Annales Agriculturae Fenniae

Maatalouden
tutkimuskeskuksen
aikakauskirja

Journal of the
Agricultural
Research
Centre

Vol. 22, 2

Annales Agriculturae Fenniae

JULKAISIJA — PUBLISHER

Maatalouden tutkimuskeskus
Agricultural Research Centre

Ilmestyy 4—6 numeroa vuodessa
Issued as 4—6 numbers a year

ISSN 0570-1538

ALASARJAT — SECTIONS

Agrogeologia et -chimica — Maa ja lannoitus ISSN 0358-139X
Agricultura — Peltoviljely ISSN 0358-1403
Horticultura — Puutarhaviljely ISSN 0358-1411
Phytopathologia — Kasvitaudit ISSN 0358-142X
Animalia nocentia — Tuhoeläimet ISSN 0517-8436
Animalia domestica — Kotieläimet ISSN 0358-1438

JAKELU JA VAIHTO

Maatalouden tutkimuskeskus, Kirjasto, 31600 Jokioinen

DISTRIBUTION AND EXCHANGE

Agricultural Research Centre, Library, SF-31600 Jokioinen

This journal is selectively referred by Automatic Subject Citation Alert, Bibliography and Index of Geology — American Geological Institute, Biological Abstracts of Bioscience Information Service, Bulletin Signaletique — Bibliographie des Sciences de la Terre, Chemical Abstracts, Current Contents, Entomological Abstracts, Informascience — Centre National de la Recherce Scientifique, Referativnyj Zhurnal, Review of Applied Entomology (Series A. Agricultural) — Commonwealth Institute of Entomology.

ANNALES AGRICULTURAE FENNIAE VOL. 22: 77—85 (1983)

Seria PHYTOPATHOLOGIA N. 93 — Sarja KASVITAUDIT n:o 93

SURVIVAL OF SOME PLANT PATHOGENS DURING INDUSTRIAL-SCALE COMPOSTING OF WASTES FROM A FOOD PROCESSING PLANT

AARRE YLIMÄKI, ANNELI TOIVIAINEN, HEIKKI KALLIO and ELISA TIKANMÄKI

YLIMÄKI, A., TOIVIAINEN, A., KALLIO, H. & TIKANMÄKI, E. 1983. **Survival of some plant pathogens during industrial-scale composting of wastes from a food processing plant.** Ann. Agric. Fenn. 22: 77—85. (Agric. Res. Centre, Inst. Pl. Path. SF-31600 Jokioinen, Finland.)

The survival of some plant pathogens in compost windrows prepared on an industrial scale in winter, spring and autumn by a convenience food factory was studied during six-month composting periods. Attention was paid mainly to pathogens of potatoes and other vegetables, the raw materials used by the food industry.

At the beginning of composting, the *Plasmodiophora brassicae* content of all the examined windrows was relatively high and decreased significantly by the end of composting, when the pathogen did not appear at all in most cases. In view of the control of club rot, these results can be regarded as excellent, because *Brassica nigra,* the test plant cultivated to indicate the fungus, is more susceptible than the crucifers normally grown in Finland to the races of *Plasmodiophora brassicae.* According to the experiment, exposure to temperature of 70 °C for approx. one week is sufficient to eradicate *Plasmodiophora* spores, provided that the moisture content and the pH are optimal (i.e. moisture content 60—80 % and alkaline pH). A three-week exposure to a temperature of 60—65 °C did not suffice for an equally good result.

Of the other micro-organisms, particular attention was paid to the parasitic fungi common in vegetables: *Rhizoctonia solani, Botrytis cinerea* and *Fusarium* species. The first, which forms sclerotia was, as expected, the most difficult to eradicate. It disappeared, however, and became practically insignificant under the same conditions as *Plasmodiophora brassicae.*

In order to obtain as complete an eradication of plant pathogens as possible, it is very important that the compost is occasionally turned, because only in that way will the fungi surviving in the surface layer of the pile become influenced by the high temperature in the centre.

No live nematode inhabitants of plants were discovered in the samples; they had all been destroyed at the temperature of over 50 °C prevailing in all the compost windrows.

Index words: composting, food prosessing plant wastes, plant pathogens, *Plasmodiophora, Rhizoctonia, Botrytis, Fusarium.*

INTRODUCTION

The growing attention paid to various forms of environmental damage has increased the necessity of investigating problems involved in the disposal and reclamation of wastes. For example the increased use of sewage for irrigation in the U.S.A. has caused plant disease problems (COOKE

1956). In Finland too, a risk of spreading plant pathogens has followed the disposal of vegetable rinsing water and sludge onto cultivated fields. As some sectors of the food industry produce considerable amounts of sludge each year, studies on its reclamation by composting have been started (KALLIO and TIKANMÄKI 1982). From an ecological point of view composting would be an ideal treatment for wastes, provided it were efficient enough, safe and economical. Composting techniques as well as hygiene with respect to human and animal pathogens has been relatively widely studied. In contrast, there is little information on the survival of plant pathogens during composting (HOITINK et al. 1976).

The aim of the present study was to investigate from the point of view of plant protection the usability of compost prepared on as industrial scale in the growing media of plants. Above all, the extent of eradication of plant pathogens during the composting process was investigated. In the first place, attention was paid to pathogens of potatoes and other vegetables, the raw material of the food industry. When using composted soil, the most dangerous pathogens are those which are able to contaminate the soil for a long time or permanently, and are difficult to destroy in the soil. Therefore, the survival of club rot *(Plasmodiophora brassicae)* on cruciferous vegetables during composting was followed with particular interest. Some other plant pathogens were also determined and their survival was followed.

MATERIAL AND METHODS

In a study on the composting of biologically decomposable waste, carried out in connection with the waste disposal system of a food processing plant, the following raw materials were used: sludge from an effluent treatment plant, peat-bound broiler breeder manure and broiler chicken feathers from a slaughter-house. Crushed pine bark was used as a siccative during composting. In the preparation of the compost the following treatments were used as variables: type of mixing and aeration, covering of piles against rain, addition of urea, and stabilization with limestone or calcium oxide. Changes in temperature, pH, dry matter content, conductivity, nitrogen balance and carbon content were recorded in compost windrows prepared outdoors in winter, spring and autumn (KALLIO and TIKANMÄKI 1982).

Samples. For determination of micro-organisms, samples were taken from 20 cm below the surface of each windrow, or from immediately below the frozen layer in winter windrows where the surface was frozen. This was repeated four times, i.e. at the time of preparation of the pile, after 2 weeks, 4 weeks, and about six months. The six-month samples were taken from both 20 cm below the surface and from the centre of the windrow. A one-litre sample was taken from each location, each sample comprising 50 sub-samples. The codes of the piles are same as the codes used in KALLIO and TIKANMÄKI (1982).

Determination of micro-organisms. No complete record of the whole variety of micro-organisms in the compost was aimed at, but particular attention was paid to fungi such as *Plasmodiophora brassicae, Botrytis cinerea, Rhizoctonia solani,* and *Fusarium* spp. known as plant pathogens.

Club rot *(Plasmodiophora brassicae)*

The *Plasmodiophora brassicae* content of the compost samples was assayed using *Brassica nigra* L. as the test plant as it has proven to be very susceptible to the Finnish races of club rot and to have genetically uniform seed material. The seed was obtained from the Swedish Seed Association, Svalöv.

Ten seeds of the test plant were sown in ø 10 cm pots filled with compost and steam-sterilized soil

mix (1:1), or simply in steamsterilized soil controls. Four replicate pots were used.

Six to seven weeks after sowing, the roots of the test plants were rinsed with water and assayed on a scale of 0—3 (SEAMAN et al. 1963). The results were calculated as disease index using WILLIAMS' (1966) scale of 0—100.

Other micro-organisms

In order to determine the other micro-organisms the compost samples were investigated by applying the blotter method (de TEMPE 1963) as follows: 10 small heaps of compost from carefully mixed compost samples were put on blotting paper saturated with sterilized water in a ø 15 cm petri dish. Five replicates were taken for each sample, making up 50 sub-samples for each windrow sample. The petri dishes were first incubated for 5—7 days at 5 °C, and then for 15 days at 20—22 °C. The fungi were isolated 3 weeks after the beginning of culturing. Nematode assays were carried out at the Institute of Pest Investigation of the Agricultural Research Centre.

RESULTS AND DISCUSSION

Although several fungus species were discovered in the compost samples (Tables 4—6), in the present study attention was paid to the destruction of parasitic fungi of the cultivated plants used as raw material by the food industry in compost windrows prepared during different seasons. At the same time, factors causing the destruction were investigated. The availability of oxygen, temperature, moisture content and acidity were recorded as factors regulating the type and quantity of micro-organisms. (KALLIO and TIKANMÄKI 1982)

Club rot

The *Plasmodiophora brassicae* content of the material was relatively high at compost preparation, whereas the disease index ranged between 20 and 100 (Tables 1—3) at different composting times.

In all the windrows, the *Plasmodiophora* activity of the material used was relatively high at the beginning, but decreased during composting to negligible levels, and in most windrows disappeared altogether.

The temperature in **winter windrows** C_1 usually

Table 1. Occurrence of club rot *(Plasmodiophora brassicae)* in winter windrows (C_1).

Windrow		Disease index 0—100				
		Weeks of composting				
		beginning	2	4	25	
					surface	centre
Open, without pipe ($A_1B_1C_1$)	I	79,6	2,5	0,6	0,0	0,0
	II	58,2	0,0	0,0	0,0	0,0
Open, with pipe ($A_1B_2C_1$)	I	99,0	0,0	0,0	73,0	0,0
	II	21,3	0,0	0,0	0,0	0,0
Covered, without pipe ($A_2B_1C_1$)	I	2,0	0,6	0,0	1,3	1,3
	II	93,3	3,3	18,2	0,0	0,0
Covered, with pipe ($A_2B_2C_1$)	I	70,7	0,0	0,0	0,0	9,4
	II	90,3	0,0	0,0	0,0	0,0

remained below 60 °C and the moisture content at about 70 per cent. These conditions were not sufficient to eradicate the club rot spores totally, although the reduction was very large (Table 1). In three winter windrows ($A_1B_2C_1I$, $A_2B_2C_1I$, $A_2B_1C_1I$) liv club rot spores were isolated at the end of composting. Common to these windrows and in contrast to the other windrows were the low pH values recorded, i.e. during the first two weeks of composting the values remained below pH 6. In the other winter windrows the values recorded from the beginning were above pH 6 and rose constantly, reaching pH 7 in three weeks and remaining then between pH 7 and 8,5. In a sample of the surface layer of the windrow ($A_1B_2C_1I$) the club rot content was very high (index 73), the explanation being that the internal temperature of the windrow rose to above 40 °C for only one week and reached 63 °C only during one day. Such low temperatures lasting only a short time were not sufficient to destroy the *Plasmodiophora* spores in the surface layer of the windrow: in the centre, however, the temperatures were sufficiently high.

In **spring windrows** (C_2) the temperature rose regularly to at least 70 °C and the moisture content dropped after one week to below 50 per cent, remaining at 30—40 per cent. The pH values in all the windrows remained alkaline (pH 7—8). Except for two windrows ($A_1B_1C_2II$ and $A_2B_1C_2I$), the club rot content in the windrows decreased to nearly insignificant levels (Table 2). In contrast, in samples taken from the two windrows mentioned above, even 3rd degree club rot outgrowth appeared on the test plants, even though the temperatures remained at 60—70 °C for 3—4 weeks. Accordingly, in these windrows a 3—4 week exposure to 60—70 °C was insufficient to destroy all the club rot spores.

Table 2. Occurrence of club rot *(Plasmodiophora brassicae)* in spring windrows (C_2).

Windrow		Disease index 0—100				
		Weeks of composting				
		beginning	2	4	25	
					surface	centre
Open, without pipe ($A_1B_1C_2$)	I	6,6	0,0	1,3	0,3	0,0
	II	70,0	50,3	0,0	11,8	2,0
Open, with pipe ($A_1B_2C_2$)	I	86,0	57,1	5,9	0,7	0,3
	II	34,3	2,4	0,9	0,3	0,6
Covered, without pipe ($A_2B_1C_2$)	I	66,9	2,3	0,4	0,4	3,4
	II	42,6	21,7	0,0	0,0	0,5
Covered, with pipe ($A_2B_2C_2$)	I	74,5	0,0	0,9	0,0	0,3
	II	40,6	26,2	0,0	0,3	0,0

Autumn windrows (C_3, Table 3). The composition of two autumn windrows of formula 2, $A_1B_1C_3I$ and $A_1B_1C_3II$ was exceptional in that that no club rot could be assayed on the three first samples; six tons of calcium oxide was added to 17 m^3 of the waste mixture. On completion of composting the samples caused no club rot infection in the test plants. In most cases, the temperature in the autumn windrows exceeded 70 °C and the moisture content ranged between 50 and 70 per cent.

In the final samples of windrows of formula 3, $A_1B_1C_3I$ and $A_1B_1C_3II$, where the temperatures exceeded 70 °C for 1—2 weeks, no club rot fungi were observed, while in windrow $A_1B_1C_3III$, as well as in windrows of formula 4, $A_1B_1C_3II$ and $A_1B_1C_3III$ even 3rd degree club rot occurred in the roots of the test plants. The highest temperatures

Table 3. Occurrence of club rot *(Plasmodiophora brassicae)* in autumn windrows (C_3).

Windrow		Disease index 0—100				
		Weeks of composting				
		beginning	2	4	25	
					surface	centre
Formula 2 ($A_1B_1C_3$) (with CaO)	I	—	—	—	1,5	0,0
	II	—	—	—	0,0	0,0
Formula 3 ($A_1B_1C_3$)	I	90,0	1,6	0,3	0,0	0,0
	II	84,7	4,5	15,9	0,0	0,0
	III	100,0	3,5	57,6	14,8	4,0
Formula 4 ($A_1B_1C_3$) (with urea)	I	86,5	1,9	8,9	0,9	0,0
	II	91,0	27,2	28,3	7,8	0,9
	III	100,0	0,8	4,9	20,9	0,0

recorded in the three windrows were 60—68 °C for 1—1 ½ weeks and, accordingly, were not sufficient to eradicate the *Plasmodiophora* spores.

The destruction of *Plasmodiophora brassicae* during composting is primarily caused by the high temperature. As MARTIN (1963) observed, low temperatures (about 50 °C) lasting for 3—4 weeks did not suffice to destroy the fungi, or even to weaken them to any noticeable extent. A much better result was obtained after a three-week exposure to 60—65 °C, and when 70 °C was reached the fungi were killed as quickly as in one week. The fact that the destruction of plant pathogens in all parallel windrow samples was not quite consistent was due to clumps appearing in the compost material. The temperature in the clumps did not rise as high as elsewhere in the windrows.

Although, according to present studies, temperature seems to be the decisive factor in eradication of plant pathogens in compost, the eradication depends on several other factors as well. At least moisture content and pH seem to affect the result in such a way that the moisture content should remain at around 60—80 per cent and pH at an alkaline level.

The probable importance of other micro-organisms such as antagonists, nematodes living on fungi or bark, which have also been found to have a suppressive effect on plant diseases caused by some soil-borne fungi or nematodes (HOITINK 1980, HOITINK and POOLE 1976), were not discussed in the present study.

Brassica nigra, which was used as the test plant, is, according to earlier experience, considerably more susceptible to the races of *Plasmodiophora brassicae* occurring in Finland than the crucifers actually cultivated in Finland. Returning the properly composted soil onto fields does not, therefore cause any considerable risk of spreading club rot disease.

Other fungi causing plant diseases

After composting over six months, *Botrytis, Fusarium,* and *Rhizoctonia* could still be detected in the windrows. However, these fungi occurred in negligible quantities in all the windrows; *Rhizoctonia* was not observed (Table 4). In the samples from spring windrows no *Botrytis* was observed, and only negliable quantities of *Fusarium* and *Rhizoctonia* were found (Table 5). No *Botrytis cinerea* appeared in the autumn windrows, whereas *Fusarium* and specially *Rhizoctonia solani* occurred abundantly (Table 6).

Botrytis cinerea, a very common saprophyte on dead plants and parasite on weakened plants and stored plant products, is apparently easily and quickly eradicated even at temperatures as low as about 40 °C (HOITINK et al. 1976). On the other

Table 4. Occurrence of micro-organisms (%) in compost samples of winter windrows (C_1), a. at the beginning of composting, b. and c. at the end of composting; b. in the surface, c. in the centre.

	Without pipe (B_1)												With pipe (B_2)											
	Open (A_1)						Covered (A_2)						Open (A_1)						Covered (A_2)					
	I			II			I			II			I			II			I			II		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Acrospeira				2	4					2			14			4								4
Alternaria		6	16												2		2			2				
Apiosordaria				2												4								
Arthrobotrys	8	58	82	10	84	82	26	2	2	16	68	82	32	44	60	6	82	94	18	76	58		34	20
Ascobolus				8						20						2								4
Botryotrichum	2			14			20			44			26			8			6			58		
Botrytis		2			10				2		2			2	8					2	8			
Cephalosporium		16	6		18	20	14	24		4	62	18		6	14		12	4		38	28	2		
Chaetomium	2			18			10						4			8						4		
Chrysosporium	56	8	4	60	20	28	38	4	8	44	10	14	36	80	46	46	20	14	34	6	6	80	56	80
Cladosporium		12	32	4	16	4			12			4		14	12	2	8			6	16		14	2
Cylindrocarpon				12			2															2		
Dactylella																2								
Doratomyces	36			34			44			26			24			58			50			36		
Echinobotryum	4			2			4									4			4					
Fusarium	2		2	14	2		2			20			12		2	8		6	4			2		
Fusidium	12									4			10						4					
Geotrichum				4			12			4			8											
Gliocladium	2			4			4		14				4						4		4	4		
Graphium				6			10						12			16			16					
Humicola	12	16	8	20			10			22		4	16	50	10	32	40	32	26	6	22	4	6	30
Mortierella				4						24			18			4						20		
Mucor	72			62			24			52			46			36			26			40	2	4
Ostracoderma													2											
Papulaspora		100	96		62	14		2			6	16	2	86	76	2	88	44		92	66		100	94
Penicillium	4	2	4	10	12	12	34	14	38	12	22		6	18	10	30			4	4				
Phoma herbarum							2									4								
Pilobolus				22						20						22								
Stemphylium													2								2	2		
Syncephalastrum																						24		
Thamnidium				2												2								
Trichocladium	12			6			2			8		2	2	2		4			6				2	
Trichoderma								16	10					2	2				2	8			2	
Volutella	10			2						4			4			2								
Ascomycetes		2		4																				
Hyphae of sclerotia			8	10	24	40	4			16	2	38	2		2	16	50	48		38		4		2
Streptomycetes	2			42	2	6	4	4			10	22	4	6	14	20	14	2	4	18	26	4	4	
Unidentified	4			20	8	2	2	6		4	14		2			20	14		8			8	8	

hand *Fusarium* spp. and especially *Rhizoctonia solani* may have distinctly higher tolerance to relatively high temperatures — exceeding 60 °C — for several weeks. The present results do not support the findings by YUEN (1979) that *Rhizoctonia solani* is destroyed in three weeks when the internal pile temperature exceeds 50 °C; neither do they support those by MARTIN (1963) that the fungus is destroyed in even a short time provided the temperature in the compost is 60—67 °C. *Rhizoctonia solani* was able to remain vigorous especially in windrows prepared in autumn even though the temperature rose to about 70 °C.

On the other fungus species occurring in the composts, *Arthrobotrys, Chrysosporium, Graphium, Humicola* and *Papulaspora* species, which were the dominant fungi in the samples after composting for six months, are worthy of mention. Of these the *Humicola* species are common soil-borne fungi and are known potent decomposers of cellulose in the composting processe (KANE and MULLINS 1973). Other fungi and decomposers of cellulose occurring in the composts discussed here were *Chaetomium* spp. and the thermotolerant *Aspergillus fumigatus*.

Table 5. Occurrence of micro-organisms (%) in compost samples of spring windrows (C_2), a. at the beginning of composting, b. and c. at the end of composting; b. in the surface, c. in the centre.

	Without pipe (B_1)												With pipe (B_2)											
	Open (A_1)						Covered (A_2)						Open (A_1)						Covered (A_2)					
	I			II			I			II			I			II			I			II		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Actospeira	2												4		2				2					
Agrostalagmus										2														
Apiosordaria							8															6		
Arthrobotrys	4	98	98	2	98	58	16	4	8	2	22	26	22	96	94		76	48	8	8	12	2	8	2
Ascobolus	10		2		4	2	10		12				12		2				2		14			
Aspergillus fumigatus	6			2			8			6			8						6			4		
Botryotrichum	10	2		4				10	36	8	36	18	4			2			4	34	16	10	80	86
Chepaliophora								2								2						4		
Cephalosporium		2	18		10	4		2	14	2	12	14	6	4	14		8	18	2	12	2	2	6	10
Ceratocystis														4										
Chaetomium	2	2	2				2	20	22		8		6		4	4			2	8		2	12	28
Chrysosporium	20			22	8		12	44	60	44	14	8	34		2	14	64	70	28	18	38	22	4	8
Cladosporium	12			4			6						2			4			6		4	4		
Clamysdomyces											4	2												
Coemansia													2											
Cylindrocarpon	2												2											
Doratomyces	56			56			78	16	6	80		10	80	4		58	2		70	44	42	4		12
Echinobotryum										2			2						2			2		
Fusarium	2						10	2					6						2			2		
Fusidium				2		4		4	6	8		4			2	4			16			12		
Geotrichum							2										2	6						
Gliocladium						2		2																
Graphium	24	12	16	6	8	20	26	36	40	8	46	52	34	16	32	32	2	4	18	28	86	8	32	14
Humicola	10	4	2	6	2	2	30			12	4		18	18	14	6	10	48	24	2	2	12	4	
Mortierella				2			10			8									2			8		
Mucor	20			68	2		20	2		58	2	12	34			28		2	30			38		
Other Mucorales															4									
Paecilomyces elegans	10																		2					
Papulaspora		98	100	22	100	100	4	96	74		68	68	2	100	90	14	94	96	2	98	22		32	40
Penicillium	88			6			40	2		30	2	4	58			10			44	6	6	30	12	8
Pilobolus	4			2			10						2						10					
Rhizoctonia															6		10							
Sporotrichum				2															6			2		
Stemphylium				2																				
Thamnidium				2																				
Trichocladium				6			2		2	6			8	6			2	6	8					
Trichoderma				6		4				2												4		
Volutella	2																							
Ascomycetes	22	36	4			2	2		8				10	6	8	20				10	8	2		
Basidiomycetes					10	4	2																2	
Hyphae of sclerotia							2								4								4	8
Streptomycetes	56	4	20	62	4	8	54	6	2	52	26	24	82	4	6	56	18	26	50	2	16	46	8	6
Unidentified	12	2	62	2	20	40	12	66	44	2	30	30	10			40		2	12	94	80		2	4

Fungi living on wood and carried into these composts with the bark were *Ceratocystis, Doratomyces, Trichoderma* and *Trichocladium.*

Nematodes

In all the compost samples analyzed, nematodes living on microbes were found in abundance. Nematode species living on plants were not, however, observed live, which is natural because nematodes cannot survive in temperatures of 54—60 °C for even a day.

Table 6. Occurrence of micro-organisms (%) in compost samples of autumn windrows (C_3), a. at the beginning of composting, b. and c. at the end of composting; b. in the surface, c. in the centre.

	Without pipe (B_1) open (A_1)																							
	Formula 2 (D_1)						Formula 3 (D_2)									Formula 2 (D_3)								
	I			II			I			II			III			I			II			III		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Acrospeira																	4		2		2	2		
Agrostalagmus	2			2																				
Alternaria										2			2											
Apiosordaria				10						2			2			8			18			4		
Arthrobotrys		44	40		4	6	62	94	70	76	48	2	66	58	46	80	92	90	64	82	60	70	100	36
Ascopolus							4	42		8	16		12	4		2	20			28			80	
Aspergillus fumigatus	16			8												2								
Botryotrichum	52	6	44	36	48	88	6	2	42	2		6	4		6	14		6	4		10	8		
Botrytis		2																						
Cephaliophora	2			14																				
Cephalosporium		46	98	4	92	98	18	52	88	2	26	36	6	28	38	8	22	30	8	62	40	12	22	26
Ceratocystis							4												8			16		
Chaetomium	90	6	2	58	2	8	12		10	12	2	2	18		12	18	2		16	4	2	10		4
Chrysosporium			2			2	8	76	96	14	98	100	18	100	100	26	100	100	24	94	100	36	72	98
Cladosporium	2																							
Doratomyces	42	4	4	52			24	16		34	18	2	48	4	12	62		6	42	4		64	38	2
Echinobotryum													4			4			2					
Fusarium	4			2					4		4	2		28	30		46	46		44	14			44
Fusidium				4			14			12						2								
Gliocladium	30			2			8	6		6	10		4	34	26	6	64	10	10	40	20	12		10
Graphium	12			10			30	4		32			43	4		34	2		42			54	2	
Humarina		52	74		24			2					8				12						4	
Humicola	2			2			84	14	22	74	50	66	88	68	98	86	36	28	80	30	80	76	2	2
Mortierella		2	6							2	4				2									
Mucor	52			26			24			28	2		22			10		60	8			12		14
Ostracoderma		2					2			10									4					
Papulaspora			6	2			36	100	96	24	94	100	40	98	100	40	100	100	34	98	100	36	80	100
Penicillium	18			2			8			2	8	28	4			2	18		2			4		2
Pilobolus																						2		
Rhizoctonia		14						12	88	38	72	48	4	98	70		56	66	2	44			26	86
Stemphylium	2																							
Trichocladium							48	4	4	64	36	24	42	48	28	18	10	2	16	6	16	16	6	
Trichoderma							8	36	62	4	54	38		40			68	18		88	14	6	10	70
Trichurus				2			6					2	4	2	8	8	6		12			18	2	2
Volutella										2														
Myxomycetes														2			12	6		6				
Ascomycetes	4			4			2	2	2	2						6			2			12		
Basidiomycetes							2												2					
Hyphae of sclerotia																4						4		
Streptomycetes	32			14			20	22	10	12	34		12	26	18	38	22	18	32	32	8	26	10	12
Unidentified		2	4	10			60	28	38	46	6		52	10	6	16	12	52	20	16	22	52	28	8

Acknowledgements. — The authors are grateful to Mr. Kari Tiilikkala, Institute of Pest Investigation, Agricultural Research Centre, for his valuable help in the analyses of the nematodes.

REFERENCES

COOKE, W. B. 1956. Potential plant pathogenic fungi in sewage and polluted water. Pl. Dis. Rep. 40: 681—687.

HOITINK, H. A. J. 1980. Composted bark, a lightweight growth medium with fungicidal properties. Pl. Dis. 64: 142—147.

— & POOLE, H. A. 1976. Composted park media for control of soil-borne plant pathogens. The Intern. Plant Propagators' Soc., Comb. Proc. 26: 261—263.

—, HERR, L. J. & SCHMITTHENNER, A. F. 1976. Survival of some plant pathogens during composting of hardwood tree bark. Phytopath. 66: 1369—1972.

KALLIO, H. & TIKANMÄKI, E. 1982. Composting of sewage

sludge and other wastes from a food processing plant in Finland. Ann. Agric. Fenn. 21: 91—102.

KANE, B. E. & MULLINS, J. T. 1973. Thermophil fungi in a municipal waste compost system. Mycologia 65: 1087—1100.

MARTIN, P. 1963. Plant pathology problems on refuse composting. Intern. Res. Group on Refuse Disposal (IRGRD) Inf. Bull. 19: 211—215.

SEAMAN, W. L., WALKER, J. C. & LARSSON, R. H. 1963. A new race of *Plasmodiophora brassicae* affecting Badger Shipper cabbage. Phytopath. 53: 1426—1429.

TEMPE, J. de 1963. The blotter method for seed health testing. Proc. Intern. Seed. Test. Ass. 28,1: 133—151.

WILLIAMS, P. H. 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopath. 56: 624—626.

YUEN, G. Y. & RAABE,, R. D. 1979. Eradication of fungal plant pathogens by aerobic composting. Phytopath. 69: 922.

Manuscript received november 1982

Aarre Ylimäki and Anneli Toiviainen
Agricultural Research Centre
Institute of Plant Pathology
SF-31600 Jokioinen, Finland

H. Kallio
University of Turku
Department of Chemistry and Biochemistry
Laboratory of Food Chemistry
SF-20500 Turku 50, Finland

Elisa Tikanmäki
Saarioinen Ltd
SF-36420 Sahalahti, Finland

SELOSTUS

Eräiden kasvitaudinaiheuttajien säilyminen elintarviketeollisuuden jätteiden teollisessa kompostoinnissa

AARRE YLIMÄKI, ANNELI TOIVIAINEN, HEIKKI KALLIO JA ELISA TIKANMÄKI

Maatalouden tutkimuskeskus, Turun yliopisto ja Saarioinen Oy

Vuoden 1980 aikana selvitettiin Maatalouden tutkimuskeskuksen kasvitautiosastolla ja Saarioinen Oy:n elintarviketeollisuuslaitoksen toimesta talvella, keväällä ja syyskesällä teollisesti valmistetuissa kompostiaumoissa olevien kasvipatogeenien elossa säilymistä 6 kk kestäneen kompostoinnin aikana. Huomio kohdistettiin ensisijaisesti elintarviketeollisuuden raaka-aineenaan käyttämien perunan ja vihanneskasvien tautien aiheuttajiin.

Kaikkien tutkittujen aumojen möhöjuuripitoisuus oli kompostoinnin alussa verraten suuri ja oli se kompostoinnin päättyessä laskenut merkittävästi, useimmissa tapauksissa tautia ei tullut esiin ollenkaan. Kun möhöjuuri-sienen indikaattorikasvina käytetty *Brassica nigra* on Suomessa tavattaville *Plasmodiophora brassicae*-roduille alttiimpi kuin viljeltävät ristikukkaiskasvit, ovat tulokset möhöjuuren ennakkotorjuntaa silmällä pitäen erinomaiset. Tutkimuksen mukaan noin yhden viikon pituinen altistuminen 70 °C lämpötilalle riittää tuhoamaan möhöjuuri-itiöt edellyttäen, että kosteus ja pH-arvo ovat myös optimaaliset eli kosteus 60—80 % ja pH-arvo alkaalisella tasolla. Kolmen viikon pituinen altistuminen 60—65 °C:n lämpötilalle ei riittänyt yhtä hyvän tuloksen saavuttamiseen.

Muista pieneliöistä kiinnitettiin huomiota nimenomaan kasvimateriaaleissa yleisiin loissieniin *Rhizoctonia solani, Botrytis cinerea* ja *Fusarium*-lajit. Niistä ensiksi mainittu oli lepoasteita ja rihmastopahkoja muodostavana odotetusti muita vaikeammin tuhoutuva. Se hävisi kuitenkin käytännöllisesti katsoen merkityksettömäksi samoissa oloissa kuin möhöjuuri.

Patogeenisten sienien mahdollisimman täydellisen tuhoutumisen kannalta on erittäin tärkeätä kompostin sekoittaminen aika ajoin, koska vain siten pintaosissa olevat sienet saadaan korkeiden lämpötilojen vaikutuspiiriin. Kasveissa loisivia ankeroisia ei näytteistä tavattu elävinä ollenkaan, vaan olivat ne kaikissa kompostiaumoissa vallinneissa 50 °C:n lämpötiloissa tuhoutuneet.

ANNALES AGRICULTURAE FENNIAE VOL. 22: 86—92 (1983)

Seria ANIMALIA DOMESTICA N. 61 — Sarja KOTIELÄIMET n:o 61

UREA PHOSPHATE AS A SOURCE OF SUPPLEMENTAL PHOSPHORUS FOR POULTRY

TUOMO KIISKINEN

KIISKINEN, T. 1983. **Urea phosphate as a source of supplemental phosphorus for poultry.** Ann. Agric. Fenn. 22: 86—92. (Agric. Res. Centre, Inst. Anim. Husb., SF-31600 Jokioinen, Finland.)

Three experiments, one on laying hens and two on broiler chicks, were conducted to compare the biological availability of phosphorus from urea phosphate (UP) with that of the dicalcium phosphate (DP) commonly used in poultry rations. The contents of UP and DP in the diets varied from 0,7 to 1,4 % and from 0,8 to 1,6 %, respectively. In Experiment 3 both phosphates were used on two phosphorus levels: 0,24 and 0,40 % available phosphorus in the diets of broiler chicks. No significant differences were observed between the phosphates with respect to egg production, final body weights of broilers, efficiency of feed conversion, mortality, serum values, leg weakness or tibia ash content. In Experiment 2 on broilers the tibia ash phosphorus content tended to be slightly higher ($P < 0,01$) with UP than with DP. The average body weight of the UP groups at three weeks of age was significantly higher ($P < 0,05$) than that of the DP groups in Experiment 3. No significant difference was found in the rate of growth, mortality, leg weakness or percentage of tibia ash between phosphorus levels.

The results of these experiments suggest that urea phosphate has an equivalent availability to that of dicalcium phosphate and is a safe phosphorus supplement for practical poultry rations.

Index words: urea phosphate, dicalcium phosphate, laying hen, broiler, biological availability of phosphorus.

INTRODUCTION

Urea phosphate CO $(NH_2)_2H_3PO_4$ is a crystalline compound of urea (38 %) and phosphoric acid, containing approximately 17,5 % N and 20 % P. It has been tested on ruminants, and the absorption of nitrogen is egual to that from urea and the availability of phosphorus is equivalent to that from dicalcium phosphate (SOMMER et al. 1975). However, urea phosphate (UP) decreased the acceptability of the feed ration more than urea. In normal circumstances non-ruminants cannot utilize non-protein nitrogen (NPN). When added to diets which are adequate in essential amino acids but deficient in non-specific nitrogen, NPN compounds can be utilized for the synthesis of nonessential amino acids (ROSE et al. 1949). KIISKINEN (1977) used UP as a source of supplemental phosphorus and NPN in low-protein (barley-oat) layer rations. UP decreased the feed consumption and egg production. The concentration of UP was 2,5 %, which is unnecessarily high for practical poultry rations. SARKKINEN (1977) has described the urea phosphate process developed in Finland by Kemira Oy. This process could utilize the cheap

impure phosphoric acid produced by the fertilizer industry. This aroused interest in elucidating the availability of UP as a source of supplemental phosphorus for poultry, too. The present study was arranged to compare the biological availability of phosporus from urea phosphate with that from dicalcium phosphate (DP).

MATERIAL AND METHODS

Birds and housing

In the first experiment, 1000 laying hens (W.L.) were reared in 2-tier stair-step cages, one hen per cage (20 x 44 cm). The average room temperature was 15—16 °C and relative humidity 55 % during the experiment. The day length was 16 hours. The experiment was run between 52 and 68 weeks of age.

Eight hundred broiler chickens (Pilch) were used in Experiment 2. Day-old-chicks were randomly distributed into eight 6 m² floor pens. Each pen was supplied with two feed pans and round automatic drinkers. In the third experiment, 320 Pilch broilers were distributed into eight 3 m² pens with one feeder and waterer. In both experiments the distribution of sexes was 1:1, chicks were reared under standard conditions and slaughtered at the age of 6,5 weeks.

Experimental design

The first experiment included two treatment groups (dicalcium phosphate and urea phosphate), each with four replicates of 125 hens. The same treatments were also applied in Experiment 2 and the number of replicates was four with 100 broilers each. The third experiment had a 2 x 2 factorial design. Both the above-mentioned phosphates were used on two phosphorus levels: 0,24 and 0,40 % available phosphorus in the diet. Every treatment had two replicates of 40 chicks.

Diets and feeding

The test phosphates provided the only supplemental phosphorus in the diets (Tables 1—3). Because UP contains no calcium and its phosphorus content is higher than in DP, DP was used respectively more than Up in the experimental diets and the UP diets were supplemented with more calcium carbonate than the diets containing DP. In Experiment 3, both phosphates were used on two levels, and to produce the low P concentration (0,24 % available P) soybean meal was used as a protein and wheat starch as an energy source. There were

Table 1. Composition of the diets in Experiment 1.

	Dicalcium phosphate DP	Urea phosphate UP
	%	
Fish meal	3,5	3,5
Meat and bone meal	1,0	1,0
Soybean meal	6,5	6,5
Dried yeast	1,5	1,5
Barley	55,0	55,0
Oats	23,5	23,0
$CaCO_3$	6,8	7,5
$CaHPO_4$	1,3	—
Urea phosphate[1]	—	1,1
NaCl	0,25	0,25
Vitamin- and mineral premixes[2]	0,65	0,65
Analysis:		
Crude protein %	15,8	17,1[3]
Ether extract %	2,7	2,6
Crude fibre %	4,9	4,8
Ash %	8,6	8,2
Ca %	3,13	3,16
P %	0,63	0,85
Calc. ME MJ/kg	10,30	10,25

[1]) Phosphorus content 19,8 %, nitrogen 17,5 %
[2]) Premixes of Vaasan Mylly Oy
[3]) Approx. 1,2 % protein equivalents from UP.

small differences in ME concentrations due to the different amounts of mineral sources. The feeding of the experimental diets in Experiment 2 started at the age of two weeks. Both groups were offered the same starter diet during the first two weeks. In all experiments feeding was ad libitum. The layer diets were meals and the broiler diets pellets (3 mm).

Measurements

Daily egg production and feed consumption in four week periods were measured for each replicate of the treatments. Mortality and number of cracked eggs were also recorded. The broiler chicks were weighed individually twice, at the age of 2—3 weeks and again at 6,5 weeks. The total weight per pen was measured for day-old-chicks. In the slaughter-house the total carcass weight of each replicate (pen) was recorded. Feed consumption of each pen was measured between weighings. Mortality and leg weakness were recorded.

Table 2. Composition of the diets in Experiment 2.

	DP	UP
	%	
Fish meal	5,5	5,5
Soybean meal	15,0	15,0
Fat	3,5	3,5
Wheat	22,0	21,7
Barley	43,0	42,5
Wheat middlings	7,0	7,0
Torula yeast	0,5	0,5
$CaHPO_4$	1,6	—
Urea phosphate	—	1,3
$CaCO_3$	—	1,1
NaCl	0,25	0,25
Vitamin- and mineral premixes	0,65	0,65
Calcium lignosulphonate	1,0	1,0
	100,0	1000,0
Analysis:		
Crude protein %	20,7	22,0[1])
Ether extract %	5,0	4,4
Crude fibre %	2,7	3,6
Ash %	4,3	5,5
Calcium %	0,92	1,23
Phosphorus %	0,91	1,04
Calc. ME MJ/kg	12,0	11,9

[1]) Approx 1,4 % protein equivalents from UP.

Table 3. Composition of the diets in Experiment 3.

Diet	DP/0,24	UP/0,24	DP/0,4	DP/0,4
	%			
Soybean meal				32,0
Wheat starch[1])	64,0	63,6	634,7	62,9
Soya oil				1,0
$CaHPO_4$	0,8	—	1,6	—
Urea phosphate	—	0,7	—	1,4
$CaCO_3$	1,0	1,5	0,5	1,5
NaCl				0,45
Na_2SO_4				0,10
Vitamin- and mineral premixes				0,45
Dl-methionine				0,20
Analysis				
Crude protein %	15,5	15,7	14,9	16,5
Ether extract %	1,6	1,6	1,5	1,5
Crude fibre %	2,2	2,1	1,8	1,6
Ash %	4,0	3,7	4,2	4,0
Calcium %	0,91	0,87	0,89	0,88
Phosphorus %	0,46	0,46	0,56	0,60
Supplemented P %	0,16	0,16	0,32	0,32
Calc. available P %	0,24	0,24	0,40	0,40
Calc. NPN (urea phos.) %		0,14		0,28
Calc. ME MJ/kg[2])	12,5	12,4	12,4	12,3

[1]) A product of Raision Tehtaat: dry matter 92,8, crude protein 7,2 %, calcium 0,04 % and phosphorus 0,12 %.
[2]) ME MJ/kg 9,6 for soybean meal and 14,6 for wheat starch.

Analyses of diets, tibia and serum

The proximate analyses of the diets were carried out using standard procedures (Weende). The calcium and phosphorus contents of the diets and tibia ash were determined in the laboratories of Viljavuuspalvelu Oy and Kemira Oy. The left tibia was removed from carcasses of 20 broilers per dietary treatment in Experiment 2 and 16 broilers in Experiment 3. The tibias were ashed at 600 °C 16 hours after cleaning, crushing and ether extraction (Twisselman).

The inorganic phosphorus in the plasma of laying hens (Expt. 1) and the serum of broilers (Expt. 3) was analyzed by the method of TAUSSKY and SHORR (1953). The ammonium and urea concentrations in serum were determined by the colorimetric method of McCULLOUGH (1967) in Experiment 3. Urea was first hydrolysed to ammonium by urease. Blood samples were taken from the wing vein before slaughter. In Experiment 3 the chickens on the low level of phosphorus were, unfortunately, killed before blood sampling.

Statistical analyses

All data were examined by analysis of variance. The significant treatment differences were determined using Tukey's test (STEEL and TORREY 1960) and the t-test.

RESULTS AND DISCUSSION

Urea phosphate at a concentration of 1,1 % in the layer diet (Expt. 1) did not significantly affect egg production, feed consumption, mortality or inorganic phosphorus level in the plasma in comparison with dicalcium phosphate (Table 4).

The equivalent growth rate and feed intake of broilers were obtained with both test phosphates when used as a source of supplemental phosphorus in practical diets (Expt. 2, Table 5). No significant differences were found in mortality,

Table 4. Performance of laying hens in Experiment 1.

	DP	UP	Significance
Laying %	66,2	66,4	NS
Egg weight g	60,9	61,3	"
Daily feed cons. g/hen	119,6	121,4	"
Feed efficiency (kg/kg)	2,98	2,99	"
Cracked eggs %	0,28	0,27	"
Mortality %	1,8	2,6	"
Plasma inorg. P mg/100 ml ±SD(N)	2,77 ±0,53(12)	2,73 ±0,52(14)	"

Table 5. Performance of broiler chicks in Experiment 2.

	DP	UP	Significance
Body weight gain g (2—6,5 weeks)	1232	1227	NS
Slaughter weight g	888	891	"
Daily feed cons./chicken g (2—6,5 weeks)	85,2	84,8	"
Feed efficiency			
kg/kg wt.gain (2—2,6 weeks)	2,01	2,00	"
" (0—6,5 weeks)	1,87	1,85	"
kg/kg slaughter wt.	3,17	3,11	"
Mortality %	0,50	0,75	"
Leg weakness %	2,00	1,25	"
Tibia ash % in fat-free DM	55,0 ±0,9(20)	53,8 ±1,7(20)	"
Calcium % in tibia ash	37,4 ±0,4(20)	37,6 ±0,5(20)	"
Phosphorus % in tibia ash	17,9 ±0,2(20)	18,2 ±0,3(20)	**

** $P < 0,01$

incidence of leg weakness, tibia ash or percentage of calcium in tibia ash. The phosphorus content of tibia ash was, however, slightly higher ($P<0,01$) with the UP diet than with the DP diet.

The increase in the three week body weight of the UP groups over that of the DP groups ($P<0,05$) in Experiment 3 was apparently due to the non-protein nitrogen of UP (Table 6). The protein content of the diets was only 15 % and in certain circumstances supplementation of urea to low protein diets has produced positive chick growth response (FEATHERSTON et al. 1962, KOCI and GROM 1972, LEE and BLAIR 1972, DAVIS and MARTINDALE 1973, MILLER 1973).

The final weights and slaughter weights were also greater when chickens were fed diets with urea phosphate than with dicalcium phosphate. Mortality, incidence of leg weakness, tibia and serum values were equal on both phosphates. The highest content of UP (1,5 %) used in Experiment 3 corresponds to 0,5 % urea. A two to four times higher concentration of urea in pig and layer rations has drastically elevated the urea but not the ammonium concentration in serum (KORNEGAY et al. 1970, KIISKINEN 1977).

It was surprising that the growth rate of chickens was equal on both phosphorus levels in Experiment 3 (Table 6). However, tibia ash and its phosphorus content tended to be slightly higher on 0,40 % than on 0,24 % available phosphorus. The difference in percentage of phosphorus in tibia ash was significant ($P<0,01$). The recommendations for requirements of non-phytin phosphorus vary between 0,4 and 0,55 % in broiler diets (ANON. 1981).

The differences in feed consumption between phosphates and phosphorus levels ($P<0,05$) were more obviously due to differences in content of mineral material and ME concentration than in the acceptability of the diets. For the same reason,

Table 6. Perfomance of broiler chicks in Experiment 3.

	Phosphate				Average			Average		
	DP		UP							
	Available P %									
	0,24	0,40	0,24	0,40	DP	UP	Signi-ficance	0,24 % $P_{av.}$	0,40 % $P_{av.}$	Signi-ficance
Body weight (3 weeks) g	504[a]	509[ab]	516	522[b]	507	519	*	510	516	NS
SD	46	58	51	53	53	52		49	56	
Body weight (6,5) weeks) g	1475	1509	1523	1510	1493	1517	NS	1500	1510	NS
SD	189	195	183	179	192	181		187	186	
Slaughter weight g	893	882	912	892	887	90	NS	903	887	NS
Daily feed cons.g (3—6,5 weeks)	37,0	38,3	39,0	41,4	37,7	40,2	*	38,0	39,9	*
Daily feed cons.g (3—6,5 weeks)	92,06	95,8	96,1	100,3	94,2	98,2	*	94,4	98,1	*
Feed conv. kg/kg w.g.	2,07	2,10	2,08	2,16	2,09	2,12	NS	2,08	2,13	NS
Feed conv. kg/kg sl. wt.	3,50	3,51	3,54	3,67	3,51	3,61	NS	3,52	3,59	NS
Mortality %	7,5	1,3	3,8	6,3	4,4	5,0	NS	5,6	3,8	NS
Leg weakness %	8,8	11,3	8,8	12,5	10,0	10,6	NS	8,8	11,9	NS
Tibia ash %	52,4	52,8	52,4	52,8	52,6	52,6	NS	52,4	52,8	NS
SD	2,1	1,6	1,2	1,2	1,9	1,2		1,7	1,4	
P % in tibia ash	18,1[a]	18,4[b]	18,3[ab]	18,4[b]	18,2	18,3	NS	18,2	18,4	**
SD	0,19	0,30	0,21	0,24	0,27	0,23		0,20	0,27	
Serum inorg. P mg/100 ml	—	4,25	—	4,17						
SD	—	0,38	—	0,27						
Serum urea mg/100 ml	—	4,63	—	4,58						
SD	—	2,91	—	2,84						
Serum NH_4	—	4,58	—	4,59						
SD		1,44		2,44						

a—b, * = $P<0,05$ ** = $P<0,01$

there were respective differences in feed efficiency.

The results of these experiments clearly show that phosphorus is as available to poultry in urea phosphate as in dicalcium phosphate. No evidence was found that the urea part of UP had any detrimental effect on the performance of the chicks and laying hens. Because the addition of 1 % or more of urea to practical diets can reduce the weight gain of chicks (MARCH and BIELY 1971, SIBBALD and HAMILTON 1975) and because of earlier experiences with urea phosphate (KIISKINEN 1977) the use of UP should be limited to 1,5—2 % on the diet.

REFERENCES

ANON. European Federation News W.P.S.A. 1981. Mineral Requirements for Poultry/Recommendations in European Countries. W. Poult. Sci. J. 37: 127—138.

DAVIS, R. H. & MARTINDALE, C. H. 1973. The performance of laying hens fed on rearing and laying diets containing urea. Br. Poult. Sci. 14: 153—160.

FEATHERSTON, W. R., BIRD, H. R. & HARPER, A. E. 1962. Effectiveness of urea and ammonium nitrogen for the synthesis of dispensable amino acids by the chick. J. Nutr. 78: 198—206.

KIISKINEN, T. 1977. Ei-proteiinityppi yksimahaisten kotieläinten, lähinnä siipikarjan ravitsemuksessa. Maatalouden tutkimuskeskus, Kotieläinhoidon tutkimuslaitoksen tiedote 9, 65 p.

KOCI, S. & CROM, A. 1972. Mocovina ako zdroj nebielkovinoveho dusika vo výkrme kurciat. Ved. Práce, Hydinárstvo, 12: 63—74.

KORNEGAY, E. T., MOSANGHIRI, V. & SNEE, R. D. 1970. Urea and amino acid supplementation of swine diets. J. Nutr. 100: 330—340.

MARCH, B. E. & BIELY, J. 1971. Urea tolerance in growing and adult chickens. Poult. Sci. 50: 1077—1080.

MCCULLOUGH, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clin. Chem. Acta 17: 297—304.

MILLER, D. 1973. Chick growth response from nitrogenous compounds fed with suboptimal level of protein. Poult. Sci. 52: 1059-1064.

LEE, D. J. W. & BLAIR, R. 1972. Effects on chick growth of adding various non-protein nitrogen sources or dried autoclaved poultry manure to diets containing crystalline essential amino acids. Br. Poult. Sci. 13: 243—249.

ROSE, W. C., SMITH, L. C., WOMACH, M. & SHANE, M. 1949. The utilization of the nitrogen of ammonium salts, urea and certain other compounds in the synthesis of non-essential amino acids in vivo. J. Biol. Chem. 181: 307—316.

SARKKINEN, K. 1977. Production of urea phosphate and its use in the feeding of animals. Int. Congr. on Phosphorus Compounds. Rabat, October 17.—21. 1977: 433—441.

SIBBALD, I. R. & HAMILTON, R. M. G. 1975. Urea tolerance in chicks. Can. J. Anim. Sci. 55: 167—168.

SOMMER, A., HERCEG, J. & PATEK, V. 1975. Výskum vyuzitia fostâtu mocoviny vo vyzive vykrmoveho dobytka. Zivocisna vyroba (Praha) 20: 23—32.

STEEL, R. G. & TORRIE, J. H. 1960. Principles and procedures of statistics. 481 p. New York.

TAUSSKY, H. H. & SHORR, E. 1953. A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem. 202: 675—685.

Manuscript received November 1982

Tuomo Kiiskinen
Agricultural Research Centre
Institute of Animal Husbandry
SF-31600 Jokioinen, Finland

SELOSTUS

Ureafosfaatti siipikarjarehujen lisäfosforin lähteenä

TUOMO KIISKINEN

Maatalouden tutkimuskeskus

Ureafosfaatti, joka kiteytetään ureasta ja fosforihaposta, sisältää typpeä noin 17,5 ja fosforia 20 %. Sen valmistuksessa voidaan käyttää halpaa, epäpuhdasta fosforihappoa ja sen vuoksi ureafosfaatin valmistus myös rehuseoksissa käytettäväksi on kiinnostanut lannoiteteollisuutta. Ureafosfaatin ureaosa ei kuitenkaan käytännön rehuseoksissa tule hyväksikäytetyksi yksimahaisten kotieläinten aineenvaihdunnassa.

Ureafosfaatin käyttökelpoisuutta verrattiin dikalsiumfosfaatin fosforiin munivilla kanoilla ja broilereilla suoritetuissa kokeissa. Molempia fosfaatteja käytettiin siten, että lisättyä fosforia tuli yhtä paljon seoksiin. Ryhmien munantuotanto- ja kasvutulokset olivat täysin yhteneväiset. Myös veren fosforiarvojen sekä sääriluun tuhkapitoisuuden ja sen fosforipitoisuuden perusteella fosforin käyttökelpoisuutta voidaan em. fosfaateissa pitää samanarvoisena. Koska ureafosfaatti ei huonontanut rehun maittavuutta eikä nostanut veren urea- ja ammoniumpitoisuutta, sitä voidaan pitää käytetyissä rajoissa turvallisena lisäfosforin lähteenä siipikarjan rehuseoksissa.

ANNALES AGRICULTURAE FENNIAE VOL. 22: 93—103 (1983)
Seria ANIMALIA DOMESTICA N. 62 — Sarja KOTIELÄIMET n:o 62

NUTRITIONAL AND TOXICOLOGICAL EVALUATION OF PEKILO AN EXPERIMENT WITH HENS DURING TWO LAYING AND BREEDING PERIODS

TUOMO KIISKINEN and PER ANDERSSON

KIISKINEN, T. & ANDERSSON, P. 1983. **Nutritional and toxicological evaluation of Pekilo. An experiment with hens during two laying and breeding periods.** Ann. Agric. Fenn. 22: 93—103. (Agric. Res. Centre, Inst. Anim. Husb., SF-31600 Jokioinen, Finland.)

Pekilo, a dried microfungus *(Paecilomyces varioti)* cultivated in sulphite spent liquor, replaced 0 (PO), 50 (P50) and 100 % (P100) of supplemented protein (fish meal and soybean meal) in the diets of two successive generations of hens. The total numbers of hens and cocks in this study were 1600 and 270 birds respectively. The laying periods lasted for 10 and 6 months and 5 test hatchings were performed during each laying period.

The use of Pekilo reduced the weight gain of young chicks (0—8 weeks) by 2—4 % ($P < 0,01$). At the end of the growing periods (18 weeks) the differences between treatments were small and insignificant with respect to growing chicks of laying strain. In addition Pekilo had no significant effect on the relative weight gains of hens and cocks. The hen-day egg production values in the first generation were 45,4 (PO), 45,5 (P50) and 46,5 g (P100) and in the second generation 41,4, 40,0 and 39,4 g respectively. No statistically significant differences were found in the egg production parameters.

Pekilo had no negative effect on the acceptability of the rations. The birds consumed feed chiefly depending on the energy concentration of the diet. Because of their lower metabolizable energy (ME) concentration, the Pekilo diets were consumed more than the Pekilo-free diets during the growing and laying periods. Feed conversion of laying hens in the Pekilo groups was therefore inferior to the Pekilo-free groups and the difference between the PO and P100 groups was significant ($P < 0,05$) in the second generation.

Pekilo did not significantly affect mortality, fertility or hatchability. No differences in the incidence of malformations were found in dead embryos. The frequency of primarily abnormal spermatocytes increased significantly in the sperm of the second generation when the content of Pekilo increased but the changes were too small to affect fertility.

Index words: chick, hen, cock, single cell protein, Pekilo, growth, laying, fertility, hatchability.

INTRODUCTION

Numerous studies have shown that 10—15 % of dried yeasts can be included in poultry feed rations, chiefly to replace soybean meal (van WEERDEN 1972, D'MELLO 1973, TIEWS et al. 1974, VOGT et al. 1974, WALDROUP and HAZEN 1975, BRENNE 1976, SHANNON et al. 1976, MORDENTI et al. 1978, VOGT et al. 1978, SUCCI et al. 1980). Pekilo, a Finnish single cell

product, is a microfungus (*Paecilomyces varioti*) which is cultivated in sulphite spent liquor. It has been tested on many animal species in Finland and several other countries and generally the results on poultry have been compatible with the results of the above-mentioned studies on yeasts (POUTIAINEN 1973, DELIC and LAZOR 1979, KIISKINEN 1979, KORNIEWICZ and PLONKA 1979, SURDZHIISKA and VLADIMIROVA 1979). Most of the studies with single cell proteins (SCP) have been short-term experiments in laying hens and broilers. Relatively few long-term experiments have been performed. YOSHIDA (1975) reported a Jananese multi-generation experiment with 15 % yeast grown on n-paraffin in the diets of five generations of hens. No detrimental effects on the production, fertility, hatchability or viability of birds were found and no evidence was obtained suggesting that the yeast feeding is detrimental to the production of meat and eggs of good quality.

In order to obtain additional information regarding the feeding value and availability of Pekilo for poultry a long-term study was conducted with two generations of laying hens and cocks. In the planning of the study the requirements of the FDA (Food and Drug Administration, USA) for tests of this kind were followed as far as possible.

MATERIAL AND METHODS

Animals and housing. White Leghorn hens and cocks (strain SK 12) were used. The first generation was kept in an old poultry house where both the size of the experimental groups and the number of replicates were limited. The chicks were grown in litter floor pens 10 chicks/m², up to the age of 18 weeks when the hens were moved into two-hen cages (600 cm²/hen). The cocks were transferred later, at the age of 25 weeks. The laying period lasted about 10 months (10 × 28 days). The room temperature varied from 15 to 25 °C and the relative humidity from 40 to 85 % depending on the season. The day length was 23 hours for the first two weeks after which it was reduced to 10 hours and maintained at this level until 20 weeks. It was then increased by 20 minutes a week to a maximum of 16 hours. The light intensity during the laying period was 10 lux.

The second generation was hatched and grown in a new poultry house at Jokioinen. The birds were in three-tier batteries. During the growing period (0—18 weeks) the housing density was 18 chicks/m². In the house for layers and cocks the densities were 4 hens/cage (530 cm²/hen) and 2 cocks/cage (1170 cm²/cock). The laying period lasted 6 months (6 × 28 days). The temperature in the chick room was initially 34 °C and was reduced by 3 °C a week to 20 °C. The relative humidity varied between 35 and 45 %. The average room temperature for laying hens and cocks was 17 °C and the relative humidity 40—45 %. The light programme was approximately the same as for the first generation. The day length during the growing period was 9 hours and the light intensity during the laying period 8 lux.

Experimental groups

Group 1. Pekilo level 0; protein supplements: fish meal, soybean meal
Group 2. Pekilo level 50; one half of the fish and soybean meal was replaced with Pekilo
Group 3. Pekilo level 100; fish and soybean meal totally replaced with Pekilo
Size of the groups:
1st generation: each group comprised 250 chicks (5 × 50), 150 laying hens (5 × 30) and 40 cocks.
2nd generation: each group comprised 448 chicks (8 × 56), 384 laying hens (8 × 48) and 50 cocks.

Diets and feeding. The formulae of the experimental diets are presented in Table 1. Because of the relatively low metabolizable energy

(ME) value of Pekilo, an attempt was made to reduce the differences in energy concentration in the chicken starter feeds (0—8 weeks) by supplementing them with animal fat. Larger amounts of methionine were also added to the starters containing Pekilo. Because Pekilo, like other SCP products, is comparably rich in available phosphorus, extra calcium was added in the form of calcium carbonate to balance the Ca-P relationship in starter and grower feeds containing Pekilo. Diets for breeding cocks contained less calcium carbonate than feeds for hens. All diets were in meal form and were given *ad libitum.*

Experimental procedures. The birds were weighed individually at the age of 4, 8 and 19 weeks as well as at the end of the laying (breeding) period. The average weight of day old chicks was calculated from the total weight of a pen. The egg laying and feed consumption of the replicates was checked during a period of 28 days.

Experimental hatchings were carried out five times in each generation. Artificial insemination was used. All cocks which gave sperm were used in every insemination. All the first generation hens and one third of the second generation hens were inseminated. About 0,1 ml sperm was injected per hen. Between inseminations the volume of sperm was measured two or three times and two or three samples were taken for measurement of sperm density. One sample was taken for the calculation of abnormal spermatocytes. The density of sperms (x 10^9/ml) was determined using the cell counter in the institute of Microbiology of the University of Helsinki. For this measurement the sperms were diluted to a concentration of 0,1 % in physiological saline. The glass plate preparations for the microscopic counting of abnormal spermatocytes were dyed with 2 % eosin and 10 % nigrosin solutions. The counting was carried out in the Hautjärvi clinic of the College of Veterinary Medicine.

Dead birds were registered and some were sent for diagnosis to the National Veterinary Institute. At the end of the growing and laying periods five hens were sent for post mortem examination to the same Institute. Dead embryos from all hatchings were investigated for malformations. Chicks from all hatchings were checked for one week after hatching.

The height of albumen and Haugh unit (HU) was determined with an Ames HU micrometer and the specific weight of the eggs using NaCl solutions. The measurements were performed three times for the first generation and twice for the second generation, except specific weight, which was only determined once.

At the end of each laying period blood samples were taken from the wing vein of 16 and 20 hens per group in the first and second generations, respectively. The following determinations were performed: Hb, haematocrite, serum total proteins, serum urea and serum urate. Serum analyses were done in a private laboratory (Yhtyneet Kliiniset Laboratoriot Oy).

The proximate feed analysis (Weende) was done for every protein supplement and each lot of feed mixtures. The amino acids of each protein supplement and a common sample of each diet were analyzed using a gaschromatograph (Hewlett Packard 5710, 3 m column 3 % SE-30 on gaschrom Q) after hydrolysis in 6 N HCl satured with nitrogen gas (110 °C for 20 hours). Total nucleic acids in Pekilo were determined in the Technical Research Centre of Finland. Calcium and phosphorus were analysed in Pekilo, fish meal and the rations of the first generation (at the laboratory of Viljavuuspalvelu Oy). The ME concentrations of the layers' diets of the first generation were determined with eight hens on each, using total collection of faeces.

The results were statistically evaluated using analysis of variance. The differences between treatment means were tested by the Tukey- and t-test (STEEL and TORRIE 1960).

RESULTS AND DISCUSSION

According to the analyses, the mixing of the diets was technically succesful (Table 2). The Pekilo lot no 2 used in the growing and laying rations of the second generation contained considerably more protein than lot no 1 (Table 3). Thus the protein content of the Pekilo diets was higher than expected, but lowering the amount of Pekilo at this stage would not have been wise from the point of view of the experiment. According to the ME determination of the laying diets ME content decreased about 30 KJ per percentage unit increase in Pekilo. The methionine concentration in Pekilo and the laying rations and the cystine content in all used protein sources and diets were lower than the table values and calculated concentrations (Tables 3 and 4). Apparently cystine was destroyed during the hydrolysis of the samples.

The weight gain in the chickens was similar in both generations (Table 5). The mean weight of chicks at the age of 8 weeks decreased as the Pekilo level increased. The difference between the PO and P100 levels was significant ($P<0,01$) in both generations and between P0 and P50 in the second generation ($P<0,05$). At the end of the growing period the differences between the groups were small, and only in the second generation was the difference between P0 and P100 significant ($P<0,05$). Pekilo had no significant effect on the changes in weight during the laying (breeding) period (Table 5). The Pekilo contents were very high and will hardly ever be

Table 1. Components and calculated contents of the experimental diets (%).

	0—8weeks			9—18 weeks			Laying hens (cocks)		
Pekilo level	0	50 (2nd)	100 (2nd)	0	50	100	0	50	100
Fish meal	8	4	—	4	2	—	3	1,5	—
Soybean meal	14	7	—	6	3	—	10	5	—
Pekilo	—	12,5	25	—	6	12	—	7	14
Wheat	10	10	10	—	—	—	—	—	—
Barley	36	34	32	54	54	54	54	54	54
Oats	24,4	24,2 (23,9)	24 (23,3)	29,7	28,5	27,3	20,6 (26,1)	20,1 (25,6)	19,6 (25,1)
Grass meal	4	4	4	3	3	3	3	3	3
Soya oil	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Animal fat	—	0,4 (0,7)	1,8 (1,5)	—	—	—	—	—	—
Dicalcium phosphate	2	2	2	1,3	1,3	1,3	1,5	1,5	1,5
Limestone flour	0,2	0,5	0,8	0,6	0,8	1,0	6,5 (1,0)	6,5 (1,0)	6,5 (1,0)
Sodium chloride	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4
Vitamin premix[1)]	0,15	0,15	0,15	0,15	0,15	0,15	0,15	0,15	0,15
Trace element premix[2)]	0,3	0,3	0,3	0,25	0,25	0,25	0,25	0,25	0,25
DL-methionine	0,06	0,08 (0,10)	0,10 (0,14)	0,06	0,06	0,06	0,06	0,06	0,06
Calculated:									
Crude protein	20,4	20,2	20,2	15,4	15,4	15,4	15,4 (16,1)	15,5 (16,2)	15,4 (16,1)
ME MU/kg[3)]	10,96	10,85 (10,92)	10,80 (10,97)	10,94	10,85	10,76	10,21 (10,80)	10,17 (10,77)	10,13 (10,73)
Methionine	0,45	0,44 (0,46)	0,43 (0,46)	0,35	0,33	0,31	0,33	0,32	0,31
Lysine	1,16	1,12	1,09	0,78	0,76	0,75	0,79	0,78	0,77
Calcium	0,97	0,98	1,06	0,81	0,83	0,87	3,11 (1,00)	3,06 (0,95)	3,02 (0,90)
Phosphorus	0,86	0,89	0,92	0,66	0,68	0,70	0,66	0,71	0,75
Available Phosphorus	0,61	0,64	0,67	0,41	0,42	0,44	0,41	0,46	0,51

[1)]Supplies per kg: Vit. A 15 000 IU., vit D_3 1800 IU., vit. E 20 mg, vit. K 1,0 mg, vit. B_2 3,5 mg, vit. B_6 1 mg, vit B_{12} 0,015 mg, niacin 18 mg, folic acid 0,24 mg, choline 500 mg.

[2)]Supplies mg per kg: Fe 20, Zn 45, Mn 48, Cu 4, Co 0,6, J 0,5, Se 0,1

[3)]Pekilo's ME content 10,5 MU (2,5 Mcal)/kg

available in practice. In spite of this, the effects of Pekilo on weight gain of chicks were relatively slight. An unbalanced selection of protein sources is usually unfavourable in animal production. Some studies with poultry have shown that the availability of protein and amino acids in yeasts grown on hydrocarbon is inferior to that in soybean meal (WALDROUP and FLYNN 1975, EL

Table 2. Analysis and determinations of the diets.

	0—8 weeks			9—18 weeks			Laying hens			Cocks		
Pekilo level	0	50	100	0	50	100	0	50	100	0	50	100
1st generation												
Dry matter (%)	88,9	89,4	90,0	88,4	88,6	88,7	88,8	88,8	89,0	88,1	88,1	88,4
% in dry matter												
Crude protein	23,0	23,4	22,8	17,5	18,0	17,8	17,2	16,8	17,0	17,7	17,7	17,6
Ether extract	3,9	4,2	4,1	3,9	3,6	3,5	3,7	3,5	3,3	3,9	3,7	3,5
Crude fiber	6,1	6,4	7,1	6,2	6,3	6,2	5,9	6,6	6,7	5,9	6,2	6,6
NFE	60,4	59,5	59,6	66,9	66,4	66,8	63,4	63,3	63,1	66,4	66,4	66,6
Ash	6,6	6,5	6,4	5,5	5,7	5,7	9,8	9,8	9,9	6,1	6,0	5,7
Calcium	0,96	0,96	0,98	0,71	0,85	0,89	3,13	3,22	3,17	1,07	0,99	0,84
Phosphorus	0,90	0,97	0,95	0,73	0,68	0,78	0,72	0,72	0,81	0,77	0,81	0,82
ME_{N33} MJ/kg	—	—	—	—	—	—	11,05	10,95	10,63	—	—	—
2nd generation												
Dry matter (%)	87,7	87,9	88,2	87,8	87,8	88,1	88,0	88,2	88,6	87,8	88,0	88,3
% in dry matter												
Crude protein	22,3	22,3	21,7	17,0	17,6	18,0	17,0	17,3	18,3	17,9	18,2	18,5
Ether extract	5,0	5,3	5,6	4,3	4,0	3,8	3,6	3,4	3,2	4,0	3,8	3,7
Crude fiber	6,4	7,0	7,3	7,1	7,2	7,4	6,9	7,2	7,5	7,5	7,3	7,4
NFE	59,9	59,0	59,0	65,8	65,4	64,9	62,2	61,8	60,9	63,9	66,3	64,4
Ash	6,4	6,4	6,4	5,8	5,8	5,9	10,3	10,3	10,1	6,7	6,4	5,9

Table 3. Chemical analyses of the protein sources.

	Pekilo		Fish meal	Soybean meal
	1st	2nd		
Dry matter %	91,8	91,1	91,1	88,1
% in dry matter				
Crude protein	50,8	56,9	70,2	49,9
Ether extract	1,5	1,4	10,8	2,5
Crude fiber	8,0	6,0	0,5	6,4
NFE	34,1	29,4		34,7
Ash	5,6	6,3	14,5	6,5
Calcium	0,31	0,25	2,38	—
Phosphorus	1,24	2,57	2,27	—
Amino acids g/16 g N				
Methionine	1,1		3,0	1,4
Cystine	0,4		1,0	1,1
Lysine	5,9		7,2	6,2
Arginine	5,6		5,6	7,2
Histidine	1,6		2,5	2,3
Leucine	6,3		7,7	7,9
Isoleucine	4,0		4,5	4,7
Phenylalanine	3,6		3,9	4,3
Tyrosine	3,1		3,2	3,4
Threonine	3,9		4,4	4,3
Valine	4,5		5,2	5,0
Glycine	4,6		5,8	4,3
[1]Total nucleic acids (%)	8,1	10,0		

[1] OGUR & ROSEN, 1950. Arch. Biochem. 25: 262—267

BOUSHY and ROODBEEN 1980). Some investigations with Pekilo have indicated that it does not provide all the supplementary protein necessary for small chicks (BRENNE 1976, BJØRNSTAD 1977, KORNIEWICZ and PLONKA 1979). In this light, the 2—4 % lower body weights of the Pekilo groups compared to the control group at the age of 8 weeks is quite understandable. Later the differences diminished and were negligible at the end of the growing period.

Consumption of the starter feed (0—8 weeks) did not differ between treatments, because the ME concentration of the diets were at the same level (Table 6). With regard to the grower feed (9—18 weeks) the consumption of the Pekilo diets was to some extent higher than that of the Pekilo-free diets. The difference between the treatments was significant ($P<0,05$) in the second generation. The higher intake of feed and the higher protein content of Pekilo diets during the growing period caused higher protein consumption in the Pekilo groups than in the Pekilo-free group.

Table 4. Analyzed amino acids percentages in the diets.

	0—8 weeks			9—18 weeks			Laying hens		
Pekilo level	0	50	100	0	50	100	0	50	100
Methionine	0,42	0,43	0,42	0,33	0,34	0,34	0,25	0,24	0,24
Cystine	0,24	0,22	0,21	0,19	0,20	0,20	0,20	0,19	0,18
Lysine	1,05	1,01	0,98	0,65	0,66	0,63	0,68	0,64	0,66
Arginine	1,21	1,09	1,06	0,84	0,86	0,86	0,87	0,73	0,72
Histidine	0,41	0,36	0,34	0,25	0,22	0,24	0,27	0,25	0,25
Leucine	1,50	1,38	1,36	1,13	1,17	1,05	1,09	0,97	0,94
Isoleucine	0,86	0,80	0,81	0,72	0,74	0,71	0,65	0,56	0,46
Phenylalanine	0,91	0,80	0,81	0,84	0,85	0,77	0,67	0,58	0,56
Tyrosine	0,69	0,63	0,63	0,55	0,58	0,54	0,52	0,47	0,47
Treonine	0,77	0,78	0,77	0,59	0,61	0,56	0,60	0,55	0,52
Valine	0,99	1,00	0,97	0,85	0,89	0,75	0,78	0,69	0,61
Glycine	0,96	0,90	0,89	0,72	0,72	0,66	0,70	0,64	0,62

Table 5. Mean bodyweight and mortality of birds.

	1st generation				2nd generation			
Pekilo level	0	50	100	SE	0	50	100	SE
Mean weight g								
One day	40,9	41,0	41,1		38,7	39,4	39,7	
4 weeks	272^{c}	270^{c}	257^{c}	1,0	298^{c}	300^{c}	277^{d}	1,0
8 weeks	730^{c}	718^{c}	698^{d}	3,1	771^{c}	746^{d}	739^{d}	3,6
Hens 18 weeks	1435	1415	1425	5,0	1483^{a}	1474^{ab}	1456^{b}	4,1
" at end of laying period	2107	2085	2068	12,7	2001	2040	2006	7,8
Mean change in weight g	672	670	643	10,1	518^{c}	566^{d}	550^{cd}	5,7
" " " " %	46,7	47,2	45,1	0,66	35,1	38,4	37,6	0,37
Cocks 18 weeks	1946	2026	1963	14,8	2017	2035	1996	17,0
" at end of experiment	2443	2531	2413	20,5	2129	2170	2145	18,7
Mean change in weight g	497	505	450	16,9	112	135	149	16,1
" " " " %	25,6	25,3	23,1	0,89	6,1	7,0	7,6	0,81
Mortality (%)								
0—8 weeks	—	0,4	0,8		0,4	1,6	1,0	
9—18 weeks	—	0,9	—		0,5	—	0,7	
Hens laying period	2,7	2,7	2,7		8,6	7,3	9,4	
Cocks (dead + culled)	24,4	17,9	20,0		8,5	8,5	10,6	

a—b $P<0,05$

The values not followed by the same index letter in each row of a generation differ significantly. If no letters are used the differences are non-significant.

c—d $P<0,01$

Table 6. Feed consumption of chicks and pullets.

	1st generation				2nd generation			
Pekilo level	0	50	100	SE	0	50	100	SE
Feed (kg/chicken)								
0—8 weeks	1,96	2,01	1,97	0,011	2,18	2,26	2,18	0,016
9—18 weeks	5,60	5,62	5,78	0,042	$5,53^{a}$	$5,64^{b}$	$5,79^{c}$	0,027
Feed (g/chicken/day)								
0—8 weeks	35,7	36,6	35,9	0,20	37,5	39,0	37,6	0,27
9—18 weeks	80,0	80,2	82,6	0,60	$79,0^{a}$	$80,6^{a}$	$82,7^{b}$	0,38
ME (KJ/chicken/day)								
0—8 weeks	391	397	388	2,2	411^{a}	426^{b}	412^{a}	2,9
9—18 weeks	875	870	889	6,5	864^{a}	875^{ab}	890^{b}	4,1
Crude protein (g/chicken/day)								
0—8 weeks	7,3	7,7	7,4	0,04	7,3	7,6	7,2	0,05
9—18 weeks	12,4	12,8	13,0	0,09	$11,8^{a}$	$12,5^{ab}$	$13,1^{b}$	0,06
Methionine (mg/chicken/day)								
0—8 weeks	150	157	151	0,8	158	168	158	1,1
9—18 weeks	264	273	281	2,0	261^{a}	274^{ab}	281^{b}	1,3
Lysine (mg/chicken/day)								
0—8 weeks	375	370	352	2,0	394^{a}	394^{a}	368^{b}	2,7
9—18 weeks	520	529	520	3,9	514^{a}	532^{b}	521^{ab}	2,4

a—b—c $P<0,05$ See Table 5

Pekilo had no effect on the age at the start of laying (Table 7). The differences in egg production were not statistically significant between the treatments. Feed consumption in the Pekilo groups was higher than in the Pekilo-free group. As a result of this, feed effeciency on the Pekilo diets was inferior to the Pekilo-free diet and the difference between the P0 and P100 groups in the second generation was significant ($P<0,05$).

The efficiency of metabolizable energy and protein were significantly impaired in the Pekilo diets of the second generation. This can be explained on the basis of the Pekilo's high protein content during the second laying period. This elevated the protein content of the Pekilo diets, and thus the protein intake, to an unnecessarily high level, causing an unfavourable energy-protein relationship in the laying diets containing Pekilo in the second generation. The cocks had

Table 7. Egg production, feed consumption and efficiency in hens, feed consumption in cocks.

	1st generation				2nd generation			
Pekilo level	0	50	100	SE	0	50	100	SE
Production								
Age at 50 % laying (days)	170	168	171	0,9	170	172	169	0,5
Laying percentage	79,3	79,7	81,0	0,59	76,7	75,2	74,4	0,40
Mean egg weight (g)	57,5	57,1	57,4	0,28	54,0	53,2	53,0	0,26
Hen-day prod. (g)	45,4	45,5	46,5	0,24	41,4	40,0	39,4	0,29
Feed consumption								
g/hen/day	$124,4^{a}$	$126,6^{ab}$	$130,0^{b}$	0,61	120,4	123,3	124,1	0,55
ME KJ/hen/day	1368	1367	1372	6,2	1324	1331	1315	5,6
Crude protein/g/hen/day	19,0	19,0	19,6	0,09	$18,1^{ac}$	$18,9^{bc}$	$20,1^{d}$	0,11
Feed efficiency								
kg/kg egg	2,74	2,80	2,81	0,016	$2,91^{a}$	$3,08^{ab}$	$3,14^{b}$	0,017
MJ/kg egg	30,5	30,2	29,8	0,16	$32,0^{a}$	$33,3^{b}$	$33,3^{b}$	0,17
Crude protein g/kg egg	419	420	426	2,4	437^{c}	472^{d}	512^{e}	3,4
Feed consumption in cocks								
kg/cock	30,1	31,1	31,6	—	19,2	19,4	20,0	—
g/cock/day	113,2	116,9	118,8	—	99,5	100,5	103,6	—

a—b, $P<0,05$
c—d—e, $P<0,01$ See Table 5

the same kind of tendency in feed consumption as the hens (Table 7). The higher feed intake of the cocks of the second generation was partly caused by their longer period on litter.

Although the differences in rate of egg production were not significant there was a tendency towards lower egg production and egg weight when Pekilo was included in the diets of the second generation. This may be related to the change in Pekilo and its different quality (protein content). The lower Hb values in the Pekilo groups than in the control group in the second generation support the possibility of a slight disturbance. In the earlier Finnish experiments Pekilo or Torula yeast (*Candida utilis*) replaced all soybean meal in layers' diet without a negative effect on the laying rate, but some decrease in egg weight was found (POUTIAINEN 1973, KIISKINEN 1979).

Pekilo had no effect on the consistency of the egg albumen expressed as the height of albumen and Haugh unit or on the specific weight of the egg, which represents the thickness of the shell (Table 12). In general SCP has no detrimental effects on HU or shell quality (LUND 1973, POUTIAINEN 1973, YOSHIDA 1975, WALDROUP and HAZEN 1975, VOGT et al. 1975, 1978, KIISKINEN 1979).

Table 8. Data of experimental hatchings.

	1st generation			2nd generation		
Pekilo level	0	50	100	0	50	100
Number of hatchings	5	5	5	5	5	5
Total number of eggs	1711	1689	1725	1541	1555	1492
Fertility (%)	92,6	94,1	93,0	88,9	89,5	87,8
Hatchability (%)	87,0	88,1	88,5	83,3	84,4	80,4
Mean weight of day old chicks (g)	37,7	37,6	38,3	36,0	34,9	35,9
Mean weight at one week of age (g)	$78,9^{a}$	$75,7^{b}$	$75,8^{b}$	75,6	74,6	74,9
Mortality during the first week (%)	0,6	1,7	1,1	1,9	1,7	1,7

a—b, $P < 0,05$ See Table 5

Table 9. Data of sperm quality tests.

	1st generation			2nd generation		
Pekilo level	0	50	100	0	50	100
Number of cocks	31	31	32	45	44	41
Sperm volume ml	0,38	0,39	0,44	0,29	0,30	0,36
SD	0,11	0,12	0,15	0,11	0,12	0,20
Sperm density $\times 10^9$/ml	3,77	3,62	3,51	3,21	3,65	3,59
SD	1,34	1,63	1,33	1,34	1,70	1,57
Normal spermatocytes (%)	88,1	86,0	89,0	$87,3^{a}$	$82,8^{ab}$	$82,1^{b}$
SD	7,3	5,2	5,3	7,1	9,6	8,7
Prim. abn. spermatocytes (%)	$7,7^{ab}$	$10,1^{a}$	$7,2^{b}$	$4,4^{ac}$	$7,4^{bcd}$	$8,7^{bd}$
SD	4,2	4,1	4,2	2,6	5,1	5,8
Sec. abn. spermatocytes (%)	4,2	3,9	3,7	8,3	9,8	9,2
SD	5,0	3,3	3,9	7,7	7,4	6,2
Total abn. spermatocytes (%)	11,9	14,0	10,9	$12,7^{a}$	$17,2^{ab}$	$17,9^{b}$
SD	7,3	5,2	5,2	$7,1^{a}$	9,6	8,7

a—b $P < 0,05$
c—d $P < 0,01$ See Table 5

There were no significant differences between the treatments in fertility, hatchability or mean weight of day old chicks (Table 8). The uneven conditions in the poultry house for the first generation may have resulted in the lower weight ($P<0,05$) and higher mortality of the chicks in the Pekilo groups during the first week after hatching. A long-term study involving the feeding of pigs with Pekilo has recently been performed and no detrimental effects on the reproductive performance of the sows or vitality and growth of the piglets could be found when Pekilo was the sole source of supplementary protein (ALAVIUHKOLA and SUOMI 1981).

No significant differences were found in the volume or density of sperm (Table 9). The percentage of primarily abnormal spermatocytes increased in the Pekilo groups of the second generation compared to the Pekilo-free group. These differences were statistically significant (P0/P50 $P<0,05$, P0/P100 $P<0,01$). As a result of this, also the difference in the number of total abnormal spermatocytes and normal spermatocytes was also significant between the P0 and P100 groups ($P<0,05$). In the first generation, the number of primarily abnormal spermatocytes was higher in the P50 group than in the other groups but the difference was significant only with respect to the P100 group ($P<0,05$).

Table 10. Data of teratogenic investigations.

Pekilo level	0	50	100
1st generation			
Dead embryos	49	63	49
" " (%)	2,9	3,7	2,8
Abnormalities	1 (cysta)	1 (enceph-alocoele)	—
2nd generation			
Dead embryos	53	54	58
" " (%)	3,5	3,5	3,9
Abnormalities	9	10	6
— cheilosehisis	2	—	—
— encephalocoele	5	4	4
— sehistosoma	—	3	1
— dipygus	—	1	—
— 4 legs	—	1	—
— cross bill	1	—	—
— back	—	1	—
— dwarf	1	—	—
— perosis	—	—	1

The higher frequency of primarily abnormal spermatocytes in the Pekilo groups is an interesting phenomenon as such, but changes of the size found in this study cannot be expected to affect fertility.

Mortality was not significantly influenced by dietary treatment (Tables 5 and 7). Nor has the mortality of chicks or hens increased significantly in the other studies with Pekilo (POUTIAINEN 1973, BRENNE 1976, KORNIEWICZ and PLONKA 1979, KIISKINEN 1979). Among the embryos dead in shell or still inside the shell, two cases of

Table 11. Blood and serum values of laying hens.

	1st generation				2nd generation			
Pekilo level	0	50	100		0	50	100	
n	16	16	16		20	20	20	
Hb (g/l)	14,1	14,6	14,2	NS	15,3	14,3	14,2	NS
SD	1,9	1,5	1,5		2,0	1,5	1,6	
Haematocrite (%)	32,9	31,6	31,7	NS	32,8	30,8	31,7	NS
SD	3,1	2,3	2,1		3,0	1,9	3,0	
Serum								
proteins (g/l)	45,7	44,4	45,8	NS	48,1	48,5	45,4	NS
SD	3,8	2,4	2,7		7,2	6,0	7,4	
urea (µmol/l)	2,06	2,29	2,27	NS	1,32	1,73	1,30	NS
SD	0,61	0,49	0,57		0,92	0,84	0,89	
urate (µmol/l)	279	274	251	NS	261	273	295	NS
SD	59	84	74		87	105	126	

NS = non-significant

Table 12. Results of egg quality tests.

	1st generation			2nd generation		
Pekilo level	0	50	100	0	50	100
N	361	352	341	229	218	214
Height of albumen	7,3	7,5	7,5	7,6	7,6	7,6
S.D.	1,0	1,0	0,9	1,1	1,1	1,1
Haugh unit	86,2	87,4	87,3	90,0	89,9	89,8
S.D.	5,9	5,7	5,3	6,3	6,4	6,0
N	332	325	338	114	120	120
Spec. weight	1,0840	1,0841	1,0837	1,0814	1,0823	1,0818
S.D.	0,0045	0,0046	0,0044	0,0055	0,0060	0,0050

malformation were found in the first generation (Table 10). In the second generation the number of malformations was considerably higher. However, there were no differences between the experimental groups. In the post mortem examinations of the dead birds, some cases of cannibalism, nephrosis and Marek's disease were found in each experimental group. No significant lesions were found in the post mortem examinations of birds killed at the end of the growing and laying periods.

No significant differences in blood and serum values were found between the treatments (Table 11). The variation in the values was large and the trend in uric acid concentration was opposite in the generations. Changes in concentration should have been checked with particular birds. No notable changes in serum uric acid concentration can be expected however, because the hen is uricotelic and therefore capable of disposing of excess nucleic acids. The elevation of serum uric acid with the Pekilo diets of the second generation was more likely due to the higher protein intake of those groups than to nucleic acids. On the basis of results of this study and taking into consideration the high amounts of Pekilo used, this SCP product can be regarded as a suitable and safe protein source for poultry.

REFERENCES

ALAVIUHKOLA, T. & SUOMI, K. 1981. Long-term study on the effects of Pekilo protein on the reproductive performance of sows. Ann. Agric. Fenn. 20: 10—14.

BJØRNSTAD, J. 1977. Biological evaluation of protein quality with particular reference to the availability of amino acids. Techn. Rep. No 253. Norwegian Agr. Res. Council. Oslo.

BRENNE, T. 1976. Encelleprotein i foringa av slaktesvin og kyllinger. Norges Landbrukshøgskole Meld. nr 179. 65 p.

DELIC, I. & LAZOR, M. 1979. Pekilo-protein in animal feeding. Res. Rep. Pekilo-symp. Tampere 1978. Inst. Anim. Husb. Rep. N:o 12: 97—105.

D'MELLO, J. P. F. 1973. Amino acid supplementation of hydrocarbongrown yeast in diets for young chicks. Nutr. Rep. Intern. 8: 105—109.

EL BOUSHY, A. R. & ROODBEEN, A. E. 1980. Amino-acid availability in Lavera yeast compared with soybean meal and herring meal. Poult. Sci. 59: 115—118.

KIISKINEN, T. 1979. Pekilo in the feeding of poultry. The results of the experiments carried out in Finland. Res. Rep. Pekilo-symp. Tampere 1978. Inst. Anim. Husb. N:o 12: 133—143.

KORNIEWICZ, A. & PLONKA, S. 1979. Pekilo-protein in poultry

-and swine feeding in Poland. Res. Rep. Pekilo-symp. Tampere 1978. Inst. Anim. Husb. Rep. N:o 12: 71—77.

LUND, S. 1973. Sulfittgjaer som proteinkilde til verpehøner. Inst. for fjørfe og pelsdyr. Stensiltrykk nr 51. 7 p.

MORDENTI, A., QUAGLIO, G. L. & TOCCHINI, M. 1978. Research on the use of SCP (Toprina) in turkey nutrition. Proc. Abstr. W. Poult. Congr. Rio de Janeiro. p. 1636—1642.

POUTIAINEN, E. 1973. Pekilo-proteiini munivien kanojen ja broilereiden ruokinnassa. Siipikarja N:o 9: 247—255.

SHANNON, D. W. F., MCNAB, J. M. & ANDERSON, G. B. 1976. Use of an n-paraffin-grown yeast in diets for replacement pullets and laying hens. J. Sci. Food Agric. 27: 471—476.

STEEL, R. G. D. & TORRIE, J. H. 1960. Principles and procedures of statistics. 481 p. New York.

SUCCI, G., PIALORSI, S., DIFIORE, L. & CARDINI, G. 1980. The use of methanol-grown yeast LI-70 in feeds for broilers. Poult. Sci. 59: 1471—1479.

SURDZHIISKA, S. & VLADIMIROVA, L. 1979. Pekilo — a protein source of the broiler feed mixture. Res. Rep. Pekilo-symp. Tampere 1978. Inst. Anim. Husb. Rep. N:o 12: 129—132.

TIEWS, J., GROPP, J., SCHULZ, V., ERBERSDOBLER, H, & BECK, H. 1974. Alkanheten in der Geflügelernährung. 3. Mitt. Z. Tierphysiol., Tierernähr. u. Futtermittelk. 34: 86—113.

WALDROUP, P. W. & FLYNN, N. F. 1975. Comparison of the nutritive value of yeast grown on hydrocarbon feedstocks under varying processing conditions. Poult. Sci. 54: 1129—1133.

WEERDEN, E. J. van., SHACKLADY, C. A., WAL, P. & SCHUTTE, J. B. van der, 1972. Metabolizable energy of yeast grown on pure n-paraffins and its use in rations for broiler chicks. Arch. Geflügelk. 36: 1—4.

VOGT, H., STUTE, K. & HARNISCH, S. 1976. Die Verwendung von Molkenhefe im Geflügelfutter. Arch. Geflügelk. 40: 205—212.

—, KRIEG, R. & HARNISCH, S. 1978. Alkanhefe VITON im Geflügelfutter Arch. Geflügelk. 42: 201—207.

YOSHIDA, M. 1975. Yeast grown on n-paraffin as future poultry feed. W. Poult. Sci. J. 31: 221—234.

Manuscript received October 1982

Tuomo Kiiskinen
Agricultural Research Centre
Institute of Animal Husbandry
SF-31600 Jokioinen, Finland

Per Andersson
National Veterinary Institute
SF-00100 Helsinki 10, Finland

SELOSTUS

Pekilon soveltuvuus siipikarjan valkuaisrehuksi

TUOMO KIISKINEN ja PER ANDERSSON

Maatalouden tutkimuskeskus ja Valtion eläinlääketieteellinen laitos

Kotimaisen mikrobivalkuaistuotteen, Pekilon valmistusprosessia yritetään myydä ulkomaille. Sen vuoksi tarvittiin tietoa Pekilon pitkäaikaisen käytön vaikutuksista siipikarjan tuotannossa. Suoritetussa tutkimuksessa Pekiloa käytettiin kanan valkuaisrehuna kahden sukupolven ajan. Kolmatta sukupolvea pidettiin kuoriutumisen jälkeen yksi viikko tarkkailussa. Vertailuryhmän rehun kalajauhosta ja soijasta korvattiin 50 ja 100 % Pekilolla. Pekilon määrät olivat poikasrehussa (0—8 vk) 0, 12,5 ja 25; kasvatusrehussa (9—18 vk) 0, 6 ja 12 sekä munitus-(siitos-) rehussa 0,7 ja 14 %. Ensimmäisen sukupolven ryhmissä oli 250 poikasta, 150 kanaa ja 40 kukkoa sekä toisessa sukupolvessa 450 poikasta, 384 kanaa ja 50 kukkoa ryhmää kohden. Ensimmäisen sukupolven muninta- (siitos) kausi kesti 10 kk ja toisen 6 kk. Eläinten lisäämisessä käytettiin keinosiemennystä. Kummallakin sukupolvella suoritettiin 5 koehaudontaa.

Pekilon käyttö alensi alle 8-viikkoisten poikasten kasvua 2—4 %. Kasvatuskauden lopussa (18 vk) ryhmien väliset painoerot olivat merkityksettömiä. Päivittäinen munantuotos oli ensimmäisessä sukupolvessa 45,4 (Pekilo 0), 45,5 (P 50) ja 46,5 g (P 100) sekä toisessa sukupolvessa vastaavasti 41,4, 40,0, 39,4 g. Erot eivät olleet tilastolliseti merkitseviä.

Pekiloryhmien rehunkulutus oli kasvatus- ja munitus- (siitos-) kaudella suurempi kuin vertailuryhmän, koska rehun energiapitoisuus oli niillä alhaisempi. Rehun hyväksikäyttö oli siten Pekiloryhmissä vertailuryhmää heikompi. Erityisesti tämä korostui toisessa sukupolvessa, jolloin Pekilo oli toista erää, jonka valkuaispitoisuus oli huomattavasti korkeampi kuin ensimmäisen.

Ryhmien välillä ei ollut merkitseviä eroja kuolleisuudessa, hedelmällisyydessä ja haudontatuloksessa. Primäärisesti epänormaalien siittiöiden määrän lisääntyminen toisen sukupolven Pekiloryhmien kukkojen spermassa ei ollut sitä luokkaa, että se vaikuttaisi hedelmällisyyttä heikentävästi. Kuolleiden sikiöiden tutkimusten ja ruhonavausten perusteella ei Pekilon todettu lisäävän epämuodostumien esiintymistä.

Näiden koetulosten mukaan, ottaen samalla huomioon suuri käyttömäärä, Pekiloa voidaan pitää sopivana ja turvallisena siipikarjan valkuaisrehuna.

ANNALES AGRICULTURAE FENNIAE VOL. 22: 104—109 (1983)

Seria PHYTOPATHOLOGIA N. 94 — Sarja KASVITAUDIT n:o 94

VIRAL DISEASES OCCURRING ON *RIBES* SPECIES IN FINLAND.

KATRI BREMER

BREMER KATRI, 1983. **Viral diseases occurring on *Ribes* species in Finland.** Ann. Agric. Fenn. 22: 104—109. (Agric. Res. Centre, Inst. Pl. Path. SF-31600 Jokioinen, Finland.)

The reversion disease, which is transmitted by the gall mite *Cecidophyides ribis* is the most common and important viral disease in black currants. A severe strain of the reversion virus occurs rarely.

The vein-banding virus transmitted by aphids is very common in red currant and gooseberry cultivations started with uncertified plants. Other viruses, infectious variegation, tomato black ring, raspberry ring spot and cucumber mosaic viruses have been found sporadically.

Index words: reversion, vein-banding virus, infectious variegation, tomato black ring, raspberry ring spot, cucumber mosaic, *Ribes alpinum, R. nigrum, R. rubrum, R. spicatum,* currant viruses.

Reversion disease and gall mites used to be common in black currant cultivations (BREMER and HEIKINHEIMO 1980). A certificate scheme to produce and distribute healthy, genuine propagation material from berry plants was thus introduced during the 1970s (BREMER and YLIMÄKI 1978). Field surveys and virus indexing carried out for this scheme showed that several virus diseases infect currants and gooseberries. This is a repot of the results of the surveys and virus testing.

METHODS

Test plants were grown in sterilized soil and kept in an insect-proof glasshouse.

Viruses were transmitted by bottle grafting or by sap transmission. Small branches were grafted during late summer and autumn onto the following virusfree test plants: black currant cvs. Amos Black, Baldwin and Öjebyn, and gooseberry seedlings of cv. Kaunisrannan punainen. Black currant test plants were propagated by cuttings from healthy mother plants. Sometimes seedlings of the red currant cv. Jonkheer van Tets were also used.

Sap expressed from very young leaves and mixed with 2 % nicotine base was used as an inoculum. Test plants for sap transmission tests were: *Chenopodium quinoa, Nicotiana tabacum* and *Petunia hybrida.* Test plants were dusted with carborundum before inoculation.

Fig 1. Deformed and healthy flowers of a black currant infected by the reversion disease.

Fig 2. A yellow line pattern in *R. alpinum* infected by the reversion disease in the wild.

Fig 3. First symptoms of the reversion dislase.

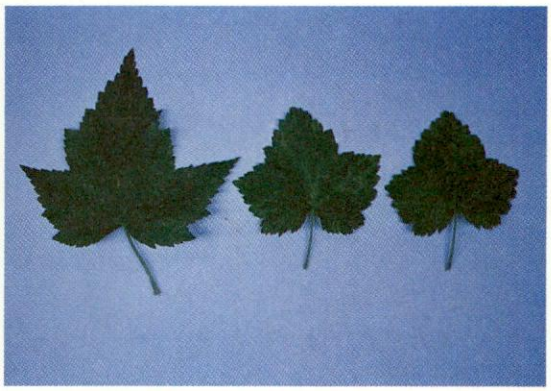


Fig 4. Spots caused by raspberry ringspot virus on black currant leaves.

Fig. 5. Spots caused by the tomato black ring virus on a black currant leaf.

Double-diffusion presipitin tests were made on 0,8 % agarose gel prepared in 0,01 M phosphate buffer and 0,02 % NaN_3. Wells 5 mm in diameter were spaced with 6 mm between the centres.

Antisera were kindly supplied by Drs A.F. Murant and A.T. Jones, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland and M. Christensen, Plantevaerncentret, Lyngby, Denmark.

Electron microscope preparations were made as follows: small pieces of leaves were prefixed with 2,5 % glutaraldehyde in 0,2 M sodium phosphate buffer (ph 7,2) for 2 h at 5 °C. Then the tissue pieces were washed in the same buffer with 0,2 M sucrose, postfixed for 2 h with 1 % osmium tetroxide in 0,1 M sodium phosphate buffer, ph 7,2. The sections were obtained from samples embedded in Epon 812 and stained with uranyl acetate and lead citrate.

Reversion disease

Reversion virus strains with mild leaf symptoms have been very common in Finland. Bushes infected with a virulent strain which causes deformation of flowers and leaves (Fig. 1) have been found in Finland in only four localities, which are very far apart: one in western Finland, two in the southwest and one near the eastern border. Reversion disease symptoms (reverted leaves and deformed flowers) were also observed in wild plants of *Ribes alpinum* and *Ribes spicatum*. A yellow line patterns sometimes occurred in the leaves of *Ribes alpinum* in the wild in autumn (Fig. 2). The reversion disease was transmissible by grafting from these wild plants onto black currant test plants.

The causal agent of the reversion disease is not known. Thin sections from flowers, leaves and veins of the leaves from infected bushes were therefore inspected under a transmission electron microscope. No virus or mycoplasma-like particles were seen, though thin sections were made at different times of the year from different parts of several infected plants.

Vein-banding virus

During field surveys, symptoms of the vein-banding virus were observed in nearly all inspected red currant and gooseberry fields planted with noncertified plants. Symptoms of yellow vein banding along the main veins or vein clearing often occurred only in segments of the leaves. The symptoms were quite clear during the whole summer and the infected leaves or segments of leaves turned yellow earlier than healthy leaves. Symptoms in gooseberries were similar to those in red currant. Veinbanding symptoms also occurred in black currants but they were faint or latent.

Aphids, which transmit the vein-banding virus (TRESH 1970), are common in Finland (VAPPULA 1962).

In our test, the *Cryptomyzus ribis* aphid transmitted the vein-banding virus from a naturally infected gooseberry bush to gooseberry and red currant seedlings as well as to black currant plants (cv. Öjebyn).

C. ribis also transmitted the virus from a naturally infected red currant bush (cv. Red Dutch) to seedlings of Jonkheer van Tets red currant.

The vein-banding virus was also transmitted by grafting from a naturally infected gooseberry onto red currant and gooseberry seedlings and from them back to gooseberry seedlings. Graft transmissions also succeeded from red currant onto gooseberry seedlings and onto black currant plants (cvs. Öjebyn and Amos Black) and from them back to the gooseberry seedlings.

R. alpinum seedlings also became infected after a graft transmission from a diseased red currant or gooseberry bush. The symptoms in *R. alpinum* were faint but clear in spring and early summer. The virus could be transmitted by grafting from *R. alpinum* back to red currant and gooseberry seedlings.

Attempts to transmit the vein-banding virus by sap failed. Furthermore, the vein-banding virus particles were not found in thin sections inspected under a transmission electron microscope.

Infectious variegation

Black currant bushes with symptoms of bright yellow flecks in the leaf lamina and sometimes yellow banding along the veins were found in three localities: two in southern Finland and one near Tornio in northern Finland. Similar symptoms were also found on a sample of black currant twigs from a farm near Helsinki. These symptoms were similar to those of the infectious variegation (TRESH 1970).

Grafts were made from the white currant bush onto black currant test plants (cvs. Baldwin, Amos Black and Öjebyn), two red currants (cv. Red

Dutch) and the white currant cv. White Jüterbog.

Symptoms appeared slowly in Baldwin and Amos Black. One year after grafting a few yellow lines near veins and a few yellow flecks appeared on a few leaves. Two years later, more white-yellow flecks developed on many leaves. The youngest leaves never displayed any symptoms. When a twig from an infected, but symptomless Baldwin black currant was grafted onto a Red Dutch red currant or a White Jüterbog, the symptoms appeared very clearly in them. Attempts to transmit infectious variegation disease by sap did not succeed.

Red and white currant plants developed very many, bright by coloured whitish-yellow flecks between veins 1—2 years after grafting. Flecks remained the same through out the summer in the leaves inside the bush, whereus symptoms in leaves which received plenty of sun faded during the summer.

Raspberry ring spot virus

Green-yellowish ring spots, spots and short lines were noticed in the leaves of black currant bushes in the field (Fig. 4). Similar symptoms were also seen in cuttings brought from the same field to root in a glasshouse in March-April. Later in summer, the symptoms disappeared. This disease could be transmitted by grafting from black currants onto black currant cvs. Amos Black and Öjebyn.

Sap transmission from young black currant leaves with ring spot symptoms succeeded in spring when a 2 % nicotine base was used as a buffer. Yellow flecks, which slowly turned necrotic, developed in *C. quinoa* leaves in about 10 days. Systemic mottle and distortion of the top appeared later.

In the gel double-diffusion test, unpurified sap from *C. quinoa* reacted with antiserum to raspberry ring spot virus. One precipitation line was produced. A positive reaction was obtained with undiluted antiserum from Denmark but not with the antiserum from Scotland.

Raspberry ring spot virus symptoms were seen in two fields and the virus was also isolated from both places. In one experimental field, symptoms of the virus occurred in many bushes of the cvs. Erkheikki, Kangasfors, Cotwold cross, Sunderbyn II and Öjebyn.

Tomato black ring virus

While testing black currant plants from one nursery, symptoms different from those caused by the raspberry ring spot virus developed on the leaves of black currant test cvs. Amos Black, Baldwin and Öjebyn.

Large, concentric, angular, chlorotic ring spots appeared on young leaves early in the spring (Fig. 5). Later in June, the symptoms disappeared. A new grafting from a diseased bush onto a new test plant was necessary to produce the symptoms.

The virus was transmissable via sap from young leaves with symptoms to *C. quinoa.* Small, yellow local spots developed on the leaves of *C. quinoa,* and systemic mottle also appeared later.

In the gel-diffusion test, sap from infected *C. quinoa* reacted positively with antiserum from Scotland against tomato black ring virus.

Tomato black ring virus was found in several black currant bushes of cvs. Sunderbyn I and Öjebyn in one field.

Cucumber mosaic virus

The cucumber mosaic virus was isolated by sap and aphid transmission from one black currant bush. Some branches of the bush had leaves with yellow-green mottle. The virus was transmitted from young mottled leaves by the aphid *Myzus persicae* and by sap into *C. quinoa* and *N. tabacum,* which developed typical symptoms of cucumber mosaic. A graft transmission from the infected black currant was made onto gooseberry seedlings. The following year, yellow spots developed on the gooseberry leaves. No back-grafts were attempted.

Two diseases with virus-like symptoms

Red currant virus. Grafting from one symptomless red currant bush produced symptoms consisting

of small, yellow dots which later turned necrotic, and severe crinkling of the young leaves on seedlings of Jonkheer van Tets red currant. Symptoms became masked later in the summer. Sap transmission of the disease from young red currant leaves was not succesful.

Black currant virus

After graft transmission from a symptomless black currant cv. Brödtorp onto black currant test plants, Amos Black and Baldwin, a yellowish-green, faint mosaic appeared on their leaves. *R. alpinum* and gooseberry seedlings could also be infected by graft transmission. Symptoms were very weak in *R. alpinum* and gooseberry. After back-grafting onto black currant plants, normal, strong symptoms developed on the leaves of Amos Black. The attempts to transmit the disease by sap failed.

DISCUSSION

The reversion and vein-banding virus diseases seem to be the only economically important virus diseases in *Ribes* crops in Finland. The use of certified propagation material from currants and gooseberries has already reduced the occurrence of virus diseases, and this development will continue.

R. alpinum, which is susceptible to several currant viruses and is naturally infected by the reversion disease, can be a source of re-infection for currant fields. *R. alpinum* grows wild in southern and central Finland and is common (HIITONEN 1933).

The infectious variegation virus infects, according to the literature (TRESH 1970), only black currant, but in the present tests the virus was transmissible to black, white and red currants, which all displayed typical symptoms. Therefore the name infectious variegation is used here for this disease, though it is not possible to identify viral diseases only on the basis of their symptoms.

Sap transmissible, soil-borne viruses like raspberry ring spot and tomato black ring viruses might occur in currants, at least in plant nurseries, more commonly than is known. TAPIO (1972) found tomato black ring virus in plant nursery soil. These viruses might easily be overlooked in currants even with virustesting. Clear symptoms are visible for only a short time in the wild and also in a cool, well-shaded glasshouse. Sap transmission does not always succeed. Careful testing of mother plants for the certificate scheme is therefore necessary.

REFERENCES

BREMER, K. & HEIKINHEIMO, O. 1980. Problems of the reversion disease of *Ribes* in Finland. Acta Hortic. 95: 87—91.

— & YLIMÄKI, A. 1978. A certificate system to produce and distribute virus tested propagation material from berry plants in Finland. Ann. Agric. Fenn. 17: 42—44.

HIITONEN, J. 1933. Suomen kasvio. 771 p. Helsinki.

TAPIO, E. 1972. The appearance of soil-borne viruses in Finnish plant nurseries. Maatal. tiet. Aikak. 44: 81—92.

TRESH, J. M. 1970. Virus and viruslike diseases of gooseberry and currant. In "Virus diseases of small fruits and grapevines." 290 p. Berkeley.

VAPPULA, N. 1962. Suomen viljelykasvien tuhoeläinlajisto. Ann. Agric. Fenn. 1, suppl. 1: 1—275.

Manuscript received February 1983

Katri Bremer
Agricultural Research Centre
Institute of Plant Pathology
SF-31600 Jokioinen

SELOSTUS

Ribes-suvulla Suomessa esiintyvät virustaudit

KATRI BREMER

Maatalouden tutkimuskeskus

Viljelyksillä tehtyjen havaintojen ja virustautitestausten mukaan äkämäpunkin levittämä suonenkatoviroosi (reversion virus disease) on ollut yleisin ja haitallisin virustauti mustaherukassa. Paitsi mustaherukasta se on tavattu pari kertaa punaherukasta ja luonnonvaraisista *R. alpinum* ja *R. spicatum*-kasveista. Tarkastettujen käyttötaimien lisääntyvä käyttö on vähentänyt suonenkatoviroosin samoin kuin muidenkin virustautien esiintymistä. Suonenkatovirus esiintyy voimakkuudeltaan erilaisina rotuina. Kukat turmeleva ja siten sadon täysin tuhoava rotu ei ole meillä yleinen, sitä on tavattu vain neljältä, tosin maan eri puolilla sijaitsevalta viljelykseltä.

Kirvojen levittämä suonikloroosivirus (vein banding virus) on ollut hyvin yleinen sekä punaherukka- että karviaisviljelyksillä. Mustaherukassa tämä virus on lähes piilevä, mutta jonkun kerran se on tullut esille mustaherukasta testauksissa.

Tarttuva kirjavuusvirustauti (infectious variegation), jota ei ole todettu meillä aikaisemmin, on tavattu kolmelta paikkakunnalta, mustaherukasta ja valkoherukasta. Ymppäämällä tauti voitiin siirtää myös punaherukkaan. Muualla tauti tunnetaan vain mustaherukasta.

Vadelman rengaslaikkuvirus (raspberry ringspot), jota ei ole meillä aikaisemmin todettu herukoista, on eristetty kahden paikkakunnan mustaherukoista sekä mehussa että ymppäämällä. Samoin myös tomaatin mustalaikkuvirus, jota ei ole aikaisemmin eristetty herukoista.

Kurkun mosaiikkivirus (cucumber mosaic) tavattiin yhdeltä viljelykseltä mustaherukkapensaasta, josta se voitiin siirtää persikkakirvan avulla sekä ymppäämällä testikasveihin.

Merkityksellisiä näistä taudeista ovat suonenkato- ja suonikloroosivirustauti. Ne alentavat herukoiden marjasatoa huomattavasti. Tervetaimituotannossa on kuitenkin syytä testata ydinkasveista pois kaikki ankeroislevintäiset virukset. Esim. vadelman rengaslaikku- ja tomaatin mustalaikkuvirukset eivät aiheuta sanottavia vaurioita herukoille, mutta jos ne kulkeutuvat herukoiden taimien mukana ja pesiytyvät maahan, on niistä suurta haittaa esim. vadelmalle ja eräille koristekasveille, joissa ne aiheuttavat ankaran taudin.

ANNALES AGRICULTURAE FENNIAE VOL. 22: 110—114. (1983)
Seria PHYTOPATHOLOGIA N. 95 — Sarja KASVITAUDIT n:o 95

FUSARIUMS OF THE POTATO IN FINLAND VII
YIELD DECREASE CAUSED BY PATHOGENS OF POTATO DRY ROTS AND GANGRENE

ESKO SEPPÄNEN

SEPPÄNEN, E. 1983. **Fusariums of the potato in Finland VII. Yield decrease caused by pathogens of potato dry rots and gangrene.** Ann. Agric. Fenn. 22: 110—114. (Agric. Res. Centre, Inst. Pl. Path. SF-31600 Jokioinen, Finland.)

The influence of seed tuber infection by different pathogens on the tuber yield of some cvs. was studied by infecting each tuber 2 to 3 weeks before planting. Preliminary trials were carried out to elucidate the importance of soil-borne seed infection.

Fusarium sulphureum caused a greater decrease in yield than the other fungi: *F. avenaceum, F. solani* var. *coeruleum, F. culmorum* and *Phoma exigua* var. *foveata.* This may be due to its better utilization of the environmental conditions prevailing during sprouting and in soil just after planting. The yield decrease seems to vary according to varietal resistance. Powder seed dressing just before planting is hardly necessary against soil-borne contamination.

Index words: potato, dry rot, *Fusarium avenaceum, F. culmorum, F. sulphureum, F.solani* var. *coeruleum,* gangrene, *Phoma exigua* var. *foveata,* yield decrease.

INTRODUCTION

The importance of potato dry rots and gangrene is principally in the damage caused during storage. Only one of the highly pathogenic fungi of potato tubers, *Fusarium avenaceum,* is reported as a pathogen of potato wilt (McLEAN and WALKER 1941), and is considered a relatively weak pathogen compared with *F. oxysporum* and *F. solani* var. *eumartii. Phoma exigua* var. *foveata,* a pathogen of gangrene, is known as a partly systemic parasite of potato, but it does not essentially disturb the growth of the potato.

The ample occurrence of dry rots and gangrene in seed tubers gives rise to a decrease in yield because of the weakened ability of the seed tubers to produce a flourishing crop. PÄTZOLD and GÄHRE (1972) inoculated seed tubers with *F. solani* var. *coeruleum* and planted them. Infection caused delayed emergence, poor growth, reduced yield and tuber malformation. There was a large variation in the decrease in yield between the 24 cvs. in the trials. The yield of the most susceptible cvs. decreased about 50 % and the average decrease was about 30 %. Mist spray treatment with thiabendazol raised yields by an average of 20 percentage units, i.e. to 90 % of the mean yield of non-infected tubers. Rather similar results have been reported for a number of seed dressing trials.

OLOFSSON (1976) used naturally infected seed material and obtained various (0—16 %) yield increases by powder seed dressing with benomyl. BÅNG (1978) obtained fairly similar results using naturally infected seed. She utilized powder treatment on some tubers and mist spray treatment on others (cf. NIELSEN 1978). SEPPÄNEN (1977) used seed tubers with heavy artificial infection (separately with *Fusarium solani* var. *coeruleum*, *F. sulphureum* and *Phoma exigua* var. *foveata*) and ascertained average yield losses of 20—40 % compared with non-infected seed tubers.

In 1979—82 we carried out some small scale field trials to find out whether late infection with the main pathogens of dry rots and gangrene influence the yields and whether the varietal resistance is of any importance.

MATERIAL AND METHODS

In the present work there were three questions to be answered, concerning influence of separate pathogens on the yields of Bintje, the influence of two pathogens, *Fusarium sulphureum* and *Phoma exigua* var. *foveata,* on the yields of cultivars with different resistance to the fungi, and the influence of powder seed dressing on possible wounding and soil-borne infection during and after planting in cv. Bintje.

The growing procedures were standard trial practices. Attention was paid only to the quantity of the yields.

The infection material was from the same isolates used in the study of optimal growth temperatures of the fungi in tubers of Bintje (SEPPÄNEN 1980, 1981 a). The selection of cvs. of different resistance was based on the first results of varietal resistance (SEPPÄNEN 1980, 1981 b).

Tubers 40—50 mm in size were infected using an adaptation of the method developed by LANGTON (1971). A wound 5 mm in diameter and 2 mm deep was made with a cork borer at the mid-point between the heel and rose ends of the tuber. The mixture, pure cultures of the fungus including the remaining agar, was used as inoculum. The wounds were filled with the inoculum and left uncovered, except in the case of *F. avenaceum* tubers, which were incubated in plastic bags. The tubers were incubated for two to three weeks at 6—10 °C and 90—95 % RH. Only tubers successfully infected were used, planting exactly the same number per plot.

In the trials with powder treatment, half of the inoculated tubers were dressed with thiabendazol (Tecto 10 %; 1 kg/ton) and the second half was left without dressing. Before planting, they were all damaged in the same way. The damage was caused by a "stone-box" method and it was intended to correspond to the rather rough "tuber treatment" caused by automatic potato planters.

The trials were preliminary and the areas of the plots only 10 m² with three replicates; the plots of the powder treatment trial, however, were 20 m² with four replicates. Observations were made on emergence and growth during the season. The main attention was paid to the quantity of the yields.

The results were treated using standard statistical methods; variance analysis and LSD values were calculated.

RESULTS AND DISCUSSION

Seed tuber infection of separate fungi decreased tuber yields to various extents (Table 1). The decrease was highest with *Fusarium sulphureum,* an average of 20—30 % for four years' trials.

Phoma exigua var. *foveata, F. avenaceum* and *F. solani* var. *coeruleum* infections decreased yields by about 10 % on average and *F. culmorum* had hardly any effect on the yield.

In 1980—82 we studied the influence of varietal resistance on yield decrease. These results (Table 2) supported the results of the yield decrease studies presented above; the mean decrease in yield of susceptible cvs. was fairly similar. The yield decreases of a moderately susceptible cv. (Saturna) and those of moderately resistant cvs. (Sabina and Tuomas) were less,

Table 1. The influence of seed tuber infection by different pathogens on the tuber yield of Bintje. Every tuber was inoculated two to three weeks before planting.

Pathogen	Tuber yields tons/ha				Mean of relative yields
	1979	1980	1981	1982	
Control	27,4 a	26,0 a	51,7 a	27,0 a	100
Phoma exigua v. *foveata*	20,3 b	25,9 a	47,6 ab	23,3 ab	88
Fusarium avenaceum	24,3 a	22,4 a	45,4 b	21,9 b	86
F. culmorum	26,7 a	28,0 a	44,8 b	—	(97)
F. solani v. *coeruleum*	24,7 a	27,8 a	46,3 b	21,4 b	92
F. sulphureum	16,9 c	24,9 a	45,8 b	16,9 c	78
F	$15,47^{xxx}$	1,33	$3,18^{x}$	$7,95^{xx}$	
LSD	3,2	—	4,3	4,1	

Table 2. Influence of varietal resistance on the decrease in tuber yield. Seed tubers inoculated 2—3 weeks before planting with *Fusarium sulphureum* or *Phoma exigua* var. *foveata*. H. Tuomas = Hankkijan Tuomas

1980			1981			1982			Mean of relative yields
F. sulphureum									
SABINA	control	20,6 a	H. TUOMAS	control	29,9 a	H. TUOMAS	control	15,1 a	(100)
SABINA	infected	17,3 a (84)	H. TUOMAS	infected	25,4 b (85)	H. TUOMAS	infected	14,9 a (99)	(89)
SATURNA	control	24,6 a	SATURNA	control	42,7 a	SATURNA	control	19,1 a	(100)
SATURNA	infected	20,2 b (82)	SATURNA	infected	38,9 b (91)	SATURNA	infected	14,7 b (77)	(83)
SANNA	control	33,6 a	BINTJE	control	48,2 a	BINTJE	control	26,8 a	(100)
SANNA	infected	28,3 b (84)	BINTJE	infected	44,0 b (91)	BINTJE	infected	16,5 b (62)	(79)
P.e.v. foveata									
H. TUOMAS	control	22,6 a	H. TUOMAS	control	29,9 a	H. TUOMAS	control	15,1 a	(100)
H. TUOMAS	infected	20,7 a (91)	H. TUOMAS	infected	25,9 b (87)	H. TUOMAS	infected	15,3 a (101)	(93)
SATURNA	control	27,5 a	SATURNA	control	42,7 a	SATURNA	control	19,1 a	(100)
SATURNA	infected	24,0 a (94)	SATURNA	infected	39,1 b (92)	SATURNA	infected	17,3 a (91)	(92)
STINA	control	30,5 a	BINTJE	control	48,2 a	BINTJE	control	26,8 a	(100)
STINA	infected	25,5 b (84)	BINTJE	infected	43,6 b (90)	BINTJE	infected	24,2 a (90)	(88)

Table 3. Effect of powder seed dressing on tuber yield of highly contaminated Bintje potatoes that were bruised using a 'stone-box' method on planting day.

	1979	1980	1981	1982	Mean ratio
Fusarium sulphureum infected					
Control	27,2	15,4 a	32,8	19,9 a	100
Powder treatment	28,1	18,3 b	40,1	24,4 b	117
F	0,14	7,81^{x}	3,81	21,88^{xxx}	
LSD	—	2,5	—	2,8	
Phoma exigua var. *foveata* infected					
Control	26,1	21,0	37,4	19,1	100
Powder treatment	28,1	21,6	40,4	20,4	106
F	0,98	0,25	1,35	0,51	
LSD	—	—	—	—	

referring with regard to the importance of their resistance. The yield differences were significant with moderately resistant cvs. in two cases out of six, with moderately susceptible cvs. in four cases out of six and with susceptible ones in five cases out of six. The yield variation within any given treatment was rather high and, as a consequence, yield differences of about 10 % were often not significant. In every case results obtained must be seen as trends.

The reasons for the highest yield decreases caused by *F. sulphureum* are its generally high pathogenicity and better tolerance of environmental conditions during incubation at low temperature and in soil during the first weeks of the growth season. Low temperature conditions were unfavourable to *F. avenaceum* and yield decrease became rather low; even the cv. Bintje was highly susceptible to it. In the light of these results, a seed tuber infection of 5 to 10 % has no essential influence on the coming yield.

A preliminary trial series carried out in 1979—82 showed the possible necessity for powder treatment against infections occurring during and just after planting (Table 3). Major and minor increases in tuber yield were obtained in every trial; 17 % in seed infected with *Fusarium sulphureum* and 6 % in seed infected with *Phoma exigua* var. *foveata,* on average. However, the yield increase was significant in only two of the eight trials. It must be borne in mind that in the present study we used a highly susceptible cultivar (Bintje), every tuber was infected, and the ''stone-box, treatment'' caused more bruises than potato planters usually do. The treatment is therefore hardly likely to be needed often in practical potato production. On the other hand, there is a strong tendency to develop planters that handle seed tubers as carefully as possible.

REFERENCES

BÅNG, U. 1978. Kemisk bekämpning av lagringsrötor i potatis. Resultat från svenska undersökningar. Nordisk Växtskyddskonferens, Ruissalo 11.—13.12.1978: 10—16.

MCLEAN, J.G. & WALKER, J.C. 1941. A comparison of *Fusarium avenaceum, F. oxysporum* and *F. solani* var. *eumartii* in relation to potato wilt in Wisconsin. J. Agric. Res. (Washington) 63: 495—525.

NIELSEN, A.F. 1978. Afsvampning af laeggekartofler og virkningen mod lagersygdomme på datterknoldene. Nordisk Växtskyddskonferens, Ruissalo 11.—13.1978: 7—9.

OLOFSSON, J. 1976. Viktiga sjukdomar i potatislager. Växtskyddsnotiser 40: 40—55.

PÄTZOLD, C. & GEHRE, H. 1972. Auswirkung von experimentellen *Fusarium*-Infectionen des Pflanzgutes und von

Desinfectionsmassnahmen auf Entwicklung und Ertrag von Kartoffelsorten. Landbauforschung Völkenrode 22: 129—132.

SEPPÄNEN, E. 1977. *Fusarium*- ja *Phoma*-lajien aiheuttamien perunan varastotautien kemiallisen torjunnan mahdollisuuksista. Kasvitautien tutk.lait. tiedote 30: 13—25.

— 1980. Studies on potato gangrene in Finland. Ann. Agric. Fenn. 19: 173—179.

— 1981 a. Fusariums of the potato in Finland II. On the growth optima of *Fusarium* species in tubers of cv. Bintje. Ann. Agric. Fenn. 20: 157—176.

— 1981 b. Fusariums of the potato in Finland III. Varietal resistance of potato tubers to some *Fusarium* species. Ann. Agric. Fenn. 20: 177—183.

Manuscript received January 1983

Esko Seppänen
Agricultural Research Centre
Institute of Plant Pathology
SF-31600 Jokioinen, Finland

SELOSTUS

Siemenperunan *Fusarium*- ja *Phoma*-infektion vaikutus satoon

ESKO SEPPÄNEN

Maatalouden tutkimuskeskus

Tavoitteena oli selvittää varastotauteja aiheuttavien sienten vaikutusta Bintjen satoon, lajikealttiuden merkitystä sadon alenemiseen ja jauhepeittauksen merkitystä käytettäessä voimakkaan sienitartunnan saanutta siementä. Kahden ensinmainitun kysymyksen selvittämiseksi istutusmukulat tartutettiin, yksi infektio mukulaa kohti, 2—3 viikkoa ennen istutusta. Jauhepeittauksen merkitystä selvitettäessä osa siemenmukuloista peitattiin ennen istutusta ja molemmat erät käsiteltiin ns. kivilaatikkomenetelmällä, joka vastasi automaatti-istutuskoneessa tapahtuvaa vioittumista. Näin käsitellyt siemenerät viljeltiin tavanomaisin keinoin. Vain mukulasadot selvitettiin.

Eniten alensi satoa *Fusarium sulphureum*, 20—30 %, *F. avenaceum*, *F. solani* v. *coeruleum* ja *Phoma exigua* var. *foveata* aiheuttivat n. 10 prosentin sadon alennuksen. *F. culmorum* tartutuksella tuskin on vaikutusta satoon. *F. sulphureum*in muita suurempi satoa alentava vaikutus perustunee sen parempaan mukautumiseen maassa istutusaikana vallitseviin lämpö- ja kosteusoloihin. Lajikekestävyydellä näyttää olevan vaikutusta sadon alennuksen suuruuteen, vaikkakaan saadut tulokset eivät sitä kiistattomasti todista.

Jauhepeittaus kohotti satoja joka kokeessa, mutta vain kahdessa kokeessa kahdeksasta ero oli tilastollisesti merkitsevä. Kun kokeet on tehty erittäin alttiilla lajikkeella, joka oli kokonaan tartutettu ja kun vioituskäsittely rikkoi mukuloita kenties pahemmin kuin istutuskone, voidaan päätellä, että jauhepeittaus lisätoimenpiteenä tulee kysymykseen vain poikkeuksellisen saastuneiden siemenerien kohdalla.

ANNALES AGRICULTURAE FENNIAE VOL. 22: 115—119 (1983)

Seria PHYTOPATHOLOGIA N. 96 — Sarja KASVITAUDIT n:o 96

FUSARIUMS OF THE POTATO IN FINLAND VIII
OCCURRENCE OF THE PATHOGENS CAUSING POTATO DRY ROT AND GANGRENE

ESKO SEPPÄNEN

SEPPÄNEN, E. 1983. **Fusariums of the potato in Finland VIII. Occurrence of the pathogens causing potato dry rot and gangrene.** Ann. Agric. Fenn. 22: 115—119. (Agric. Res. Centre, Inst. Pl. Path., SF-31600 Jokioinen, Finland.)

The incidence of potato dry rots and gangrene, and their causal pathogens, was studied from tuber samples collected in 1979, '80 and '81. Visual observations were made on the occurrence of bacterial soft rot, tuber blight and mechanical damage.

The total incidence of tuber rots was about 10 %, nearly half being caused by *Fusarium* spp., a quarter by gangrene and the rest by bacterial soft rot and tuber blight. Mechanical damage was very common.

Fusarium avenaceum, F. solani var. *coeruleum* and *Phoma exigua* var. *foveata* were the dominant pathogens, each causing about 25—30 % of the total loss. Of the other pathogens of dry rot only *F. sulphureum, F. culmorum, F. oxysporum* and *F. sambucinum* need be named but they are of little importance. *P. exigua* v. *exigua* is a noteworthy pathogen causing gangrene.

Index words: potato, dry rot, *Fusarium* spp., gangrene, *Phoma* spp. incidence, occurrence.

INTRODUCTION

The occurrence of pathogens causing dry rots and gangrene of potatoes has been under investigation by a number of workers in different countries. Usually only the pathogens of dry rots have been under investigation; the species of *Fusarium* have been isolated, identified and their relative incidence studied (Table 1). These investigations have been carried out in more or less different ways and with varying accuracy but they probably give a good idea of the incidence of each fungus in different countries or areas. There are three dominant species: *Fusarium solani* v. *coeruleum* has been the most common in the United Kingdom, West Germany and Scandinavia, *F. sulphureum* is clearly dominant in East Germany and France, and *F. avenaceum* seems to be of minor importance but quite common in France and Scandinavia. The other species are of relatively little importance. The importance of pathogens of gangrene compared with Fusariums has been studied in a few works only. According to BOYD (1972) gangrene was more important than the dry rots in the United Kingdom. In Sweden OLOFSSON (1976) considered that at least 70 % of infections were caused by *Phoma exigua* var. *foveata* (cf. NEDSTAM 1973).

In Finland the first figures of the incidence of *Fusarium* species (Table 1) were based on the relative frequency of isolates in material collected at random, partly from imported seed stocks, and partly from native stocks in 1975—79. In order to confirm the result and to compare the importance of gangrene and dry rots an other study was carried out. The material collected in 1979 was common to both these studies but the findings are presented in different ways.

Table 1. The relative incidence of *Fusarium* species according to studies carried out in different countries.

Pathogen	McKee UK 1952	Langerfeld FRG 1970	Nedstam Sweden 1973 Bintje	Nedstam Sweden 1973 Dianella	Olofsson Sweden 1976	Janke GDR 1976	Götz & Pett GDR 1977	Tivoli & Jouan France 1981*	Seppänen Finland 1981
F. solani v. *coeruleum*	91	45	83	9	36	3	13	12	30
F. sulphureum	1	21	1	21		69	79	39	10
F. sambucinum	<1		8	4	9				5
F. sambucinum v. *coeruleum*						1			<1
E. avenaceum	6	3	6	48		2	4	19	30
F. arthrosporioides				3		1			
F. culmorum	<1	6			4	18	2	6	15
F. oxysporum		2				2		2	5
F. oxysporum v. *redolens*		1							<1
F. tricinctum	1					1			<1
F. graminearum								9	<1
F. sporotrichioides						2			<1
F. solani		1				1			<1
F. trichothecioides									<1
F. roseum type X								9	

*) The Latin names used by Tivoli and Jouan have been transformed according to Booth (1971).

MATERIAL AND METHODS

Material. Samples of 400—500 tubers each were collected from harvested stocks in 1979, 1980 and 1981. In 1979, 19 samples originated from the seed potato centre and 56 samples from Raision Tehtaat Oy, a company producing different kinds of processed potatoes. In 1980, all 67 samples were from different areas of Hämeen Peruna Oy, the leading producer of potato starch in Finland, and in 1981 (partly in spring 1982) from a wide area in southeastern Finland. The material may be considered territorially representative and it includes samples from stocks used for seed, processing and table potatoes. The varietal distribution was not good, Saturna being sampled much more often than Record, the most commonly grown cv. in Finland. On the other hand cv. Frila is no longer of importance in Finnish potato production. On the whole the material was not representative but it was sufficiently large and varied to give the required approximate information on the incidence and importance of different pathogens.

Methods. At first 100 tubers were chosen at random and analysed for the occurrence of different diseases and pathogens. Only visual observations were made on tuber blight, bacterial soft rot and mechanical damage. Detailed analyses were carried out on tubers showing symptoms of dry rot and gangrene. The pathogens were identified using conventional methods. The tubers were cut into pieces, disinfected and placed on moist filter paper at room temperature. Within a few days the fungi had grown sufficiently for isolation. The isolates were grown on PDA (Difco) or on PSA (prepared according to Booth 1971), and only the latter was used for identification of Fusariums, which was done as in an earlier paper (Seppänen 1981). The *Phoma* spp. were identified with the thiophanate-methyl test of Tichelaar (1974).

To obtain information on the occurrence of latent infection a hundred apparently healthy tubers were selected from each sample and treated with the so called 'knack test'. Four wounds 5 mm in diameter and 10 mm deep, were made in each tuber, using a sterilized spike for each tuber. After the treatment the tubers were incubated for two mounths under normal storage conditions, about 5 °C and 90—95 % RH. The analyses of the tubers and the identification of pathogens

were carried out as presented above. The incidence of different pathogens was calculated in accordance with their occurrence in separate tubers. Where one tuber included more than one pathogen causal to the rot, both pathogens were counted as having equal incidence in the tuber. This method may put too much emphasis on weak pathogens with respect to the stronger ones, but hardly causes noteworthy error in the results. In the calculation of the results, the size of the stocks from which the samples were taken was not considered.

RESULTS AND DISCUSSION

Results are presented according to the year (Table 2) and the cultivar (Table 3), although comparisons between them are hardly justified for shortness of the material.

The figures of visual observations on bacterial soft rot and tuber blight indicate that these diseases are not insignificant as storage diseases, even though they are of minor importance compared with gangrene and dry rot. With regard to the tuber blight we must bear in mind that the material was collected during years when late blight was of minor importance. On the other hand, during these three years the harvest seasons were generally more rainy than normal, which possible caused an increase in the incidence of bacterial soft rot. The incidence of tuber rots caused by wound parasites (Table 2) might be still higher in view of the high incidence of mechanical damage, different kinds of wounds and scuffing. According to a rough estimate an average of 15—20 % of the tubers were useless as table potatoes.

Fusarium dry rot was about twice as common as gangrene, the average of rotted tubers being nearly 5 % (i.e. about half of all storage rots). The figures indicate a decreasing incidence of dry rot, caused by a decreased occurence of *Fusarium solani* var. *coeruleum*. Actually, the situation is hardly as bright as these results indicate. *Fusarium avenaceum* seems to be at least as common as *F. solani* var. *coeruleum*, and these fungi together with *P. exigua* var. *foveata* are the principal pathogens of gangrene and dry rots. *F. sulphureum* was rather rare, only 1 % of the total. Other *Fusarium* spp. comprised *F. culmorum*, *F. oxysporum* and *F. sambucinum*, in approximately

Table 2. Incidence of bacterial soft rot, tuber blight, dry rot and gangrene in tuber samples collected in 1979—81, and percentage importance of the pathogens of dry rot and gangrene.

Year	No. of samples	Soft rot	Tuber blight	Dry rot	Gang-rene	*Fusarium* *avena-ceum*	*solani* var. *coeruleum*	*sulph-ureum*	other spp.	*Phoma exigua* var. *exigua*	var. *foveata*
1979	75	1,3	0,5	7,4	3,2	28	32	1	4	8	27
1980	67	2,1	1,9	3,2	2,6	26	18	1	11	16	28
1981	23	0,2	1,1	1,3	2,4	28	4	1	2	21	42
	165										
	Mean	1,5	1,2	4,8	2,8	27	22	1	7	13	30
		14	12	47	27	47	39	2	12	30	70
				100				100			100

equal amounts. The occurrence of gangrene has been rather even in different years (2—3 % of tubers, Table 2). With regard to the relations between the species, *Phoma exigua* var. *exigua* and *P.e.* v. *foveata,* the latter has been dominant. The percentage of gangrene infected tubers as well as the relative incidence of the pathogens varied widely in separate samples.

If we compare these results with the earlier knowledge presented in Table 1, we can see that in principle they are roughly the same, even if the percentage figures vary to some extent. *F. avenaceum* and *F. solani* var. *coeruleum* are the most important and all the others are of little importance. Naturally, the results may vary according to year, cultivar and the health of the seed stock as GÖTZ (1980) emphasized. Perhaps the moist harvest season have been a reason for the common occurrence of *F. avenaceum,* the fungi favouring very moist conditions. A comparison of these results with those reported from different countries (Table 1) shows that only the result reported by NEDSTAM (1973) from cv. Dianella is similar to any degree. In the other countries *F. solani* var. *coeruleum* or *F. sulphureum* has been the dominant species.

With regard to the incidence of different diseases or pathogens in different cultivars there are actually only a few striking exceptions from the average (Table 3). Frila had more gangrene and Pito more dry rot infections than the others but because of the small number of samples in question (14) only a few highly diseased samples may increase the percentage of rots to a level many times higher. Thus, and considering the different origins of the samples, the results do not give evidence of a typical disease of any cultivar.

The incidence of latent infections varied greatly (0—60 %). The knack-test used is a typical *Phoma* test; latent infections were five times higher than those in the conventional analyses (table 4). The figure for dry rot infections was at the same level as that in the conventional analyses.

Table 3. Incidence of the diseases and pathogens in different cultivars.

Cultivar	No. of samples	Soft rot	Tuber blight	Dry rot	Gang-rene	*Fusarium*				*Phoma exigua*	
						avena-ceum	*solani* var. *coeruleum*	*sulph-ureum*	other spp.	var. *exigua*	var. *foveata*
Saturna	59	1,2	2,2	2,5	2,1	18	20	0	12	21	29
Record	41	1,0	0,4	4,9	2,4	33	25	1	3	7	31
Pito	14	2,2	0,9	15,4	2,2	20	37	4	13	6	20
Frila	14	2,4	0,1	6,4	7,3	21	23	1	1	21	33
Others	37	1,8	0,8	3,9	3,1	46	22	1	3	8	20

Table 4. Incidence of dry rot and gangrene and their causal agents (%) in tubers treated with the "knack-test".

Year	No. of samples	Dry rot	Gangrene	*Fusarium*				*Phoma exigua*	
				avena-ceum	*solani* var. *coeruleum*	*sulph-ureum*	other spp.	var. *exigua*	var. *foveata*
1979	75	9,0	18,4	14	11	2	3	6	64
1980	67	3,6	11,3	16	4	0	4	26	50
1981	23	1,0	8,0	7	4	1	1	10	79
	165								
	Mean	5,7	14,1	14	7	1	3	15	60

REFERENCES

BOOTH, C. 1971 The genus Fusarium. 237 p. London and Reading.

BOYD, A. E. W. 1972. Potato storage diseases. Rev. Pl. Path. 51: 297—321.

GÖTZ, J. 1980. Probleme der Resistenzprüfung von Kartoffeln gegenüber der Fusarium-Trockenfäule. Arch. Phytopath. Pfl.schutz 16: 251—258.

— & PETT, B. 1977. Zum Auftreten pathogener Fusarien an Kartoffelknollen in der DDR. Nachr.bl. Pfl.schutz DDR 31: 7—8.

JANKE, C. 1976. Untersuchungen über die in Lagerhäusern der DDR Trockenfäule an Kartoffeln verursachenden *Fusarium*-Arten. Arch. Phytopath. Pfl.schutz 12: 379—391.

LANGERFELD, E. 1970. Lagerfäulen an Kartoffeln 1969/70. Nachr.bl. Deut. Pfl.schutzd. 22: 109—110.

MCKEE, R. K. 1952, Dry rot disease of the potato. II. Fungi causing dry rot of seed potatoes in Britain. Ann. Appl. Biol. 39: 38—43.

NEDSTAM, B. 1973. Fusarium rötor på potatis. Växtskyddsnotiser 37: 50—54.

OLOFSSON, J. 1976. Viktiga sjukdomar i potatislager. Växtskyddsnotiser 40: 40—55.

SEPPÄNEN, E. 1981. Fusariums of the potato in Finland I. On the *Fusarium* species causing dry rot in potatoes. Ann. Agric. Fenn. 20: 156—160.

TICHELAAR, G. M. 1974. The use of thiophanate-methyl for distinguishing between the two *Phoma* varieties causing gangrene of potatoes. Neth. J. Pl. Path. 80: 169—170.

TIVOLI, B. & JOUAN, B. 1981. Inventaire, fréquence et agressivité des différentes ou variétés de *Fusarium* responsables de la pourriture sèche des tubercules de pomme de terre. Agronomie 1: 787—794.

Manuscript received February 1983

Esko Seppänen
Agricultural Research Centre
Institute of Plant Pathology
SF-31600 Jokioinen, Finland

SELOSTUS

Perunan varastotautien merkitys ja niiden aiheuttajat

ESKO SEPPÄNEN

Maatalouden tutkimuskeskus

Perunan varastotautien merkitystä ja niiden aiheuttajia sekä näiden suhteellista merkitystä selvitettiin vuosina 1979, -80 ja -81 maan eri puolilta kootusta yhteensä 165 mukulanäytettä käsittävästä aineistosta. Yksityiskohtaisen tutkimuksen kohteena olivat *Fusarium*- ja *Phoma*-lajit; taudinaiheuttajat eristettiin, puhtaaksiviljeltiin ja määritettiin tavanmukaisin menetelmin. Märkämädän ja mukularuton samoin kuin mekaanisten vioitusten esiintymisestä tehtiin vain silmävaraiset havainnot. Tulokset on esitetty prosenttilukuina.

Aineisto ei ole edustava maamme koko perunantuotanto huomioon ottaen, mutta antanee puutteellisenakin oikean kuvan *Fusarium*- ja *Phoma*-lajien merkityksestä ja keskinäisistä esiintymissuhteista.

Erilaisten tautien pilaamia mukuloita oli yhteensä kymmenisen prosenttia, tästä noin puolet eli 5 prosenttiyksikköä *Fusarium*-sienten pilaamia, noin neljännes eli 2—3 prosenttiyksikköä *Phoma*-lajien ja suunnilleen saman verran märkämädän ja mukularuton pilaamia yhteensä. Mekaaniset vioitukset, pääasiallisesti erilaiset haavat ja kuoriutuminen, olivat erittäin yleisiä; noin 15—20 % mukuloista oli niiden takia ruokaperunaksi kelpaamattomia.

Fusarium- ja *Phoma*-lajeista olivat yleisimmät *F. avenaceum*, *F. solani* var. *coeruleum* ja *P. exigua* var. *foveata*, kukin niistä on syynä noin 25—30 %:n varastotappioista. Muista lajeista vain *P. exigua* var. *exigua* on taloudellisesti merkittävä. Ottaen huomioon mekaanisten vioitusten runsauden varastotappiot saattaisivat olla huomattavasti suuremmatkin. Tätä tukee tutkimuksessa todettu piilevien Phoma-infektioiden yleisyys. Lajikkeiden erityisestä alttiudesta tietylle taudille ei tehty tutkimus antanut selviä viitteitä.

ANNALES AGRICULTURAE FENNIAE VOL. 22: 120—121 (1983)

Seria PHYTOPATHOLOGIA N. 97 — Sarja KASVITAUDIT n:o 97

RESEARCH NOTE

DIFFERENT TOLERANCES OF 14 *FUSARIUM* SPP. TO THIOPHANATE-METHYL

ESKO SEPPÄNEN

SEPPÄNEN, E. 1983. **Different tolerances of 14 *Fusarium* spp. to thiophanate-methyl.** Ann. Agric. Fenn. 22: 120—121. (Agric. Res. Centre, Inst. Pl. Path., SF-31600 Jokioinen, Finland.)

The behaviour of 14 *Fusarium* spp. on different agars with increasing doses of thiophanate-methyl was studied. The tolerance of different species varied over a wide range, *F. avenaceum* being the most tolerant and *F. oxysporum* indicating highest sensitivity.

Index words: Fusarium spp., growth rate, tolerance, thiophanate-methyl.

The identification of fungi is largely based on the use of different substrates and on the regulation of incubation conditions. To distinguish *Phoma exigua* var. *foveata* from var. *exigua,* TICHELAAR (1974) developed a rapid method by adding 25 ppm thiophanate-methyl to malt agar, which caused the increased formation of yellow crystals. We studied whether the method would be useful in identifying *Fusarium* species. We used a control and three different levels of thiophanate-methyl — 25, 50 and 75 ppm — added to malt agar, potato dextrose agar and potato sucrose agar. Fourteen *Fusarium* species, pathogens of potato tubers (SEPPÄNEN 1981), were used (one to ten isolates of each). The dishes were incubated for four days in the dark at a temperature of 22 ± 1 °C. Attention was paid to the growth rate (GR) and colour of the colonies grown on agars with different levels of thiophanate-methyl.

The results obtained on different agars and separate tests were very similar. Increasing thiophanate-methyl contents of the agars naturally decreased the growth rate of the fungi, and

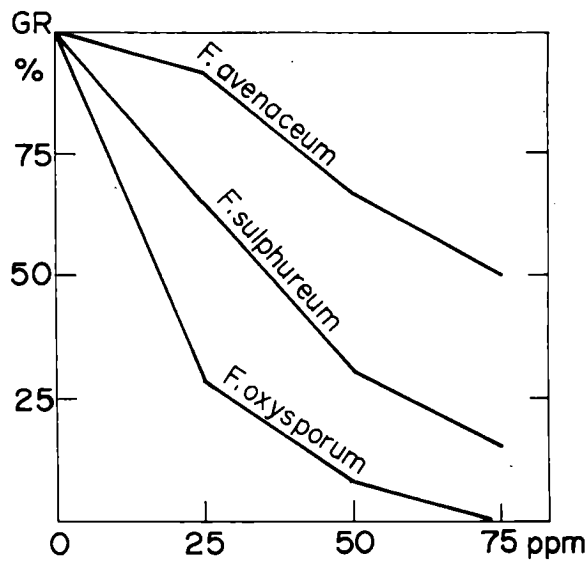


Fig. 1. Differences in tolerance of three *Fusarium* species to thiophanate-methyl. The curves are the means of three tests carried out on potato dextrose agar. See text.

changed the colour of the colonies. Especially in those fungi capable of producing red pigment, the red colour became stronger and even turned to yellow.

A more noteworthy result was that there were signified differences in the tolerances of the fungi (Fig. 1). The tolerance of *F. avenaceum, F. acuminatum* and *F. tricinctum* was highest. Besides *F. oxysporum,* the species *F. oxysporum* var. *redolens* and *F. sporotrichioides* were most sensitive. The other species, *F. sulphureum, F. trichothecioides, F. sambucinum, F. sambucinum* var. *coeruleum, F. culmorum, F. graminearum, F. solani* and *F. solani* var. *coeruleum,* formed an intermediate group.

The separate isolates of a given species behaved fairly similarly and it appears to be possible to develop a useful method for identification of certain *Fusarium* species.

REFERENCES

SEPPÄNEN, E. 1981. Fusariums of the potato in Finland I. On the *Fusarium* species causing dry rot in potatoes. Ann. Agric. Fenn. 20: 156—160.

TICHELAAR, G. M. 1974. The use of thiophanate-methyl for distinguishing between the two *Phoma* varieties causing gangrene of potatoes. Neth. J. Pl. Path. 80: 169—170.

Manuscript received February 1983

Esko Seppänen
Agricultural Research Centre
Institute of Plant Pathology
SF-31600 Jokioinen, Finland

SELOSTUS

Fusarium-lajien tiofanaatti-metyyli -toleranssin vaihtelusta

ESKO SEPPÄNEN

Maatalouden tutkimuskeskus

Kasvatettaessa 14 *Fusarium*-lajia erilaisilla ravintoalustoilla, jotka sisälsivät 0, 25, 50 tai 75 ppm tiofanaatti-metyyliä todettiin, että niiden sietokyky oli merkitsevästi erilainen. Kestävin oli *Fusarium avenaceum* ja alttein *F. oxysporum.*

ANNALES AGRICULTURAE FENNIAE VOL. 22: 122—136 (1983)

Seria AGROGEOLOGIA ET -CHIMICA N. 116 — Sarja MAA JA LANNOITUS n:o 116

SELENIUM IN FINNISH AGRICULTURAL SOILS

TOIVO YLÄRANTA

YLÄRANTA, T. 1983. **Selenium in Finnish agricultural soils.** Ann. Agric. Fenn. 22: 122—136. (Agric. Res. Centre, Inst. Soil Sci., SF-31600 Jokioinen, Finland.)

The mean total selenium content of 93 mineral soil samples taken from the plough layer in the most important agricultural areas of Finland was 0,209 mg/kg dry soil, ranging from 0,050 to 0,633 mg/kg. The mean selenium content of coarse mineral soils was 0,172 mg/kg and that for clay soils 0,290 mg/kg. The highest selenium contents, which reached 1,28 mg/kg, were found in organogenic soils. The mean selenium content of organogenic soils taken from the plough layer was 0,464 mg/kg.

The selenium contents of soils taken from the plough layer and the deeper layer at the same location were of the same order of magnitude.

On average, 2,9—4,8 % of the total selenium present in the plough layer and deeper layer was extractable into hot water.

The total selenium content of the mineral soil samples correlated closely with the clay fraction and organic carbon contents of the soil, and in the case of samples from the deeper layer also with the aluminium extractable into acid 0,2 M $(NH_4)_2C_2O_4$ solution.

In the multiple regression analysis the variations in the selenium content of mineral soils extractable into hot water were best explained by the total selenium and organic carbon contents of the soils, and in the case of plough layer samples also by the $pH(CaCl_2)$.

The variations in the selenium content of mineral soils extractable into acid 0,2 M $(NH_4)_2C_2O_4$ solution were best explained by the total selenium content of the soils and by the aluminium extractable into the same solution.

From the results of this study it would appear that selenium and its fractions in mineral soil samples taken from Finnish agricultural soils are most closely linked with the clay fraction and organic carbon contents of the soils and with the aluminium extractable into acid ammonium oxalate solution.

Index words: selenium determination, total selenium content of agricultural soils, water-extractable selenium, ammonium oxalate-extractable selenium.

INTRODUCTION

The selenium content of soil is naturally of the same order as the type of rock from which the main soil component is derived. The average selenium content of igneous rocks is low, only 0,09 mg/kg (GOLDSCHMIDT 1954, p. 532), while that of sedimentary rocks such as shales may be as high as several milligrams per kilogram (FLEMING and WALSH 1957, LAKIN 1961, REUTER 1975).

The selenium content of metamorphic rocks is, on average, higher than that of igneous rocks but lower than of shales. Thus soils derived mainly from igneous rocks can be taken to have the lowest selenium content, soils derived from metamorphic rocks to have a moderate selenium content and soils formed from sedimentary rocks to have the highest selenium content.

The Finnish bedrock is composed of slightly more than 60 % igneous and only about 25 % of sedimentary rocks. Migmatites, which are mixed rocks usually containing granites and gneisses, account for around 22 % (SIMONEN 1964).

KOLJONEN (1973 a, b, c, 1975) has reported a mean selenium content of 0,025—9,9 mg/kg in most common rocks in Finland.

According to SWAINE (1955, p. 91) the selenium content of agricultural soils in different parts of the world usually ranges between 0,1 and 2,0 mg/kg. The several thousand soil samples analysed since then in different parts of the world have not essentially changed this finding (ROSENFELD and BEATH 1964, p. 41—46, WELLS 1967, FLEMING 1968, PATEL and MEHTA 1970, MISRA and TRIPATHI 1972, LÉVESQUE 1974 a, GUPTA and WINTER 1975, ROBINSON 1976, DOYLE and FLETCHER 1977, FRANK et al. 1979, ELSOKKARY 1980).

In Finland, Sweden and Denmark the selenium content of agricultural soil averages 0,2—0,6 mg/kg (LINDBERG and BINGEFORS 1970, BISBJERG 1972, HAMDY and GISSEL-NIELSEN 1976 a, SIPPOLA 1979).

In Finland KOLJONEN (1974, 1975) has analysed almost 100 soil samples and found a selenium content of <0,01—0,50 mg/kg, which is slightly lower than the figure reported by SIPPOLA (1979), though not all the samples were taken from agricultural soil.

In selecting the method of digestion to be used in determining the selenium contents of soil samples, account must be taken of the fact that decomposition of the sample requires caution as certain selenium compounds readily volatilise, e.g. at high temperatures. The formation of volatile selenium compounds in media containing hydrogen halides can be exploited. The separation of selenium by distillation of selenium tetrabromide, $SeBr_4$, from the analysis matrix has been used extensively (e.g. KOLJONEN 1973 d).

The soil sample, usually 0,5—5 g is most conveniently decomposed by fusing with $NaOH$-Na_2O_2 (KRONBORG and STEINNES 1975), by wet digestion with HNO_3-$HClO_4$ (or HNO_3-H_2SO_4) (LÉVESQUE and VENDETTE 1971, CLINTON 1977, ELSOKKARY and ØIEN 1977), wet digestion with H_2SO_4-$HClO_4$ in the presence of sodium molybdate and ascorbic acid (XIAO-QUAN et al. 1982) or by treating with HNO_3-$HClO_4$-HF (GOLEMBESKI 1975, BAJO 1978). If the soil contains a large amount of organic matter care should be taken to ensure that the solution used for digestion does not become discolored due to carbonisation of the organic matter. Darkening of the solution indicates reducing conditions and hence selenium losses.

The advantages of using perchloric acid in the wet digestion method are its high oxidising power and the suitable maximum temperature (200 °C), at which the volatilization of selenium is small. Not all methods of digestion yield the total selenium content of the soil. The HNO_3-H_2SO_4 treatment used by STEINNES (1977) effectively decomposes the organic fraction, but decomposition of silicates is not complete. The results obtained with this treatment are therefore about 5 % below those obtained with alkali fusion reported by KRONBORG and STEINNES (1975).

The selenium fraction of the soil most readily available to plants is considered to be the water-extractable selenium. Nevertheless, plants use only a small part of the water-extractable fraction (LÉVESQUE 1974 b).

The sorption of selenium in the soil depends on the soil type, oxidation-reduction conditions and on the physical and chemical properties of the soil in general. Selenium may be present in the soil as selenide (oxidation state —2), elemental selenium with an oxidation state of zero, selenite (+4), selenate (+6) and as organic selenium. High soil pH favours the oxidation of selenite to selenate (GEERING et al. 1968), which is sorbed by the soil

considerably more weakly than selenite (CARY and GISSEL-NIELSEN 1973, YLÄRANTA 1983 b).

Clay minerals are important as sorbers of selenite (CARY and GISSEL-NIELSEN 1973). Selenium applied in the form of fertilizer is taken up by plants more effectively from fine sandy soil than from clay soil (GISSEL-NIELSEN 1971). The native selenium content of clay soils is usually higher than that of coarse mineral soils (WELLS 1967, PATEL and MEHTA 1970, SIPPOLA 1979). Thus the uptake of selenium by plants increases as the clay fraction of the soil increases (GISSEL-NIELSEN 1975). The capacity of organic matter for retaining selenium is even greater than that of clay minerals, though the mode of retention differs (CARY et al. 1967, HAMDY and GISSEL-NIELSEN 1976 b). In organic fractions the selenium forms complexes with organic compounds and may appear in amino acids and proteins found in soil micro-organisms.

In addition to investigating the selenium contents of Finnish agricultural soils and studying the proportion of selenium fractions in them, this study was aimed at shedding more light on the dependence of selenium content on some of the main soil factors.

The choice of method for decomposing the soil sample is influenced by the selenium determination method to be used. This study employed the hydride method, for which it was first necessary to look at the behaviour of selenium during the decomposition of various soil samples.

MATERIAL AND METHODS

I Decomposition of soil samples

BAJO (1978) has proposed a good method for decomposing the soil samples for the determination of selenium. In this study the volatilization of selenium during digestion was investigated using two mineral soils and one organogenic soil with additions of $Na_2{}^{75}SeO_3$. The soil samples were taken from the plough layer (0—20 cm) of agricultural soil. The particle size composition of the inorganic matter of the mineral soils was determined using the pipette method of ELONEN (1971):

Particle size (Ø)	Clay soil	Fine sandy soil
<0,002 mm	60,3 %	3,4 %
0,002—0,02	29,0	10,0
0,02 —0,06	4,0	48,0
0,06 —0,2	4,5	36,4
0,2 —2	2,2	2,2

The organogenic soil was Carex peat, which had a degree of humification of H_6 of the von POST scale. The clay soil was found to contain 1,8 % organic carbon, the fine sandy soil 2,9 % and the Carex peat 32,1 % as analysed by Leco CR-12 carbon determinator (SIPPOLA 1982).

For the determination of total selenium the clay soil and fine sandy soil were decomposed as follows, modified from BAJO's (1978) method: 1 g of air dried soil (Ø ≤ 0,2 mm) was weighed into a 120 ml teflon dish. To this was added 25 ml of concentrated HF (Merck, product number 338), 5 ml concentrated HNO_3 (Merck 456) containing 69 nCi of ^{75}Se and 12 ng of inactive selenium in the form of sodium selenite (Radiochemical Centre, Amersham, England) and 5 ml of concentrated $HClO_4$ (Merck 519). The mixtures were allowed to stand overnight and then evaporated almost to dryness on a sand bath. The residues in the bowls were treated with 10 ml of 6 M HCl and heated on an electric hotplate to the boiling point.

To decompose the peat soil 0,5 g of sample was treated with 10 ml HF, 20 ml HNO_3 and 5 ml $HClO_4$. The decomposition took the same course as for the mineral soils. Five decompositions were performed for each soil type.

After cooling, the residues were transferred in 5 ml portions into 10 ml glass measuring tubes. The

dishes were finally rinsed with small amounts of 6 M HCl so as to give six measuring tubes per sample.

The radioactivity in the tubes was measured immediately on a Wallac GTL 500 Automatic Gamma Sample Counter (Scaler/Timer SC-23, Pulse High Analyser AS-12, Programmer PP-67) using a 2 × 2'' NaI(Tl) well crystal (SCDA-4) detector.

The range 70—190 keV (photopeak 136 keV) was selected for measurement of the radioactivity of the samples. ^{75}Se-labelled HNO_3 solution added to the combustion solution in 5 ml batches was used as the standard. Most of the radioactivity, viz. over 90 % of that measured, was contained in the first two measuring tubes, which gave an average of 25 000 impulses during one minute's measurement. The other sample tubes were measured for 5—60 min, during which time 4 000—40 000 impulses were recorded. The background reading was around 70 impulses per minute.

Since selenium must have the oxidation state +4 in order to be reduced in the hydride method for measurement as hydrogen selenide, H_2Se (BRODIE 1977, YLÄRANTA 1983 a), the reduction of sodium selenate to selenium(IV) was also studied using the digestion methods described above.

1 ml of either sodium selenite (Merck 6607) or sodium selenate solution (BDH 10262) containing 500 ng Se in 6 M HCl was measured into the teflon dish. Five parallel dishes were prepared for both selenite and selenate solutions. The selenium solutions were subjected to digestion treatment in the same way as the soil samples. The decomposition residue was finally transferred to a 50 ml graduated flask, which was then filled up to the mark with 6 M HCl. The selenium content was determined using the hydride method (YLÄRANTA 1983 a).

II Selenium content of agricultural soils

Samples of agricultural soils were collected from 112 points in different parts of Finland south of the line Oulu-Kajaani-Lieksa (Fig. 1). The soil samples were taken from the plough layer (c. 0—20 cm) and from below the plough layer in the same place (c. 20—40 cm). Plough layer samples totalled 112, while samples from below the plough layer totalled 93. Most of the samples were taken in spring 1979 from ploughed fields prior to tilling. The size of the samples was 15 l. The sampling sites were chosen to be at least 200 m from any busy roads and well away from any industrial installation that might have had a major effect on the selenium content of the soil.

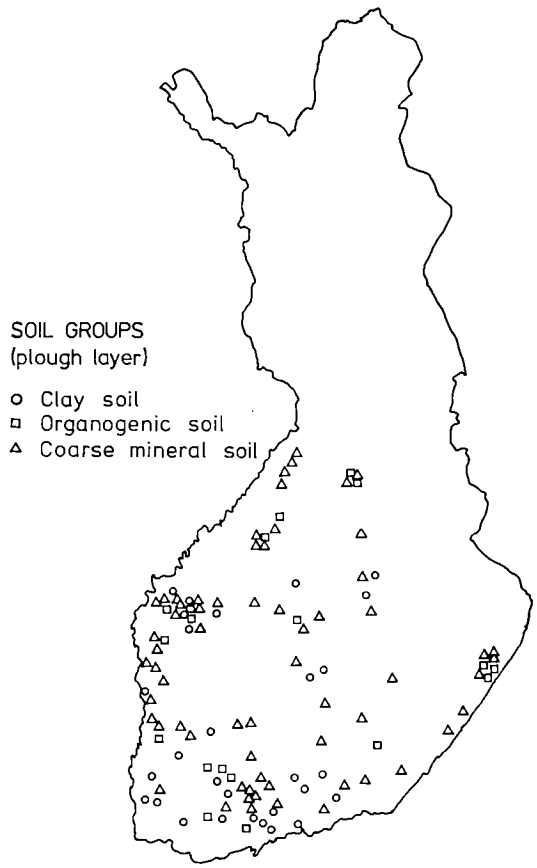


Fig. 1. Location of the sampling points.

The samples were spread on plastic sheet and dried at room temperature (19—21 °C). The air-dried samples were ground mechanically using a revolving wooden disc until the soil passed through a carbon steel sieve with circular mesh of diameter 2 mm. Following this treatment the soil samples were mixed and 0,5 l samples removed for analysis.

The particle size composition of the inorganic matter of the mineral soils was determined using the pipette method of ELONEN (1971). Organic carbon was determined using a modification (TARES and SIPPOLA 1978) of ALTEN's wet digestion method. pH($CaCl_2$) values were measured using soil suspensions made by mixing 25 ml of soil with 62,5 ml of 0,01 M $CaCl_2$ solution. The pH($CaCl_2$) was measured two hours after addition of the $CaCl_2$ solution.

For the determination of total selenium 15 ml of soil was ground in an agate mortar until 50 % of the ground sample passed through a 60 μm sieve, the remaining particles having a diameter of less than 0,2 mm. The total selenium was decomposed by treatment with HF-HNO_3-$HClO_4$, modified from BAJO's (1978) method. The weight of air-dried mineral soil used for analysis was 1 g, while that of air-dried peat was 0,5 g. At least two decompositions were performed for each soil sample.

In the determination of selenium extractable in hot water, a 25 ml soil sample was weighed out for extraction with 100 ml of de-ionised water. The soil suspensions were boiled in a 250 ml boiling flask for 30 min (YLÄRANTA 1982).

The Se, Fe and Al extractable in acid ammonium oxalate solution, 0,2 M $(NH_4)_2C_2O_4$, 0,1 M $C_2H_2O_4$, pH 3,3, were determined after shaking for 17 h (end over end 0,5 r/s). For this extraction 10 ml of soil and 100 ml extractant were used. Each 10 ml soil batch was also weighed.

The water-extractable selenium and ammonium oxalate-extractable selenium were determined using the hydride method by treating 20 ml of filtrate by dry ashing with $Mg(NO_3)_2$ as for plant matter (YLÄRANTA 1983 a). The selenium determinations were performed using a hydride apparatus developed from that described by SIEMER and HAGEMANN (1975) connected to a Perkin-Elmer 5000 atomic absorption spectrophotometer. The hydride apparatus is described in detail in an earlier publication (YLÄRANTA 1983 a).

Selenium determinations were made on 2 ml aliquots of solution. At least three determinations were carried out for each sample solution. 1 ml of Se(IV) solution, containing about 50 % of the selenium in the sample, was added to the sample in the hydride generator and the determination repeated. This simple method of addition to reveal any interference with the measurement is made possible by the fact that the standard curve for selenium is a straight line over a very wide range of concentration. None of the samples was found to change the detection of the amount of selenium added. The selenium determinations were usually carried out four hours after preparation of the analysis solutions. Some of the total selenium determinations were also performed after the solutions had been allowed to stand for 24 hours. According to the measurements, allowing the sample to stand for a few days does not alter the result.

Ammonium oxalate-extractable iron was determined by diluting the filtrates 1:10 with water and then using a Perkin-Elmer 5000 atomic absorption spectrophotometer with an air-acetylene flame. Ammonium oxalate-extractable aluminium was determined by diluting the filtrates 1:2 and 1:5 with extractant and using a Techtron AA-4 atomic absorption spectrophotometer with a nitrous oxide-acetylene flame. A series of standard solutions containing iron, aluminium, silicon and manganese in the ratio 5:5:5:1 was prepared for these determinations (RAAD et al. 1969).

The dry matter content of the air-dried samples was determined from 10 g soil samples ($\varnothing \leq 2$ mm) and from 1 g soil samples ($\varnothing \leq 0,2$ mm). The soil sample was weighed into a quartz dish and dried for 17 h at 105 °C.

I Decomposition of soil samples

Measurements carried out on the clay soil tubes following digestion gave (101,1 ± 1,3) % of the ^{75}Se added to the dishes in HNO_3 solution, (100,3 ± 0,6) % of that added to the fine sandy soils and (98 ± 2) % of that added to the Carex peat soils, all at the 99 % confidence level. The selenite solution added to the digestion mixture can never represent all the different forms of selenium in the soil. Nevertheless, the results can be taken to indicate that there is no great danger of losing selenium from the sample during digestion. However, the digestion solution must at no stage be evaporated completely to dryness, since according to BAJO (1978) this may lead to loss of selenium.

The results of four parallel determinations carried out using the hydride method show that the mean selenium content of the clay soil was 0,203 mg/kg of dry matter, that of the fine sandy soil was 0,164 mg/kg and that of the peat soil 0,557 mg/kg.

Using the modification of the digestion method chosen for both mineral soils and organogenic soils with selenium(IV) and selenium(VI) solutions showed that in every case the amount of selenium in the 6 M HCl solution corresponded with that added. This indicates that the selenium in the measurement solution is Se(IV) and thus entirely measurable using the hydride method. BAJO (1978) reported that following his digestion method and before treatment with HCl the selenium was present as both Se(IV) and Se(VI). In the applications studied the digestion residue is boiled in 6 M HCl in order to reduce all the selenium present in the solution to Se(IV). YLÄRANTA (1983 a) found that Se(VI) is reduced by boiling in 6 M HCl to form that can be measured using the hydride method.

According to AGEMIAN and BEDEK (1980), digestion with a nitric-perchloric acid mixture followed by digestion with hydrofluoric acid in the presence of permanganate and persulphate liberates selenium from organic complexes, unlike the method of BAJO (1978). In this study, four parallel digestions were carried out on 1 g samples of the mineral soils and 0,5 g samples of the peat soil using both the digestion methods described in this study and the method recommended by AGEMIAN and BEDEK (1980) for the fine sandy soil, clay soil and peat soil described earlier without any statistically significant differences in the selenium contents. AGEMIAN and BEDEK (1980) added in the final step of the method into the teflon beaker 10 ml of (1 + 1) hydrochloric acid to dissolve the digestion residue. They did not boil the solution. In this study the boiling was necessary to reduce all Se(VI) to Se(IV).

Organic constituents of soil extracts also appear to inhibit hydride production (WORKMAN and SOLTANPOUR 1980). In addition, all the selenium in the filtrate must be in the form of Se(IV) for hydride determination. For this reason, 20 ml batches of soil extract were subjected to dry ashing with $Mg(NO_3)_2$ and the residue dissolved in acid and treated as for plant matter in selenium analysis (YLÄRANTA 1983 a).

II Selenium content of agricultural soils

The 112 plough layer samples contained 93 mineral soils (Table 1). Nineteen samples contained more than 12 % organic carbon and were therefore classed as organogenic soils. Twenty-nine of the mineral soil samples contained more than 30 % clay and were thus classed as clay soils. There were 64 samples of coarse mineral soil. Eighty of the samples taken from deeper down (20—40 cm) were mineral soils, of which 26 were clay and the other 54 coarse mineral soils. There were 13 samples of organogenic soil.

The mean selenium content of mineral soil samples taken from the plough layer was 0,209

Table 1. Soil sample means ($\bar{x}$) and standard deviations (s) for pH($CaCl_2$), organic carbon content (%), clay content of inorganic material (%), total selenium content (mg/kg), hot water and acid ammonium oxalate-extractable selenium content (μg/kg).

Soil group	Number of samples	pH($CaCl_2$)		Organic C (%)		Clay (%)		Total Se (mg/kg) Mean		Range	Extractable Se (μg/kg) Hot water		Amm. oxal.	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s		$\bar{x}$	s	$\bar{x}$	s
Plough layer														
1. Mineral soils	93	5,0	0,57	3,3	1,8	22	17	0,209	0,108	0,050—0,633	9,0	4,1	54	26
Clay soils	29	5,1	0,58	3,6	1,9	43	11	0,290	0,120	0,131—0,633	11	3,5	66	31
Coarse mineral soils	64	4,9	0,57	3,1	1,8	13	8,7	0,172	0,078	0,050—0,489	7,9	4,0	48	21
2. Organogenic soils	19	4,5	0,59	23	7,9	—	—	0,464	0,239	0,212—1,281	18	8,5	60	27
Deeper layer														
1. Mineral soils	80	4,9	0,56	2,1	1,4	22	16	0,197	0,115	0,040—0,654	6,5	3,8	49	26
Clay soils	26	5,0	0,65	2,4	1,1	41	8,9	0,275	0,125	0,140—0,654	8,5	3,2	64	31
Coarse mineral soils	54	4,8	0,51	2,0	1,5	13	9,1	0,159	0,089	0,040—0,560	5,6	3,7	42	20
2. Organogenic soils	13	4,2	0,44	30	6,3	—	—	0,575	0,178	0,329—0,982	17	5,9	61	22

mg/kg. The mean selenium content of coarse mineral soils, 0,172 mg/kg, was considerably lower than the value of 0,290 mg/kg obtained for clay soil. The highest selenium content obtained, 0,633 mg/kg, was in clay soil, while the lowest, 0,050 mg/kg, came from coarse mineral soil. The selenium contents of the mineral soils were of the same order of magnitude as those reported by SIPPOLA (1979).

The selenium contents of samples taken from the plough layer were about the same as those taken from deeper down in the case of the mineral soils (Table 1). The correlation between the total selenium contents of the upper and lower of the mineral soils was highly significant ($r = 0{,}926^{***}$), which does not support the claim by KOLJONEN (1974, 1975) that farming activity increases the selenium content of the soil. In Canada, FRANK et al. (1979) also failed to find any increases in the selenium content of agricultural soil as a result of the application of sewage sludge. It is known that there is little downward movement of selenium in acid or neutral soils (GISSEL-NIELSEN and BISBJERG 1970, GISSEL-NIELSEN 1976, GISSEL-NIELSEN and HAMDY 1977, YLÄRANTA 1982), and so the fact that the selenium contents of the upper and lower soil layers are roughly the same cannot be explained by the leaching of selenium.

In arid and semi-arid regions, where there may be much more readily soluble selenium than in humid areas, selenium may accumulate in the topmost soil layers as a result of evaporation. In the same regions the decay of plants with high selenium contents raises the selenium content of the topmost soil layers (RAVIKOVITCH and MARGOLIN 1957, SINGH and KUMAR 1976).

The highest selenium contents, some of them over 1 mg/kg, were found in organogenic soils. The mean selenium content of plough layer samples was 0,464 mg/kg and that of samples from deeper down was 0,575 mg/kg. SIPPOLA (1979) reported a considerably lower mean selenium content of 0,169 mg/kg from 55 samples of organogenic topsoil.

Organogenic soil samples taken from deeper down contained more selenium than those taken from the plough layer. However, the difference in selenium contents was not statistically significant, and can probably be explained by the differences in organic carbon contents. The mean organic carbon content of the plough layer samples was 23 %, while that of samples from deeper down was 30 %.

The reason for the low organic carbon content of the plough layer may be the application of clay to peat soils, which has been a general practice in Finland. Since the "volume weight" of soils with

a high content of organic matter is low, the selenium contents are high when calculated per unit mass. The mean selenium content of the surface Carex peat samples studied was 0,46 mg/kg, while their content of air-dried, "ground" (Ø ≤ 2 mm) peat was 0,22 mg/l. The volume weight of peat in its natural state is even lower than that of a sample treated in the laboratory (ERVIÖ 1970). The volume weight of many mineral soils is almost unity (SIPPOLA and TARES 1978), which means that their selenium content is almost the same whether expressed per unit mass or per unit volume. It is important to consider the volume from the point of view of selenium uptake by plants, since a plant uses the same volume of soil irrespective of whether the substrate is clay, coarse mineral soil or organogenic soil.

The small number of samples used in this study is insufficient for a regional breakdown of the selenium contents of Finnish agricultural soils. However, classification of the mineral matter in the soils showed that more selenium had accumulated in clay soil than in coarse mineral soil, the selenium content of the latter being slightly lower. As the selenium content of the mineral soils differs from that of the organogenic soils, the variation in soil type in different parts of Finland affects the selenium contents of agricultural soil. Of the 250 soil samples studied by SIPPOLA (1979), the lowest selenium content, on average 121 μg/kg, was found in samples from northern Finland. Low selenium contents were also found in soil samples taken from central Finland. The highest selenium content, 301 μg/kg, was found in soil samples taken from southern Finland, most of which were clay. Despite the differences in selenium contents between different agricultural soils, the selenium contents of timothy were very low, mean value less than 10 μg/kg of dry matter, throughout Finland.

In 28 samples of Danish soil analysed by HAMDY and GISSEL-NIELSEN (1976 a) the selenium extractable into cold water represented 2,4 % of the total selenium, ranging from 1,7 to 5,5 %. In this study mean values for the proportion of the total selenium extractable into hot water from plough layer samples were 3,8 % for clay soils, 4,6 % for coarse mineral soils and 3,9 % for organogenic soils. The mean proportion of selenium extractable from deeper soil samples was smaller than for plough layer samples (Table 1).

The selenium(IV) available to plants is known to bind strongly with active iron oxides in the soil (GEERING et al. 1968, CARY and ALLAWAY 1969). Acid ammonium oxalate solution probably extracts the iron selenites (JOHN et al. 1976).

Acid ammonium oxalate solution extracted about six times more selenium from mineral soil samples taken from the plough layer than did hot water. The amount of selenium extracted corresponds to between one-third and one-fourth of the selenium content of the soils.

The fact that acid ammonium oxalate solution preferentially extracts from the soil active colloid fractions containing iron, aluminium and silicon (TAMM 1922) perhaps explains the small amounts of selenium extracted from organogenic soils, which were only three times those extracted into hot water.

The differences between the amounts of selenium extracted from plough layer samples and from deeper soils samples were small.

To get an idea of the effect of the various soil factors on the total selenium content of the soil and on the contents of selenium extractable into hot water and acid ammonium oxalate solution, a multiple regression analysis was performed on the mineral soil samples taken from the plough layer and from deeper down. The analysis was performed by means of a VAX — 11/780 data processing system using the program "Regression" provided in the SPSS software (KIM and KOHOUT 1975). Because of the small number of samples, this analysis was not carried out for organogenic soils or for clay soils and coarse mineral soils separately. In addition to the small number of samples, assessment of the selenium situation in organogenic soils was made more difficult by the earlier practice in Finland of spreading clay on peat soils, a measure which may have affected the physical and chemical properties of the soils.

Samples for which any one variable measured was outside the mean ±99,9 % confidence limits for this variable were rejected from the analysis. This criterion led to the rejection of 6 plough layer samples and 4 samples from deeper down, leaving for the regression analyses 87 samples from the plough layer and 76 samples taken from deeper down.

In no case did the absolute value of the simple correlations between the independent variable selected for the regression analyses exceed 0,50. The extractable selenium, iron and aluminium contents calculated per unit mass correlated most closely with the dependent variables, and so only the contents calculated per unit mass were used in the following regression analyses.

The computer program used is designed for stepwise processing. Variables with an F value in the equation significant at least the 5 % level were chosen for the equation model.

The correctness of the equation models chosen was checked by studying the residuals, i.e. difference between what is actually observed and what is predicted by the regression equation, in other words the amount which the regression equation has not been able to explain. The computer program "Regression" output the residuals, the plot of standardised residuals (residual/standard error of regression) and the plot of standardised residuals against predicted standardised dependent variable. Direct examination of the residuals and the overall pattern of the scatter plot gave no indication of any "abnormalities" requiring correction in the valid regression equations.

An attempt was made to explain the dependent variables by means of the variables chosen from among the following set of independent variables:

x_1 = clay fraction content, % of dry (105 °C) soil
x_2 = organic carbon content, % of dry soil
x_3 = soil pH($CaCl_2$)
x_4 = total selenium content, mg/kg dry soil
x_5 = content of aluminium extractable into acid ammonium oxalate solution, mg/kg dry soil
x_6 = content of iron extractable into acid ammonium oxalate solution, mg/kg dry soil

Only the clay content of inorganic material was measured (Table 1). In calculating the clay content of the soil samples, the organic matter was assumed to have a carbon content of 58 %. The amount of organic matter in the soil is thus 100/58 × organic C %.

The mean amount of aluminium extractable from the plough layer into acid ammonium oxalate solution was 2800 mg/kg dry soil (standard deviation 1300 mg/kg), while that for iron was 8000 mg/kg (4200 mg/kg). The corresponding figures for the deeper soil layer were 2400 mg/kg (1200 mg/kg) for aluminium and 8500 mg/kg (4900 mg/kg) for iron.

An attempt was made to explain the total selenium content of mineral soils (Y) by means of the variables x_1, x_2, x_5 and x_6.

PATEL and MEHTA (1970) and HAMDY and GISSEL-NIELSEN (1976 a) also took pH as a independent variable for the total selenium content of the soil. This was not done in the present study as the soil pH changes rapidly as a result of cultivation methods without any change in the soil's selenium content.

The clay fraction content, organic carbon content and extractable aluminium content of the soils explained to a statistically significant extent (P = 0,001), 58,1 % (R^2 = 0,581) of the variation in the total selenium content of the plough layer:

$$Y = 3{,}70 \times 10^{-2} + 3{,}48 \times 10^{-3}x_1 + 9{,}97 \times 10^{-3}x_2 + 1{,}84 \times 10^{-5}x_5 \; (F = 37{,}87^{***})$$

R^2 = 0,581

S (standard error of estimate of the regression equation) = 0,0522

n (number of samples) = 87

$s_{byl.25}$ (standard error of the unstandardised regression coefficient) = 0,00038

$s_{by2.15}$ = 0,00386

$s_{by5.12}$ = 0,00000

In the deeper layer soils the clay fraction content, organic carbon content and acid ammonium oxalate-extractable aluminium content explained 56,4 % of the variation in the total selenium content of the soils:

$Y = 2{,}53 \times 10^{-2} + 3{,}40 \times 10^{-3}x_1 + 1{,}99 \times 10^{-2}x_2 + 1{,}80 \times 10^{-5}x_5 (F = 30{,}56^{***})$

$R^2 = 0{,}564$

$S = 0{,}058$

$n = 76$

$s_{by1.25} = 0{,}00046$

$s_{by2.15} = 0{,}00564$

$s_{by5.12} = 0{,}00001$

The selenium content of mineral soils is closely dependent on the clay fraction content of these soils since in the plough layer this latter variable explained 50,6 % of the variation in the selenium content and 43,3 % of this variation in the deeper soils (Table 2). WELLS (1967), PATEL and MEHTA (1970), FRANK et al. (1979) and SIPPOLA (1979) have reported that clay soils contain more selenium than any other mineral soils.

Table 2. Coefficients of partical correlation ($r_{yx...}$) between total selenium content (mg/kg) of the soil and other soil variables, and the standardized regression coefficients (BETA) between the variables.

		Clay (%)		Organic C (%)		Extractable Al (mg/kg)	
	n	$r_{y1.25}$	BETA	$r_{y2.15}$	BETA	$r_{y5.12}$	BETA
Plough layer	87	0,711***	0,658	0,274**	0,195	0,401***	0,299
Deeper layer	76	0,658***	0,594	0,387***	0,298	0,333**	0,251

*** significant at the 0,1 % level

** significant at the 1 % level

In the 28 samples of Danish mineral topsoils reported by HAMDY and GISSEL-NIELSEN (1976 a), the combination of organic matter and total iron was most significant and accounted for about 54 % of the total variation in the selenium content. Their regression analysis also included the clay fraction content of the soil, though this was of little significance in explaining the selenium content of the soils. On the other hand, it should be remembered that the mean clay content of the mineral fraction of the soil samples studied by HAMDY and GISSEL-NIELSEN (1976 a) was only 6,9 %, with the highest contents 12—15 %, so that the variation in clay fraction content in the soil samples was also small, as were the contents of organic matter.

MISRA and TRIPATHI (1972) reported that the selenium content of 106 samples of Indian topsoil, mean Se 0,41 mg/kg soil, was closely correlated with the content of organic matter in the soils. In the material studied the organic carbon content of the soils explained only 7,5 % of the variation in the selenium content of the plough layer and 15 % of the variation in the selenium content of soil from deeper down (Table 2).

According to WELLS (1967), the average content of selenium in topsoil is greater than that of the parent rocks, and indicates a marked accumulation of the element during soil formation. This accumulation arises from the high retention of selenium by clay-sized particles, especially kaolin, gibbsite and ferric iron oxides.

In the material studied by LÉVESQUE (1974 a), consisting of 234 samples from 54 Canadian soil profiles, the selenium distribution was closely associated with both organic carbon and ammonium oxalate-extractable iron and aluminium. In this study the acid ammonium oxalate-extractable aluminium explained 16 % of the variation in selenium content of the plough layer and 11 % of that in the deeper layer (Table 2). However, unlike the results reported by LÉVESQUE (1974 a) the amount of iron extractable into acid ammonium oxalate solution was of no significance in explaining the total selenium content of the soils.

An attempt was made to explain the amount of selenium extractable from mineral soils into hot water using the variables x_1, x_2, x_3, x_4, x_5 and x_6.

The total selenium content, organic carbon content and soil pH($CaCl_2$) together explained 41,7 % of the variations in water-extractable

selenium (Y) present in the plough layer samples:

$Y = -4{,}61 + 20{,}5x_4 + 0{,}637x_2 + 1{,}47x_3$
$(F = 19{,}55^{***})$
$R^2 = 0{,}417$
$S = 2{,}69$
$n = 87$
$s_{by4.23} = 3{,}90$
$s_{by2.34} = 0{,}206$
$s_{by3.24} = 0{,}521$

The total selenium content and organic carbon content explained 42,0 % of the variations in the selenium extractable from the deeper layer into hot water:

$Y = 1{,}75 + 17{,}7x_4 + 0{,}619x_2 (F = 26{,}06^{***})$
$R^2 = 0{,}420$
$S = 2{,}41$
$n = 76$
$s_{by4.1} = 3{,}66$
$s_{by2.4} = 0{,}245$

Table 3. Coefficients of partial correlation ($r_{yx...}$) between content of hot water-extractable selenium (mg/kg) of the soil and other soil variables, and the standardized regression coefficients (BETA) between the variables.

		Se (mg/kg)		Organic C (%)		$pH(CaCl_2)$	
	n	$r_{y4.23}$	BETA	$r_{y2.34}$	BETA	$r_{y3.24}$	BETA
Plough layer	87	0,502***	0,470	0,323***	0,285	0,297**	0,247
Deeper layer	76	0,494***	0,488	0,286**	0,256	—	—

*** significant at the 0,1 % level
** significant at the 1 % level

The selenium content of plough layer samples explained 25,2 % and the selenium content of soil samples from deeper down 24,4 % of the selenium extractable from the soils into hot water (Table 3). The organic carbon content of samples from the plough layer and from deeper down, and the $pH(CaCl_2)$ of the plough layer samples each explained only about 10 % of the variations in water-extractable selenium. MISRA and TRIPATHI (1972) also reported a close correlation between the soil's organic carbon content and water-soluble selenium. In their material the organic carbon contents were 0,11—0,92 %, while water-soluble selenium accounted for 4,09—20,0 % of total selenium.

In the material studied by HAMDY and GISSEL-NIELSEN (1976 a) none of the variables pH, silt, clay, organic matter, total iron or acid-soluble iron correlated statistically significantly with the amount of selenium extractable into cold water.

SINGH and KUMAR (1976) reported a close correlation between the clay fraction content and total iron content, on the one hand, and the selenium content extractable into hot water, which ranged from 3,1 % to as high as 36,0 % of total selenium, on the other.

An attempt was made to explain the amount of selenium extractable into acid ammonium oxalate (Y) using the variables x_1, x_2, x_3, x_4, x_5 and x_6.

The total selenium content of the soils and the aluminium extractable into ammonium oxalate solution explained 63,6 % of the variations in the selenium extractable from the plough layer into acid ammonium oxalate solution:

$Y = 2{,}57 + 139x_4 + 7{,}50 \times 10^{-3}x_5$
$(F = 72{,}65^{***})$
$R^2 = 0{,}636$
$S = 12{,}8$
$n = 87$
$s_{by4.5} = 18{,}5$
$s_{by5.4} = 0{,}00114$

Table 4. Coefficients of partial correlation ($r_{yx...}$) between content of acid ammonium oxalate-extractable selenium (mg/kg) of the soil and other soil variables, and the standardized regression coefficients (BETA) between the variables.

		Se (mg/kg)		Extractable Al (mg/kg)	
	n	$r_{y4.5}$	BETA	$r_{y5.4}$	BETA
Plough layer	87	0,637***	0,525	0,585***	0,458
Deeper layer	76	0,657***	0,607	0,387***	0,293

*** significant at the 0,1 % level

The corresponding independent variables — the total selenium content and aluminium extractable into ammonium oxalate — explained 55,0 % of the variations in the selenium extractable from the deeper soil layer into acid ammonium oxalate solution:

$Y = 6{,}69 + 149x_4 + 5{,}17 \times 10^{-3}x_5$ ($F = 43{,}96$***)

$R^2 = 0{,}550$

$S = 14{,}4$

$n = 76$

$s_{by4.5} = 20{,}2$

$s_{by5.4} = 0{,}00145$

The total selenium content of the soil samples taken from the plough layer explained 40,6 % of the selenium extractable into acid ammonium oxalate solution, while the corresponding figure for the deeper soil layer was 43,2 % (Table 4). This finding is not surprising in view of the fact that acid ammonium oxalate solution extracts up to one-third of the total selenium from mineral soils (Table 1).

The results of this study suggest that selenium is more clearly connected with the aluminium extractable into acid ammonium oxalate solution rather than the iron, since the aluminium extractable into ammonium oxalate solution explained 34,2 % of the selenium extracted by the same solution from the plough layer, the figure for the deeper layers being 15 %. In the adsorption studies conducted by JOHN et al. (1976) selenite was retained by acid ammonium oxalate solution, Tamm's solution, extractable Al_2O_3 and Fe_2O_3, though more strongly by Fe_2O_3. Hydrous iron selenites are the best-known poorly soluble selenium compounds in the soil (ALLAWAY et al. 1967, GEERING et al. 1968), so that the finding that extractable aluminium is of greater significance than extractable iron in this study conflicts with the available data.

REFERENCES

AGEMIAN, H. & BEDEK, E. 1980. A semi-automated method for the determination of total arsenic and selenium in soils and sediments. Anal. Chim. Acta 119: 323—330.

ALLAWAY, W. H., CARY, E. E. & EHLIG, C. F. 1967. The cycling of low lewels of selenium in soils, plants and animals. Symposium: Selenium in biomedicine p. 273-296. Ed. MUTH, O.H. 445 p. Westport.

BAJO, S. 1978. Volatilization of arsenic (III, V), antimony (III, V) and selenium (IV, VI) from mixtures of hydrogen fluoride and perchloric acid solution: application to silicate analysis. Anal. Chem. 50: 649—651.

BISBJERG, B. 1972. Studies on selenium in plants and soils. Risø Report 200. 150 p.

BRODIE, K. G. 1977. A comparative study: Determining arsenic and selenium by AAS. Amer. Lab. 11: 73—79.

CARY, E. E., WIECZOREK, G. A. & ALLAWAY, W. H. 1967. Reactions of selenite-selenium added to soils that produce low-selenium forages. Soil Sci. Soc. Amer. Proc. 31: 21—26.

— & ALLAWAY, W. H. 1969. The stability of different forms of selenium applied to low-selenium soils. Soil Sci. Soc. Amer. Proc. 33: 571—574.

— & GISSEL-NIELSEN, G. 1973. Effect of fertilizer anions on the solubility of native and applied selenium in soil. Soil Sci. Soc. Amer. Proc. 37: 590—593.

CLINTON, O. E. 1977. Determination of selenium in blood and plant material by hydride generation and atomic-absorption spectroscopy. Analyst 102: 187—192.

DOYLE, P. J. & FLETCHER, W. K. 1977. Influence of soil parent material on the selenium content of wheat from west-central Saskatchewan. Can. J. Plant. Sci. 57: 859—864.

ELONEN, P. 1971. Particle-size analysis of soil. Acta Agr. Fenn. 122: 1—122.

ELSOKKARY, I. H. 1980. Selenium distribution, chemical fractionation and adsorption in some Egyptian alluvial and lacustrine soils. Z. Pfl.ernähr. Bodenkunde 143: 74—83.

— & ØIEN, A. 1977. Determination of Se in soils. Acta Agric. Scand. 27: 285—288.

ERVIÖ, R. 1970. The importance of soil bulk density in soil testing. Ann. Agric. Fenn. 9: 278—286.

FLEMING, G. A. 1968. Cobalt, selenium and molybdenum in Irish soils. Rep. Welsh Soils Discuss. Group 9: 41—56.

— & WALSH, T. 1957. Selenium occurrence in certain Irish soils and its toxic effects on animals. Proc. R. I. Acad. 58, Sect. B: 151—166, plate XVII.

FRANK, R., STONEFIELD, K. I. & SUDA, P. 1979. Metals in agricultural soils of Ontario. II. Can. J. Soil Sci. 59: 99—103.

GEERING, H. R., CARY, E. E., JONES, L. H. P. & ALLAWAY, W. H. 1968. Solubility and redox criteria for the possible forms of selenium in soils. Soil Sci. Soc. Amer. Proc. 32: 35—40.

GISSEL-NIELSEN, G. 1971. Influence of pH and texture of the soil on plant uptake of added selenium. J. Agric. Food Chem. 19: 1165—1167.

— 1975. Selenium concentration in Danish forage crops. Acta Agric. Scand. 25: 216—220.

— 1976. Selenium in soils and plants. Proc. Symp. Selenium-tellurium in the environment. Notre Dame, May 11.—13. 1976: 10—25.

— & BISBJERG, B. 1970. The uptake of applied selenium by agricultural plants. 2. The utilization of various selenium compounds. Plant and Soil 32: 382—396.

— & HAMDY, A. A. 1977. Leaching of added selenium in soils low in native selenium. Z. Pfl.ernähr. Bodenkunde. 140: 193—198.

GOLDSCHMIDT, V. M. 1954. Geochemistry. 730 p. Oxford.

GOLEMBESKI, T. 1975. Determination of sub microgram amounts of selenium in rocks by atomic-absorption spectroscopy. Talanta 22: 547—549.

GUPTA, U. C. & WINTER, K. A. 1975. Selenium content of soils and crops and the effects of lime and sulfur on plant selenium. Can. J. Soil Sci. 55: 161—166.

HAMDY, A. A. & GISSEL-NIELSEN, G. 1976 a. Relationships between soil factors and selenium content of Danish soils and plants. Risø Report 349. 13 p.

— & GISSEL-NIELSEN, G. 1976 b. Fractionation of soil selenium. Z. Pfl.ernähr. Bodenkunde 139: 697—703.

— & GISSEL-NIELSEN G. 1977. Fixation of selenium by clay minerals and iron oxides. Z. Pfl.ernähr. Bodenkunde 140: 63—70.

JOHN, M. K., SAUNDERS, W. M. H. & WATKINSON, J. H. 1976. Selenium adsorption by New Zealand soils. I. Relative adsorption of selenite by representative soils and the relationship to soil properties. N. Z. J. Agric. Res. 19: 143—151.

KIM, J.-O. & KOHOUT, F. J. 1975. Multiple regression analysis: subprogram regression. Statistical package for the social sciences. p. 320—367. 2nd Ed. New York.

KOLJONEN, T. 1973 a. Selenium in certain igneous rocks. Bull. Geol. Soc. Finl. 45: 9—22.

— 1973 b. Selenium in certain metamorphic rocks. Bull. Geol. Soc. Finl. 45: 107—117.

— 1973 c. Selenium in certain sedimentary rocks. Bull. Geol. Soc. Finl. 45: 119—123.

— 1973 d. Determination of selenium in rocks, soils, and plants. Suomen Kemistilehti B 46: 133—138.

— 1974. Selenium in certain Finnish sediments. Bull. Geol. Soc. Finl. 46: 15—21.

— 1975. The behavior of selenium in Finnish soils. Ann. Agric. Fenn. 14: 240—247.

KRONBORG, O. J. & STEINNES, E. 1975. Simultaneous determination of arsenic and selenium in soil by neutron-activation analysis. Analyst 100: 835—837.

LAKIN, H. W. 1961. Geochemistry of selenium in relation to agriculture. USDA Agric. Handb. 200: 1—65.

LÉVESQUE, M. 1974 a. Selenium distribution in Canadian soil profiles. Can. J. Soil Sci. 54: 63—68.

— 1974 b. Some aspects of selenium relationships in Eastern Canadian soils and plants. Can. J. Soil Sci. 54: 205—214.

— & VENDETTE, E. D. 1971. Selenium determination in soil and plant materials. Can. J. Soil Sci. 51: 85—93.

LINDBERG, P. & BINGEFORS, S. 1970. Selenium levels of forages and soils in different regions of Sweden. Acta Agric. Scand. 20: 133—136.

MISRA, S. G. & TRIPATHI, N. 1972. Note on selenium status of surface soils. Indian J. Agric. Sci. 42: 182—183.

PATEL, C. A. & MEHTA, B. V. 1970. Selenium status of soils and common fodders in Gujarat. Indian J. Agric. Sci. 40: 389—399.

RAAD, A. T., PROTZ, R. & THOMAS, R. L. 1969. Determination of Na-dithionite and NH_4-oxalate extractable Fe, Al and Mn in soils by atomic absorption spectroscopy. Can. J. Soil Sci. 49: 89—94.

RAVIKOVITCH, S. & MARGOLIN, M. 1957. Selenium in soils and plants. Kvatim 7: 41—52.

REUTER, D. J. 1975. Selenium in soils and plants: a review in relation to selenium deficiency in South Australia. Agric. Rec. 2: 44—50.

ROBINSON, M. F. 1976. The moonstone: more about selenium. J. Hum. Nutr. 30: 79—91.

ROSENFELD, I. & BEATH, O. A. 1964. Selenium: geobotany, biochemistry, toxicity, and nutrition. 411 p. New York.

SIEMER, D. D. & HAGEMANN, L. 1975. An improved hydride

generation-atomic absorption apparatus for selenium determination. Anal. Lett. 8: 323—337.

SIMONEN, A. 1964. Kallioperä. Suomen geologia. p. 49—124. Ed. RANKAMA, K. 414 p. Helsinki.

SINGH, M. & KUMAR, P. 1976. Selenium distribution in soils of bio-climatic zones of Haryana. J. Indian Soc. Soil Sci. 24: 62—67.

SIPPOLA, J. 1979. Selenium content of soils and timothy (*Phleum Pratense* L.) in Finland. Ann. Agric. Fenn. 18: 182—187.

— 1982. A comparison between a dry-combustion method and a rapid wet-combustion method for determining soil organic carbon. Ann. Agric. Fenn. 21: 146—148.

— & TARES, T. 1978. The soluble content of mineral elements in cultivated Finnish soils. Acta Agric. Scand. Suppl. 20: 11—25.

STEINNES, E. 1977. A neutron activation method for the simultaneous determination of arsenic, mercury and selenium in soil. Acta Agric. Scand. 27: 110—112.

SWAINE, D. J. 1955. The trace element content of soils. Techn. Commun. Commonw. Bur. Soils 48: 1—157.

TAMM, O. 1922. Eine Methode zur Bestimmung der anorganischen Komponenten des Gelkomplexes im Boden. Statens Skogsförsöksanstalt, Medd. 19: 387—404.

TARES, T. & SIPPOLA, J. 1978. Changes in pH, in electrical conductivity and in the extractable amounts of mineral elements in soil, and the utilization and losses of the elements in some field experiments. Acta Agric. Scand. Suppl. 20: 90—113.

WELLS, N. 1967. Selenium in horizons of soil profiles. N. Z. J. Sci. 10: 142—179.

WORKMAN, S. M. & SOLTANPOUR, P. N. 1980. Importance of prereducing selenium(VI) to selenium(IV) and decomposing organic matter in soil extracts prior to determination of selenium using hydride generation. Soil Sci. Soc. Am. J. 44: 1331—1333.

XIAO-QUAN, S., LONG-ZHU, J. & ZHE-MING, N. 1982. Determination of selenium in soil digests by graphite furnace atomic absorption spectrometry after extraction with 1,2-diamino-4-nitrobenzene. Atomic Spectroscopy 3: 41—44.

YLÄRANTA, T. 1982. Volatilization and leaching of selenium added to soils. Ann. Agric. Fenn. 21: 103—113.

— 1983 a. The hydride method for measuring the selenium content of plants. Ann. Agric. Fenn. 22: 18—28.

— 1983 b. Sorption of selenite and selenate in the soil. Ann. Agric. Fenn. 22: 29—39.

Manuscript received February 1983

Toivo Yläranta
Agricultural Research Centre
Institute of Soil Science
SF - 31600 Jokioinen, Finland

SELOSTUS

Suomen viljelymaiden seleeni

TOIVO YLÄRANTA

Maatalouden tutkimuskeskus

Seleeniä esiintyy kaikkialla maankuoressa, mutta sen pitoisuus on vähäinen ja ylittää harvoin 100 mg/kg. Suomen kallioperässä yleisten magmakivien seleenipitoisuus on pieni, keskimäärin vain 0,09 mg/kg. Harvinaisemmat sedimenttikivet sisältävät seleeniä huomattavasti enemmän.

Maaperän ja saastumattomien viljelymaiden seleenipitoisuus on luonnollisesti samaa suuruusluokkaa kuin niiden kivilajien, joista maaperän pääaines koostuu. Tämän perusteella voidaan otaksua, että Suomen viljelymaissa on vähän seleeniä.

Viljelymaiden seleenipitoisuuden selvittämiseksi kerättiin maanäyteitä 112 paikasta linjan Oulu-Kajaani-Lieksa eteläpuolelta (Kuva 1). Maanäytteet otettiin viljelymaiden muokkauskerroksesta (noin 0—20 cm) ja samasta kohdasta muokkauskerroksen alapuolelta, noin 20—40 cm:n syvyydestä. Muokkauskerroksesta kertyi 112 näytettä ja syvemmästä maakerroksesta 93 näytettä.

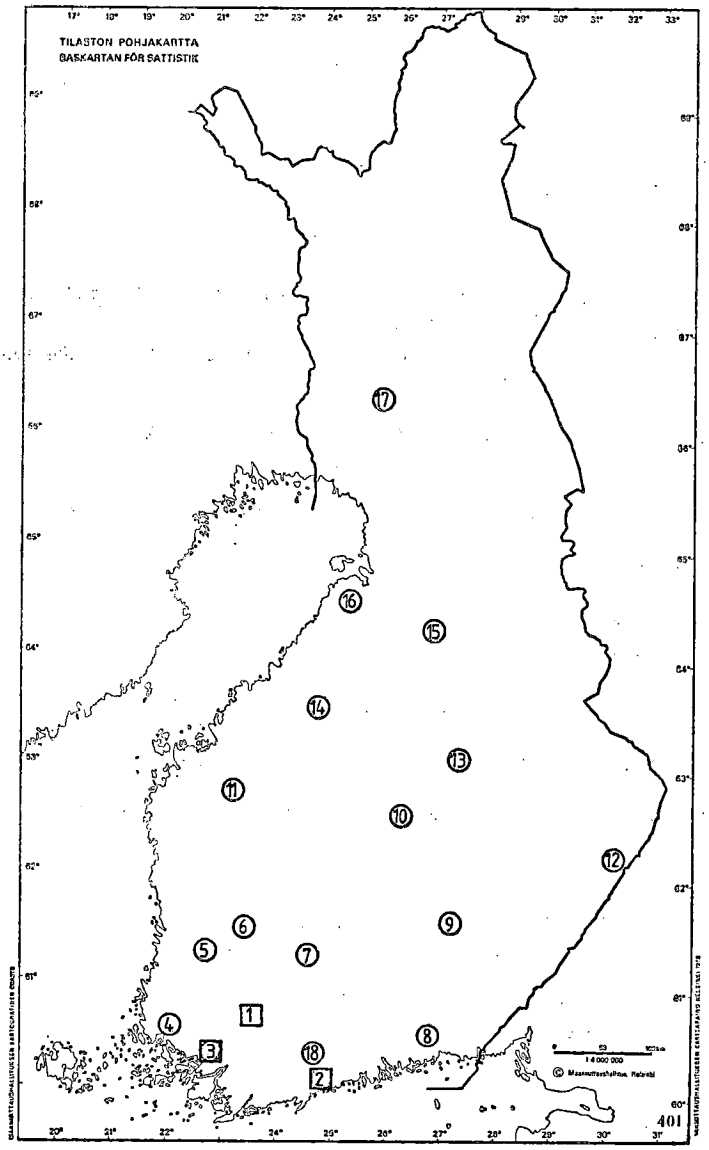
Muokkauskerroksen kivennäismaanäytteissä oli seleeniä 0,050—0,633 mg/kg kuivaa maata, keskiarvo 0,209 mg/kg. Karkeiden kivennäismaiden keskimääräinen seleenipitoisuus oli 0,172 mg/kg ja savimaiden 0,290 mg/kg. Suurimmat seleenipitoisuudet, jopa 1,28 mg/kg, analysoitiin eloperäisistä maista. Muokkauskerroksen ja syvemmän maakerroksen seleenipitoisuuksissa ei ollut suuria eroja.

Kun viljelymaiden seleenipitoisuus vaihtelee maapallolla yleisimmin 0,1 ja 2,0 mg/kg välillä, ei Suomen viljelymaista mitattuja seleenipitoisuuksia voida pitää poikkeuksellisen pieninä.

Seleenin vähäinen uuttuneisuus veteen, keskimäärin vain 4 % kokonaisseleenistä, on tyypillistä humidisille alueille. Sen sijaan semiaridisilla ja aridisilla alueilla saattaa vesiliukoista seleeniä olla kymmeniäkin prosentteja maiden kokonaisseleenistä.

Tutkimuksen perusteella näyttää seleeni ja sen fraktiot Suomen kivennäismaissa liittyvän kiinteimmin savekseen, orgaaniseen hiileen ja happamaan ammoniumoksalaattiliuokseen uuttuvaan alumiiniin.

CONTENTS



INSTITUTES, EXPERIMENTAL STATIONS AND BUREAUS OF THE AGRICULTURAL RESEARCH CENTRE

1. Administrative Bureau, Information, Library, Institutes of Soil Science, Agricultural Chemistry and Physics, Plant Husbandry, Plant Breeding, Animal Husbandry, Animal Breeding; Bureau for Local Experiments, Central Laboratory, Computing Service (JOKIOINEN) — 2. Institutes of Plant Pathology and Pest Investigation; Pesticide Regulation Unit (VANTAA) — 3. Institute of Horticulture (PIIKKIÖ) — 4. South-West Exp. Sta. (MIETOINEN) — 5. Satakunta Exp. Sta. (KOKEMÄKI) — 6. Sata-Häme Exp. Sta. (MOUHIJÄRVI) — 7. Häme Exp. Sta. (PÄLKÄNE) — 8. Kymenlaakso Exp. Sta. (ANJALA) — 9. South Savo Exp. Sta. (MIKKELI) — 10. Central Finland Exp. Sta. (LAUKAA) — 11. South-Pohjanmaa Exp. Sta. (YLISTARO) — 12. Karelia Exp. Sta. (TOHMAJÄRVI) — 13. North Savo Exp. Sta. (MAANINKA) — 14. Central Pohjanmaa Exp. Sta. (TOHOLAMPI) — 15. Kainuu Exp. Sta. (VAALA) — 16. North Pohjanmaa Exp. Sta. (RUUKKI) — 17. Lapland Exp. Sta. (ROVANIEMI) — 18. Swine Research Exp. Sta. (HYVINKÄÄ).

SISÄLTÖ — CONTENTS

Helsinki 1983. Government Printing Centre

ISSN 0570-1538