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ANNALES AGRICULTURAE FENNIAE, VOL. 27: 99—105 (1988)

Seria ANIMALIA NOCENTIA N. 127 — Sarja TUHOELÄIMET n:o 127

NON-CHEMICAL CONTROL OF CARROT RUST FLY IN FINLAND

S. KETTUNEN, I. HAVUKKALA, J. K. HOLOPAINEN and T. KNUUTTILA

KETTUNEN, S., HAVUKKALA, I., HOLOPAINEN, J. K. & KNUUTTILA, T. 1988. **Non-chemical control of carrot rust fly in Finland.** Ann. Agric. Fenn. 27: 99—105. (Agric. Res. Centre, Dept. Pest Inv., SF-31600 Jokioinen, Finland.)

The effects of carrot cultivars, ash sprinkled as an oviposition barrier, onion as a companion plant and crop rotation on carrot rust fly were studied in small garden plots. Five cultivars were tested. Variety Sytan received fewer eggs than others, while Chantenay showed significantly less root damage compared to the variety Nantes 20 Notabene. Ash sprinkled on carrot rows reduced egg numbers to ca. 1/3 of the control. Growing onion as a companion plant was not effective. Crop rotation seems an effective method for carrot rust fly control: establishment of a new carrot plot reduces root damage significantly. A novel combination of applying crop rotation together with a small trap crop at the previous cultivation site (to reduce pest movement to the new site) is suggested.

Index words: carrot, carrot rust fly, Psilidae, *Psila rosae*, oviposition, root damage, non-chemical control, resistance, ash, companion plant, onion, crop rotation, trap crop.

INTRODUCTION

The carrot rust fly *Psila rosae* F. (Diptera, Psilidae) can be a harmful pest of carrot in home gardens and small-scale cultivation in Finland. As this insect often occurs very locally and its populations vary considerably each year, it is difficult to estimate the need for chemical control, which may in turn lead to the unnecessary use of pesticides. Another problem is that some pesticides are easily absorbed by carrot oils and pesticide degradation is thus retarded. Diazinon (SUETT 1971), fensulfothion (GETZIN and ARCHER 1983) and trichloronate (TIITTANEN and VARIS 1970) can persist for a long time in carrots, and phorate persists in the soil (SUETT 1976). These difficulties have led to the search for non-chemical, alternative methods for controlling the pest.

In Finland commercial carrot growers seldom encounter problems with this pest. Non-chemical methods have been primarily developed for home gardens where damage has been the most severe and where pesticides may be applied with insufficient expertise. In addition, in home gardens control methods which are not totally reliable can be used without great economic losses (MARKKULA and TIITTANEN 1982).

Traditional methods such as crop rotation,

companion plants and the timing of seed and harvest have been employed against carrot fly.

Less susceptible carrot cultivars have also been developed (ELLIS et al. 1978, 1984). Different repellents and attractants have been separated and studied (STÄDLER 1977, RYAN and GUERIN 1982). In this study the efficiency of sprinkling ash, growing onion as a companion plant and the susceptibility of five carrot cultivars to carrot fly were tested.

MATERIAL AND METHODS

The field experiments were conducted in central Finland (Kuopio, Karttula and Vesanto) (62° 23' N) in summer 1983 and in western Finland (Nakkila) (61° 23' N) in summer 1985. In 1983 carrots were grown in loam soil mixed with peat and fertilized chemically (Puutarhan Y-lannos 1, 10 kg/a). On May 13 the seed was sown in 5-m-long double rows (8 cm apart). At a distance of 30 cm on both sides of test rows one extra row of carrots was sown. All test treatments had four replications. The crop was harvested on September 13. In Nakkila carrot was sown in organic soil on June 2 and harvested on October 12.

Sprinkling wood ash (birch and pine) on the ground around the plants was tested as a natural method for carrot rust fly control. Ash was spread 0.5 cm thick and 16 cm wide over the carrot rows twice during the growing period (June 23 and August 8). Growing onion as companion plant with carrot was also attempted. Instead of carrot rows, one row of onion was planted on both sides of the test rows. For the comparison rows no control methods were used. The carrot variety cultivated was Nantes 20 Notabene.

In the other part of the experiment the susceptibility of five carrot cultivars to *Psila rosae* was assessed. Four varieties were cultivated in the trial at Kuopio: a commonly grown early variety, Nantes 20 Notabene; an autumn variety, Nantes Duke Notabene; Nantes Clause's Sytan Original, which has resistance to carrot rust fly (ELLIS et al. 1982, 1984) and a short rooted variety, Suko sp. Baby. The varieties Nantes 20 Notabene and Chantenay (Lontoontori) were grown in Nakkila.

Nantes 20 and Sytan were also grown in two home gardens in Karttula and Vesanto. The test rows were 2 m long double rows 8 cm apart with four replications 30 cm apart. Both areas had large *Psila rosae* populations together with extensive root damage and crop losses in the previous years. In Vesanto two replications of each variety were on an old carrot field and two on an adjacent new cultivation site.

In Nakkila there were two adjacent carrot rows, 20 meters long, of the varieties Nantes 20 Notabene and Chantenay (Lontoontori). Carrot had been cultivated at the site for several years.

Flies were monitored in Kuopio by four yellow water traps (Pioneer Cont. Ltd, Feltham, Pats. Pend. England, no. 40 A, Ø 17 cm, see FORSBERG 1981). In the home gardens two yellow sticky traps at each site were used (REBELL E:FA. CH-8820 Wädenswil; 15 × 21 cm) (FORSBERG 1981).

On the basis of yellow trap catches, the oviposition of carrot fly adults was observed during the first generation (27.VI—25.VII) in Kuopio and during the second generation (25.VIII—15.IX) in Karttula. In the 4 double rows 5 plants ($n = 20$ at each date) were selected and a soil sample was taken from the soil surface around the plants (Ø 5 cm). The eggs were separated by the flotation method (HUGHES and SALTER 1959) and counted by a stereo microscope. In Kuopio 36 soil samples per treatment were taken, in Karttula 28.

At harvest time the percentage of damaged roots was counted for each area (Kuopio: n = 2941 carrots, Vesanto: n = 475, Karttula: n = 810, Nakkila: n = 2 × 100). In Vesanto, where the worst damage occurred, also the percentage of root area damaged by larvae was assessed using the scale 0, 1, 5, 10, 15, 20, . . ., 100 %

In Kuopio and Karttula the counts of eggs laid on different cultivars and carrots protected by various methods were tested by χ^2-tests. Yates' correction was applied when needed. The crops in Kuopio were handled by the analysis of variance between cultivars and control methods. A comparable analysis of variance was used for testing the crops in Karttula and Vesanto. The damage in the home gardens was tested by the χ^2-four-field test and damage percentages in Vesanto were handled by analysis of variance using arcsin-transformations to the %-values. Root damage in Nakkila was tested by χ^2-tests.

RESULTS

In Kuopio most of the eggs were found in the first half of July. Soil type made it difficult to locate the eggs, which were mostly found singly or in groups of 2—3 eggs. On carrots treated with ash only 6 eggs were found, the control plot 22.5 (the average of two control plots) and on the plot with onions 20 eggs (χ^2-test: $\chi^2 = 11.206$, df = 2, $P < 0.01$). The experiment was disturbed by the weak growth of onions. Oviposition on the variety Sytan was less than on the other varieties (Fig. 1), but in Kuopio less eggs were laid than in Karttula (total eggs = 90, $\chi^2 = 9.548$, df = 3, $P < 0.025$ and total eggs = 138, $\chi^2 = 47.54$, df = 1, $P < 0.001$, respectively).

Only 0.6 % (n = 2941) of carrots in Kuopio were damaged, precluding statistical comparisons between the control methods or tested varieties. In Vesanto the percent of damage was high, 21.9 % (n = 475) and in Karttula 4.7 % (n = 810), with no statistical differences between the Sytan and Nantes 20-varieties. In Vesanto the percentages of damaged surface on each carrot were also analyzed. Again, no differences between the varieties were found. On the old cultivation site more of the carrots were damaged (29.5 %, n = 241 carrots) than on the new site (14.1 %, n = 234). The amount of surface damaged was statistically significantly less on the new site than on the old plot (Fig. 2, analysis of variance on the percentages, F = 17.7, df = 1, 471, $P < 0.001$).

In Nakkila the percentage of damaged roots in Nantes 20 Notabene was 23 % and significantly less in Chantenay, 2 % (n = 100, $\chi^2 = 20.16$, df = 1, $P < 0.001$).

Field tests in southern Finland (Tikkurila) conducted in 1923 suggested that the Chante-

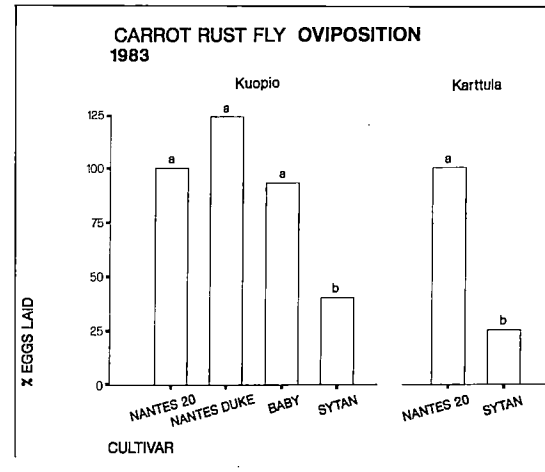

Fig. 1. The relative numbers of carrot rust fly eggs laid on various carrot cultivars in central Finland. The total number of eggs was 90 and 138 for Kuopio and Karttula, respectively. Different letters on top of the bars denote statistically significant differences ($P < 0.05$, χ^2-test).

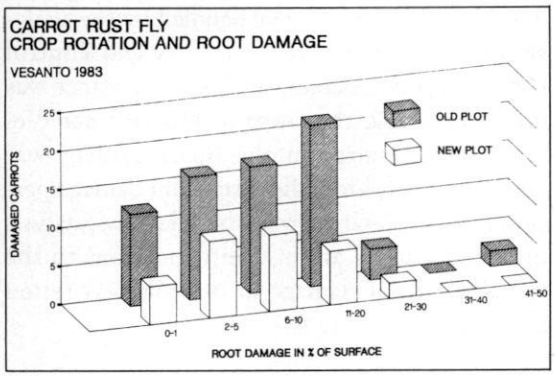

Fig. 2. Root damage in an old carrot field and an adjacent newly cultivated plot (pooled data from varieties Nantes 20 and Sytan, n = 475 carrots) in a home garden in central Finland.

nay-type variety is more heavily damaged than Nantes (PAKALEN 1947); however the exact identity of the varieties tested is not known.

The average undamaged carrot crop in Kuopio (kg/100 m ± S.E., n = 4) of untreated rows was 408 ± 47 kg, with ash treatment 391 ± 47 kg and with onion as a companion plant 365 ± 28 kg. The growing density in Kuopio was 14.4 carrots/meter, in Vesanto 14.8 and in Karttula 25.3, respectively.

DISCUSSION

Oviposition and root damage

In our experiment Sytan grew slower than Nantes 20, which could affect its attractiveness to the carrot rust fly. Sytan produced a smaller yield also in the official Finnish variety tests of 1974—1976 (PESSALA 1979). Landing of gravid females is affected by the hue of carrot leaves (BRUNEL and LANGOUET 1970) and leaf volatiles (STÄDLER 1977). Leaf hue varies with cultivar and the age of seedlings (BRUNEL et al. 1981). Leaf volatiles also influence oviposition (STÄDLER 1977). The smaller number of eggs laid on Sytan could be due to its less attractant colour, or to smaller concentrations of methyl-eugenol or other attractive substances emanating from smaller plants. GUERIN and STÄDLER (1984) found that Sytan foliage induced less egg-laying than Danvers, and suggested that contact chemicals are also involved in oviposition preference.

Although the oviposition on Sytan was ca. 40 % less than on Nantes 20, no differences were found in the amount of root damage. However, larvae may have moved between the test rows which were at a 30 cm distance from each other. Carrot fly larvae can move even 60 cm in the soil (JONES and COAKER 1980). In addition, the amount of damage in Kuopio was too small (0.6 % of the carrots) to show differences. ELLIS et al. (1984) tested Sytan and Danvers in five seasons and observed that 40—67 % fewer larvae were found in Sytan. Larval development was slower in Sytan and Sytan was also found to survive the carrot fly attack better than Danvers, suggesting more effective antibiosis.

The ultimate reason for the lesser susceptibility of Chantenay is unknown. The autumn variety employed has a shorter main root than the faster growing summer variety Nantes; ELLIS et al. (1978) have reported that varieties with shorter roots are more resistant to root damage. In addition to smaller size at oviposition time, Chantenay may contain more chlorogenic acid, which is known to correlate with resistance (COLE 1985). Resistant varieties also release less root volatiles (GUERING and RYAN 1984).

The reduction of oviposition on carrots by ash treatment parallels results on cabbage root fly (HAVUKKALA 1982). Ash also increased the mortality of *Delia radicum* -larvae and reduced

their penetration ability in the soil (HAVUKKALA et al. 1984). Ash may also affect the suitability of soil for carrot rust fly oviposition, since soil humidity and soil type control the selection of egg-laying site (BOHLEN 1967).

Crop rotation and trap crops

Crop rotation is one of the most commonly recommended cultural methods against the carrot fly. *Psila rosae* overwinters as pupae in the soil near the host plant and if carrots are grown on the same site, the flies can cause damage also during the following year. In central Europe the damage can be reduced by moving the cultivation site every year (SANT 1961, FREULER et al. 1982).

In Karttula and Vesanto the carrots were clearly much more damaged (4.7 % and 21.9 %, respectively) than in Kuopio (0.6 %). One reason could be the lack of crop rotation. In the home gardens carrots had been cultivated in the same place for many years, but in Kuopio no carrots had been grown in the area for at least two years. DABROWSKI and LEGUTOWSKA (1976) noticed that when carrot was grown for two consecutive years in the same place larval damage increased to 15 % in the second year. With crop rotation only 2 % of the carrots were damaged.

In Vesanto damage was significantly greater at the old cultivation site compared to the new, although the sites were adjacent to each other with no apparent differences in the growth of the plants. This suggests small movement distances by females. In a mark-recapture study by STÄDLER (1972) recaptured flies flew only 15 to 30 metres, staying i nearby bushes and trees. Flight distances may be small especially in the presence of host plants at the eclosion site (STÄDLER, pers. comm.), as adults accumulate at field edges adjacent to eclosion sites (WAINHOUSE and COAKER 1981), particularly in nettle-growing areas.

If young females are effectively arrested by even small plots of a host plant, this might be utilized in a crop rotation program by shifting the main cultivation area, but sowing some carrot also at the earlier site as a kind of trap crop. This would reduce the number of migrating adults reaching the crop at the new site. This novel way of combining crop rotation and trap crops might be applicable to several other pests with limited migration distances and pronounced arrestment responses at host patches. Such responses of patch-restricted search have recently been elucidated from several insects (STANTON 1983).

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SELOSTUS

Porkkanakärpäsen luonnonmukainen torjunta

KETTUNEN, S., HAVUKKALA, I., HOLOPAINEN, J. K. ja KNUUTTILA, T.

Kuopion yliopisto ja Maatalouden tutkimuskeskus

Porkkanakärpänen on porkkanakempin ohella harmillinen tuholainen etenkin pienpalstoilla ja kotipuutarhoissa. Se esiintyy satunnaisesti, mutta sitä voi paikoitellen olla runsaastikin. Ammattiviljelmillä tuhot ovat yleensä vähäisiä.

Tämä tutkimus kuuluu osana Maatalouden tutkimuskeskuksen tuhoeläinosastolla tehtyihin selvityksiin biologisten ja bioteknisten menetelmien kehittämiseksi torjunta-aineiden avuksi ja niiden sijaan avomaan viljelyksille.

Kenttäkokein selvitettiin porkkanalajikkeiden alttiutta porkkanakärpäsen tuhoille, tuhkan käyttöä munintaesteenä ja sipulin tehoa seurakasvina. Kaksi lajiketta, Sytan ja Lontoontori (Chantenay), osoittautuivat osittain vastustuskyisiksi. Mekaanisena munintaesteenä puutuhka siroteltuna porkkanariveihin vähensi porkkanakärpästen munintaa selvästi. Sipuli seurakasvina tehosi huonosti. Paras munintaeste olisi suojaverkko, jolloin porkkanakempinkin vioitukselta vältyttäisiin. Kiertoviljely suuremmilla aloilla on hyvä keino: palstan siirtäminen lyhyenkin matkaa uuteen paikkaan vähentää oleellisesti juuristotuhoja. Kotipuutarhoissa välivuoden pitäminen auttaa. Tuholaisen siirtymistä uudelle viljelyalueelle voidaan kasvinvuorotusta käytettäessä yrittää estää myös kylvämällä vähän porkkanaa vanhalle palstalle pyydyskasvustoksi.

ANNALES AGRICULTURAE FENNIAE, VOL. 27: 107—116 (1988)

Seria PHYTOPATHOLOGIA N. 106 — Sarja KASVITAUDIT n:o 106

BIOLOGICAL CONTROL OF *BOTRYTIS CINEREA* AND *RHIZOCTONIA SOLANI* IN LETTUCE BY *STREPTOMYCES* SP.

RISTO TAHVONEN and MARJA-LEENA LAHDENPERÄ

TAHVONEN, R. & LAHDENPERÄ, M.-L. 1988. **Biological control of *Botrytis cinerea* and *Rhizoctonia solani* in lettuce by *Streptomyces* sp.** Ann. Agric. Fenn. 27: 107—116. (Agric. Res. Centre, Dept. Plant Path., SF-31600 Jokioinen, Finland.)

The suitability of a *Streptomyces* strain isolated from light-coloured *Sphagnum* peat for controlling *Botrytis cinerea* and *Rhizoctonia solani* on lettuce was studied on a peat substrate in a greenhouse. Either the seedlings or substrate were severely infected by the fungus. The decrease in yield was considerable. Treatment of the seedlings with a spore suspension prepared from the *Streptomyces* isolate significantly reduced yield losses caused by *B. cinerea* but had no effect on those caused by *R. solani*.

Index words: *Streptomyces* sp., *Botrytis cinerea, Rhizoctonia solani,* biological control, lettuce.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a major greenhouse crop in Finland. It is cultivated by planting seedlings grown under artificial light at the end of January or beginning of February when the natural lighting conditions are not suitable for plant growth. Under such conditions, *Botrytis cinerea* Pers. ex Fr. is the most common and serious fungal pathogen that affects both seedlings after planting and the final crop (FLETCHER 1984). Although *B. cinerea* is the most serious disease of lettuce in greenhouse cultivation in Finland, present regulations prohibit chemical control even on small seedlings at the beginning of the growing season (BLOMQVIST et al. 1987). Usually, the control of *B. cinerea* on lettuce can be successfully accomplished by suitable cultivation techniques such as growing the seedlings under optimum conditions, covering the substrate with perforated white plastic sheeting, and planting the seedlings into the top of the substrate so that the root collar remains dry during cultivation. In this case the fungal pathogen is only a problem at the end of the harvesting stage.

Rhizoctonia solani Kuhn, which causes lettuce bottom rot, has appeared as a new disease on certain crops in Finland since the end of the 1970's, and in some areas has caused considerable financial losses. The disease has been most common in the Helsinki area and in Teuva. It is not possible to control the fungus during cultivation because those chemical control

methods which have been used succesfully in many countries (FLETCHER 1984) are forbidden in Finland.

Streptomyces sp., isolated from light-coloured *Sphagnum* peat, has proved to be strongly antagonistic, both *in vitro* and *in vivo*, to a number of fungal plant pathogens (TAHVONEN 1982). *B. cinerea* causes damage to lettuce especially close to the ground and during the harvesting stage when there is usually sufficient moisture for the growth of most microbes. It was thus thought that biological control by *Streptomyces* would also be possible as in preliminary *in vitro* experiments, *B. cinerea* has proved extremely sensitive to the antibiotic excreted by *Streptomyces* isolates. Experiments in the control of *Botrytis* and *Rhizoctonia* were carried out at the Department of Plant Pathology, at the University of Helsinki and at the Department of Plant Pathology, at the Agricultural Research Centre, during 1981—1984.

MATERIAL AND METHODS

Lettuce plants were grown in new, fertilized, light-coloured *Sphagnum fuscum* peat in a greenhouse in accordance with current techniques for growing lettuce in Finland (FORSELL 1972). The size of the plots varied, depending on the experiment, from 1—2 m^2. Seedlings were planted at a spacing of 20 × 20 cm. Plots were separated from the underlying soil by plastic sheeting, and from neighbouring plots by a 20 cm-high barrier. The lettuce variety was Ostinata. Peat-pot seedlings were planted without the white plastic covers usually used in early cultivation, the edges of the pots being below the surface of the substrate. By this method the danger of *B. cinerea* infection is usually greater.

The control of *B. cinerea* and *R. solani* in lettuce was studied by *Streptomyces griseoviridis* Anderson et al. isolated from light-coloured *Sphagnum* peat. A spore suspension of the *Streptomyces* isolate, prepared by growing the microbes on agar was used at a concentration of 10^8 CFU/ml (TAHVONEN 1982) in the initial experiments (Table 1). Seedlings were sprayed with about 0.5 ml suspension/seedling just before planting, and then the seedlings and surrounding soil with 25 ml suspension/m^2 immediately after planting. The soil surface in one of the experimental treatments was sprayed again on two occasions, at an interval of two weeks, including the planting date. Every second row of seedlings was inoculated on two occasions, at 2-week intervals, with a suspension of *B. cinerea* following planting. The inoculum (1 PDA plate/200 ml) was pipetted at the dose of 1 ml/seedling over a semicircular area (radius 1—2 cm) around the base of the seedling.

A standard preparation containing 10^8—10^9 CFU/ml was used in the subsequent experiments. *Streptomyces* isolate was grown in a nutrient solution (4 g yeast extract, 10 g malt extract and 4 g glucose/l) in Rhoux bottles. After transfer, the bottles were either shaken 2—4 times a day for 3—4 days, or kept on a water-bath shaker for 24 h. The bottles were maintained in a horizontal position, broad side down, and the microbe formed a dense mass of conidia on the surface of the liquid within 4—7 days. The conidial mass was separated from the liquid by centrifugation.

The conidia and mycelial mass was washed twice with sterilized water, and finally separated by centrifugation to yield a porridge-like mass which was stored at –20 °C. The standard preparation contained 1 g of this mass/100 ml H_2O. The dilutions used in treating the seedlings and soil were treated with it as shown in the tables.

Table 1. Effect of the suspension of *Streptomyces* sp. spores on *Botrytis cinerea* and *Rhizoctonia solani* on lettuce, and the damage caused by these pathogens. Preliminary experiments, 1981.

Plant inoculation		Untreated	Treatment of the plants with *Streptomyces*		F-value
			at planting[2)]	at planting[2)]+ on two occasions at an interval of two weeks	
Infection of the plants with *Botrytis*	yield (g/plant)	100 (110)	130	138	11.34**
	disease index, 0—4	2.22	1.45	1.43	6.93*
Uninfected plants growing next to ones infected with *Botrytis*	yield (g/plant)	100 (158)	108	109	<1
	disease index, 0—4	0.93	0.63	0.48	1.57
Infection of the plants with *Rhizoctonia*	yield (g/plant)	100 (157)	117	93	<1
	disease index, 0—4	1.60	1.53	1.28	<1
Uninfected plants growing next to ones infected with *Rhizoctonia*	yield (g/plant)	100 (176)	98	97	<1
	disease index, 0—4	0.57	0.60	0.39	<1

1) Infection with the pathogen done immediately after planting, and on two occasions at an interval of two weeks, with 1 ml of the fungal suspension (1 PDA dish/200 ml) over a semicircular area at the base of every seedlings at a distance of 1—2 cm from the roots.

2) Seedlings sprayed with the suspension of *Streptomyces* spores before planting, and spraying of the seedlings and soil immediately after planting.

In the experiments seedlings were sprayed with dilutions made from the above preparation, and/or the treatment was repeated during the growing season. The dilutions and amounts used in spraying are given in the tables. The experiment shown in Table 4 also included treatment with vinclozoline fungicide which was used as a comparison for the results of biological control.

In the experiments presented in Table 2 and 3, *B. cinerea* was allowed to spread naturally onto the plants. In the experiment shown in

Table 2. Effect of the standard *Streptomyces* preparation on the lettuce yield and the abundance of *Botrytis cinera* in winter 1983.

	Untreated	Wetting the seedlings before planting with a 1 % dilution of the preparation, 1/1000 seedlings				Spraying + respraying[1]
		4	8	20	4 + spraying[1]	
yield (g/plant)	100 (119)	107	116	116	105	105
disease index, 0—4	1.46	0.88	1.03	1.01	1.02	1.25
dead and severely infected, %	25.0	8.3	11.7	10.0	13.0	16.7

F-value: yield = 0.60, disease index = 0.93, dead and severely infected = 2.12

$LSD_{t0.05}$ dead and severely infected = 14.3

1) Spraying of the plants and soil surface immediately after planting with a 1 % dilution of the preparation, 1 dl/m², and the same treatment in the respraying two weeks after planting.

Table 3. Effect of the standard *Streptomyces* preparation on the lettuce yield and the abundance of *Botrytis cinerea* in winter 1984.

Streptomyces sp. Isolate No.	Wetting the seedlings (1/100 seedlings) with the diluted preparation (%)				Spraying the plants immediately after planting, 0.1 %,
	8 1/0.1 %	8 1/1.0 %	20 1/0.1 %	20 1/1.0 %	0.1 l/m²
	Yield, untreated 100 (123 g/plant)				
6	105	119	104	104	93
78	107	111	107	103	118
87	115	114	123	112	123
$\bar{x}$	109	115	111	106	111
	B. cinerea abundance 0—3, untreated 1.18				
6	1.19	0.85	1.47	1.28	1.58
78	1.10	1.07	1.20	1.07	0.98
87	1.27	0.95	0.85	0.87	0.96
$\bar{x}$	1.19	0.96	1.17	1.07	1.22

$LSD_{t0.05}$: yield = 23, disease index = 0.69

Table 4. Effect of the standard *Streptomyces* preparation and vinclozoline on the lettuce yield and the abundance of *Botrytis cinerea* in winter 1984.

Mode of application	*Streptomyces* isolate No.	*B. cinerea*-%	Disease index	Yield (g/plant)
Untreated		34.7	1.18	100 (136)
Seedlings treatment[1)]	6	22.7	0.96	98
—»—	61	25.3	1.07	89
—»—	65	36.0	1.16	95
Soil treatment[2)]	6	24.0	1.09	91
—»—	61	20.0	1.04	88
—»—	65	14.7	0.75	94
Seedling treatment, vinclozoline[3)]		5.3	0.49	106
	F-value	<1	<1	2.04

1) 1 % dilution of the standard preparation, 4 l/1000 seedlings, just before planting

2) Spraying the soil surface following planting with a 1 % dilution of the standard preparation, 1 dl/m^2

3) 0.05 % dilution of Ronilan, 4 l/1000 seedlings, just before planting

Table 4 seedlings were inoculated in the germling stage, 10 days after sowing, by wetting the plants with a conidial and mycelial suspension made by mixing a petri dish (∅ 9 cm) containing two week-old fungus, without agar, with 200 ml of water.

Experiments similar to those in the control of *B. cinerea* were also carried out for the control of *R. solani.* In these experiments inoculation was performed about one week before planting by moistening the substrate with the fungal suspension (PDA dish/5 l/m^2). In the initial experiments, the soil surrounding the lettuce seedlings was inoculated after planting, as for *B. cinerea.* Since the treatments were not effective against *R. solani,* only the results of the preliminary experiment, and those of one experiment, are presented in the tables.

Weight of the merchantable crop and degree of plant infection were determined at the end of the growing using the scale 0—3 or 0—4, where 0 is healthy and the highest value indicates either a dead plant or one completely spoilt by the fungus. The effects of the treatments were tested by analysis of variance and the effect of the fungus on the yield by linear regression in which the healthiest experimental treatment in each experiment was given the yield percentage 100.

RESULTS

B. cinerea caused typical rotting of the lettuce head during maturation (Fig. 2). The degree of fungal infection varied considerably, by tens of percent, between different replicates in the same experimental treatment, despite all plots being subjected to exactly the same growing conditions. Compared to the treatments with healthy plants the largest yield reductions were on the order of 50 %. There was an extremely significant ($R = -0.695$) negative correlation between crop weight and frequency of *B. cinerea* (Fig. 1).

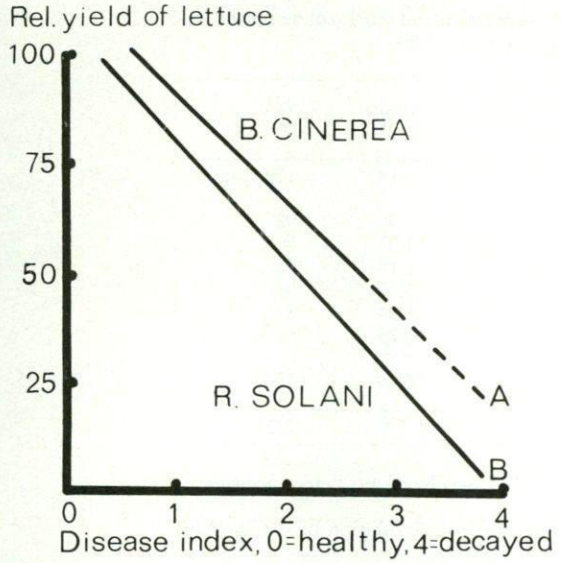

Fig. 1. The effect of *Botrytis cinerea* (A) and *Rhizoctonia solani* (B) abundance on the size of the lettuce yield.
A: $y = -24.5x + 116.8$, $r = -0.695$, $N = 71$
B: $y = -27.2x + 107.7$, $r = -0.962$, $N = 52$

Fig. 2. Spoilage caused by *Botrytis cinerea* to full-grown lettuce heads.

In the preliminary experiments (Table 1), treatment of the seedlings before and after planting with the suspension of *Streptomyces* spores increased the yield by 30 %, and considerably decreased infection of the yield by *B. cinerea* in plants artificially infected by the fungus.

The trend was the same for the uninfected plants, although the result was not statistically significant. Additional treatments carried out twice at an interval of two weeks after planting did not further increase the yield in comparison to the seedling treatment. In the follow-up study in which treatments were performed by a stable preparation, treatment of the seedling with a suspension of *Streptomyces* spores before planting increased the yields in 1982 and 1983 by 2—23 % (on the average 11 %). However, owing to the variation between the replications the result was not statistically significant. The proportion of dead and severely infected plants among those treated in the 1982 experiment (Table 2) was smaller than that for untreated ones (Fig. 4). Spraying the surface of the substrate gave a similar or poorer result in the control of *B. cinerea* than when the seedlings were only treated before planting. Since the seedlings grew very vigorously in the 1984 experiment and were only slightly damaged by *B. cinerea*, the effect on the yield was small. The variation in the frequency of *B. cinerea* in this experiment was great. The number of plants either moderately of severely infected in the different replications in the untreated seedlings varied from 8 to 40 %. The corresponding values for the treated seedlings were 0—40 %, except for one plot which was 76 %. Treating the seedlings with fungicide before planting appeared to be very effective in the control of *B. cinerea* — 35 % of the untreated plants were infected by the fungus compared with 5 % of those treated with vinclozoline.

R. solani initially caused reddish-brown or brown rotting (Fig. 3) at the base of the plants and on the outermost leaves in contact with the soil. The lettuce heads were eventually completely spoilt. Fungal mycelia were clearly visible to the naked eye on the lettuce heads and on the surface of the substrate. This pathogen severely reduced lettuce yield. The coefficient of correlation between the degree of infection of the yield and the weight of the yield was -0.962, i.e. the degree of infection completely explained the decrease in the yield (Fig. 1).

In the preliminary experiments, *Streptomyces*

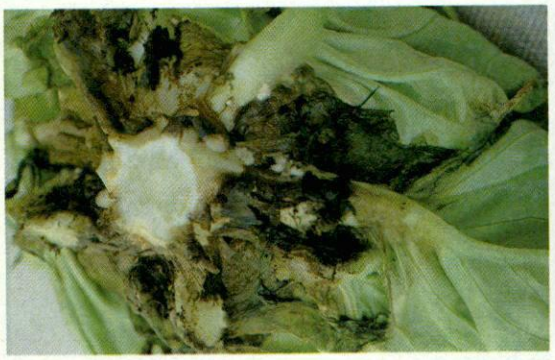

Fig. 3. Damage caused by *Rhizoctonia solani* to lettuce.

treatment slightly increased the yield and decreased the frequency of the pathogen, although the result was not statistically significant (Table 1). The amount of infection by *R. solani* was small. *Streptomyces* preparation had no effect on *R. solani* infection on lettuce in the follow-up experiments in which the pathogen was sprayed onto the substrate before planting (Table 5). Three experiments of the type shown in Table 5 were carried out. However, the results are not included in tabular form because they were always approximately the same. In these experiments, the fungal pathogen almost completely prevented the production of a merchantable crop.

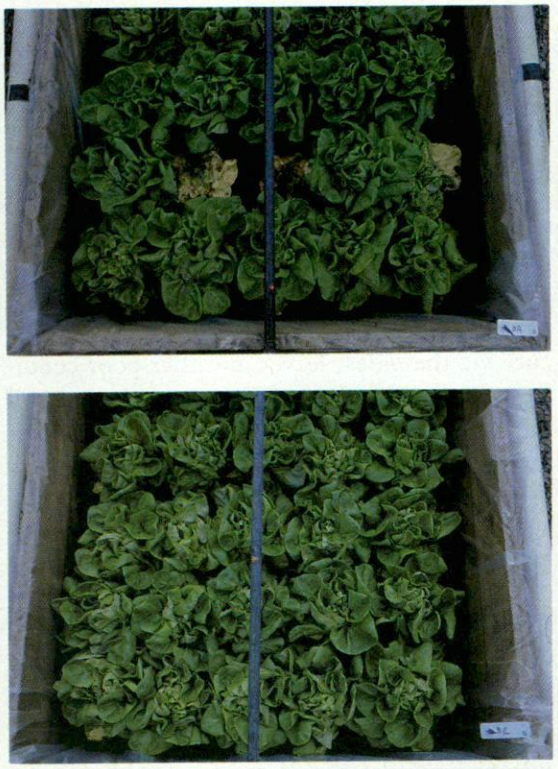

Fig. 4. A. An untreated lettuce plot with a large number of dead lettuce and plants severely damaged by *Botrytis cinerea.*

B. A plot with lettuce seedlings treated before planting with the suspension of *Streptomyces* spores.

Table 5. Effect of the standard *Streptomyces* preparation on the yield and the amount of *Rhizoctonia solani* on lettuce in autumn 1982.

Treatment	Dilution of the standard preparation	Disease index, 0—4	Yield 100 = healthy plants (110 g/plant)
Seedlings before planting *)	1 %	3.5	15
	5 %	3.4	6
The substrate after planting *)	0.1 %	3.4	15
	1 %	3.1	28
The substrate after planting + 2 additional treatments at an interval of two weeks *)	1 %	3.2	23
Untreated		3.3	19
	F-value:	<1	1.8

*) Spraying of the seedlings and seedlings and substrate, 1 dl/m^2

DISCUSSION

Treating lettuce seedlings with a suspension of *Streptomyces* spores significantly reduced the frequency of *B. cinerea* on the yield, and correspondingly increased the size of the merchantable crop in a number of the experiments. Such a result by biological control is, in a way, theoretically rather surprising since *B. cinerea* usually infects the above-ground parts of lettuce via the oldest leaves in rather poor condition (FLETCHER 1984). *Streptomyces* are soil microbes hence it would be expected that their capacity to control pathogens on the above-ground plant parts would be rather limited. When treating plants with the *Streptomyces* suspension it is natural that the spores are spread over all parts of the plant and especially on the surface of the substrate. Since the oldest leaves of the plants are in contact with the peat and, being protected by the younger leaves represent a rather moist environment, such conditions could be considered favourable for the growth of *Streptomyces* and production of the antibiotic and thus restrict the growth of *B. cinerea*. The old, dead leaves may also act as a growth substrate for the antagonist, and hence prevent or reduce infection by *Botrytis*.

Treatment of the seedlings was found to stimulate plant growth in the same experiments. Fast and uninterrupted growth of the plant is also important with regard to the control of *B. cinerea* (FLETCHER 1984). *Streptomyces* bacteria have also been found to have a stimulating effect on other plants (MERRIMAN et al. 1974a, 1974b). This growth-stimulating effect may also be a factor contributing to the reduction in *B. cinerea* and increase in the yield. However, it is *B. cinerea* that plays the decisive role in determining the size of the yield, as shown by the extremely significant negative correlation ($r = -0.69$) between the size of the yield and the degree of infection.

The *Streptomyces* spore suspension had no effect on bottom rot caused by *R. solani*, although good or satisfactory results have been obtained with this preparation in the control of *Rhizoctonia* damping-off on cabbage (TAHVONEN 1982). Antagonists of the *Streptomyces* species have also been found to have an effect on *R. solani* in other studies, too (YDIN et al. 1955, 1965, MERRIMAN et al. 1974a, 1974b). The type of *R. solani* which occurs on lettuce in Finland differs decisively from that found on cruciferae. Its growth rate on PDA medium is about double that of the strains isolated from cruciferous plants. The anastomosis group of the *R. solani* isolated from lettuce was AM-1, while that for crucifers was AM-2 (HOLLO 1985). In addition, the fungus grew as mycelia visible to the naked eye on the surface of the peat medium. No such observation has been recorded for *R. solani* strains isolated from crucifers. It would thus appear from the above that the *R. solani* on lettuce grows too fast for successful biological control by *Streptomyces*.

There are relatively few references to the biological control of *B. cinerea* in the literature. Studies have been carried out on apples, strawberries and wine with different species of *Trichoderma* (TRANSMO and RAA 1977, DUBUS et al. 1978, TRANSMO and YSTAAS 1980, TRANSMO 1981, DUBUS 1982). *Actinomycetes* species have also been used in the biological control of *B. cinerea* on wine. The result has been as good as that obtained with folpet fungicide (PANAIOTOV et al. 1977).

Cultivation techniques are crucial in the control of *B. cinerea* on lettuce (FLETCHER 1984), and the prospects of achieving control by modern methods are good. Present-day heating and moistureregulation systems, the use of perforated plastic sheeting as a cover for the substrate during planting, containerized seedlings which permit planting at a depth that keeps the root collar dry, the production of seedlings which are not overly large for planting and good illumination, normally ensure suffi-

cient protection against *B. cinerea*. However, the risk of infection by this pathogen is great in the type of old-fashioned greenhouses which are still widely in use. This is especially the case during the first planting in the winter when light conditions are poor. Therefore the need for the development of biological control methods is considerable, especially since chemical control is prohibited by strict legislature concerning fungicide residues. These experiments indicate that *Streptomyces* would be a promising antagonist. However, the method still requires further development especially in the case of a particular commercial application which has proved to be as effective in seed dusting as the spore-suspension preparation used in the present experiments (TAHVONEN 1986).

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SELOSTUS

Botrytis cinerea ja *Rhizoctonia solani* -sienien biologinen torjunta salaatilta *Streptomyces* -mikrobilla

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Maatalouden tutkimuskeskus ja Kemira Oy

Vaaleasta rahkaturpeesta eristettyä *Streptomyces* sp. bakteeria käytettiin torjuntapieneliönä salaatinharmaahomeen (*Botrytis cinerea*) ja salaatinseittimädän (*Rhizoctonia solani*) haittojen vähentämiseksi. Kasvualustan pinta ja taimet ruiskutettiin istutuksen jälkeen tai pelkät taimet käsiteltiin ennen istutusta *Streptomyces* -itiösuspensiolla. Taimet tai kasvualusta keinosaastutettiin taudinaiheuttajalla tasaisen infektion varmistamiseksi kokeissa.

Taimien käsittely ennen istutusta tai taimien ja maan käsittely heti istutuksen jälkeen vähensivät salaatinharmaahomeen aiheuttamia vioituksia ja lisäsivät satoa. Lisäkäsittelyt kasvukauden aikana eivät parantaneet torjuntatulosta. Taimien kemiallinen käsittely vinklotsoliini-valmisteella ennen istutusta antoi paremman torjuntatuloksen kuin biologinen torjunta. *Streptomyces* -käsittelyllä ei ollut vaikutusta salaatinseittimädän torjunnassa.

Suoritettujen kokeiden perusteella biologisella torjunnalla voidaan vähentää salaatinharmaahomeen haittoja, mutta tulos ei ole tehtyjen kokeiden perusteella riittävän hyvä käytäntöä ajatellen. Kokeessa mukana ollut kemiallinen torjuntaruiskutus taimille ennen istutusta oli riittävän tehokas harmaahomeen torjumiseksi, mutta menetelmä vaatii vielä jatkotutkimuksia mm. jäämäkokeiden muodossa. Tehdyt harmaahomeen torjuntakokeet osoittivat epäsuorasti, että taudinaiheuttajan merkittävä leviämistie on istutettavat taimet, jolloin myös torjuntatoimet on kohdistettava istutushetkeen tai ajankohtaan juuri ennen istutusta.

ANNALES AGRICULTURAE FENNIAE VOL. 27: 117—124 (1988)

Seria AGROGEOLOGIA ET -CHIMICA N. 150 — MAA JA LANNOITUS n:o 150

LIME AND BARK ASH FOR RED CLOVER

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HUOKUNA, E., HIIVOLA, S.-L., SIMOJOKI, P. & ETTALA, E. 1988. **Lime and bark ash for red clover.** Ann. Agric. Fenn. 27: 117—124. (Agric. Res. Centre, South Savo Res. Sta., SF-50600 Mikkeli, Finland.)

According to a study carried out in 1978—81 at the research stations of the Agricultural Research Centre in North Savo (PSA), South Ostrobothnia (EPO), Central Finland (KES) and South Savo (ESA), 5 t/ha of dolomitic lime mixed with the top soil layer raised the pH of soil in the surface layer (0—5 cm) by 0.3, in the 5—10 cm layer by 0.3 and in the 10—20 cm layer by 0.1 units. The respective figures after treatment with bark ash were 0.4, 0.4 and 0.1 pH units.

Lime spread on the soil surface increased the pH of the highest layer by an average of 0.4 and ash by 0.7 units. Measurements were carried out three years after the application.

The treatments increased the calcium and magnesium contents of the soil significantly. Liming decreased the potash figure in the fertility study.

The acidity of soil before treatment had a crucial effect on the dry matter yield of clover. Clover hardly grew in acidic soil. All treatments, even surface liming, improved the yield considerably, but in those sites where the soil pH was at least 5.7, no amount of lime improved the yield. On the contrary, lime spread on the surface caused a distinct reduction in yield during the first year, but it levelled out in the second year. In most cases, ash had a slightly more favourable effect than dolomitic lime.

Clover grew well when the surface layer of soil (down to 5 cm) had a sufficiently high pH; the bottom layer could be acidic. Liming increased the magnesium content of the yield to some extent and decreased the potassium content. These treatments had very little effect on the calcium and phosphorus contents.

Lime and particularly ash increased the clover yield and its protein content.

Index words: lime, bark ash, red clover.

INTRODUCTION

The acidity and nutrient contents of Finnish fields are, on average, sufficient for the growth of red clover (KURKI 1982). There are, however, many fields where, much as it would be desirable, clover cannot be cultivated mainly because the plough layer is too acidic, being less than pH 5.7 (VIRTANEN 1931). The present cultivation methods (HUOKUNA and LAPIO-

LAHTI 1980) and acid rain (SOVERI 1985) increase the acidity of soil rather rapidly. Observations and preliminary studies in the fields of experimental farms have shown that conventionally cultivated fields, especially those with coarse fine sand soil, will contain spots where a low pH and a scarcity of certain nutrients prevent the growth of clover (HUOKUNA 1978).

In accordance with indications given by preliminary research, this study was carried out to establish the quantities of dolomitic lime and bark ash that would be needed and the way they should be applied to improve the growth of red clover in various kinds of soil.

MATERIAL AND METHODS

The study was carried out in 1978—81 at the following research stations of the Agricultural Research Centre: North Savo (PSA), South Ostrobothnia (EPO), Central Finland (KES) and South Savo (ESA). The soils and their fertility at the outset of the experiment were:

Place and trial soil		humus %	pH	Ca	K	Mg	P
				mg/l of soil			
PSA	fine sand	5	5.7	1100	260	80	15.0
EPO 1	silty fine sand	5	4.8	280	200	55	5.5
EPO 2	humous fine sand	15	4.9	660	150	85	5.9
KES	silt	6	6.3	1500	100	250	17.5
ESA	fine sand	6	6.5	1900	75	120	11.5

The fields were sown in 1978. The experimental design was split-plot with four replications. Each plot measured 2.5 × 10 m. In the main plots, 0 and 5 t/ha of both lime and ash (in ESA there was additionally 10 t/ha of both) were mixed with the top soil. The surfaces of the plots were spread with 0, 1.5 and 5 t/ha of dolomitic lime and bark ash. The lime treatments were carried out simultaneously before the clover was sown.

The contents of the dolomitic lime were (g/kg) Ca 208, Mg 83 and its neutralization capacity was 34.5 %. The corresponding figures for bark ash were Ca 294, Mg 26, K 44, P 14 and S 7. There the neutralization capacity of Ca was 35 %. In addition, the ash contained (mg/kg) Zn 1650, Cu 139, B 296, Mo 2.7 and Co 170.

The red clover, variety Tepa, was sown as such (10 kg/ha) after inoculation with Valio's rhizobium. Barley was used as nurse crop. In the years when ley was grown, 400 kg/ha of PK fertilizer (containing micronutrients) was applied annually.

During the experiment the weather did not differ much from long-time averages at any of the places. There were no long dry periods or excessively heavy rains. The mean temperature in May—September was about 12.5 °C, the rainfall 300 mm and the effective temperature sum (> 5 °) about 1200 °C.

The crop was harvested twice annually, the first time when the clover started flowering at the beginning of July and the second time at the end of August. The mineral contents of the feed yields were analysed at the Central Laboratory of the Agricultural Research Centre, but the laboratory did not then have the capacity to handle more than the samples of a few experiments. After the end of the experiment in 1981, the nutrients and pH were determined by layer (0—5, 5—10 and 10—20 cm) from one

plot replication of each experimental site. The analysis took place at the Department of Soil Science of the Agricultural Research Centre. The variance analysis of the yield results was calculated for each experiment by the Computing Center of the Agricultural Research Centre.

RESULTS

Effect of treatments on soil acidity and nutrient content

With respect to top soil acidity, the experimental fields made up two clearly distinguishable groups. The EPO plots were in acidic land, pH 4.8 and 4.9. In other experiments the corresponding figure was at least 5.7. The 5 t/ha of lime and the same quantity of ash mixed with the top soil layer increased the pH of the soil layer (as measured in the autumn of the second year) in the 0—5 cm layer by 0.3 and 0.4; in the 5—10 cm layer by 0.3 and 0.4 and in the 10—20 cm layer by 0.1 and 0.1 units, respectively (Table 1). The highest rise took place in the two EPO experiments.

After spreading the surfaces of the EPO, KES and PSA fields with 1.5 t/ha of dolomitic lime, the pH of the 0—5 cm surface layer increased by an average of 0.2 units. A five-tonne application increased the figure by 0.4 units. Bark ash was somewhat more effective in removing acidity, as the corresponding figures were 0.2 and 0.7. In the ESA field the result was the opposite, lime decreasing the pH of surface soil by 0.5 and ash by 0.6 units. An attempt was made to mix the initial liming with the whole depth of top soil by harrowing. Only rarely, however, was the effect of the treatments felt even in the 5—10 cm layer; there was no effect at all in the bottom layer of the top soil at the depth of 10—20 cm.

Table 1. The effect of initial lime and ash (5 t/ha) treatments on the changes (+-) in pH and content of some minerals (unit mg/l) in different soil layers. Average of all experiments in 1981.

	Treatment	Layer 0—5 cm	5—10 cm	10—20 cm
pH	Lime	+ 0.3	+ 0.3	+ 0.1
	Ash	+ 0.4	+ 0.4	+ 0.1
Ca	Lime	+ 284	+ 252	+ 81
	Ash	+ 440	+ 388	+ 79
Mg	Lime	+ 69	+ 74	+ 15
	Ash	+ 20	+ 17	+ 2
K	Lime	- 18	- 11	- 9
	Ash	+ 12	+ 10	+ 4
P	Lime	+ 1.5	+ 2.7	+ 2.1
	Ash	+ 3.6	+ 4.6	+ 3.8

Without the initial treatment, the Ca content of the top soil layer was 950—1350 mg/l in the EPO field and 1600—1800 mg/l in the other sites. A five-tonne lime application increased it by an average of 300 and ash by 440 units (Table 1). Spreading the surface with 1.5 t/ha of lime enhanced the Ca figure on average 210 units and five tonnes 480 units. The corresponding changes caused by ash were 290 and 1100 units (Table 2). The Mg figures increased by 35 and 120 mg/l with lime and by 30 and 60 mg/l with ash. In the deeper, 5—10 cm layer, the effect of surface treatments could be observed in both the Ca and Mg figures, but the trend was non-uniform, and the average figures were about one third of those above. In all cases, the potassium content of the top soil decreased in plots treated with dolomitic lime.

Table 2. The effect of surface spreading of lime and ash on the changes (+-) in the Ca and Mg content (unit mg/l) of the upper layer (0—5 cm) of the soil. Average of all experiments in 1981.

Treatment	Ca	Mg
Lime 1.5 t/ha	+ 210	+ 35
Ash —"—	+ 290	+ 30
Lime 5 t/ha	+ 480	+ 120
Ash —"—	+ 1100	+ 60

Yields

No differences were detected in the colour and luxuriance of the plant stands on the various experimental sites. Nor was there any difference in wintering, except in EPO, where the untreated plant stands and those that had received 1.5 t/ha of lime or ash wintered clearly more poorly than those that had received 5 t/ha.

There were considerable differences in yield between the different experiments, although the yields that are usually obtained from the well tended fields of all these experimental sites are almost the same. In acidic soil, pH 4.9, the yield in plot 0 was very low, and the initial treatments and surface application increased the yields substantially (Fig. 1). The yield, which in the first year was 5300 kg/ha, rose to 7800 kg/ha in the second year. In both experiments, the highest yields were obtained in the second year. By contrast, in all experiments where the soil pH was 5.7 or higher (ESA, PSA and KES), the yield was relatively high (8700 kg/ha) in the first year, but fell sharply in the second year. The initial treatments did not cause any statistically significant changes to yields. In-

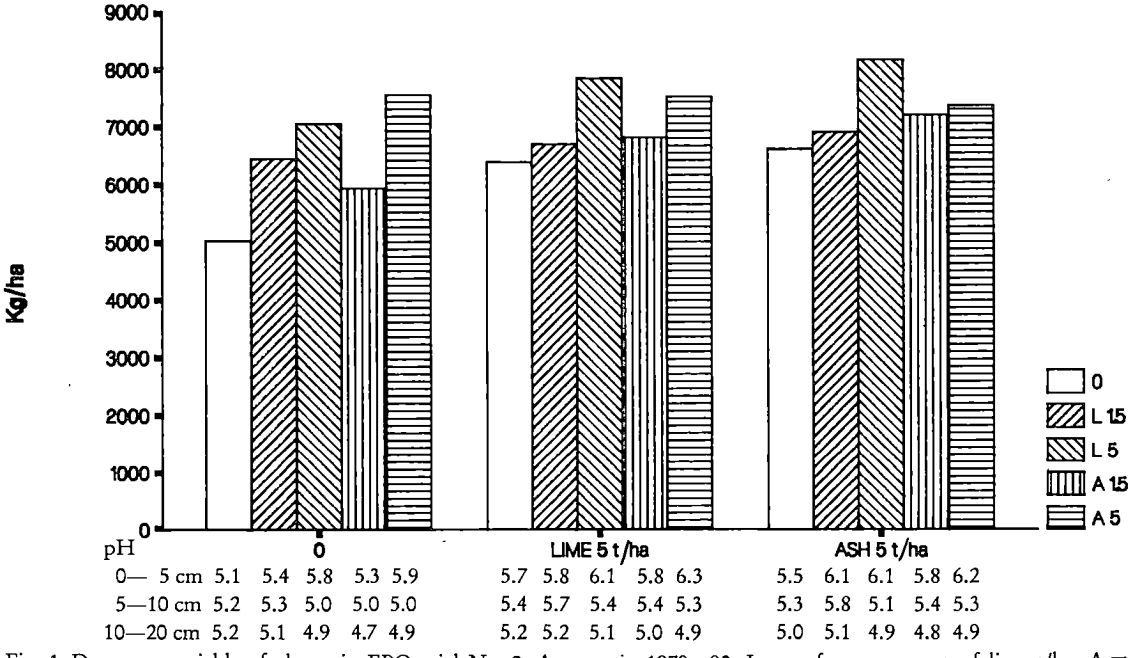

Fig. 1. Dry matter yields of clover in EPO trial Nr. 2. Average in 1979—80. L = surface treatment of lime t/ha. A = surface treatment of bark ash t/ha. In the bottom are pH figures of soil in different layers in 1981.

Table 3. The effect of lime and ash on the dry matter yield of clover in the first year in soils with a high pH (over 5.7). The ESA, KES and PSA experiments, on average, in 1979.

	Initial treatments			
	0	Lime 5 t/ha	Ash 5 t/ha	Average
Surface spreading				
0	7480 a	7420 a	7500 a	7470 a
Lime 5 t/ha	6740 b	6610 b	6670 b	6670 b
Ash —"—	7990 a	8040 a	8230 a	8090 c
Average	7400	7360	7470	7410

F-values initial treatments 0.04^{0} NS, surface treatments 19.57^{xxx}, interaction 0.08^{0} NS. LSD in surface treatments 500 kg/ha.

Table 4. The effect of lime and ash on the dry matter yield of clover in the second year. The ESA and PSA experiments on average. The yield level in ESA 4800 and in PSA 6200 kg/ha.

	Initial treatments			
	0	Lime 5 t/ha	Ash 5 t/ha	Average
Surface spreading				
0	5260	5240	5690	5400
Lime 5 t/ha	5270	5770	5710	5580
Ash 5 t/ha	5290	5590	5620	5500
Average	5270	5530	5670	5490

Table 5. The effect of lime and ash (5 t/ha) on the protein content and protein yields. PSA 1980.

Surface spreading	Initial treatment: 0			Lime			Ash			Average yield kg/ha
	Protein-%		Yield kg/ha	Protein-%		Yield kg/ha	Protein-%		Yield kg/ha	
	1. cut	2. cut		1. cut	2. cut		1. cut	2. cut		
0	9.3	16.6	580	7.7	18.3	490	9.1	18.0	620	560
Lime	9.5	16.4	640	9.3	18.5	640	9.6	19.8	660	650
Ash	10.3	19.1	660	10.6	17.1	710	9.6	18.9	640	670
Average			630			610			640	630

stead, the application of dolomitic lime to the surface decreased the yield at all these sites in the first year (Table 3). This finding was no longer observed in the experiments in ESA and PSA in the second year (Table 4). In the KES experiment the same trend continued, but the yields were already so low that the result seems uncertain. Ash had a more favourable effect than lime in these high-pH soils.

Nutrient contents of yield

Nitrogen determinations of the yield were only performed on the PSA and KES samples in 1980. The lime and ash spread on the surface increased the N-content of clover, and the effect was clearly visible in protein yields (Table 5).

The EPO (2 experiments), ESA and PSA samples were analysed for Ca, Mg, P and K in 1980. The PSA samples were analysed in 1979, too. The EPO and ESA samples were similar in spite of the acidity of the site, whereas the PSA yield had clearly higher K contents (level 33—49 g/kg) and clearly lower Ca (6.3—9.0), P (1.7—3.3) and Mg (1.9—2.6) contents than the EPO and ESA samples. The clearest consistent difference was in the P content of the main crop which was 0.7 units lower compared with the aftermath (Table 6). The K content of the crop showed a similar change in most cases.

The effect of the treatments on the minerals determined in the yield was slight. No consistent variation could be observed in the Ca and P contents (Table 7). The Mg content was somewhat higher in the plot with initial liming than in the 0 or ash plots. The K content of the yield decreased a little in the limed area when compared with the unlimed section. However, ash treatment increased it.

Table 6. Mineral content of clover yield in different experiments, EPO and ESA in 1979, PSA in 1979 and 1980. Unit g/kg herbage DM.

		Ca	Mg	K	P
EPO 1	first yield	12.8	3.3	35	2.5
—"—	second —"—	12.0	3.4	32	2.5
EPO 2	first —"—	11.5	3.2	32	2.4
—"—	second —"—	12.0	3.4	33	3.2
ESA	first —"—	11.5	3.0	28	2.3
—"—	second —"—	12.3	3.5	31	2.9
PSA 79	first —"—	8.6	2.5	35	2.1
—"—	second —"—	9.0	2.4	45	3.0
—"— 80	first —"—	6.5	1.9	45	1.7
—"—	second —"—	8.0	2.4	57	2.2

Table 7. The content of calcium and magnesium (g/kg DM) in red clover after different treatments. Average of EPO and ESA experiments in 1981.

Surface spreading	Initial treatments: 0		Lime 5 t/ha		Ash 5 t/ha	
	1. yield	2. yield	1. yield	2. yield	1. yield	2. yield
	Calcium					
0	12.2	11.8	12.2	11.6	12.3	12.5
Lime 1.5 t/ha	11.5	12.5	12.3	12.2	12.4	11.8
—"— 5 —"—	11.1	12.5	11.9	13.1	12.4	13.4
Ash 1.5 —"—	11.7	12.7	11.9	12.6	11.5	12.9
—"— 5—"—	12.3	12.7	12.0	12.8	12.4	12.9
Average	11.8	12.4	12.1	12.5	12.2	12.7
	Magnesium					
0	3.16	2.90	3.73	3.48	3.24	3.04
Lime 1.5 t/ha	3.13	3.11	3.76	3.76	3.26	3.10
—"— 5 —"—	3.35	3.75	3.75	4.12	3.36	3.71
Ash 1.5 —"—	2.98	3.21	3.53	3.76	2.97	3.15
—"— 5 —"—	3.15	3.15	3.46	3.65	3.07	2.95
Average	3.15	3.22	3.65	3.75	3.18	3.19

DISCUSSION

In most of these experiments, every treatment increased the pH of the top soil layer (0—5 cm). The measurement performed in the coarse coarse fine sand of ESA was an exception: both lime and ash made the top soil slightly more acidic during the three years. This may be a result of the intensified binding of nitrogen, which makes the top soil in particular more acid (MENGEL and STEFFENS 1982).

In a preliminary study of this experiment, red clover did not grow in coarse fine sand when the soil pH was 5.5, Ca 500, K 180, Mg 50 and P 4.3 mg/l. It grew satisfactorily when lime and ash treatment had changed the respective figures to 5.65, 650, 280, 70 and 6.0. Additional lime made it grow well and then the figures were 5.85, 1000, 210, 75 and 6.4. In the experiments of the actual study the growth of clover was meagre at pH 5.0. Only at pH 5.7 was the growth more or less normal, but in some cases the yield continued to increase after the treatments that raised the pH to 6.2. The results conform exactly to VIRTANEN's (1931) observations. When the threshold was exceeded, the growth of clover could not be improved even with large amounts of lime or ash (in ESA 15 and in KES 20 t/ha).

A review of the fertility figures of the experimental plots implies that soil acidity is crucial to clover. The calcium content also plays an important role, but the threshold limits of other nutrients are very low. However, the clo-

ver did not require removal of acidity from the whole depth of its roots, a sufficient alkalinity in the 5 cm layer being enough to make it grow better. Surface liming had a favourable effect on acidic soil (EPO) but caused a reduction in yield in experiments where the pH was over 5.7. The slight reduction in yield caused by the surface liming of ley has been attributed to the evaporation of ammonia nitrogen and the binding of phosphorus into a form resisting dissolution (SAARELA et al. 1981). In this case the probable reason for the reduction in yield is the binding of phosphorus. The favourable effect of ash, particularly in the area of high pH, is probably due to suitably soluble micronutrients which were not contained in dolomitic lime.

The treatments had a very slight effect on the Ca content of the yield, although for example in the EPO experiments the soil Ca contents were low. Even higher quantities of lime did not increase the Ca content of clover significantly. The Mg supply of the experimental plots was also below the target value of 150 mg/l (JOKINEN 1981). Nevertheless, in the EPO and ESA experiments the Mg content of clover was almost the same as in red clover usually and clearly above the target (2 g/kg) established for feed. The figure was further substantially increased by ample liming, no matter whether it was mixed with soil or spread on the surface (Table 6).

The PSA result was an exception: the Ca and Mg contents of the yield were 30—50 % lower than in EPO and in ESA even though there was a satisfactory amount of these nutrients in the soil (Table 5). This result may be caused by some soil properties which could not be established in this study.

The K content of the clover yield was also exceptional in the PSA field. It was already higher than elsewhere (35—45 g/kg) in the first year, but in the second year it rose so high that it could even be dangerous to animals (65 g/kg). The supply of potash in the soil was high, and liming reduced the K content of the yield. Probably the liming increased the solubility of K, so that it leached beyond reach of the clover roots.

Both lime and ash increased the clover yield and its protein content (Table 4). The result of the two experiments is further supported by NIEMI's (1978) observation that lime and ash increase the intensity of nitrogen binding.

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SELOSTUS

Kalkki ja kuorituhka apilankasvun parantajana

ERKKI HUOKUNA, SIRKKA-LIISA HIIVOLA, PAAVO SIMOJOKI ja ELSI ETTALA

Maatalouden tutkimuskeskus

Maatalouden tutkimuskeskuksen Pohjois-Savon (PSA), Etelä-Pohjanmaan (EPO), Keski-Suomen (KES) ja Etelä-Savon (ESA) tutkimusasemilla v. 1978—81 tehdyn tutkimuksen mukaan 5 t/ha dolomiittikalkkia sekoitettuna ruokamultakerrokseen nosti mullan pH-lukua maan pintakerroksessa (0—5 cm) 0.3, kerroksessa 5—10 cm 0.3 sekä kerroksessa 10—20 cm 0.1 yksikköä. Puunkuorituhkan aiheuttamat vastaavat luvut olivat 0.4, 0.4 ja 0.1 pH-yksikköä. Pintaan levitetty kalkki nosti ylimmän kerroksen pH-lukua keskimäärin 0.4 ja tuhka 0.7 yksikköä. Mittaukset tehtiin kolmen vuoden kuluttua levityksestä.

Käsittelyt lisäsivät merkitsevästi maan kalsium- ja magnesiumpitoisuutta. Kalkitus alensi viljavuustutkimuksessa kalilukua. Apilan kuiva-ainesatoon vaikutti ratkaisevasti maan happamuus ennen käsittelyä. Happamassa maassa (pH 5) ei apilan juuri kasvanut. Kaikki käsittelyt, jopa pintakalkituskin lisäsivät satoa merkitsevästi. Sensijaan niissä paikoissa, joissa maan pH oli vähintään 5.7, ei millään kalkkimäärillä saatu sadonlisäystä. Päinvastoin aivan pinnalle annettu kalkki aikaansai selvän sadonalennuksen ensimmäisenä satovuonna, mutta se tasaantui jo seuraavana. Tuhkan vaikutus oli useimmissa tapauksissa hieman edullisempi kuin dolomiittikalkin.

Apila viihtyi jo, kun maan 5 cm:n pintakerroksen pH oli saatettu riittävän korkeaksi, pohjakerros sai olla hapankin. Kalkitus nosti jonkin verran sadon magnesiumpitoisuutta ja alensi sen kaliumpitoisuutta. Kalsium- ja fosforipitoisuuteen oli näillä käsittelyillä hyvin pieni vaikutus.

Kalkki ja varsinkin tuhka nostivat apilan valkuaispitoisuutta ja satoa.

ANNALES AGRICULTURAE FENNIAE, VOL. 27: 125—130 (1988)

Seria AGRICULTURA N. 87 — Sarja PELTOVILJELY n:o 87

COMPARISON OF BARLEY STARCH AND AGAR GELATINIZED MEDIA IN REGENERATION OF BARLEY SOMATIC CELLS

MAARIT ISOKORPI and SEPPO SORVARI

ISOKORPI, M. & SORVARI, S. **Comparison of barley starch and agar gelatinized media in regeneration of barley somatic cells.** Ann. Agric. Fenn. 27: 125—130. (Agric. Res. Centre, Dept. Plant Breed. SF-31600 Jokioinen, Finland.)

The regeneration capacity of somatic cells of *Hordeum vulgare* cv. 'Arra' seedlings was studied in barley starch and agar gelatinized nutrient media. In a starch media that contained 2 mg/l 2,4-D mesocotyl originated calli multiple meristemoids developed. Green shoots developed when these meristemoids were subcultured into a hormone-free medium. The callus from agar gelatinized nutrient media also contained green areas after eight weeks' culture but when these areas were subcultured into a hormone-free medium only roots developed.

Index words: *Hordeum vulgare,* somatic cell culture, regeneration, gelatine agent, barley starch, agar.

Abbreviations: IAA: indole-3-acetic acid
BAP: 6-benzylaminopurine
2,4-D: 2,4-dichlorophenoxyacetic acid

INTRODUCTION

Callus formation and plant regeneration is generally considered more difficult in monocotyledonous than in dicotyledonous plants. However, whole plants have been regenerated in a number of cereals (GREEN and PHILLIPS 1975, DALE and DEAMBROGIO 1979, 1980, SEARS and DECKARD 1982). Although agar contains inhibitory substances (KOHLENBACH and WERNICKE 1978) it is one of the most commonly used gelatine agents in the nutrient media of tissue culture. The negative influence of agar is obvious especially in the initial stages of anther culture (SORVARI 1986a). Barley starch has been succesfully used in barley anther cultures (SORVARI 1986a, b, SORVARI and SCHIEDER 1987) and has proved to be very advantageous in potato tuber disc culture, too (SORVARI 1986c).

The purpose of this work was to study the regeneration of the somatic cells of barley in a barley starch gelatinized nutrient media and to compare it to a nutrient media gelatinized with agar.

MATERIAL AND METHODS

Plant material

Seeds of 6-rowed *Hordeum vulgare* cv. 'Arra' (spring type) were dehusked and then surface sterilized as follows:

1) rinsed in 94 % ethanol for 5 s
2) rinsed in 70 % ethanol for 5 s
3) rinsed with sterile distilled water for 5 s
4) washed with 5 % sodium hypochlorite with two drops of Tween 80 for 30 min in a shaker.
5) washed twice with sterile distilled water

After sterilization, seeds were allowed to imbibe in sterile distilled water for 24 hours in darkness at + 25 °C. The seeds were then germinated at 25 °C in daylight in sterile test tubes containing 10 ml of half strength N_6-medium (CHU 1978) solidified with 0.7 % agar. After three days seedlings (shoot and one seminal root from each seedling) were cut into 2 mm long segments and placed onto the solid culture medium for callus initiation.

Culture media and conditions of incubation

The composition of the nutrient medium used (Table 1) was based on the N_6-medium (CHU 1978). It was modified with following hormones:

IA: IAA 1 mg/l and BAP 1 mg/l
IB: no hormones
IC: 2,4-D 0.5 mg/l
ID: 2,4-D 1.0 mg/l
IE: 2,4-D 2.0 mg/l

Table 1. Composition of nutrient media used in callus induction and growth.

		Nutrient media				
		IA	IB	IC	ID	IE
Macro nutrients (mg/l)	KNO_3			2830		
	KH_2PO_4			400		
	$(NH_4)_2SO_4$			463		
	$MgSO_4 \cdot 7H_2O$			185		
	$CaCl_2 \cdot 2H_2O$			166		
Micro nutrients (mg/l)	H_3BO_3			1.6		
	$MnSO_4 \cdot 4H_2O$			4.4		
	$FeSO_4 \cdot 7H_2O$			27.8		
	$Na_2EDTA \cdot 2H_2O$			37.2		
	$ZnSO_4 \cdot 7H_2O$			1.5		
	KJ			0.8		
Organic supplements (mg/l)	Thiamine. HCl			1.0		
	Glycine			2.0		
	Pyridoxine. HCl			0.5		
	Nicotinic acid			0.5		
Hormones (mg/l)	IAA	1.0	–	–	–	–
	BAP	1.0	–	–	–	–
	2,4-D	–	–	0.5	1.0	2.0
Other components g/l	Sucrose			30		
	Agar or			7		
	Barley starch			60		
	pH			5.8		

Gelatinization of the culture medium was obtained with barley starch (60 g/l) and agar (7 g/l).

The gelatinized medium was autoclaved at 121 °C for 15 minutes and then poured into ∅ 90 mm petridishes.

Due to the tendency of starch media to soften polyester nets were laid onto the media in order to prevent the tissue from sinking into the medium.

Cultures were incubated in phytotrons under low intensity fluorescent light (4000 lux) for a 14-hour photoperiod at a constant temperature of 25 °C.

After four weeks the cultures were transferred onto fresh media and callus growth visually estimated.

RESULTS

Only tissue originated from root and mesocotyle was able to form a callus in the media that contained 2,4-D (Table 2). No callus formed on hormone-free culture media or on those which contained IAA and BAP. In all cultures leaf-originated tissue turned necrotic and died after one weeks' culture. Callus formation was best in root tips and mesocotyle pieces which were taken 2 mm above the point of seminal root attachment. Callus originated from root segments was soft, watery and generally translucent. However calli from mesocotyle pieces could be divided into two types, according to whether they were cultured in agar or barley starch gelatinized nutrient media. Agar gelatinized media produced a soft, watery and translucent callus. However, in barley starch gelatinized media the callus was generally white, more compact and nodular to varying extents. After eight weeks' culture on a medium containing 2 mg/l of 2,4-D, green meristemoids were observed on the callus surface (Figs. 1 and 2). When these meristemoids were subcultured into a hormone-free medium they developed green

Table 2. Callus growth of explants from barley seedlings both in barley starch and agar gelatinized nutrient media. Growth was visually estimated on an arbitrary scale of 0, +, ++, +++

Source of explant	Distance from the base (mm)	Nutrient media (see Table 1): IA	IB	IC	ID	IE
cotyledon	6—50	0	0	0	0	0
mesocotyle	4	0	0	+	+	+
	2	0	0	++	+++	+++
	0	0	0	++	++	++
seminal root	0— 2	0	0	0	0	+
	4— 8	0	0	+	+	+
	10—12	0	0	+	+	++
	14—16	0	0	++	++	++

* 0 no callus growth
\+ restricted growth
++ medium growth
+++ maximum growth

Table 3. Regeneration of shoots and roots from mesocotyle callus in IE-medium gelatinized with agar or barley starch.

Renegeration from mesocotyle callus	Nutrient media (see Table 1): IE-agar	IE-barley starch
shoot	no	yes
roots	yes	yes

shoots (Table 3) and in some cases also roots (Fig. 3). The formation of meristemoids was best in the barley starch media which contained 2 mg/l 2,4-D.

In agar gelatinized media these calli from mesocotyle pieces also contained green meristemoids. However they were not able to form shoots after transfer into a hormone-free medium (Fig. 4).

Fig. 1. A general view of the mesocotyle callus with multiple green meristemoids after eight weeks culture in a barley starch medium containing, 2,4-D 2 mg/l (× 3).

Fig. 2. A magnification of the previous picture showing multiple meristemoids with developing leaves (× 20).

Fig. 3. Green shoots and roots developing from isolated meristemoids in hormone free barley starch medium.

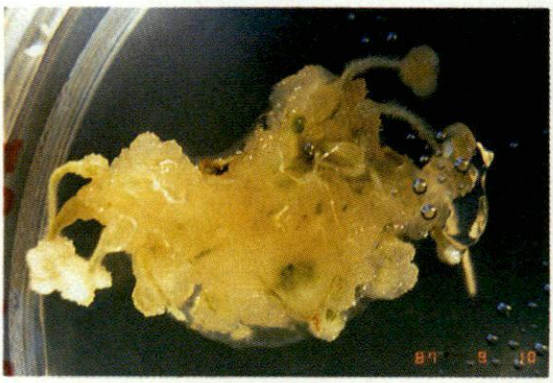

Fig. 4. A mesocotyle callus with regenerated roots two weeks after transfer to hormone free medium (× 3).

DISCUSSION

Callus growth was best in root tips and in the mesocotyle region containing the crown. These tissues contain actively dividing meristematic cells which obviously explains why their callus-forming capacity is much better as compared to that of other tissues e.g. leaf segments, which

formed no callus at all. DALE and DEAMBROGIO (1979) were also able to induce callus only from the basal intercalary meristem region of the leaf sheath, but from the more differentiated parts no callus formation was obtained.

The recalcitrance of monocot leaf tissue in callus formation is still obscure but there are clearly two different systems between monocots and dicots because in dicots, such as potato, a callus is easily obtained from leaf tissue directly (WEBB et al. 1983) or from leaf tissue derived protoplasts (THOMAS 1981). Leaves would be a convenient tissue for obtaining protoplasts in cereals, too, but the induction of protoplast division requires further basic study. This difficulty is also reflected by the recalcitrance of cereals leaves to form callus (POTRYKUS et al. 1976).

In this study 2,4-D was found to be necessary for callus induction and growth. IAA and BAP did not induce callus growth at all; the presence of these hormones turned the cultured tissue necrotic. In the hormone-free medium there was also no callus formation either, but the cultured tissue did not become necrotic as in the culture media containing IAA and BAP. On the contrary these two hormones together with barley starch were advantageous for embryoid and callus formation in barley anther culture (SORVARI 1986b).

In cereals regeneration has generally been very difficult compared to the regeneration capacity of dicotyledons. Another problem with cereals has been a rather high amount of albinism in regenerated plants (DALE and DEAMBROGIO 1980, YE et al. 1985). The reasons for albinism are not completely clear, but the composition of the nutrient medium has a strong influence on it (CLAPHAM 1973). In barley anther culture as well, barley starch proved to be superior to agar in the regeneration of green plantlets (SORVARI 1986a, b and SORVARI and SCHIEDER 1987).

The promotive effect of barley starch on differentiation can be confirmed also in the regeneration of the somatic cells of barley. In cultures where agar was used as a gelatine agent only roots were obtained. The regeneration of somatic cells has been obtained in other laboratories also by agar-based media in monocots (e.g. HANZEL et al. 1985 and HUNSINGER 1987), but the differentiation of albino plantlets could not be avoided (HANZEL et al. 1985). The plants regenerated in starch media were all green.

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SELOSTUS

Ohratärkkelyksen ja agarin vaikutus ohran regeneraatiokykyyn

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Kasvien regenerointi yksisirkkaisilla, erityisesti heinäkasveilla, on huomattavasti vaikeampaa kuin kaksisirkkaisilla. Tupakkakasvien solukkoviljelytutkimuksissa on havaittu ravintoalustojen kiinteyttämiseen yleisesti käytetyn agarin sisältävän inhibiittoreita, jotka mahdollisesti estävät erilaistumisprosessin. Tämän tutkimuksen tarkoituksena oli selvittää ohratärkkelyksen ja agarin vaikutuksia ohran regeneraatioon somaattisesta kallussolukosta. Ohratärkkelysalustalla verson tyviosasta muodostuneen valkoisen kalluksen pinnalle kehittyi kahdeksan viikon viljelyn jälkeen useita meristemoideja, jotka hormoonittomalla ohratärkkelysalustalla muodostivat vihreitä versoja sekä juuria. Agaralustalla kallus oli vetistä ja haurasta, ja vaikka siihenkin muodostui vihertäviä alueita, kehittyi näistä hormoonittomalla agaralustalla vain juuria.

Ohratärkkelyspohjainen alusta osoittautui selvästi agarpohjaista paremmaksi ohran versojen regeneraatiossa. Ohratärkkelyksen vaikutuksen perusteita ei vielä tunneta. Tärkkelysalustan pehmeneminen viljelyn edetessä viittaa entsymaattiseen toimintaan ja tärkkelyksen merkitykseen ravitsevana tekijänä.

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Seria AGRICULTURA N. 88 — Sarja PELTOVILJELY n:o 88

THE EFFECT OF FROST AND HERBICIDE TREATMENT ON SOME FIELD CROPS AND WEEDS

LEILA-RIITTA ERVIÖ

ERVIÖ, L.-R. 1988. **The effect of frost and herbicide treatment on some field crops and weeds.** Ann. Agric. Fenn. 27: 131—140. (Agric. Res. Centre, Dept. Crop. Sci., SF-31600 Jokioinen, Finland.)

The effect of frost and herbicide application on spring barley, oats, pea and the weeds *Chenopodium album, Tripleurospermum inodorum* and *Sinapis alba* was studied in pot trials. Frost treatment was applied in a climate room at temperatures of -4 °C or -8 °C. Field trials were used in winter rye and spring barley.

Frost treatment at the 3—4-leaf stage caused visible injuries in barley and oats. Barley recovered well and there was no decrease in yield. Frost damage was more severe in oats. The harder frost temperature led to a reduction in yield components and to a decrease of 15 % in the yield of oats.

Pea and weeds were severely damaged by frost. The treatment reduced the number and weight of plants per pot.

Herbicide application combined with frost treatment caused no significant changes in the yields of barley and oats. Pea suffered more from combined treatment which reduced the number and weight of pea plants per pot by 25 % and 45,5 % on average, respectively. Basagran MCPA was slightly more aggressive to pea than mixture of Basagran 480 and Bladex.

Frost occurring during weed control did not weaken the effect of the herbicides on weeds in this study.

Index words: effect of frost, effect of herbicide treatment, cereals, pea, weeds, *Chenopodium album* L., *Sinapis alba* L., *Tripleurospermum inodorum* Schulz Bip.

INTRODUCTION

Night frosts often occur under Finnish conditions concurrently with herbicide applications on spring sown crops. The weather during spraying time may alter the selectivity of a herbicide thus causing risk of injuries to the crop. Particularly frost, which as such damages plants, may increase injuries to crops during herbicide treatment. However, there are little possibilities to postpone applications during the period of night frosts. Despite low night temperatures, day temperatures may be high and consequently weeds and crops pass the suitable spraying stage very quickly.

The effect of frost and herbicide application on some field crops and weeds was studied at the Department of Crop Science of the Agricultural Research Centre in 1982—84. The study was supported by The Academy of Finland.

MATERIAL AND METHODS

The study was carried out as field trials and as pot trials in a greenhouse. The crops studied in the field were winter rye and spring barley. Plants in the pot trials were spring barley, oats and pea and the weeds *Chenopodium album* L., *Sinapis alba* L. and *Tripleurospermum inodorum* Schulz Bip. *S. alba*, although not a weed species, was chosen as a representative of cruciferous weeds because like *Sinapis arvensis* it has high competitive ability and is easy to grow in pots.

Pot trials

Pots used for the trials had volumes of 3 litres and surface areas of 176 cm^2. Six plants of cereals, pea and *S. alba* and ten plants of the other weeds were grown per pot.

The temperature regime before the frost may have an influence on the response of crops to frost. Therefore three groups of plants were grown at three different temperatures in a greenhouse before the frost and herbicide treatments. The temperatures were chosen according to the weather conditions prevailing in Finland during the juvenile stages of spring sown crops. The night/day temperatures were 10 °/24 °, 0 °/24 °C and 0 °/15 °C, respectively.

The herbicides used in different stands were as follows:

Spring barley and *S. alba*	Actril S 3 l/ha (a.i. MCPA 235/dichlorprop 184/ioxynil 38/bromoxynil 24 g/l)
	Mepro Special 3,5 l/ha (MCPA 261/mecoprop 181/dicamba 30 g/l
	Basagran MCPA 4 l/ha (bentazone 250/MCPA 125 g/l)
	Glean 20 DF 20 g and 40 g/ha (chlorsulfuron 200 g/kg)
Pea	Basagran MCPA 3,5 l/ha
	Basagran 480 1,5 l/ha+Bladex 1,0 l/ha (bentazone 480 g/l+cyanazine 500 g/l)

C. album and Actril S 4 l/ha and *T. inodorum* Basagran MCPA 4 l/ha

Frost treatment of plants was applied in climate rooms 6 or 12 hours before or 12 hours after herbicide application. During the frost treatment temperature was lowered stepwise over two hours from +5 °C to -4 °C or -8 °C, where plants were kept for one hour. After treatment temperature was raised again over two hours to +5 °C, and further over three hours to +18 °C. After frost and herbicide treatments plants were grown to maturity in a greenhouse where the night/day temperature was regulated to 10 °/20 °C.

Plants were treated and harvested at certain developmental stages. Time of treatment was dependent of the temperature regime by which plants were grown. Cereals were treated at the 3—4-leaf stage, pea at the height of 5 cm, and weeds when they had 2—4 true leaves.

Barley and oats were harvested after maturity. The number and fresh weight of culms, ears and grains were determined. The length of ear-bearing culms was measured.

Pea and weeds were harvested pot by pot as soon as 60 % of plants were flowering. An exception was *T. inodorum* which did not begin flowering at all during the study period, and was harvested at the rosette stage. The number of pea plants as well as the number of branches, flowers and pods per pot were counted. Plants were weighed and their length measured. Weeds/pot were counted and weighed.

Field trials

In 1982 and 1984 the effect of frost and herbicide application on winter rye and spring barley was studied in the field using a split-plot design with four replicas. The soil was sandy clay and each crop received the fertilization recommended in practical farming.

Always after a night frost, rye and barley were sprayed at nine o'clock the next morning.

Soil surface temperature inside the stand was recorded during the treatment period. The spraying period of rye was ten days in 1982 and five days in 1984 (Table 12). Barley was treated on three dates in both years.

The herbicides used on rye were Actril S 4,0 and 6,0 l/ha and Mepro Special 3,5 and 5,3 l/ha. Barley was sprayed with 3,0 l/ha of Actril S and 4,0 l/ha of Basagran MCPA.

RESULTS

The effect of frost in pot trials

Frost treatment caused visible injuries in barley and oats. Leaves were droopy after the treatment and some of them wilted. It was difficult to separate frost damage from the symptoms caused by herbicide injury. No damage could be detected on plant parts that developed after the treatments.

When assessed on a percent scale one week after the treatment, the damage in barley varied from 1 to 10 % (0 = no injuries). However, the variation was random; neither the temperature conditions during the juvenile stages of plants nor the degree of the frost appeared to be important.

No harmful effect due to frost could be detected in the yield of barley (Table 2). The only significant difference between treatments was in the weight of culms which increased in frost treated plants. The differences in the ear and grain yield between frost treatments were not significant.

Oats suffered from frost more than barley did. The damage was clearly more severe at the temperature of -8 °C than at -4 °C (Table 1). Herbicide treatment did not increase the injuries at either temperature. The harmful effect of frost was also seen in the yield components of oats. The number and weight of culms and panicles were smallest when oats were exposed to -8 °C. This resulted in decreased grain yield (Table 3).

The different temperature regimes before frost treatment did not significantly effect

Table 1. Average damage to plants assessed on a 0—100 % scale (0 = no injuries) one week after treatment.

Growth temperature/ herbicide treatment		Frost -4 °C	Frost -8 °C
Oats			
10 °/24 °C	untreated	13	79
	herbicide treatment	4	49
0 °/24 °C	untreated	3	85
	herbicide treatment	2	32
0 °/15 °C	untreated	3	80
	herbicide treatment	1	33
Pea			
10 °/24 °C	untreated	29	97
	herbicide treatment	73	99
0 °/24 °C	untreated	3	65
	herbicide treatment	32	90
0 °/15 °C	untreated	0	33
	herbicide treatment	38	31
T. inodorum			
10 °/24 °C	untreated	13	100
	herbicide treatment	100	100
0 °/24 °C	untreated	1	43
	herbicide treatment	100	100
0 °/15 °C	untreated	8	43
	herbicide treatment	100	100
C. album			
10 °/24 °C	untreated	8	100
	herbicide treatment	99	100
0 °/24 °C	untreated	3	48
	herbicide treatment	100	100
0 °/15 °C	untreated	0	60
	herbicide treatment	94	96
S. alba			
10 °/24 °C	untreated	84	88
	herbicide treatment	100	100
0 °/24 °C	untreated	56	38
	herbicide treatment	100	100
0 °/15 °C	untreated	18	44
	herbicide treatment	91	100

Table 2. The effect of frost temperature on spring barley.

Treatment	Culms g/pot
No frost	29.1[a]
Frost -4 °C	33.5[ab]
Frost -8 °C	34.4[b]
F-value	3.20*

[a-b] Figures with the same superscript letter in the same column are not significantly different. * $P \leq 0.05$.

yields of barley and oats. Similarly the timing of frost treatment in relation to herbicide application was not significant.

Pea was severely injured by frost treatment (Table 1). Part of the branches and leaves wilted and some plants even died (Table 4). Especially those plants grown in warm conditions during their juvenile stages suffered from the harder frost temperature (Tables 1 and 5). This was seen in the decrease of the number and weight

Table 3. The effect of frost temperature on oats.

Treatment	Culms/pot number	Culms/pot g	Panicles/pot number	Panicles/pot g	Grain yield g/pot
No frost	20[b]	96.1[a]	18[ab]	37.7[b]	24.6[b]
Frost -4 °C	20[b]	99.4[b]	19[b]	39.6[b]	24.5[b]
Frost -8 °C	18[a]	89.8[a]	16[a]	32.9[a]	20.8[a]
F-value	8.24***	89.80***	49.69***	32.90***	55.70***

[a-b] as Table 2. *** $P \leq 0.001$

Table 4. The effect of frost temperature on pea.

Treatment	Plants/pot number	g	Branches/ pot	Flowers/ pot	Pods/ pot	Average length cm
No frost	6[b]	51.7[b]	14[b]	29[b]	9[c]	35[b]
Frost -4 °C	6[b]	39.3[b]	14[b]	24[b]	4[b]	30[b]
Frost -8 °C	4[a]	20.9[a]	10[a]	13[a]	2[a]	19[a]
F-value	169.49***	107.98***	25.28***	84.44***	23.43***	96.25***

[a-c] as Table 2. ***$P \leq 0.001$

Table 5. The effect of growing temperature in the juvenile stage on the response of pea to the frost treatment.

Growth temperature/ treatment	Plants/pot number	Plants/pot weight g	Branches/ pot	Flowers/ pot	Pods/ pot	Average length cm
10 °/24 °C						
No frost	6	57.3	14	30	19	33
Frost -4 °C	6	41.9	16	21	5	29
Frost -8 °C	1	3.2	2	3	0	8
0 °/24 °C						
No frost	6	40.8	14	20	5	31
Frost -4 °C	6	33.9	13	23	5	27
Frost -8 °C	5	25.5	14	16	2	24
0 °/15 °C						
No frost	6	57.1	16	37	4	40
Frost -4 °C	5	42.0	11	28	3	34
Frost -8 °C	5	33.8	13	19	3	27
F-value	83.85***	31.84***	91.86***	9.05***	9.67***	27.84***

***$P \leq 0.001$

Table 6. The effect of frost and herbicide treatment on *T. inodorum.*

Treatment	Plants/ pot	Weight g/pot	Average plant length cm
Untreated, no frost	10	8.6	7
Untreated, frost	8	5.7	5
Herbicide treatment, frost	0	0.0	0
F-value	1115.03***	489.[illegible]***	231.08***

***P ≤ 0.001

Table 7. The effect of frost and herbicide treatment on *C. album.*

Treatment	Plants/ pot	Weight g/pot	rage plant length cm
Untreated, no frost	10	6.4	18
Untreated, frost	7	4.9	15
Herbicide treatment, frost	0	0.0	0
F-value	785.99***	480.91***	664.60***

***P ≤ 0.001

of plants and in lower values of the different yield components.

All weeds studied were susceptible to frost treatment which decreased their biomass production and killed some plants (Tables 6—8). The interaction between early growth temperature and the temperature during frost treatment was seen in the number and length of *T. inodorum.* Plants grown in cool conditions survived better after the frost. This interaction was not shown in the case of the other two weed species.

Herbicide treatment in pot trials

Herbicide application combined with frost treatment caused no significant decrease in the yield of barley and oats in comparison to control (Tables 9—10). On the contrary, some herbicides increased the number and weight of the culms of barley but this did not increase the grain yield.

Oats produced the lowest amount of culms and panicles after exposure to frost without herbicide treatment (Table 10). Differences in grain yield between treatments with two dosages of chlorsulfuron were insignificant.

Table 8. The effect of frost and herbicide treatment on *S. alba.*

Treatment	Weight of stems g/pot	Weight of pods g/pot
Untreated, no frost	6.4	0.7
Untreated, frost	5.8	0.9
Herbicide treatment, frost	1.0	0.2

Pea suffered from the combination of frost and herbicide treatments. They decreased the number and weight of pea plants as well as the number of flowers and pods (Table 11). Basagran MCPA was slightly more aggressive to pea than the mixture of Basagran 480 and Bladex.

Herbicide application destroyed all the plants of *T. inodorum* and *C. album*, but a few plants of *S. alba* survived the treatment (Tables 6—8). It was obvious that night frost occurring at the time of herbicide application did not weaken the efficacy of the compounds used in this study.

Table 9. The effect of herbicide treatment on spring barley in connection with frost.

Treatment	kg, l/ha	Culms/pot number	Culms/pot weight g	Ears g/pot	Grain yield g/pot
Untreated, no frost		11[a]	29.8[ab]	11.3[a]	9.2[ab]
Untreated, frost		11[a]	31.1[b]	12.1[ab]	9.2[ab]
Actril S	3.0	11[a]	37.0[c]	13.0[ab]	8.7[ab]
Mepro Special	3.5	12[b]	40.3[c]	13.6[b]	8.4[a]
Basagran MCPA	4.0	12[b]	37.8[c]	13.1[ab]	8.7[ab]
Glean 20 DF	0.02	11[a]	26.9[ab]	11.9[ab]	9.5[ab]
Glean 20 DF	0.04	11[a]	24.7[a]	11.9[b]	9.7[b]
F-value		3.28**	24.78***	3.01**	2.62*

[a-c] as Table 2. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table 10. The effect of herbicide treatment on oats in connection with frost.

Treatment/dosage	Culms number/pot	Panicles number/pot
Untreated, no frost	20[b]	18[b]
Untreated, frost	17[a]	15[a]
Glean 20 DF 20 g/ha	20[b]	18[b]
Glean 20 DF 40 g/ha	19[b]	18[b]
F-value	5.01**	4.66**

[a-b] as Table 2. ** $P \leq 0.01$

Table 11. The effect of herbicide treatment on pea in connection with frost.

Treatment, l/ha		Plants/pot number	g	Flowers number/pot	Pods number/pot	Average plant length cm
Untreated, no frost		6[b]	51.7[c]	29[b]	9[b]	35[c]
Untreated, frost		5[a]	41.9[bc]	23[b]	4[a]	31[bc]
Basagran MCPA	3.5	4[a]	24.0[a]	15[a]	2[a]	21[a]
Basagran+Bladex	1.5+1.0	5[a]	32.7[b]	20[ab]	3[a]	26[ab]
F-value		3.76*	10.58***	6.20***	12.02***	7.69***

[a-c] as Table 2 *** $P \leq 0.001$ *** $P \leq 0.001$

Field trials

May 1982 was cool during the spraying period of rye but only mild night frosts occurred (Table 12). In 1984 the temperature varied considerably. The early and late parts of May were warm with maximum temperatures of above +20 °C. On the other hand, the spraying period of rye was cool with night frosts.

Barley was treated with herbicides in June. The month was generally warmer in 1984 than 1982. In both years, frost was recorded only on a few nights when the temperature ranged from -0.6 °C to -4.8 °C.

No visible damage was observed in crops after night frosts. Herbicide application after a frost had no significant effect on the yield of crops compared with untreated plots although some variation between the yields was observed (Table 13).

The efficacy of herbicides on weeds was good or moderate in barley as shown:

	Weeds/m^2	Weed weight g/m^2
Untreated	20	5.2
MCPA/diklorprop/ ioxynil/bromoxynil	3	0.2
Bentazone/MCPA	11	1.2

Weeds in rye died usually during the growing season and could not be collected for later assessment.

Table 12. Temperatures in May and June during herbicide treatments in 1982 and 1984.

1982	Temperature °C			1984	Temperature °C		
Date	max	min[1]	min[2]	at spraying	max	min[1]	min[2]	at spraying
MAY								
4	10.3	2.0	–[3]		13.0	7.3	–[3]	
5	13.0	3.4	2.1	3.0	15.1	7.2	–	
6	19.4	7.3	5.5	12.0	12.7	2.5	–	
7	10.6	6.1	–		13.3	–5.0	5.0	7.0
8	17.3	6.3	–		10.2	1.7	2.0	
9	15.4	2.0	–		6.4	–5.7	–4.0	2.0
10	13.7	6.4	4.5	6.0	4.5	–5.4	–4.0	–2.0
11	12.0	3.4	1.3	6.0	9.5	–6.0	–6.0	3.0
12	13.6	2.1	–1.0	3.0	13.2	–6.5	–4.5	6.0
13	8.0	3.1	1.1	4.0	16.0	–5.2	–3.0	
14	10.6	3.8	1.6	6.0	18.8	–3.8	–2.0	
15	9.0	1.1	–1.7	8.0	22.1	–2.0	–	
16	10.4	0.4	–2.5	3.0	23.3	8.3	–	
17	14.3	–2.0	–5.2	6.0	27.7	5.7	–	
18	10.6	5.4	–		25.0	7.2	–	
JUNE								
9	9.0	0.4	–		14.7	–1.2	–	
10	9.0	–1.2	–3.8	7.0	10.6	–2.4	–	
11	13.9	–1.3	–4.8	9.0	12.2	–4.7	–4.7	10.0
12	10.3	2.8	–		13.5	–3.3	–2.0	10.0
13	8.8	4.4	–		16.8	8.2	8.0	13.0
14	12.1	4.8	–		11.8	8.5	8.0	
15	12.8	2.6	–		17.1	8.5	8.0	
16	9.2	6.2	–		20.3	2.8	–	
17	8.0	5.2	–		20.1	3.6	–	
18	13.5	6.1	–		21.3	4.3	–	
19	15.8	6.2	–		19.7	4.0	–	
20	18.4	7.9	–		19.0	6.7	–	
21	16.9	3.2	–0.6	17.0	21.0	0.9	–	
22	14.6	8.2	–		17.7	10.6	–	

[1] on soil surface [2] in crop canopy [3] — not measured

Mean temperatures: May 8.5 °C 12.6 °C
June 11.2 °C 13.1 °C

Table 13. The effect of herbicide application on the yield of rye and barley in connection with frost in 1982 and 1984.

Crop/treatment		Yields kg/ha 1982	1984
RYE		n.s.	n.s.
Untreated		2540	2120
Actril S	4,0 l/ha	2600	2230
	6,0 "	2520	2070
Mepro Special	3,5 l/ha	2440	2600
	5,3 "	2350	2390
BARLEY		n.s.	n.s.
Untreated		4360	2670
Actril S	3,0 l/ha	4550	2250
Basagran MCPA	4,0 l/ha	4300	3070

DISCUSSION

The growth conditions of pot trials in the greenhouse and climate room were not natural for field crops and weeds. The frost treatment employed in this study was also rough on plants compared to field conditions as temperature and light in the climate room were regulated stepwise.

On the other hand, this method made it possible to study some special factors which could not be controlled outdoors. This was quite apparent in the field trials included in the study. They yielded relatively little information on the interaction between frost and herbicide application because of the few and mostly mild frosts during spraying time. Furthermore there were difficulties regarding the application of herbicides at the proper developmental stage of a crop as the occurrence of frost was random.

In the years when the period for spraying was long (e.g., barley in 1982) the developmental stage of the crop differed between early and late applications. The tolerance of cereals to herbicides decreases at the stage of stem elongation and injuries could thus be expected. However, this could not be shown in the present study.

Temparature conditions around the time of herbicide application may affect the efficacy and selectivity of herbicides (a.o. THONKE 1978, DAVIES et al. 1979). According to several studies, many of them increase in phytotoxicity by a rising temperature (HÅKANSSON and SVENSSON 1974, THONKE 1984, LALLUKKA 1976). There are also some results from investigations as well as practical experience about the weakening of herbicidal activity in cool conditions (THONKE 1978). ELBAEK JENSEN (1984) showed that low temperature weakened the efficacy of bentazone, chlorsulfuron and 2,4-D on *S. alba* in climate rooms. On the other hand, night frosts in Swedish field conditions did not affect herbicidal efficacy and selectivity of some phenoxyacetic acid herbicides used on winter wheat (WALLGREN 1984).

The efficacy of herbicide application on weeds did not decline by frost in this study. All weeds studied seemed to be very susceptible to frost and were destroyed by herbicide application. In this regard, the results differed from some other reports where the tolerance of *C. album* and *T. inodorum* to bentazone was higher in cool than in warm conditions (BEHREND and MENCK 1973, DAVIES et al. 1979, SKUTERUD 1984). On the other hand, the effect of temperature is always in interaction with other environmental factors and thus variation in phytotoxicity in different circumstances is understandable.

The trials with barley in this study included many herbicides, even a mixture with dicamba (Mepro Special) which in certain circumstances has proved to be aggressive on barley (LALLUKKA 1976). This time no injuries causing significant yield decrease could be detected from that product (Table 9). A high temperature at spraying time seems to be more harmful to barley than frost.

Oats were suspectible to frost treatment. The number of culms and panicles decreased due to frost but not by herbicide application in this study (Table 10). The harmful effect of frost and herbicide treatment on oats has been earlier shown by RADEMACHER (1954).

Pea was harvested before maturity because of its long growing time. The reduction in pod number caused by frost and herbicide treatment is likely to result in yield reduction (Table 11).

The mixture of bentazone and MCPA was aggressive to pea. The amount of MCPA in this compound is too high in relation to the tolerance of pea. This has been earlier stated in many other reports (a.o. JUNNILA 1984).

Temperature during the juvenile stages of plants had a smaller effect on the frost tolerance of plants than expected. This was import-

ant only in the case of pea which tolerated frost better when grown earlier in cool (0 °C/15 °C) or partly cool (0 °/24 °C) conditions (Table 5).

Field trials were carried out with winter rye and spring barley. The results (Table 13) are in agreement with some earlier investigations (LALLUKKA 1975, ELBAEK JENSEN 1984, WALLGREN 1984) and supported the information obtained from pot trials with barley in this study. Herbicide application after night frost did not cause any injuries in winter cereals nor in barley, or did so only occassionally. Possible disturbances in plant growth after herbicide treatment had no effect on final grain yield.

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SELOSTUS

Hallan vaikutus herbisidiruiskutuksen valikoivuuteen ja torjunnan tehoon

LEILA-RIITTA ERVIÖ

Maatalouden tutkimuskeskus

Hallan ja samaan aikaan suoritetun herbisidikäsittelyn vaikutusta ohraan, kauraan ja herneeseen sekä jauhosavikkaan (*Chenopodium album* L.), valkosinappiin (*Sinapis alba* L.) ja saunakukkaan (*Tripleurospermum inodorum* Schulz Bip.) tutkittiin vuosina 1982—84 astiakokeissa kasvihuoneessa. Kenttäkokeet järjestettiin rukiilla ja ohralla. Hallakäsittely tehtiin kasvukammiossa -4 °C:n tai -8 °C:n lämpötilassa.

Pelkkä hallakäsittely vioitti kasveja enemmän kuin herbisidit. Viljelykasveista kestävin oli ohra, joka vioittui pakkasesta vain ohimenevästi eikä sadonalennusta ilmennyt. Kaura kärsi pakkasesta, joka vähensi sen satoa 15 %. Herne ja tutkitut rikkakasvit vioittuivat herkästi pakkasesta. Käsittely hävitti joitakin yksilöitä kokonaan sekä pienensi jäljelle jääneiden painoa ja kasvinosien lukumäärää. Painon vähennys oli herneellä 60, saunakukalla 34, jauhosavikalla 23 ja valkosinapilla 10 %.

Kasvien alkukehityksen aikana vallinneen lämpötilan merkitys näkyi herneen ja saunakukan hallankestävyydessä. Viileässä kasvaneet yksilöt vioittuivat hallasta lievimmin.

Herbisidikäsittely ei lisännyt ohran ja kauran vioitusta. Herne kärsi sekä pakkasesta että sen yhteydessä tehdystä herbisidiruiskutuksesta. Kasvien lukumäärä astiaa kohti väheni herbisidiruiskutuksen vaikutuksesta keskimäärin 25 % ja niiden paino 46 %. Basagran MCPA oli herneelle lievästi aggressiivisempi kuin yhdistelmä Basagran 480 + Bladex.

Herbisidikäsittelyt hävittivät rikkakasvit tehokkaasti astiakokeissa. Kenttäkokeissakaan ei hallan havaittu heikentäneen herbisidien torjuntavaikutusta. Ruis ja ohra eivät kenttäkokeissa vioittuneet yöhallojen ja herbisidikäsittelyjen vuoksi.

ANNALES AGRICULTURAE FENNIAE, VOL. 27: 141—151 (1988)

Seria AGRICULTURA N. 89 — Sarja PELTOVILJELY n:o 89

INITIAL DEVELOPMENT OF THREE BLUE LUPIN *(LUPINUS ANGUSTIFOLIUS* L.) VARIETIES IN NORTHERN FINLAND

TADEUSZ ANISZEWSKI

ANISZEWSKI, T. 1988. **Initial development of three blue lupin *(Lupinus angustifolius* L.) varieties in northern Finland.** Ann. Agric Fenn. 27: 141—151 (Agric. Res. Centre, Kainuu Res. Sta. 92810 Pelsonsuo, Finland.)

Sprouting of the varieties researched was weak on average (61 % with a variation from 29 % to 93 %) although high grade elite seeds were used in sowing. During the seedling period damage of the varieties was not a problem. The number of vernalized plants in the field experiment was very small (average 2.6 % with a variation from 0.7 % to 6.2 %).

Temperature sum and rainfall did not affect blue lupin's initial growth development, while sunny and half cloudy days had a clear effect.

In the extreme growth conditions of northern Finland where difficulties in initial growth development (in sprouting) can be observed the periods of sprouting and seedling had however a completely neutral relationship with the green matter of blue lupin. However this neutrality could not be proved in relation to the crude protein harvest of green matter.

Index words: acclimatization, blue lupin, *Lupinus angustifolius* L., neutrality of variety, extremity of variety, initial stage of growth development, sprouting, seedling, vernalization, phenology.

INTRODUCTION

Recently, interest in alternative crops has increased in Scandinavia and some European countries (JACOBSEN 1988). Finland, too, has begun research into the possibilities for the cultivation of lupin, characterized by its biological fixation of nitrogen and modesty in relation to growing conditions (ANISZEWSKI 1988a). The possibilities of lupin cultivation in Finland were first studied by PITKÄNEN (1939) and VALLE (1938, 1941). Observations on the success of lupin growing during 1938—1943 and 1945—1953 were made by the Department of Plant Breeding of the Agricultural Research Centre (1938—1943, 1945—1953). Since the 1950's no research on lupin had been conducted in Finland until 1983, when the present research was started. One year later, an extensive research project was implemented on the cultivation techniques of lupins, in which the present research also had a special and

independent role.

The chief aim of the research was to compare the thriving and adaptability of blue lupin varieties in the climatic conditions of northern Finland. The results of the trials, as regards the formation of dry matter, have been reported in the pilot article elsewhere (ANISZEWSKI 1988b). This paper, however, will only deal with neutrality and extremity of the initial stage of growth development of blue lupin varieties.

MATERIAL AND METHODS

During 1983—1986, field trials with blue lupin were conducted in Posio (66° 12″ N and 27° 13″ E), and managed in 1983—1984 by the Central Finland Research Station, and by the Kainuu Research Station of the Agricultural Research Centre in 1985 and 1986. This was the northernmost test of lupin cultivars ever conducted. The varieties studied were the following three new varieties of the narrow-leaved lupin (*Lupinus angustifolius* L.): var. Mirela, var. Kazan and var. Remik. The methods applied in this research were those used by STUCZYNSKA (1968) in the study of *Lupinus elegans* L.'s thriving and adaptability and, where the phenological aspect was concerned, the methods used by REEVERS et al. (1977) and BOUNDY et al. (1982) were applied. The material, methods, experimental site, trial establishment, experimental material, empirical measurements and observations as well as statistical analysis used in this study are described in detail elsewhere (ANISZEWSKI 1988b).

RESULTS

Sprouting

Sprouting (of the lupin varieties) was weak on average (61 %) and fluctuated considerably in different years (Figs. 1 and 2). The relative sprouting of var. Mirela was inversely proportional to that of var. Kazan. The same relationship prevailed for var. Mirela and var. Remik, while, on the other hand, sprouting of var. Kazan and var. Remik was directly proportional (Table 1, Fig. 3). When the periods of field emergence among the blue lupin varieties are compared, there is a direct proportion between var. Mirela and var. Kazan as well as between var. Mirela and var. Remik, which is contrary to the point of view of relative sprouting. The relationship between var. Kazan and var. Remik is also directly proportional (Table 2, Fig. 3).

Seedling

On the average, 4.3 % of plants died between the sprouting and seedling periods (Fig. 4, Tables 3a and 3b). This is a comparatively small percentage when compared with the plant damage that occurred during the period of sprouting. The damage which occurred during the seedling period fluctuated from 2.2 % (1986) to 7.4 % (1983). On the basis of the comparison for sprouting, it can be said that varieties which sustain great damage during sprouting on average produce the best seedling.

On the one hand, the seedling of var. Mirela and var. Kazan, and, on the other hand, that of var. Mirela and var. Remik, was inversely proportional while the seedling between var. Kazan and var. Remik was directly proportional while the seedling between var. Kazan and var.

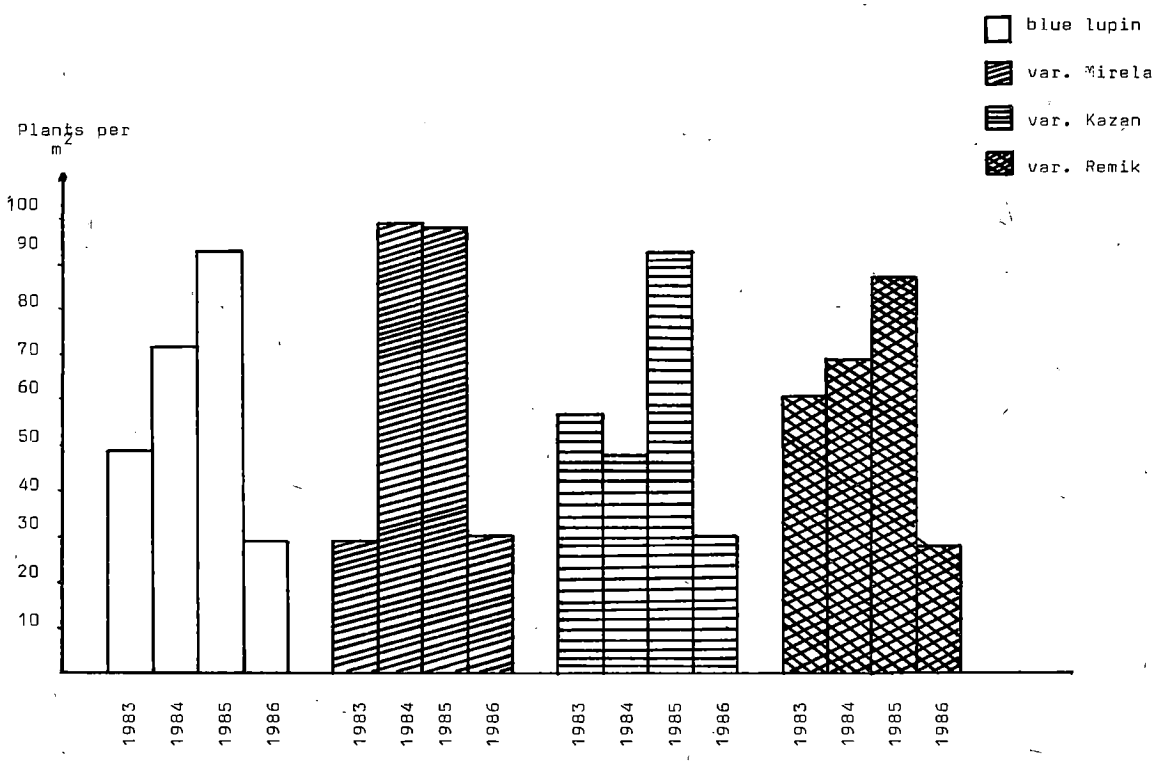

Fig. 1. Sprouting of blue lupin varieties in Posio during 1983—1986.

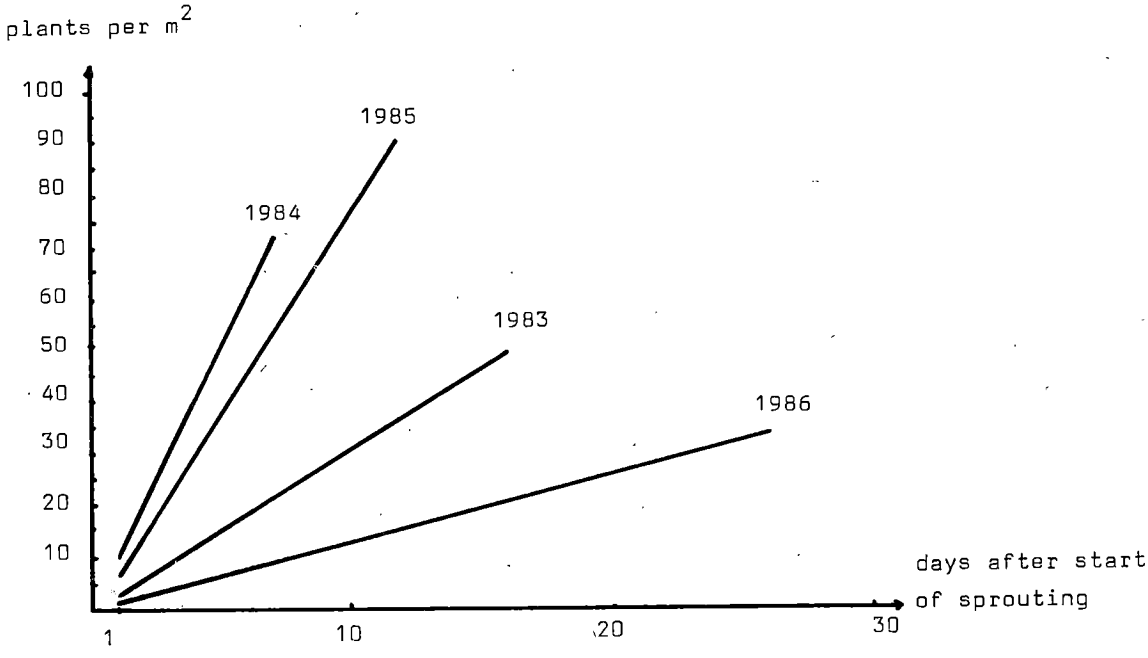

Fig. 2. Sprouting of blue lupin in Posio during 1983—1986.

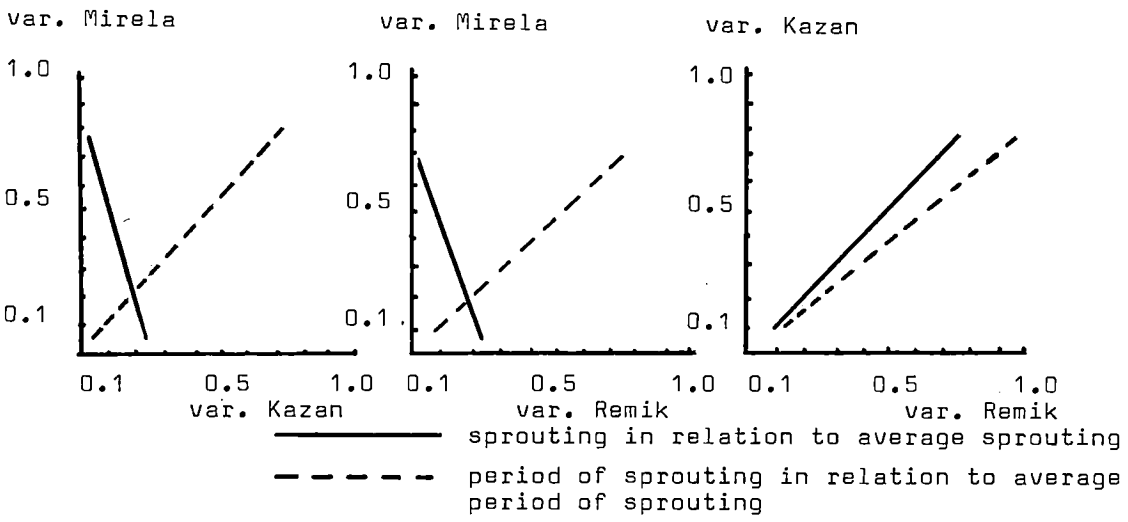

Fig. 3. Relative sprouting and relative period of sprouting of the blue lupin varieties in Posio during 1983—1986.

Table 1. Sprouting of blue lupin varieties in Posio during 1983—1986.

Variety	Year and sprouting (%)				
	1983	1984	1985	1986	1983—1986 ($\bar{x}$)
var. Mirela	29	99	98	30	64
var. Kazan	57	48	93	30	57
var. Remik	61	69	87	28	61
1983—1986 ($\bar{x}$)	49	72	93	29	61

Table 2. Sprouting period (days) of blue lupin varieties in Posio during 1983—1986.

Variety	Years and days				
	1983	1984	1985	1986	1983—1986 ($\bar{x}$)
var. Mirela	17	7	14	25	16
var. Kazan	14	6	10	24	14
var. Remik	17	8	13	30	17
1983—1986 ($\bar{x}$)	16	7	12	26	16

Table 3a. Seedling of blue lupin varieties in Posio during 1983—1986. ($z = \frac{a}{b} \times 100$ % ; a = seedling, b = sprouting).

Variety	Year and seedling (z)				
	1983	1984	1985	1986	1983—1986 ($\bar{z}$)
	%				
var. Mirela	82.8	100.0	93.9	93.3	92.5
var. Kazan	98.2	100.0	94.6	100.0	98.2
var. Remik	96.7	91.3	97.7	100.0	96.4
1983—1986 ($\bar{z}$)	92.6	97.1	95.4	97.8	95.7

Table 3b. Seedling of blue lupin varieties in Posio during 1983—1986. ($v = \frac{a}{c} \times 100\ \%$; a = seedling, c = sowing seeds net).

Variety	Year and seedling (v)				
	1983	1984	1985	1986	1983—1986 ($\bar{v}$)
			%		
var. Mirela	24.0	99.0	92.0	28.0	60.8
var. Kazan	56.0	48.0	88.0	30.0	55.5
var. Remik	59.0	63.0	85.0	29.0	59.0
1983—1986 ($\bar{v}$)	46.3	70.0	88.3	29.0	58.4

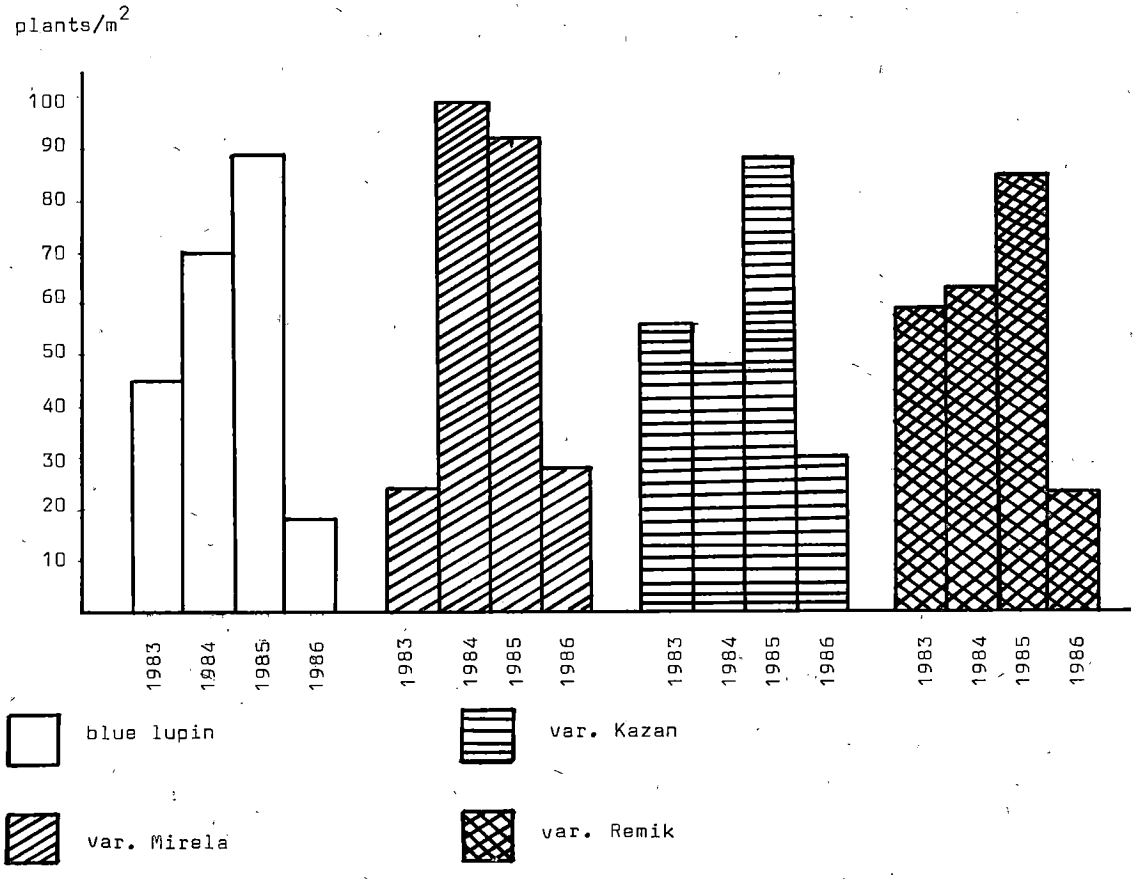

Fig. 4. Seedling of blue lupin in Posio during 1983—1986.

Remik was directly proportional (Fig. 5). When comparing the seedling periods among the blue lupin varieties, it can be said that var. Mirela and var. Kazan, and var. Mirela and var. Remik are both directly proportional in their mutual interdependence. There is also nothing exceptional about the var. Kazan and var. Remik relationship.

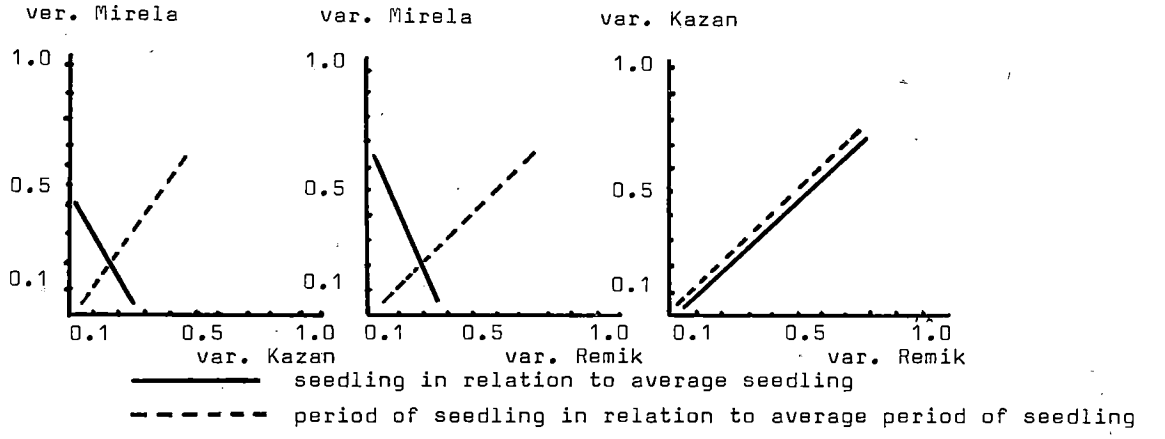

Fig. 5. Relative seedling and relative period of seedling of blue lupin varieties in Posio during 1983—1986.

Vernalization

Comparatively few vernalized lupin plants have been empirically observed in the trials (Fig. 6). On average, during 1983—1986, only 2.6 % of all the blue lupin seedlings vernalized. Plants vernalized the most in 1983 (6.2 %) and the least in 1984 (0.7 %). Varietal differences have been observed.

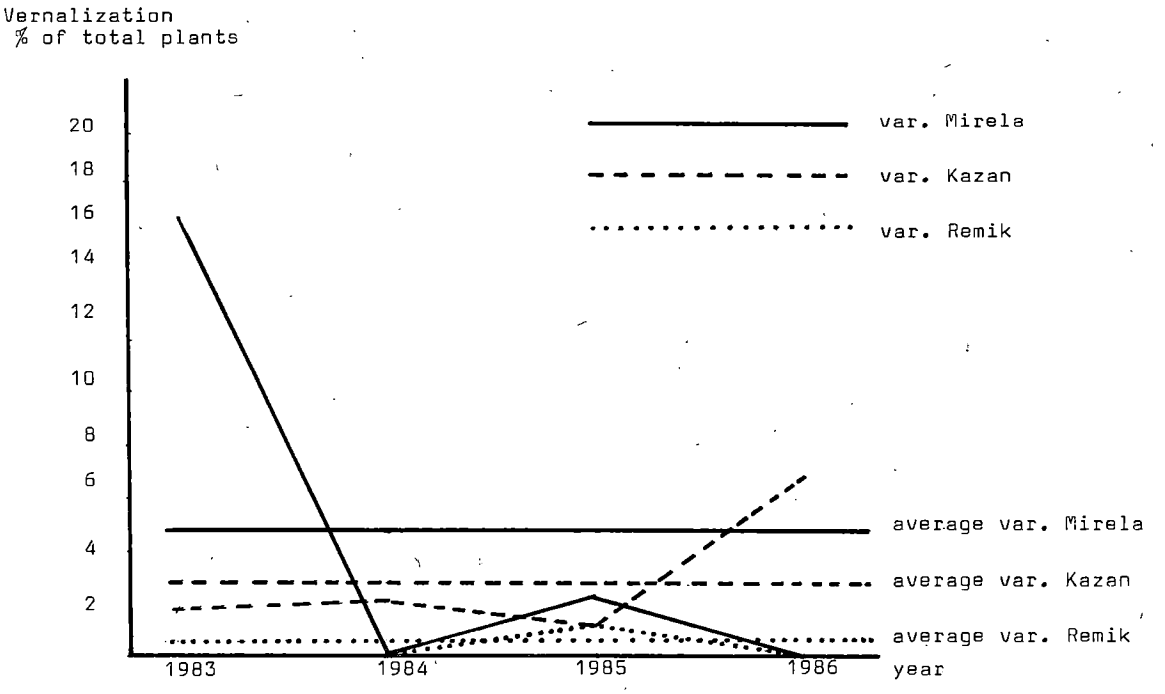

Fig. 6. Vernalization of blue lupin varieties in Posio 1983—1986.

The effect of environment

Results of the growth measurements and environmental factors of the trial have been described earlier (ANISZEWSKI 1988b). Statistical analysis indicated that effective, average and plus average temperature sum up to the 30th growth day had no effect on the sprouting of blue lupin, nor on its seedling or vernalization (Table 4). In addition precipitation up to the 30th growth day did not correlate with these. Sunny and half cloudy days, on the other hand, had an effect on both its sprouting as well as on its seedling. As for vernalization there was no effect whatsoever.

Interdependence during initial development

Statistical analysis (Table 5) clearly shows that sprouting affects the green matter (Pearson's $r = 0.631^{***}$) and crude protein harvest of green matter ($r = 0.801^{***}$) more than seedling (correspondingly $r = 0.627^{***}$ and $r = 0.785^{***}$). Fig. 7 describes, apart from the effect of sprouting, the effect of seedling on green matter and on the crude protein harvest of green matter. During vernalization there is no effect either on green matter or on crude protein harvest of green matter. The periods of field emergence and seedling also have no effect on green matter. In all probability, there is some sort of effect on the crude protein harvest of green matter (statistical significance on the level of $P = 0.05$).

Table 4. Effect of environmental factors on the initial stage of blue lupin growth development in Posio during 1983—1986.

Factor of initial development		Correlation (Pearson's r)			Regression ($y = a + bx$)		
		Spro	Seedl	Vernal	Spro	Seedl	Vernal
$0.32x_1$	Effective temperature sum (x_1)	−0.29°	−0.27°	−0.18°			
$0.32x_2$	Average temperature sum (x_2)	−0.29°	−0.27°	−0.18°			
$0.315x_2$	Plus average temperature sum (x_3)	−0.28°	−0.27°	−0.19°			
$0.21y_1$	Precipitation (x_3)	−0.25°	−0.27°	+0.29°			
$0.34z_1$	Sunny days (z_1)	$+0.58^{***}$	$+0.55^{***}$	+0.20°	$y=3.66+0.33z_1$		$y=11.28+0.02z_1$
$0.31a_1$	Half cloudy days (a_1)	$+0.6^{***}$	$+0.58^{***}$	+0.07°	$y=5.15+0.02a_1$		$y=5.37+0.02a_1$

Spro = Sprouting
Seedl = Seedling
Vernal = Vernalization

For definitions of x_1, x_2, x_3, y_1, z_1 and a_1 see ANISZEWSKI (1988 b).

Table 5. Interdependence of the initial stage of blue lupin growth development in Posio during 1983—1986.

Interdependence of stage	Correlation (Pearson's r)						
	Sprop	Sprod	Seedlp	Seedld	Vernal	Greenm	Crupr
Sprop		-0.675^{***}	$+0.994^{***}$	-0.558^{***}	−0.234°	$+0.631^{***}$	$+0.801^{***}$
Sprod			-0.672^{***}	$+0.827^{***}$	+0.05°	−0.298°	-0.375^{*}
Seedlp				-0.575^{***}	−0.277°	$+0.627^{***}$	$+0.785^{***}$
Seedld					+0.092°	−0.188°	−0.219°
Vernal						−0.219°	−0.054°

Sprop = Sprouting (plants)
Sprod = Sprouting (days)
Seedlp = Seedling (plants)
Seedld = Seedling (days)
Vernal = Vernalization
Greenm = Green matter harvest
Crupr = Crude protein harvest of green matter

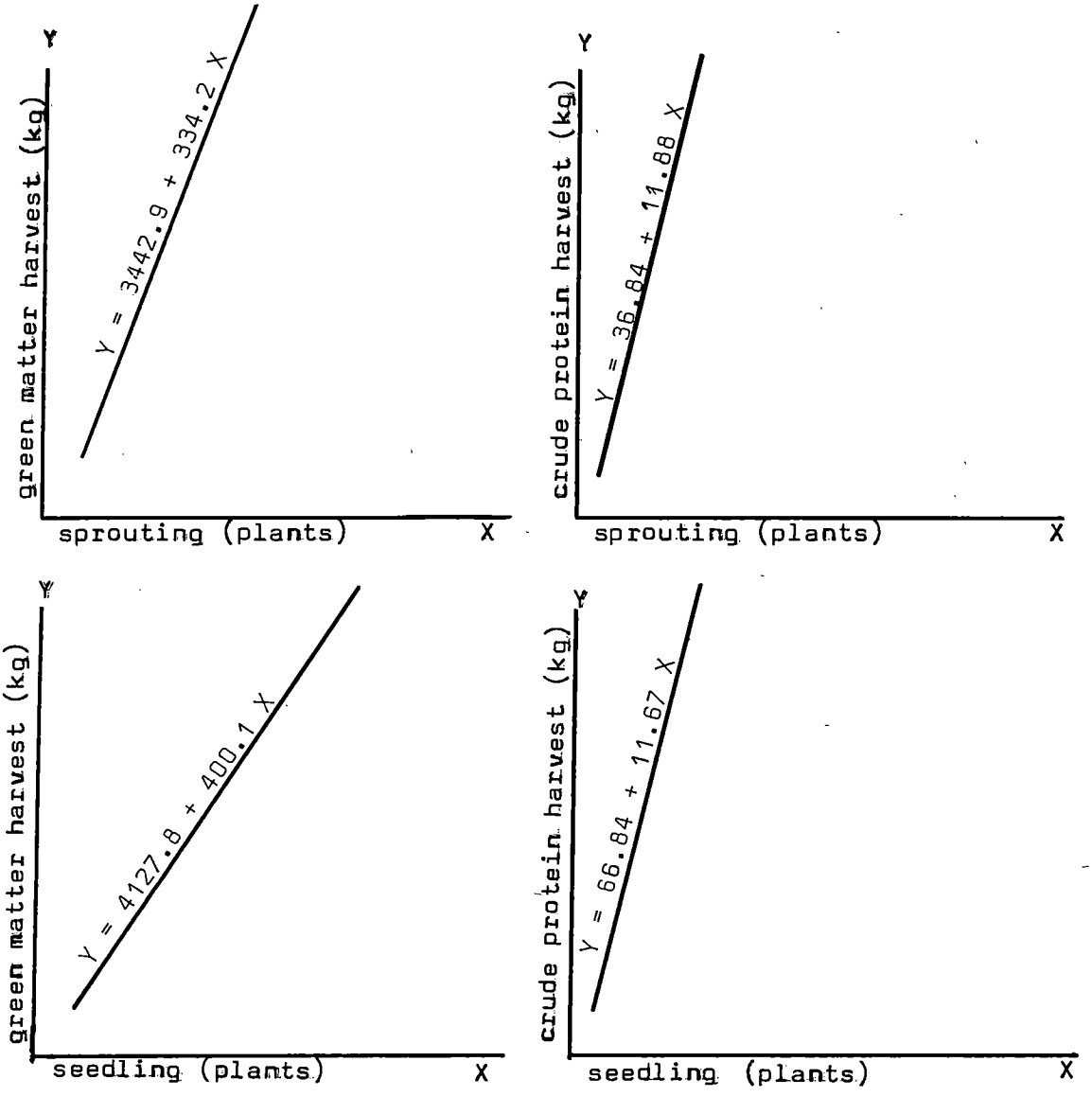

Fig. 7. Dependence between initial development and harvest of blue lupin in Posio during 1983—1986.

DISCUSSION

Sprouting differences among the varieties in different years are surprising considering the fact that high grade elite seeds were used and that sowing was carried out according to the method of PAPROCKI (1983). For example, in the study of blue lupin conducted by GARSIDE (1979) in Australia (41° 35″ S), plant damage during sprouting was not observed at all, and DJUBIN reported (1981) that early sowings carried out during a cold spring sometimes caused damage to lupin sprouts. In the present comparative study on the sprouting of blue

lupin varieties it appeared that var. Mirela is on the average a more resistant variety than var. Kazan and var. Remik are with regard to seed vigour. The ecological development of sprouting in var. Mirela is also clearly different in comparison with the other varieties. The results of this field trial, however, do not provide an explanation as to why this is so. It is possible that either a genetic peculiarity (gene mark) or the effect of protective substances (alkaloids) maybe involved. MIKOLAJCZYK and WROBLEWSKA (1983) have mentioned the genetic similarities of the varieties researched.

Both WEIER et al. (1974) and ZURZYCKI and MICHNIEWICZ (1977) consider the significance of vernalization in plant physiology to be extremely important. KOROVIN (1984) has stated that vernalization accelerates the development of blue lupin biomass. DJUBIN (1981) has mentioned the fact that there is a connection in the damage of sprouts and vernalization. In our study, sprout damage was great and the number of vernalized plants was small. MIKOLAJCZYK and WROBLEWSKA (1983) have presented that the above mentioned varieties do not need vernalization at all.

Earlier studies of blue lupin (GARSIDE 1979, DJUBIN 1981) have reported that lupin's initial stage of growth development is dependent upon air temperature. KOROVIN (1984) mentions the fact that the soil's average temperature has a significant effect on the initial stage of blue lupin's growth development. In our research, however, different air temperature sums had no effect on the initial stage of growth development. It became clear in our research that the amount of sunny and half cloudy days had a clear effect on the initial stage of blue lupin's growth development. The results of our study are not in direct contradition with contention of SWIECICKI and SWIECICKI (1981), according to which blue lupin reacts weakly to cloudiness.

The present results show that despite clear difficulties during the initial stage of growth development (sprouting), the periods of sprouting and seedling have a neutral relationship with the green matter of the blue lupin varieties studied. The same stage of neutrality cannot, however, be proved in the relationship of crude protein harvest of green matter. In connection with the above neutrality, the results show that sprouting of the varieties in the growth conditions of northern Finland is at the varietal extremity.

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SELOSTUS

Kolmen sinilupiinilajikkeen (*Lupinus angustifolius* L.) alkukehitys Pohjois-Suomessa

TADEUSZ ANISZEWSKI

Maatalouden tutkimuskeskus

Vuosina 1983—1986 tutkittiin kolmen sinilupiinilajikkeen (Mirela, Kazan ja Remik) viihtyvyyttä ja sopeutumista Pohjois-Suomen (66° 12″ N ja 27° 13″ E) kasvuoloihin. Tutkimusongelmana on ollut sinilupiinin alkukehitys, sen orastuminen, taimettuminen ja jarovisointi sekä niiden vuorovaikutus viher- ja raakavalkuaissatoihin.

Tutkittujen lajikkeiden orastuminen oli keskimääräisesti heikko (61 %, vaihtelua oli 29 %:sta 93 %:iin), vaikka kylvöön käytettiin hyvälaatuista valiosiementä. Taimettumisvaiheessa lajikkeiden kuolleisuus ei ollut enää ongelma. Jarovisoituneiden yksilöiden määrä oli peltotutkimuksessa hyvin pieni (keskimääräisesti 2.6 % luvun vaihdellessa 0.7 %:sta 6.2 %:iin).

Lämpötilasumma ja sademäärä eivät vaikuttaneet sinilupiinin alkukehitykseen. Sen sijaan siihen on ollut selvä vaikutus aurinkoisten ja puolipilvisten päivien määrällä.

Pohjois-Suomen äärirajallisissa kasvuoloissa, joissa on havaittavissa vaikeuksia lupiinin alkukehityksessä (orastumisessa), orastumisaika samoin kuin taimettumisaika ovat kuitenkin täydellisessä neutraalisuhteessa sinilupiinin viher-satoon. Tätä neutraalisuusastetta ei kuitenkaan pystytty todistamaan suhteessa raaka-valkuaissatoon.

ANNALES AGRICULTURAE FENNIAE, VOL. 27: 153—162 (1988)

Seria ANIMALIA DOMESTICA N. 84 — Sarja KOTIELÄIMET n:o 84

VITAMIN E STATUS AND PRODUCTIVITY OF PIGS FED WITH FRESH OR STORED BARLEY WITH OR WITHOUT VITAMIN E SUPPLEMENTATION

JOUKO TYÖPPÖNEN, TIMO ALAVIUHKOLA and KAIJA SUOMI

TYÖPPÖNEN, J., ALAVIUHKOLA, T. & SUOMI, K. 1988. **Vitamin E status and productivity of pigs fed with fresh or stored barley with or without vitamin E supplementation.** Ann. Agric. Fenn. 27: 153—162. (Coll. Veter. Med., Dept. Biochem., SF-00551 Helsinki, Finland and Agric. Res. Centre, Swine Res. Sta. SF-05840 Hyvinkää, Finland.)

Growing pigs were fed with freshly harvested or stored barley harvested the year before supplemented with different doses of vitamin E (0 to 60 mg dl-α-tocopheryl acetate; α-Tac/kg diet). Barley age had no significant effect on vitamin E content in plasma, liver or heart muscle in the pigs. Similarly the same vitamin E supplements used in the two diets resulted in identical vitamin E levels in plasma, liver and heart muscle of the pigs. Thus the endogenous vitamin E in the fresh and stored barley as well as vitamin E additives in these diets were absorbed similarly. No harmful effects were found by feeding the pigs freshly harvested barley. Production indices and meat quality were similar in all groups, independent of barley age or amount of supplemented vitamin E. However, the basal diets provided only a barely sufficient vitamin E intake, as estimated from tissue vitamin E levels in the pigs. Dietary vitamin E supplementation of 40 mg α-Tac/kg more than doubled plasma vitamin E concentrations, and liver and heart muscle vitamin E levels were elevated over three times as compared to pigs fed the basal diets. By the dietary vitamin E supplementation level of 40 mg vitamin E/kg, which is slightly higher than previous recommendations, plasma vitamin E concentration was kept constant at 0.3 mg vitamin E/g lipid and tissues contained a safety marigin of this vitamin. The observed vitamin E levels in the tissues of the pigs were relatively low as compared to other species fed the same amounts of dietary vitamin E. Alpha-tocopherol accounted for 90 % of the observed vitamin E isomers in the tissues. The remainder consisted of γ-tocopherol and traces of α-tocotrienol.

Index words: growing pigs, vitamin E requirement, tocopherols, barley, productivity.

INTRODUCTION

Sufficient vitamin E intake is essential for pigs as well as for other domestic animals in order to prevent diseases caused by vitamin E deficiency (LANNEK and LINDBERG 1975). It is now evident that the main function of vitamin E in animals is to act as a free radical scavenger to prevent initiations of autocatalyzed peroxidation reactions in membrane phospholipids (McCAY and KING 1980). Selenium in the enzyme glutathione peroxidase (GSH-Px) acts

synergistically as an intracellular antioxidant and reduces the peroxidated fatty acids in membrane lipids to hydroxy fatty acids (ROTRUCK et al. 1973). The requirements of these antioxidant substances for pigs have been previously estimated (BENGTSSON et al. 1978a, b, HAKKARAINEN et al. 1978a, b).

According to a general opinion, pigs should not be fed newly harvested grain. One of the reasons for this is that the vitamin E content in the fresh grain is thought to be lower or less available in newly harvested grain than in stored grain. Previous investigations have shown that the final content of vitamin E in barley is reached several weeks before harvesting and thereafter remains at a relatively constant level if properly stored (GREEN 1958, KIVIMÄE and CARPENA 1973, POHJANHEIMO et al. 1975). There is some evidence of the existence of an 'after ripening process' in harvested barley, but this phenomenon lacks significance if harvesting and the subsequent storage conditions are satisfactory (HAKKARAINEN et al. 1983a, b).

The aim of present experiments was to determine if some factors in freshly harvested barley would decrease the absorption of vitamin E in pigs, or if the vitamin E requirement of pigs would increase by the feeding of such barley. If this were the case, tissue vitamin E levels should be lower in pigs fed fresh barley as compared to pigs fed stored barley, provided that the vitamin E level in both grains is the same.

MATERIAL AND METHODS

Experiment 1

Six groups of healthy pigs with 16 pigs per group were fed from 25 kg body weight to slaughter with two different basal diets composed of freshly harvested barley or stored barley of the same variety harvested the year before (Table 1). Four of the groups were fed a diet composed of fresh barley supplemented with 0, 20, 40 or 60 mg α-Tac/kg diet. The two remaining groups were fed a diet of stored barley with supplements of 0 and 40 mg α-Tac/kg diet. Before the experimental period begun, all pigs received a vitamin E supplemented feed up to 25 kg body weight (Table 1). During the experiment, weight gain and other production indices were weekly registered and state of health of the pigs were observed. At slaughter, the meat quality was determined and heart muscle samples were collected for vitamin E analyses.

Table 1. Composition of basal feeds.

Ingredient	Before exp. (g/kg)	Exp. feed (g/kg)
Barley	410[a]	850[b]
Oats	405[a]	–
Soybean	70	80
Fish meal	80	40
Mineral and vitamin mix[c]	35	30
Total	1000	1000

[a] Stored grain
[b] Stored or newly harvested barley
[c] Contained per kg experimental diet: $CaHPO_4$ $2H_2O$ 12.9 g, $CaCO_3$ 8.6 g, NaCl 3.1 g, MgO 0.57 g, $FeSO_4$ $7H_2O$ 0.45 g, ZnO 0.1 g, MnO_2 56 mg, CuO 27 mg, $CoSO_4$ $6H_2O$ 3.1 mg, KI 0.3 mg, SeO_2 0.1 mg, dl-α-tocopheryl acetate 35 mg (not included in experimental diets), retinol 2.6 mg, cholecalciferol 0.042 mg, thiamin 1.7 mg, riboflavin 6.0 mg, pyridoxine 3.4 mg, nicotinic acid 34.5 mg, pantothenic acid 12.9 mg, cyanocobalamin 0.017 mg.

Experiment 2

Four groups of pigs with 36 pigs per group were fed from 25 kg body weight to slaughter the same basal diets based on fresh and stored barley as in Experiment 1 (Table 1). The two lots of barley were harvested from the same field. Variety, fertilization and handling were also identical for each year. Two groups received a fresh or stored barley diet without vitamin E supplements, whereas the two other groups were fed the same diets with supplements of 40 mg α-Tac/kg. Before starting the experiment, the pigs were fed up to 25 kg body weight with a vitamin E supplemented feed (Table 1). At the start of the experiment, blood was sampled from 5 randomly chosen pigs for the determinations of total lipid and vitamin E contents. During the experimental feeding period, productivity indices were registered weekly. After 30 days on the experimental diets, blood samples were collected for vitamin E analyses. At slaughter, samples of blood, liver and heart muscle were collected for the determination of vitamin E content. Meat quality was also determined. Vitamin E content in plasma, liver and heart muscle samples were analyzed by high-performance liquid chromatography with fluorescence detection as described by HAKKARAINEN et al. (1984). Plasma total lipids were analyzed by the method of EPSTEIN et al. (1972).

RESULTS

Experiment 1

The vitamin E content (α-tocopherol; α-T + α-tocotrienol; α-T3) in fresh barley was 36 mg/kg and that in stored barley 31 mg/kg. In pigs fed fresh barley without vitamin E supplement, the vitamin E (α-T) content in heart muscle (mean of two pooled samples) was 5.2 μg/g. The fresh barley diet supplemented with 20, 40 and 60 mg α-Tac/kg resulted in mean heart muscle α-T concentrations of 6.8, 7.5 and 9.3 μg/g, respectively. Pigs fed stored barley supplemented with 0 or 40 mg α-Tac/kg resulted in mean heart muscle vitamin E levels of 3.1 and 8.5 μg/g, respectively. Neither productivity of the pigs nor meat quality were affected by feeding pigs diets based on fresh or stored barley (Table 2). The effects of dietary vitamin E supplementations on these parameters remained insignificant as well.

Table 2. Daily gain, feed conversion and carcass quality in Experiment 1. Mean values of 16 pigs per group are presented.

Diet	Fresh barley				Stored barley	
Vitamin E suppl. (mg/kg)	0	20	40	60	0	40
Daily gain (g)	736	735	746	748	735	787
Feed conversion (FU/kg gain)	2.63	2.63	2.59	2.62	2.63	2.48
Side fat (mm)	16.8	15.4	17.4	16.6	18.3	16.3
Colour of lean (ELL degr.)	40	35	40	39	36	37

Experiment 2

The vitamin E content (α-T) in fresh and stored barley was equal, i.e. 11.9 mg/kg. The relative content of fatty acids was also similar in fresh and stored barley: Myristic acid 0.2 and 0.3 %, palmitic acid 20.9 and 22.5 %, stearic acid 1.1 and 1.2 %, oleic acid 15.4 and 15.1 %, linoleic acid 55.7 and 54.5 %, and linolenic acid 6.7 and 6.4 %, respectively. The contents of free fatty acids of the total fat content in fresh and stored barley were 6.5 and 9.8 %, respectively. At the start of the experiment, the plasma α-T content in 5 randomly chosen pigs was 0.86 ± 0.33 mg/l. The mean total lipid content in the plasma of the same pigs was 2.66 ± 0.61 g/l. Alpha-tocopherol content in plasma was 0.31 ± 0.05 if expressed as mg/g lipid. There were no significant differences in plasma vitamin E levels of the pigs fed fresh or stored barley at the same vitamin E supplementation level (Table 3). In vitamin E supplemented groups, plasma vitamin E levels were from 2 to 2.5 times higher compared to the corresponding unsupplemented groups.

As concerns α-T levels analyzed in liver and in heart muscle, no significant differences were found between the groups fed fresh or stored barley diets supplemented with the same amount of vitamin E (Table 4). The addition of 40 mg α-Tac/kg into the diet increased the vitamin E content in liver and heart muscle over three times as compared to that found in pigs fed the basal diets.

Plasma samples almost exclusively contained α-T as shown in Table 5. In vitamin E supplemented groups the relative content of α-T in plasma samples was higher as compared to pigs fed with the basal diets. In plasma samples taken on the 30th day of the experiment, a peak in the chromatogram that co-eluted with α-tocotrienol (α-T3) was observed. In unsupplemented groups it accounted for 5—10 % of the area of the α-T peak and in vitamin E supple-

Table 3. Plasma lipid and vitamin E content. Mean with standard deviations of 10 pigs are presented. I = 30th day of experiment. II = slaughtered pigs.

Diet		Fresh barley		Stored barley	
Vitamin E suppl. (mg/kg)		0	40	0	40
Total lipids (g/l)	I	2.84 ±0.58	3.39 ±0.86	3.30 ±0.57	3.39 ±0.93
	II	3.89[a] ±0.58	3.60[a] ±0.61	3.86 ±0.47	3.78[b] ±0.86
α-tocopherol (mg/l)	I	0.50 ±0.18	1.34 ±0.46	0.60 ±0.17	1.47 ±0.62
	II	0.58[a] ±0.25	1.13[a] ±0.46	0.51 ±0.19	1.43[b] ±0.65
α-tocopherol (mg/g lipid)	I	0.18 ±0.05	0.41*** ±0.16	0.19 ±0.06	0.44*** ±0.15
	II	0.15[a] ±0.06	0.31[a]*** ±0.08	0.14 ±0.04	0.37[b]*** ±0.11
Correlation lipid/α-toc.	I	r = 0.600 NS	r = 0.153 NS	r = -0.014 NS	r = 0.634 P < 0.01
	II	r = 0.582 NS	r = 0.905 P < 0.01	r = 0.615 P < 0.05	r = 0.828 P < 0.001

[a]N = 7
[b]N = 9
NS = Not significant
***P < 0.001 as compared to group 0

Table 4. Vitamin E content in liver and heart muscle. Mean with standard deviations of 6 pigs are presented.

Diet	Fresh barley		Stored barley	
Vitamin E suppl. (mg/kg)	0	40	0	40
Liver α-tocopherol (μg/g)	1.40 ±0.29	5.72*** ±2.18	1.75 ±0.48	4.88*** ±1.14
Heart muscle α-tocopherol (μg/g)	2.20 ±0.30	7.11*** ±2.51	2.52 ±0.66	6.37*** ±1.86

***$P < 0.001$ as compared to group 0

Table 5. Relative distribution of vitamin E isomers in plasma, liver and heart muscle. Mean with standard deviation of 10 plasma samples and 6 tissue samples per group are presented. I = 30th day of experiment. II = slaughtered pigs.

Diet		Fresh barley		Stored barley	
Vitamin E suppl. (mg/kg)		0	40	0	40
			α-tocopherol (%)		
Plasma	I	96.4 ± 1.7	98.3 ± 0.4	96.2 ± 1.1	98.3 ± 0.6
	II	97.6[a] ± 1.7	> 99[a]	97.4 ± 1.2	> 99[b]
Liver		94.6 ± 1.4	98.3 ± 0.7	97.3 ± 1.7	98.9 ± 0.4
Heart muscle		93.4 ± 1.6	97.9 ± 1.0	96.9 ± 0.9	98.7 ± 0.3
			γ-tocopherol (%)		
Plasma	I	3.6 ± 1.7	1.7 ± 0.4	3.8 ± 1.1	1.6 ± 0.6
	II	2.4[a] ± 1.7	< 1.0[a]	2.7 ± 1.2	< 1.0[b]
Liver		5.4 ± 1.4	1.7 ± 0.7	2.7 ± 1.7	1.2 ± 0.4
Heart muscle		6.6 ± 1.6	2.1 ± 1.0	3.1 ± 0.9	1.3 ± 0.3

[a]N = 7
[b]N = 9

mented groups the area of the α-T3 peak was 1 to 3 % as compared to α-T. In slaughterhouse samples the plasma α-T3 peak was not observed.

The distribution of α-T and γ-T in liver and heart muscle samples was much the same as that in plasma (Table 5). In pigs fed stored barley the relative content of α-T in the tissues was slightly higher compared to pigs fed fresh barley. In the tissue samples of unsupplemented pigs, traces of α-T3 peak were found.

As in the first experiment, productivity and meat quality were similar in all pigs. Thus, the type of barley or vitamin E supplement had no effect on these parameters (Table 6).

Table 6. Daily gain, feed conversion and carcass quality in Experiment 2. Mean values of 36 pigs per group are presented.

Diet	Fresh barley		Stored barley	
Vitamin E suppl. (mg/kg)	0	40	0	40
Daily gain (g)	826	818[a]	830[a]	811
Feed conversion (FU/kg gain)	2.70	2.71	2.69	2.76
Side fat (mm)	16.5	16.2	15.9	17.1
Colour of lean (ELL degr.)	33	33	33	32
pH 24 h	5.50	5.42	5.48	5.49

[a]N = 35

DISCUSSION

The lowest dietary vitamin E level with sufficient selenium intake that prevents vitamin E and selenium deficiency syndrome (VESD) in pigs has been estimated to be 5 mg α-T/kg feed (HAKKARAINEN et al. 1978a). A supplement of 15 mg α-Tac/kg to a vitamin E deficient diet was shown to maintain a constant vitamin E level in the body fats of pigs (HAKKARAINEN et al. 1978b).

In the present study, 0.1 mg selenium was added per kg feed which thus ensured an adequate selenium intake for the pigs (Table 1). The content of vitamin E in the basal diets originated almost exclusively from barley which composed 85 % of the feeds. In Experiment 1, the analyzed content of α-T + α-T3 in fresh barley was 36 mg/kg and in stored barley 31 mg/kg. Because α-isomers account for 60 % of the total vitamin E content in barley (HAKKARAINEN et al. 1983b) the total vitamin E contents in fresh and stored barley were calculated to be 60 mg/kg and 52 mg/kg, respectively. In Experiment 2, the α-T content in both fresh and stored barley was 11.9 mg/kg. In barley, the relative content of α-T is 14.5 % (HAKKARAINEN et al. 1983b). Thus the total vitamin E content in both types of barley used in Experiment 2 was 82 mg/kg. The total biological activity of vitamin E in barley has been shown to be 37 % as compared to the activity of α-Tac (HAKKARAINEN et al. 1984). Consequently, vitamin E activity in Experiment 1 was 22 mg/kg in fresh barley and 19 mg/kg in stored barley, and finally in the basal diets 19 mg/kg and 16 mg/kg, respectively, as expressed in α-Tac equivalents. Similarly, in Experiment 2 dietary vitamin E activities as α-Tac equivalents were calculated to be 30 mg/kg in both barley samples and 26 mg/kg in the basal diets.

The analyzed α-T levels in plasma were similar to some previously reported vitamin E levels found in pigs fed the same dietary doses of vitamin E (FORSETH 1977, CHRISTENSEN 1980, McMURRAY and RICE 1982, YEN et al. 1985). The importance of the simultaneous expression of vitamin E and lipid content in plasma has been stressed because of their significant mutual correlation (HORWITT et al. 1972). In the present study, this correlation was only partly observed and was stronger at the end of the experiment as compared to samples taken on the 30th day of the experiment (Table 3). If plasma α-T were expressed as mg/g lipid, then the basal feeding groups showed a decreasing tendency in their plasma α-T levels from 25 kg body weight to slaughter (Fig. 1), as the content of total lipids in plasma increased with increasing age of the pigs. The decreasing ten-

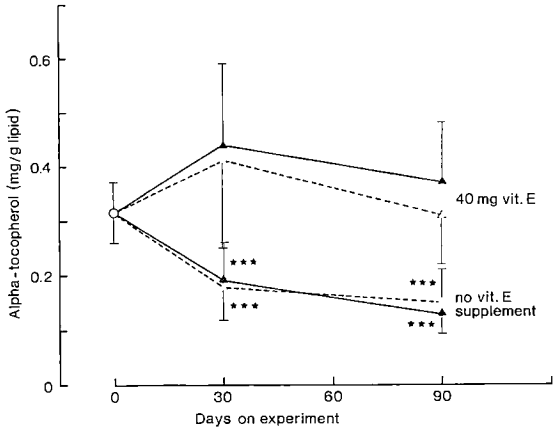

Fig. 1. Plasma α-tocopherol levels in growing pigs fed with fresh (Δ---Δ) or stored (◄——►) barley with or without vitamin E supplement of 40 mg dl-α-tocopheryl acetate/kg diet. Mean with standard deviation of 5 to 10 pigs are given. ***$P < 0.001$ as compared to vitamin E supplemented group fed with same type of barley.

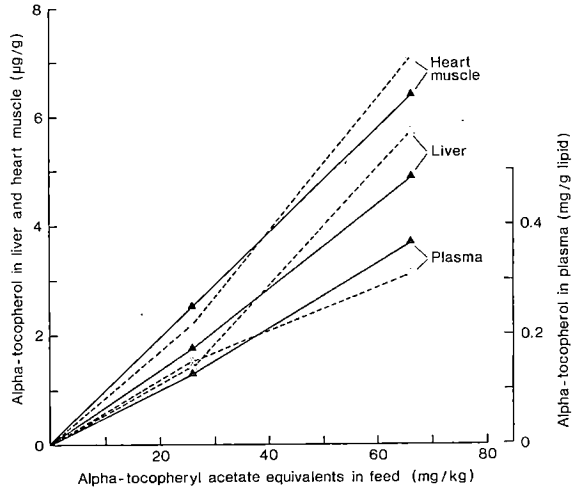

Fig. 2. Responses of plasma, liver and heart muscle to different doses of dietary vitamin E after 3 month feeding. Results represent mean values of 6 to 10 pigs. Feeding with fresh barley (Δ---Δ) or stored barley (◄——►).

dency in plasma vitamin E level was interpreted as a sign of an only barely sufficient vitamin E content in the basal feeds although their calculated vitamin E content in α-Tac equivalents was 26 mg/kg, which should be sufficient according to previous recommendations. In groups that were supplemented with 40 mg α-Tac/kg feed, plasma α-T levels were significantly higher and maintained their initial level of 0.3 mg/g lipid throughout the experiment.

The lowest risk-free plasma vitamin E level in humans has been estimated to be 0.8 mg/g lipid (HORWITT et al. 1972). In the mink, this plasma vitamin E index at the lowest level observed in healthy animals was about 2 mg/g lipid (TYÖPPÖNEN et al. 1984). In horses, which have as low a serum lipid content as pigs, the lowest mean plasma vitamin E level with low-vitamin E feeding was 0.5 mg/g lipid (RONEUS et al. 1986). In view of the plasma vitamin E levels in the other species mentioned above, the vitamin E levels observed in pig plasma are low, especially in the groups fed basal diets. In these pigs, the vitamin E/lipid ratio in the plasma of slaughterhouse samples was only 0.1—0.2 mg vitamin E/g lipid (Fig. 1). Supplementation of the basal diets with 40 mg α-Tac/kg elevated the vitamin E/lipid ratio significantly to 0.3—0.4 mg vitamin E/g lipid.

The vitamin E levels in the liver and heart muscle of the pigs fed basal feeds were relatively low and had very limited reserves for possible increased demands of this vitamin (Fig. 2). At the studied dietary range, an increase of vitamin E of about three-fold caused a linear response in the analyzed tissues (Fig. 2). In experiments with mink (TYÖPPÖNEN et al. 1984) and horse (RONÈUS et al. 1986) the nutritional adequacy of vitamin E within reasonable marigin of safety was defined to lie on the upper part of the initial steep part of a dietary dose/tissue response line. For horses this meant about 150 mg vitamin E/kg feed and for minks about 70 mg/kg. In the present study, the highest dietary vitamin E level was 66 mg/kg but the top of the initial linear proportion of the dose/response line was evidently not yet reached (Fig. 2). On the basis of the vitamin E levels analyzed in plasma and tissue, it was concluded that the supplement of 40 mg α-Tac employed in these diets provided the pigs with an adequate vitamin E status.

There were no significant differences in the absorption of vitamin E from fresh or stored barley (Figs. 1 and 2). The transport of α-T from the blood to the tissues seemed to be slightly more efficient in pigs fed fresh barley as compared to the groups fed stored barley. In pigs fed fresh barley, plasma α-T levels were lower and tissue α-T levels higher as compared to pigs fed stored barley (Fig. 2).

The relative content of α-T in plasma and tissues was higher in groups which received supplemental vitamin E as compared to pigs fed the basal diets (Table 5). The same observation has previously been made in the chick (HAKKARAINEN et al. 1984), the mink (TYÖPPÖNEN et al. 1984), the horse (RONÈUS et al. 1986) and in the cow (HAKKARAINEN et al. 1987). The reason for the above phenomenon is probably due to the increased dietary content of α-T in relation to other dietary vitamin E isomers. In plasma, the proportion of α-T increased further with the increased age of pigs. The content of α-T in liver was slightly higher as compared to that in heart muscle (Table 5).

Barley contains a wide variety of tocopherol and tocotrienol isomers and the relative content of α-T is only 14.5 % of the total vitamin E content in barley (HAKKARAINEN et al. 1983b). The most dominant vitamin E isomer in barley is α-T3 which makes up 45 % of all isomers as expressed in weight units. Although a considerable biopotency is assigned to α-T3 (37 % as compared to α-Tac, BIERI and MCKENNA 1981) α-T was almost exclusively found in the tissues of pigs that ingested this grain. The same finding has been previously made in the pig (MCMURRAY and RICE 1982) the chick (HAKKARAINEN et al. 1984) the mink (TYÖPPÖNEN et al. 1984) the horse (RONÈUS et al. 1986) and in the cow (HAKKARAINEN et al. 1987). The reasons for this finding and the possible ways for vitamin E isomers to express their biological activities is discussed elsewhere (HAKKARAINEN et al. 1984).

In the present study, no harmful effects were found by feeding fresh barley to growing pigs. Although the basal diets were only barely sufficient in vitamin E as shown by vitamin E levels found in the tissues of the pigs, even these diets provided a productivity similar to that achieved with the vitamin E-supplemented diets. However, there might be situations when a higher-than-normal dietary level of vitamin E is necessary to meet the requirements and prevent deficiency. Some unexpected factors that might induce a relative vitamin E deficiency are, for instance decreased function of other parts of the antioxidative defence system, poor quality of dietary fats, or increased oxygen metabolism (GRANT 1966, ULLREY 1981, MCMURRAY and RICE 1982, THODE JENSEN et al. 1983, HAKKARAINEN et al. 1987). It should also be remembered that the vitamin E content in grain can vary considerably depending on harvesting and storage conditions, and thereby the basal vitamin E content in the feed can be very low (MCMURRAY and RICE 1982, HAKKARAINEN et al. 1983a, b).

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SELOSTUS

Vastakorjatulla tai säilötyllä ohralla ruokittujen sikojen E-vitamiinitasot ja tuottavuus sekä E-vitamiinilisän vaikutus näihin tekijöihin

JOUKO TYÖPPÖNEN, TIMO ALAVIUHKOLA ja KAIJA SUOMI

Eläinlääketieteellinen korkeakoulu ja Maatalouden tutkimuskeskus

Kasvavia sikoja ruokittiin vastakorjatulla tai edellisvuotisella ohralla, johon oli lisätty eri tasoja E-vitamiinia (0—60 mg dl-α-tokoferyliasetaattia; α-Tac/kg rehua). Ohran iällä ei ollut merkittävää vaikutusta sikojen plasman, maksan tai sydänlihaksen E-vitamiinipitoisuuteen. Samansuuruiset E-vitamiinilisät näissä rehuissa johtivat myös samansuuruisiin E-vitamiinipitoisuuksiin sikojen elimissä. Vastakorjatun ja edellisvuotisen ohran endogeeninen E-vitamiini ja myös näihin rehuihin lisätty α-Tac absorboituivat siis samalla tavoin tutkituissa sioissa. Vastakorjatun ohran syötöstä ei todettu haittavaikutuksia, vaan tuotosta ja lihan laatua kuvaavat suureet olivat samanlaiset kaikilla sioilla, riippumatta syötetyn ohran iästä tai siihen lisätyn E-vitamiinin määrästä. Kudoksista mitattujen E-vitamiinipitoisuuksien perusteella näytti kuitenkin pelkän perusrehun syöttö antavan sioille vain niukasti riittävän E-vitamiinisuojan. Kun perusrehuun lisättiin 40 mg α-Tac/kg, sikojen plasman E-vitamiinitasot kaksinkertaistuivat sekä maksan ja sydänlihaksen E-vitamiinipitoisuudet yli kolminkertaistuivat verrattuna perusrehulla olleiden ryhmien E-vitamiiniarvoihin. Käyttämällä E-vitamiinin lisäystasoa 40 mg α-Tac/kg, joka on hieman enemmän kun nykyiset suositukset, plasman E-vitamiinitaso pysyi koko kasvatuskauden ajan vakiotasolla 0.3 mg α-T/g lipidiä. Tällöin myös kudokset sisälsivät E-vitamiinia tietyn turvallisuustason. Sikojen kudoksista mitatut E-vitamiinitasot olivat suhteellisen alhaiset verrattuna muihin eläinlajeihin, joita on ruokittu samoilla E-vitamiinitasoilla kuin tässä kokeessa. Alfa-tokoferolin osuus sikojen kudoksissa oli yli 90 % E-vitamiinin kokonaismäärästä. Jäännös koostui γ-tokoferolista sekä osittain myös pienestä määrästä α-tokotrienolia.

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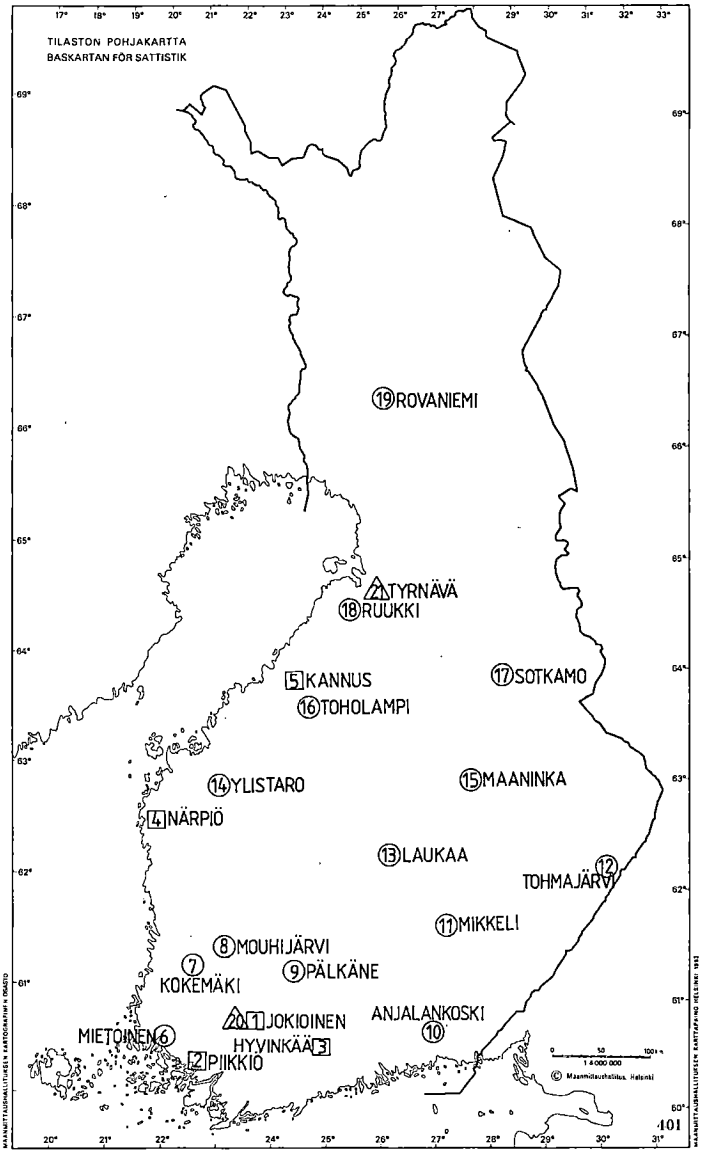

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