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## EFFECT OF THE PLACEMENT TECHNIQUE AND AMOUNT OF FERTILIZER ON SPRING WHEAT AND BARLEY GROWN ON CLAY SOILS I. EFFECT ON GRAIN YIELD

MARTTI ESALA and GÖTHE LARPES

ESALA M. & LARPES G. 1986. Effect of the placement technique and amount of fertilizer on spring wheat and barley grown on clay soils. I. Effect on grain yield. *Ann. Agric. Fenn.* 25: 159—167. (Agric. Res. Centre, Dept. Agric. Chem. Phys., SF-31600 Jokioinen, Finland.)

A series of 12-year field experiments were conducted to compare the effect of the placement and broadcasting techniques on 250, 500, 750 and 1000 kg/ha dressings of compound fertilizer (20-4-8) on yields of spring wheat and barley. The experiments also included an unfertilized treatment. The experiments were carried out on sandy clay and clay loam in southern Finland.

Compared to broadcasting, placement of these amounts of fertilizer resulted in yield increases of 630, 680, 440 and 260 kg/ha i.e. 21,9, 17,8, 9,8 and 5,3 % for barley, and respectively 360, 360, 260 and 150 kg/ha, i.e. 13,5, 10,6, 6,6 and 3,7 % for spring wheat. Higher yield increases were obtained by fertilizer placement on sandy clay than on clay loam. Yield increases were highest using nitrogen applications of 50 and 100 kg per hectare.

Barley made better use of the increased fertilizer applications than spring wheat. By increased fertilizer dressings the yields were higher on sandy clay than on clay loam.

Higher yield increases obtained by fertilizer placement on sandy clay correlated with a high precipitation deficiency in early summer.

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Index words: fertilizer placement, fertilizer broadcasting, fertilizer dressing, clay soils, wheat, barley.

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### INTRODUCTION

During a dry summer fertilizer broadcasted onto the soil surface remains outside the scope of the plant's roots (KAILA and HÄNNINEN 1961, NIEMINEN et al. 1967, KÄHÄRI and ELONEN 1969, KAILA and ELONEN 1971, ELONEN 1979). In order to solve this problem the method and machinery for placing fertilizer in bands a few centimeters below and to the

side of the seed rows were developed in Finland in the 1960's. The method has been shown to lead to better growth and nutrient uptake when the first part of the summer is dry, because the nutrients placed into the moist soil are better available to the plant roots (AURA 1967, KAILA and ELONEN 1970, LYGSTAD 1977, LINDEMAN 1978). Nowadays, almost all of the

machines sold for fertilization and seeding in Finland are combine drills or drills suitable for fertilizer placement.

Fertilizer placement has been shown to lead almost without exception, to better growth and development of cereals, to higher yields, and better yield quality. These profitable effects have been observed to be mostly caused by a faster and better uptake of nitrogen by the crop (NIEMINEN et al. 1967, AURA 1967, KIVI 1969, KAILA and ELONEN 1970, PESSI et al. 1970, LYGSTAD 1977, ELONEN 1981).

In addition the uptake of phosphorus and potassium by plants has been observed to increase when these nutrients are placed, although not in amounts as high as in the

uptake of nitrogen. Placement of phosphorus has resulted in yield increases only when the plant available phosphorus status of the soil has been low. Placement of potassium has not been found to bring about yield increases (DUNCAN and OHLROGGE 1957, MILLER and OHLROGGE 1957, AURA 1967, PESSI et al. 1970, MCLEOD et al. 1975).

Because the placement of fertilizers has been shown to affect the need for a fertilizer rate, a series of experiments was arranged during 1969—80 by Agricultural Research Centre of Finland to compare the broadcasting and band placement techniques on spring cereals using different amounts of fertilizer.

## MATERIAL AND METHODS

### Field experiments

The experiments were carried out in Tikkurila, located near Helsinki on the southern coast of Finland.

Broadcasting and placement of fertilizer were compared using four levels of fertilizer: 250, 500, 750, 1000 kg per hectare respectively of compound fertilizer (20-4-8). The experiments also included an unfertilized plot. The amounts of nutrients (kg/ha) contained in the different levels of compound fertilizer were as follows:

Compound fertilizer	N	P	K
250	50	11	21
500	100	22	42
750	150	33	62
1000	200	44	83

The crops studied in the experiments were spring wheat (varieties: 1969 Svenno, 1970 Veka and 1971—80 Tähti) and spring barley (1969 Karri, 1970—80 Pomo).

The experiments were conducted on sandy clay and clay loam. Texture, organic matter content, pH and nutritional status of the soils are presented in Table 1. Soil pH and exchangeable calcium content were higher in sandy clay soil than in the sandy clays of southern Finland on average (KURKI 1982). In clay loam soil these values were slightly lower than in clay loams of southern Finland on average. The exchangeable potassium contents were lower and readily soluble phosphorus contents were higher than in corresponding soils in southern Finland on average.

There were two replicates of spring wheat and two replicates of barley on both soils. The experimental method employed was split-plot with fertilizer amounts in the main plots and fertilization methods in the sub-plots. The amount of fertilizer and fertilization method treatment remained the same on the same plot throughout the experimental period. The crop species were exchanged yearly.

The fertilizer was broadcasted after preliminary harrowing and before final seed-bed

Table 1. Soil texture, organic matter content and nutritional status of the experimental areas.

Percentage of	Sandy clay	Clay loam
clay (< 0,002 mm)	38	37
silt (0,002 - 0,02 "	20	28
fine sand (0,02 - 0,2 "	36	30
coarse sand (0,2 - 2 "	6	5
Content of organic matter, %	5,2	5,6
Soil pH (water)	6,5	5,6
Amount of nutrients soluble in acid (pH 4,65) ammonium acetate		
Ca, mg/l	3300	1850
K "	150	230
P "	18,2	12,4

preparation with an S-tooth harrow. Broadcasting was done by a Juko fertilizer drill driving the coulters up. The fertilizer was band placed before seeding using the same machine. The distance of the fertilizer rows was 15,5 cm and the depth of placement about 8 cm. The seed was sown towards the fertilizer rows with a Juko sowing drill. No difference has been found between separate and combined fertilizer and seed drilling in earlier experiments unless separate drilling causes a worse seed-bed (LARPES 1970, LARPES et al. 1970, EKEBERG 1977). In addition, the direction of the seed rows when compared with that of the fertilizer rows has not been shown to influence crop growth and yield.

The other cultivation operations were carried out according to the modern cultivation techniques as much as possible. The combine harvested yields were weighed fresh and calculated to a 15 % moisture content according to a grain moisture content determination at 105 °C. The straw was removed from the plots. In autumn 1971 the experimental areas were limed with two tons of limestone per hectare.

### Weather conditions

Weather observations were made from a climatic station situated 300—400 meters from the experimental area. The mean temperature during May—August of 1969—79 was 0,3 °C

higher than that during the normal period 1931—60 (Table 2). The summers of 1974, 1976, 1977 and 1978 were considerably cooler than the summers in the normal period and the summers of 1970, 1972, 1973 and 1975 were correspondingly warmer than those of the normal period.

Precipitation deficiency was calculated as the difference between the potential evapotranspiration and precipitation. The potential evapotranspiration was calculated from the class-A-pan evaporation observations by the following formula of VAKKILAINEN (1982, p. 54—56):

$$PET = 0,05 + 0,18 \ln t,$$

where PET = potential evapotranspiration

t = time in days calculated from 1st of May so that for example, the 1st of May = 1 and the 3rd of June = 34

VAKKILAINEN (1982) found this formula to be most suitable for calculating potential evapotranspiration from class-A-pan evaporation observations in southern Finland. The climatic station was discontinued in autumn 1979, thus the data for 1980 is missing.

According to these observations, the former part of the experimental period, i.e. 1969, 1970, 1971, 1973 and 1975 was drier, while 1972, 1974 and the final years of the experimental period, i.e. 1976—79 were more humid than the normal period.

Statistical analyses were carried out using a VAX-11/780 computer and SPSS software (NIE et al. 1975, JENKINS 1981).

Table 2. The monthly mean temperatures and precipitation deficiencies from May—August in Tikkurila and the corresponding temperature values from the normal period 1931—60. The temperature observations from summer 1980 are from the Seutula observation station about 8 km from the experimental fields (ANON. 1969—79, ANON. 1980).

	Mean temperature °C					Precipitation deficiency mm				
	V	VI	VII	VIII	V—VIII	V	VI	VII	VIII	V—VIII
1969	8,7	15,6	16,5	16,1	14,2	23	110	54	91	278
1970	9,5	16,7	16,4	15,4	14,5	40	128	—6	66	228
1971	10,5	14,1	17,0	15,5	14,3	70	98	123	27	318
1972	9,3	16,5	20,0	16,6	15,6	14	61	45	—98	22
1973	10,2	17,0	20,1	15,0	15,6	23	109	151	73	356
1974	7,2	14,6	15,9	14,7	13,1	17	50	15	6	88
1975	11,7	13,6	17,8	16,3	14,9	15	99	109	57	280
1976	10,8	13,0	15,6	14,8	13,6	49	48	31	45	173
1977	9,6	14,2	14,6	14,4	13,2	24	50	—49	40	65
1978	10,5	14,7	15,6	13,7	13,6	63	64	25	—60	92
1979	10,7	16,0	14,9	16,1	14,4	38	64	—23	29	108
1980	7,0	16,8	16,4	15,8	14,0	—	—	—	—	—
1969—										
1979	9,9	15,1	16,8	15,3	14,3	34	80	43	25	182
1931—										
1960	9,3	14,3	17,0	15,4	14,0	—	—	—	—	—

## RESULTS

The highest fertilizer application resulted in the highest yields for both spring wheat and barley on both soil types.

Fertilizer placement caused yield increases every year. The greatest yield increases, 16 % for spring wheat and 31 % for barley, were obtained as a mean of both soil types and all fertilizer amounts in 1970. The smallest increases, less than 1 % for both plant species, were obtained in 1976.

The yield increases as averages of the 12 years were as follows:

Amount of fertilizer (20-4-8) kg/ha	Yield increases by fertilizer placement					
	spring wheat		barley		average	
	kg/ha	%	kg/ha	%	kg/ha	%
250	360	13,5	630	21,9	500	17,8
500	360	10,6	680	17,8	520	14,4
750	260	6,6	440	9,8	350	8,3
1000	150	3,7	260	5,3	200	4,6

The effect of fertilizer placement was best at a low (250 kg/ha) or a normal (500 kg/ha) fertilization level. Barley was able to profit

more from fertilizer placement and increased amounts of fertilizer than spring wheat (Figs. 1 and 2). By the nitrogen fertilization level of 100 kg per hectare which corresponds to that practiced in southern Finland, the placement technique increased barley yields by 680 kg/ha, on average. Yield increases varied from 90 to 1350 kg/ha. The corresponding yield increases for wheat varied from —10 to 1000 kg/ha with an average of 360 kg/ha.

A regression function of the 2nd stage was found to best describe the influence of fertilization on yield. The regression functions calculated for the compound fertilizer amounts of 250—1000 kg per hectare are expressed in Figures 1 and 2.

The analyses of variance calculated from the whole data showed the difference between the two fertilization methods to be statistically significant ( $P < 0,001$  %) on sandy clay and nonsignificant on clay loam for both plants. Fertilizer placement as well as an increased amount of fertilizer resulted in higher yield increases on sandy clay than on clay loam.

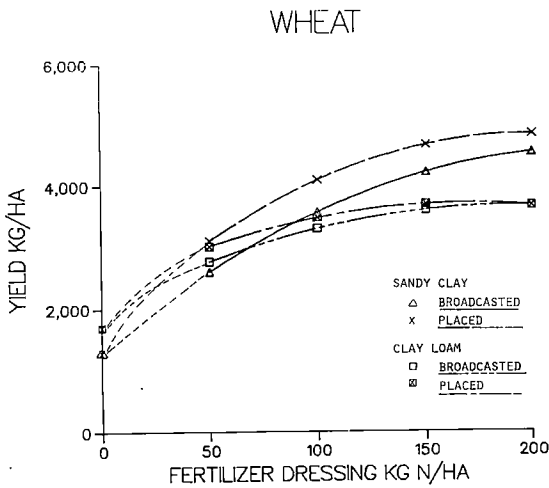


Fig. 1. The effect of fertilizer placement and broadcasting on the yield of spring wheat.

**SANDY CLAY**

broadcasted:  $y = -0,0636N^2 + 28,86N + 1326$   $R^2 = 0,53^{***}$   
 placed:  $y = -0,0824N^2 + 32,25N + 1697$   $R^2 = 0,47^{***}$

**CLAY LOAM**

broadcasted:  $y = -0,0474N^2 + 17,81N + 2001$   $R^2 = 0,10^{***}$   
 placed:  $y = -0,0500N^2 + 16,83N + 2301$   $R^2 = 0,06^{***}$

The fertilization levels, where the placement effect was the best, were calculated from the yield function curves (Figs. 1 and 2) by subtracting and derivating. The results were as follows (sandy clay soil):

	wheat	barley
Nitrogen fertilization level where the placement effect was the best, kg/ha	90	89
Corresponding increase in grain yield obtained by placement technique, kg/ha	520	900

The yield increases achieved by a larger amount of fertilizer diminished faster by placement than by broadcasting. According to the field observations, this was caused by

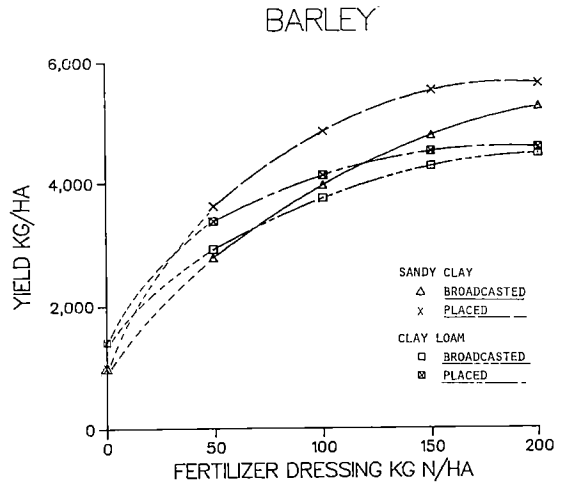


Fig. 2. The effect of fertilizer placement and broadcasting on the yield of barley.

**SANDY CLAY**

broadcasted:  $y = -0,0703N^2 + 34,09N + 1260$   $R^2 = 0,46^{***}$   
 placed:  $y = -0,1120N^2 + 41,52N + 1825$   $R^2 = 0,42^{***}$

**CLAY LOAM**

broadcasted:  $y = -0,0620N^2 + 25,96N + 1778$   $R^2 = 0,16^{***}$   
 placed:  $y = -0,0690N^2 + 25,29N + 2286$   $R^2 = 0,12^{***}$

improved growth and more lodging during certain years as a result of better utilization of nitrogen on plots where the fertilizer was placed.

The yield increases obtained by fertilizer placement on sandy clay were dependent on the moisture conditions in early summer (Figs. 3 and 4). The precipitation and precipitation deficiency of May, June, July, August in different combinations, as well as of one and two months after sowing were plotted against the yield increases obtained by fertilizer placement. The precipitation deficiency of May + June explained the highest portion of the yield increases. The r squares were  $0,77^{***}$  for wheat and  $0,67^{**}$  for barley. The average data of sowing in the experiments was the 13th of May.

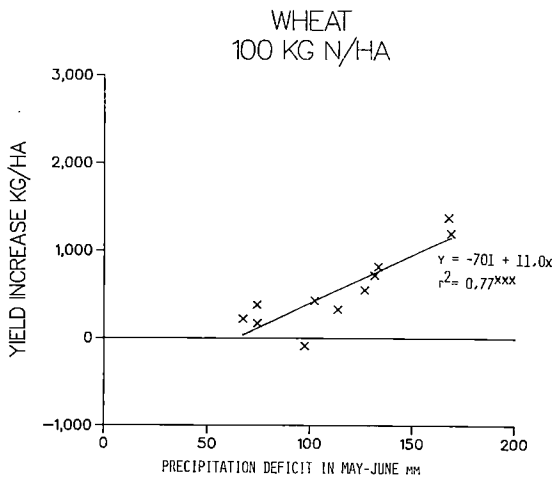


Fig. 3. The effect of precipitation deficiency in May—June on the yield increases obtained by placement of 100 kg/ha nitrogen as compound fertilizer (20-4-8) on sandy clay in spring wheat.

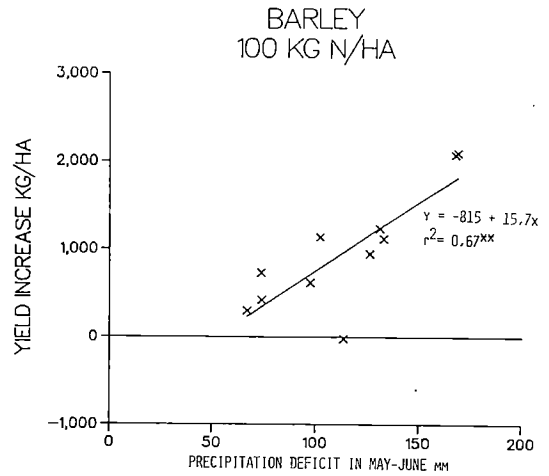


Fig. 4. The effect of precipitation deficiency in May—June on the yield increases obtained by placement of 100 kg/ha nitrogen as compound fertilizer (20-4-8) on sandy clay in barley.

## DISCUSSION

The results of this series of experiments confirm the positive results obtained with fertilizer placement from experiments carried out in Finland during the 1960's. Yield increases achieved by fertilizer placement in Swedish and Norwegian experiments have generally been smaller.

The yield increases achieved with fertilizer placement in a 10-year series of experiments carried out near Helsinki at the 1960's were on average 415 kg/ha, i.e. 16,1 % with nitrogen applications from 30 to 100 kg/ha as compound fertilizers (8-5-7 and 15-8-12). The relative yield increases for barley were of the same magnitude as those for spring wheat, but the absolute yield increases were higher for barley than for spring wheat (ELONEN et al. 1967, NIEMINEN et al. 1967, KARA et al. 1970, ELONEN and KARA 1972). Similar results have been achieved in other experiments conducted near Helsinki in the 1960's. Yield increases achieved by LARPES (1968) with spring wheat on clay soil

during 1965—67 were 17 % on average using a nitrogen application of 80 kg/ha. In one series of experiments in southwestern Finland, the yield increases obtained by fertilizer placement for spring wheat in 1967 were 380 kg/ha as an average of four experiments, and in 1968 they were 310 kg/ha as an average of seven experiments (KÖYLIJÄRVI 1969). The yield increases obtained by KIVI and HOVINEN (1969) with spring wheat in 1967 and 1968 were 8—15 % in the experiments carried out in middle Finland and 3—8 % in southern Finland. 400 and 800 kg per hectare compound fertilizer (8-5-7) was used in these experiments.

In Sweden the yield increases obtained by fertilizer placement in spring cereals have averaged 8—10 % in the eastern and northern parts of the country and 4—5 % in the southern Sweden (HUHTAPALO 1981). The 141 experiments carried out in southern Norway resulted in yield increases of 140 kg/ha, i.e. 4 % on average when the amount of



fertilizer nitrogen applied varied from 40 to 120 kg/ha (LYNGSTAD 1977). The yield increases from 86 experiments carried out on clay soils were 180 kg/ha on average.

KARA et al. (1970) obtained yield increases twice as high with fertilizer placement compared to broadcasting when the amount of fertilizer nitrogen was increased from 30 to 80 kg/ha as compound fertilizer (8-5-7). The results of LARPES (1968) from 1965 and 1967 were similar. The yield increases in the series of experiments discussed in this publication remained almost constant with the corresponding amounts of fertilizer.

These experiments were carried out at only one research station, which limits adaptation of the results to larger areas. However, on sandy clay soil a positive correlation was found between the deficiency in precipitation and the yield increases obtained by fertilizer placement. The precipitation deficiency of May—June was 114 mm as an average of the first 11 experimental years. Statistics for a longer period are available from Jokioinen weather observation station located about 100 km northwest of Tikkurila. At this station the average precipitation deficiency in May—June during 1962—83 was 92,8 mm (ANSALEHTO et al. 1984, p. 30) suggesting a yield increase of 320 kg/ha for wheat and 640 kg/ha for barley on the same soil type with a nitrogen dressing of 100 kg/ha. In Norway LYNGSTAD (1977) observed a negative correlation between yield increases obtained by fertilizer placement and precipitation during the growing season.

The biological optimum for fertilization approached the highest amount of compound fertilizer applied in these experiments, i.e. 1000 kg per hectare (200 kg N/ha). In practice, however, a reasonable application is usually

lower because lodging, which causes yield decreases and difficulties in combining, is less on experimental plots than on normal fields.

The yield increases obtained by the placement of fertilizer compared to those of broadcasting probably were mostly the result of better efficiency in the use of nitrogen by the crops as the soils had good P and K status.

Yield increases obtained by placement compared to broadcasting were greatest employing the nitrogen fertilizer amounts of between 50 to 100 kg/ha, i.e. the application commonly in use in Finland.

The placement of fertilizer resulted in greater yield increases on sandy clay than on clay loam soil compared to broadcasting. The inferior water economy of the crops due to the poorer structure of clay loam probably limited growth on this soil. This prevented the plants from making full use of the better availability of the nutrients placed. The field observations showed a good establishment and early growth of the crops grown on clay loam. However, if the latter part of summer was dry, this led to higher evapotranspiration and lack of water at the grain filling period. In the same situation the crops receiving broadcasted fertilization had smaller canopies leading to a better ability to conserve water because of smaller amounts of evapotranspiration.

Spring wheat was able to use the natural nutrient resources of the unfertilized plots better than barley. This resulted on average in 370 kg/ha higher yields of spring wheat than barley on these plots. Barley, on the other hand, made use of the higher fertilizer applications and better availability of the placed nutrients better than spring wheat did, responding with higher yields on these treatments.

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# SELOSTUS

## Eri ravinnemäärien sijoitus kevätvehnälle ja ohralle savimailla I. Vaikutus satoon

MARTTI ESALA ja GÖTHE LARPES

Maatalouden tutkimuskeskus

Maatalouden tutkimuskeskuksen maanviljelyskemian ja -fysiikan osastolla järjestettiin vuosina 1969—80 kenttäkoesarja, jonka tarkoituksena oli selvittää sijoituslannoituksen vaikutusta lannoituksen tarpeeseen. Kokeissa verrattiin sijoituslannoitusta ja pintalannoitusta keskenään lannoitemäärillä 250, 500, 750 ja 1000 kg typpirikasta Y-lannosta hehtaarille. Lisäksi kokeissa oli lannoittamaton koejäsen. Koekasveina olivat ohra ja kevätvehnä. Kokeet järjestettiin hietasavella ja hiesavella ja ne jatkuivat samoilla paikoilla yhtämittaisesti 12 vuotta.

Sijoituslannoitus tuotti pintalannoitukseen verrattuna huomattavan suuria sadonlisäyksiä, jotka riippuivat lannoitustasosta seuraavasti: ohralla sadonlisäys oli lannoitemäärillä 250, 500, 750 ja 1000 kg Ytr/ha vastaavasti 630, 680, 440 ja 260 kg/ha eli 21,9, 17,8, 9,8 ja 5,3 %. Kevätvehnän sadonlisäys oli vastaavilla lannoitemäärillä 360, 360, 260 ja 150 kg/ha eli 13,5, 10,6, 6,6 ja 3,7 %. Sijoituslannoituksen tuottamat sadonlisäykset olivat hietasavella suurempia kuin hiesavella. Ilmeisesti hiesaven huonompi rakenne aiheutti sen, että kasvit hyötyivät vähemmän lannoitteen sijoittami-

sesta. Lannoitteen sijoittamisen hyöty oli suurin typpilannoitemäärillä 50 ja 100 kg/ha. Sijoituslannoituksella saadut sadonlisäykset olivat samaa suuruusluokkaa kuin aikaisemmissa Etelä-Suomessa tehdyissä kokeissa. Ruotsalaisissa ja norjalaisissa kokeissa on ilmeisesti kosteammasta alkukesästä johtuen saatu sijoituslannoituksella pienempiä sadonlisäyksiä kuin suomalaisissa tutkimuksissa.

Suurimmalla käytetyllä lannoitemäärällä saatiin sekä kevätvehnästä että ohrasta molemmilla lannoitustavoilla suurin sato. Lannoitemäärän lisäämisen tuottamat sadonlisäykset olivat hietasavella suurempia kuin hiesavella.

Touko—elokuun sademäärän ja sadannan vajuksen vaikutusta 100 kg/ha typpitasolla sijoituksella hietasavella saatuun sadonlisäykseen tutkittiin regressioanalyysillä kuukausittain sekä eri kuukausien yhdistelminä. Vastaavasti tutkittiin myös näiden tekijöiden vaikutusta laskettuna yhden ja kahden kuukauden kuluttua kylvöstä. Touko—kesäkuun sadannan vajuus selitti lannoitteen sijoituksella saatuja sadonlisäyksiä parhaiten. Saponlisäys oli sitä suurempi, mitä kuivempi oli alkukesä.

EFFECT OF THE PLACEMENT TECHNIQUE AND AMOUNT OF FERTILIZER  
ON SPRING WHEAT AND BARLEY GROWN ON CLAY SOILS  
II. EFFECT ON THE QUALITY AND MINERAL CONTENTS OF GRAIN YIELD

MARTTI ESALA and GÖTHE LARPES

ESALA M. & LARPES G. 1986. Effect of the placement technique and amount of fertilizer on spring wheat and barley grown on clay soils. II. Effect on the quality and mineral contents of grain yield. *Ann. Agric. Fenn.* 25: 169—175. (Agric. Res. Centre, Dept. Agric. Chem. Phys., SF-31600 Jokioinen, Finland.)

The effect of the placement and broadcasting of 250, 500, 750 and 1000 kg/ha of compound fertilizer (20-4-8) on the mineral contents and quality of grain yields of spring wheat and barley was investigated in a 12-year series of experiments. The experiments also included an unfertilized treatment. The experiments were carried out on sandy clay and clay loam.

Grain moisture content was lowest with applications of 50—100 kg/ha nitrogen. Fertilizer placement resulted in lower grain moisture contents than broadcasting as a result of a more even and faster maturation of the crops.

Neither the fertilizer application level nor the method of application employed had any effect on the hectoliter weight of the crops. The method of application had no effect on grain size, but an increased fertilizer level resulted in an increase in grain size. Grain protein content was higher the higher the fertilizer application. The application method had no statistically significant influence on grain protein content.

The application method had no effect on the mineral contents of the grain yield. The increase in fertilizer amount resulted in higher potassium and manganese contents in spring wheat and higher copper, zinc, manganese and boron contents in barley. The zinc content in spring wheat and the calcium content in barley was lowest with the application of 250 kg/ha fertilizer.

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Index words: fertilizer placement, fertilizer broadcasting, fertilizer dressing, clay soils, wheat, barley, yield quality, mineral contents in grain.

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## INTRODUCTION

The method and machinery for placing fertilizer for cereals in bands a few centimeters below and to the side of seed rows was developed in Finland in the 1960's, and has been widely accepted by Finnish farmers.

The beneficial effects of the placement of

fertilizer have been shown to be especially due to the better uptake of nitrogen by the crop (NIEMINEN et al. 1967, AURA 1967, KIVI 1969, KAILA and ELONEN 1970, PESSI et al. 1970, LYGSTAD 1977, ELONEN 1981).

In addition, the uptake of phosphorus and

potassium has been found to increase when these nutrients are placed. However, yield increases have been found only by phosphorus in the situations when the soil phosphorus status has been low (DUNCAN and OHLROGGE 1957, MILLER and OHLROGGE 1957, AURA 1967, PESSI et al. 1970, MCLEOD et al. 1975).

A series of experiments was carried out during 1969—80 at the Finnish Agricultural Re-

search Centre to compare the effect of the broadcasting and band placement techniques on spring cereals using different amounts of fertilizer. The yield results of this series were reported in an earlier paper by ESALA and LARPES (1986). The present paper deals with the yield quality and mineral contents of the grain in the above experiments.

## MATERIAL AND METHODS

Broadcasting and placement were compared using four amounts of compound fertilizer (20-4-8): 250, 500, 750, 1000 kg per hectare. The experiments also included an unfertilized plot. The crops studied in the experiments were spring wheat and barley. The experiments were carried out on sandy clay and clay loam. A detailed description of the experiments including soil properties is presented elsewhere (ESALA and LARPES 1986).

A 1 kg sample was obtained from the yield of each plot after harvesting. Another sample was collected for grain moisture content determinations. Hectoliter weight, 1000-grain weight and nitrogen content were determined from dried sample every year. P, K, Ca, Mg, Cu, Fe, Zn, Mn and B contents were analysed

from barley yields during 1974—80. The same elements were determined from wheat yields in 1975 and 1977—80.

The nitrogen content of the grain yield was determined by the Kjeldahl-method. For P, K, Ca, Mg, Cu, Fe, Mn and Zn determinations the samples were first ashed and the ash was dissolved in HCl. P was determined in the extractate by the vanadate method and the other above mentioned elements were determined by atomic absorption spectrometry. Boron was determined by the azomethine-H method (SIPPOLA and ERVIÖ 1977).

Statistical analyses were carried out using a VAX-11/780 computer and SPSS software (NIE et al. 1975).

## RESULTS

### Yield quality

Grain moisture content is, of course, dependent on harvest time. As the crops of the whole experiment were harvested at the same time, the differences in grain moisture content can be considered to represent differences in grain maturity between the plots (Fig. 1).

The differences in the grain moisture

contents between the plots were greater for barley than for wheat. The grain moisture content of barley was lower in the plot dressed with 50 or 100 kg N/ha than in the other plots. The placement of fertilizer resulted in lower grain moisture contents than that by broadcasting. In barley dressed with 50 kg N/ha the difference was 4,5 percentages in favor of fertilizer placement. According to field obser-

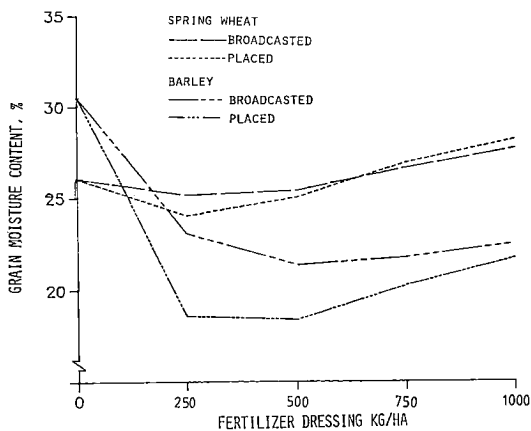


Fig. 1. Effect of application method on the grain moisture content of spring wheat and barley.

variations this difference in grain maturity resulted from late tillering of the plots where fertilizer was broadcasted. The difference between the dressing methods was statistically significant ( $P < 0,001$  %) when the nitrogen dressing was 50 or 100 kg/ha and insignificant with a nitrogen dressing of 150 or 200 kg/ha.

The differences between the treatments in the grain moisture content of spring wheat were smaller than for those of barley. Fertilizer nitrogen amounts greater than 100 kg/ha resulted in later ripening. The differences between the dressing methods were statistically insignificant whereas the influence of the amount of fertilizer was statistically significant ( $P < 0,01$  %).

Neither fertilizer amount nor dressing method had any statistically significant effect on the hectoliter weight of the crops (Fig. 2). However, the hectoliter weight of barley was about 4 kilograms lower in the unfertilized treatments than by nitrogen fertilization with 50 and 100 kg/ha.

The 1000-grain weight of spring wheat increased linearly from 29,8 to 36,0 grams when the dressing was increased from 0 to 1000 kg of compound fertilizer per hectare (Fig. 3). The 1000-grain weight of barley increased respect-

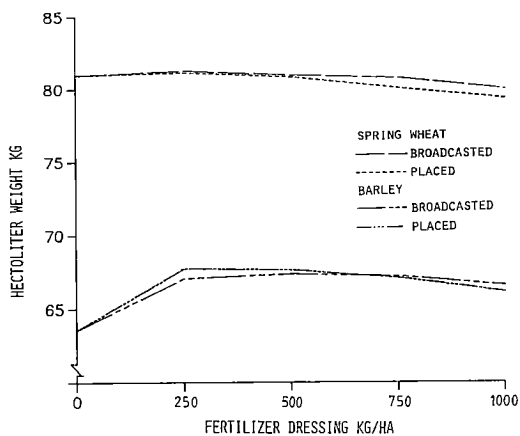


Fig. 2. Effect of application method on the hectoliter weight of spring wheat and barley.

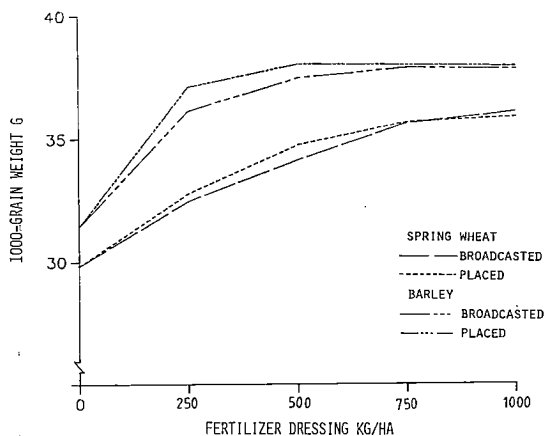


Fig. 3. Effect of application method on the 1000-grain weight of spring wheat and barley.

ively from 31,4 to 37,9 grams. However, fertilizer applied in amounts greater than 500 kg/ha had no effect on 1000-grain weight of barley. The effect of fertilizer amount on the 1000-grain weight of both crops was statistically significant ( $P < 0,001$  %). The 1000-grain weight was slightly higher by placement than by the broadcast technique, however, the differences between the two methods were not statistically significant.

Grain protein content was calculated by multiplying the nitrogen content of wheat by a

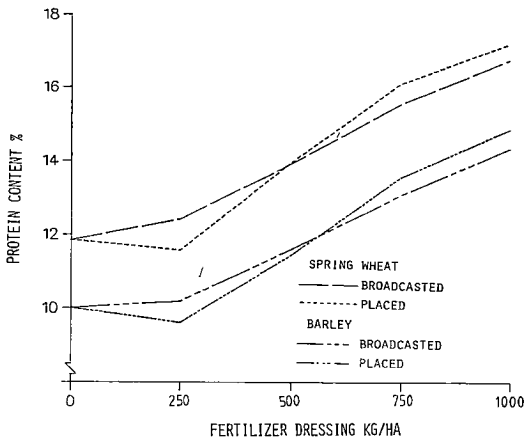


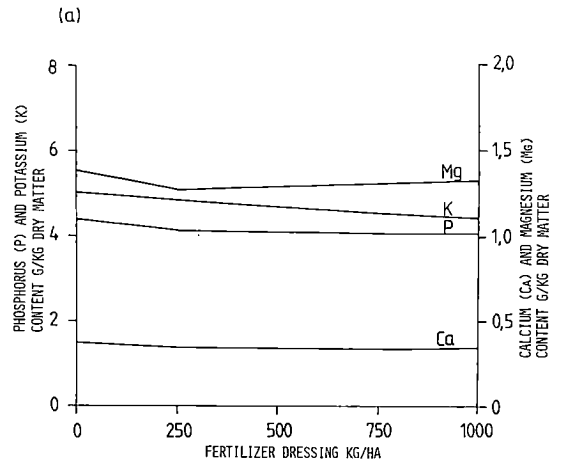
Fig. 4. Effect of application method on the protein content of spring wheat and barley.

factor of 5,7 and that of barley by a factor of 6,25. The protein content of both crops increased clearly as a function of fertilizer amount (Fig. 4) and this influence was significant ( $P < 0,001$ ). With a nitrogen dressing of 50 kg/ha the protein content of spring wheat was 0,8 percentages lower and that of barley 0,6 percentages lower when fertilizer was placed compared broadcasting. However, with nitrogen dressings of 150 and 200 kg/ha placement resulted in 0,5—0,6 percentages higher protein contents compared to the broadcasting method. These differences were, however, statistically insignificant.

### Mineral contents of the grain

The mineral contents of the grain yield were determined for barley during 1974—80 and for spring wheat in 1975 and during 1977—80.

The method of application had no clear statistically significant effect on the mineral contents of the grain yield, nor did the amount of fertilizer generally affect the phosphorus, potassium, calcium or magnesium contents of the grain (Fig. 5). However, the decrease in the potassium content of spring wheat grain was



(a)

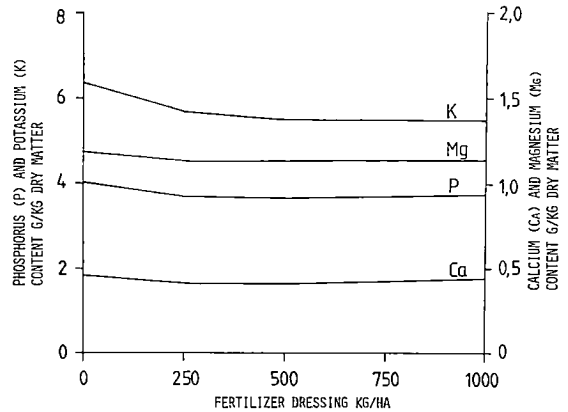


Fig. 5. Effect of amount of fertilizer on the contents of phosphorus, potassium, calcium and magnesium in the seeds of spring wheat (a) and barley (b).

significant ( $P < 0,001$ ). Likewise the calcium content of barley was significantly affected ( $P < 0,01$ ), being lowest for the crops dressed with 250 kg compound fertilizer per hectare and slightly higher in the unfertilized crops and at the higher fertilizer levels. In addition the potassium content of unfertilized barley grain was 0,6—0,7 g/kg dry matter higher than of the grain from the fertilized plots, however, this effect was not statistically significant.

The general effect of fertilizer amount on the trace element contents of Cu, Fe, Zn, Mn

and B of spring wheat was not significantly affected with the exception of zinc and manganese contents (Table 1). The zinc content of spring wheat was 37,7 mg/kg dry matter in the unfertilized plots and declined to 32,4 mg/kg with a dressing of 250 kg compound fertilizer per hectare. When the amount of fertilizer was increased the zinc content rose linearly to 39,3 mg/kg in the plots fertilized with 1000 kg compound fertilizer per hectare.

The manganese content of spring wheat increased linearly from 27,9 mg/kg in the unfertilized plots to 38,8 mg/kg in the plots dressed with 1000 kg compound fertilizer per hectare. These effects were statistically significant ( $P < 0,001$ ). Higher levels of fertilizer increased the copper, zinc, manganese and boron contents of barley statistically significantly ( $P < 0,001$ ).

Table 1. Effect of the amount of fertilizer on the contents of copper, iron, zinc, manganese and boron in the grain of spring wheat and barley in mg/kg dry matter.

Amount of fertilizer (20-4-8) kg/ha	Spring wheat					Barley				
	Cu	Fe	Zn	Mn	B	Cu	Fe	Zn	Mn	B
0	6,48	36,8	37,7	27,9	1,56	7,65	33,6	25,4	9,9	1,47
250	5,74	34,0	32,4	28,7	1,57	7,31	30,6	25,1	10,0	1,44
500	5,48	36,1	34,7	32,3	1,62	7,96	31,8	28,5	11,8	1,45
750	5,35	37,9	36,9	34,9	1,67	8,50	32,8	31,5	13,2	1,55
1000	5,62	39,2	39,3	38,8	1,73	8,74	32,7	34,4	14,9	1,66
P	0,05	0,05	0,01	0,01	0,05	0,001	0,05	0,001	0,001	0,001

## DISCUSSION

In earlier Finnish studies grain moisture content at harvest has been 1—3 percentages lower in plots where fertilizer was placed compared to broadcasting (LARPES 1966, ELONEN et al. 1967, NIEMINEN et al. 1967, KARA et al. 1970). These investigators reported the same results with spring wheat and barley. The results discussed in the present paper were of the same magnitude (1,1 percentages) for spring wheat but higher (4,5 percentages) for barley.

Grain size and volume weight has not been shown in the present study nor in former Finnish or Nordic experiments to be affected by fertilizer placement compared to broadcasting (NIEMINEN et al. 1967, ELONEN et al. 1967, KIVI and HOVINEN 1969, KARA et al. 1970, LYGSTAD 1977).

The protein content of the grain was not affected significantly by the application method

in the present series of experiments. However, fertilizer placement did result in a slight decrease when 50 kg/ha nitrogen was applied, and slightly increased at higher application levels. In the experiments of NIEMINEN et al. (1967) and KARA et al. (1970) a statistically significant ( $P < 0,05$ ) decrease of 1,6 percentages in the grain protein content of spring wheat was observed when fertilizer was placed compared to broadcasting. The decrease in the protein content of barley was 0,5 percentages and that of oats 0,3 percentages respectively, but these effects were statistically insignificant. Moreover, in the above-mentioned experiments increased amounts of fertilizer applied resulted in a compensation of the decrease in grain protein content at the lower fertilizer application level.

The protein content of the grain was slightly



lower, although not significantly lower at the fertilizer application level of 50 kg/ha nitrogen. The crops probably utilized the nitrogen fertilizer for developing as good a yield potential as possible. Later the crops had less nitrogen for protein synthesis in relation to their yield potential than at the other fertilizer levels. At the higher fertilizer applications the crops probably still had more nitrogen for achieving an elevated protein content even in the higher-yielding plots where fertilizer was placed compared to broadcasting. As a result of both a higher yield and a higher protein content, the protein yield rose by a factor of five when the amount of nitrogen application was increased from 0 to 200 kg/ha.

Mineral element contents have not been analyzed in former Nordic experiments investigating the placement and broadcasting of fertilizer. For example, PESSI et al. (1974) studied the effect of fertilizer dressing on the mineral element content of grain. The mineral contents of the grain reported from these two investigations are of the same order of magnitude although there were some differences in the methods of analysis employed.

PESSI et al. (1974) did not find any influence due to fertilizer rate on the mineral content of spring wheat and barley grown on clay soils, except that the zinc content decreased with increasing amounts of fertilizer. However, the potassium, zinc and manganese contents of the grain of spring wheat and the calcium, copper, zinc, manganese and boron contents of barley grain were found to be significantly affected by the amount of fertilizer in the present experiments. Except for the potassium content of spring wheat, the contents of these mineral elements increased with an increasing amount of fertilizer.

It could be expected that the mineral element contents of grain would decrease with higher yields obtained by increased applications of fertilizer. This did not occur, however. The mineral element contents remained at constant levels with increased applications and, on the contrary, the contents of some mineral elements were even higher with increasing fertilizer application. Higher yields thus take up more of these elements from the soil and accordingly accelerates the removal of these elements from the soil.

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## SELOSTUS

### Eri ravinnemäärien sijoitus kevätvehnälle ja ohralle savimailla II. Vaikutus sadon laatuun ja kivennäisainepitoisuuteen

MARTTI ESALA ja GÖTHE LARPES

Maatalouden tutkimuskeskus

Maatalouden tutkimuskeskuksen maanviljelyskemian ja -fyysiikan osastolla järjestettiin vuosina 1969—80 kenttäkoesarja, jonka tarkoituksena oli selvittää sijoituslannoituksen vaikutusta lannoituksen tarpeeseen. Kokeissa verrattiin sijoituslannoitusta ja pintalannoitusta keskenään lannoitemäärillä 250, 500, 750 ja 1000 kg typpirikasta Y-lannosta hehtaarille. Lisäksi kokeissa oli lannoittamaton koejäsen. Koekasveina olivat ohra ja kevätvehnä. Kokeet järjestettiin hietasavella ja hiesavella.

Puintikosteus oli alhaisin 50—100 kg N/ha käytettäessä. Sijoituslannoitetut kasvut tueltuivat nopeammin ja tasaisemmin, mistä oli seurauksena niiden alhaisempi puintikosteus kuin pintalannoitetuissa kasvustoissa.

Hehtolitrain painoon ei lannoitemäärä eikä lannoitustapa vaikuttanut. Lannoitustavalla ei ollut vaikutusta myöskään siementen kokoon, mutta lannoituksen lisääminen johti

tuhannen siemenen painon suurenemiseen.

Siementen valkuaispitoisuus kohosi lannoitusta lisättäessä. Lannoitustavalla ei ollut selvää, tilastollisesti merkitsevää vaikutusta siementen valkuaisainepitoisuuteen, vaikka pitoisuus oli sijoituslannoitusta käytettäessä alhaisella lannoitustasolla vähän alempi ja korkeammilla lannoitustasoilla vähän korkeampi kuin pintalannoitusta käytettäessä.

Lannoitustapa ei vaikuttanut siementen kivennäisainepitoisuuksiin. Lannoitusta lisättäessä kohosivat kevätvehnän siementen kalium ja mangaanipitoisuudet sekä ohran siementen kupari-, sinkki-, mangaani- ja booripitoisuudet tilastollisesti vähintään merkitsevästi. Kevätvehnän siementen sinkkipitoisuus ja ohran siementen kalsiumpitoisuus oli alhaisin 250 kg Ytr/ha käytettäessä. Muiden kivennäisainepitoisuuksiin siemenissä ei lannoitemäärällä ollut tilastollisesti merkitsevää vaikutusta.

## MARGARINE, BUTTER, HONEY AND VEGETABLE OILS AS SOURCES OF ORGANOCHLORINE COMPOUNDS IN THE FINNISH DIET

RAIJA MOILANEN, JORMA KUMPULAINEN and HEIKKI PYYSALO

MOILANEN, R., KUMPULAINEN, J. & PYYSALO, H. 1986. Margarine, butter, honey and vegetable oils as sources of organochlorine compounds in the Finnish diet. Ann. Agric. Fenn. 25: 177—185. (Agric. Res. Centre, Centr. Lab., SF-31600 Jokioinen, Finland.)

Residue levels of neutral organochlorine compounds were analysed in 13 samples of turnip rape (*Brassica campestris*) seed samples and 10 rape (*Brassica napus*) seed samples collected from different areas of Southern Finland during 1978—1984. Organochlorine compounds were also analysed in sunflower and soy oils used in addition to turnip rape oil by the margarine industry. Samples of margarines, butter, honey and beeswax were also analysed. Residues of DDT-, PCB-, hexachlorobenzene- (HCB), hexachlorocyclohexane- (HCH), heptachlor- and its epoxide-, chlordanes-, toxaphene-, mirex-, kepone-, aldrine-, and dieldrine-compounds were determined by GLC-mass spectrometry employing the selected ion monitoring (SIM) technique. Only low residues of DDT-, PCB-, HCB-, HCH- and heptachlor-compounds were detected and none of the estimated daily intakes of these compounds exceeded 1 % of the acceptable daily intake (ADI) limits set by FAO/WHO. Residues of the other compounds were below the limit of detection.

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Index words: organochlorine compounds, rape, turnip rape, butter, vegetable oils, margarine, honey, beeswax, Finnish diet.

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## INTRODUCTION

The use of organochlorine compounds in Finland is relatively small in scale (TIITTANEN 1983). Use of PCB-compounds is practically nonexistent and DDT-compounds were banned in Finland, as in most countries, in the early 1970's.

Technical grade hexachlorocyclohexane (HCH) is composed of a mixture of stereoisomers (alpha-, beta-, gamma- and delta-HCH). Technical HCH and preparations containing about 99 % gamma-HCH (lindane)

have been widely employed in agricultural and veterinary practice. Lindane is used in Finland as an insecticide for cruciferous plants, such as rape and turnip rape. Its annual consumption with endosulphane (1,9 tons/year) is lower than that of most other European countries (TIITTANEN 1983).

Heptachlor, a component of technical chlordane mixtures, is used by the plywood industry as an insecticide and fungicide in preparing plywood for export to tropical countries, and

the annual Finnish consumption of heptachlor is approximately 60 tons. As only a small part of this may be lost in processing or in the burning of waste materials heptachlor cannot be regarded as a pesticide commonly used in agriculture.

Although most of the compounds in question have been banned in Finland, they are used as insecticides elsewhere including the Eastern European countries. Via long distance transport residues of these compounds can be found in areas where they have never been employed (JANSSON et al. 1979, WICKSTRÖM et al. 1981, MOILANEN et al. 1982, PYYSALO et al. 1981, 1983, HELLE et al. 1983).

Due to the lipophilic nature of most neutral organochlorine compounds, they enter into the food chain by accumulating into fats such as vegetable oils and the tissue fats of marine and terrestrial animals. Being on the top of the food chain humans are very heavily exposed to these compounds and relatively high concentrations have been found in human milk (VUORI et al. 1977, WICKSTRÖM et al. 1983) and in human adipose tissue (MUSSALO-RAUHAMAA et al. 1984). It is therefore important to monitor the contents of organochlorine compounds in fats used for human consumption in order to estimate the intake trends of these compounds.

In Finland the cultivated area of rape and turnip rape in 1983 was 61 000 ha. The 1983 harvest approximated 106 million kg and consisted mostly of turnip rape (93 %). Rape and turnip rape are used as feed for domestic animals because of their high protein content (about 38 %) and energy value (fat content 40—45 %). Sixty to seventy percent of oil-based animal feed concentrates have been imported to Finland in recent years.

Rape and turnip rape are the main flowers for honey collection in many areas, thus it is of interest to determine how pesticide residues in those plants accumulate in honey. Very few

reports are available on residues of organochlorine compounds in honey (DZILINSKI 1975) nor has honey been studied earlier in Finland.

The margarine and oil industry also use rape and turnip rape oils; the margarine industry uses about 26 % of total rape oil and about 30 % of total imported soy and sunflower oils. The raw materials used in the manufacture of Finnish margarine are presented in Table 1.

Table 1. Raw materials used by the Finnish margarine industry.

Raw material	Quantity t	%
Rape and turnip rape oil	7975	26,2
Soy and sunflower oil	9233	30,3
Cocoa	1333	4,4
Other vegetable fats and oils	2375	7,8
Fish oil	2262	7,4
Tallow and lard	7264	23,9
Total	30442	100,0

According to National Food Balance Sheets 41,5 thousand tons of vegetable oils, mostly corn and sunflower oils, were imported in 1983 and 28,5 thousand tons of vegetable oils other than margarine or butter-margarine mixture were used for human consumption representing 16,1 g/d per person.

The residues of organochlorine compounds in butter, vegetable oils and fats have been studied in many countries (KALRA et al. 1983, FAO/WHO 1982, NORE'N et al. 1982). TYLLINEN et al. 1975 have reported levels of PCB and DDT in Finnish butter in 1975. There are no recent estimates available, however, on the average levels of intake of organochlorine residues from vegetable oils, butter, or margarines in Finland.

The object of this work was to investigate the residue levels of organochlorine compounds in samples of rape and turnip rape seeds, honey, beeswax, vegetable oil, margarine and butter, and thus determine the average daily intake of organochlorine compounds by the Finnish population from these food sources.

## MATERIAL AND METHODS

### Sample collection

Rape and turnip rape seeds representing the most important cultivation areas in Southern Finland were collected. Rape samples were collected from Jokioinen yearly during 1978—1984, from Anjala, Mietoinen and Tikkurila in 1979, from Tikkurila in 1980, Anjala in 1982 and Mietoinen in 1984. Turnip rape samples were collected from Jokioinen yearly during 1978—1984 and from Tikkurila, Mietoinen and Anjala in 1980. Altogether thirteen individual turnip rape and then rape samples of 1 kg each representing four sub-samples collected from a 15 m<sup>2</sup> area (to be used primarily for official cultivar selection experiments during 1978—1984) were analysed.

Imported sunflower and soy oil samples were both raw and purified oils used as raw materials by the oil and margarine industry. Samples of these oils were also purchased from a local Jokioinen retail store. Sunflower oil was imported from Hungary. Soy oil was extracted and purified by a Finnish Company (Raisio Ltd) from soybeans imported from the USA. The oil was separated from the soybeans by pressing at 85 °C and the residual oil extracted from the seed into hexane.

Ten butter samples were prepared from milk collected during mid-August and mid-December 1984 at two-week intervals. Margarine samples were bought from retail stores in 1984. Seven honey samples and five beeswax samples were collected in the summer of 1983.

### Preparation of samples

Vegetable oil, margarine, butter, honey and beeswax samples were extracted and cleaned up by the method of VEIEROV and AHARONSON (1980) employing acetone-hexane-diethyl

ether extraction followed by purification with concentrated sulphuric acid. Fat content was determined by the method of AOAC (HORWITZ 1980). Rape and turnip rape seed samples were ground thoroughly before extraction. Extraction of 10 g of the seed samples was performed by the soxhlet method for six hours with 100 ml of acetone:n-hexane:diethyl ether:petrole ether (5,5 : 2,5 : 1 : 9). Fat was then collected and weighed. Cleanup was by sulphuric acid as for vegetable oils, margarines and butter.

### Chromatographic analysis

Analyses of concentrated extracts were carried out by GLC-MS employing the selected ion monitoring technique using a Hewlett Packard 5970 gas chromatograph-mass spectrometer equipped with an OV-101 fused silica column (25 m, i.d. 0,32 mm). The GLC oven was temperature programmed from 120 °C to 275 °C, 16 °C per minute.

PCB-concentrations were determined as the sum of the ten most prominent signals ( $m/e = 326, 360$ ) and expressed as Clophen A 60 standard mixture. DDT was expressed as the sum of  $p,p'$ -DDT,  $p,p'$ -DDE and  $p,p'$ -DDD ( $m/e = 237$  and  $248$ ). Other monitoring ions were 272, 274, 237 and 270 for heptachlor, mirex and kepone, 351 for heptachlor epoxide, 181, 183, 219, 109 for alpha-, beta-, gamma- and delta HCH, 263 for aldrin, dieldrin and chlordanes and 307, 341, 377, 413 for toxaphenes. Retention times of the identified compounds were identical to those of reference compounds kindly supplied by the US Environmental Protection Agency. 2,4,6-trichlorophenyl was used as an internal standard. Recovery was 85—100 % for all the compounds studied.

## RESULTS AND DISCUSSION

The results for rape and turnip rape seed samples are presented in Table 2. The time trends of organochlorine compounds in rape and turnip rape seeds during 1978—1984 are presented in Figure 1.

Chlordanes, toxaphenes, mirex, kepone, aldrine, dieldrine and heptachlor epoxide were not detected in any of the samples. The detection limit of these compounds was 1  $\mu\text{g}/\text{kg}$ .

Gamma-HCH was the main HCH residue in rape and turnip rape seeds. In some samples low beta-HCH residues (below 20 % of the total HCH content) were also found.

In terms of residue levels no significant differences between the sample collection sites of rape and turnip rape were found. Residues of DDT and HCH seem to have decreased during 1978—1984. A slight decreasing trend was also seen in PCB and lindane residues, but not as clearly as that for DDT and HCB residues.

Only four honey samples contained PCB residues (0,1—0,2  $\text{mg}/\text{kg}$ ). No other residues of organochlorine compounds were found in honey and beeswax samples, which indicates that the organochlorine pesticide residues in rape or turnip rape do not accumulate in honey or beeswax.

The effect of purification is quite evident when comparing the higher residues in raw oils with those of purified oils (Table 3). The residues of HCB were quite low in all of the samples analysed. In soy and sunflower oils, butter and margarines gamma-HCH was the main HCH component.

Table 2. Organochlorine compounds in rape and turnip rape seed samples during 1978—1984 ( $\mu\text{g}/\text{kg}$  fat).

Compound	Mean	SD	Minimum	Maximum	N
$\Sigma$ PCB	4,83	1,99	2,0	9,0	23
$\Sigma$ DDT	0,61	0,85	0,0	3,0	23
Heptachlor	1,47	1,44	0,0	4,2	23
HCB	1,52	1,03	0,5	4,8	23
$\gamma$ -HCH	11,41	5,04	1,5	24,0	23

A comparison of 1984 results of rape and turnip rape seeds with results of imported raw soy and sunflower oils indicates that residues of DDT, lindane and heptachlor are lower in rape and turnip rape on the basis of fat content. Residues of PCB and HCB are slightly higher in rape and turnip rape seeds, than in soy and sunflower oil. Lindane and heptachlor residues are higher in sunflower oil than in soy oil.

Residues are slightly lower in margarines than in butter (Table 4). The relatively high concentration of PCB in margarines compared with PCB levels in vegetable oils may have accumulated from animal fat which is also a component of margarine.

The residues of PCB- and DDT-compounds in butter found in this study are of the same levels as those reported in an earlier Finnish study (TYLLINEN et al. 1975). Results on organochlorine compounds in butter from other countries (Table 5) indicate considerable differences in pesticide concentrations between countries. The highest mean level of DDT (4770  $\mu\text{g}/\text{kg}$ ) has been reported from India

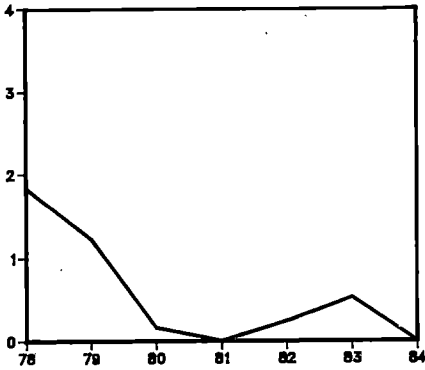
Table 3. Mean residues of organochlorine compounds ( $\mu\text{g}/\text{kg}$ ) in soy and sunflower oils.

Sample	$\Sigma$ PCB	$\Sigma$ DDT	HCB	$\gamma$ -HCH	Heptachlor
Sunflower oils:					
bottled	0,8	0,2	0,2	8,0	5,0
purified	1,4	3,0	0,4	12,0	6,0
raw	3,5	10,2	0,6	20,0	8,0
Soy oils:					
bottled	0,8	0,2	0,0	2,0	1,0
purified	1,3	2,1	0,5	2,0	1,2
raw	3,0	8,1	0,8	14,0	3,2

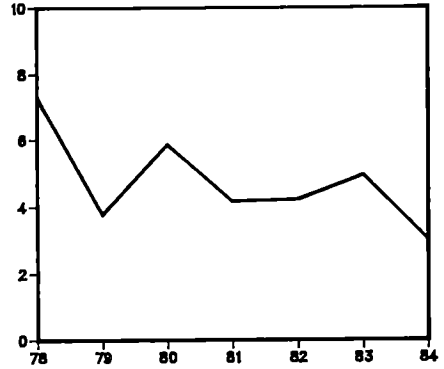
Table 4. Mean residues of organochlorine compounds ( $\mu\text{g}/\text{kg}$ ) in Finnish butter and margarines.

Sample	$\Sigma$ PCB	$\Sigma$ DDT	HCB	$\gamma$ -HCH	Heptachlor
Butter	64,3	1,0	9,7	15,1	5,2
Margarines	55,1	0,0	0,8	5,7	5,0

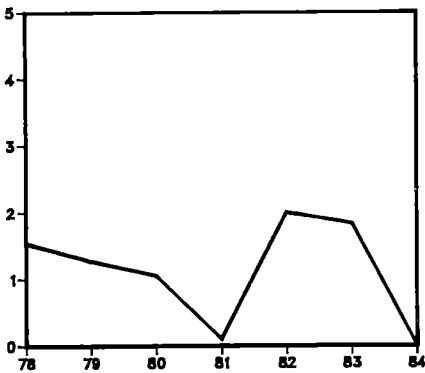
RESIDUES OF DDT DURING 1978–1984



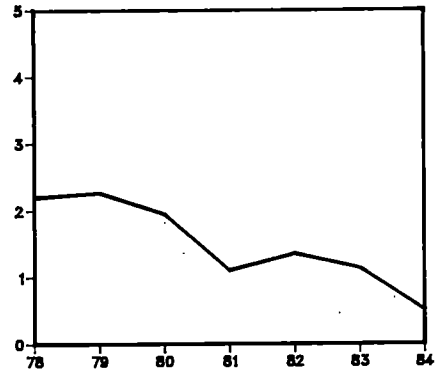
RESIDUES OF PCB DURING 1978–1984



RESIDUES OF HEPTACHLOR DURING 1978–1984



RESIDUES OF HCB DURING 1978–1984



RESIDUES OF LINDANE DURING 1978–1984

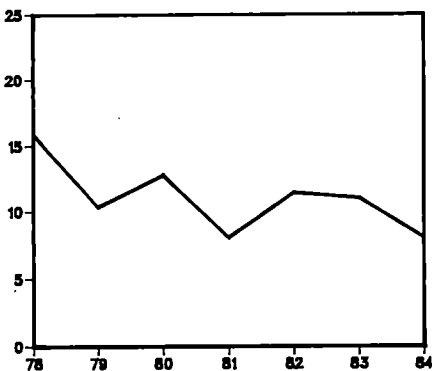


Fig. 1. Time trends of organochlorine compounds in rape and turnip rape seeds during 1978–1984. Concentrations are expressed as µg/kg fat.

whereas the lowest reported level (1 µg/kg) is that of the present study. Pesticide residues in Finnish butter are generally low, while all the other countries reported higher DDT and HCH levels in butter. Only Swedish investigators reported slightly lower HCB levels than those of Finnish butter. In several cases PCB was not reported. If not separated in the analytical procedure, PCB can interfere with the determination of certain other pesticides. The levels of PCB were slightly higher in Finnish butter (64 µg/kg) than in Danish butter (50 µg/kg). Heptachlor or its epoxide and toxaphene were not reported in many cases. The level of heptachlor in Finnish butter was lower than that of Swiss (6 µg/kg), Italian (60 µg/kg) or Japanese butter (15–24 µg/kg) (ANON. 1982).

Table 5. Mean levels of organochlorine compounds in butter from various countries,  $\mu\text{g}/\text{kg}$ .

Country	year	PCB	DDT	HCB	HCH	Reference
Italy	1973		478		53	CAMPANINI et al. 1980
Ireland	1971—72		50		21	DOMNEY et al. 1975
UK	1972—76				40—70	WHO/FAO 1982
India	1977		4770			DHALIWAL & KALRA 1978
India	1978—81		3310		1890	KALRA et al. 1983
Guatemala	1979		600			WHO/FAO 1982
Iran	1976—77		120		110	HASHEMY-TONKABONY & ASSADI-LANGAROODI 1979
France	1975		51	50	230	MATHIEU et al. 1977
France	1976		30	34	230	MATHIEU et al. 1977
Switzerland	1971—77			18—40	53—85	WHO/FAO 1982
Germany, Federal Rep.	1977				22	WHO/FAO 1982
Denmark	1972—78	30—80		20—30	20—40	WHO/FAO 1982
Denmark	1974—75	50	30	50	20	VOLDUM-CLAUSEN 1977
Denmark	1975—76	50	30	50	20	VOLDUM-CLAUSEN 1977
Sweden	1971—73	40	40	18	59	NOREN et al. 1982
Sweden	1975—76	30	17	8	28	NOREN et al. 1982
Finland	1972		65			TYLLINEN et al. 1975
Finland	1973—74		76			TYLLINEN et al. 1975
Finland	1984	64	1	9.7	15	Present study

Table 6. Residue limits and ADI for butter

Compound	ADI (FAO/WHO) mg/kg body weight	Residue limits mg/kg fat	
		FAO/WHO	USA
$\Sigma$ DDT	0,0050	1,25	
$\gamma$ -HCH (Lindane)	0,0100	0,1	
heptachlor and its epoxide	0,0005	0,01	
aldrine and dieldrine	0,0001	0,15	
HCB	0,0006	0,5	
$\Sigma$ PCB			2,5
$\Sigma$ HCH			0,3

Feed concentrates may be the main source of pesticide contamination in butter; approximately 20 % of feed concentrates have been imported to Finland in recent years. Part of the PCB and DDT found in butter may also originate from atmospheric long-distance transport.

Limits for residues in dairy products recommended by the FAO/WHO Expert Committee on Pesticide Residues are presented in Table 6 (ANON. 1978, 1979). These limits do not include total HCH isomers and PCB. In the USA, administrative guideline limits have been established for HCH and PCB in dairy products (DUGGAN 1978). Acceptable daily

intakes (ADI) for humans are also presented in Table 6. Comparison of the organochlorine compound levels in Finnish butter and margarines with the residue concentration limits indicate much lower contents in the products studied.

The calculated average daily intakes of organochlorine compounds from butter, margarines and vegetable oils for a Finnish man weighing 70 kg is presented in Table 7. The calculated average daily intakes of all the studied organochlorine compounds from these sources are lower than 1 % of the ADI values of these compounds, respectively.



Table 7. The average consumption of butter, margarines and vegetable oils, their concentrations of organochlorine compounds and their estimated intakes in Finland.

	Butter	Margarines	Vegetable oils	Total	per kg body wt.	FAO/WHO ADI ( $\mu\text{g}/\text{kg}$ )	Intake /ADI (%)
<i>Consumption</i>							
— total (g/d)	30,1	19,5	16,1				
— fat (g/d)	26,5	16,9	16,1				
<i>Concentrations</i> ( $\mu\text{g}/\text{kg}$ )							
$\Sigma$ PCB	64,3	55,1	0,8				
$\Sigma$ DDT	1,0	0,0	0,2				
$\Sigma$ HCH	15,0	5,7	5,0				
HCB	9,7	0,8	0,1				
Heptachlor	5,2	5,0	3,0				
<i>Intakes</i> ( $\mu\text{g}/\text{d}/70$ kg)							
$\Sigma$ PCB	1,94	1,07	0,013	3,023	0,043		
$\Sigma$ DDT	0,03	0,00	0,003	0,033	0,001	5,0	0,002
$\Sigma$ HCH	0,45	0,11	0,081	0,641	0,009	10,0	0,090
HCB	0,29	0,02	0,002	0,308	0,004	0,6	0,600
Heptachlor	0,16	0,10	0,048	0,306	0,004	0,5	0,800

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## SELOSTUS

### Margariini, voi, hunaja ja kasviöljyt organoklooriyhdisteiden lähteinä suomalaisessa ravinnossa.

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Maatalouden tutkimuskeskus ja Valtion teknillinen tutkimuskeskus

Tutkimuksen tarkoituksena oli selvittää kalarasvoja lukuunottamatta elintarvikekasvojen eräiden raaka-aineiden sekä valmiiden tuotteiden ja hunajan neutraalien organoklooriyhdisteiden jäämäpitoisuuksia ja arvioida mainittujen elintarvikkeiden merkitystä organoklooriyhdisteiden lähteinä suomalaisessa ravinnossa.

Neutraalien organoklooriyhdisteiden jäämäpitoisuudet määritettiin rapsi- ja rypsinäytteistä, jotka oli kerätty eri puolilta Etelä-Suomea vuosina 1978—1984. Organokloorijäämäpitoisuudet määritettiin lisäksi auringonkukka- ja soijaöljyistä, joita käytetään rapsiöljyn lisäksi margariinien valmistuksessa raaka-aineena. Jäämäpitoisuudet määritettiin myös margariini-, voi-, hunaja- ja mehiläisvahanäytteistä.

DDT- ja PCB-, heksaklooribentseeni-, heksakloorisykloheksaani-, heptakloori- ja sen epoksidi-, klordaani-,

toksafeeni-, mireksi-, keponi-, aldiini- ja dieldriiniyhdisteet määritettiin massaspektrometrisesti käyttäen valittujen ionien monitorointitekniikkaa.

Tulokset osoittivat, että kotimaisen rypsi- ja rapsiöljyn organokloorijäämäpitoisuudet ovat vähäisiä ja keskimäärin laskeneet ajan funktiona aikana 1978—1984. Suomalaisen voinin organokloorijäämäpitoisuudet osoittautuivat kirjallisuusvertailussa muiden kuin PCB:n osalta huomattavasti pienemmiksi kuin muissa maissa tuotetun voinin vastaavat pitoisuudet. Kaikkien tutkittujen näytetyyppien keskimääräiset jäämäpitoisuudet olivat vähäisiä eikä minkään tutkitun yhdisteen keskimääräinen saanti tutkituista elintarvikerasvoista yhteensä ylittänyt 1 % FAO/WHO:n suurimmas-  
ta sallitusta päiväsaannista.

THE FUNGI ON WINTERED BRANCHES OF OUTDOOR  
ROSES IN FINLAND

KAIHO MÄKELÄ

MÄKELÄ, K. 1986. The fungi on wintered branches of outdoor roses in Finland. Ann. Agric. Fenn. 25: 187—197. (Agr. Res. Centre, Dept. Pl. Path. SF-31600 Jokioinen, Finland.)

The material investigated comprised a total of 143 samples representing 56 varieties of roses from three localities in southern Finland. Samples were taken from dead branches removed during spring pruning.

The fungi were grown by the moist chamber method and identified microscopically.

Roses weakened by severe winters were found to be susceptible to destructive diseases. The sample material included some 15 potential pathogens of canker and dieback in roses, the most common being *Botrytis cinerea*, found in 89 % of the samples. The destructive pathogens of stem canker, *Coniothyrium fuckelii* occurred in 48 %, and its perfect stage *Leptosphaeria coniothyrium* in some 3 % of the samples. *Gnomonia rubi* was found sporadically. The occurrence of *Alternaria* spp., *Cryptosporium minimum*, *Cylindrocarpon* spp., *Fusarium* spp. and *Phoma* spp. was fairly common (20—40 %).

On the other hand *Hainesia lythri*, *Seimatosporium lichenicola* and *Truncatella angustata* were rather rare (8—13 %). *Coniella fragariae*, *Diplodia rosarum* and *Phomopsis* sp. occurred sporadically. These pathogens were of little importance in causing disease in roses.

Some 70 species and genera of fungi were identified in the samples the majority being saprophytes, which are common everywhere. Rare fungi, occurring in less than 2 % of the samples, accounted for some 45 % of the fungi.

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Index words: canker and dieback of outdoor roses, *Coniothyrium fuckelii*, *Gnomonia rubi*, *Botrytis cinerea*.

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## INTRODUCTION

Roses are valued ornamental plants in parks and gardens in Finland. Rose plants are imported to Finland in varying numbers annually, for example between 880 to 1 462 million plants were imported between 1975 and 1979 (METSOLA 1981). The selection of varieties is wide; wide-ranging 140 different

rose varieties were imported in 1974 (METSOLA 1976).

The long and often severe winter poses the most serious threat to outdoor roses. At the Agricultural Research Centre's Department of Horticulture in Piikkiö three overwintering tests with roses were carried out during 1972—

1979. Seventy-seven percent the roses survived the first winter, 49 % the second and 31 % the third winter. During the exceptionally severe winter of 1978 all varieties including some 600 bushes, were almost completely destroyed: only 1,2 % of the plants survived (KALLIO 1980).

Winter damage to roses is aggravated by various fungal injuries (GROVE 1937, SCHNEIDER et al. 1967, GORLENKO 1969, WITTMAN 1982). The most common and destructive of these fungi are the species *Coniothyrium*, *C. funkelii* and *C. wernsdorffiae*, the perfect stage of *Leptosphaeria coniothyrium* (WATERMAN 1930, GREEN 1934, WESTCOTT 1934, BAKKER 1946, SCHMIDT 1954, PROTSENKO 1959, ITRAMA 1968, GORLENKO 1969, IIDA et al. 1980, PUNITHALINGAM 1980). Other less common pathogens include *Gnomonia rubi* (DOWSON 1924, RAMSBOTTOM 1925, SCHNEIDER et al. 1969), *Botrytis cinerea* (DEACON

1934, GLASER et al. 1981), *Alternaria* sp. (GLASER et al. 1981), *Coniella fragariae* (SUTTON 1980), *Cryptosporium minimum* (GRIEVE 1932, GROVE 1937, CONNERS 1954), *Cylindrocladium scoparium* (RAMSBOTTOM 1925, MILLER 1954, STOREY 1964, DOMSCH et al. 1980), *Diplodia rosarum* (GROVE 1937, MILLER 1954), *Hainesia lythri* (SHEAR and DODGE 1921, GROVE 1937, SUTTON 1980), *Phomopsis* spp. (GROVE 1935, SUTTON 1980, GRASSO and ROSA 1982), *Seimatosporium* spp. (SCHOE-MAKER 1964, BROCKMANN 1975, SUTTON 1980) and *Truncatella* spp. (CUBA 1961, SUTTON 1980).

The species and frequency of various fungi on the shoots of dead, wintered outdoor roses were studied at the Agricultural Research Centre, Department of Plant Pathology from 1976 to 1982.

## MATERIAL AND METHODS

In 1976, 1977 and 1979 sample material was gathered from roses planted in parks in Helsinki; in 1978 from the University of Helsinki Botanical Gardens, in 1978 and 1981 from rose experiments at the Horticultural Institute of Lepaa (in Hattula) and in 1981 and 1982 from the Häme Research Station (in Pälkäne) of the Agricultural Research Centre. The plants were either one or two years old. Samples were taken from branches removed during spring pruning.

A total of 143 samples were examined and the number of samples varied between 9 and 27 annually and by locality. The samples represented 56 rose varieties, of which the most common were *Europeana*, *Queen Elisabeth*, *Buismans Triumph* and *Peace*. Varieties represented only by one or two samples accounted for two thirds of the total.

Twenty-five percent of the varieties examined were included in the study for 3 to 4 years. Seventy-five percent of the varieties were included from 1 to 2 years only. Varieties common to the various localities were even less frequent with only 5 % of the varieties occurring in all three localities.

Consequently the results are presented as a whole without specifying variety, year or locality.

Sections of the dead branches were cultured in Petri dishes (Ø 15 cm) using the moist chamber method. The dishes were kept at +10 °C for two weeks, then at room temperature for one week, and again at +10 °C for several weeks if necessary. During this time the fungi were examined using a stereomicroscope, a light microscope and by microphotography.

## Weather conditions

In the winter of 1978 December was very cold and the soil froze under the thin layer of snow causing, roses to die in exceptional numbers. In the winter of 1980, alternating snowfall and rainfall, along with cold spells during the early winter caused damage by water and ice-scorch.

In late winter the plants were at the mercy of the sun, the cold and the drying wind. Consequently roses suffered serious winter damage. During other winters roses overwintered reasonably well (Meteorol. Yearb. Finl. 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982).

## RESULTS

A total of 71 species and genera of fungi (Table 1) were identified in the samples. Some 6 % of the fungi remained unidentified. The number of fungi in the samples was 8,4 on average (range 3—20) in the entire material. The largest

number of species, 13,7 on average (range 9—20), was found in samples from the Häme Research Station in 1981 and the lowest number of species, 4,6 on average (range 3—8) occurred in the Helsinki samples in 1978.

Table 1. Frequency of fungi on wintered branches of rose samples investigated in 1976—1982.

Fungi	Fungi % of samples investigated
<b>MYXOMYCETES</b>	
Physarales	
<i>Didymium</i> spp.	4,9
<b>ZYGOMYCOTINA</b>	
Mucorales	
<i>Actinomucor</i> sp.	2,1
<i>Mucor</i> spp.	3,5
<i>Rhizopus nigricans</i> Ehrenb.	5,6
<b>ASCOMYCOTINA</b>	
Pyrenomycetes, Sphaeriales	
<i>Ceratocystis</i> sp.	2,1
<i>Chaetomium</i> spp.	21,0
<i>C. elatum</i> Kunze ex Fr.	
<i>C. olivaceum</i> Cooke ex Ellis	
<i>Gnomonia rubi</i> (Rehm) Winter	9,8
<i>Nectria cinnabarina</i> (Tode ex Fr.) Fr.	2,8
<i>Melanospora</i> sp.	0,7
<i>Scopinella</i> sp.	0,7
Loculoascomycetes, Pleosporales	
<i>Leptosphaeria coniothyrium</i> Sacc.	2,8
Discomycetes	3,5
Unidentified Ascomycotina	4,9
<b>BASIDIOMYCOTINA</b>	
Holobasidiomycetidae	
<i>Rhizoctonia</i> sp.	2,1
<b>DEUTEROMYCOTINA</b>	
Hyphomycetes	
<i>Acremoniella atra</i> (Corda). Sacc.	13,3
<i>A. verrucosa</i> Fogn.	0,7
<i>Acremonium</i> spp.	14,7
<i>Alternaria</i> spp.	35,7

Fungi	Fungi % of samples investigated
<i>Alysidium resinae</i> (Fr.) M. B. Ellis var <i>microsporus</i> Sutton	10,5
<i>Arthrinium phaeospermum</i> (Corda) M. B. Ellis	2,1
<i>Arthrobotrys suberba</i> Corda	3,5
<i>Aspergillus</i> spp.	13,3
<i>Botrytis cinerea</i> Pers. ex Fr.	89,3
<i>Chrysosporium</i> sp.	1,4
<i>Cladosporium</i> spp.	72,0
<i>Cylindrocarpon</i> spp.	39,9
<i>C. destructans</i> (Zinssm.) Scholten	37,8
<i>Dendryphion nanum</i> (Nees ex Fr.) Hughes	0,7
<i>Doratomyces stemonites</i> (Pers. ex Fr.) Morton et G. Smith	14,7
<i>Echinobotryum</i> state of <i>Doratomyces stemonites</i>	2,1
<i>Epicoccum purpurascens</i> Ehrenb. ex Schlecht	27,3
<i>Fusarium</i> spp.	32,8
<i>F. avenaceum</i> (Fr.) Sacc.	18,5
<i>F. culmorum</i> (W. G. Smith)	
<i>F. oxysporum</i> Schlecht	
<i>F. redolens</i> Wollenw.	
<i>Fusidium</i> sp.	0,7
<i>Geotrichum candidum</i> Link.	0,7
<i>Gliocladium</i> sp.	7,0
<i>Conatobotrys simplex</i> Cda	0,7
<i>Graphium</i> sp.	4,2
<i>Gyoefferfella entomobryoides</i> (Boerema & von Arx) Marvanova	0,7
<i>Humicola grisea</i> Traaen	2,1
<i>Monodictys levis</i> (Wiltsh) Hughes	1,4
<i>Oidiodendron</i> sp.	3,5
<i>Ostracoderma</i> state of <i>Peziza ostracoderma</i> Korf	9,1
<i>Papulaspora rubida</i> Hotson	11,2
<i>Penicillium</i> spp.	39,9
<i>Stachybotrus aurantia</i> Barron	0,7
<i>Torula herbarum</i> (Pers.) Link. ex S. F. Gray	0,7
<i>Trichocladium asperum</i> Harz	1,4
<i>T. opacum</i> (Corda) Hughes	0,7
<i>Trichoderma viride</i> Pers. ex Fr.	36,4
<i>Trichothecium roseum</i> Link ex Fr.	31,5
<i>Ulocladium consortiale</i> (Thüm.) Simmons	23,1
<i>Verticillium ternatum</i> (Nees ex Pers.) Link.	1,4
Unidentified Hyphomycetes	3,5
Coelomycetes	
Melanconiales	
<i>Colletotrichum dematium</i> (Pers. ex Fr.) Grove	0,7
<i>Cryptosporium minimum</i> Laub.	19,6
<i>Hainesia lythri</i> (Desm.) Hohn.	9,1
<i>Seimatosporium lichenicola</i> (Corda) Shoemaker ex Müller	13,3
<i>Truncatella angustata</i> (Pers. ex Link.) Hughes	7,7
Sphaeropsidales	
<i>Ascochyta</i> sp.	1,4
<i>Camarosporium</i> spp.	6,3
<i>C. rosae</i> Grove	4,9
<i>Coniella fragariae</i> (Oud.) Sutton	0,7
<i>Coniothyrium fuckelii</i> Sacc.	47,6
<i>Diplodia rosarum</i> Fr.	0,7
<i>Discosia artocreas</i> (Tode) Fr.	0,7
<i>Phoma</i> spp.	25,2
<i>Phomopsis</i> sp.	4,2
Unidentified Sphaeropsidales	9,8
Unidentified other fungi	39,9
<b>BACTERIA</b>	
Actinomycetales	
<i>Streptomyces</i> spp.	57,3

## Fungi causing canker diseases

The sample material included some 15 species and genera of fungi which, according to the literature, are potential pathogens on rose shoots. *Alternaria* species, mainly *A. alternata*, was fairly common. *Botrytis cinerea* grew on almost all samples.

*Coniella fragariae* was found in one 'Olala' sample from Pälkäne in 1982 (Fig. 7).

*Coniothyrium fuckelii* occurred in almost half of the samples examined (Figs. 1—4). The material included very little *Leptosphaeria coniothyrium*, the perfect stage of *C. fuckelii*.

Occurrence of *Cryptosporium minimum* was very common in 1979 in Helsinki and in 1982 in Pälkäne (Figs. 5—6).

*Cylindrocarpon* species, mainly *C. destructans*, were frequent in the samples (Figs. 8—10).

*Diplodia rosarum* occurred in one 'Cordula' sample from Pälkäne in 1982 (Fig. 27).

The *Fusarium* species occurred in one third of the samples, the most common being *F. avenaceum* and the other species being *F. oxysporum*, *F. culmorum* and *F. redolens*.

*Gnomonia rubi* was commonly found (in 13 out of 24 samples) on roses from the Häme Research Station in 1982 and also on one sample from Helsinki in 1976. The dark perithecia of the fungus grew under the bark of the dead branches, with only the perithecia neck protruding (Figs. 13—15). Fungus size varied widely; perithecia 889—1219 × 442—865 μm, asci 60—97 × 9,2—12,3 μm, ascospores 19—27 × 4,2—6,9 μm.

*Hainesia lythri* was only found in 1976 and

1981 (Figs. 11—12). The *Phoma* species occurred in one quarter of the samples (Fig. 16). The *Phomopsis* species were found infrequently in the samples and only in those from the Häme Research Station in 1981 and 1982 (Figs. 17—19). *Seimatosporium lichenicola* was uncommon in the samples (Figs. 20—23). The occurrence of *Truncatella angustata* was infrequent in 1976, 1981 and 1982 (Figs. 24—26).

## Saprophytes

A large number of the fungi growing on the dead rose branches were common saprophytes which are not important pathogens. The most common were the species *Cladosporium*, *Chaetomium* and *Penicillium*, *Epicoccum purpurascens*, *Tricoderma viride*, *Trichothecium roseum* and *Ulocladium consortiale*. The frequency of the fungi varied between 21 % and 72 % of samples. Fungi occurring in only a few samples accounted for some 50 % of all the fungi identified and some represented rare species.

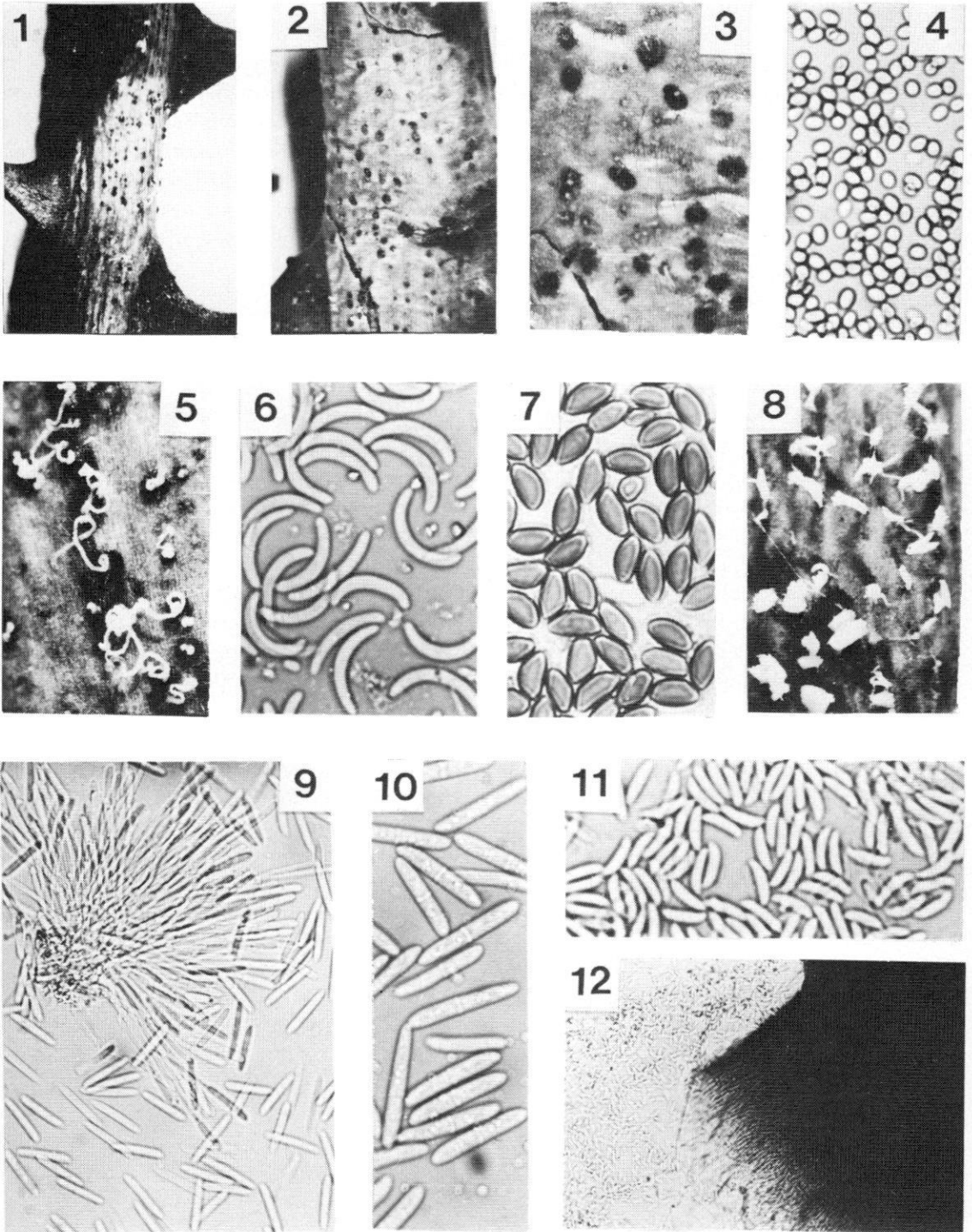
Occurrence of *Alysidium resinae* var. *microsporus* was uncommon in the samples from Pälkäne in 1981 and 1982 (Figs. 28—30).

*Camarosporium rosae* occurred infrequently in the samples from Helsinki in 1976 and 1977 (Fig. 31).

*Gyoerffyella entomobryoides* was encountered in one 'Molde' sample from Pälkäne in 1982 (Fig. 32, cf. INGOLD 1974).

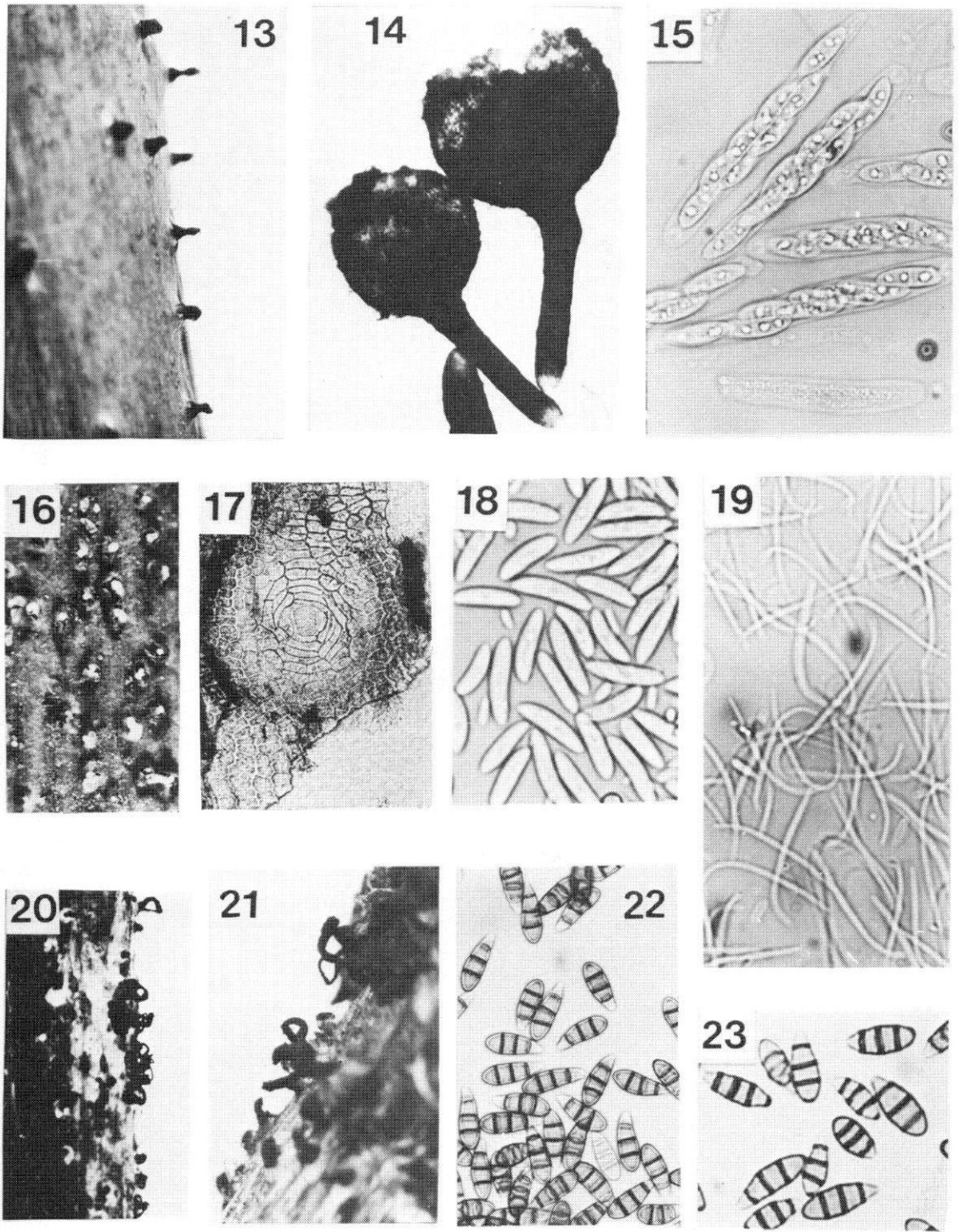
*Trichocladium opacum* was found in one 'Andalusien' sample from Pälkäne in 1982 (Figs. 33—44).



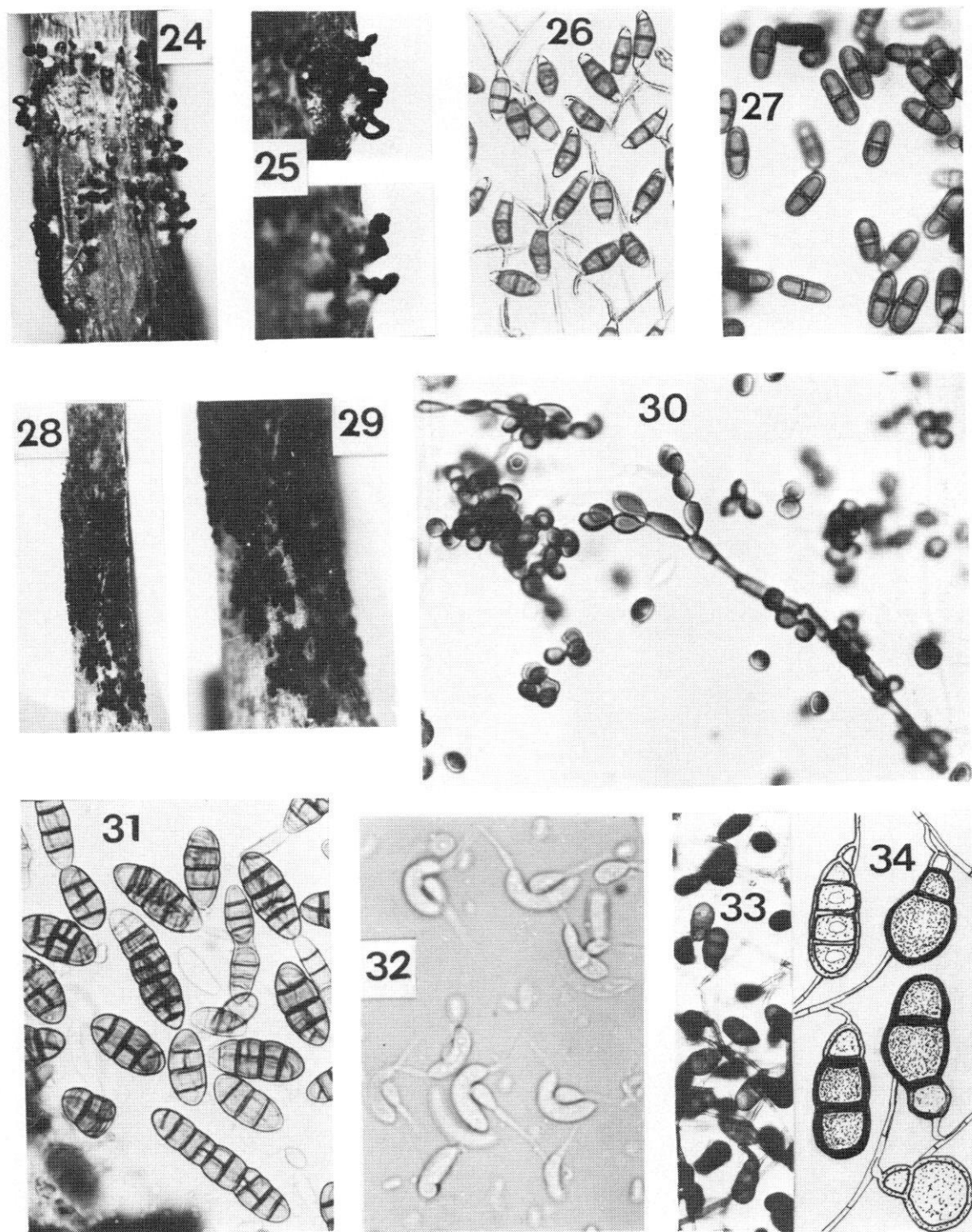


Figs. 1—12. 1, 2, 3, 4: *Coniothrium fuckelii*. 5, 6: *Cryptosporium minimum*. 7: *Coniella fragariae*. 8, 9, 10: *Cylandrocarpon destructans*. 11, 12: *Hainesia lythri*.

Materials: In the branches of outdoor roses at Pälkäne in 1982, with the exception of figures 2 and 4 at Hattula in 1981. 1:  $\times 3$ . 2:  $\times 6$ . 3, 5, 8:  $\times 15$ . 4:  $\times 550$ . 6, 10:  $\times 700$ . 7:  $\times 900$ . 9, 12:  $\times 300$ . 11:  $\times 1200$ .



Figs. 13—23. 13, 14, 15: *Gnomonia rubi*. 16: *Phoma* sp. 17, 18, 19: *Phomopsis* spp. 20, 21, 22, 23: *Seimatosporium lichenicola*.  
 Materials: In the branches of outdoor roses at Pällkäne in 1982, with the exception of the figure 17 at Hattula in 1981. 13, 21:  $\times 6$ . 14:  $\times 50$ . 15:  $\times 600$ . 16:  $\times 15$ . 17:  $\times 200$ . 18, 19:  $\times 1000$ . 20:  $\times 3$ . 22:  $\times 500$ . 23:  $\times 750$ .



Figs. 24—34. 24, 25, 26: *Truncatella angustata*. 27: *Diplodia rosarum*. 28, 29, 30: *Alysidium resiniae* var. *microsporus*. 31: *Camarosporium rosae*. 32: *Gyoeffiyella entomobryoides*. 33, 34: *Trichocladium opacum*.

Materials: In the branches of outdoor roses at Pälkäne in 1982. 24, 29:  $\times 3$ . 25:  $\times 6$ . 26:  $\times 500$ . 27:  $\times 700$ . 28:  $\times 1\ 1/2$ . 30:  $\times 750$ . 31:  $\times 400$ . 32:  $\times 300$ . 33:  $\times 250$ . 34:  $\times 650$ .

## DISCUSSION

Roses are generally short-lived because they suffer due to the severe winters in Finland (KALLIO 1980). Roses weakened by the winter climate have also been found to be susceptible to destructive diseases in other countries (GROVE 1937, SCHNEIDER et al. 1967, GORLENKO 1969, WITTMAN 1982). On the other hand, it is likely that the severity of the Finnish winter eliminates some rose diseases.

Since rose plants are imported to Finland (METSOLA 1976, 1981) it is reasonable to expect that the fungal species are carried over with the plants, and also that the pathogens are mainly the same as in the producing countries. When compared with the reference literature, the results of this research seem to confirm this hypothesis.

The occurrence of *Coniothyrium fuckelii* was very common in this study. The fungus is widespread and is possibly the most destructive cause of stem canker of the *Rosa* (WHITE et al. 1936, ITRAMA 1968, MATTA et al. 1976, IIDA et al. 1980, GLASER et al. 1981, HORST 1983). On the other hand, the samples did not include *C. wernsdorffiae*, a branch canker, which has been widely spread both in Europe (VAN PAETEREN 1926, PROTSENKO 1959, GORLENKO 1969, STAHL and UMGELTER 1976, WITTMAN 1982) and in North America (DRAYTON 1926, WESTCOTT 1934) since the

1920's. Both species have also been found in Scandinavia (GJAERUM et al. 1985).

The occurrence of *Gnomonia rubi* is interesting. The fungus is not mentioned in Scandinavia (GJAERUM et al. 1985), however the fungus on the *Rosa* and *Rubus* species has been encountered since the 1920's in North America as well in Europe (DOWSON 1924, RAMSBOTTOM 1925, SCHNEIDER et al. 1969). Climatic factors seem to have favoured the growth of the fungus. During the research period winter damage was considerable in spring 1981 and because of the cold and the rain the whole growing season was disadvantageous.

*Botrytis cinerea* was very common. The fungus is mentioned as a canker pathogen in the rose in the Narvik area, for instance (DEACON 1931) and Poland (GLASER et al. 1981).

Of the *Fusarium* species *F. avenaceum* was the most common, occurring one third of the samples. The *Fusarium* species are common pathogens in Finland in several plants and this is also likely to apply to roses.

Other fungi, which have been mentioned in the literature as pathogens on rose shoots, remain rather insignificant pathogens simply due to their relatively restricted occurrence in the samples.

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## SELOSTUS

### Talven aikana kuolleiden ryhmäruusujen sienistö

KAIHO MÄKELÄ

Maatalouden tutkimuskeskus

Tutkimus tehtiin vuosina 1976—1982. Talvikaudet 1978—79 ja 1980—81 olivat epäedullisia ja ruusut kärsivät pahoja talvivaurioita. Muina vuosina ruusut talvehtivat kohtalaisesti.

Aineisto käsitti 143 ryhmäruusunäytettä. Ne edustivat 56 lajiketta, joista yleisimpiä olivat *Europeana*, *Queen Elisabeth*, *Buismans Triumph* ja *Peace*. Näytteet olivat peräisin Helsingistä, Hattulasta ja Pälkäneeltä. Ne saatiin kevät-leikkausten yhteydessä poistetuista kuolleista versoista.

Sienet kasvatettiin kosteuskammion menetelmällä ja määritettiin mikroskooppisesti.

Ankarien talvien heikentämät ruusut olivat alttiita versotautien tuhoille. Myös viileät ja sateiset kesät lisäsivät sairastumisalttiutta. Mahdollisia ruusun versotautien aiheuttajia esiintyi noin 15 sienisuvun edustajissa. Yleisin, 89

% näytteistä, oli *Botrytis cinerea*. Vaarallisimpana pidettyä *Conothyrium fuckelii*ta todettiin 48 % ja sen suvullista astetta *Leptosphaeria coniothyrii*nia vain alle 3 % näytteistä. *Gnomonia rubi* löytyi satunnaisesti. *Alternaria*-, *Cryptosporium*-, *Cylindrocarpon*-, *Fusarium*- ja *Phoma*-lajeja esiintyi 20—40 %. Näiden sienten joukossa olivat tärkeimmät ruusun versotautien aiheuttajat. Jokseenkin harvinaisia (8—13 %) olivat *Hainesia lythri*, *Seimatosporium lichenicola* ja *Truncatella angustata*. Vain yksittäisissä näytteissä todettiin *Coniella fragariae*, *Diplodia rosarum*, *Phomopsis*- ja *Rhizoctonia*-lajeja. Näiden sienten merkitys ruusun versotautien aiheuttajina oli vähäinen.

Näytteistä määritettiin noin 70 sienilajia ja sukua. Valtaosa oli kaikkialla yleisiä saprofyyttejä. Harvinaisia, alle 2 % näytteistä, oli todetuista sienistä noin 45 %.

## OCCURRENCE OF WINGED APHIDS ON POTATO PLANTS AND PRESSURE FOR POTATO VIRUS Y TRANSMISSION IN FINLAND

SIRPA KURPPA and PERTTI RAJALA

KURPPA, S. & RAJALA, P. 1986. Occurrence of winged aphids on potato plants and pressure for potato virus Y transmission in Finland. Ann. Agric. Fenn. 25: 199—214. (Agric. Res. Centre, Dept. Pest Inv., SF-31600 Jokioinen, Finland.)

The focus of this study was species of winged aphids which have been shown to be variably important in the transmission of potato viruses. Aphid specimens were trapped using yellow water tray set in 9 different areas throughout the country during 1981—1983. Fluctuations in the abundance of aphids during the three experimental years were great and aphids were generally less numerous in the north. The most abundant aphid was *Rhopalosiphum padi*, forming about 50 % of the total catch and dominating in the north. Aphids arrive from cereals in the middle of the growing season. Due to their copiousness they cause remarkable vector pressure, even though *R. padi* is basically an inefficient viral vector. Summer migrants of *Aphis fabae* occur variably throughout the growing season, devoid of a clear population peak. *Aphis frangulae-nasturtii* spring migrants appear at the beginning of potato growth and other aphids occur thereafter. The effect of the *Aphis* species on vector pressure is substantial on a local basis. Occasionally *Acyrtosiphon pisum* and *Phorodon humuli* occur abundantly then causing additional risk of viral transmission. Because many of these aphids as well as various *Aphis* populations are foreign migrants monitoring by suction traps set along the Finnish coast has been suggested.

Index words: virus vectors, vector pressure, potato virus Y, *Rhopalosiphum padi*, *Aphis frangulae*, *Aphis nasturtii*, *Aphis fabae*, *Sitobion avenae*, *Phorodon humuli*, *Acyrtosiphon pisum*.

## INTRODUCTION

Previous documentation on aphid populations in Finnish potato fields has been based on counting and observation of apterous aphids. LIRO (1926) has reported *Macrosiphum euphorbiae* Thos., and JAMALAINEN (1946) has mentioned *Myzus persicae* Sulz., *Aulacorthum circumflexum* Buckt., *Aulacorthum solani* Kalt. and *Aphis frangulae* Kalt. More recently HAGMAN (1978) and KURPPA (1981) have documented the species *M. persicae*, *A. solani*,

*M. euphorbiae*, *A. frangulae*, *Aphis nasturtii* Kalt. and *Aphis fabae* Scop.

*A. frangulae* and *A. nasturtii* were found to be the most common species in the southern Finland by HEIE and HEIKINHEIMO (1963) and by HAGMAN (1978). *M. persicae* and *M. euphorbiae* were scattered throughout the potato cultivation regions and *A. solani* occurred in the northern areas especially (HAGMAN 1978). Occurrence of the same

species has been reported to be common in potato cultivation areas in Sweden (SIGVALD 1977).

Many other aphid species occasionally infest potato fields (SIGVALD 1982, VAN HARTEN 1983) though their host plants are different. Some species transmit viruses (SIGVALD 1982, VAN HARTEN 1983). For instance, HAGMAN (1978) has shown that *Rhopalosiphum padi* (L.), the most common cereal aphid in Finland, was easily able to insert its stylet into potato plant, but when caged it was sustained in the plant for less than one day. SIGVALD (1982) has shown *R. padi* to have a major role in the transmission of potato viruses in northern Sweden.

Recently VAN HARTEN (1982) and SIGVALD (1984) have introduced methods of calculating vector pressure indexes for the transmission of potato virus Y (PVY) and have presented values of vector efficiency factors for different aphid species. De BOKX and PIRON (1984) have related the occurrence of aphids to

PVY transmission and have identified viruses from live aphids caught in potato fields.

The yellow water tray, a simple and inexpensive piece of equipment, was chosen for this study. It is widely used for aphid monitoring on farms (SIGVALD 1982, VAN HARTEN 1983, de BOKX and PIRON 1984). Apterous aphids were first trapped using yellow water trays by MOERICKE (1951) and have since then been modified. Trays of different sizes, shapes and colors have been introduced (A'BROOK 1973, MOERICKE 1969) but no standard has been fixed.

Vector aphids have not been systematically monitored in Finland though viral transmission in potato fields has been common. This study concerning the local and seasonal occurrence of winged aphids has aimed to provide a basis for approximations of vector pressure. The local vector pressure indexes of PVY<sup>o</sup> and PVY<sup>n</sup> have been calculated for 7 different areas.

## MATERIAL AND METHODS

In a 1978 preliminary survey conducted at the University of Helsinki Experimental Farm in Viikki (Fig. 1) three yellow water trays were placed 20 m apart on a potato plot (about 0.5 ha) situated in a large field (open area about 30 ha).

During 1981–83, yellow trays were distributed to fields within the major potato cultivating areas of the country (Fig. 1). In the Helsinki area, in the south, one tray was set in the same field used in 1978, located about 1 km from the coast of the Gulf of Finland. The second tray was set in an inland area (surrounded by forest) about 15 km from the coast. In the Tyrnävä area located in the north, one tray was situated near the coast of the Gulf of Bothnia and a second tray was set beside the Seed Potato Center, about 15 km from the

coast. One tray only was set in each of the other monitored areas. Several potato cultivars were grown. Potato cultivars emerged on about the 8th of June in the south and around the 25th of June in the far north. Throughout the growing season there was about a 2-week time difference in the rate of potato growth in the respective areas. This variable was taken to account when calculating local vector pressure.

The yellow trays employed were square in shape, with dimensions of 7 × 45 × 40 cm, and made of polystyren (thickness 2 mm). The reflection spectrum of the tray surface was 570–580 nm, as measured by a spectrophotometer (CO-8) at the Otaniemi Technical College (reflection from magnesium oxide was used as a reference). The yellow water trays were placed at a minimum of 50 m from the



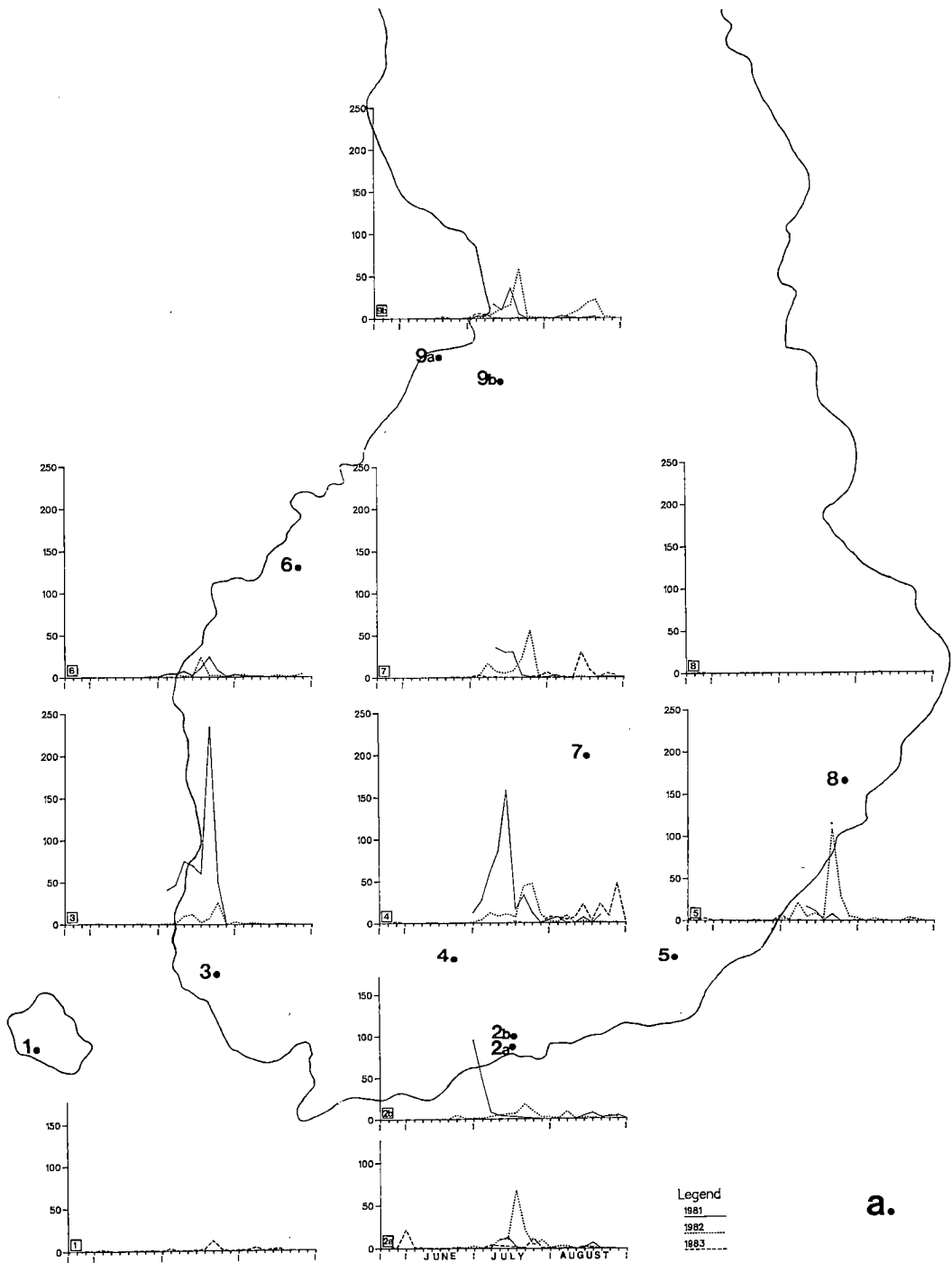
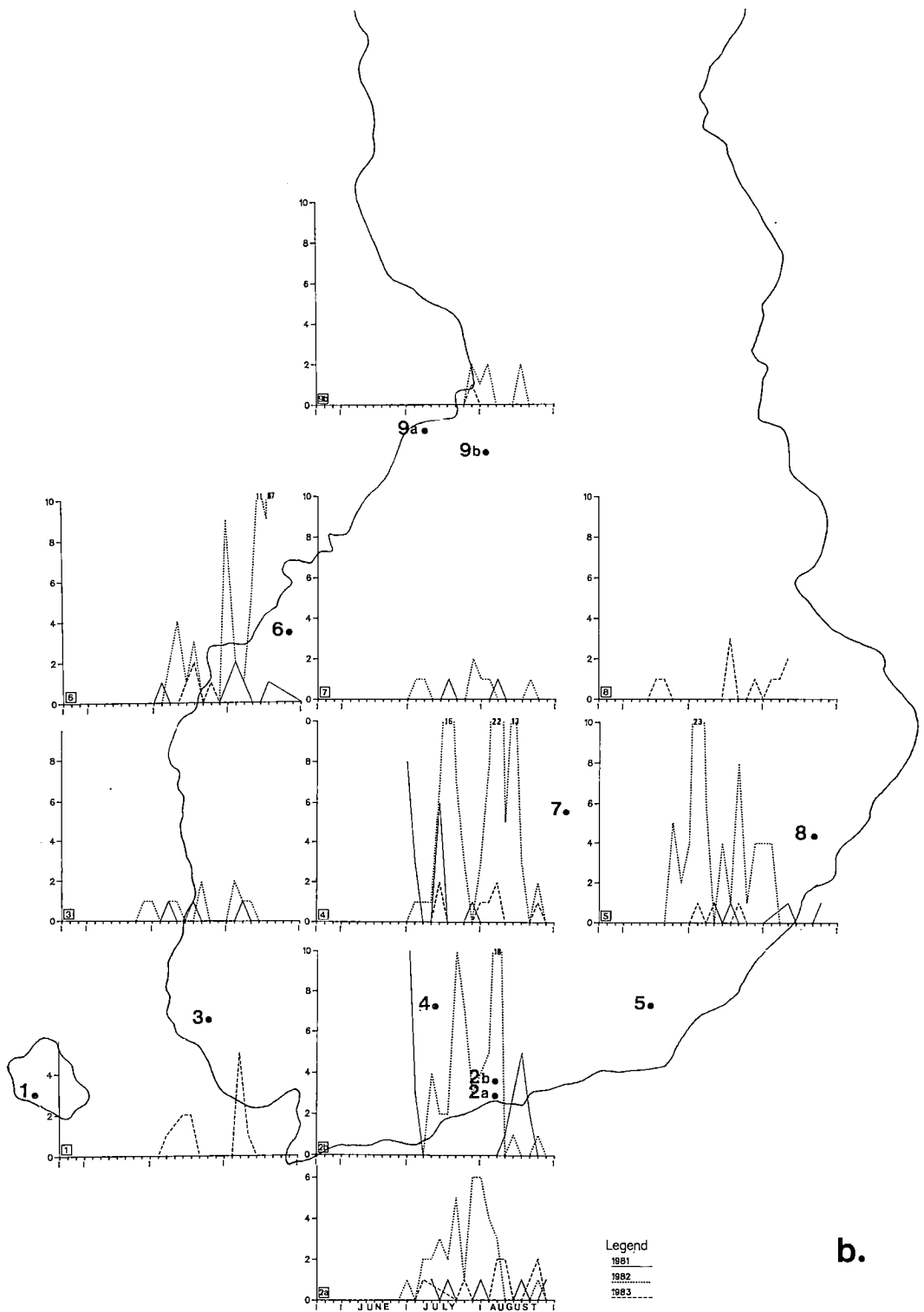
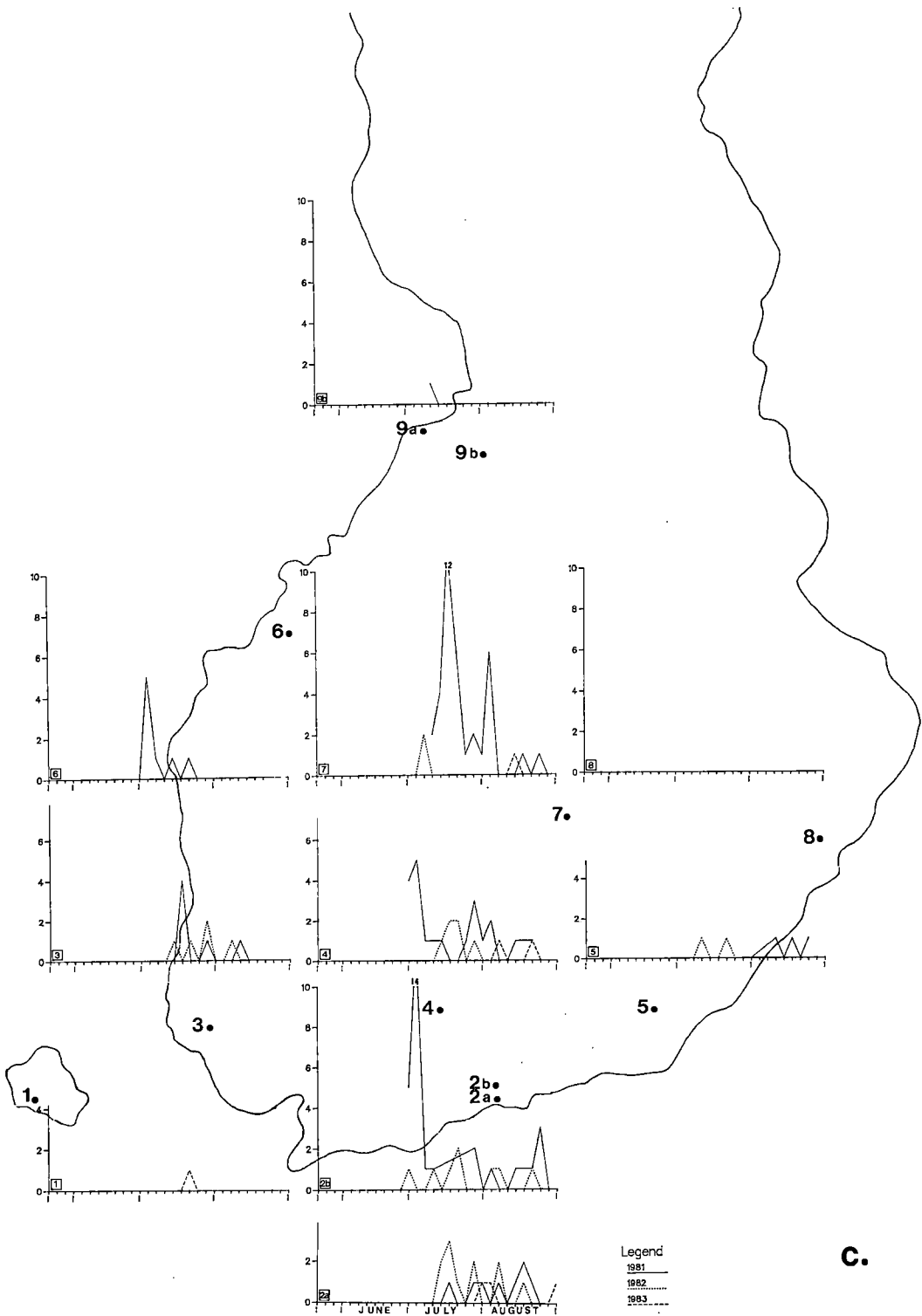


Fig. 1. Aphids catches in yellow water traps set in potato fields in different localities, in 1981—83, a) *R. padi*, b) *A. fabae*, c) *A. frangulae-nasturtii*, d) *S. avenae*. Y-axis number of aphids per yellow water trap. X-axis time between 20th May to 31th August, fine scale 3,5 days, coarse scale 1 month. Localities: 1. Ahvenanmaa, Maarianhamina, 2. Helsinki, coastal site (2a), inland site (2b), 3. Mietoinen, 4. Loppi 1981—82, Jokioinen 1983, 5. Anjala 1981—82, Joutseno 1983, 6. Jepua 1981—82, Kauhava 1983, 7. Laukaa, 8. Kitee, 9. Tyrnävä, coastal site (9a), inland site (9b).

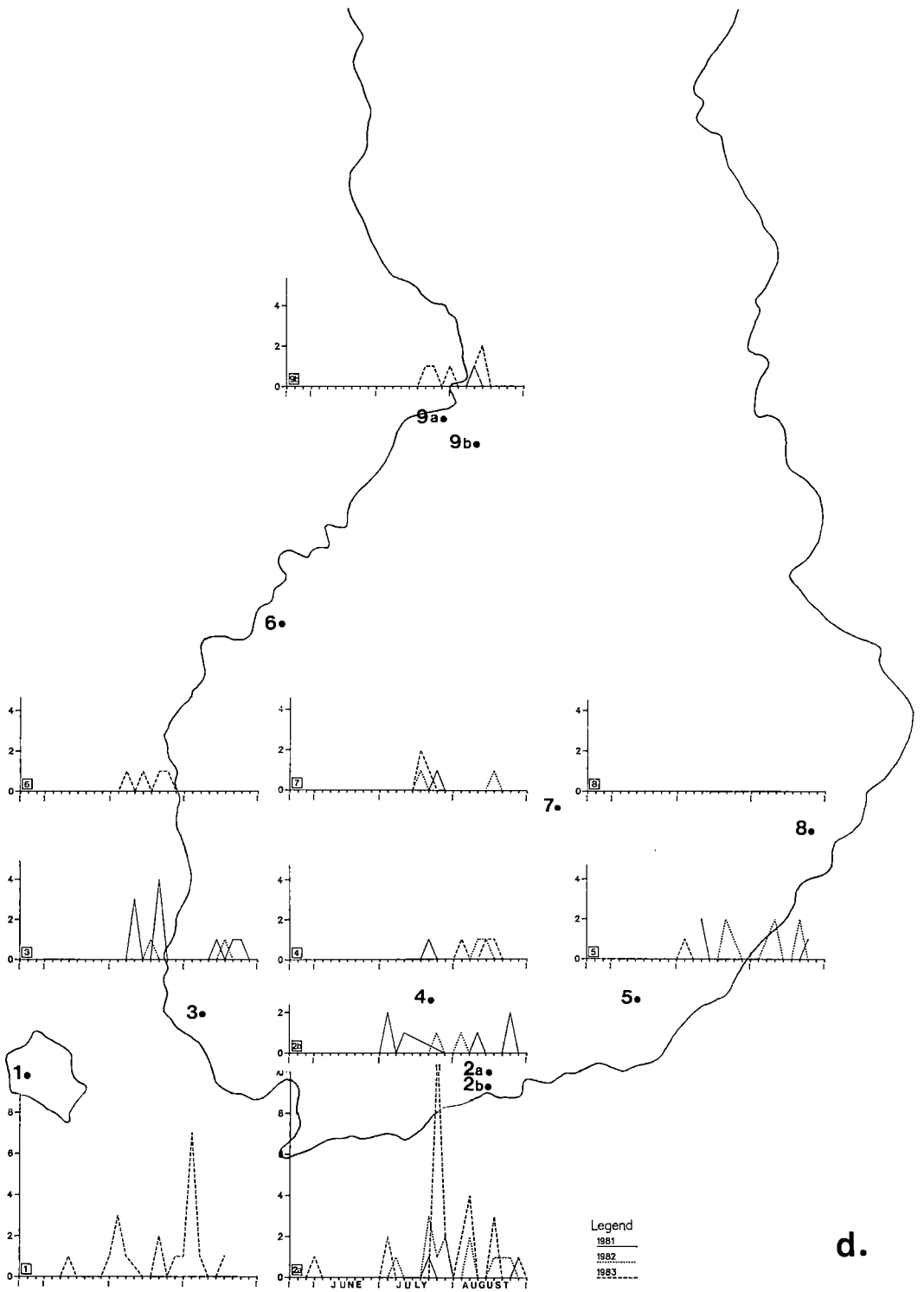


b.



Legend  
 1981 ———  
 1982 .....  
 1983 - - - -

C.



d.

edge of the potato fields. They were set upon wooden frames at the height of about 60—70 cm. The trays were filled with tap water to which a few drops of detergent had been added. Water was changed or added when necessary. Some trays had to be covered with wire nets to keep out birds.

Aphids were thus trapped from June 15th to the end of July 1978, and from June 15th to the end of August in 1981—1983. Captured aphids, hereafter referred to as the catch, were removed from the trays daily in 1978 and twice a week in 1981—83, and then placed into a mixture of water, glycerol and ethanol (20:5:75).

In 1978 all aphids occurring in significant numbers were identified by species. During 1981—83 *R. padi*, *Sitobion avenae* Fabr., *Metopolophium dirhodum* Walk., *M. persicae*, *A. solani*, *M. euphorbiae*, *A. fabae*, *Phorodon humuli* Schrank, *Acyrtosiphon pisum* Harris and *Brachycaudus helichrysi* Kalt. were identified. *A. frangulae* and *A. nasturtii* were identified as a complex of two species. The data obtained was analysed using the analysis of covariance and significance of the variations was tested with Tukey's t-test.

The vector pressure index for potato virus Y was calculated by two methods (VAN HARTEN 1983, SIGVALD 1984). The first has been especially developed for PVY<sup>n</sup> and the second specially for PVY<sup>o</sup>. PVY<sup>n</sup> is more common in Finland (KURPPA 1983).

The factors in brackets were not included in the original methods. The factor used for the efficiency of *A. frangulae-nasturtii* was matched to the factors of the other related aphids included in VAN HARTEN's method. The

Factors describing vector efficiencies of different aphids are as follows:

	PVY <sup>n</sup> VAN HARTEN (1983)	PVY <sup>o</sup> SIGVALD (1984)
<i>M. persicae</i>	1,0	1,0
<i>A. pisum</i>	0,05	0,8
<i>A. frangulae-nasturtii</i>	(0,1)	0,3
<i>A. fabae</i>	0,1	0,2
<i>A. solani</i>	0,1	0,1
<i>B. helichrysi</i>	0,01	0,1
<i>M. euphorbiae</i>	0,1	0,1
<i>M. dirhodum</i>	0,01	(0,01)
<i>P. humuli</i>	0,15	0,1
<i>R. padi</i>	0,02	0,1
<i>S. avenae</i>	(0,01)	0,01

factor for *S. avenae* was equalized to that used for *M. dirhodum* by VAN HARTEN (1983). SIGVALD (1984) suggested the factor of 0,1 for all other aphid species occurring occasionally. However, 0,01 has been used in the present study for *M. dirhodum* to equalize it to the factor SIGVALD assigned to *S. avenae* and to that for PVY<sup>n</sup>. The factors of 0,1 were employed for *A. solani* in both methods. These factors were rendered equal to those for *M. euphorbiae*.

Accumulation to the vector pressure index was initiated following emergence of potato plants in the second week of June in the southern areas, one week later in the central areas and two weeks later in the northernmost area in East Bothnia. Age resistance was evaluated only by SIGVALD's (1985) method. The potato sensitivity factor was 1,0 up to 25 days from emergence decreasing weekly thereafter to 0,8, 0,6, 0,4, 0,2, 0,1 and 0,0, respectively. VAN HARTEN (1983) mentions about age resistance, but does not give any exact values to be used in calculations.

## RESULTS

### Species of winged aphids encountered in Helsinki in 1978

In 1978, 20 species of aphids were identified from the catch of Helsinki (Table 1). The numerical distribution of aphids caught in the three trays were matched well with *R. padi* being the most frequent species followed by *A. frangulae-nasturtii* and *A. fabae*, respectively.

The total number of aphids per day as well as the number of *R. padi* per day peaked on July 16th, occurring about 36 days after the emergence of the potato plant. The number of *A. fabae* first peaked on June 25th and again on July 16th. The frequency of *A. frangulae-nasturtii* followed a slightly similar trend. The other species were encountered during short periods throughout the growing season or occasionally. The group of aphids that has not been accounted for included numerous occasional species.

### Occurrence of winged aphids in potato fields throughout the country during 1981—83

The number of aphids in all monitored areas peaked between July 7th and 19th, during 1981—1982. On July 10th, 1981 the local maximum number of aphids occurred in Loppi, an inland area in the south (310 aphids per catch). On July 12th, 1982, the highest local maximum was found on the southern coast in Helsinki (427 aphids per catch). An additional peak occurred in Tyrnävä, northern East Bothnia on August 2nd, 1982. In 1983, no real peak in the numbers of aphids was found.

In the Tyrnävä area there was no significant difference between coastal and inland catches. In the Helsinki area catches from the trays 14 km set apart differed greatly. The inland catch better resembled catches 80 km further north than catches from the Helsinki coast (Fig. 1 a—d).

Table 1. Number of winged aphids trapped in yellow water tray, in Helsinki, 1978.

Aphid species	Period of 5 days <sup>a</sup>								Total
	1	2	3	4	5	6	7	8	
<i>Aphis frangulae, nasturtii</i>	3	11	5	6	3	10	13	7	58
<i>Aphis fabae</i>	—	6	41	17	5	8	31	21	129
<i>Aphis idaei</i>	—	—	—	—	—	1	8	9	18
<i>Acyrtosiphon pisum</i>	—	—	—	—	—	—	—	1	1
<i>Aulacorthum solani</i>	—	—	—	—	—	—	—	1	1
<i>Brevicoryne brassicae</i>	—	—	1	1	—	—	—	—	2
<i>Cavariella aegopodii</i>	—	—	8	14	—	—	—	1	23
<i>Euceraphis</i> spp.	—	—	—	—	1	1	3	2	7
<i>Hyalopterus pruni</i>	—	—	—	—	2	—	2	5	9
<i>Hyperomyzus lactucae</i>	—	—	1	—	—	—	1	—	2
<i>Macrosiphum euphorbiae</i>	—	—	—	—	3	3	5	9	20
<i>Macrosiphum rosae</i>	1	3	4	9	—	1	3	2	23
<i>Megoura viciae</i>	—	—	—	—	—	—	—	4	4
<i>Metopolophium dirhodum</i>	—	1	—	2	1	1	—	—	5
<i>Myzaphis rosarum</i>	—	1	27	63	1	—	—	3	95
<i>Myzocallis</i> spp.	—	—	—	—	1	2	3	—	6
<i>Myzus persicae</i>	—	1	1	9	—	1	—	—	12
<i>Phorodon humuli</i>	—	—	2	14	—	—	—	—	16
<i>Rhopalosiphum padi</i>	5	1	16	121	142	35	127	5	452
<i>Sitobion avenae</i>	2	—	3	2	1	—	1	—	9
Others	3	10	29	20	5	9	20	38	134
Total	14	34	138	278	165	72	217	51	969

<sup>a</sup> 1 = June 15th—19th 5  
 2 = June 20th—24th 6  
 3 = June 25th—29th 7  
 4 = June 30th—July 4th 8  
 5 = July 5th—9th  
 6 = July 10th—14th  
 7 = July 15th—19th  
 8 = July 20th—25th

In 1981 the total mean of the number of aphids per catch throughout the growing season was 24. The local number of aphids throughout the growing season was highest in Loppi (mean 52 per catch) and lowest in Tyrnävä (mean 10 per catch).

In 1982 the total mean per catch was 37 aphids and the number of aphids was highest in Helsinki (mean 112 aphids per catch) and lowest in Tyrnävä (mean 9 aphids per catch). The number of aphids caught in most areas (Tyrnävä, Jepua, Laukaa, Anjala and Mietoinen) was significantly lower than that of Helsinki ( $P = 0,05$ ).

In 1983 the total mean was only 7 aphids per catch. The highest local mean, 18 aphids per catch, was recorded in Kitee, in eastern Finland. The number of aphids recorded in Tyrnävä, Laukaa, Kauhava and Joutseno was significantly lower than in Kitee ( $P = 0,05$ ).

Of the total catch over the experienced years, *R. padi* was found to be the most frequent. Other commonly occurring aphids from the most to least frequent were: *A. fabae*, *A. frangulae-nasturtii* and *S. avenae*. *P. humuli* occurred abundantly in 1982 and a few *A. pisum* were found in 1983.

### 1. *R. padi*

The number of *R. padi* peaked in all monitored areas between July 7th—17th, 1981, and between July 16th—23rd, 1982 (Fig. 1a). On July 17th, 1981 the highest maximum number for 1981 was in Mietoinen, in the southwestern Finland (235 *R. padi* per catch). On July 19th, 1982 the highest maximum number for 1982 was found in Anjala in southeastern Finland (114 *R. padi* per catch). In 1983, *R. padi* occurred least frequently, no real peaks were found in areas other than Jokioinen, a southern inland area. The peak occurred on August 26th (47 aphids per catch).

In 1981 the number of *R. padi* trapped

throughout the growing season was significantly higher in the southwestern areas of Mietoinen and Loppi than in the southeastern areas of Helsinki and Anjala ( $P = 0,05$ ). The mean number of aphids per catch in Mietoinen was 32 and that of Helsinki 2. No significant differences in the number of *R. padi* caught throughout the growing season was noticed among the monitored areas in 1982. The total mean was 6 aphids per catch. Low numbers of *R. padi* caught in all of the monitored areas in 1983 (total mean 1,5 aphids per catch) were not statistically treated.

### 2. *A. fabae*

The highest maximum number of *A. fabae* (67 aphids per catch) was found in Jepua, in central East Bothnia (Fig. 1b) on August 29th, 1982. However, no single dominant peak of its occurrence was detected in any of the monitored areas. *A. fabae* was less frequent in the other years. The highest numbers were 8 aphids per catch at the end of June 1981 in Loppi and 5 aphids per catch received at the beginning of August 1983 in Maarianhamina.

The number of *A. fabae* throughout the growing season was significantly higher in Helsinki (Fig. 2b) than in Tyrnävä and Laukaa, in 1981 ( $P = 0,05$ ). The number of Jepua, in central East Bothnia, were significantly higher than in the surrounding areas of Tyrnävä, Laukaa and Mietoinen, in 1982 ( $P = 0,05$ ). The numbers of *A. fabae* in 1983 were too low to show any essential differences.

### 3. *A. frangulae-nasturtii*

*A. frangulae-nasturtii* occurred rather frequently in 1981. The highest maximum number, 14 aphids per catch, was caught near Helsinki (2 b), in southern Finland (Fig. 1 c) on July 17th. The maximum numbers of *A. frangulae-nastur-*

*tii* were obtained from all the monitored areas between June 29th and July 17th. In 1982 and 1983 *A. frangulae-nasturtii* occurred occasionally and no peak of abundance was found.

In 1981 the number of *A. frangulae-nasturtii* throughout the growing season was significantly higher in Laukaa compared to Tyrnävä in the north and the southern areas Anjala and Mietoinen ( $P = 0,05$ ). The numbers in Helsinki were highest in 1982 and significantly higher than in Tyrnävä, Jepua, Laukaa and Anjala ( $P = 0,05$ ). No essential differences in the numbers of *A. frangulae-nasturtii* between the areas were encountered, in 1983.

#### 4. *S. avenae*

*S. avenae* was found on occasion throughout the growing seasons of 1981 and 1982. In 1983, a peak of 11 aphids per catch occurred on July 22nd (Fig. 1d). The numbers of *S. avenae* were highest in the south, in Helsinki. The number of aphids in Helsinki differed significantly from the numbers in the areas to the west and north: Mietoinen, Loppi, Laukaa and Tyrnävä, in 1982 and 1983 ( $P = 0,05$ ).

#### 5. Other aphid species

In 1982 a great number (maximum 160 aphids per catch) of *P. humuli* was found in the south, in Helsinki (Table 2.). In July, the aphid was frequent in the catches of all the other areas except Tyrnävä, but in lower numbers (less than 30 aphids per catch).

On June 20th, 1982. *A. pisum* appeared in the southwestern island of Maarianhamina (Table 2.). From then on 1 to 5 *A. pisum* were present in each catch during July. Already, about a week after its first appearance *A. pisum* was found in East Bothnia and even in the central inland area. Later, a couple of *A. pisum* per catch were occasionally present in all the

other areas except Mietoinen and Tyrnävä. That year the number of *A. pisum* reached up to 2,5 % of the total number of aphids caught in all the monitored areas.

*M. persicae* occurred a few times in most of the monitored areas (Table 2.). In 1982, this species was even found in East Bothnia. *A. solani* was most frequent in the northernmost areas, but was encountered in Helsinki as well. In addition, *M. euphorbiae* and *B. helichrysi* were found, but very seldom.

#### Vector pressure

Vector pressure index values varied greatly in different years and in the different areas. Every year the pressure was lowest in northern East Bothnia (Fig. 2). The highest vector pressure in each of the three different years was found every time from different areas. The vector pressure in the Helsinki inland area resembled more the vector pressure in the area northwards than the vector pressure in the Helsinki coastline site.

*R. padi* was the most important aphid in the formation of vector pressure in the north (Fig. 2.). *A. fabae* had a great share in vector pressure of the central areas. *A. frangulae-nasturtii* formed over 65—67 % of the vector pressure in central Finland and had a significant share in the vector pressure in all the other areas in 1981. In the other two years *A. frangulae-nasturtii* was noticeable but much less important. The share of *M. persicae* in vector pressure was significant in the south in 1981 and 1983, but also in the central areas and even in the northernmost East Bothnia in 1982. The effect of the migration of *P. humuli* on vector pressure was great in 1982. The only area where this effect was not noticed was in northern East Bothnia. In 1983 the effect of the abundance of *A. pisum* was noticed in the vector pressure even in East Bothnia.

The vector pressures calculated for PVY°



Table 2. Aphids occasionally encountered in potato fields from different localities in Finland.

Aphid sp. Year	Locality of the potato field from south to north*								
	1.	2.	3.	4.	5.	6.	7.	8.	9.
<i>M. persicae</i>									
1981		July 14th Aug. 4th	—	—	—	—	—	—	—
1982		—	July 23rd	July 23rd	—	July 2nd	—	—	June 25th Aug. 23rd
1983	—	July 18th Aug. 5th Aug. 26th	—	Aug. 18th	—	—	—	—	—
<i>A. solani</i>									
1981		July 10th	—	—	July 10th July 14th	—	—	—	Aug. 25th
1982		July 23rd July 30th	July 12th	—	—	July 2nd July 17th July 19th	—	—	—
1983	—	—	—	Aug. 2nd	—	—	—	—	June 17th June 24th Aug. 11th
<i>P. humuli</i>									
1982		July 9th— 19th July 26th— Aug. 6th	July 9th July 19th 26th Aug. 2nd Aug. 27th	July 9th— Aug. 6th	June 28th July 5th— 19th July 26th— Aug. 2nd	July 5th July 16th 19th	July 2nd	—	—
<i>A. pisum</i>									
1983	June 20th July 1st— July 29th Aug. 8th— 15th	July 22th July 29th Aug. 5th	—	July 25th— Aug. 2nd Aug. 8th	July 22nd	July 5th July 18th— July 22nd	July 7th	—	—
* Localities:									
1. Maarianhamina (1983)	4. Loppi (1981—82)	6. Jepua (1981—82)							
2. Helsinki (1981—83)	Jokioinen (1983)	Kauhava (1983)							
3. Mietoinen (1981—83)	5. Anjala (1981—82)	7. Laukaa (1981—83)							
	Joutseno (1983)	9. Tyrnävä (1981—83)							

and PVY<sup>n</sup> by the two methods (VAN HARTEN 1983, SIGVALD 1985) were remarkably unequal (Fig. 2). The high total vector pressure indexes of PVY<sup>o</sup> (SIGVALD 1985) throughout the country in 1981, and in Anjala, Laukaa and Tyrnävä, in 1982, resulted from high numbers of *R. padi* having a fairly high efficiency for PVY<sup>o</sup>. In 1981, the vector pressure index of PVY<sup>o</sup> was increased by the effect of *A. frangulae-nasturtii*. In 1982, the southern visitor, *P. humuli*, caused a great increase in the vector pressure for PVY<sup>n</sup>, counted by VAN

HARTEN's (1983) method, in all of the other areas except Tyrnävä. The effect of these aphids in the PVY<sup>o</sup> pressure index was eliminated by the age resistance of potato. In 1982 in Jepua the vector pressure for PVY<sup>n</sup> was increased remarkably because of the late, high abundance *A. fabae*. In 1983 the effect of the occurrence of *A. pisum* became dominant in most of the areas for the pressure of PVY<sup>o</sup>. The occurrence of the aphid was low, but the high efficiency factor and early appearance made it significant.

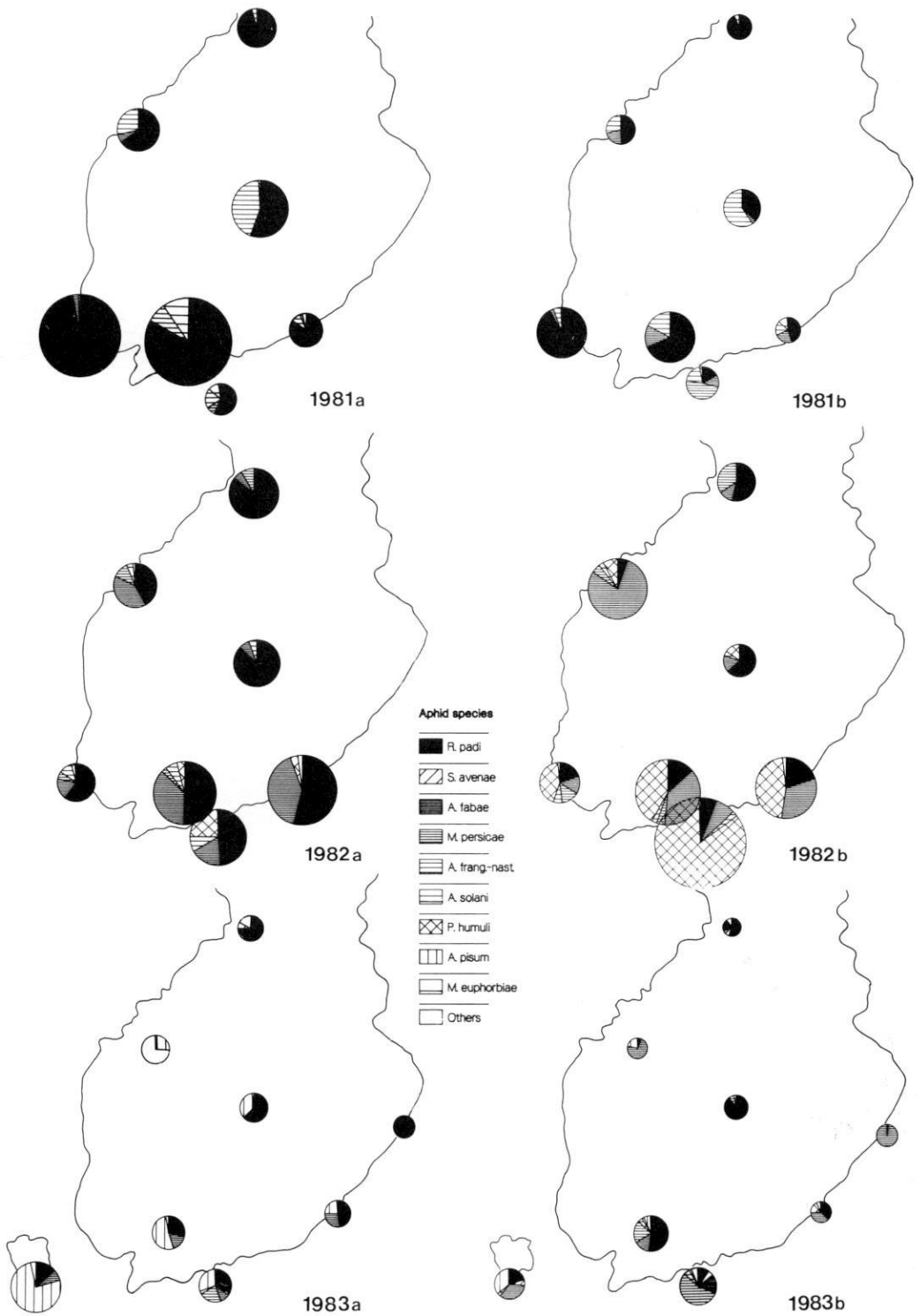


Fig. 2. Vector pressures counted by the occurrence of the different aphid species in various localities, in 1981—83. a) Vector pressure of PVY<sup>o</sup> (SIGVALD 1984), b) Vector pressure of PVY<sup>i</sup> (VAN HARTEN 1983). The surface area inside the circle is proportional to total vector pressure and the sectors describe the relative effect of the occurrence of the different aphid species in forming vector pressure.

## DISCUSSION

All the identified species are known to be abundant in Finland (HULDEN and HEIKINHEIMO 1984). High variations in the numbers of *R. padi* on cereals (RAUTAPÄÄ 1976) were reflected in the numerical variations of the aphid on the potato plant. Summer migrants of *R. padi* arrived at potato fields in the middle of July, two to three weeks after the time when the population peak of the aphid normally occurs on cereals (RAUTAPÄÄ 1976, LEATHER and LEHTI 1982). *R. padi* migrants were then caught in high numbers by suction traps also (WIKTELIUS 1981, LEATHER and LEHTI 1982, WIKTELIUS and EKBOM 1985). After feeding on potato plants the aphids disappeared obviously flying to some *Gramineae*-plant, many of which have been shown suitable for *R. padi* to feed on (RAUTAPÄÄ 1970).

The summer migrants of *R. padi* appear on the potato plant about one month after its emergence. Up to this time the potato has been suggested to be very sensitive to PVY<sup>o</sup> transmission (SIGVALD 1981) though the plant has just begun to acquire some age resistance. Being so abundant the summer migrants of *R. padi* were found to have an important role in forming of PVY<sup>o</sup> vector pressure. On the contrary, the low numbers of *R. padi*, that fly onto the potato during autumn migration, occur too late to have any importance.

The abundance of *R. padi* on cereals and the rate of population growth seem to be valuable indicators when attempting to predict the occurrence of the aphid on the potato. Concurrent time with the observations of *R. padi* the abundance of *S. avenae* might also be noted. If numerous and flying onto the potato plant, *S. avenae* is worth recognising as a cause of a slight additional increase in viral pressure of the potato. However, the intensity of viral transmission ultimately depends on the occurrence of the viral sources in the potato field and its surroundings (e. g. SIGVALD 1982, de BOKX

and PIRON 1984).

The abundance of winged *A. frangulae-nasturtii* peaked on the potato in the beginning of July; fairly late to refer to it as a typical spring migration. HAGMAN (1978) has showed *Chamaenerion angustifolium* to serve as an alternative host for alate fundatrigenae of *A. frangulae-nasturtii* emigrating from *Framnus* sp. in June when the potato has not yet emerged. Because the offspring of alates on *C. angustifolium* are apterae, the migrants appearing on to the potato are suggested to fly from *Framnus* sp. Possibly, they are alate offspring of the second or third generation on the winter host. The aphids appearing on the potato in August most obviously belong to autumn migrants developed on the potato or some other plant.

SHANDS and SIMPSON (1971) found the third generation of *A. frangulae* to bear the most offspring which develop winged spring migrants but HAGMAN (1978) reported the migrants to be fundatrigenae. Variabilities in the appearance of alate offspring have been shown with *R. padi* (WIKTELIUS 1984). The generation in which alate spring emigrants of *R. padi* are developed is earlier the higher the population density in a particular bird cherry tree. An equal phenomenon, if true for *A. frangulae-nasturtii*, might explain the differences in previous observations. Thus, potato would receive the spring immigrants of *A. frangulae-nasturtii*, if the primary host plants around the particular potato fields were abundant and the density of the overwintered aphid population on them was fairly low. If the fundatrix population was high, the fundatrigenae would develop to alates and would emigrate to alternative hosts (for instance *C. angustifolium*), before emergence of the potato.

The rarity of *Framnus* sp. is most likely to restrict the occurrence of *A. frangulae-nasturtii* in the northern and eastern parts of the

country. To make a reliable prediction of the occurrence of this aphid complex in the other areas requires a complicated system of monitoring.

The density of *A. fabae* is in some years very high, especially in the western and central areas, and the aphid tends to arrive in the potato fields at the end of the season. *A. fabae*, though an inefficient vector of PVY<sup>n</sup>, causes a great risk of viral transmission, because the potato is supposed to be sensitive as late as the end of the season (VAN HARTEN 1983). The abundance of *A. fabae* on the potato seems to depend primarily on the density of aphid populations in the approximate neighborhood of the potato field. Predictions on aphid numbers can be based merely on strictly local observations.

*A. pisum* and *P. humuli* are distributed from the south or southeast. Obviously these aphids, as well as some *Aphis* species are foreign migrants. A simple way to predict their appearance would be to set suction traps, or other suitable traps, on the southern and western coasts of Finland. The immigrants would appear in these traps a few days before appearing in substantial numbers in potato fields. This can be concluded by comparing the dates of the first appearance of the aphids in

Maarianhamina or Helsinki to those of the other areas in this study. A few days notice would provide sufficient time to advise farmers to spray oil or insecticide or to destroy the haulms of their potato crop, if it is late in the season.

The vector pressure for PVY<sup>n</sup> was lowest in the north, and there a smaller number of aphid species took part in the formation of the pressure. The vector pressure value, counted by VAN HARTEN's (1983) method, actually gives the maximum vector pressure of the season, as the mature resistance is not taken to account. Necessarily, more information about the development of the mature resistance is needed in practice. In formation of the vector pressure *R. padi* is the most important in the area of the Seed Potato Center at Tyrnävä as well as on the other side of the Gulf of Bothnia (SIGVALD 1977). Occasional visitors, potentially creating a great danger of viral transmission seem to occur frequently in the south outside the main seed potato cultivation area. However, monitoring of the appearance of these aphids and their migrations domestically is necessary. Checking for the presence of virus in them, as did de BOKX and PIRON (1984), might be helpful in restricting the introduction of the viruses to the important seed potato areas.

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## SELOSTUS

### Siivellisten kirvojen esiintyminen ja siitä aiheutuva perunan Y-viruksen levintävaara Suomen perunapelloilla.

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Perunakasvustossa elävät kirvalajit tunnetaan meillä varsin tarkkaan, mutta lentävien, perunalle satunnaisesti laskeutuvien, virustautien kantajina tunnettujen kirvalajien merkitystä ei ole tarkasteltu. Näiden siivekkäiden kirvojen lajistoa ja yleisyyttä sekä kirvojen aiheuttamaa perunan Y-viruksen levintävaaraa oli tarkoitus selvittää tässä tutkimuksessa.

Kirvalajit määritettiin ensin Helsingin yliopiston koetilalla Viikissä tehdyssä kenttäkokeessa. Kasvustoon asetetuista keltamaloista löydettiin toistuvasti kahdenkymmenen eri kirvalajin siivekkäitä yksilöitä. Kun vektorilajeina tunnettujen kirvojen esiintymistä koko perunanviljelyalueella seurattiin vuosina 1981—83, kirvojen esiintymisessä havaittiin suuria vuosittaisia ja alueittaisia vaihteluja. Kes-

kimäärin kirvoja esiintyi selvästi vähemmän pohjoisimmilla alueilla, Pohjanmaalla.

Viljojen tärkein kirvalaji, tuomikirva, *Rhopalosiphum padi*, on keskimäärin yleisin perunapelloilla lentävistä lajeista (yli 50 % keltamaljojen saaliista). Tuomikirvojen yleisyys perunalla määräytyy sen perusteella, miten populaatiot kehittyvät viljoissa kevätkesän aikana. Tuomikirva tulee perunapelloille noin viikon kuluttua siitä, kun sen populaatio on viljoilla saavuttanut huippunsa. Lisää tuomikirvoja tulee perunalle kunnes kaikki kirvat ovat poistuneet viljoilta.

Perunakasvustoon tulevat tuomikirvat lentävät perunalta edelleen muihin kasvustoihin. Toinen lievä tuomikirvan esiintymishuippu saatetaan havaita vielä elokuun lopulla kirvojen siirtyessä talvi-isäntäkasveihin, mutta virusten levinnän kannalta kuitenkin vain ensimmäisellä esiintymällä on merkitystä. Kun myös viljakirvaa, *Sitobion avenae*, tulee perunalle mikäli kirvojen määrä läheisillä viljapelloilla on runsas, pitäisi kirvojen esiintymisennusteet viljaa ja perunaa varten yhdistää. Kirvojen esiintyminen viljoissa olisi perustana perunaa varten laadittaville ennusteille.

Seuraavaksi tärkeimmät perunalta siivekkäinä tavattavat lajit ovat perunalla elävät paatsaman perunakirvat, lajipari *Aphis frangulae-nasturtii* ja papukirva, *A. fabae*. Paatsaman

perunakirvoja tulee perunapelloille heinäkuun alussa, ilmeisesti suoraan talvi-isäntäkasveilta, *Framnus*-lajeilta. Papukirvaa tavataan perunapelloilla läpi kasvukauden. *Aphis*-lajien esiintymisen ennustaminen näyttää niiden moni-isäntäisyyden ja suurten esiintymävaihtelujen tähden vaativan runsaasti lisätyötä. Humalakirvan, *Phorodon humuli*, ja hernekirvan, *Acyrtosiphon pisum*, satunnaisia runsaita esiintymiä havaitaan maan eteläosissa ja rannikoilla. Kirvat ovat todennäköisimmin peräisin Suomea eteläisimmiltä alueilta ja niiden esiintymistä voitaisiin ennakoida pyydyttämällä kirvoja rannikoille sijoitettuihin imupyydyksiin.

Kirvojen aiheuttama Y-viruksen leviämisaara Suomen perunanviljelyalueella näyttää pienenevän pohjoiseen päin siirryttäessä. Siemenperunakeskuksen alueella viruksia levittää yleensä vain 2—3 kirvalajia, joista merkittävin on tuomikirva. Etelämpänä levintävaara aiheutuu useiden eri kirvalajien esiintymisestä ja eteläiset lajit, kuten humalakirva ja hernekirva aiheuttavat levintävaaran ajoittaista hyvin voimakasta kasvua. Mikäli Etelä-Suomen perunakasvustojen nopea taantuminen halutaan välttää, joudutaan kiinnittämään erityistä huomiota satunnaisten virusta levittävien kirvalajien esiintymiseen.

## COMPARISON OF DRY MATTER CONTENTS IN GRASS SILAGES AS DETERMINED BY OVEN DRYING AND GAS CHROMATOGRAPHIC WATER ANALYSIS

LEA HUIDA, HANNA VÄÄTÄINEN and MARTTI LAMPILA

HUIDA, L., VÄÄTÄINEN, H. & LAMPILA, M. 1986. Comparison of dry matter contents in grass silages as determined by oven drying and gas chromatographic water analysis. *Ann. Agric. Fenn.* 25: 215—230. (Agric. Res. Centre, Inst. Anim. Husb., 31600 Jokioinen, Finland.)

The dry matter contents of 437 grass silage samples determined by gas chromatographic water analysis and by oven drying of 2—4 h at 80 °C and then for 15 h at 105 °C were compared. On an average, oven drying gave 1,42 %-units lower dry matter values than the gas chromatographic method and the difference increased with rising pH. To examine the reasons for the difference, the concentrations of volatile compounds in samples were analysed before and after oven drying. The gas chromatographic method for the analysis of formic acid is also reported.

Volatility of acetic, propionic, butyric, isovaleric, valeric and lactic acids was found to be in relation to pH and to decrease with a rise in pH. pH had an opposite effect on the volatility of ammonia nitrogen. Formic acid was analysed in 166 samples of which 156 were of low pH, hence the effect of pH was not clearly apparent. Ethanol was completely lost from all samples regardless of pH. Examination by pH classes,  $\text{pH} < 4,2$ ,  $4,2 \leq \text{pH} \leq 4,5$  and  $\text{pH} > 4,5$ , showed that with rising pH the volatility of lactic acid decreased relatively strongest, the percentages of its volatility being on average 31,2, 24,3 and 15,6, respectively. The common volatility of fatty acids excluding formic acid followed the mean volatility of acetic acid and was from the lowest pH class to the highest 89,1, 93,1 and 86,0 %, respectively. The mean volatility formic acid was over 90 % in all pH classes. The volatility of ammonia nitrogen increased with rising pH from 82,8 to 85,3 % and further to 92,9 %. Acetic acid and lactic acid were, in quantity, the most important compounds affecting the error of oven dry matter, forming 87 % of the total amount of volatiles in samples of  $\text{pH} \leq 4,5$ . The corresponding part in samples of  $\text{pH} > 4,5$  decreased to 71 %, indicating the increasing effect of the other volatile fatty acids and ammonia nitrogen upon the error. The oven dry matter value plus the amount of lost volatiles corresponded in all pH classes fairly well to the dry matter value measured by gas chromatography, indicating this method to be satisfactory for the analysis of water in grass silages. The variation of the difference between the sum and dry matter value obtained by water analysis was fairly large, however.

For oven dry matter, correction equations based on the percentages of volatility of volatile fatty acids, lactic acid, ethyl alcohol and ammonia nitrogen are presented. Because the volatility of lactic acid was observed to be dependent on pH, the equations are presented according to pH classes.

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Index words: corrected oven dry matter, gas chromatographic water analysis, gas chromatographic formic acid analysis, grass silage.

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## INTRODUCTION

The loss of volatile compounds in silage by oven drying causes an error in dry matter contents which has been attempted to correct in different ways. AERTS et al. (1974), DULPHY et al. (1975) and HAIGH and HOPKINS (1977) obtained 5—10 % greater dry matter contents employing toluene distillation than by oven drying. Using dry-distillation, WEISSBACH and BERG (1977) observed that the dry matter content obtained by toluene distillation was 4 % greater than the true dry matter. They claimed that the error was caused by inaccurate correction for volatile compounds in the toluene distillate and the presence of carbon

dioxide. In addition, the previously omitted ethyl alcohol content of the distillate has been criticized (AERTS et al. 1974, HENDERSON 1978, PORTER et al. 1984).

Gas chromatographic water analysis (HUIDA 1982) has been used in our laboratory for the determination of dry matter content in grass silage. By analysing the concentrations of known volatile compounds evaporated during oven drying, we have tried to confirm the validity of the gas chromatographic method and to explain the differences in the results obtained by the methods compared.

## MATERIAL AND METHODS

Analyses were carried out on 437 grass silage samples collected during the winter of 1983—1984 and 1984—1985 from different farm silos. A total of 344 samples were taken from silages treated with formic acid or a mixture based on formic acid, 66 samples from silages treated with a nonformic acid-containing additive and 27 samples from untreated silages. For the analyses, 5 portions of the chopped fresh silage were weighed and analysed as follows:

- 1) For oven dry matter determinations, a sample of 200 g was weighed in a preweighed aluminium container with net bottom covered by soft paper and dried in a forced-air oven for 2—4 h at 80 °C, then for 15 h at 105 °C.
- 2) For gas chromatographic water analyses, a sample of 30 g was extracted with absolutic ethyl alcohol as earlier described (HUIDA 1982). Dry matter thus measured is termed "extracted dry matter" in this paper.
- 3) For the determination of acetic, propionic, butyric, isovaleric, valeric and lactic acids, ethyl alcohol and ammonia nitrogen, a sample of 30 g was extracted with 270 ml of water in Waring blender two times for 5 min cooling in

between. Volatile fatty acids and ethyl alcohol were determined from a filtrated water extract by gas chromatography, employing methods developed in our laboratory (HUIDA 1973, 1982). Lactic acid and ammonia nitrogen were determined photometrically according to the methods of BAKER and SUMMERSON (1941) and MCCULLOUGH (1967), respectively.

- 4) For the determination of formic acid, the water extract was concentrated as follows: 10 ml of extract was alkalized to pH 9,5 with 1 N NaOH in a flask and evaporated to dryness in a rotavapor. The residue was dissolved into 2 ml of water, then pipetted with a pasteur pipette into a tube and evaporated to dryness. The flask was rinsed once with one ml of water to the same tube and evaporated to dryness. Two drops of water, 0,2 g of NaHSO<sub>4</sub> and 2,0 ml of ethyl ether were then added into the tube. The tube was closed tightly with a screw cap and shaken in a vortex mixer for one min. Formic acid was measured by gas chromatography from ethyl ether extract. The instruments and parameters are shown in Table 1. Standard solutions using mixtures of formic



Table 1. Instruments and parameters in the gas chromatographic formic acid determination.

Gas chromatograph	Hewlett Packard Model 5890
Column	Hewlett Packard Series 530 $\mu$ Carbowax 20 M, length 10 m Thermal conductivity, 150 °C
Detector, temperature	250 °C
Injector, temperature	250 °C
Carrier gas, flow rate	Helium, 12 ml/min
Injection volume	1,60—1,70 $\mu$ l
Oven temperature program	60 °C for 3 min, then first rate 4°/min to 100 °C, then immediately second rate 20°/min to 150 °C for 3 min
Integrator	Hewlett Packard Model 3392A

acid and acetic acid were pretreated as sample extracts and corresponded to the concentration range of sample solutions.

Formic acid eluted after acetic acid in 7 min. The separation is shown in Figure 1.

After sample injection, 1  $\mu$ l of ethyl ether was injected to clean the column. If the

integration did not end immediately after the formic acid peak, it was ended by ether injection. At the injection end of column, a glass capillar replaced twice a week was used to prevent column blocking. Calibration was performed by the external standard method. The sample injection was carried out twice and the volume of the injection was checked with a magnifying lens before and after injections. The relative deviation of the results of two replicates (the difference of replicates as per cent of the mean) in the data of 80 samples averaged 4,8 %. If the deviation was greater than 10 %, the injection was repeated.

5) To estimate the loss of volatile compounds by oven drying, 30 g of fresh silage was weighed in a glass dish, in which the sample was dried as described in section 1 above, simultaneously with portions of the same samples weighed in aluminium containers for oven dry matter determination. After drying, 100 ml of water was added into the dish and left on the sample for one hour. The mixture was transferred into a blender, diluted with water to 300 g and extracted two times for 5 min cooling in between. Formic acid and other volatile fatty acids, ethyl alcohol, lactic acid and ammonia nitrogen were measured from this filtrated extract.

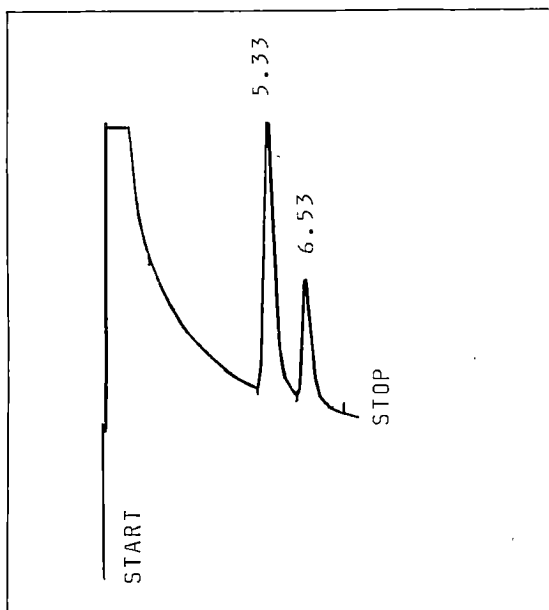


Fig. 1. The separation of acetic acid and formic acid. Concentration of acetic acid 0,029 %, retention time 5,33; concentration of formic acid 0,020 %, retention time 6,53. Integrator parameters: Att 2  $\uparrow$  = 5, chart speed 0,5.

## RESULTS AND DISCUSSION

There was a close relationship ( $r = 0,980$ ,  $P < 0,001$ ) between dry matter values obtained by the different methods (Fig. 2). The dry matter determined by gas chromatography was on the average 1,42 %-units or 6,9 % greater than the oven dry matter; the values being 21,89 % and 20,47 %, respectively (Table 2).

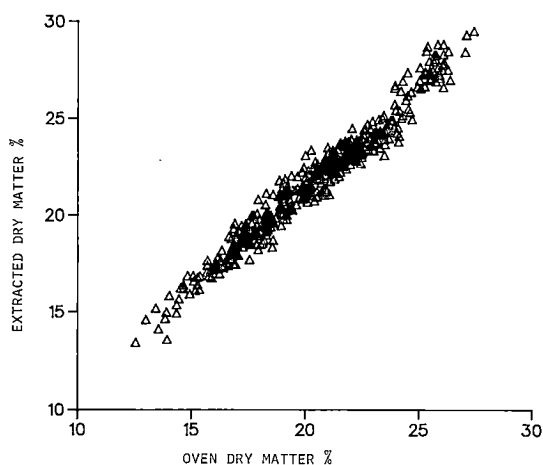


Fig. 2. Relationship between extracted dry matter and oven dry matter.  $n = 437$ .

Table 2. Dry matter values obtained by different methods, pH and the percentage concentrations of volatile compounds in fresh sample before drying.  $n = 437$ . In the case of formic acid  $n = 166$ .

	Mean	S.D.
Extracted dry matter, %	21,89	3,15
Oven dry matter, %	20,47	3,05
Difference, %-units	1,42	0,64
Total amount of volatiles, %	2,23	0,54
Loss in oven drying %-units	1,24	0,44
pH	4,19	0,40
Concentration before drying, %		
Ethanol	0,16	0,12
Formic acid	0,22	0,13
Acetic acid	0,56	0,33
Propionic acid	0,03	0,06
Butyric acid	0,04	0,15
Isovaleric plus valeric acid	0,01	0,04
Lactic acid	1,32	0,64
Ammonium nitrogen	0,03	0,04

All other volatiles were analysed from 437 silage samples except formic acid which was analysed from only 166 samples. About four out of five silage samples treated with a formic acid additive contained formic acid, which in quantity appeared to be, after lactic acid and acetic acid, together with ethanol, the most important volatile lost during oven drying. The results of 166 samples may, however, give a fairly reliable estimate of how much formic acid contributes to the difference between dry matter contents. Samples ranged in pH value from 3,70 to 5,99, with a mean of 4,19 (Table 2, Fig. 3).

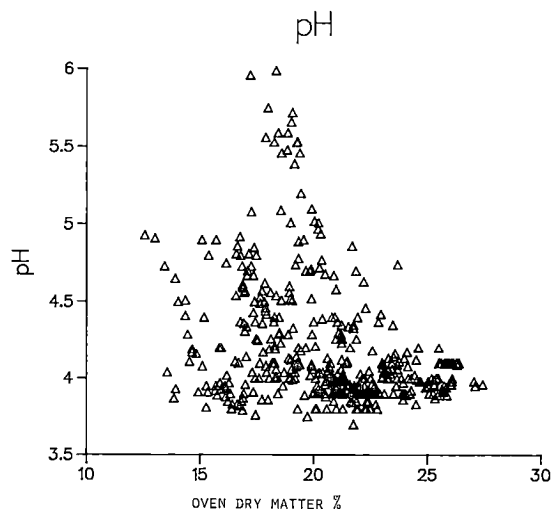


Fig. 3. Variation of pH related to oven dry matter.  $n = 437$ .

Lactic acid and acetic acid with the mean concentrations of 1,32 and 0,56 %, respectively, amounted to 84 % of the mean total concentration of analysed volatiles (2,23 %, Table 2). In the samples analysed for formic acid, the corresponding amount was 82 %. Formic acid with lactic acid and acetic acid made up 92 % of the total concentration of volatile compounds. All samples contained

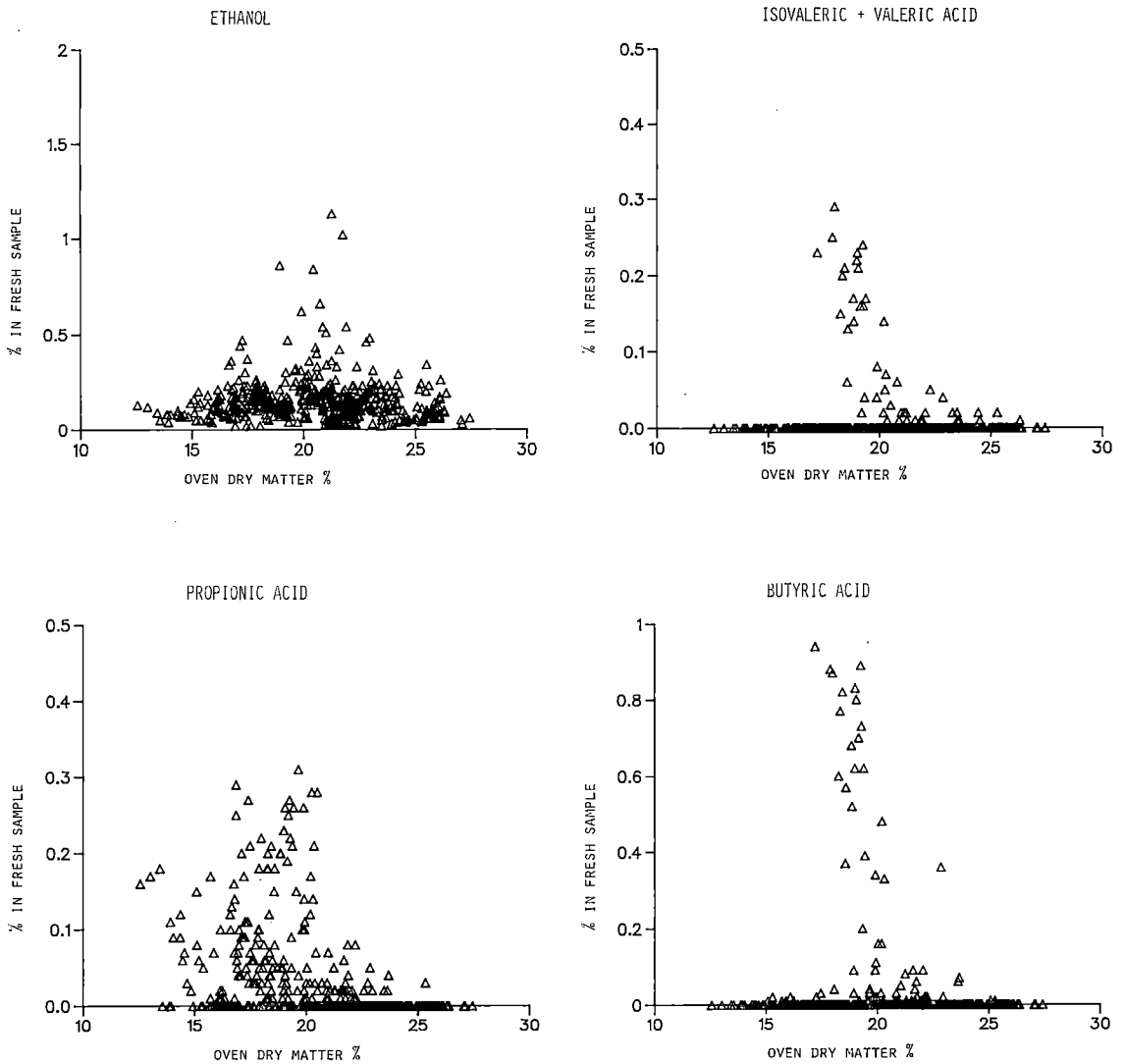


Fig. 4. The percentages of different volatiles in fresh sample in relation to oven dry matter.

ethanol (Fig. 4) but its content remained fairly low, on the average 0,16 % in fresh sample. The butyric acid content in low quality silage samples rose to nearly 1 % (Fig. 4). Butyric acid was found, however, only in every sixth sample, with a mean content of 0,04 %. Propionic acid was found in most samples, the highest concentration being 0,3 %, with a mean of 0,03 %. Isovaleric and valeric acids together were encountered only in every ninth sample, with a

maximum concentration of 0,3 % and a mean of 0,01 %. Thus, these fatty acids contributed on the average only slightly to the error in dry matter contents caused by evaporation. In some cases they may be of importance. Ammonia nitrogen was found in all samples (Fig. 4). Its average content was 0,04 % in fresh sample (Table 2).

As a consequence of strong fermentation other volatiles occur in silages besides ethanol,

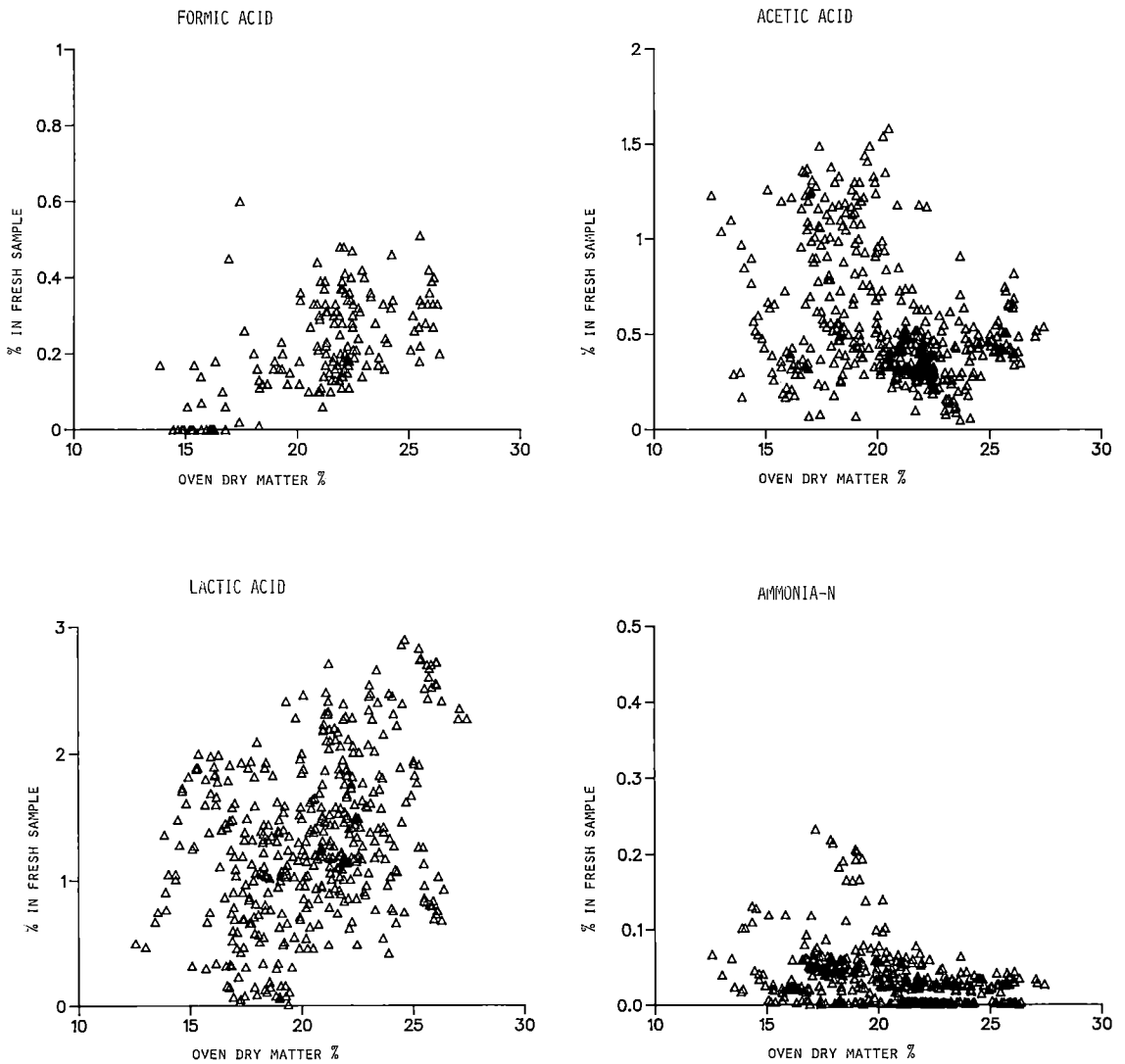


Fig. 4. Continued.

volatile fatty acids, lactic acid and ammonia nitrogen, but their content is obviously small. WEISSBACH and BERG (1977) did not find any formic acid in their samples, and the amount of the ethanol together with the lactic, acetic, propionic, butyric, isobutyric, valeric and isovaleric acids accounted for 98,3 % of the total content of volatile compounds.

The loss of fatty acids and ammonia nitrogen during oven drying has been found to be

related to the pH of the sample. When pH rises the loss of fatty acids decreases and the loss of ammonia nitrogen increases (BERG and WEISSBACH 1976, DULPHY et al. 1975, NØRGAARD PEDERSEN and MØLLER 1965). In the data of BERG and WEISSBACH (1976) which included 83 silage samples of heterogenic raw material, the pH ranged from 3,1 to 6,9. The variation of pH explained 69 % of the loss of acetic acid and 68 % of butyric acid during

oven drying for 48 h at 105 °C. Ammonia had an equal coefficient of determination of 68 %. The pH did not have a corresponding effect on the loss of lactic acid (BERG and WEISSBACH 1976, DULPHY et al. 1975). Ethanol is reported to evaporate by 99—100 %, regardless of pH (DULPHY et al. 1975, NØRGAARD PEDERSEN 1981, PORTER et al. 1984). In the treated material of 437 grass silage samples, the percentages of volatilities correlated ( $P < 0,001$ ) with pH in volatile fatty acids, lactic acid and ammonia nitrogen (Table 3).

Contrarily to the data in the literature, the correlation coefficients indicate that the loss of lactic acid and that of fatty acids were related to the pH of silage and decreased with high pH ( $r = -0,282$ ,  $P < 0,001$ ). As expected, ammonia nitrogen correlated positively with pH ( $r = 0,285$ ,  $P < 0,001$ ). Ethanol evaporated from all samples completely. In samples analysed for formic acid ( $n = 166$ ) no statistically significant correlation between volatility of formic acid and pH existed. Most of the samples in this group consisted of good quality silage with a pH value  $< 4,2$ , which obviously contributed to the results.

Table 3. The correlation between the loss of different volatiles, %, and pH. Correlation is calculated from the samples containing more than 0,0 % of a volatile.

	r	n
Ethanol	0	437
Formic acid	N.S.	146
Acetic acid	-0,212***	437
Propionic acid	-0,630***	181
Butyric acid	-0,843***	73
Isovaleric, valeric acid	-0,759***	48
Lactic acid	-0,282***	437
Ammonia nitrogen	0,285***	437

\* $P < 0,05$ , \*\* $P < 0,01$ , \*\*\* $P < 0,001$

### Classified material

Since significant correlations were found, the material was divided for further examination into three pH classes,  $pH < 4,2$ ,  $4,2 \leq pH \leq 4,5$  and  $pH > 4,5$ , according to the quality classification principle for silages suggested by WIERINGA (1966).

The average of dry matter values, determined by gas chromatography and oven drying, decreased with increasing pH (Table 4). The mean difference between dry matter values in different pH classes by oven drying was,

Table 4. The mean dry matter values obtained by different methods and the concentrations of volatiles before drying, in three pH classes.

	pH < 4,2 n = 307, formic acid n = 156		4,2 ≤ pH ≤ 4,5 n = 52, formic acid n = 5		pH > 4,5 n = 78, formic acid n = 5	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Extracted dry matter, %	22,61	3,19	20,61	2,36	19,89	2,20
Oven dry matter, %	21,26	3,02	19,16	2,30	18,24	2,04
Difference, %-units	1,36	0,61	1,44	0,73	1,65	0,59
Volatiles, total %	2,18	0,58	2,44	0,49	2,31	0,37
Lost in oven drying total %-units	1,13	0,41	1,34	0,38	1,59	0,36
pH	3,98	0,10	4,35	0,08	4,91	0,38
Concentrations in fresh sample, %						
Ethanol	0,14	0,10	0,19	0,19	0,20	0,12
Formic acid	0,23	0,13	0,03	0,04	0,10	0,05
Acetic acid	0,40	0,17	0,73	0,30	1,07	0,25
Propionic acid	0,01	0,02	0,04	0,05	0,14	0,08
Butyric acid	0,00	0,01	0,01	0,05	0,19	0,30
Isovaleric plus valeric acid	0,00	0,00	0,01	0,01	0,05	0,08
Lactic acid	1,49	0,57	1,41	0,49	0,57	0,41
Ammonia nitrogen	0,02	0,02	0,05	0,02	0,08	0,06

however, greater than by gas chromatography. Hence, the difference between dry matter values increased from 1,36 % in the low pH class to 1,65 in the high pH class. A similar change was found by HAIGH and HOPKINS (1977) and WILSON et al. (1964) in comparing dry matter values obtained by oven drying and toluene distillation.

In the middle pH class, the concentration of acetic acid differed noticeably and the concentrations of ethanol and lactic acid differed to some degree from corresponding concentrations in the low pH class. The concentration of lactic acid in the middle pH class was only 0,08 % units lower than that in the low pH class, in which the total amount of volatiles averaged 2,18 % in a fresh sample. In the middle pH class, the total amount of volatiles was highest, 2,44 %, with acetic and lactic acid constituting 88 % of it. The corresponding shares of acetic and lactic acids were 87 in the low pH class and 71 % in the high pH class, respectively.

The total mean volatility of acetic, propionic, butyric and isovaleric plus valeric acids in the whole material was 89,0 %, which agrees with the result obtained by PORTER et al. (1984) and that of WILSON et al. (1964) with grass silages (Table 5). DULPHY et al. (1975) and NØRGAARD PEDERSEN and MØLLER (1965) reported 7 and 20 %-units lower volatilities, depending partly on the temperature by oven drying, which was 20—25 °C

lower. With the very heterogenic sample material, WEISSBACH and BERG (1977) found the volatility of fatty acids to be 100 % by oven drying in two phases (Table 5).

In the present material, the volatility of fatty acids was on an average in the whole data and in pH classes equal to the volatility of acetic acid (Table 5 and 6) or it was 88,8 %, which deviates only one per cent unit from the volatility of acetic acid found by McDONALD and DEWAR (1960) with grass silages (Table 5). The variation of volatility with rising pH was small compared to that presented by BERG and WEISSBACH (1976), DULPHY et al. (1975) and NØRGAARD PEDERSEN and MØLLER (1965), who reported that the loss of volatile fatty acids decreases 30—40 %-units from a low pH < 4,0 to a high pH > 4,8 (Table 6).

Like the other fatty acids, formic acid evaporated for the most part, on the average 92,0. PORTER et al. (1984) suggested a correction factor of 0,67 for formic acid in fresh sample by oven drying at 100 °C 16 h, which corresponds to a 25 % lower volatility compared to our results.

Some ammonia nitrogen was found in many samples after oven drying. Its volatility averaged 84,9 %, being 15 %-units lower than reported by PORTER et al. (1984) and WEISSBACH and BERG (1977), but 23 %-units higher than the value found by DULPHY et al. (1975) (Table 5). Volatility increased by 10 per cent

Table 5. The mean volatility of different compounds in silage by oven drying.

Ethanol	Formic acid	Acetic acid	Propionic acid	Butyric acid	Isovaleric plus valeric acid	Volatile fatty acids	Lactic acid	Amm-N	Oven drying	References
100	92,0	88,8	94,9	91,8	88,1	89,0	27,6	84,9	2—4 h at 80 °C then 15 h at 105 °C	Present material
90						8			48 h at 105 °C	BERG & WEISSBACH 1976
100						83,0	14,2	61,6	48 h at 105 °C	DULPHY et al. 1975
		87,9		89,4		8,7			18 h at 100 °C	McDONALD & DEWAR 1960
						32,5			22 h at 103 °C	MO & Tjørnholm 1978
						70,2	4		20 h at 80 °C	NØRGAARD PEDERSEN & MØLLER 1965
99	67					89	41	100	16 h at 100 °C	PORTER et al. 1984
100						100	8	100	16 h at 100 °C	WILSON et al. 1964

Table 6. The variation of the loss of volatiles with different pH by oven drying. Temperature and time by oven drying are the same as in Table 4.

pH	Ethanol n	Formic acid n	Acetic acid n	Propionic acid n	Butyric acid n	Isovaleric plus vale- ric acid n	Volatile fatty acids n	Lactic acid n	Ammonia-N n	References									
< 4,2	100,0	307	91,8	138	89,0	307	99,7	64	99,7	33	100,0	9	89,1	307	31,2	307	82,8	307	Present material
4,2 ≤ pH ≤ 4,5	100,0	51	93,3	3	92,7	52	97,4	40	98,5	6	100,0	10	93,1	52	24,3	52	85,3	52	
> 4,5	100,0	78	98,3	5	85,4	78	89,5	77	83,0	34	80,3	29	86,0	78	15,6	78	92,9	78	
< 4,0	90				95		84						94		8				BERG & WEISSBACH 1976
4,5—5,0	90				74		72						75		8		47		
> 5,0	90				48		57						52		8		74		
3,8	100												92,4		14,2		43,2		DULPHY et al. 1975
4,4	100												78,1		14,2		71,3		
5,0	100												63,8		14,2		99,5		
3,15—4,00													80,86						NØRGAARD PEDERSEN & MØLLER 1965
4,01—4,75													73,44						
4,76—7,23													52,47						

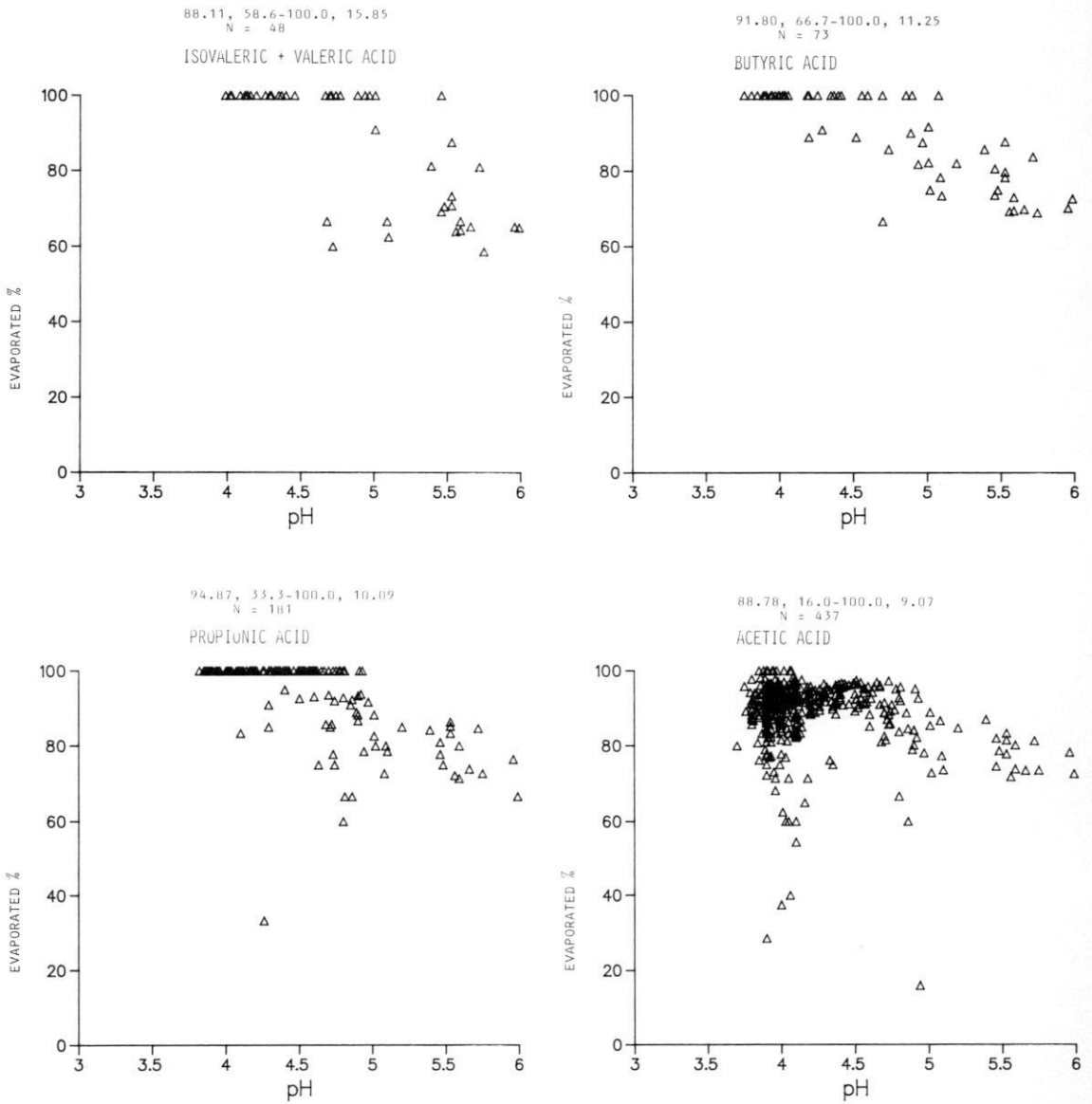


Fig. 5. The volatility of different compounds by oven drying in relation to pH. The mean volatility, its range and standard deviation are mentioned above the figure.

units from the lowest pH class to the highest (Table 6). The pH did not affect the volatility of ammonia nitrogen as much as reported by BERG and WEISSBACH (1976) and DULPHY et al. (1975). The total loss of ammonia nitrogen in the present study was on the average greater than that in their data (Table 5).

The loss of lactic acid averaged 27,6 % in the whole data (Table 5). The volatility of lactic acid decreased relatively strongest with rising pH, which was not demonstrated by BERG and WEISSBACH (1976) and DULPHY et al. (1975). The lactic acid content of silages of good quality averaged 1,49 % in a fresh sample, from



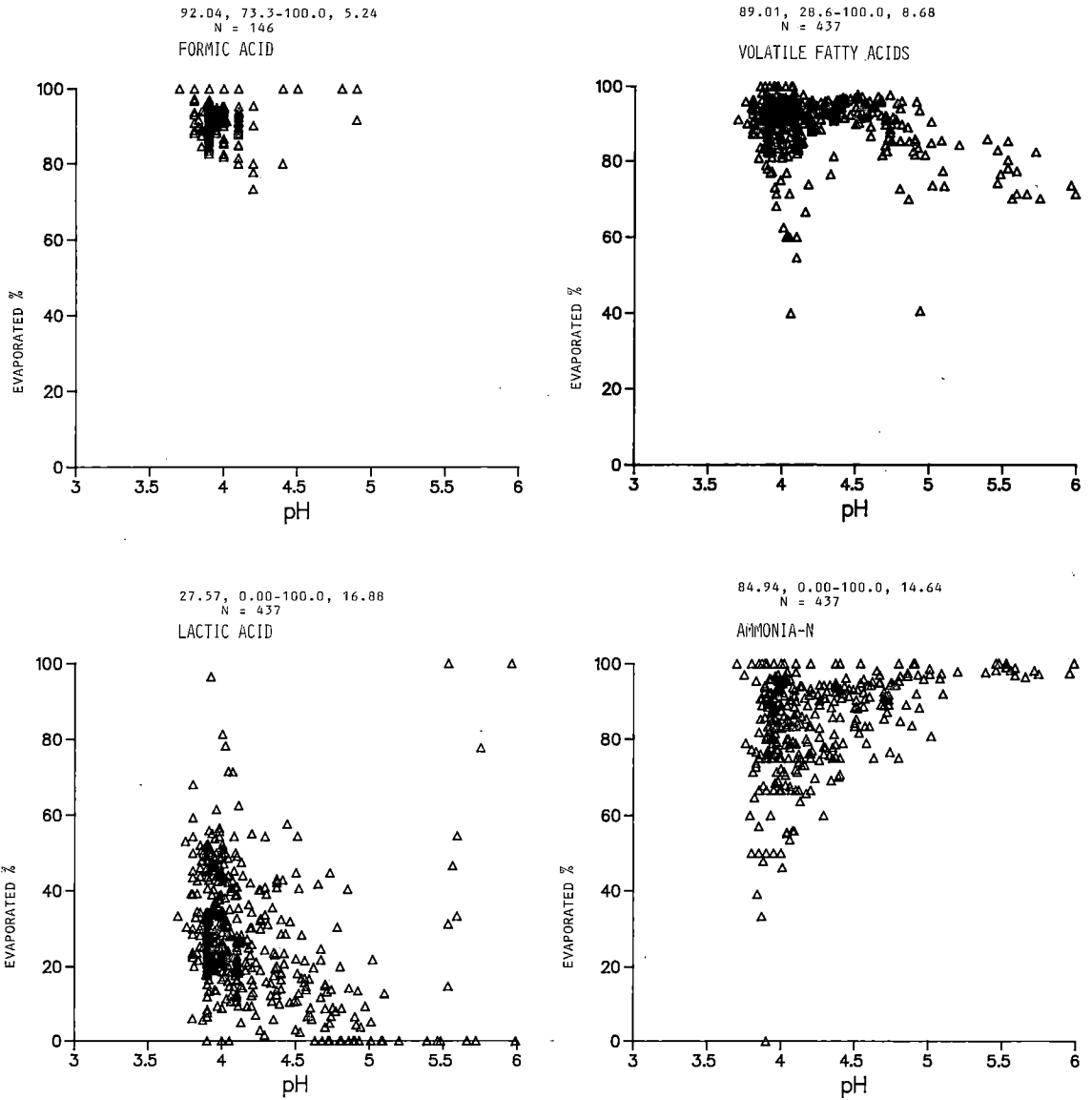


Fig. 5. Continued.

which 31,2 % volatilized. Silages of medium quality still contained 1,41 % lactic acid, with a mean volatility of 24,5 %. The mean content of lactic acid in silages of poor quality was considerably lower, 0,57 % in a fresh sample and volatility was 15,6 %, only a half of that in the low pH class (Table 6). The variation of

the volatility of different compounds by oven drying in relation to pH is shown in Figure 5.

Presuming that the amounts of analysed compounds cover all volatiles lost during oven drying, the oven dry matter value plus the sum of lost volatiles should equal the dry matter value obtained by gas chromatography. By

Table 7. Comparison of dry matter values obtained by gas chromatography with oven dry matter values corrected for the amount of lost volatiles. n = 437.

	pH < 4,2		4,2 ≤ pH ≤ 4,5		pH > 4,5		pH < 4,2, samples analysed for formic acid n = 156	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Extracted dry matter, %	22,61	3,19	20,61	2,36	19,89	2,20	22,52	3,00
Oven dry matter plus volatiles, %	22,39	3,12	20,51	2,29	19,83	2,08	22,31	2,98
Difference	0,22	0,54	0,10	0,64	0,06	0,58	0,21	0,47

adding the amount of lost volatiles to the oven dry matter value, on the average 0,22, 0,10 and 0,06 %-units lower dry matter values were obtained than by the gas chromatographic method (Table 7). In the lowest pH class the mean difference, 0,22 %-units, was equal for the whole data and for the group of silages which were analysed for formic acid.

The mean difference between the corrected oven dry matter and extracted dry matter was relatively small and decreased with rising pH. The variation of the difference was, however, large (Fig. 6).

The errors in dry matter value due to losses of different volatiles are avoided by employing a method which determines the water content of silage directly. In this way inaccuracies and

errors, caused by the correction of oven dry matter with mean losses of different compounds can be avoided. The correction factors include the variation of volatility and are not necessarily valid in individual cases. The errors in gas chromatographic analysis depend on failures in extraction of water and in measurement, and can thus be controlled. The complete extraction of water from silages of different raw material is a subject for further study. The gas chromatographic method is, however, too laborious compared to oven drying for dry matter analysis. Therefore estimates for correction factors which enable the use of oven drying in dry matter analysis are suggested.

### The correction of oven dry matter

Several means for the correction of oven dry matter have been suggested (DULPHY et al. 1975, EKERN 1972, LINGVALL and ERICSSON 1981, NØRGAARD PEDERSEN 1981, PORTER et al. 1984, WEISSBACH and BERG 1977). The method in use in Finland corrects the oven dry matter with volatility factors by adding to the value 80 % of a concentration of acetic acid and 100 % of propionic, butyric, isovaleric and valeric acids (JARL and HELLEDAY 1948, NORDFELDT 1955, ULVESLI and BREIREM 1960, Table 8, equation 1). This correction is limited and underestimates dry matter values of silages in the low pH class which contain high concentrations of lactic acid and formic acid

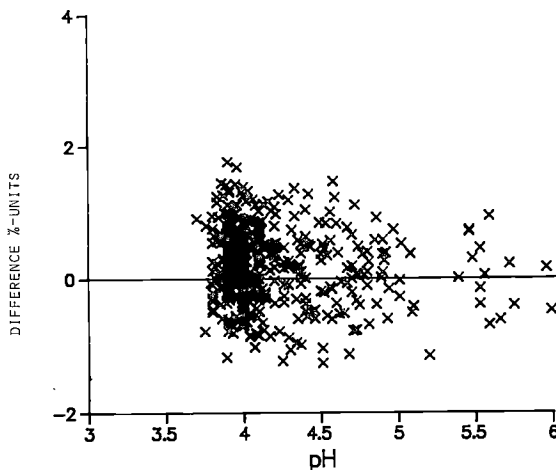


Fig. 6. The difference between dry matter obtained by gas chromatography and oven dry matter corrected for the amounts of lost volatiles in relation to pH. n = 437.

Table 8. The correction of oven dry matter value with the percentages of different lost volatiles in fresh sample.

The correction used at present:

$$1) \text{ ODM} + 0,8 \times \text{AA} + 100 \times (\text{PA} + \text{BA} + \text{IVA})$$

The correction according to separate volatility percentages of single volatiles in different pH classes:

$$\begin{aligned} 2a) \text{ pH} < 4,2: & \text{ ODM} + \text{E} + 0,9178 \times \text{FA} + 0,8877 \times \text{AA} + 0,9974 \times \text{PA} + 0,9966 \times \text{BA} + \text{IVA} + 0,3116 \times \text{LA} + 0,8284 \times \text{AN} \\ 2b) 4,2 \leq \text{pH} \leq 4,5: & \text{ ODM} + \text{E} + 0,9333 \times \text{FA} + 0,9268 \times \text{AA} + 0,9742 \times \text{PA} + 0,9849 \times \text{BA} + \text{IVA} + 0,2428 \times \text{LA} + 0,8529 \times \text{AN} \\ 2c) \text{ pH} > 4,5: & \text{ ODM} + \text{E} + 0,9833 \times \text{FA} + 0,8537 \times \text{AA} + 0,8950 \times \text{PA} + 0,8299 \times \text{BA} + 0,8033 \times \text{IVA} + 0,1564 \times \text{LA} + 0,9293 \times \text{AN} \end{aligned}$$

The correction according to volatility percentages in different pH classes; common mean volatility percentage for acetic, propionic, butyric, isovaleric and valeric acids:

$$\begin{aligned} 3a) \text{ pH} < 4,2: & \text{ ODM} + \text{E} + 0,9178 \times \text{FA} + 0,8907 \times (\text{AA} + \text{PA} + \text{BA} + \text{IVA}) + 0,3116 \times \text{LA} + 0,8284 \times \text{AN} \\ 3b) 4,2 \leq \text{pH} \leq 4,5: & \text{ ODM} + \text{E} + 0,9333 \times \text{FA} + 0,9313 \times (\text{AA} + \text{PA} + \text{BA} + \text{IVA}) + 0,2428 \times \text{LA} + 0,8529 \times \text{AN} \\ 3c) \text{ pH} > 4,5: & \text{ ODM} + \text{E} + 0,9833 \times \text{FA} + 0,8600 \times (\text{AA} + \text{PA} + \text{BA} + \text{IVA}) + 0,1564 \times \text{LA} + 0,9293 \times \text{AN} \end{aligned}$$

The correction according to the mean percentage of volatility obtained from the whole data:

$$4) \text{ ODM} + \text{E} + 0,9204 \times \text{FA} + 0,8901 \times (\text{AA} + \text{PA} + \text{BA} + \text{IVA}) + 0,2757 \times \text{LA} + 0,8494 \times \text{AN}$$

ODM = Oven dry matter

E = Ethanol

FA = Formic acid

AA = Acetic acid

PA = Propionic acid

BA = Butyric acid

IVA = Isovaleric plus valeric acid

LA = Lactic acid

AN = Ammonia nitrogen

Table 9. The percentages of different volatiles of the total lost amount by oven drying.  $n = 156$ ,  $\text{pH} < 4,2$ , all samples are analysed for formic acid.

	Mean	n <sup>*)</sup>
Ethanol	12,7	156
Formic acid	23,0	138
Acetic acid	31,3	156
Propionic acid	1,7	29
Butyric acid	1,6	21
Isovaleric + valeric acid	0,7	1
Lactic acid	34,7	156
Ammonia nitrogen	0,4	156

<sup>\*)</sup> only those samples containing the volatile in question are involved.

that originate in additives (Table 9 and 10). Disregarded amounts of ethyl alcohol increase underestimation further. Ammonia nitrogen, on the other hand, is small in magnitude.

To find a reliable means for correction, dry matter was calculated by three different formulas (Table 8):

1) according to separate percentages of volatility for every single volatile in different pH classes: equations 2a—2c.

2) as above, but replacing the separate percentages of volatility of acetic, propionic, butyric, isovaleric and valeric acids by their common percentage of volatility: equations 3a—3c.

3) using the mean percentages of volatility obtained from the whole data: equation 4.

Dry matter values calculated according to the formula 1 (equations 2a—2c, Table 8) corresponded well to those obtained by the gas chromatographic method (Table 10). Corrected oven dry matter explained 96,73 % of the variation of extracted dry matter, the correlation being 0,984 ( $P < 0,001$ ) (Table 11). Treating acetic, propionic, butyric, isovaleric and valeric acids together, by the second formula, neither affected the mean corrected dry matter values (Table 10) nor weakened the explained degree in regression (Table 11). Therefore equations 3a—3c and 2a—2c are equally suitable for the correction of oven dry matter. Use of equations containing eight factors, does not cause problems when calcu-

Table 10. Correction of oven dry matter by volatile percentages by different formulas and the difference between corrected values and dry matters measured by gas chromatography in pH classes.

	$\text{pH} < 4,2$ $n = 307$		$4,2 \leq \text{pH} \leq 4,5$ $n = 52$		$\text{pH} > 4,5$ $n = 78$	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
1) Present correction	21,59	3,02	19,86	2,19	19,47	2,14
Correction by volatility percentages in pH classes:						
2) Separate %	22,35	3,09	20,48	2,31	19,84	2,14
3) Common %	22,35	3,09	20,48	2,31	19,85	2,15
4) Correction by mean volatility %	22,29	3,08	20,49	2,32	19,96	2,15
5) Extracted dry matter	22,61	3,19	20,61	2,36	19,89	2,20
Differences:						
5-1	1,03	0,57	0,80	0,63	0,41	0,63
5-2	0,26	0,55	0,13	0,60	0,04	0,60
5-3	0,27	0,55	0,13	0,60	0,03	0,60
5-4	0,32	0,55	0,12	0,60	0,07	0,59

Table 11. Oven dry matter values corrected by different ways as explainers of gas chromatographic dry matter.  $n = 437$

	S.D. of estimate	r	r <sup>2</sup> %
$y = 1,020^*$ (correction equation 2a-2c) -0,215	+0,57	0,984	96,73
$y = 1,020^*$ (correction equation 3a-3c) -0,218	+0,57	0,983	96,72
$y = 1,026^*$ (correction equation 4) -0,340	+0,58	0,983	96,59

lating with an electronic computer, on the other hand, manual calculation may favor the use of simple equations 3a—3c. Bearing in mind the pH of the sample in selecting volatility factors is more reliable than correction by mean factors in the whole data (formula 3, Tables 8 and 10). The differences between correction methods are quite small, because the mean

volatilities do not noticeably differ, excluding lactic acid. The deviation of lactic acid is, however, clear and while lactic acid together with acetic acid are, in quantity, the most important factors contributing to correction, it is thus important to take into consideration the variations in their volatilities (formulas 1 and 2).

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## SELOSTUS

### Uunikuivatuksella ja kaasukromatografisella vesianalyysillä määritettyjen nurmisäilörehujen kuiva-ainepitoisuuksien vertailu.

LEA HUIDA, HANNA VÄÄTÄINEN ja MARTTI LAMPILA

Maatalouden tutkimuskeskus

Kaasukromatografisella vesianalyysillä määritettyä 437 säilörehunäytteen kuiva-ainetta verrattiin uunikuivatuksella 2—4 t 80 °C ja sen jälkeen 15 t 105 °C saatuun kuiva-aineeseen. Uunikuivatuksella saatiin keskimäärin 1,42 %-yksikköä alhaisempia kuiva-ainepitoisuuksia kuin kaasukromatografisella menetelmällä. Erotus kasvoi pH:n kohotessa. Eron syiden selvittämiseksi haihtuvien yhdisteiden pitoisuudet määritettiin näytteistä sekä ennen kuivausta että kuivauksen jälkeen. Muurahaishappopitoisuuden analysoinnissa käytetty kaasukromatografinen menetelmä on selostettu.

Etikka-, propioni-, voi-, isovaleriaana- ja valerianahappojen ja myös maitohapon haihdunnan havaittiin olevan suhteessa näytteen pH:hon ja heikkenevän pH:n kohotessa. Ammoniumtyypen haihduntaan pH:lla oli päinvastainen vaikutus. Muurahaishappo analysoitiin vain 166 näytteestä, joista 156:ssa pH oli alle 4,2, joten pH:n vaikutus ei tullut selvästi esiin. Etanoli haihtui kaikista näytteistä 100 %:sesti pH:sta riippumatta. pH-luokittaisessa tarkastelussa (luokat: pH < 4,2, 4,2 < pH < 4,5, pH > 4,5) havaittiin maitohapon haihdunnan heikkenevän suhteellisesti voimakkaimmin pH:n kohotessa, haihdunta-%:ien ollessa keskimäärin eri pH-luokissa 31,2, 24,3 ja 15,6 %. Lukuunottamatta muurahaishappoa, haihtuvien rasvahappojen haihdunnan yhteismäärä noudatteli eri pH-luokissa etikkahapon keskimääräistä haihduntaa ja oli alimmasta pH-luokasta kor-

keimpaan 89,1, 93,1 ja 86,0 %. Muurahaishappo haihtui keskimäärin yli 90 %:sesti kaikissa pH-luokissa. Ammoniumtyypen haihdunta voimistui pH:n kohotessa 82,8 %:sta 85,3 %:iin ja edelleen 92,9 %:iin.

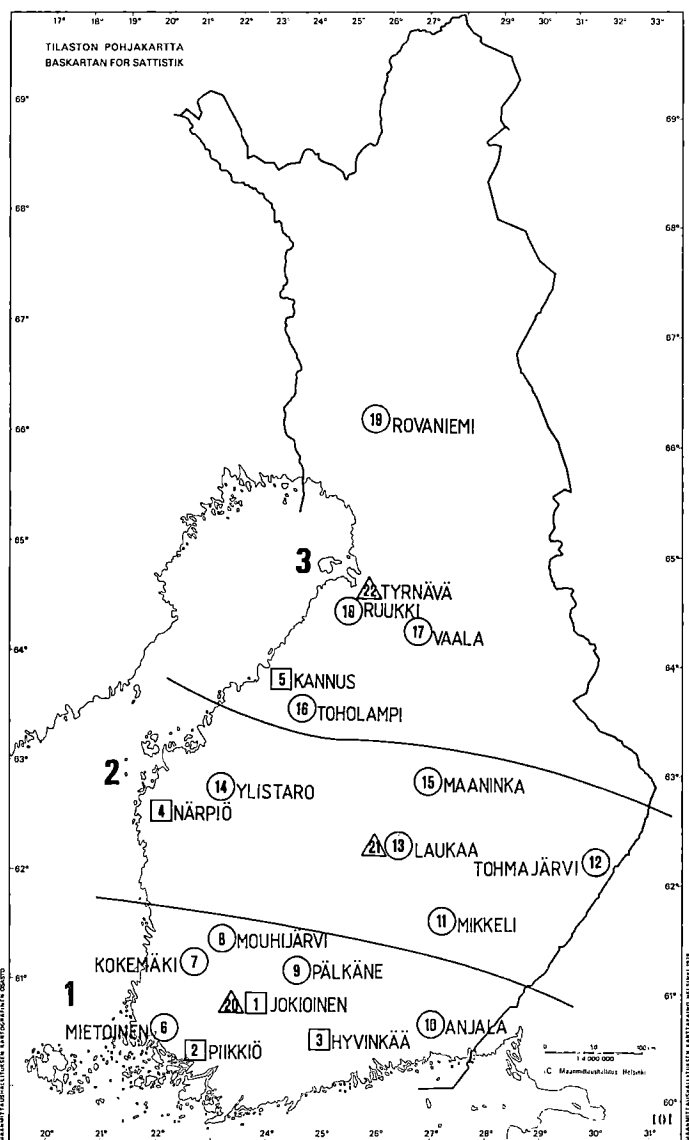
Määrällisesti tärkeimmät uunikuiva-ainepitoisuuden virheeseen vaikuttavat yhdisteet olivat etikka- ja maitohappo, jotka yhdessä muodostivat näytteissä pH ≤ 4,5 87 % tuoreen näytteen haihtuvien aineiden kokonaismäärästä. Näytteissä pH > 4,5 vastaava osuus oli 71 %, mikä merkitsee muiden haihtuvien rasvahappojen ja ammoniumtyypen merkityksen korostumista pH:n kohotessa.

Haihtuneen ainemäärän ja uunikuivauksella saadun kuiva-ainepitoisuuden summa vastasi kaikissa pH-luokissa melko hyvin kaasukromatografisella menetelmällä saatua kuiva-ainepitoisuutta, mikä osoitti kaasukromatografisen menetelmän melko luotettavasti mittaavan näytteen vesipitoisuuden. Summan ja vesimäärityksellä saadun kuiva-ainepitoisuuden välisessä erossa oli kuitenkin melko laajaa vaihtelua.

Eri yhdisteiden haihdunta-%:hin perustuen esitetään uunikuiva-ainepitoisuudelle korjausyhtälöt, joissa on haihtuvien rasvahappojen lisäksi otettu huomioon maitohappo, etanoli sekä ammoniumtyyppi. Korjausyhtälöt on esitetty pH-luokittain erityisesti maitohapon haihdunnan pH-sidonaisuuden takia.

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