is more important than the amount, and some of the water stability is related to physical factors. As discussed by De Ploey & Poesen (1985), the aggregate stability is more closely related to organic matter for soils containing less than 2% humus.

Organic matter analyses

Organic matter is assessed by content of carbon, nitrogen and loss on ignition, which here is corrected by 2% according to the clay content of 25%.

Table 12. Chemical soil analyses for rotation, fertilizer treatment and aggregate fraction.

Rotation/treatment/fraction	Organic cont. %	Total C %	Total N %
I a < 0.6	5.3	2.96	0.30
I a 0.6-2	5.1	3.17	0.33
I a 2-6	5.3	3.18	0.33
I a > 6	5.2	3.04	0.31
I b < 0.6	5.4	3.05	0.32
I b 0.6-2	5.8	3.17	0.34
I b 2-6	5.4	3.10	0.33
I b > 6	5.5	3.16	0.33
I c < 0.6	5.8	3.40	0.35
I c 0.6-2	6.4	3.42	0.35
I c 2-6	6.3	3.46	0.36
I c > 6	6.4	3.43	0.35
I d < 0.6	5.8	3.26	0.34
I d 0.6-2	5.7	3.33	0.34
I d 2-6	5.8	3.33	0.35
I d > 6	5.9	3.30	0.34
II a < 0.6	5.7	2.98	0.31
II a 0.6-2	5.9	3.08	0.33
II a 2-6	5.9	3.12	0.33
II a > 6	5.6	3.06	0.33
II b < 0.6	4.7	3.04	0.33
II b 0.6-2	6.3	2.99	0.33
II b 2-6	5.8	3.02	0.33
II b > 6	5.9	3.10	0.34

Table 12 continue

Rotation/treatment/fraction	Organic cont. %	Total C %	Total N %
II c < 0.6	6.1	3.20	0.34
II c 0.6-2	5.9	3.19	0.34
II c 2-6	6.0	3.22	0.34
II c > 6	6.0	3.18	0.34
II d < 0.6	5.2	2.94	0.32
II d 0.6-2	5.5	2.99	0.33
II d 2-6	5.5	2.96	0.32
II d > 6	5.6	3.03	0.33
III a < 0.6	5.7	3.29	0.34
III a 0.6-2	5.8	3.04	0.31
III a 2-6	6.2	3.28	0.33
III a > 6	6.0	3.20	0.33
III b < 0.6	6.1	3.38	0.34
III b 0.6-2	6.3	3.41	0.35
III b 2-6	6.4	3.44	0.35
III b > 6	5.9	3.37	0.35
III c < 0.6	6.2	3.49	0.35
III c 0.6-2	6.6	3.62	0.36
III c 2-6	6.7	3.55	0.36
III c > 6	6.7	3.61	0.36
III d < 0.6	6.2	3.39	0.35
III d 0.6-2	6.3	3.35	0.34
III d 2-6	6.4	3.41	0.35
III d > 6	6.2	3.38	0.34
IV a < 0.6	6.6	3.58	0.35
IV a 0.6-2	6.1	3.46	0.34
IV a 2-6	6.5	3.50	0.35
IV a > 6	6.2	3.33	0.34
IV b < 0.6	7.1	3.77	0.38
IV b 0.6-2	6.7	3.64	0.36
IV b 2-6	7.0	3.66	0.36
IV b > 6	7.1	3.73	0.37
IV c < 0.6	9.2	3.89	0.38
IV c 0.6-2	9.2	3.78	0.37
IV c 2-6	9.3	3.84	0.39
IV c > 6	9.1	3.85	0.38
IV d < 0.6	9.6	4.03	0.39
IV d 0.6-2	9.4	3.79	0.38
IV d 2-6	9.1	3.81	0.37
IV d > 6	9.6	4.17	0.40

Table 13. Total C (g/100g), means for treatments and rotations

	Treatment			
	a	b	c	d
I	3.09	3.12	3.43	3.31
II	3.06	3.04	3.20	2.98
III	3.20	3.40	3.57	3.38
IV	3.47	3.70	3.84	3.95

Table 14. Total C (g/100g), means for each aggregate fraction

	Aggregate fraction			
	<0.6	0.6-2	2-6	>6
Total C	3.33	3.34	3.37	3.37

The long-term effects of manure, straw incorporation and crop rotation on Total C and Total N in the soil of this experiment are published by Uhlen (1991). The figures presented here follow the same trend as found for the effects of rotations and treatments. No significant difference in the Tot.-C content in the different aggregate fractions was found.

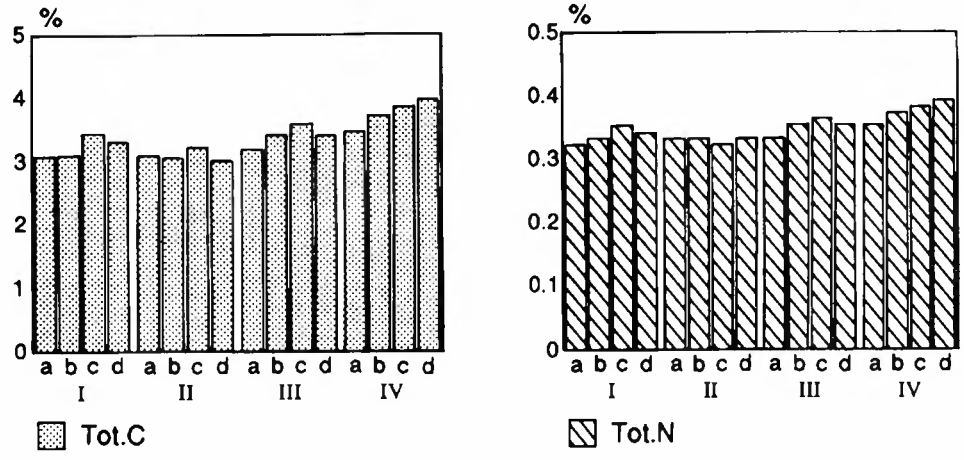


Figure 3. Amount of C (Total C) and N (Total N) for rotations and treatments

As discussed above, the correlation between soil organic content and aggregate stability is not thought to be particularly good. The diagram shows trend lines between Total C and

aggregate stability.

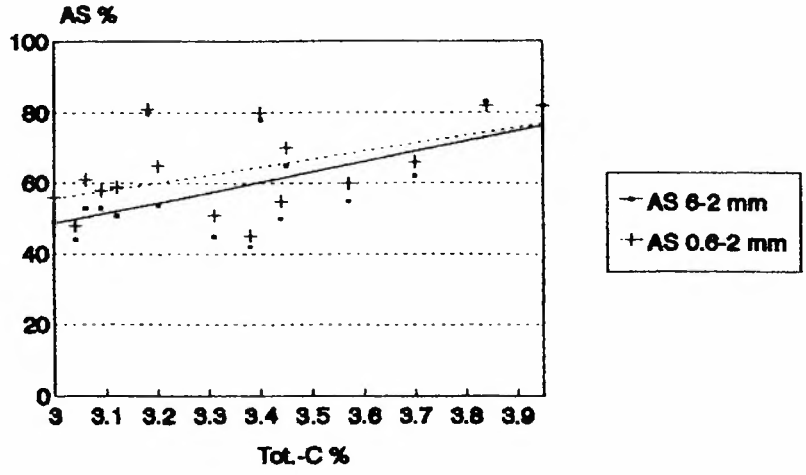


Figure 4. Plot of aggregate stability vs. Total C and lines showing an increasing trend in aggregate stability as the C-content increases

In order to investigate further the relationship between C-content and aggregate stability, Tot.C analyses taken in 1984 from each plots were used in a linear regression. The calculations were done within each rotation. The analyses of variance showed significant correlations between Tot.C and aggregate stability. The coefficients of determination are, however, low, indicating that the aggregate stability is influenced by other factors.

Y = aggregate stability in the 6-2 mm fraction.

Rotation		R^2
I	Y = ÷3.0 + 15.8 Tot.C	0.20
II	Y = 19.3 + 7.6 Tot.C	0.16
III	Y = 9.4 + 13.8 Tot.C	0.21
IV	Y = 48.2 + 8.5 Tot.C	0.24

Y = aggregate stability in the 0.6-2 mm fraction

Rotation		R^2
I	Y = 22.7 + 10.3 Tot.C	0.12
II	Y = 2.4 + 13.9 Tot.C	0.28
III	Y = 15.8 + 13.9 Tot.C	0.36
II	Y = 59.8 + 5.6 Tot.C	0.20

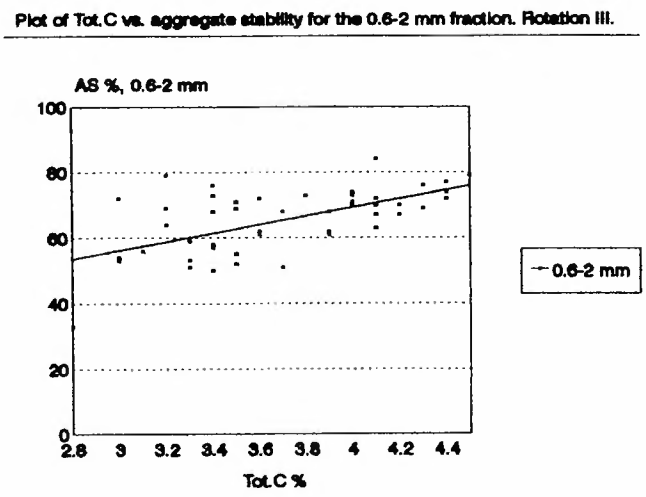


Figure 5. Aggregate stability for the 0.6-2 mm fraction versus Tot.C in soil for rotation III. Tot.C samples from each plot (48) from 1984. Aggregate stability measured in 1992

CONCLUSION

A high aggregate stability renders the soil less susceptible to erosion by water. Soil management to increase the aggregate stability should be used as part of a strategy for soil erosion control. The addition of organic materials to the soil is important in order to increase or maintain the aggregate stability. The best way to increase the humus content is to grow ley, which not only leaves plant residues but also retards the decomposition of organic matter. Rotations with ley give the soil a higher aggregate stability than arable rotations. A rotation of 4 years ley + 2 years grain has resulted in a very high aggregate stability, whereas 2 years of ley + 4 years of grain has had a much smaller effect.

Manure applied once in the six years' periods at a rate of 60 t/ha (9% dry matter) has given a significant increase in aggregate stability. Incorporation of straw has had a weak, hardly significant positive effect compared with straw removal. The lowest aggregate stability was found in the rotation with grain and row crops. This may be due to a more mechanical destruction by raindrops, tillage and harvesting.

Aggregate stability is correlated, although weakly, with Total C content. Aggregate stability is influenced by mineral content, especially clay content, amount and type of organic matter as well as by biological activity in the soil. Correlation with Total C cannot be expected to be very good on soils with a relatively high humus content. Soil erodibility is an important input in soil erosion models. This investigation suggests that aggregate stability is an important parameter to describe soil erodibility. In addition, the previuos land use must be considered, as this will influence the present soil structure.

REFERENCES

Alberts, E.E. & R.C. Wendt 1985. Influence of soybean and corn cropping on soil aggregate size and stability. Soil Sci. Soc. Am. J. 49:1534-1537.

Bryan, R. B. 1977. Assessment of soil erodibility: new approaches and directions. In: T.S. Toy, Erosion. Research Techniques, Erodibility and Sediment Yield. Geo Books. Norwich.

Børresen, T. & G. Uhlen 1991. Jorderosjon og fosfortap ved overflateavrenning i feltlysimeter i Ås vinteren 1989/90. Norsk Landbruksforskning 5: 47-54.

Chow, T.L., J.L. Daigle, I. Ghanem & H. Cormier 1990. Effect of potatoe cropping practices in water runoff and soil erosion. Can. J. Soil Sci. 70: 137-148.

De Ploey, J. & J. Poesen 1985. Aggregate stability, runoff generation and interrill erosion. In: K.S. Richards, R.R. Arnett & S. Ellis (eds.), Geomorphology and Soils. Pp. 99-120. George Allen & Unwin. London.

Haraldsen, T.K., A.H. Aastveit, R. Eltun & G. Guttormsen 1992. Avrenningsundersøkelser i SFL 1991. Statens fagtjeneste for landbruket. Faginfo nr. 8.

Kok, H. & D.K. McCool 1990. Quantifying freeze/thaw-induced variability of soil strength. Trans.ASAE 33(2): 501-506.

Laflen, J.M., L.J. Lane & G. Foster 1991 WEPP. A new generation of erosion prediction technology. J. Soil and Water Conservation 46: 34-38.

Lehrsh, G.A. & P.M. Jolley 1992. Temporal changes in wet aggregate stability. Trans. ASAE 35 (2): 493-498.

Low, A.J. 1955. Improvements in the structural state of soils under leys. Journal of Soil Science 6: 179-199.

Lundekvam, H. 1992. Resultat frå nyare forsøk med erosjon. Foredrag SFFL-konferanse Kolbotn 17.-18. september 1992. Manuscript.

Meyer, L. D. 1985. Interrill erosion rates and sediment characteristics. In: El-Swaify et al. (eds.), Soil Erosion and Conservation. Soil Conservation Society of America. Iowa.

Meyer, L.D. & W.C. Harmon 1992. Soil erosion varies during the crop year. Trans. ASAE 35(2): 459-464.

Morgan, R.P.C. 1986. Soil degradation and soil erosion in the loamy belt of northern Europe. In: G. Chisci & R.P.C. Morgan (eds.), Soil erosion in the European Community. A.A. Balkema, Rotterdam.

Njøs, A. 1967. Virkninger av ulike vekstomløp på fysiske forhold i jorda. Nordiske Jordbrugsforskeres Forening 49:107.

Njøs, A. & T. Børresen 1991. Long-term experiment with straw management, stubble cultivation, autumn and spring plowing on a clay soil in S.E. Norway. Soil & Tillage Research 21: 53-66.

Renard, K.G., G.R. Foster, G.A. Weesies & J.P. Porter 1991. RUSLE. Revised universal soil loss equation. J. Soil and Water Conservation January-February 1991: 30-33.

Skidmore, E. L. & J. B. Layton 1992. Dry-soil aggregate stability as influenced by selected soil properties. Soil Sci. Soc. Am. J. 56: 557-561.

Skøien, S. 1989. Tillage effects on soil erosion and runoff losses of nitrogen and phosporus. Rainfall simulation. Informasjon fra SFFL nr. 6. Ås.

Skøien, S. 1989. Jordas eroderbarhet. Informasjon fra SFFL nr. 6. Ås.

Tisdall, J.M. & J.M. Oades 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33: 141-163.

Uhlen, G. 1974. Omløpsforsøk. Resultater fra 10 lokale forsøk på Sør-Østlandet. Plantedyrkingsmøte NLH 7.-8. mars 1974.

Uhlen, G. 1981. Virkning av eng i omløpet, husdyrgjødsel og halmnedpløying på kornavling og på jordas moldinnhold. Institutt for jordkultur Serie B 1/81. Ås.

Uhlen, G. 1991. Long-term Effects of Ferilizers, Manure, Straw,and Crop Rotation on Total-N and Total-C in Soil. Acta Agric. Scand. 41:119-127.

Zobeck, T.M. 1991. Soil properties affecting wind erosion. J. Soil and Water Conservation 46: 112-118.

Zobeck, T.M. & T.W. Popham 1992. Influence of microrelief, aggregate size, and precipitation on soil crust properties. Trans. ASAE 35 (2): 487-492.

Effect of broadcasted and fertigated N and raised beds on yield and freeze injury of the red raspberry (*Rubus idaeus* L.)

R. NESTBY[1] & K.L. KONGSRUD[2]
[1]The Norwegian State Agricultural Research Stations, Kvithamar Research Station, Stjørdal, Norway
[2]The Norwegian State Agricultural Research Stations, Apelsvoll Research Station, section Kise, Nes på Hedmark, Norway

Nestby, R. & K.L. Kongsrud 1993. Effect of broadcasted and fertigated N and raised beds on yield and freeze injury of the red raspberry (*Rubus idaeus* L.). Norwegian Journal of Agricultural Sciences 7: 249-259. ISSN 0801-5341.

In experiments with the red raspberry cv. 'Veten', it was found that fertigation resulted in better exploitation of nitrogen than broadcasting. Fertigated plants had a higher leaf nitrogen content than those treated with nitrogen by broadcasting, and thus increased primocane height and higher yields. Depending on location and year, higher fertilization rates increased the danger of freeze injury and gave subsequent reductions in yield. A negative correlation was found between freeze tolerance and cane growth parameters and between freeze tolerance and leaf nitrogen. Plants on flat beds displayed more freeze injury than plants on raised beds; fertilization in the autumn tended to have a negative yield effect on plants on the flat, but not on plants on raised beds. The fruits were smaller and were less likely to rot on raised beds than on the flat beds.

Key words: Flat beds, freeze tolerance, N-fertilizing, raised beds, red raspberry, 'Veten', yield

Rolf Nestby, Kvithamar Research Station, N-7500, Stjørdal, Norway

The red raspberry cultivar 'Veten' was released in 1964 and since then has become the main cultivar in Norway (Hjeltnes 1963). It has a relatively low freeze tolerance, and will normally yield successfully only in districts with midwinter min. temperatures of not lower than -25°C and late spring min. temperatures of above -7°C (Nestby 1992). The extent of freeze injury is dependent not only on the temperature, but also on the accessibility of nitrogen in the growth period and on growth (Van Adrichem 1966, Ljones & Sakshaug 1967, Pacholak 1978a & b). It is also known that poor drainage, apart from reducing the yield, also increases the freeze injury.

This experiment was conducted in order to discover what effects different levels of broadcasted and/or fertigated nitrogen would have on yield, freeze tolerance and growth parameters. These treatments were applied on both raised and flat beds, in order to investigate whether the expected improvement in root conditions afforded by raised beds,

had any influence on yield and freeze injury. Any positive effects of new cultivation methods in increasing the yield and reducing the freeze injury will have an impact on the economics of raspberry production, and will provide the potential for expanding the production.

MATERIAL AND METHODS

Origin of plant material

The plants were bought as Norwegian Government controlled certified material. During the second year in the field approximately 10 % of the plants were found to produce crumbly fruit; this was assumed to be caused by a somatic mutation. The canes with crumbly berries were not harvested, and for the statistics the plots were corrected to an equal number of fruiting canes.

Planting system and experimental design

Plants were spaced 0.5 m apart and planted in rows in May 1987, with a distance of 3.5 m between the rows. Later the density was maintained at 10 canes per metre; the Gjerde method was used for supporting the canes (Øydvin 1970). The canes were topped each spring at 160 cm.

Three research fields were used in the experiments. Two fields were located at Kvithamar Research Station (63° 40' N; 10° 11' E) on soils that were on an almost horizontal plane. One of these fields located at Hammer, was of a relatively poorly drained loam with 14.3% organic material, and the other, located at Værnes, was of on a moderately to well-drained silt loam with 3.8% organic material (Solbakken 1987). The third research field was located on a southeast slope at Apelsvoll Research Station, substation Kise (60° 47' N, 10° 49' E) on a well-drained gravely clay with 6.0% organic material.

Each field was arranged as a split-plot design with two replicates. In each replicate N-fertigation [$(Ca(NO_3)_2$] and bedtype were applied to four main plots, randomizing the two fertigation levels within the two rows raised 20 cm and within the two rows on flat land. To each main plot N-fertilizer [$Ca(NO_3)_2$] was broadcast to three subplots. The subplots were 4.0 m long and N-fertilizer was broadcast on an area measuring 4.0 x 1.0 m^2. The different treatments were as follows:

Method of fertilizing	Fertilizing per 1000 m^2
broadcasting	N0 = 0 kg N
	N1 = 4 kg N in the spring
	N2 = 4 kg N in spring and autumn
fertigation	N0 = 0 kg N
	N1 = 4 kg N distributed in June/July/August

The N1 and N2 levels for broadcasted fertilizer and the N1 level for fertigation were adjusted for 1990 and 1991 to 6 kg N at the Kise location and to 8 kg N at the Hammer and Værnes locations. The reason for this was that the medium levels of N produced too low a cane growth from the start of the experiment, and the levels had to be adjusted to provoke the effects of high levels of N. The degree of adjustment was based on leaf N percentages at each location. The rows which were not fertigated were watered, by drip irrigation, to the same soil water tension as the fertigated rows measured by Jet-fill tensiometers at the Kvithamar fields, and by measurement of precipitation and potential evaporation at Kise.

The six fertilizing levels referred to in the tables are as follows:

Fertilizing level	Kg N per 1000 m^2 Broadcasted (B)		Fertigated[1] (F)
	Spring (S)	Autumn (A)	
0	0	0	0
8F	0	0	4(6 or 8)
8B	4(6 or 8)	0	0
16BF (8S+8A)	4(6 or 8)	0	4(6 or 8)
16B (8+8)S	4(6 or 8)	4(6 or 8)	0
24BF (8S+8S+8A)		4(6 or 8)	4(6 or 8)4(6 or 8)

[1] The levels in parentheses are at Kise and Kvithamar respectively after the adjustment of fertilizer level in 1990.

Fertilizer other than N was added to obtain optimal levels according to analysis of both soil and leaves.

Registrations based on canes and leaves

The yield was weighed as marketable and rotted, and the fruit size was calculated on the basis of weighing 50 fruits from every plot at each harvest. Cane height was recorded by measuring 10 normally developed canes, and cane diameter was calculated on the basis of measuring the diameter in the middle of each of 10 canes per plot. In the spring of all years pruned primocanes and frutocanes were weighed on each plot. Canes pruned between the rows during summer were left on the plot. The freeze injury was scored each May when the laterals were approximately 10 cm. All normally developed canes of the plot were included in the rating. The injury was determined on the basis of a 0-9 scoring system. Score 9 indicated 0-10% injury of the buds, with 10% increase in the injury with each reduction in score by one point, a score of zero indicated 91-100% injury. There was no recording of freeze injury on the canes at Kise. Every year in the last week of August leaf samples were collected at each plot for analysis of leaf N (the Kjeldahl analysis), as a percentage of leaf dry matter.

Soil registrations

Soil sampling for determination of soil-water-air ratios, was carried out following the

procedure of Peerlkamp & Bockel (1960), using steel rings with an inside diameter of 58 mm and a height of 38 mm, giving a volume of 100 cm^3. Four undisturbed soil samples were taken in October from each plot in one replicate, alternating between years, to calculate material, pore and air volume. Samples were not taken at Kise because the soil contained too much stone.

Also, in October of each year, 20 samples were taken with a soil auger from a depth of 20 cm in each plot for the determination of NH_4 and NO_3.

Statistical evaluation

The main effects of the treatments and their interactions were analysed using the SAS procedure, GLM. Correlation coefficients were computed with the SAS procedure, CORR. Graphics were made by the SAS procedures GCHART, GPLOT and GREPLAY (SAS Inst. Inc., 1987).

RESULTS AND DISCUSSION

Effects on yield

Fertilizing with nitrogen affected yield taken as an average of three locations and three years (Table 1). However, no interaction was found between year and level of fertilizer, but there was an interaction between year and location, between location and bed type and between location and fetilization method (Table 2). From Table 1 it can be seen that on raised beds, the yield on fertigated plots was higher than that on broadcasted plots, and that the lowest yield on fertigated plots (8F) was comparable with the highest yield on the broadcast-treated plots (16B). However, there was no increase in yield when the amount of fertilizer was changed from 16BF to 24BF. The same tendency was observed on flat beds, but there the highest yield was achieved with no fertilizing together with 8F and 16BF.

Table 1. Effects on yield of the red raspberry cv. 'Veten' in kg/1000 m^2, fruit size in grams per berry and rotted berries in percentage of total yield, of broadcasted (B) in spring (S) and autumn (A) and fertigated (F) nitrogen [$Ca(NO_3)_2$] , as an average of three locations and three years

	Bed type					
	Raised bed	Flat bed	Raised bed	Flat bed	Raised bed	Flat bed
	Yield		Fruit size		Rot	
0	678	870	3.0	3.4	5.8	4.0
8F	812	897	3.2	3.4	5.2	6.8
8B (S)	738	772	3.1	3.3	5.6	5.9
16BF (S)	873	841	3.2	3.4	5.3	6.7
16B (S+A)	794	751	3.3	3.4	5.6	4.9
24BF (S+A)	888	784	3.2	3.2	4.6	6.5
Mean	797	819	3.1	3.4	5.4	5.8
Standard error	59	57	0.1	0.1	1.2	0.7
P	0.0096	0.0726	0.0354	0.0959	0.9490	0.0003

Table 2. F values and probabilities (P) for main effects and interactions of yield, fruit size and percentage rotted fruits of the red raspberry cv. 'Veten' as an average of three locations and three years. F-B indicates the fertilizing method (fertigate/broadcast)

Interaction		Yield		Fruit size		Rot	
		F	P	F	P	F	P
Location	x Bed type	8.50	0.0003	12.72	0.0001	0.05	0.9517
	x F-B	4.80	0.0092	7.07	0.0011	1.56	0.2133
	x Nitrogen level	1.72	0.0791	3.77	0.0001	0.59	0.8204
Nitrogen	x Bed type	3.23	0.0082	2.99	0.0131	2.31	0.0458
Year	x Nitrogen level	1.15	0.3252	0.39	0.9518	0.46	0.9142
	x Location	34.09	0.0001	16.03	0.0001	48.44	0.0001
Bed type	x F-B	2.60	0.1085	2.21	0.1390	9.41	0.0025
F-B		11.40	0.0009	0.38	0.5386	2.25	0.1352
Bed type		2.14	0.1450	29.96	0.0001	0.19	0.6651

The reason for the differences in yield response between fertigation and broadcasting and the different reactions on bed type can be explained by different levels of accessibility of nitrogen to the plants, depending on treatment. Figure 1 showes that there were such differences in accessibility, and it is clearly shown that fertigation gave a higher leaf nitrogen content than broadcasting. This stronger and more efficient fertilizing treatment gave an increase in the leaf nitrogen, which in all years resulted in higher yields along with an increase in the primocane length and diameter, but for the cane yield there was an increase in 1991 only. The increase in primocane length without increased weight of cane yield is in accordance with Ljones & Sakshaug (1967), with the exception of the year 1991 when both the primocane length and the cane yield increased when leaf nitrogen increased. These factors were positively correlated with yield (Table 3). Also, the adjustment in fertilizer level in 1990 gave rise to longer canes measured in the spring of 1991 and 1992 than those of the previous years. The increased fertilization also tended to result in a larger primocane diameter, but there were no differences in reaction between fertigation and broadcasting. Despite the higher fertilizing level in 1990, and thus the longer and thicker canes than those found in the previous year, the weight of pruned canes in the autumn was less than that of the year before, and there was no evident effect of fertilizer treatment on cane yield. Therefore, the reason for the lower cane yield was probably that the number of canes was less than that of the year before, indicating that the number of canes in 1990 was dependent on factors influencing the plants in 1989. One of these factors could have been the amount of accessible nitrogen, since the number of pruned canes in the autumn of 1991 was strongly influenced by fertilization and nitrogen accessibility. The effect on cane number in 1991 may therefore have been an effect of fertilization in 1990.

In some cases, especially on flat beds, increased nitrogen accessibility reduced the yield. Some of that reduction must have been caused by freeze injury, since there was a negative correlation between freeze tolerance and leaf nitrogen (Table 3). Ljones & Sakshaug (1967) found very little connection between nitrogen accessibility and top freeze. This is not necessarily in contradiction to the results in this experiment, since the freeze injury here was rated on the basis of whole canes and not just the top.

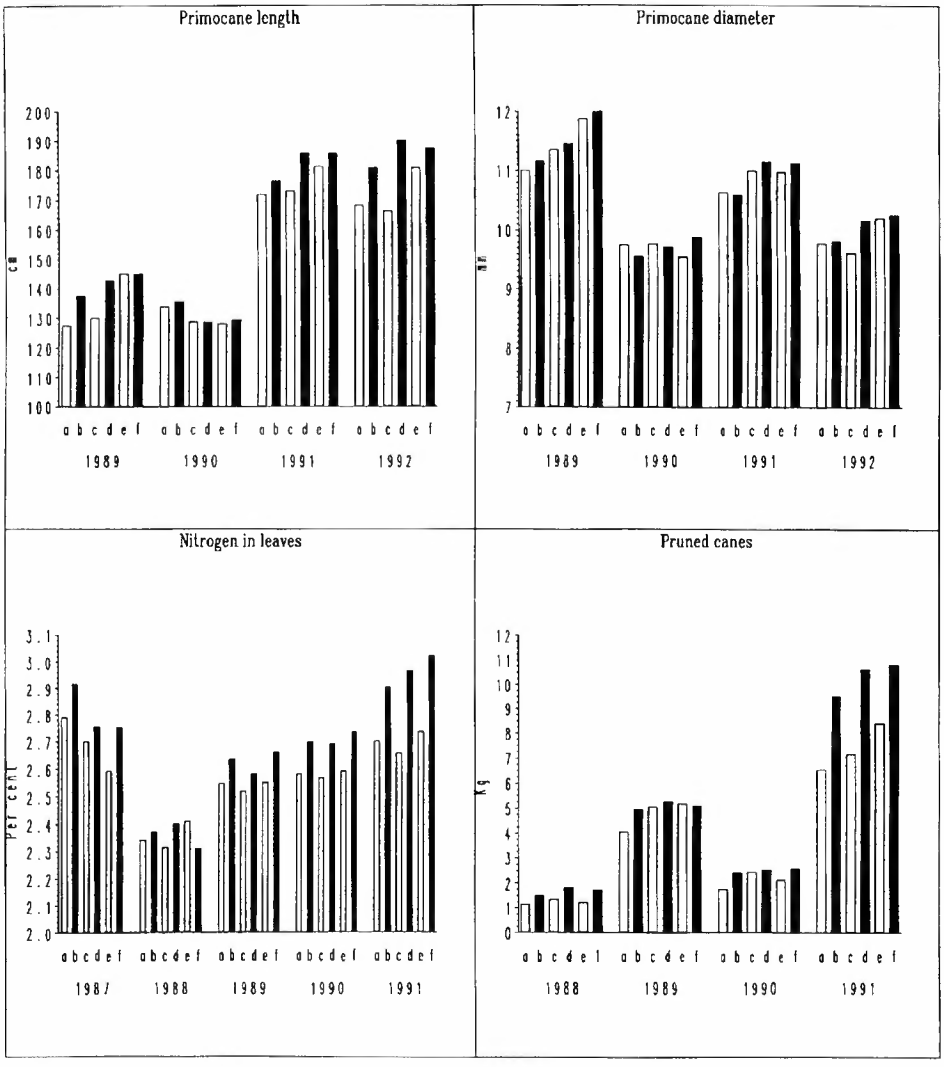


Figure 1. Effect of nitrogen levels from 0 to 24 (a-f) kg/ 1000 m^2 broadcasted (white bar) or broadcasted/fertigated (black bar) on cane parameters, and percentage of nitrogen in leaves of the red raspberry cv. 'Veten' in the years 1987 to 1992. Codes for nitrogen levels: a = 0 ,b = 8, c = 8, d = 16, e = 16, f = 24

The positive effect of raised beds on freeze tolerance could be an effect of the air volume in the soil, which tended to be higher in 1990 and was higher in 1991 ($p = 0.0157$) on raised than on flat beds.

Table 3. Correlation coefficients between yield or freeze tolerance and growth parameters, percentage of nitrogen in leaf dry matter, soil parameters and yield the previous year, of the red raspberry cv. 'Veten', calculated on the basis of three years except for those years in parentheses

Parameter	Yield	Freeze tolerance
Pruned canes	0.65***	-0.61***
Primocane length	0.61***	-0.31***
Primocane diameter	0.44**	-0.60***
Leaf nitrogen	0.37***	-0.43***
Soil sol. nitrogen	-0.46* (1991)	0.51* (1991)
Soil material volume	0.43**	-0.57** (1991)
Soil air volume	-0.42**	0.20

*, **, *** are significant at, respectively, p=0.05, p=0.01, p=0.001

Broadcasting may also have led to the access of nitrogen late in the season when the weather was dry during spring and summer, with unwanted late growth and increased danger of freeze injury. There was a tendency toward reduced yield on flat beds with broadcasting at the end of August (Table 1), which supports this theory. The table also indicates that the yield increased with higher accessions of nitrogen on raised bed than on flat beds, an interaction that was significant (Table 2). The reason of this could possibly be that the nitrogen was transported much faster out of the root zone on raised beds compared with flat beds, indicated by a tendency to a lower level of soluble nitrogen ($p = 0.0698$) in the soil on raised beds and a lower cane yield ($p = 0.0415$) in 1991. These effects were not obvious in 1989 and 1990, however.

In effect, this suggests that fertilizing with nitrogen should be carried out by fertigation or by some other means of solution in water, and that raised beds generally need more nitrogen than flat beds. Also, it was found that fertilizing in the autumn had no effect or a negative effect on flat land, and no effect or a positive effect on raised beds, depending on the level of fertilization.

Effects on fruit size

Effects on fruit size were negligible (Table 1), but there were differences within the raised beds; the smallest size was obtained when no nitrogen was added. At 8B the fruit size was 0.1 g larger and at all other nitrogen levels the fruit size was additional 0.1 g larger, with the exception of at 16B which gave an 0.2 g larger fruit size than no fertilization. Flat beds produced 0.3 g larger fruits than raised beds and the interaction between bed type and nitrogen level was therefore significant, but not the interaction between bed type and fertilizing method (Table 2). The reason for raised beds having to be fertilized in order to increase the berry size, while this was not necessary on flat land, must have been the greater loss of nitrogen, or a combination of less water and nitrogen, as a result of better drainage conditions on raised beds than on flat land.

In conclusion, to achieve the same fruit size on raised and flat beds, the raised beds demand more nitrogen.

Effects on fruit rot

There were differences in incidence of fruit rot within both bed types and between fertilizing levels (Table 1), but they were significant only on the flat beds. The difference in incidence between the two bed types was not significant, and there was no interaction between bed type and location or bed type and fertilizing method (Table 2). More rotted fruit were found on flat beds with fertilizing than on those without fertilizing, and there tended to be most rotted fruits on the fertigated plots. On raised beds more rotted fruits were produced without fertilization than by fertilization at the highest rate, and there was a tendency for fertigated plots to produce fewer rotted berries than broadcasted plots. The effect of fertilizer on raised beds was the opposite of that on flat beds. The reason for this is not obvious, and it is likely that a combination of factors are involved. There may be an optimum level for nitrogen in the plant that keeps the rot at a low level. That optimum level was reached on flat beds without fertilizing, and on raised bed at the highest fertilizing level. This theory was supported by a statistical analysis which indicated that for the percentage of leaf nitrogen there was a difference between fertigation methods ($p = 0.0205$),and an interaction between fertilizing method and bed type ($P=0.0001$). Also, increased rotting may have been an effect of more shading in the row because of increased cane growth ($p = 0.0009$) on the fertigated plots compared with growth on broadcasted plots, and longer primocanes on flat than on raised beds (Fig. 1 & Fig. 2). The reduced fruit rot in rows on raised beds may also have been a result of better aeration than that on flat land.

Optimal level of leaf nitrogen

Figure 3 shows that as an average of 1990 and 1991, the freeze injury at Hammer increased with increasing leaf nitrogen, but within limits did not reduce yield. At Værnes fertigation tended to reduce the yield on the flat beds in 1990 and 1991, but to increase it on the raised beds. The freeze injury was more severe on flat than on raised beds (Fig. 2). In Figure 3 it is shown that the freeze injury increased exponentially with an increase in leaf nitrogen. Also, the yield began to flatten out at leaf nitrogen levels higher than approximately 3.1%. Within the limits of nitrogen accessibility in this experiment, the enhanced accessibility of nitrogen by fertigation did not increase the freeze injury to such an extent that the positive nitrogen effect on yield was reduced. At Kise in 1990 and 1991 fertigation had a negative effect on yield on flat land, and an effect close to that achieved by broadcasting on raised beds. Figure 3 shows that there was an exponential reaction on yield as a result of an increase in the leaf nitrogen content. The largest yield was achieved at a nitrogen level close to 2.5%. Small changes in increasing the percentage beyond 2.8% resulted in severe reductions in yield. In 1991 there was a severe yield reduction, primarly as a result of freeze injury, but some of the effect was probably caused by a injury caused by the raspberry cane midge (*Resseliella theobaldi* Barnes) in the autumn of 1990.

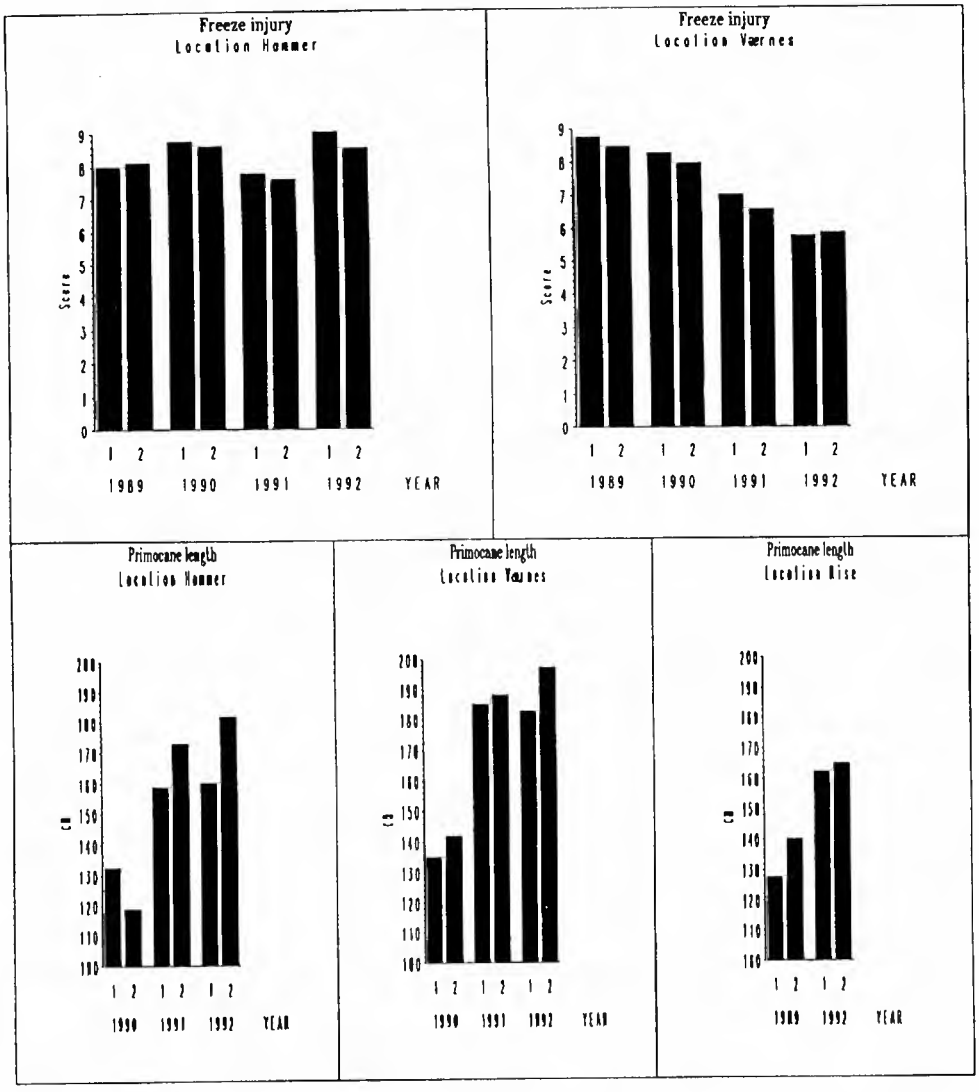


Figure 2. Freeze injury of the red raspberry cv. 'Veten' rated (0-9) at two locations and primocane length in centimetres at three locations affected by raised (1) and flat beds (2) in the years 1989 to 1992. A rating of 9 is equal to no injury, while 0 is a total injury

In effect, this means that more fertigated nitrogen could be applied on raised beds than on flat beds at locations exposed to freeze injury. At locations with a relatively minor chance of freeze injury, a leaf nitrogen content up to 3.5% can be recommended; the best yield was achieved with a combination of fertilizing with 8 kg/1000 m^2 nitrogen broadcasted in the spring plus 8 kg/1000 m^2 nitrogen added by divided fertigation through June, July and August. At a location with a moderate freeze injury problem, the leaf nitrogen percentage should not exceed 3.2; the best yield result was achieved by using 8 kg/1000 m^2 fertigated

N on the flat beds and a combination of 8 kg/1000 m^2 broadcasted N in the spring plus 8 kg/1000 m^2 fertigated N on raised beds. At a location with a major risk of severe freeze injury, the leaf nitrogen percentage should not be higher than 2.8; the best yield result was achieved by fertigation with 6 kg/1000 m^2 on raised beds and 0-6 kg on flat beds. In the cases where there was a combination of 8 kg broadcasted N and 8 kg fertigated N, it would be reasonable to expect a similar effect by adding all nitrogen by fertigation at a level between 8 and 16 kg/1000 m^2.

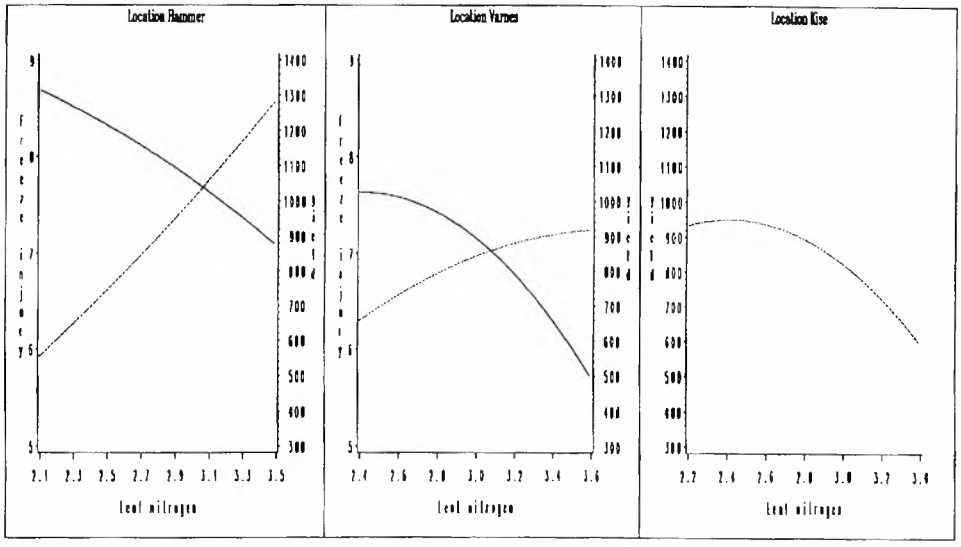


Figure 3. Effect on the red raspberry cv. 'Veten' of percentage of leaf nitrogen on freeze injury (—) and yield (---) in kg/1000 m^2, rating of 9 of freeze injury indicates no injury and 0 indicates total injury

AKNOWLEDGEMENTS

The authors thanks Dr. Hugh Daubeny, Vancouver Research Station, British Columbia (Canada), for a thorough professional and linguistic review of the report, and Odd Slettan, Kvithamar Research Station and Arild Bergersen, Apelsvoll Research Station, section Kise, for technical assistance.

REFERENCES

Hjeltnes, A. 1963. Sortsforsøk med bringebær 1954-1962. Frukt og bær 16: 19-25. (In Norwegian)

Ljones, B. & Sakshaug, K. 1967. Nitrogen effects on composition and yield components of raspberry cultivars. Meld. Norg. LandbrHøgsk. 46(12): 1-19.

Nestby, R. 1992. Screening red raspberry germplasm for freeze tolerance in Norway. Acta Agric. Scand. 42: 189-196.

Pacholak, E. 1978a. The effect of fertilazation on raspberry tolerance of low temperature. Roczniki Akademii Rrolniczej w Poznaniu, Ogrodnictwo 98(7): 173-186. (English summary).

Pacholak, E. 1978b. Factors affecting frost tolerance of raspberry canes. Acta Horticulturae 81: 97-101.

Peerlkamp, P.K. & Bockel, P. 1960. Moisture retention curves. Proceedings of the Technical Meetings of the committee for Hydrological Research T.N.O. 5, pp. 122-138.

SAS Institute Inc. 1987. Sas User's Guide:Statistics, 1987 end., SAS Inc., Cary, NC.

Solbakken, E. 1987. Jordsmonnkartlegging på SF Kvithamar og Værnes prestegård, Stjørdal kommune. Norwegian Institute of Land Inventory, report no. 16, pp. 29-30 & 46-48.

Van Adrichem, M.C.J. 1966. An association between late summer growth rate and fall frost injury in red raspberries. Hort. Sci.1: 94-95.

Øydvin, J. 1970. Gjerdemetoden - en ny oppbindingsmetode for bringebær som viser gode resultat. Njøs Research Station; Anniversary report, 'Statens forsøksgard Njøs 1920-1979', pp. 98-116. (English summary).

Performance of 'Red Gravenstein', 'Summerred' and 'Aroma' on apple rootstocks M9, M26 and MM106 over 14 years

JONAS YSTAAS & ODDMUND FRØYNES
The Norwegian State Agricultural Research Stations, Ullensvang Research Station, Lofthus, Norway

Ystaas, J. & O. Frøynes. 1993. Performance of 'Red Gravenstein', 'Summerred' and 'Aroma' on apple rootstocks M9, M26 and MM106 over 14 years. Norwegian Journal of Agricultural Sciences 7: 261-266. ISSN 0801-5341.

A rootstock trial was conducted over a period of 14 years to evaluate the performance of 'Red Gravenstein', 'Summerred' and 'Aroma' on M9, M26 and MM106. It was found that tree vigour of all the cultivars was significantly affected by the rootstocks. The mean trunk circumference of the vigorous cultivar 'Red Gravenstein' on M9 and M26 as a percentage of those on MM106 was 52 and 80 respectively. The less vigorous cultivars 'Summerred' and 'Aroma' had the same vigour on M26 and MM106, while trees on M9 had 64% and 72% of the vigour of MM106, respectively. Trees on M26 had the highest cumulative yields and those on M9 the lowest. Cropping efficiency was, however, significantly higher on M9 than on M26 and MM106. Fruit size was larger on M26 and M9 than on MM106, while apples on M9 had the highest content of soluble solids. It was concluded that M9 should be recommended for intensive planting systems in high density apple orchards when soil management preventing competition from grass and weeds is provided.

Key words: Apple, rootstock, yield, yield efficiency, fruit size, fruit quality

Jonas Ystaas, Ullensvang Research Station, N-5774 Lofthus, Norway

The choice of rootstocks affects the apple tree in many ways; e.g. tree vigour, tree size, precocity, yield, fruit size, fruit quality, anchorage and winter hardiness. The transition of fruit production from high standard trees to small trees in intensive planting systems has brought the selection of dwarfing rootstocks into focus. Many experiments with apple rootstocks have been carried out in different countries to find the rootstocks best adapted to the prevailing climatic and soil conditions (Ferree & Carlson 1987). In Norway rootstock trials carried out in the 1960s established MM106 as a precocious, high-yielding and semi-vigorous rootstock (Brandstveit 1973; Haugse 1967; Husabø 1970). The trend towards more intensive planting systems, however, prompted the search for more dwarfing rootstocks. A rootstock trial with the dwarfing M9 and M26 rootstocks, using MM106 as a standard to three of the most important commercially grown apple cultivars in Norway was carried out at Ullensvang Research Station, Lofthus, western Norway at 60°N, during the period 1975-88. Preliminary results have been published previously (Ystaas 1988).

MATERIAL AND METHODS

A trial with the apple rootstocks M9, M26 and MM106 to the main commercial cultivars 'Red Gravenstein', 'Summerred' and 'Aroma' was planted in spring 1975. The plant material was one-year-old trees without feathers. The experimental design was a randomized complete block with four replicates, and two trees on each plot. The trees were planted with a distance of 4.5 m between rows and 3 m within the rows (740 trees/ha). The trees were staked and trained with a central leader as free spindle. The height of the trees was kept at 2.5 m by pruning. The soil was loamy sand high in organic matter (7%). Soil management combined frequently mown grass in the alleyways with 1-m-wide herbicide strips along the tree rows. Annual data on trunk girth, yield and fruit weight were recorded. Random samples of 20 apples from each plot were kept in cold storage at 4°C for four weeks until fruit quality examination took place. The content of soluble solids was measured by means of an Atago digital refractometer. No thinning programme was carried out on the trees.

RESULTS AND DISCUSSION

Tree vigour

Tree vigour as measured by trunk circumference of 14-year-old trees at the end of the experiment was significantly affected by rootstocks (Tables 1, 2 and 3). Tree vigour induced by M9 compared to MM106 was 52%, 64% and 72% for 'Red Gravenstein', 'Summerred' and 'Aroma', respectively. Trees of 'Red Gravenstein' on M26 had 80% of the vigour of those on MM106. These findings are in accordance with the results reported by Parry (1977) and Christensen (1973) classifying M9 as dwarfing, M26 as semi-dwarfing and MM106 as semi-vigorous rootstocks. In contrast to the vigorous cultivar 'Red Gravenstein', 'Summerred' and 'Aroma', both cultivars of moderate vigour, showed no difference in tree vigour on M26 and MM106; a relationship demonstrating interaction between cultivars and rootstocks.

Table 1. The effects of rootstocks M9, M26 and MM106 on trunk girth of 14-year-old trees, accumulated yield, yield efficiency and average fruit weight and soluble solids of 'Red Gravenstein' apples over 12 cropping years

Rootstock	Trunk girth cm	Cumulative yield kg/tree	Yield efficiency kg/cm^2	Fruit weight g	Soluble solids percent
M9	25.6	155.3	2.98	162	11.5
M26	39.7	232.7	1.94	172	11.0
MM106	49.7	186.6	0.99	156	11.2
LSD (P = 0.05)	11.5	NS	0.90	NS	NS

Table 2. The effects of rootstocks M9, M26 and MM106 on trunk girth of 14-year-old trees, accumulated yield, yield efficiency and average fruit weight and soluble solids of 'Summerred' apples over 12 cropping years

Rootstock	Trunk girth cm	Cumulative yield kg/tree	Yield efficiency kg/cm^2	Fruit weight g	Soluble solids percent
M9	22.0	154.8	4.02	122	11.9
M26	34.8	244.7	2.56	129	11.6
MM106	34.2	245.9	2.63	120	11.3
LSD (P =0.05)	3.5	75.4	0.80	NS	0.3

Table 3. The effects of rootstocks M9, M26 and MM106 on trunk girth of 14-year-old trees, accumulated yield, yield efficiency and average fruit weight and soluble solids of 'Aroma' apples over 12 cropping years

Rootstock	Trunk girth cm	Cumulative yield kg/tree	Yield efficiency kg/cm^2	Fruit weight g	Soluble solids percent
M9	23.3	170.3	4.02	161	12.3
M26	33.3	206.2	2.34	164	12.5
MM106	32.2	172.8	2.12	155	12.4
LSD (P =0.05)	9.8	NS	1.14	NS	NS

Yield

The trees on M9, M26 and MM106 were all precocious. The first crop was obtained in the third season (Figs. 1, 2 and 3). Precocity is a very important trait of rootstocks that is used in an intensive planting system (Jackson 1989; Wertheim 1989). The buildup of cumulative yield, however, was influenced by rootstock vigour and canopy size as reported by Callesen (1989). Trees on M9 did not utilize the space allotted as rapidly as those on M26 and MM106. 'Summerred' trees on M9 had a significantly smaller accumulated yield over 12 cropping years than trees on M26 and MM106 which were on the same level (Table 2). In 'Red Gravenstein' and 'Aroma' any significant rootstock effect on accumulated yield was not revealed (Tables 1 and 3). However, trees on M26 of both cultivars had the highest yield.

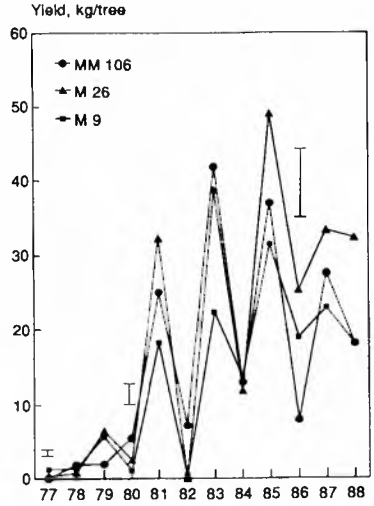


Fig. 1. Yield of 'Red gravenstein' as affected by three rootstocks during the first 12 cropping years. Vertical bars represent LSD (P = 0.05)

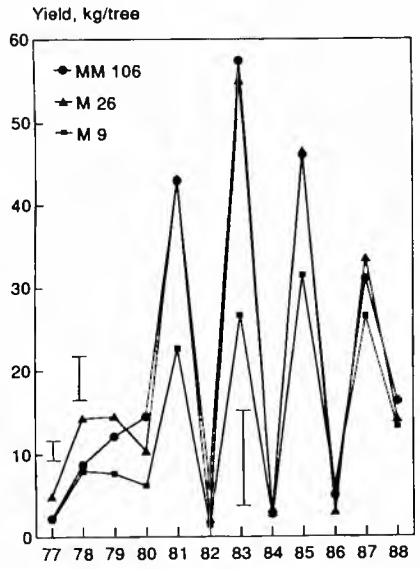


Fig. 2. Yield of 'Summerred' as affected by three rootstocks during the first 12 cropping years. Vertical bars represent LSD (P = 0.05)

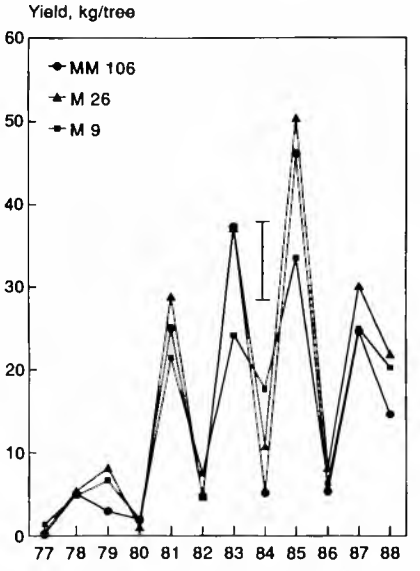


Fig. 3. Yield of 'Aroma' as affected by three rootstocks during the first 12 cropping years. Vertical bars represent LSD (P = 0.05)

Because of unfavourable growing conditions in the 1979 season, a strong biennial bearing habit was induced in all cultivars (Figs. 1, 2 and 3). The main reasons for this unfavourable development were root injuries by a winter freeze without any snow cover the preceding winter, large crop load and below normal summer temperature. If applied soon after flowering, hand and chemical thinning can promote flower induction and reduce the large difference in yields between on-years and off-years (Jonkers 1979; Meland & Gjerde 1992). No thinning programme was practised in this trial and any difference between rootstocks in suppressing biennial bearing was not detected. According to Jonkers (1979) dwarf rootstocks can reduce biennial bearing a little. In a rootstock trial with 'Gravenstein', trees on M9 produced annual crops, while trees on M26, P2 and P22 induced biennial bearing (Ystaas & Frøynes 1993).

Yield efficiency

The relationship between accumulated yield and tree size is commonly described by cumulative yield (kg per tree) divided by trunk cross-sectional area (cm^2) and named yield efficiency (Westwood et al. 1986). Trees on M9 of the vigorous cultivar 'Red Gravenstein' and the moderately vigorous 'Summerred' and 'Aroma' have all significantly higher yield efficiency than trees on M26 amd MM106. Working with 'Red Gravenstein' Måge (1980) obtained similar results, while Callesen (1989) found that high yield efficiency of trees on M9 could not compete with the rapid volume buildup of trees on M26.

Fruit size and content of soluble solids

Fruit weight was not significantly affected by rootstocks (Tables 1, 2 and 3). Trees on M26, however, produced the largest apples for all cultivars. The trend was similar for 'Red Gravenstein', 'Summerred' and 'Aroma'; fruit size decreased in the following order; M26 > M9 > MM106. This finding is in accordance with results reported by Christensen (1973),

Måge (1980) and Ystaas & Kvåle (1989).

Under climatic conditions like those prevailing in the fruit districts of Norway, Kvåle (1963) found the soluble solid content of apples to be a simple and good criterion for fruit quality. The content of soluble solids in 'Summerred' apples was significantly affected by rootstocks (Table 2). Apples of 'Summerred' on M9 as well as 'Red Gravenstein' apples from trees on M9 had the highest content of soluble solids. The content of soluble solids in apples of all the rootstock-cultivar combinations tested, however, was well above the threshold limit of acceptable eating quality of 10.8% (Kvåle 1973).

Winter hardiness

During the experimental period a test winter occurred in 1978/79, with temperatures down to -15.5°C without any snow-cover. The experimental trees of the three cultivars on M9, M26 and MM106 did not suffer any loss. Under similar conditions killing of apple trees by root frost was reported by Måge (1980a) from the Sogn area, western Norway, separating the winter hardiness of rootstocks in the following order: M26 > M9 > MM106. This is in accordance with the view held by Ferree & Carlson (1987) that M26 is the most winter hardy of the Malling rootstocks now used commercially.

CONCLUSIONS

The high yield efficiency of apple trees on rootstock M9 indicates that M9 is the best choice of rootstocks for 'Red Gravenstein', 'Summerred' and 'Aroma' in high density planting systems (1500-2000 trees/ha). Under the soil and climatic conditions prevailing in Norway, it is mandatory to avoid competition from grass and weeds for water and nutrients in orchards on M9. In these orchards soil management applying short cut grass in the alleyways and strips free from vegetation along tree rows combined with drip irrigation is necessary to be successful. If these requirements cannot be met, a semi-dwarf rootstock like M26 should be preferred, especially on sandy soils.

REFERENCES

Brandstveit, T. 1973. Grunnstammeforsøk med 'Gravenstein', 'Ingrid Marie' og 'James Grieve'. Nokre vanleg brukte stammer i samanlikning med fire MM-stammer. Forskning og forsøk i landbruket 24: 463-474.

Callesen, O. 1989. Ten years results with apple rootstocks M27, M9 and M26 at two spacings. Tidsskr. Planteavl 93: 267-274.

Christensen, J. Vittrup 1973. Grundstammer til æbletræer på let og svær jord. Tidsskr. Planteavl 77: 540-546.

Ferree, D.C. & R.F. Carlson. 1987. Apple rootstocks. In: R.C. Rom & R.F. Carlson (eds.) Rootstocks for fruit crops. John Wiley & Sons, New York. pp. 107-143.

Haugse, L. 1967. Grunnstammeforsøk med Gravenstein, Ingrid Marie og James Grieve. Nokre vanleg brukte stammer i samanlikning med fire MM-stammer. Forskning og forsøk i landbruket 18: 153-163.

Husabø, P. 1970. Forsøk med eplegrunnstammer. II. M- og MM-stammer i forsøk med 3 sortar. Forskning og forsøk i landbruket 21: 381-390.

Jackson, J.E. 1989. World-wide development of high density planting in research and practice. Acta Horticulturae 243: 17-27.

Jonkers, H. 1979. Biennial bearing in apple and pear: a literature survey. Scientia Horticulturae 11: 303-317.

Kvåle, A. 1963. Kan fruktkvalitet mælast?. Frukt og Bær 1963: 34-40.

Kvåle, A. 1973. Grenseverdiar for sukkerinnhald i eple til friskkonsum. Forskning og forsøk i landbruket 24: 693-697.

Meland, M. & B. Gjerde 1992. The effect of handthinning on return bloom of 'Summerred' and 'Aroma' apples. Acta Horticulturae (in press).

Måge, F. 1980. Grunnstammeforsøk med eplesorten 'Raud Gravenstein'. Forskning og forsøk i landbruket 31: 409-415.

Måge, F. 1980a. Rotfrost hos frukttre i Sogn vinteren 1978-79. Frukt og Bær 1980: 16-24.

Parry, M.S. 1977. Field comparisons of M26 and other dwarfing apple rootstocks on a diversity of sites. Journal of Horticultural Science 52: 59-73.

Ystaas, J. 1988. Aktuelle grunnstammer til Raud Gravenstein, Summerred og Aroma. Årsmelding Ullensvang forskingsstasjon 1987: 5-8.

Ystaas, J. & A. Kvåle 1989. Utvikling mot meir intensive plantesystem for eple. Resultat oppnådde i nyare norske forsøk. Frukt og Bær 1989: 23-31.

Ystaas, J. & O. Frøynes 1993. Effects of the Polish rootstocks P2 and P22 on vigour, yield and fruit characteristics of the apple cultivar 'Gravenstein'. Acta Agric. Scand. Section B: Soil and Plant Science (in press).

Wertheim, S.J. 1989. Preliminary results of trials with dwarfing apple and pear rootstocks. Acta Horticulturae 243: 59-70.

Westwood, M.N., P.B. Lombard, Scott Robbins, & H.O. Bjornstad 1986. Tree size and performance of young apple trees of nine cultivars on several growth-controlling rootstocks. HortScience 21: 1365-1367.

NORWEGIAN JOURNAL OF AGRICULTURAL SCIENCES
INSTRUCTIONS TO AUTHORS

THE MANUSCRIPT

The manuscript shall be typewritten on one side of the paper only. It shall be double spaced and have margins of at least three centimetres. Each of the following elements of the manuscript shall begin on a new page: (1) the title, (2) abstract, (3) the text, (4) summary, (5) list of references, (6) tables, (7) figure legends.

The pages shall be numbered consecutively beginning with the title page.

Articles will usually be organized as follows: (1) introduction, (2) materials and methods, (3) results, (4) discussion and (5) summary. Up to three grades of headings can be used to divide up the text. Articles must not exceed 20 manuscript pages, and two copies should be submitted to the managing editor.

TITLE PAGE

The title page shall contain:

1. A precise but brief title. A subtitle may be used, but also this should be brief.
2. A short title of not more than 40 letters and spaces for use as a running headline.
3. The full names of all authors.
4. The name and adress of the institution and/or departments in which the research was carried out. Names of institutions shall be in English.

ABSTRACT AND KEYWORDS

Use only keywords listed in Agrovoc. The abstract must not exeed 150 words, corresponding to 10 lines in print. The abstract shall briefly describe the purpose/question of the experiment/research project, the method, results and the principal conclusions drawn. Use only standard abbreviations in the abstract.

Do not use more then 10 keywords and list them alphabetically. Indicate the name and adress of the author to whom correspondence, proofs and offprints should be sent.

ACKNOWLEDGEMENTS

Acknowledgement shall be made only to persons who have contributed substantially to the results of the research. Authors are responsible for ensuring that individuals named are recognized as being identified with the results and conclusions described in the article.

TABLES

Each table shall be typed double spaced on a separate sheet of paper. They shall be numbered consecutively with Arabic numerals and have a concise descriptive heading. Abbreviations in tables shall be explained in footnotes, using the following symbols in this order: [1]), [2]), [3]), [4]), [5]).

Avoid horizontal and vertical rules (lines) in tables. Tables shall be self explanatory as far as possible without the reader having to refer to the text.

FIGURES

All illustrations, line drawings as well as photographs, shall be considered as figures. Figures shall be numbered consecutively with Arabic numerals. Letters, numerals and symbols must stand out clearly and be in relation to each other. Make sure they are large enough to take reduction. Before preparing line drawings the author should decide wether they are to be of 1 column, 1½ columns, or 2 colums width so that lettering, etc., after reduction, will be the same size on all drawings. Photographs should be submitted as near to their printed size as possible. If enlargement or reduction is significant in an photograph, the scale should be given on the back and not in the legend. The legend should make the general meaning comprehensible without reference to the text. Figure legends shall be typewritten together on one or more sheets of paper.

REFERENCES

Reference indicators in the text will follow the Harvard style, with the author's name and the year of publication: Høeg (1971), or (Høeg 1971). The text reference indicator to a work by two authors is given by naming both authors: Oen & Vestrheim (1985) or (Oen & Vestrheim 1985). If three or more authors, the text reference indicator is given by adding et al. to the first named author: Aase et al. (1977) or (Aase et al. 1977).

In the list of references authors' names are listed alphabetically. Two or more works by the same author are listed chronologically and if published in the same year must be distinguished by the appendage a. b. etc. after the year of publication. The corresponding appendage must also appear in the text indicator.

Høeg, O.A. 1971. Vitenskapelig forfatterskap. 2. utg. Universitetsforlaget, Oslo. 131 s.

Oen, H. & S. Vestrheim 1985. Detection of non-volatile acids in sweet cherry fruits. Acta agriculturae scandinavia 35: 145-152.

Strømnes, R. 1983. Maskinell markberedning og manuell planting. Landbrukets årbok 1984: 265-278.

Uhlen G. 1968. Nitrogengjødsling til ettårig raigras. Jord og avling 10(3): 5-8.

Aase, K.F., F. Sundstøl & K. Myhr 1977. Forsøk med strandrøyr og nokre andre grasartar. Forskning og forsøk i landbruket 27: 575-604.

Notice that:

- Only the first named author's initials and surname are inverted.
- The ampersand symbol is used between author's names.
- The date after the author's name is the year of publication.
- The issue number is given in parentheses after the volume number and only in cases where each issue of a particular volume begins on p. 1.
- A colon is used before page numbers in journal articles.
- The year of publication suffices where the volume number is missing.
- In references to a book, the name of the publisher and the place of publication are given after the title of the book. If more than one edition of a book has been published, the edition used must be indicated. The number of pages should be given.
- It is recommended not to abbreviate the titles of periodicals, but in cases where this has nevertheless been done, abbreviations should be in accordance with the World List of Scientific Periodicals and/or BUCOP British Union Catalogue of Periodicals.

ABBREVIATIONS

Use standard abbreviations where available, otherwise abbreviations given in the text should be explained at their first mention. Quantities and units of measurement shall be in accordance with «Systéme International d'Unites» (SI).

PROOFS

Page proofs will be sent to the author for proofreading. They should be read immediately and returned to the journal's editorial office. Printer's mistakes should be marked with a blue pen and any possible changes made by the author against the manuscript in red. The second proof will be checked at the editorial office.

OFFPRINTS

An order form for offprints, plus price list, will be sent to authors along with the first proofs. The author(s) will receive 50 offprints free of charge. This form should be filled out and returned with the first proofs to the editorial office.

Norwegian Journal of Agricultural Sciences

Vol. 7 1993 No. 2

Content Page

Statens fagtjeneste for landbruket, Moerveien 12, 1430 Ås, Norge
Norwegian Agricultural Advisory Service, Moerveien 12, N-1430 Ås, Norway

Indre Smaalenenes trykkeri A/S, Mysen