Annales Agriculturae Fenniae

Maatalouden
tutkimuskeskuksen
aikakauskirja

Vol. 5, 4

Journal of the
Agricultural
Research
Centre

Helsinki 1966

ANNALES AGRICULTURAE FENNIAE

Maatalouden tutkimuskeskuksen aikakauskirja
Journal of the Agricultural Research Centre

Ilmestyy 4—6 numeroa vuodessa; ajoittain lisänidoksia
Issued as 4—6 numbers yearly and occasional supplements

SARJAT — SERIES

Agrogeologia, -chimica et -physica
— Maaperä, lannoitus ja muokkaus
Agricultura — Kasvinviljely
Horticultura — Puutarhanviljely
Phytopathologia — Kasvitaudit
Animalia domestica — Kotieläimet
Animalia nocentia — Tuhoeläimet

JAKELU JA VAIHTOTILAUKSET
DISTRIBUTION AND EXCHANGE

Maatalouden tutkimuskeskus, kirjasto, Tikkurila
Agricultural Research Centre, Library, Tikkurila, Finland

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 279—297 (1966)
Seria ANIMALIA DOMESTICA N. 21 — Sarja KOTIELÄIMET n:o 21

SYSTEMATIC DIFFERENCES IN THE COMPOSITION OF THE BOVINE RUMEN FLUID BETWEEN DIFFERENT PARTS OF THE RUMEN

MARTTI LAMPILA and ESKO POUTIAINEN
Agricultural Research Centre, Department of Animal Husbandry, Tikkurila, Finland

Received September 8, 1966

The heterogeneity of the rumen contents has been observed repeatedly in studies on the physiology of the rumen. It has attracted interest mainly because of the consequent difficulty in obtaining samples representative of the entire rumen contents.

When average values representing conditions in the reticulo-rumen are needed, it seems, according to BRYANT's (1964) studies on pH and volatile fatty acids (VFA), that in the case of cows on pasture the values yielded by samples from the central part of the rumen may be used. DAVEY (1965) came to the same conclusion as regards the concentration of volatile fatty acids in the rumen contents of cows on pasture.

Between the upper and lower parts (dorsal and ventral parts) of the contents BRYANT (loc. cit.) observed regular differences in pH and VFA concentration of the same order of magnitude as were found by LAMPILA (1964) in cows on indoor feeding. Both on pasture and at indoor feeding, according to the results presented by the latter (LAMPILA 1955, 1964), the pH of the contents in the upper part of the rumen seems to exhibit a strong tendency to fall far below the level which is optimal for the microbial flora; weakening of the fermentation and of microbial protein synthesis is therefore likely under certain feeding conditions. The stratification and its consequences may thus be of considerable significance from the physiological point of view as well.

LAMPILA (1964, p. 64) has considered some of the causes responsible for the heterogeneity. He calls attention to the fact that the free fluid in the rumen serves to extract and carry the substances present or formed in the solid ingesta to the absorption surface. For such transport to take place, the concentration of the substance to be transported must be lower in the free fluid than in that retained by the food particles. Since it is known that the »reservoir» of free fluid is located in the lower part of the rumen, the difference in the ratios of free fluid and solids accounts for the difference in the concentration of volatile fatty acids observed between the upper and lower parts of the rumen contents. The continuous influx of abundant saliva, together with imbibed water, may also contribute to the generation of differences in concentration, depending on the rate of influx and on the mixing of the fluid with the rumen contents.

As a result of the numerous factors simultaneously influencing the stratification and its consequences, particularly the mixing, which tends to cancel out the effect of stratification, the het-

10073—66

erogeneity often appears to be quite random. It is thus only when comparatively large numbers of samples are available that one can attempt to decide whether there is any consistency in the differences observable between different parts of the rumen contents.

During the last few years we have carried out studies on samples collected from various parts of the rumen contents. In the present paper we shall therefore present a compilation of the results illustrating the differences between different parts of the contents, with reference to those determinations which are most numerously represented in our data.

Experimental procedures

Experimental animals and their feeding

As experimental animals, altogether four Ayrshire cows with rumen fistulas were used, whose live weights ranged from 505 to 546 kg when the animals were in normal condition. Fistulation was made according to STODDARD et al. (1951). The experiments concerning the distribution of polyethylene glycol, potassium and sodium were performed with two cows, and the other studies were made with two other cows employed previously.

Throughout the experiments the animals were on indoor feeding. They were fed twice a day, the daily ration being given in two equal parts at 12-hour intervals. Water was freely available.

The rations mostly consisted of various combinations of hay and concentrate mixture. The amount of hay given daily varied from 3.6 to 11.2 kg and the amount of concentrate mixture from 0 to 9.3 kg. The amount of hay was highest when hay was the sole feed. In some experiments grass silage was also included in the feed and in others, beets.

Dosage of polyethylene glycol

The reference substance used in this study was »Polyaethylenglykol 4 000 pract.», manufactured by Fluka A. G. It was administered in aqueous solution into the rumen through the fistula by means of a funnel with an extension consisting of a perforated metal tube, which helped to distribute the dose uniformly over the rumen contents. The dose varied from 200 to 300 g, and was administered one hour prior to the first sampling, which was done immediately before feeding was commenced.

Sampling and preparation of samples

Samples were taken at regular intervals at periods covering the time between two feeds, usually from four different points in the rumen contents. One of these points was in the upper part of the contents close to the fistula aperture, the second approximately in the centre of the ingesta, and the other two were located on the floor of the rumen in the dorsal sac and in the ventral sac, respectively.

The samples representing the upper part of the contents were taken at a point 10—20 cm cranially of the fistula opening. From a layer about 15 cm in thickness below the surface of the ingesta solid matter was taken with forceps, and the fluid expressed from it by hand immediately upon removal was used for the sample.

The sampling point representing the central part of the contents was located in the ventral region of the rumen, in the central longitudinal plane of the animal's body and approximately half-way between the surface of the ingesta and the floor of the rumen. In side view, this point approximately coincided with the lower end of the 13th rib (see SISSON and GROSSMAN 1956, p. 461, Fig. 390). Solid ingesta were taken from this point with a pair of forceps having long, fluted jaws. Matter from the upper layers was also caught between the jaws, but such matter was discarded. The fluid expressed from the solid ingesta was used.

The sampling point representing the lower part of the rumen was located on the floor of the ventral part, in the central longitudinal plane of the animal and in the same transverse plane as the central sampling point described above.

Fluid samples from this point were obtained by means of a brass tube (7/8 in. dia.) having two 1-cm holes about 5 cm from its lower end. The tube was fitted with a rubber plunger, which could be moved by means of a rod to open or close the holes. The holes were opened after the tube had been kept at the sampling point for a short while.

The fourth sampling point, i.e., the point representing the lower fore part of the rumen, was located on the floor of the dorsal sac between the reticulum and the anterior pillar *(Pila cranialis;* see SISSON and GROSSMAN 1956, p. 460, Fig. 389a). This point was located by probing with the sampling tube. Fluid samples were taken with the sampling tube described above.

The samples were cooled, immediately after collection, by immersing the beakers in cold water. Short-time storage was in a refrigerator at +4° C, and prolonged storage in a deep-freezer. Prior to analysis, and likewise before prolonged storage, the solid matter was separated by centrifuging the samples for 20 minutes at 4 000 r.p.m. with a Wifug centrifuge, type H. In the volatile fatty acid determinations some of the samples were clarified by allowing them to stand so that the solid matter settled.

pH measurements of the rumen contents

The pH was measured by an *in vivo* method as described by LAMPILA (1964). The measuring instrument has been described by LAMPILA (1955). Measurements were made at the same points from which samples of the rumen contents were withdrawn.

Analytical methods

P E G. — The polyethylene glycol determinations were made according to HYDEN's (1956) method. A nephelometer (Nephelometer Head of EEL, with Unigalvo Type 20 Galvanometer) was used for measuring turbidities.

P o t a s s i u m. — Potassium was determined with a flame photometer directly from the diluted rumen fluid after clarification by centrifuging.

S o d i u m. — The sodium concentration was determined electrometrically from the clarified rumen fluid, employing Beckman's Na electrode and a Model 76 pH meter with expanded scale (POUTIAINEN and LAMPILA 1966).

NH_3. — CONWAY's (1957) microdiffusion method was used for the determination of ammonia. The ammonia was liberated from a 1 ml sample in the »standard unit» by adding 1 ml of saturated potassium carbonate solution. The dishes were kept for three hours at room temperature (about 20° C) and the ammonia bound by the boric acid was then titrated with 0.01 N sulphuric acid.

V F A. — The amount of total volatile fatty acids was determined by FRIEDEMANN's (1938) steam distillation method as described earlier (LAMPILA 1964, p. 29).

Results and discussion

Distribution of PEG in the rumen

The concentrations of polyethylene glycol in the central, lower and lower fore part of the rumen are presented in Figs. 1—3, respectively, as ratios of the value found simultaneously in the upper part of the rumen. The linear regressions of the ratios on the concentration of PEG in the upper part as well as the correlation between the two variables in each case were calculated; the results are presented in Table 1. Tests revealed that the deviation of the regressions from linearity was not statistically significant in any instance ($P > 0.05$).

As can be seen from the values in Table 1, the ratio turned out to be independent of the concentration of PEG in the upper part, and comparisons of the concentrations in the different parts may therefore be made by comparing the means of the ratios. These means and the findings concerning the statistical significance of their differences have been entered in Table 2.

Table 1. Results of calculations concerning relationships between the concentrations in different parts of the rumen contents. [1])

Taulukko 1. Pötsin sisällön eri osien konsentraatioiden keskeistä riippuvuutta koskevien laskelmien tulokset [1])

Object of investigation	Part of the rumen contents	Degrees of freedom	Correlation coefficient	Constant term ± S. E.	Regression coefficient ± S. E.	t-value and statistical significance [2])
PEG	Central part	268	0.043	0.972 ± 0.007	—0.0000037 ± 0.0000053	0.71
	Lower part	268	0.068	0.927 ± 0.008	—0.0000071 ± 0.0000063	1.12
	Lower fore part	268	0.084	0.928 ± 0.010	—0.0000102 ± 0.0000074	1.38
Na	Central part	268	0.002	1.003 ± 0.004	0.0000013 ± 0.0000403	0.03
	Lower part	268	0.036	0.990 ± 0.008	0.0000419 ± 0.0000714	0.59
	Lower fore part	268	0.157**	0.966 ± 0.011	0.0002606 ± 0.0001002	2.60**
K	Central part	268	0.263***	0.951 ± 0.006	0.000532 ± 0.000119	4.47***
	Lower part	268	0.326***	0.898 ± 0.009	0.001032 ± 0.000182	5.65***
	Lower fore part	268	0.312***	0.898 ± 0.010	0.001061 ± 0.000198	5.37***
VFA	Lower part	49	—0.480***	1.008 ± 0.081	—0.00225 ± 0.00059	3.83***
	Lower fore part	39	—0.519***	1.095 ± 0.113	—0.00313 ± 0.00083	3.79***
NH_3	Lower part	170	0.830***	2.000 ± 0.021	0.645 ± 0.033	19.39***
pH	Central part	217	0.801***	3.231 ± 0.150	0.496 ± 0.025	19.70***
	Lower part	217	0.560***	4.396 ± 0.193	0.324 ± 0.033	9.96***
	Lower fore part	217	0.541***	4.473 ± 0.207	0.331 ± 0.035	9.48***

[1]) The independent variable (x) is in all instances the concentration in the upper part of the contents. The value of the dependent variable (y) is obtained as relative concentration with respect to the value of the independent variable, except for ammonia and pH, where the absolute values are given.

[2]) *** : $P < 0.001$
** : $P < 0.01$

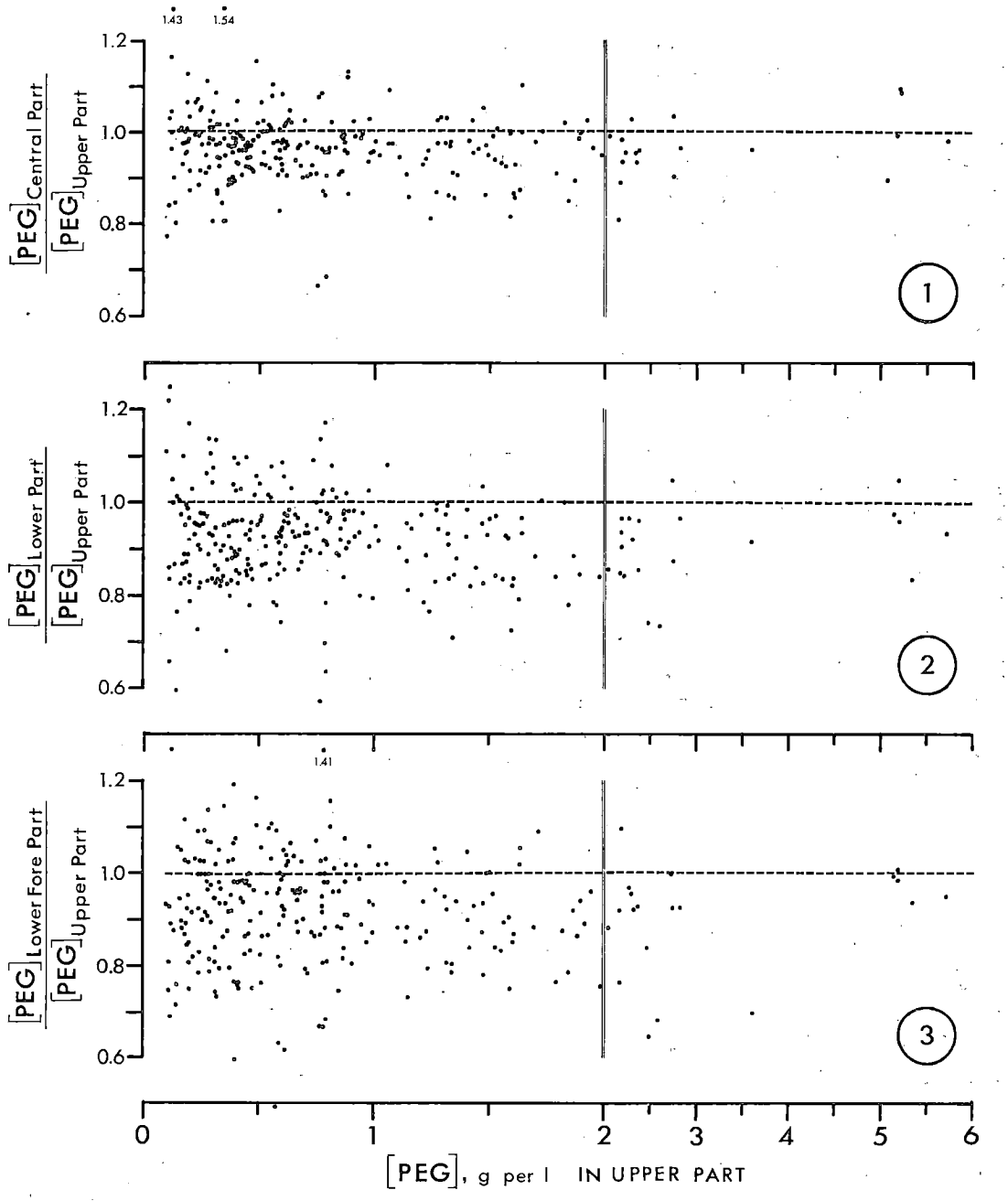
Table 2. Mean relative concentrations in different parts of the rumen contents as determined by simultaneous comparison, expressed in relation to the concentrations in the upper part of the contents [1])

Taulukko 2. Keskimääräiset suhteelliset konsentraatiot pötsinsisällön eri osissa määritettynä parivertailulla yläosan konsentraatiota vertailukohteena käyttäen [1]).

Object of investigation	No. of observations per part	Average value of the ratio ± S. E. [1])				Statistical significance of difference by t-test [2])
		Upper part (U)	Central part (C)	Lower part (L)	Lower fore part (LF)	
PEG	270	1.000	0.969 ± 0.005	0.920 ± 0.006	0.919 ± 0.007	U > C*** C > L*** U > L*** C > LF*** U > LF*** L > LF
Na	270	1.000	1.003 ± 0.001	0.995 ± 0.002	0.993 ± 0.003	U < C** C > L*** U > L** C > LF*** U > LF** L > LF
K	270	1.000	0.974 ± 0.003	0.942 ± 0.004	0.943 ± 0.004	U > C*** C > L*** U > L*** C > LF*** U > LF*** L < LF
VFA	51	1.000		0.698 ± 0.009		U > L***
	41	1.000			0.668 ± 0.013	U > LF***
pH	219	5.93 ± 0.030	6.17 ± 0.011	6.32 ± 0.014	6.43 ± 0.015	U < C*** C < L*** U < L*** C < LF*** U < LF*** L < LF***

[1]) The figures for pH refer to absolute, and not relative values

[2]) *** : $P < 0.001$
** : $P < 0.01$



Figs. 1—3. Relative concentration of polyethylene glycol (PEG) in the rumen fluid in the central part of the contents (Fig. 1), in the lower part (Fig. 2) and in the lower fore part (Fig. 3), plotted against the simultaneous absolute concentration in the fluid from the upper part of the rumen contents.

Kuvat 1—3. Polyetylenglykolin (PEG) suhteellinen konsentraatio pötsinesteessä sisällön keskiosassa (1), alaosassa (2) ja etualaosassa (3) verrattuna yläosan samanaikaiseen konsentraatioon.

The mean values reveal that the concentrations in the free fluid in the central, lower and lower fore parts of the contents amounted to 96.9, 92.0 and 91.9 per cent, respectively, of that recorded in the upper part at the same time. All differences between the different parts except the very small one between the lower and lower fore parts are statistically highly significant.

Since the indicator concerned is a water-soluble substance which is neither formed nor decomposed in the rumen nor absorbed from it through the wall, the differences in concentration reveal the manner in which fluid introduced into the rumen becomes mixed with that already present there. The figures indicate rather clearly that the introduced fluid at first sinks to the lower parts of the rumen contents, the consequence being that the concentration of the indicator substance is on an average 8 per cent lower there than in the upper part.

HYDEN (1961) has reported significant differences in PEG concentration between samples drawn from five different parts of the sheep's rumen. The mean value found for polyethylene glycol was higher in the central rumen and lower in the cranial dorsal sac than in the other parts investigated. No differences between different parts of the rumen were found with respect to Na, K, Cl or P concentration. However, the number of analyses from each part of the rumen was comparatively small, in addition to which it is only natural that the differences should be smaller in the sheep's rumen than in the cow's, on account of the smaller volumes involved.

CORBETT et al. (1959) made comparisons of PEG concentration in the liquid and solid phases of the rumen contents. They demonstrated that the concentration of PEG in the fluid was very similar in the two types of samples and displayed comparatively small variations. The samples had been derived from six different parts of the rumen contents of two Friesian heifers. However, no calculations were made concerning the differences in concentration between sampling points.

Saliva accounts for the greater part of the fluid entering the rumen (BAILEY 1961), and variations in its rate of secretion may therefore influence the magnitude of the difference in distribution of PEG. If the ingress of fluid were completely inhibited, the indicator concentration should become equalized and be the same in all parts of the rumen. Starting from this theoretical zero point, the concentration difference will obviously increase up to a certain limit, accordingly to the rate of influx of fluid, provided that mixing of the fluid invariably takes place in the same manner.

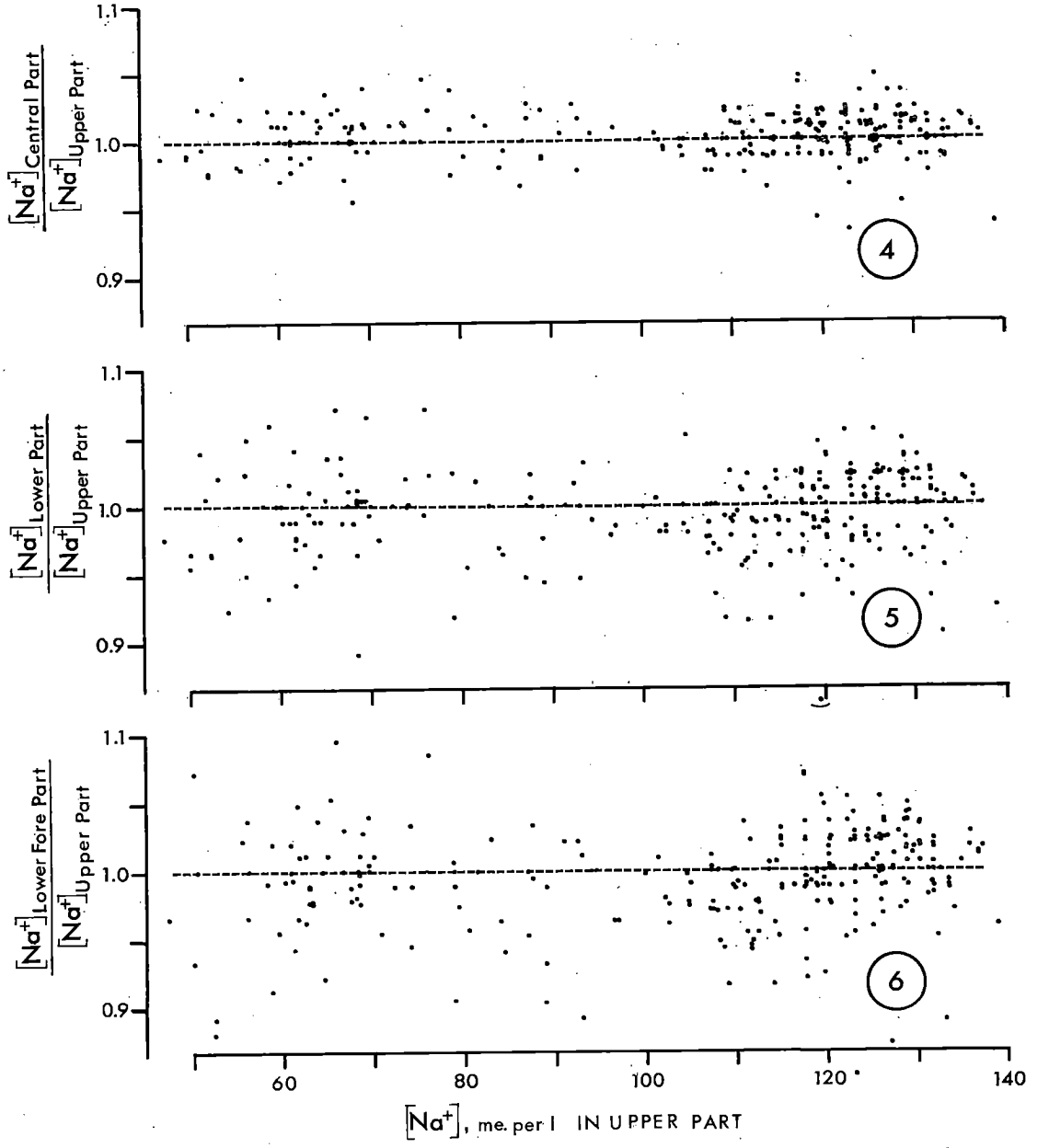
In the present experiments the amount of dry matter the animals received per day was 10.3 kg on an average, varying from 6 to 14 kg. The rate of liquid flow through the rumen at most abundant feeding was about twice that occurring at the lowest rate of ingestion. It is thus likely that some scattering of the relative values around their means has been introduced by this difference.

The sampling point in the lower fore part of the contents was located closest to, and that in the lower part farthest from, the cardia. One might thus have expected a difference in concentration to be established between the two points. In actual fact, however, the minor deviation observed, which does not amount to a statistically significant difference, suggests that very rapid mixing of fluids takes place between these two areas.

Distribution of sodium in the rumen

The concentrations of sodium in the central, lower and lower fore parts of the rumen, relative to that found at the same time in the upper part of the rumen, are presented in Figs. 4—6. The linear regression of the relative concentration on the concentration in the upper part and the correlation between the two variables have been calculated in each instance; the results are presented in Table 1.

Two of the regressions in question displayed no statistically significant deviation from linearity, while a deviation barely reaching a statistically significant level ($P < 0.05$) was established with respect to the concentration in the lower part of the rumen. Furthermore, a slight, yet statistically significant, positive correlation ($P < 0.01$) exists between the relative concentration in the lower fore part of the rumen and the absolute concentration in its upper part (Table 1). In conjunction with the slight non-linearity mentioned above, this has some influence on the conclusions that may be drawn from the mean values of the concentrations.



Figs. 4—6. Relative concentration of sodium in the central part of the rumen contents (Fig. 4), in the lower part (Fig. 5), and in the lower fore part (Fig. 6), plotted against the simultaneous absolute concentration in the fluid from the upper part of the contents.

Kuvat 4—6. Natriumin suhteellinen konsentraatio pötsinesteessä sisällön keskiosassa (4), alaosassa (5) ja etualaosassa (6) verrattuna yläosan samanaikaiseen konsentraatioon.

The data entered in Table 2 reveal that the means of the relative concentrations calculated for the different points under comparison are rather close to unity. In other words, the sodium is evenly distributed in all parts of the rumen contents. Owing to the great number of determinations, however, the existence of minor differences between different parts could still be established at a statistically significant level ($P < 0.01$ or $P < 0.001$) in all but one case.

The differences in concentration are of no practical importance, but the fact of their statistical significance allows some observations to be made concerning the probable functioning of the system. In the first place, it is interesting to note that the sodium content is slightly lower in the lower parts of the rumen than in the central and upper parts and that there is no statistically significant difference between the two lower parts. The last-mentioned fact, like the results concerning the polyethylene glycol and potassium contents, indicates that mixing between the dorsal and ventral parts in the bottom layers of the rumen contents is highly efficient.

The amount of sodium entering with the foodstuffs was noted in a previous study (LAMPILA 1965) to be only a few per cent of the total quantity of sodium entering the rumen. When the rations consisted of conventional foodstuffs, conditions were not essentially different in the present study either. However, NaCl at 50 g per day was added to most diets and at 100 g per day in some instances, in two daily doses. This somewhat increased the contribution of dietary sodium.

It is to be noted, however, that when the solid ingesta are compacted in the upper parts of the rumen contents (BALCH and JOHNSON 1950) and extracted by the free fluid (see BALCH 1958), even a relatively small quantity of sodium dissolved from the solid matter may suffice to cause the small differences observed between the concentrations in the upper and lower parts of the rumen.

Distribution of potassium in the rumen

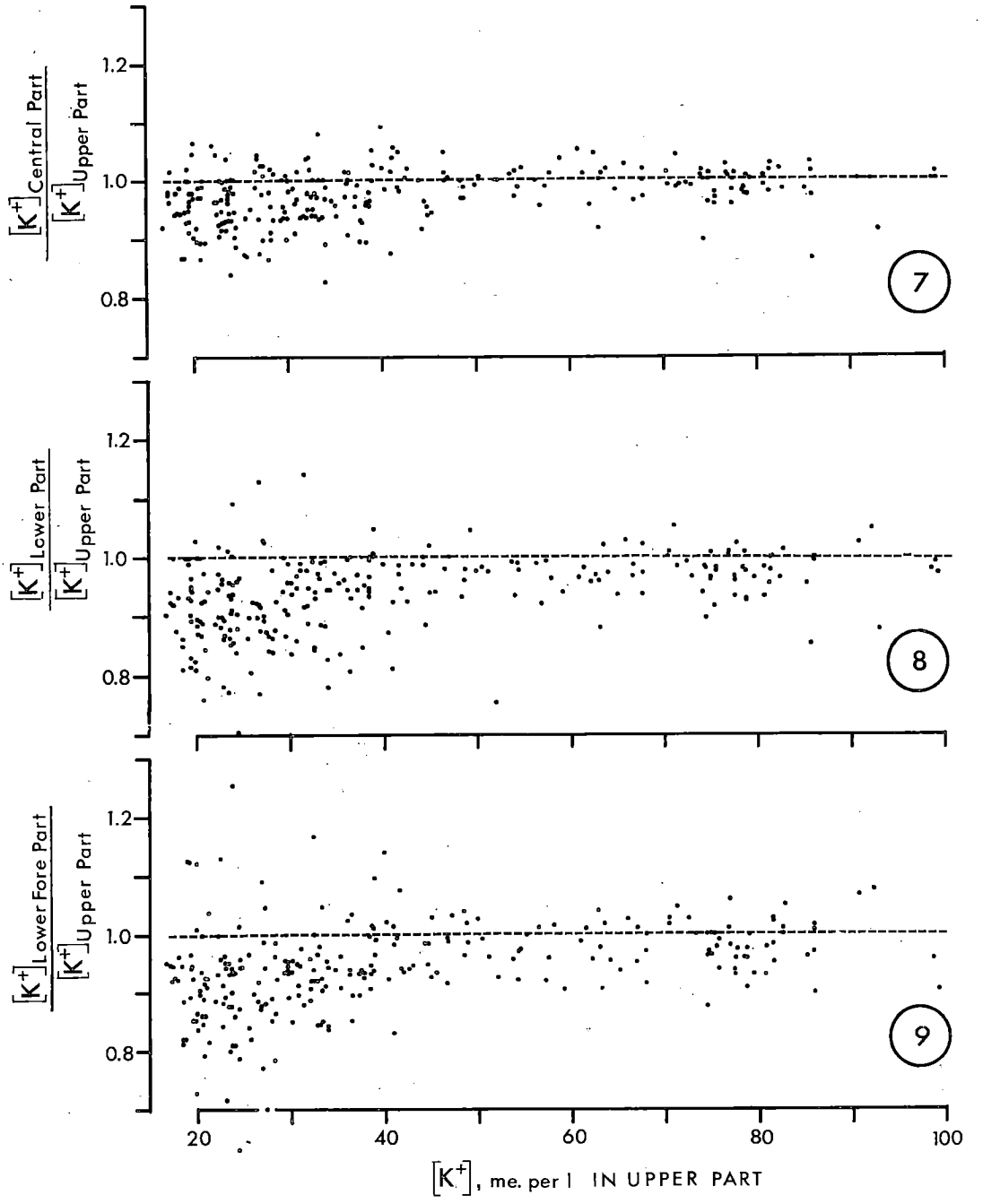
In Figs. 7—9 the relative concentrations of potassium in the central, lower and lower fore parts of the rumen contents can be seen. The same presentation has been employed here as for the PEG and sodium concentrations in the preceding figures. The linear regression and correlation data are seen in Table 1. In Table 2 the means of the relative concentrations are given for the different parts, as well as the level of statistical significance and direction of their differences.

The regressions were tested for linearity *) and the deviation from a linear regression was found to be statistically significant ($P < 0.01$ or $P < 0.001$) in all three instances. Despite this outcome of the test, the impression gained on close scrutiny of the series is that the correlation under investigation cannot be reproduced essentially better by any sufficiently simple equation of higher degree. Accordingly, it was not considered worth while to search for any such equation, and the results are treated as a linear relationship.

Table 1 reveals the existence of a statistically highly significant ($P < 0.001$) positive correlations between the relative concentration in the upper part of the rumen and those in the other parts. This implies that the differences between the average concentrations, which are statistically highly significant, with the exception of that between the lower and lower fore parts (Table 2), diminish as the potassium content in the rumen fluid increases. It therefore seemed appropriate to test the mean concentrations of the different parts for statistically significant differences with particular reference to higher concentration levels. Each series was divided into two subseries, the arbitrary limit being drawn at 40 me./l concentration in the upper part. These subseries (with $n = 166$ and $n = 104$ for the high and low level, respectively), too, display statistically significant ($P < 0.01$ or $P < 0.001$) differences between the different parts of the rumen, consistent in direction with those derived from the total series.

In endeavours to discover the causes responsible for differences in potassium concentration between different parts of the rumen contents, both the results obtained with PEG and those relating to sodium may be utilized. As can be seen from Table 2, all statistically significant dif-

*) For the test, the series of results was subdivided, according to the values of the independent variable, into four parts as closely equal in magnitude as possible; for each partial series the coefficient of linear regression was computed and these coefficients were compared.



Figs. 7—9. Relative concentration of potassium in the rumen fluid in the central part of the contents (Fig. 7), in the lower part (Fig. 8) and in the lower fore part (Fig. 9), plotted against the simultaneous absolute concentration in the fluid from the upper part of the contents.

Kuvat 7—9. Kaliumin suhteellinen konsentraatio pötsinesteessä sisällön keskiosassa (7), alaosassa (8) ja etualaosassa (9) verrattuna yläosan samanaikaiseen konsentraatioon.

ferences are in the same direction in both PEG and potassium, but the latter are smaller in every instance. The smaller differences found for potassium are primarily due to the fact that the saliva, which first reaches the contents of the lower part of the rumen, contains potassium; the dilution will therefore affect the potassium concentration of the rumen fluid relatively less than the PEG concentration.

However, such an explanation is too simplified, for in the first place the potassium concentration in the saliva may vary within rather wide limits, depending primarily on whether sodium is available in scanty or adequate amount (BAILEY and BALCH 1961). Changes in the contribution of drinking water to the quantity of fluid entering the rumen also influence the average potassium content of the incoming fluid. Furthermore, the quantity of potassium dissolved from the foodstuffs varies to some extent, according to diet, and affects the ratio prevailing in each particular instance between the concentrations of the fluid entering the rumen and that previously present in it. Obviously both the positive correlations observed and the deviations from a linear regression are mainly attributable to the combined action of these different factors.

These conclusions find further support in the results concerning the sodium concentration if it is assumed that the fluid entering the rumen at first sinks to the lower parts, as seems evident on the strength of the PEG concentrations. Apart from exceptional instances, the concentration of sodium in the saliva is higher (on an equivalent basis) than that of potassium, whereas the quantity of sodium extracted from the ingesta is smaller than the corresponding quantity of potassium. The concentration of sodium in the rumen fluid is thus more dependent on the content of the fluid entering the rumen than the potassium concentration. Correspondingly, the likelihood of differences building up between the lower and upper parts of the contents is less, as is indeed consistently indicated by the results (Table 2).

Neither here nor in the preceding consideration has any separate account been taken of the possibility that water and ions may pass through the wall of the rumen in either direction, because neither their route of entry nor their potential absorption essentially alters the character of the phenomenon in question.

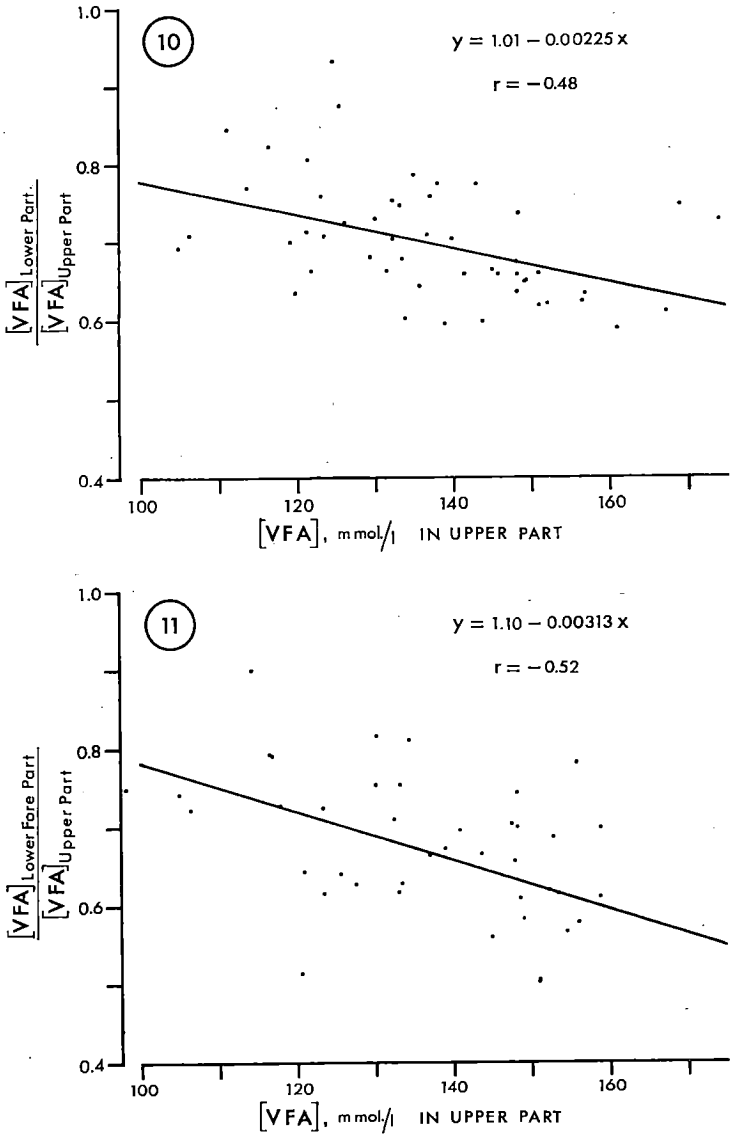
Concentration of volatile fatty acids in different parts of the rumen contents

Figs. 10 and 11 reveal that the concentration of volatile fatty acids was, without exception, lower in the lower part and lower fore part of the contents than in the upper part (both ratios less than 1). The fairly strong, and statistically highly significant, negative correlations between the relative concentrations in the lower parts and the absolute concentration in the upper part (Table 1) show that even the difference between the relative concentrations in the upper part and in the lower parts becomes greater as the acid concentration in the upper part increases. By calculation from the equations of linear regression the observation can be made that the actual concentrations at the points of measurement in the lower parts certainly increased at the same time but that their increase was relatively less than that noted in the upper part.

The test of the regressions for linearity revealed that the deviation from a linear regression was not statistically significant ($P > 0.05$) in either instances.

The values compiled in Table 2 reveal that, on the average in the entire material, the VFA concentration in the lower part and in the lower fore part of the rumen contents amounted to 70 and 67 per cent, respectively, of that in the upper part. The difference between the average concentrations is statistically highly significant ($P < 0.001$) in both instances. Since the samples from the lower part and from the lower fore part were not collected at the same time, no comparison of the acid concentrations in these two parts is possible.

On comparison of these results with the values for PEG concentration it is clear that the lower VFA level in the lower parts of the rumen contents is not attributable to the effect of dilution



Figs. 10—11. Relative concentration of volatile fatty acids (VFA) in the rumen fluid in the lower part of the contents (Fig. 10) and in the lower fore part (Fig. 11), plotted against the simultaneous absolute concentration in the upper part of the contents.

Kuvat 10—11. Haihtuvien rasvahappojen (VFA) suhteellinen konsentraatio pötsinesteessä sisällön alaosassa (10) ja etualaosassa (11) verrattuna yläosan samanaikaiseen konsentraatioon.

alone. If this were the sole cause, the difference in the concentration of volatile fatty acids between the lower parts of the rumen and the upper part should be not 30—33 per cent but only about 8 per cent, as the concentration of PEG was found to be *).

*) It has to be admitted that this comparison is not fully warranted, since the results have been derived from separate tests. However, the differences are so clearly different in magnitude that, in our opinion, the above conclusion is sufficiently well-founded. A comparison having reference to a single series of samples will be presented when the volatile fatty acids have been determined from the samples taken for PEG determinations.

The greater difference in acid content (in excess of 8 per cent) may partly be due to formation of acids at a higher rate per unit of fluid volume in the upper part than in the lower parts of the contents. If this is the case, it is obviously due to the higher density of fermenting plant matter in the upper part (BALCH and JOHNSON 1950) and to the correspondingly higher sugar content of the fluid (SMITH et al. 1956). This possibility is suggested by the relative increase of the difference with increasing concentration, unless this is caused by less efficient extraction of the acids during the period when a higher concentration is present. It should be noted that normally the acid concentration in the upper part of the contents rises to a maximum soon after the meal, when the rumen is at its fullest, the contents in the upper part consisting of a densely packed mass and the rumen containing little free fluid for extracting this mass.

On the other hand, the fact that in the upper part of the contents the pH is mostly maintained below the optimum level for fermentation (LAMPILA 1964) justifies the inference that the formation of acids in this part cannot, on the average at least, be essentially more rapid than in the lower parts of the contents. This view is supported by the experimental results of BALCH and JOHNSON (1950) and of MILES (1951), according to which, on the contrary, the fermentation of fibrous matter, at least, proceeds at a clearly higher rate in the lower than in the upper part of the rumen.

Until the combined effect of these factors is clarified, there remains the absorption of acids through the wall of the rumen to account for the great differences in acid concentration. It was pointed out before (p. 280) that both sampling points in the lower parts of the rumen were located close to the wall, on the floor of the rumen, and that the samples consisted of free fluid that ran into the sampling tube. Being freely movable, this fluid is in continuous contact with the absorption surface. One cannot say the same about the sample fluids representing the upper part, which were derived from solid ingesta by squeezing and, in a manner of speaking, represent a fluid that is »stationary», although it is constantly being exchanged as a result of the extraction process. Its communication with the absorbing surface is only possible by way of the free, extracting, fluid. Since the formation of acids and their absorption through the wall of the rumen into the blood stream is a continuous process under normal feeding conditions, it is quite obvious that the acid concentration of the free fluid must remain lower than that of the fluid retained in the plant matter (LAMPILA 1964, p. 64). It follows logically that the difference in acid concentration between the upper part and bottom layer of the contents is greater than the difference in PEG concentration between these parts, because the »reservoir» of free fluid is located in the lower part of the rumen (see e.g. BALCH 1958).

On the strength of this reasoning the differences in acid concentration due to absorption may be utilized in studying the relative rates of absorption of different acids in fistulated bovines under normal feeding conditions. Although no exact quantitative clarification is possible, one is at least in a position to draw inferences as to when the concentration relations in the rumen fluid are the same as those in the absorbed acid mixture and when some given acid is absorbed at a rate higher or lower than average. When employed in conjunction with PEG and ^{14}C-labelled acids, the method may also constitute an aid in quantitative *in vivo* studies on the formation and absorption of acids.

Ammonia concentration in the upper and lower part of the rumen contents

Samples for the determinations of ammonia content were taken simultaneously only from those sampling areas which have been referred to as the upper and lower part of the rumen contents. Fig. 12, which deviates from the preceding figures in the mode of representation employed, shows the regression of the concentration in the lower part on the concentration in the upper part as such, without conversion of the former to relative concentration values. This representa-

tion was chosen primarily because the use of relative values did not prove so suitable as before. Moreover, in contrast to the other alternative, the relationship between the variables was found to be linear in these units.

It is seen from Table 1 that there was a strong and statistically highly significant positive correlation ($r = 0.830$; $P < 0.001$) between the ammonia concentrations of the upper and lower part of the contents. The relative magnitudes of the concentrations at the two ends of the concentration range were in reverse order, as can be seen from Fig. 12. Thus it would have been inappropriate to calculate the ratio of the average concentrations in the two parts. Instead, the values indicated by dotted lines in Fig. 12 were calculated. These represent the limits within which the mean value of the concentration in the lower part can be predicted to remain (on the 95 % confidence level) for various levels of concentration in the upper part (SNEDECOR 1964, p. 139). Noting the intersection of these limits with the straight line $y = x$, the inference can be drawn that at ammonia concentration below 4 me./l in the upper part the concentration in the lower part is higher on the average. The situation is reversed when the concentration in the upper part exceeds 7 me./l.

This interesting result furnishes reason for speculation on the biological factors responsible for it. Three factors may be suggested to be of primary importance. They are (1) the flow of urea into the rumen with the saliva, (2) the stratification of the contents so that the ingested material is concentrated in the upper part, and (3) the resulting difference in pH between the upper and lower parts of the contents (see Fig. 14).

BAILEY and BALCH (1961) found that the urea N content of bovine saliva varied from 1.3 to 14.4 mg per cent. Since urea is rapidly hydrolysed to ammonia in the rumen (PEARSON and SMITH 1943), it is possible that the urea that arrives with the saliva, sinking at first in the lower part of the contents may there maintain an ammonia content higher than average when there is little ammonia in the rumen. Since the pH optimum of urease activity is in the neighbourhood of the

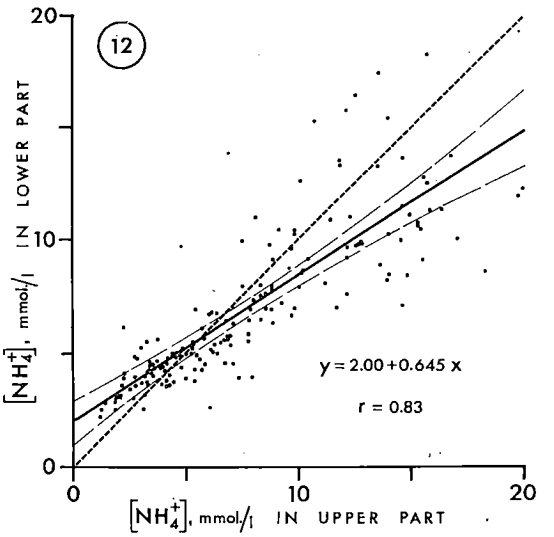


Fig. 12. Ammonia concentration in the rumen fluid from the lower part of the rumen contents, plotted against the simultaneous concentration in the upper part of the contents. Thin dotted lines indicate the 95 % confidence limits for the means.

Kuva 12. Ammoniakin konsentraatio pötsinesteessä sisällön alaosassa verrattuna yläosan samanaikaiseen konsentraatioon. Katkoviivat osoittavat 95 %:n luottamusvälin keskiarvoille.

neutral point or slightly on the alkaline side (PEARSON and SMITH loc. cit.), hydrolysis of urea, too, obviously takes place more rapidly in the lower part than in the acid ingesta in the upper part.

The abrupt rise in the ammonia concentration of the rumen contents after feeding (LAMPILA 1960) is an obvious consequence of the increase in concentration of the nitrogenous substances which are decomposed to ammonia. As can be seen from Fig. 12, the difference in ammonia concentration between the upper and lower parts undergoes a simultaneous change, so that relatively the concentration in the upper part continuously increases. Maybe the most logical explanation to account for the change is the difference in the concentration of feed material between these parts, due to stratification. It is true that the fall in pH in the upper part should slow down the rate of decomposition of protein (cf. WANNER 1956) and other nitrogenous substances (cf. ANNISON 1956, SIROTNAK et al. 1953, LEWIS 1955, HOLTENIUS 1957) to ammonia in this part

compared with the lower part, but evidently this factor is not very efficient in preventing a difference in concentration. A partial cause for this may be that the ammonia-consuming synthetic reactions are also slowed down at the same time. The results of *in vitro* tests by LAMPILA (1964, p. 50, Fig. 23) suggest that within the limits of the variation in the rumen contents the ammonia-consuming reactions may by even more strongly retarded than the reactions producing ammonia when the pH is lowered.

pH in different parts of the rumen contents

In Figs. 13—15 the pH values in the central, lower and lower fore parts of the rumen contents are plotted against the pH simultaneously observed in the upper part. In addition to the linear regression, the figures also show the limits within which the mean of the measurements or a single measured value can be predicted to fall (on the 95 % confidence level), the prediction being based on the pH in the upper part.

The results of the correlation and regression calculations are presented in Table 1. Testing of the regression for linearity revealed no statistically significant deviation ($P > 0.05$) from linear regression in any instance. The average pH values found for the different parts of the rumen contents and the levels of statistical significance of their differences are given in Table 2.

The values in the table reveal that the average pH value was lowest in the upper part of the contents, followed by the central and lower parts, while it was highest in the lower fore part. The differences between these mean values were all found to be statistically highly significant ($P < 0.001$). However, the course of the regression lines indicates that the differences between the mean values do not yet convey everything about the differences between the different parts, which diminish with increasing pH if the difference between the lower and lower fore parts is disregarded. When the upper part of the contents is used as reference, it actually seems as if the direction of the differences were reversed as the neutral point is approached. There are, however, rather few values measured in its vicinity and it is dubious whether any reliable predictions concerning the differences in this region can be made on the strength of the linear regression. Since the highest pH values are usually found when there is little fermenting matter and the contents are watery and readily miscible, it is perhaps theoretically more likely that the systematic differences between the different parts vanish.

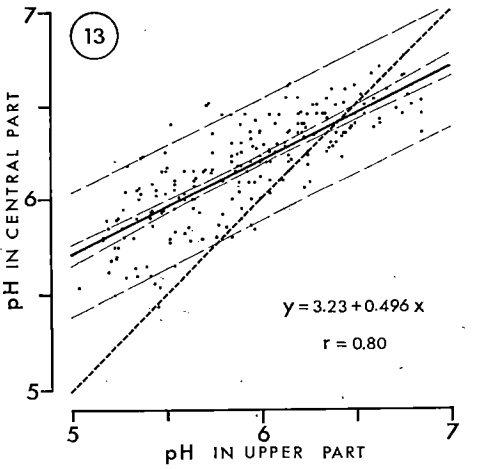
The mean pH values presented here were obtained on diets with a net energy content varying from that consistent with the maintenance requirement to a level corresponding to a milk yield of about 20 kg daily. Considering the reservation made in the following, they may thus be said to reflect the average conditions in the rumen on dairy cow diets representing the lower medium level as regards intensity of feeding. Since the measurements were mostly made at equal intervals in series covering one 12-hour feeding interval, the periodic fluctuation in pH has thus been taken into account, except in one respect. The exception is caused by the fact that the first of the four, otherwise equal, intervals of measurement (3 hours each) was bisected by an extra measurement, and that both the first and the last results in the series of measurements have been included in the means. Since both represent the condition immediately prior to commencement of feeding, at which time the pH of the rumen contents is at its highest, inclusion of these two values makes the means appear higher than they actually are. The results obtained at the middle of the first full-length interval of measurement seem to affect the means less, if at all significantly.

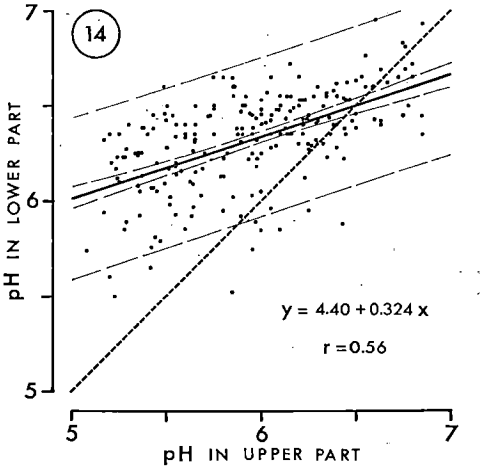
The above points should be taken into consideration when inferences are drawn from the mean values with regard to the consequences of differences in pH, i.e. concerning the activity of fermentation. BALCH and JOHNSON (1950) and MILES (1951) have shown that fibrous matter is fermented more rapidly in the lower part of the contents than in the upper part. LAMPILA (1964, pp. 43—44) has concluded from his experimental

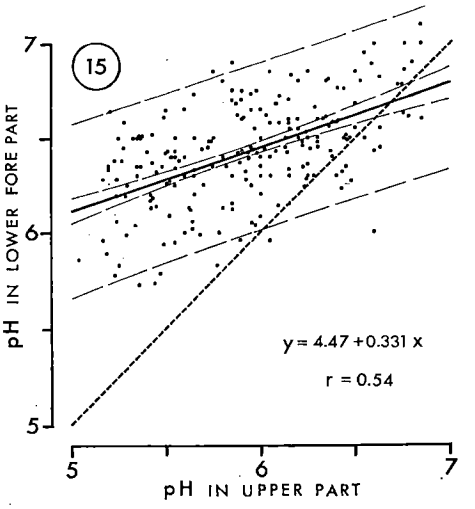
results that this difference in rate of fermentation is at least predominantly due to differences in pH, which may thus be of essential physiological significance. The regression equation determined by him (p. 46) shows that the optimum pH for fermentation is 6.45.

If the mean pH values presented here and the said regression equation are to be used to assess the differences in fermentation activity between different parts of the contents, the circumstance increasing the means pointed out above has to be kept in mind because the differences in pH increase as the pH falls. Moreover, one should note that some of the pH readings are above the optimum (6.45). These values increase the mean pH value but, naturally, they do not further increase the rate of fermentation beyond the optimum point.

The differences in the concentration of volatile fatty acids between different parts of the rumen contents can be considered the principal cause of the corresponding differences in pH. The likely causes of the former have already been discussed in the foregoing, and no closer examination of the causes responsible for the pH differences is therefore needed. The only point that should be stressed is the statistically highly significant ($P < 0.001$) difference established between the lower part and lower fore part, because no corresponding difference was evident in the PEG or mineral element concentrations. It is true that the concentration of volatile fatty acids was lower in the lower fore part than in the lower part, but since the samples were taken at different times, the potential significance of the VFA concentration remains questionable.







Figs. 13—15. pH in the central part of the rumen contents (Fig. 13), in the lower part (Fig. 14) and in the lower fore part (Fig. 15), plotted against the simultaneous value found for the upper part of the contents. The inner and outer thin dotted lines indicate the 95 % confidence limits for the means and for the individual values, respectively.

Kuvat 13—15. Sisällön pH pötsin keskiosassa (13), alaosassa (14) ja etualaosassa (15) verrattuna yläosan samanaikaiseen arvoon. Sisemmät katkoviivat osoittavat 95 %:n luottamusvälin keskiarvoille ja ulommat yksityisille arvoille.

Summary

This paper is a report of studies with fistulated cows, revealing the existence of systematic differences between different parts of the rumen contents regarding the pH of the rumen fluid and the concentrations of certain substances. The following substances were investigated in the studies: (1) polyethylene glycol, which had been introduced into the rumen for the purpose, (2) sodium, (3) potassium, (4) volatile fatty acids (VFA), and (5) ammonia. The findings made in the course of the studies were as follows.

(1) Denoting the concentration of PEG in the fluid taken from the upper part of the rumen contents with 100, the simultaneous concentration in the central part was 96.9 ± 0.5, in the lower part 92.0 ± 0.6 and in the lower fore part 91.9 ± 0.7. The level of concentration had no effect on these relative values. The differences in concentration between different parts of the contents, with the exception of that between the lower and lower fore parts, were statistically highly significant ($P < 0.001$).

(2) Denoting the sodium concentration in the fluid from the upper part of the contents with 100, the simultaneous concentration was 100.3 ± 0.1 in the central part, 99.5 ± 0.2 in the lower part and 99.3 ± 0.3 in the lower fore part. These relative values were independent of the concentration level except in the lower fore part, where they increased slightly with increasing concentration in the upper part. The differences between different parts, with the exception of that between the lower and lower fore parts, were statistically significant ($P < 0.01$ or $P < 0.001$).

(3) Denoting the potassium concentration in the fluid from the upper part of the contents with 100, the simultaneous concentration was 97.4 ± 0.3 in the central part, 94.2 ± 0.4 in the lower part and 94.3 ± 0.4 in the lower fore part. Except for the difference between the last two, the differences between the means for the different parts were statistically highly significant ($P < 0.001$). A statistically highly significant, positive correlation ($P < 0.001$) existed in all three instances between the actual concentration in the upper part of the contents and the relative concentration in the other part concerned.

(4) Denoting the VFA concentration in the fluid from the upper part of the contents with 100, the average concentration in the lower part was 69.8 ± 0.9. On separate comparison, the concentration in the lower fore part was correspondingly found to be 66.8 ± 1.3. A highly significant negative correlation was established between the relative concentrations in the lower parts and the actual concentration in the upper part ($r = -0.480$ and -0.519 for the lower and lower fore parts, respectively; $P < 0.001$ for both).

The relationship between the relative concentration in the lower part (y_1) and the absolute VFA concentration in the upper part (x, in mmol./l) is described by the equation

$$y_1 = 1.008 - 0.00225\,x$$

The relationship between the relative concentration in the lower fore part (y_2) and the absolute VFA concentration in the upper part (x, in mmol./l) is described by the equation

$$y_2 = 1.095 - 0.00313\,x$$

(5) The relationship between the ammonia contents of the fluid in the lower (y) and the upper (x) parts is described by the equation

$$y = 2.000 + 0.645\,x$$

The correlation is strong, the correlation coefficient differing from zero at a statistically highly significant level ($r = 0.830$; $P < 0.001$).

(6) The pH measurements made in series covering the 12-hour feeding interval yielded the following average values:

Part of the contents	pH
upper	5.93 ± 0.03
central	6.17 ± 0.01
lower	6.32 ± 0.01
lower fore	6.43 ± 0.02

The differences between the means for different parts of the rumen contents were statistically highly significant ($P < 0.001$) in all instances.

Denoting the pH in the upper part of the contents with x, that in the central part with y_1, that in the lower part with y_2 and that in the lower fore part with y_3, the relationship between the three latter and the former may be described by the equations

$$y_1 = 3.23 + 0.496\,x$$
$$y_2 = 4.40 + 0.324\,x$$
$$y_3 = 4.47 + 0.331\,x$$

The respective coefficients of correlation are $r = 0.801$, 0.560 and 0.541, all statistically highly significant ($P < 0.001$).

The results obtained in the studies are discussed, and from them assessments are made and conclusions drawn concerning the placement of the fluid entering the rumen and its mixing with the rumen contents, and concerning the consequences of the stratification of the ingesta.

Acknowledgements. — The authors wish to express their sincere gratitude to Mr. Erkki Mikkola, Mag. Phil., and Mr. Tauno Moisio, Mag. Phil., for valuable help rendered in the statistical treatment of the results, to Mr. Uljas Attila, M.Sc., who translated the manuscript into English, and to Mrs. Jean Margaret Perttunen, B.Sc., for revising the translation. — This research has been financed in part by a grant made by the Suomen Kulttuurirahasto (Finnish Cultural Foundation) to one of the authors (E. P.).

REFERENCES

Annison, E. F. 1956. Nitrogen metabolism in sheep. Biochem. J. 64: 705—714.

Bailey, C. B. 1961. Saliva secretion and its relation to feeding in cattle. 3. The rate of secretion of mixed saliva in the cow during eating, with an estimate of magnitude of the total daily secretion of mixed saliva. Brit. J. Nutr. 15: 443—451.

—»— & Balch, C. C. 1961. Saliva secretion and its relation to feeding in cattle. 2. The composition and rate of secretion of mixed saliva in the cow during rest. Ibid. 15: 383—402.

Balch, C. C. 1958. Digestion in the rumen. Outlook on Agriculture 2, 1: 33—43.

—»— & Johnson, V. W. 1950. Factors affecting the utilization of food by dairy cows. 2. Factors influencing the rate of breakdown of cellulose (cotton thread) in the rumen of the cow. Brit. J. Nutr. 4: 389—394.

Bryant, A. M. 1964. Variations in the pH and volatile fatty acid concentration within the bovine reticulo-rumen. N. Z. J. Agric. Res. 7, 4: 694—706.

Conway, E. J. 1957. Microdiffusion analysis and volumetric error, p. 98—104. 4th Ed. Glasgow.

Corbett, J. L., Greenhalgh, J. F. & Dand, F. E. 1959. Distribution of chromium sesquioxide and polyethylene glycol in the reticulo-rumen of cattle. Brit. J. Nutr. 13: 337—345.

Davey, A. W. F. 1965. Sampling the contents of the rumen of the dairy cow. N. Z. J. Agric. Res. 8: 409—411.

Friedemann, T. E. 1938. The identification and quantitative determination of volatile alcohols and acids. J. Biol. Chem. 123: 161—184.

Holtenius, P. 1957. Nitrite poisoning in sheep, with special reference to the detoxification of nitrite in the rumen. Acta Agric. Scand. 7: 113—163.

Hyden, S. 1956. A turbidimetric method for the determination of higher polyethylene glycols in biological materials. Ann. Agric. Coll. Sweden 22: 139—145.

—»— 1961. Determination of the amount of fluid in the reticulo-rumen of the sheep and its rate of passage to the omasum. Ibid. 27: 51—79.

Lampila, M. 1955. Preliminary studies on the variations of pH and volatile fatty acid concentration of the rumen contents of the cow. J. Sci. Agric. Soc. Finl. 27: 142—153.

—»— 1960. The effect of silage prepared in different ways upon the ammonia content of the cow's rumen ingesta. Ibid. 32: 169—175.

—»— 1964. Volatile fatty acids, pH and microbial activity in the rumen contents of the cow. Selostus: Haihtuvat rasvahapot, pH ja mikrobiston aktiviteetti lehmän pötsin sisällössä. Ann. Agric. Fenn. 3, Suppl. 3: 1—76.

—»— 1965. The passage of fluid, certain mineral elements, and volatile fatty acids from the reticulo-rumen of the cow. Selostus: Nestevirtaus ja sen mukana tapahtuva liuenneiden kivennäisaineiden sekä haihtuvien rasvahappojen poistuminen pötsi-verkkomahasta. Ibid. 4: 134—144.

Lewis, D. 1955. Amino-acid metabolism in the rumen of the sheep. Brit. J. Nutr. 9: 215—230.

Miles, J. T. 1951. Rumen digestion of some crude fiber constituents. J. Dairy Sci. 34: 492.

PEARSON, R. M. & SMITH, J. A. B. 1943. The utilization of urea in the bovine rumen. 2. The conversion of urea to ammonia. Biochem. J. 37: 148—153.

POUTIAINEN, E. & LAMPILA, M. 1966. Comparative determinations of sodium in rumen fluid, saliva and feeds with the sodium electrode and with the flame photometer. Ann. Agric. Fenn. 5: 267—278.

SIROTNAK, F. M., DOETSCH, R. N., BROWN, R. E. & SHAW, J. C. 1953. Amino acid metabolism of bovine rumen bacteria. J. Dairy Sci. 36: 1117—1123.

SISSON, S. & GROSSMAN, J. D. 1956. The anatomy of the domestic animals. 4th Ed. Philadelphia.

SMITH, P. H., SWEENEY, H. C., ROONEY, J. R., KING, K. W. & MOORE, W. E. C. 1956. Stratifications and kinetic changes in the ingesta of the bovine rumen. J. Dairy Sci. 39: 598—609.

SNEDECOR, G. W. & COCHRAN, W. G. 1964. Statistical methods. 5th Ed. Ames, U.S.A.

STODDARD, G. E., ALLEN, N. N., HALE, W. H., POPE, A. L., SORENSEN, D. K. & WINCHESTER, W. R. 1951. A permanent rumen fistula cannula for cows and sheep. J. Anim. Sci. 10: 417—423.

WARNER, A. C. I. 1956. Proteolysis by rumen microorganisms. J. Gen. Microbiol. 14: 749—762.

SELOSTUS

Järjestelmällisiä sisällön osien välisiä eroja lehmän pötsinesteen koostumuksessa

MARTTI LAMPILA ja ESKO POUTIAINEN

Maatalouden tutkimuskeskus, Kotieläinhoidon tutkimuslaitos, Tikkurila

Kirjoituksessa on selostettu fistelilehmillä suoritettuja tutkimuksia, joiden tulokset osoittavat pötsinesteen pH:ssa ja eräiden aineiden konsentraatioissa esiintyvän systemaattisia sisällön osien välisiä eroja. Tutkimuksen kohteena olleet aineet olivat: (1) polyetylenglykoli (PEG), jota oli tarkoitusta varten annostettu pötsiin, (2) natrium, (3) kalium, (4) haihtuvat rasvahapot (VFA) ja (5) ammoniakki. Tutkimuksen tulokset osoittivat seuraavaa:

(1) Kun PEG:n konsentraatiota sisällön yläosasta otetussa nesteessä merkitään 100:lla, oli samanaikainen konsentraatio keskiosassa 96.9 ± 0.5, alaosassa 92.0 ± 0.6 ja etualaosassa 91.9 ± 0.7. Konsentraatiotaso ei vaikuttanut suhteellisiin arvoihin. Sisällön osien väliset konsentraatioerot olivat alaosan ja etualaosan välistä eroa lukuun ottamatta tilastollisesti erittäin merkitsevät ($P < 0.001$).

(2) Kun merkitään natriumin konsentraatiota sisällön yläosan nesteessä 100:lla, oli samanaikainen konsentraatio keskiosassa 100.3 ± 0.1, alaosassa 99.5 ± 0.2 ja etualaosassa 99.3 ± 0.3. Suhteelliset arvot olivat konsentraatiotasosta riippumattomia, paitsi etualaosassa, jossa ne lievästi kohosivat yläosan konsentraation mukana. Osien väliset erot olivat alaosan ja etualaosan välistä eroa lukuun ottamatta tilastollisesti merkitsevät ($P < 0.01$ tai $P < 0.001$).

(3) Kun kaliumin konsentraatiota sisällön yläosan nesteessä merkitään 100:lla, oli samanaikainen konsentraatio keskiosassa 97.4 ± 0.3, alaosassa 94.2 ± 0.4 ja etualaosassa 94.3 ± 0.4. Keskiarvojen osien väliset erot olivat kahden viimeksi mainitun välistä eroa lukuun ottamatta tilastollisesti erittäin merkitsevät ($P < 0.001$). Sisällön yläosan todellisen ja muiden osien suhteellisen konsentraation välillä oli kaikissa kolmessa tapauksessa erittäin merkitsevä positiivinen korrelaatio ($P < 0.001$).

(4) Kun merkitään sisällön yläosan nesteen VFA-konsentraatiota 100:lla, oli konsentraatio alaosassa keskimäärin 69.8 ± 0.9. Erikseen verrattuna oli etualaosan konsentraatio vastaavasti 66.8 ± 1.3. Yläosan todellisen ja alaosien suhteellisen konsentraation välillä oli kummassakin tapauksessa erittäin merkitsevä negatiivinen korrelaatio ($r = -0.480$ ja -0.519, vast.; $P < 0.001$).

Alaosan suhteellisen (y_1) ja yläosan todellisen (x) VFA-konsentraation (mmol./l) keskinäistä riippuvuutta kuvaa yhtälö

$$y_1 = 1.008 - 0.00225\,x$$

Etualaosan suhteellisen (y_2) ja yläosan todellisen (x) VFA-konsentraation (mmol./l) välistä riippuvuutta kuvaa yhtälö

$$y_2 = 1.095 - 0.00313\,x$$

(5) Sisällön alaosan (y) ja yläosan (x) nesteen ammoniakkipitoisuuden keskinäistä riippuvuutta kuvaa yhtälö

$$y = 2.000 + 0.645\,x$$

Korrelaatio oli voimakas ($r = 0.830$) ja tilastollisesti erittäin merkitsevä ($P < 0.001$).

(6) 12-tuntisen ruokintavälin käsittävinä sarjoina tehdyt pH-mittaukset antoivat seuraavat keskimääräiset arvot:

Sisällön yläosa	5.93 ± 0.03
» keskiosa	6.17 ± 0.01
» alaosa	6.32 ± 0.01
» etualaosa	6.43 ± 0.02

Kaikki osien väliset keskiarvojen erot olivat tilastollisesti erittäin merkitsevät ($P < 0.001$).

Kun merkitään sisällön yläosan pH:ta x:llä, keskiosan y_1:llä, alaosan y_2:lla ja etualaosan y_3:lla, voidaan kolmen viimeksimainitun riippuvuus ensimmäisestä kuvata yhtälöillä

$$y_1 = 3.23 + 0.496\,x$$
$$y_2 = 4.40 + 0.324\,x$$
$$y_3 = 4.47 + 0.331\,x$$

Vastaavat korrelaatiokertoimet olivat 0.801, 0.560 ja 0.541. Korrelaatio oli kussakin tapauksessa erittäin merkitsevä ($P < 0.001$).

Saatuja tuloksia on tarkasteltu ja niiden perusteella on tehty arvioita ja päätelmiä, jotka koskevat pötsiin saapuvan liuoksen sijoittumista ja sekoittumista sisältöön sekä rehumaterian kerrostumisen seurausilmiöitä.

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 298—304 (1966)

Seria AGROGEOLOGIA, -CHIMICA ET -PHYSICA N. 26
Sarja MAAPERÄ, LANNOITUS JA MUOKKAUS n:o 26

READILY SOLUBLE TRACE ELEMENTS IN FINNISH SOILS

MIKKO SILLANPÄÄ and ESKO LAKANEN
Agricultural Research Centre, Department of Soil Science, Tikkurila, Finland

Received October 1, 1966

The amounts of trace elements removed yearly with crops represent only a very small proportion of the total contents of these elements in soils. The total contents are primarily dependent on the geological origin of the soil and give information about the trace element reserves in the soil. Relevant data of the total contents have been published by several investigators (e.g. SWAINE 1955, VUORINEN 1958).

The availability of trace elements is primarily dependent on their solubility, but the interpretation of the results of soluble trace element analyses is complicated by the wide variety of extracting solvents and methods used. Among the extractants used for simultaneous determination of several trace elements are 0.5 *N* acetic acid, 1 *N* neutral ammonium acetate, acid ammonium acetate, Baron's extractant, 10 % hydrochloric acid and EDTA (MITCHELL 1964 a, LAKANEN 1962, BARON 1955, SCHARRER and JUDEL 1957, VIRO 1955).

The purpose of the present study was to obtain data on the amounts of ten soluble trace elements in Finnish soils in general and on the differences existing between various soil types.

The extractant used was acid ammonium acetate (0.5N CH_3COOH, 0.5N CH_3COONH_4, pH 4.65). Since the same solvent is used in routine soil testing in Finland (VUORINEN and MÄKITIE 1955) for determining the major nutrients in soil, Ca, K, Mg and P were also determined to obtain a wider picture of the nutrient status of the soils under study.

Materials and methods

The material consisted of 481 topsoil (0—20 cm) samples from various parts of Finland. The distribution of the samples according to soil type (Table 1) is in good agreement with the larger material (VUORINEN 1958) from which the total contents of trace elements have been determined. The contents of the major nutrients found in this material likewise correspond well to the average levels of these nutrients in Finnish soils (KURKI 1963).

The soil samples were air-dried and passed through a 2 mm sieve. The soil pH was determined from a soil: water (1: 2.5) suspension with a Beckman pH meter, and calcium, potassium and phosphorus from an acid ammonium acetate extract (shaking time 1 hour in a volume

ratio 1: 10). The same extraction technique with spectrally pure chemicals was used for trace elements. These elements were separated, concentrated and determined with an ARL two meter grating spectrograph (LAKANEN 1962). Magnesium and strontium were determined directly from the extract with a Beckman DU flame photometer.

The extraction capacity of acid ammonium acetate is between those of 0.5 *N* acetic acid and *N* neutral ammonium acetate, being closer, however, to acetic acid (LAKANEN 1962).

Results and discussion

The average soluble contents of various elements in different soil types are given in Table 1 and a general picture of their distribution in the whole sample material (log.scale) in Fig. 1. Because of the excesses, of values smaller than the means of the element contents (Table 1), the maximum frequencies (Fig. 1) occur at considerably lower levels than the corresponding means. For molybdenum only the right-hand part of the distribution curve could be drawn, owing to the inadequate sensitivity of the method at very low contents.

From the present data it is not possible to draw far-reaching conclusions about the factors affecting the contents of various trace elements in different soils, owing to the complicated chemistry and behaviour of trace elements in soils. Numerous soil factors, such as parent material and total contents, texture, organic matter content, pH and moisture conditions, have been shown to affect the solubility of these elements (e.g. SILLANPÄÄ 1962 b, MITCHELL 1964).

The average contents extracted by acid ammonium acetate arranged in increasing order, are as follows: Mo, V, Co, Cu, Ni, Pb, Zn, Sr, Mn and Fe. This order corresponds relatively well to the values obtained with the acetic acid method (MITCHELL 1964 b). In comparing the soluble contents of trace elements in the present study with the corresponding total contents in soils, the order of the elements is in agreement with the results presented by various authors (e.g. GOLDSCHMIDT 1954,

Table 1. Average contents of elements extracted by acid ammonium acetate from various soil types
Taulukko 1. Happamalla ammoniumasetaatilla uuttuvien aineiden keskimääräiset pitoisuudet eri maalajeissa

Soil type *Maalaji*	n	Bulk. density g/cm³ *Tilav.p.*	pH	mg/l soil — *mg/l maata*													
				Ca	Mg	K	P	Fe	Mn	Sr	Zn	Pb	Ni	Cu	Co	V	Mo
Moraine — *Moreeni*	34	1.059	5.20	891	155	76	8.82	26.0	28.8	9.1	5.40	0.452	0.159	0.141	0.056	0.058	0.005
Sand — *Hiekka*	5	1.164	5.59	979	94	45	8.85	9.8	10.5	6.8	3.27	0.352	0.114	0.163	0.029	0.056	< 0.005
Finesand — *Hieta*	103	1.070	5.47	1 421	149	123	12.29	49.9	22.2	11.6	2.86	0.470	0.248	0.177	0.096	0.063	0.006
Silt — *Hiesu*	51	1.008	5.46	1 745	242	134	9.00	29.2	26.0	12.7	2.83	0.384	0.266	0.163	0.092	0.051	< 0.005
Clay — *Savi*	126	0.976	5.56	2 323	519	219	8.15	41.2	21.8	18.4	2.61	0.580	0.498	0.269	0.142	0.064	0.005
Gyttja clay — *Liejusavi* ...	6	0.864	4.92	885	78	121	10.24	113.6	19.4	7.4	3.18	0.360	1.534	0.319	0.322	0.071	0.010
Mould — *Multamaa* ..	57	0.654	5.04	2 165	288	101	7.34	108.8	33.7	14.4	3.34	0.700	0.517	0.263	0.260	0.110	0.017
Carex peat — *Saraturve* ...	85	0.382	4.84	1 086	262	62	6.29	157.2	27.1	11.2	3.04	0.558	0.219	0.138	0.158	0.133	0.020
Sphagnum peat — *Rahkaturve*	14	0.229	4.48	645	259	49	6.15	49.6	9.1	5.8	1.85	0.568	0.108	0.061	0.093	0.051	< 0.005
All soils — *Kaikki maat*	481	0.860	5.28	1 650	302	129	8.72	70.0	24.7	13.6	3.03	0.532	0.347	0.199	0.137	0.079	0.009

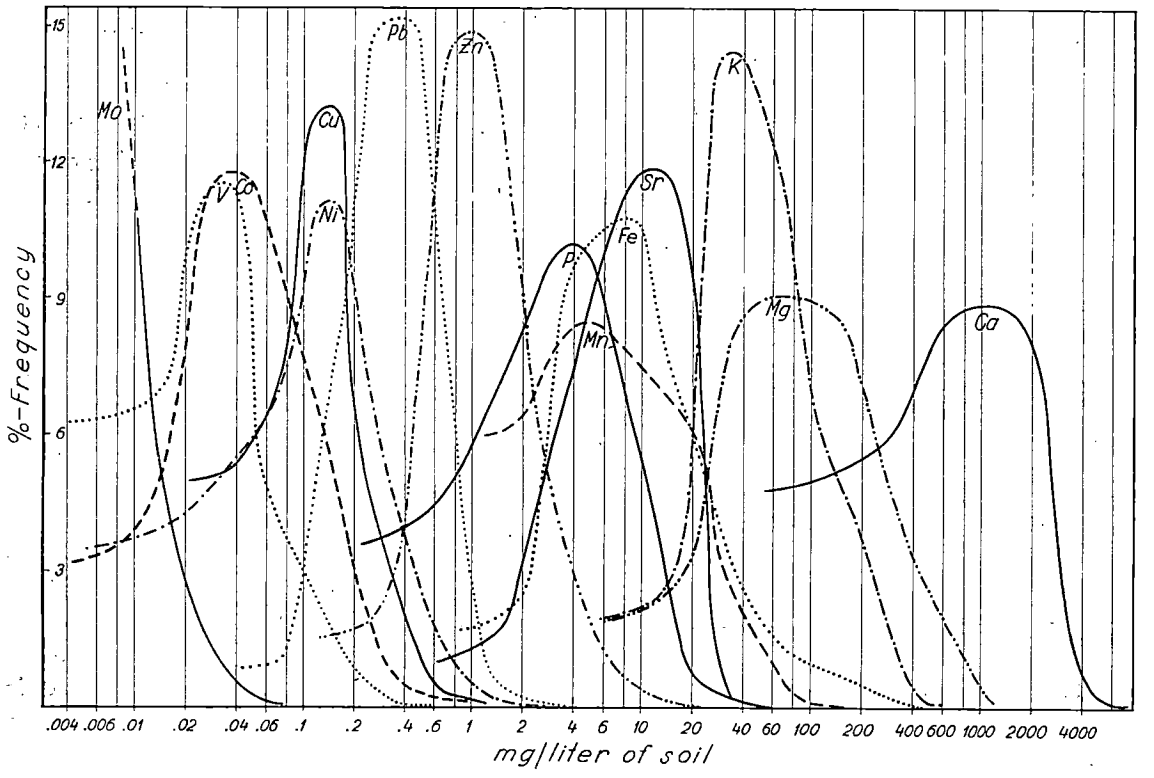


Fig. 1. General picture of the distribution of soluble elements in Finnish soils.
Kuva 1. Yleiskuva liukoisten alkuaineiden jakautumista suomalaisissa maissa.

VUORINEN 1958, MITCHELL 1964 b). The most noticeable exception is the low content of soluble vanadium, the total contents of which are usually on the same level as those of zinc and strontium. Another characteristic of acid ammonium acetate is that it extracts only the most readily soluble fraction of soil phosphorus, so that the figure obtained often amounts to less than that for iron or manganese.

In considering the results presented in Table 1, it should be noted that the concentrations are expressed on a volume basis (mg/litre of soil), which is unavoidable when soils with greatly varying bulk densities are compared. In the present material the average bulk densities of the different soil types varied by a factor of five (0.229—1.164) and that of individual samples by as much as 15 or more. Consequently, if these results were to be expressed on a weight basis (e.g. in p.p.m.), the nutrient status of peat soils would appear far too high. Particularly in Sphagnum peat soils, the nutrient status of which is known to be the lowest among Finnish soils, the ppm values would be misleadingly high. The pH values in mineral soils are around 5.5, while in organogenic soils, particularly in peats, they are considerably lower (4.5—5.0).

The soil samples under study have been divided into nine soil types [1]) representing the commonest soils in Finland. In general, the variation of soluble trace element contents is considerably wider than that of major nutrients. The internal variation within the different soil types, however, is relatively small in comparison with the total variation, as indicated by the high t-values and numerous significant differences between the contents of ten trace elements in the nine soil types (Table 2).

The results concerning trace elements given

[1]) Some of the soil types (moraine, finesand, clay) are actually groups of two or more related soil types.

Table 2. Statistical significance of the differences (t-values) between the soluble trace element contents in various soil types. Significances at 5*, 1** and 0.1*** per cent levels

Taulukko 2. Eri maalajien väliset liukoisten hivenaineiden pitoisuuksien tilastolliset erot (t-arvot). Merkitsevyydet 5, 1** ja 0.1*** prosentin tasoilla*

		Moraine *Mr*	Sand *Hk*	Finesand *Ht*	Silt *Hs*	Clay *S*	Gyttja clay *LjS*	Mould *Mm*	Carex peat *Ct*	Sph. peat *St*	
Moraine — *Mr*		•	2.645*	2.675**	0.373	2.323*	3.553***	8.384***	10.010***	0.915	Fe
		•	3.578***	1.443	0.550	1.642	2.085*	1.006	0.333	3.346**	Mn
		•	0.672	1.998*	2.702**	7.883***	0.645	3.436***	1.501	1.034	Sr
		•	1.539	3.085**	3.003**	3.440***	2.389*	1.860	3.834***	4.924***	Zn
		•	1.410	0.280	1.008	2.397*	1.340	3.546***	2.088*	1.255	Pb
Sand — *Hk*	Ni	1.117	•	5.681***	2.635*	6.239***	4.272**	10.276***	12.058***	2.397*	Fe
	Cu	0.264	•	3.079**	3.558***	3.317**	2.288*	5.710***	3.586***	0.463	Mn
	Co	2.599*	•	1.469	1.787	3.585***	0.145	2.232*	1.328	0.221	Sr
	V	0.051	•	0.346	0.366	0.557	0.067	0.048	0.843	1.659	Zn
	Mo	—	•	1.564	0.759	3.532***	0.103	4.418***	3.298***	2.173*	Pb
Finesand — *Ht*	Ni	2.649**	3.426***	•	2.007*	0.787	2.703**	6.631***	8.198***	0.777	Fe
	Cu	1.765	0.166	•	1.016	0.164	0.943	3.259**	1.200	2.713**	Mn
	Co	2.906**	4.650***	•	1.035	8.048***	1.703	2.123*	0.305	1.916	Sr
	V	0.359	0.200	•	0.073	0.704	0.565	0.569	1.628	3.941***	Zn
	Mo	0.431	3.829***	•	1.215	1.828	1.501	3.059**	1.523	1.011	Pb
Silt — *Hs*	Ni	3.138**	3.846***	0.527	•	1.566	3.379**	7.736***	9.261***	0.621	Fe
	Cu	1.129	0.000	0.757	•	1.251	1.798	1.862	0.224	3.202**	Mn
	Co	2.598*	4.342***	0.234	•	5.995***	2.111*	1.210	1.150	2.255*	Sr
	V	0.467	0.141	1.255	•	0.502	0.575	0.585	1.278	3.305**	Zn
	Mo	—	—	1.519	•	4.242***	0.659	4.845***	4.017***	1.939	Pb
Clay — *S*	Ni	8.502***	8.619***	6.383***	5.895***	•	2.964**	7.458***	9.141***	0.355	Fe
	Cu	5.198***	1.254	3.905***	4.623***	•	0.940	3.794***	1.425	2.778**	Mn
	Co	7.997***	9.891***	3.055**	3.312**	•	4.524***	3.349***	6.544***	4.266***	Sr
	V	0.376	0.206	0.021	1.303	•	1.029	0.875	1.047	3.608***	Zn
	Mo	0.000	—	0.603	—	•	3.549***	1.889	0.510	0.132	Pb
Gyttja clay — *LjS*	Ni	3.533***	3.643**	3.303**	3.258**	2.658**	•	0.652	1.334	2.890**	Fe
	Cu	5.365***	1.782	4.382***	4.883***	1.413	•	4.117***	1.928	2.136*	Mn
	Co	2.726**	2.998*	2.295*	2.337*	1.840	•	2.653**	1.499	0.407	Sr
	V	0.501	0.359	0.333	0.845	0.326	•	0.162	1.620	3.222**	Zn
	Mo	1.601	3.219*	1.438	2.079*	1.703	•	4.434***	3.310**	2.128*	Pb
Mould — *Mm*	Ni	6.434***	6.818***	4.876***	4.544***	0.331	2.595*	•	1.133	5.948***	Fe
	Cu	3.942***	1.155	2.857**	3.375**	0.180	1.397	•	1.521	4.788***	Mn
	Co	9.132***	10.188***	6.639***	6.792***	5.145***	0.625	•	2.116*	2.718**	Sr
	V	2.971**	1.486	3.446***	4.230***	3.468***	1.561	•	1.278	2.408*	Zn
	Mo	5.814***	8.081***	5.892***	6.862***	6.576***	2.422*	•	2.296*	1.328	Pb
Carex peat — *Ct*	Ni	1.581	2.440*	0.770	1.227	6.463***	3.374**	5.131***	•	7.174***	Fe
	Cu	0.106	0.293	1.682	1.112	4.881***	5.190***	3.814***	•	3.272**	Mn
	Co	6.310***	7.759***	3.196**	3.398***	0.928	1.667	3.946***	•	1.749	Sr
	V	4.173***	2.113*	4.909***	5.632***	4.942***	2.452*	1.328	•	2.610*	Zn
	Mo	5.823***	7.660***	5.829***	6.588***	6.303***	2.976**	0.930	•	0.112	Pb
Sphagnum peat — *St*	Ni	1.057	0.108	2.983***	3.341**	7.573***	3.649**	6.340***	2.207	•	
	Cu	2.932**	1.196	4.409***	3.973***	7.003***	6.954***	5.750***	2.650**	•	
	Co	0.952	1.626	0.080	0.020	1.228	2.181*	3.760***	1.553	•	
	V	0.392	0.145	0.836	0.027	0.857	0.776	3.308**	4.476***	•	
	Mo	—	—	1.326	—	—	2.061	6.024***	6.070***	•	

in Table 1 and the statistics in Table 2 are summarized in Table 3 so as to give a general picture of the relative levels of trace elements in the various soil types.

The soil types in Table 3 are arranged in order according to columns a and d. Even a glance at the figures shows that, in general, soils which are rich in some trace elements

Table 3. Summarized layout of the (9×80) statistical comparisons of average contents of ten trace elements in nine soil types. a) number of cases where the trace element content of the soil type given exceeds that in some other soil type with statistical significance; b) without reaching significance; c) is lower than in the other soil type but not significantly and d) is significantly lower

Taulukko 3. Yhdistelmä 9×80 tilastollisesta vertailusta 10:n hivenaineen pitoisuudesta 9:ssä maalajiryhmässä. a) tapausten luku, joissa annetun maalajin hivenainepitoisuus merkitsevästi ylittää jonkin muun maalajin vastaavan hivenaineen pitoisuuden; b) tapaukset, joissa ylitys ei ole merkitsevä; c) pitoisuus pienempi, mutta ei merkitsevästi, ja d) pitoisuus merkitsevästi pienempi

	a	b	c	d
Mould — *Mm*	54	16	8	2
Carex peat — *Ct*	33	20	16	11
Clay — *S*	32	17	20	11
Gyttja clay — *LjS*	29	24	14	13
Finesand — *Ht*	13	30	15	22
Silt — *Hs*	13	17	26	24
Moraine — *Mr*	11	22	17	30
Sph.peat — *St*	3	10	29	38
Sand — *Hk*	1	15	26	38

also contain others in abundance. For example, when comparing the contents of the ten trace elements in mould soils with those in the eight other soil types, the former is significantly higher in 54 out of 80 comparisons and lower in only two cases (Sr in clays and Ni in gyttja clays). In the next three soil types, the soluble trace element contents are on a relatively high level. Carex peat soils, especially, contain more V and Mo, clays more Sr and gyttja clays more Ni, Cu and Co than any other soil type. The lowest average values for any trace element are always found in Sphagnum peat or sand soils.

In mineral soils there is, in general, an inverse relationship between the soluble contents of most elements and the coarseness of soil texture, i.e. a decrease from clays to sand soils. The most noticeable exceptions to this tendency are Zn, Pb and P, for which the differences between the various soil types are smaller than for other elements. A firmer binding in the clay fraction probably has an effect on this, especially in the case of Zn and P. The gyttja clay soils, which are of postglacial origin and sedimented in salty water in the coastal lowlands of Finland, differ in many respects from other clay soils. Their sesquioxide content is usually high and owing to the presence of sulphates the pH is low. Their exhangeable alkaline and alkaline earth contents are considerably lower, while those of heavy metals, especially Fe, Ni, Cu, Co and Mo, are higher than in other clay soils.

In organogenic soils there are wide variations in the contents of various elements between the soil types. Mould soils, containing 15 to 40 per cent humus, are known to be among the best agricultural soils. This can also be seen from their high content of soluble nutrients, which is apparently due to the favourable ratios of organic to mineral matter in these soils.

With increasing organic matter the solubility of most trace elements is found to increase. In peat soils, however, the total amounts of trace elements are so limited that in spite of higher solubility the soluble amounts remain very low (SILLANPÄÄ 1962 a, b). This is especially true of Sphagnum peat soils, which contain practically no mineral material. In Carex peat soils the influence of mineral matter is more pronounced, as can be seen from their higher bulk density and considerably higher content of trace elements.

Summary

A study was conducted to obtain data on the contents of soluble trace elements in Finnish soils. The extractant used, acid ammonium acetate (0.5 N CH_3COOH, 0.5 N CH_3COONH_4, pH 4.65), was the same as is used for simultaneous determination of Ca, K and P in routine soil testing in Finland. Data of these major nutrients are also given. The results are expressed on a volume basis (mg/litre of soil).

In general, the variation of soluble trace element contents is considerably wider than that of major nutrients. The average amounts extracted increase in the following order: Mo, V, Co, Cu, Ni, Pb, Zn, P, Sr, Mn, Fe, K, Mg and Ca.

In mineral soils there is a tendency towards lower soluble contents of most elements in coarser textured soils. Among organogenic soils mould soils represent the highest and Sphagnum peat the lowest level of soluble elements. Results of statistical comparisons of the soluble contents of trace elements in various soil types are given.

REFERENCES

BARON, H. 1955. Gemeinsame Extraktion und chemische Bestimmung des leichtlöslichen Anteils der Mikronährstoffe Bor, Eisen, Kobolt, Kupfer, Mangan, Molybden und Zink in Boden. Landw. Forsch. 7: 82—89.

GOLDSCHMIDT, V. M. 1954. (Ed. Muir). Geochemistry. Oxford.

KURKI, M. 1963. Suomen peltojen viljavuudesta. Referat: Über die Fruchtbarkeit des finnischen Ackerbodens. Viljavuuspalvelu Oy. 107 p.

LAKANEN, E. 1962. On the analysis of soluble trace elements. Selostus: Liukoisten hivenaineiden analysoimisesta. Ann. Agric. Fenn. 1: 109—117.

MITCHELL, R. L. 1964 a. The spectrochemical analysis of soils, plants and related material. Commonw. Bur. Soil Sci. Techn. Comm. 44 A. 225 p.

—»— 1964 b. Trace elements in soils (Ed. Bear: Chemistry of the soil), p. 320—368. New York.

MÄKITIE, O. 1962. On the cobalt/nickel ratio in arable soils. J. Sci. Agric. Soc. Finl. 34: 91—95.

SCHARRER, K. & JUDEL, G. K. 1957. Ein spektrochemisches Analysenverfahren zur quantitativen Bestimmung von Spurenelementen in Böden, Dungemitteln und biologischen Material. Z. Anal. Chem. 139: 241—247.

SILLANPÄÄ, M. 1962 a. Trace elements in Finnish soils as related to soil texture and organic matter content. Selostus: Maan lajitekoostumuksen ja orgaanisen aineksen vaikutuksesta maan hivenaineiden pitoisuuksiin. J. Sci. Agric. Soc. Finl. 34: 34—40.

—»— 1962 b. On the effect of some soil factors on the solubility of trace elements. Selostus: Eräiden maaperätekijöiden vaikutuksesta hivenaineiden liukoisuuteen. Agrogeol. Publ. 81: 1—24.

SWAINE, D. J. 1955. The trace-element content of soils. Commonw. Bur. Soil Sci. Techn. Comm. 48: 157 p.

VIRO, P. J. 1955. Use of ethylenediaminetetraacetic acid in soil analysis: II. Determination of soil fertility. Soil Sci. 80: 69—74.

VUORINEN, J. 1958. On the amounts of minor elements in Finnish soils. J. Sci. Agric. Soc. Finl. 30: 30—35.

—»— & MÄKITIE, O. 1955. The method of soil testing in use in Finland. Selostus: Viljavuustutkimuksen analyysimenetelmästä. Agrogeol. Publ. 63: 1—44.

SELOSTUS

Liukoisista hivenaineista Suomen maalajeissa

MIKKO SILLANPÄÄ ja ESKO LAKANEN

Maatalouden tutkimuskeskus, Maantutkimuslaitos, Tikkurila

Tutkimuksen tarkoituksena oli saada yleiskuva liukoisten hivenaineiden määristä ja keskinäisistä suhteista maassa sekä vertailla niiden pitoisuuksia eri maalajeissa. Aikaisemmin on laitoksella suoritettu tutkimuksia hivenaineiden totaalimääristä maassa (VUORINEN 1958) ja selvitetty sekä totaalimäärien että liukoisuuksien riippuvuutta eri maaperätekijöistä (SILLANPÄÄ 1962 a, b).

Tutkimusaineisto, joka käsitti 481 pintamaanäytettä (0—20 cm) eri puolilta Suomea, vastasi koostumukseltaan läheisesti sekä maalajijakaantuman että pääravinteiden tason suhteen aikaisemmin julkaistuja (VUORINEN 1958, KURKI 1963) laajoja Suomen maaperää edustavia aineistoja.

Liukoisten hivenaineiden määrittämisessä käytettiin uuttonesteenä hapanta ammoniumasetaattia (pH 4.65, uuttosuhde 1:10), ts. samaa uuttonestettä ja -menetelmää kuin pääravinteiden viljavuustutkimuksessa (VUORINEN ja MÄKITIE 1955), mutta spektraalipuhtain liuoksin. Magnesium ja strontium määritettiin suoraan uutteesta Beckman DU-liekkifotometrillä, muut hivenaineet erottamisen ja rikastamisen jälkeen ARL 2-m. hilaspektrografilla (LAKANEN 1962).

Eri maalajien keskimääräiset ravinnepitoisuudet on esitetty taulukossa 1 ja yleiskuva niiden jakautumasta koko aineistossa logaritmiasteikolla kuvassa 1. Molybdeenin jakautumasta on voitu esittää vain osa, koska suuri osa Mo-pitoisuuksista on alapuolella analyysiherkkyyden. Jakautuman vinouden johdosta koko aineiston keskiarvot (taul. 1) ovat yleensä huomattavasti korkeammalla tasolla kuin vastaavien jakautumakäyrien huiput (kuva 1), jotka

kuvaavat ko. aineiden jakautuman yleisimpiä arvoja. Pääravinneanalyysien tulokset on esitetty lähinnä yleiskuvan saamiseksi koko tutkimusaineiston ravinnetasosta.

Happaman ammoniumasetaatin uuttamien hivenaineiden keskinäinen suuruusjärjestys on keskiarvojen perusteella seuraava: Mo, V, Co, Cu, Ni, Pb, Zn, Sr, Mn ja Fe, mikä on varsin hyvin sopusoinnussa Mitchell'in (1964 b) skotlantilaisista maista etikkahappouutteesta (pH 2.5) saamien tulosten kanssa. Verrattaessa esitettyjä hivenaineiden liukoisia määriä vastaaviin totaalimääriin (esim. Goldschmidt 1954, Mitchell 1964 b ja Vuorinen 1958) ilmenee niiden suuruusjärjestyksessä selvä yhdenmukaisuus. Huomattavimpana poikkeuksena on liukoisen vanadiinin alhainen pitoisuus.

Liukoisten hivenaineiden pitoisuuksien vaihtelulaajuus on yleensä huomattavasti suurempi kuin pääravinteiden. Eri maalajien sisäinen vaihtelu on kuitenkin suhteellisen pieni, mikä ilmenee mm. korkeista t-arvoista (taul. 2) ja lukuisista tilastollisesti merkitsevistä eri maalajien liukoisten hivenaineiden pitoisuuksien välisistä eroista. Taulukon 1 hivenainepitoisuuksista ja taulukossa 2 esitetyistä maalajien välisistä tilastollisista vertailuista on taulukossa 3 esitetty yhdistelmä, joka hivenaineiden laatua erittelemättä antaa yleiskuvan niiden suhteellisista määristä eri maalajeissa. Yhdistelmässä ovat maalajit sarakkeiden a ja d mukaisessa suuruusjärjestyksessä, ja siitä ilmenee mm., että maalajissa, jossa on runsaasti jotakin hivenainetta, ovat myös muiden hivenaineiden pitoisuudet korkeat.

Multamaat sisältävät liukoisia hivenaineita keskimäärin runsaimmin. Verrattaessa 10 niissä olevan hivenaineen pitoisuutta 8 muussa maalajissa oleviin vastaaviin pitoisuuksiin voidaan todeta, että 54:ssä 80:stä vertailusta multamaiden hivenainepitoisuus on merkitsevästi muita suurempi ja vain kahdessa tapauksessa (savimaiden Sr ja liejusavien Ni) merkitsevästi pienempi. Myös saraturvemaissa sekä molemmissa savimaalajiryhmissä ovat pitoisuudet varsin korkeat. Erityisesti saraturpeiden vanadiini- ja molybdeeni-, savien strontium- ja liejusavien kupari- ja kobolttipitoisuudet ovat korkeampia kuin muiden maalajien. Alhaisinta liukoisten hivenaineiden tasoa edustavat hiekka- ja rahkaturvemaat.

Kivennäismaiden hivenainepitoisuus yleensä kasvaa lajitekoostumuksen hienontuessa, ts. hiekkamaista saviin siirryttäessä. Huomattavimpia poikkeuksia tästä yleissuuntauksesta ovat sinkki ja lyijy, joiden pitoisuuksien kokonaisvaihtelukin on pienempi kuin muiden hivenaineiden. Sinkin osalta vaikuttanee tähän myös sen voimakas pidättyminen saveen. Liejusavet poikkeavat monessa suhteessa muista savimaista. Niiden seskvioksidi- ja rikkipitoisuus on yleensä korkea ja pH alhainen; vaihtuvien alkaalien ja maa-alkaalien pitoisuudet ovat alhaiset, mutta raskaiden metallien, kuten raudan, nikkelin, kuparin, koboltin ja molybdeenin, pitoisuudet ovat korkeammat kuin muiden savimaalajien.

Eloperäisten maalajien hivenainepitoisuuksissa on huomattavan suuria eroja. Esim. multamaan ja rahkaturpeiden liukoisten hivenaineiden pitoisuuksissa on tilastollisesti merkitsevä ero kaikkien muiden hivenaineiden paitsi lyijyn kohdalla. Tähän vaikuttaa ilmeisesti orgaanisen aineksen pitoisuuden suuri vaihtelu mm. siten, että se lisää hivenaineiden liukoisuutta. Toisaalta taas turvemaissa ovat hivenaineiden totaalimäärät niin pieniä, että huolimatta suuresta liukoisuusasteesta liukoiset määrät jäävät vähäisiksi (Sillanpää 1962 a, b). Tämä koskee erityisesti rahkaturpeita, joiden kivennäisainepitoisuus on erittäin vähäinen. Saraturpeissa ja multamaissa on kivennäis- ja orgaanisen aineksen suhde huomattavasti edullisempi, mikä ilmenee mm. niiden korkeammista tilavuuspainoista ja suuremmista liukoisten hivenaineiden määristä.

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 305—323 (1966)

Seria PHYTOPATHOLOGIA N. 14 — Sarja KASVITAUDIT n:o 14

VIRUS DISEASES OF CUCUMBER IN FINLAND AND CHARACTERISTICS OF THEIR CAUSAL AGENTS CUCUMBER MOSAIC AND CUCUMBER GREEN MOTTLE MOSAIC VIRUSES

ANNIKKI LINNASALMI

Agricultural Research Centre, Department of Plant Pathology, Tikkurila, Finland

Received October 12, 1966

At the present time, cultivation of cucumber (*Cucumis sativus* L.) in Finland is carried out in greenhouses throughout almost the entire country, extending as far north as the Oulu—Kemi region (65—66° N. lat.). The principal area of greenhouse cucumber production, however, is in central and southern Finland. Commercial field cultivation of this crop is, owing to the cool climate, limited to the southern and southwestern parts of the country; the northern boundary of this region passes approximately through the line Pori—Lahti (ca. 61° N. lat.).

Information about virus diseases of cucumber in Finland was given by RAINIO (1941), who reported cases from three greenhouses in central and southern Finland and named the disease »Kräusel-Krankheit». On the basis of the symptoms in cucumber as well as inoculation tests with plant sap and aphids, using as vector *Aphis gossypii* Glover, he identified the causal agent of the disease as *Cucumis* virus 1. In connexion with the counselling activities of the Department of Plant Pathology of the Agricultural Research Centre, visual observations during the course of many years have revealed several instances of cucumber virus diseases, although the causal agent of the disease was not determined (cf. JAMALAINEN 1957).

In order to find out about the present occurrence and prevalence of cucumber virus diseases in Finland, an extensive investigation was carried out throughout the whole country in the years 1962—65. Attention was given chiefly to greenhouse cultivations, but field cucumbers were also examined, principally in the main areas where this crop is grown outdoors in southwestern Finland and on the islands of Ahvenanmaa. When infected plants were discovered, the causal agent of the disease was determined on the basis of its biological, physical, and/or morphological and chemical properties. In addition, certain Wisconsin SMR cucumber cultivars, which were known to be resistant to cucumber mosaic virus, were tested for their resistance to the virus strains occurring in Finland.

Materials and methods

During the course of inspections carried out in the years 1962—65, a total of 217 greenhouse cucumber cultivations and 46 field cultivations were examined. The greenhouse cultivations examined represented about 35 % of the total number of greenhouse cucumber cultivations (according to estimates made by professional horticultural

organisations) in the country, while the field cultivations inspected made up 3—5 % of the area of commercial field cucumbers.

The greenhouse inspections were made in May—September and those on the field cultivations in July—August. From plants with symptoms indicative of possible virus infection leaf samples (a total of 54) were taken for subsequent determinations of the disease and its causal agent in laboratory and greenhouse tests. The plant testings as well as the maintenance of the virus material were carried out in a greenhouse having a mean daytime temperature of 20—25° C and night temperature about 5° lower and relative humidity of 60—70 %. Supplementary illumination in wintertime was provided by mercury vapour lamps (Osram HQL 400W/R). For insect control, the ventilators were covered with netting and furthermore sprayings with mevinphos were carried out twice weekly.

The primary testing of the samples was done by means of indicator plant tests. These inoculations, as well as all the other mechanical sap inoculations in the present work, were made with plant sap from virotic leaves, which was pressed through cheesecloth and used either as such or together with phosphate buffer (KH_2PO_4 + Na_2PO_4, 0.1 M, pH 7.0), employing carborundum (400 mesh) as abrasive. The test plants used were *Cucumis sativus* L. cv. Butcher's OE Spec., *Nicotiana glutinosa* L. and *Chenopodium quinoa* Willd. or *C. amaranticolor* Coste et Reyn.

After the primary testing, a pure virus line was isolated from each of the samples. In the case of the isolates of cucumber mosaic virus (CMV), this was done with 5—7 sap inoculation passages from local lesions through *N. glutinosa*, while for the isolates of cucumber green mottle mosaic (CGMV) similar passages were made through cucumber (Butcher's), beginning with the first leaves having systemic mosaic symptoms. The twelve CMV lines and two CGMV lines thus obtained formed the material whose detailed analysis was the basis for determining the characteristics of the types of CMV and CGMV occurring in Finland. In this paper these lines are termed isolates.

The size and shape of the virus particles were determined by electron microscopy. The material was maintained in cucumber (Butcher's).

In order to purify CMV for the electron microscopic studies, various methods were tried (TOMLINSON et al. 1959, SCOTT 1963, MURANT 1965). The method of Scott was finally selected, since it gave the best results. The purified virus mass was suspended in 0.02 M phosphate buffer, pH 7.0, or in 0.005 borate buffer, pH 9.0, and pre-fixed in a 2 % solution of formaldehyde (1 : 1) for 30 minutes (BETTO et al. 1964). To this solution was added 1 % phosphotungstic acid (PTA), pH 6.0 (1 : 1), and it was immediately sprayed onto a formvar-coated grid, after which the micrographs were made.

The electron micrographs of CGMV were made by the dip method (BRANDES 1957), dipping cucumber leaves directly into a drop of 0.5 % PTA, pH 7.0, on a formvar-coated grid. For comparison, micrographs were made in one case from palladium-shadowed dip preparations (cf. LINNASALMI 1964). A Siemens Elmiskop I electron microscope with primary magnifications of 30 000—60 000 × was used. The subsequent photographic enlargements were × 3—5, and these were used for measuring particle size. For each CMV isolate measurements were made of 300 and for each CGMV isolate of 100 particles; only negatively stained preparations were used.

For the thermal inactivation point determinations, the CMV isolates were maintained in *N. glutinosa* and the CGMV isolates in cucumber (Butcher's). The determination was made with crude plant sap pressed through cheesecloth and with the standard 10-minute heating time; the temperature was measured with a thermometer inserted in the test tube. The temperature scale for the CMV tests was 52—76° C with intervals of four degrees, and for the CGMV tests it was 86—98° C with two-degree intervals. The test plant in the CMV tests was *C. quinoa*, in the CGMV tests cucumber (Butcher's).

Serological testing of the CGMV isolates was performed with CGMV antiserum, using the slide agglutination method (MUNRO 1954).

For the determinations of pathogenicity, the CMV isolates were maintained in *N. glutinosa* and tests were carried out in the cucumber cultivars Butcher's OE Spec. and Superb OE 48, *N. glutinosa* and *C. quinoa*. In conjunction with these pathogenicity studies, the Wisconsin cucumber cultivars SMR15, SMR18 and SMR58 were tested for their resistance to the CMV isolates, using for comparison cultivar Superb OE 48. It was found that infection took place better when the test plants were kept in a cool, dark chamber (12—14° C) for 1—2 days before and after inoculation and subsequently in the greenhouse at a temperature below 18° C for the following 2—3 days. All the CMV sap inoculations were carried out in the above manner.

The CGMV isolates were maintained in cucumber (Butcher's) and their pathognicity was tested in the following plants: in the *Cucurbitaceae* plants *Cucumis sativus* (Butcher's), *Cucurbita pepo* L., *Citrullus vulgaris* Schrad., *Bryonia alba* L., in the *Solanaceae* plants *Capsicum annuum* L., *Datura stramonium* L., *Nicotiana tabacum* L. cv. White Burley, and in *C. amaranticolor (Chenopodiaceae)* and *Vigna sinensis* (L.) Endl. cv. Black Eye *(Leguminosae)*.

Inoculation in the pathogenicity tests was performed by mechanical sap inoculation. In control tests, the transmission of CMV and CGMV by means of vectors was tried. The vector species was *Myzus persicae* Sulzer, the fasting period 18 hours, acquisition time 2 minutes and inoculation period 24—36 hours.

Analyses of the amino acid composition of the protein coat were made on CMV material maintained in cucumber (Butcher's) and purified according

to the method of SCOTT (1963). Leaves were macerated in a blender in a mixture of citrate buffer and chloroform (with thioglycolic acid), pressed through cheesecloth and centrifuged at low speed. The dialysis time of the water phase against borate buffer was 24 hours. Between the three cycles of low and high speed centrifugation, the pellets were resuspended in borate buffer and finally, before protein separation, in 0.02 M phosphate buffer at pH 7.0. All the operations were performed at temperatures of 4—10° C.

CGMV material was maintained in cucumber (Butcher's) and purified as follows for the protein analyses. Deep-frozen leaves (—18° C) were macerated in a blender together with dipotassium phosphate (3 %) and differentially centrifuged 4—6 times (5 000 g 10 min., 57 000 g 60 min.). The precipitate was suspended in 0.1 M phosphate buffer at pH 7.0 and finally twice in 0.02 M phosphate buffer at pH 7.0. After each high-speed centrifugation the virus suspension was held for 2—12 hours at a temperature of —18° C.

The protein in the purified virus material was separated by the phenol method (KNIGHT 1963, p. 39). Part of the air-dried protein was hydrolysed for 24 hours in 6 N HCl (5 mg/ml) at 110° C under nitrogen. The remaining part of the protein was used as such for the tryptophan determinations.

Qualitative analyses of the amino acids were made on the acid hydrolysate, using a combination of thin-layer electrophoresis and thin-layer chromatography based on the amino acid analysis method of NYBOM (1964). The thin-layer mass consisted of cellulose MN 300, 20 × 20 cm, the electrophoretic solvent was 0.7 % formic acid, voltage 450 V, time 45 min.; the chromatographic solution was n-butanol/acetic acid/water (4 : 1 : 2), the stain was 2 % ninhydrin mixed in the solution.

Quantitative amino acid analyses were made on the HCl hydrolysate according to the method of MOORE and STEIN (cf. ANTILA et al. 1966), using auto-analyser equipment of Technicon Instruments Ltd. except for tryptophan, which was analysed by the method of SPIES and CHAMBERS (1949), using a Beckman Quartz DU Spectrophotometer for the absorption measurements.

Results

Occurrence of virus diseases

In the years of the investigation, sporadic occurrences of cucumber virus diseases were found in about 4.5 % of the greenhouse and field cultivations inspected throughout the country (Fig. 1). In ten cases the cucumber mosaic disease, caused by CMV, was observed, while there were only two cases of the cucumber green mottle mosaic, caused by CGMV; both of these

Table 1. Occurrence of virus diseases in cucumber cultivations in Finland, 1962—65
Taulukko 1. Virustautien esiintyminen kurkkuviljelmillä v. 1962—65

Province	Greenhouse cultivations			Field cultivations		
	no. inspected	virotic		no. inspected	virotic	
		no.	disease and causal agent		no.	disease and causal agent
Ahvenanmaa ..... (1)[1]	5	0		24	0	
Turku—Pori ..... (2)	9	0		19	1	cucumber mosaic, CMV
Uusimaa ..... (3)	68	2	cucumber mosaic, CMV	2	1	» »
Kymi ..... (4)	15	0		0	0	
Häme ..... (5)	36	0		0	0	
Mikkeli ..... (6)	10	0		0	0	
Vaasa ..... (7)	28	2	cucumber green mottle mosaic, CGMV	0	0	
Keski-Suomi ..... (8)	13	0		1	1	cucumber mosaic, CMV
Kuopio ..... (9)	19	1	Cucumber mosaic, CMV	0	0	
Oulu .....(10)	10	3	» »	0	0	
Lappi .....(11)	4	1	» »	0	0	
Total	217	9		46	3	

[1]) number of provinces in the map, Fig. 1 p. 308

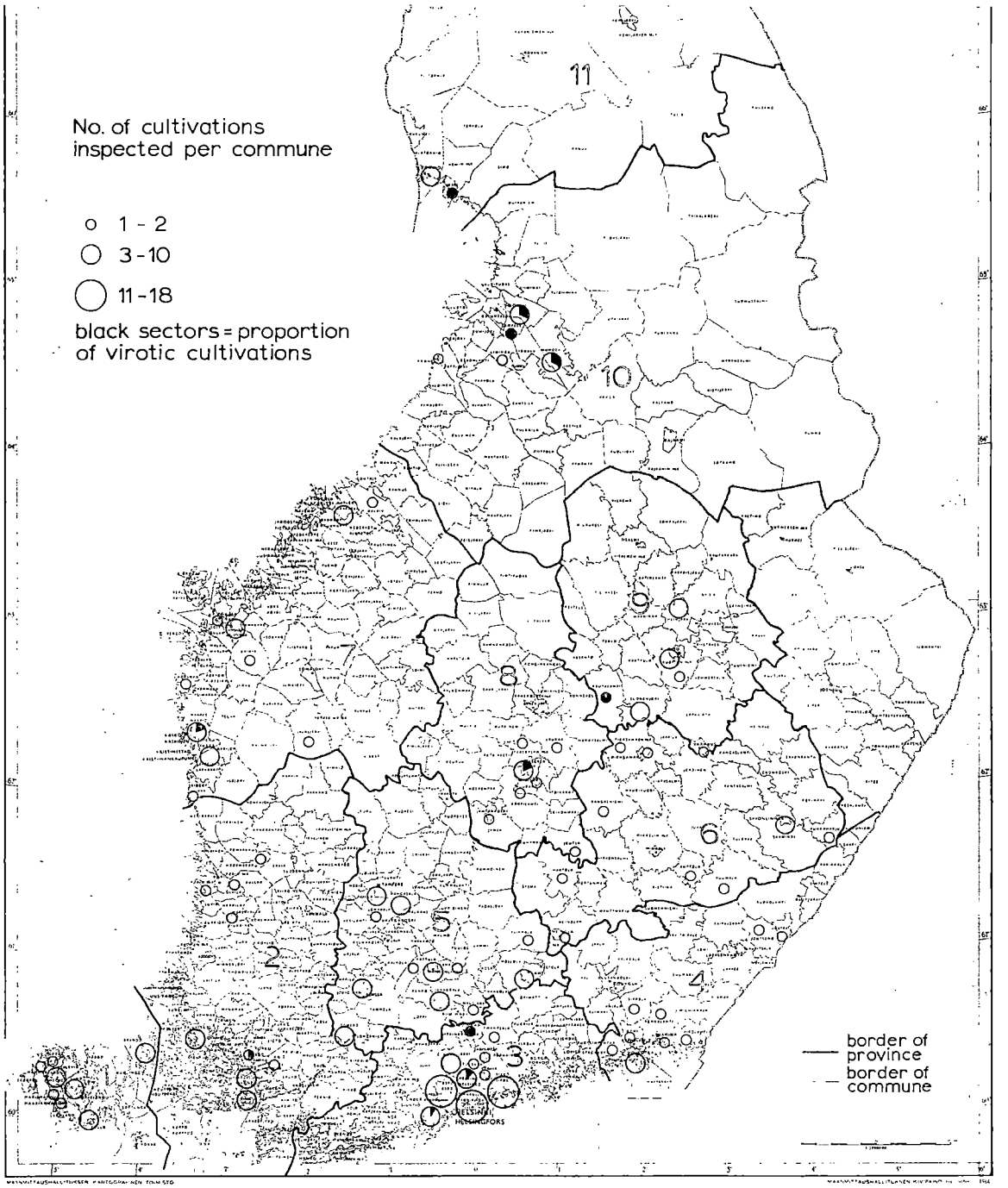


Fig. 1. Location of inspected cucumber cultivations and occurrence of virus diseases.
Kuva 1. Tarkastettujen kurkkuviljelmien sijainti ja virustautisuus.

latter occurred in the province of Vaasa (Table 1). At the time of inspection, plants infected with CMV invariably made up only a few per cent of the whole cultivation and usually occurred close to one another in groups. In both of the CGMV cases, the greenhouse cultivations at the

time of examination (in July) were completely infected.

Cucumber mosaic was encountered in the greenhouse cultivars Arla, Bestseller and Vestervang, as well as in the field cultivars Favör, Rheintraube and Superb. Cucumber green mottle mosaic occurred in the greenhouse cultivars Butcher's and President but not in the field cultivars. Arla was the favorite greenhouse cultivar, grown in about one-third of the greenhouses inspected (97 cultivations). Other rather common cultivars, in order of frequency, were Perseus (60), Rea (24) Bestseller (23), Butcher's (17), Vestervang (14) and President (10). AH 17, Topscore, Filia, Monopol, Beste von Allen and Greenspot were rarer (2—9 greenhouses). Of the field cucumbers, Favör (16) and Superb (16) were by far the most commonly grown cultivars in Finland. Reintraube, Muromi, Spångberg and Cavallius (1—6 fields) are gradually losing popularity. Since, as far as is known, none of these cultivars are resistant to CMV and CGMV, it is understandable that the few instances of virus infection tended to accumulate in the most commonly grown cultivars.

During this investigation, attention was also paid to the substrate used for cucumbers in the greenhouses as well as to whether it was fresh or had previously been used in greenhouse cultivation. It was found that about 65 % of the growers used ordinary garden soil, and about one-half of these employed fresh soil every year. In ca. 25 % of the greenhouses the substrate was mill-peat and, except in two cases, had not been used previously. The remaining 10 % of the growers used mixtures of fresh soil and mill-peat or bog-moss.

CMV infection occurred in both soil and peat cultivations, and in at least two cases it was found in plants growing in fresh mill-peat (isolates Muhos K4/64, Oulu K6/64). In one instance (Rautalampi K3/64) one chrysanthemum harvest had been obtained in the peat, while in another instance (Kemi K5/64) the soil had previously been used for growing tomatoes. The kind of substrate and its earlier use had hardly any effect on the occurrence of CMV.

On the other hand, tomatoes and chrysanthemums, which are hosts of CMV, may have been able to maintain the virus in the two above-mentioned cases. Through handling of the plants or by means of vectors, the virus could have been transferred to the cucumbers. In one greenhouse, CMV was found at the same time in both cucumbers and tomatoes (Kemi K5/64), and aphids were abundant, especially on the tomato plants. It may be mentioned that this same greenhouse was earlier found to be severely infested with peach aphids (Heikinheimo 1959). In certain north Finnish greenhouses (K3/64, K6/64, K7/64) the severe occurrence of cucumber mosaic may have been due in part to the relatively low temperatures (at night as low as 12—15° C) prevailing in the greenhouses for some two months before inspection. Such low temperatures proved to be optimal for infection by the CMV isolates (cf. p. 306).

The two greenhouses infected with CGMV (Närpiö K14/62, K16/62) were situated adjacent to one another. Each of them had used soil continuously without disinfection for at least the three preceding years. In two of the years when examinations were made (1962 and 1963) the cucumbers in these greenhouses were 100 % infected with green mottle mosaic. This was to be expected, since CGMV is known to be resistant to aging (Smith 1957) and is readily transferred from one plant to another during cultural operations. It could not be determined whether the disease had possibly been originally introduced into the greenhouse in infected seeds.

Causal agents of the virus diseases

Cucumber mosaic virus

Regardless of the cultivar, the cucumbers from which the CMV isolates originated had similar symptoms, namely leaf mottling which was especially pronounced in the young leaves (Fig. 2). In two cases, there was mild wrinkling and slight evidence of yellowish spots in field cucumbers, but the lines isolated from these plants (K25/63,

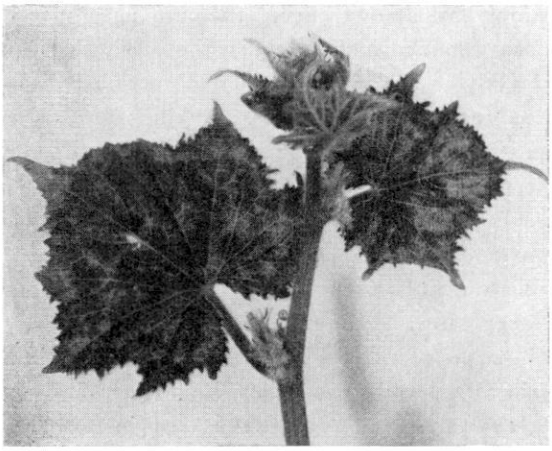


Fig. 2. Cucumber mosaic symptoms in cucumber cv. Superb OE 48, CMV isolate K25/63. Orig.
Kuva 2. Kurkun mosaiikki-oireet kurkussa.

K27/63) produced quite normal leaf mottling symptoms in tests with cucumber. In only two instances were there symptoms in the fruits, consisting of mild mottling in Superb (K25/63) and pale, round spots in Bestseller (K2/64).

The isolates obtained from tomatoes (KT144/63, KT106/64) induced typical narrow-leaf symptoms when tested in tomatoes and normal leaf mottling when tested in cucumbers.

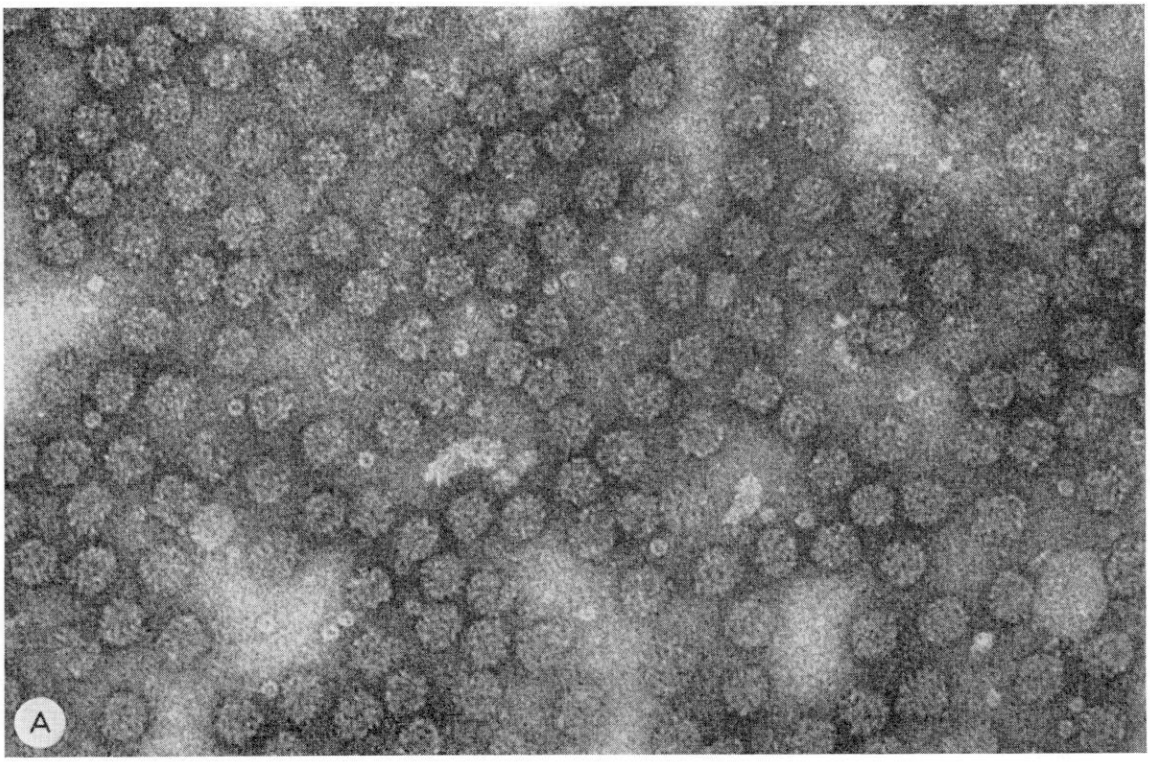
Determinations of the size and shape of the virus particles were made on the CMV isolates K31/63 and K6/64. When the material was purified according to the method of TOMLINSON et al. (1959) the electron micrographs were unsuccessful. When purified by the method of MURANT (1965), only the virus pre-fixed with formaldehyde gave fair micrographs. The best results were obtained with negatively PTA-stained preparations from material which had been purified by the procedure of SCOTT (1963) and fixed in formaldehyde (Fig. 3). The particles of both isolates were found to be similar, spherical in shape with mean diameter of K31/63 28.9 $\pm$ 4 mμ and of K6/64 28.7 $\pm$ 3 mμ. The electron micrographs also revealed that the virus material was free of visible impurities, a fact which was important to know, since this same material was used for subsequent chemical analyses (cf. KNIGHT 1963, p. 20).

The thermal inactivation point (Table 2) was determined by inoculating *C. quinoa* plants when they were at the 4- to 6-

Table 2. Characteristics of CMV isolates
Taulukko 2. CMV-isolaattien ominaisuudet

Isolate no.	Origin, cultivar	Pathogenicity				Thermal inactivation point °C
		Cucumis sativus local lesions, systemic symptoms		*Nicotiana glutinosa* local lesions, systemic symptoms	*Chenopodium quinoa* local lesions	*Chenopodium quinoa* local lesions
		Butcher's OE Spec.	Superb OE 48			
	field cucumber					
K25/63	Superb OE 48	2/3[1])	2/5	4/4	4/5	64° 2/5 68° 0/5
K27/63	Favör	3/4	5/5	15/17	4/4	72° 1/4 76° 0/4
K31/63	Rheintraube	19/22	5/5	19/25	11/11	68° 3/5 72° 0/5
	greenhouse cucumber					
K2/64	Bestseller	4/4	1/5	10/10	7/8	56° 1/4 60° 0/4
K3/64	Arla	2/5	5/5	2/4	20/20	72° 4/5 76° 0/5
K4/64	Vestervang	4/4	5/5	4/4	16/16	68° 2/4 72° 0/4
K5/64	»	3/4	5/5	2/4	5/5	72° 2/4 76° 0/4
K6/64	Arla	17/17	5/5	7/8	5/5	68° 1/5 72° 0/5
K7/64	»	4/4	5/5	1/4	20/20	74° 3/5 76° 0/5
K3/65	Bestseller	8/8	5/5	3/6	6/8	72° 4/5 76° 0/5
	greenhouse tomato					
KT144/63	Selandia	1/3	0/5	3/8	20/20	60° 2/4 64° 0/4
KT106/64	Potentat	2/4	2/5	3/8	20/20	72° 2/4 76° 0/4

[1]) No. of plants infected/no. inoculated.



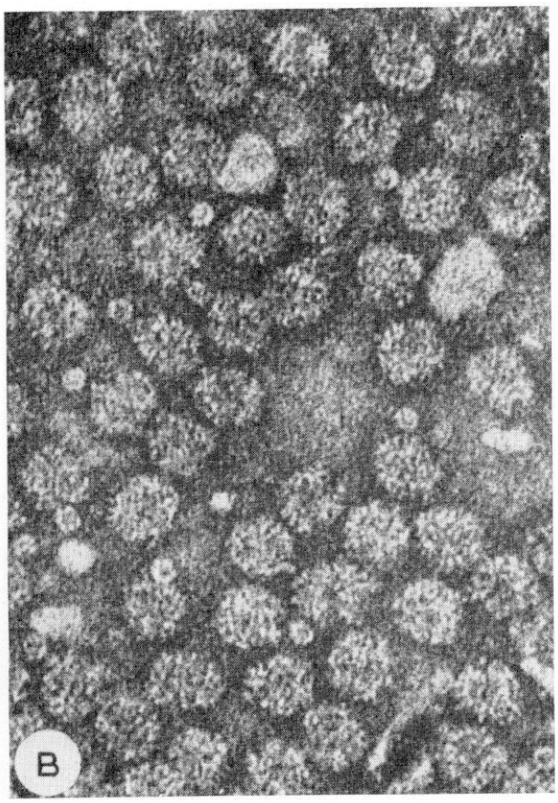


Fig. 3. CMV, isolate K6/64; fixed with 1 % formaldehyde, stained with 0.5 % PTA. A. × 210 000, B. × 300 000. Orig.

Kuva 3. CMV, isolaatti K6/64

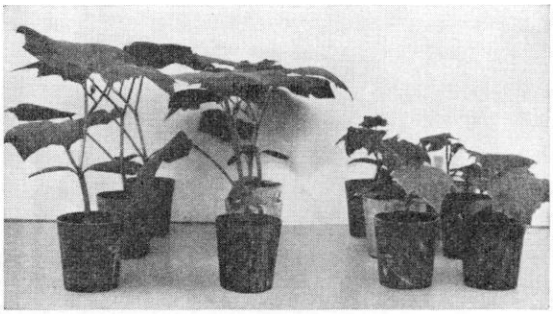


Fig. 4. Stunted growth caused by CMV, isolate K6/64 in cucumber cv. Butcher's OE Spec. Right, inoculated; left, uninoculated controls. Photo 4 weeks after inoculation. Orig.

Kuva 4. CMV:n aiheuttama kitukasvuisuus kurkussa.

leaf stage. Local lesions appeared within 5—8 days, becoming visible first in the plants inoculated with unheated control sap and later in those treated with sap which had been heated close to the inactivation point. Most of the isolates were inactivated around 70° C, while two isolates from cucumber (K25/63, K2/64) and one from tomato (KT144/63) became inactivated near the temperature of 60° C.

The results of the pathogenicity tests were as follows (Table 2).

Two cultivars of *Cucumis sativus*, Butcher's OE Spec. and Superb OE 48, were inoculated at the cotyledon stage. Pale local lesions appeared in the infected leaves 3—5 days later (extremes from 2 to 7 days), and systemic green mottling became visible — depending on the growth rate of the plant — 10—14 days later (7—20 days), beginning from the first true leaves. The growth of infected plants was stunted as compared with the healthy controls (Fig. 4). All the isolates obtained from cucumber as well as one from tomato succeeded in infecting both cucumber cultivars; the other isolate from tomato (KT144/63) infected Butcher's only slightly and Superb not at all.

Nicotiana glutinosa was inoculated at about the 4-leaf stage. Pale local lesions became visible about one week after inoculation, and systemic yellowish green mottling appeared in the new leaves about 10—14 days afterwards. Later the leaves became deformed, somewhat asymmetric, blistered and slight distorted. All the isolates caused infection in this test plant. The success of infection was somewhat more variable than in cucumber, possibly owing to genetic heterogeneity of the seed.

Chenopodium quinoa was inoculated when the seedlings had 4—6 true leaves. Local lesions began to appear about 2—4 days later as tiny translucent spots, and a week later they formed distinctly yellowish, round lesions 3—4 mm in diameter which later became necrotic. *C. quinoa* became nearly 100 % infected by all the isolates.

Inoculations using *Myzus persicae* as vector were made with one isolate from greenhouse cucumber (K3/63) and two isolates from field cucumber (K25/63, K31/63). The acquisition plant was cucumber (Butcher's), while the plants receiving the inoculations were seedlings of this cucumber and of *Cucurbita pepo* in the 1—2 true leaf stage. All three isolates caused infection in the test plants. Pale, round, ring-like lesions appeared in the cotyledons 1—2 days after inoculation, while systemic mottling symptoms developed in the true leaves about one week afterwards.

Resistance tests with the Wisconsin cultivars SMR15, SMR18 and SMR58 demonstrated that none of these cultivars were resistant to all of the isolates tested (Table 3). The isolates whose thermal inactivation point was around

Table 3. Resistance of some Wisconsin SMR cucumber cultivars to Finnish CMV isolates

Taulukko 3. Eräiden Wisconsin SMR-kurkkulajikkeiden resistenssi CMV-isolaatteihin nähden

Isolate no.	Wisconsin SMR15	Wisconsin SMR18	Wisconsin SMR58	Superb OE 48 (for comparison)
K25/63	0/5[1])	0/5	0/5	2/5
K27/63	5/5	5/5	5/5	5/5
K31/63	5/5	5/5	5/5	5/5
K2/64.....	1/5	0/5	1/5	1/5
K3/64	5/5	5/5	5/5	5/5
K4/64	5/5	5/5	5/5	5/5
K5/64	5/5	5/5	5/5	5/5
K6/64	5/5	5/5	5/5	5/5
K7/64	5/5	5/5	5/5	5/5
KT144/63 ...	1/5	0/5	0/5	0/5
KT106/64 ...	5/5	4/5	5/5	2/5

[1]) No. of plants infected/no. inoculated

72—76° produced 100 % infection in all the Wisconsin cultivars and also in Superb OE 48. On the other hand, the group of isolates having a thermal inactivation point under 70° caused less severe infection. Isolate K25/63 did not infect any of the Wisconsin cultivars and only mildly infected Superb OE 48; KT144/63 caused mild infection in SMR15, while K2/64 induced no infection in SMR18 and only slight infection in the other cultivars. These results indicate that the cucumber cultivars tested were somewhat more resistant to the group of isolates having the lower thermal inactivation point (under 70° C).

Cucumber green mottle mosaic virus

The symptoms caused by both CGMV isolates in their original host cucumbers were leaf mottling as well as pronounced crinkling and rugosity of the young leaves (Fig. 5). The mottling produced by the isolate K14/62 in Butcher's cucumber had a tinge of yellow, wnile the second isolate K16/62 caused variegation of fruit in the original host plant, the cultivar President.

Determinations of the size and shape of the virus particles, made by electron microscopy, did not reveal any differences between the two isolates. The particles of both were ca. 290 mμ long and 14 mμ in diameter, and they consisted of rigid rods with a distinct central channel (Fig. 6, Table 4).

To ascertain the thermal inactivation point, cucumber seedlings having 2—3 true leaves were inoculated. When the plants were inoculated with unheated sap or with sap which had been heated to temperatures lower than 90° C, all of them became infected, and systemic mottling appeared within a couple of weeks. The thermal inactivation point of both isolates was quite high, around 95° C (Table 4). When the sap was heated to temperatures close to the inactivation point, the inoculated plants developed symptoms later, even as much as 5 weeks after inoculation, and only some of them showed symptoms.

Serological testing with CGMV antiserum gave positive results with both isolates. The agglutination reaction of both isolates was strong (Table 4).

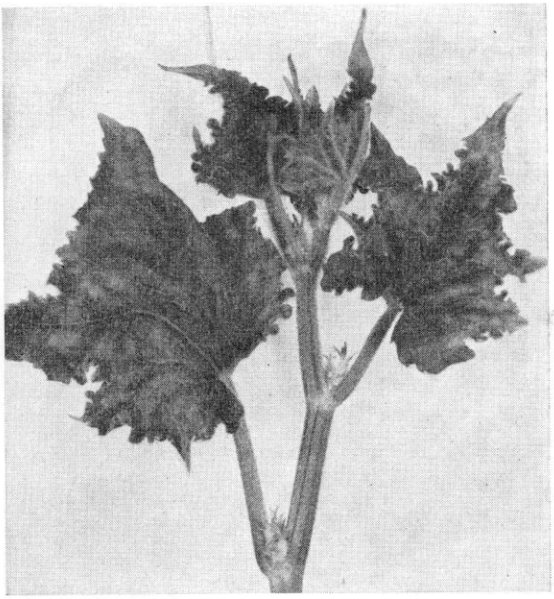


Fig. 5. Cucumber green mottle mosaic symptoms in cucumber cv. Butcher's OE Spec., CGMV isolate K14/62. Orig.

Kuva 5. Kurkun vihermosaiikki-oireet kurkussa.

Table 4. Characteristics of CGMV isolates

Taulukko 4. CGMV-isolaattien ominaisuudet

Isolate no.	Origin, cultivar	Particle size mμ		Thermal inactivation point °C	Serological test		Pathogenicity	
		length	diameter	*Cucumis sativus* Butcher's OE Spec. systemic symptoms	antiserum	normal serum	*C. sativus* Butcher's OE Spec. systemic symptoms	*Citrullus vulgaris* systemic symptoms
K14/62	Butcher's	291 ± 12	13 — 15	94° 1/4[1]) 96° 0/4	+++	—	20/20	1/2
K16/62	President	292 ± 10	13 — 15	93° 4/6 95° 0/6	+++	—	19/20	1/2

[1]) No. of plants infected/no. inoculated

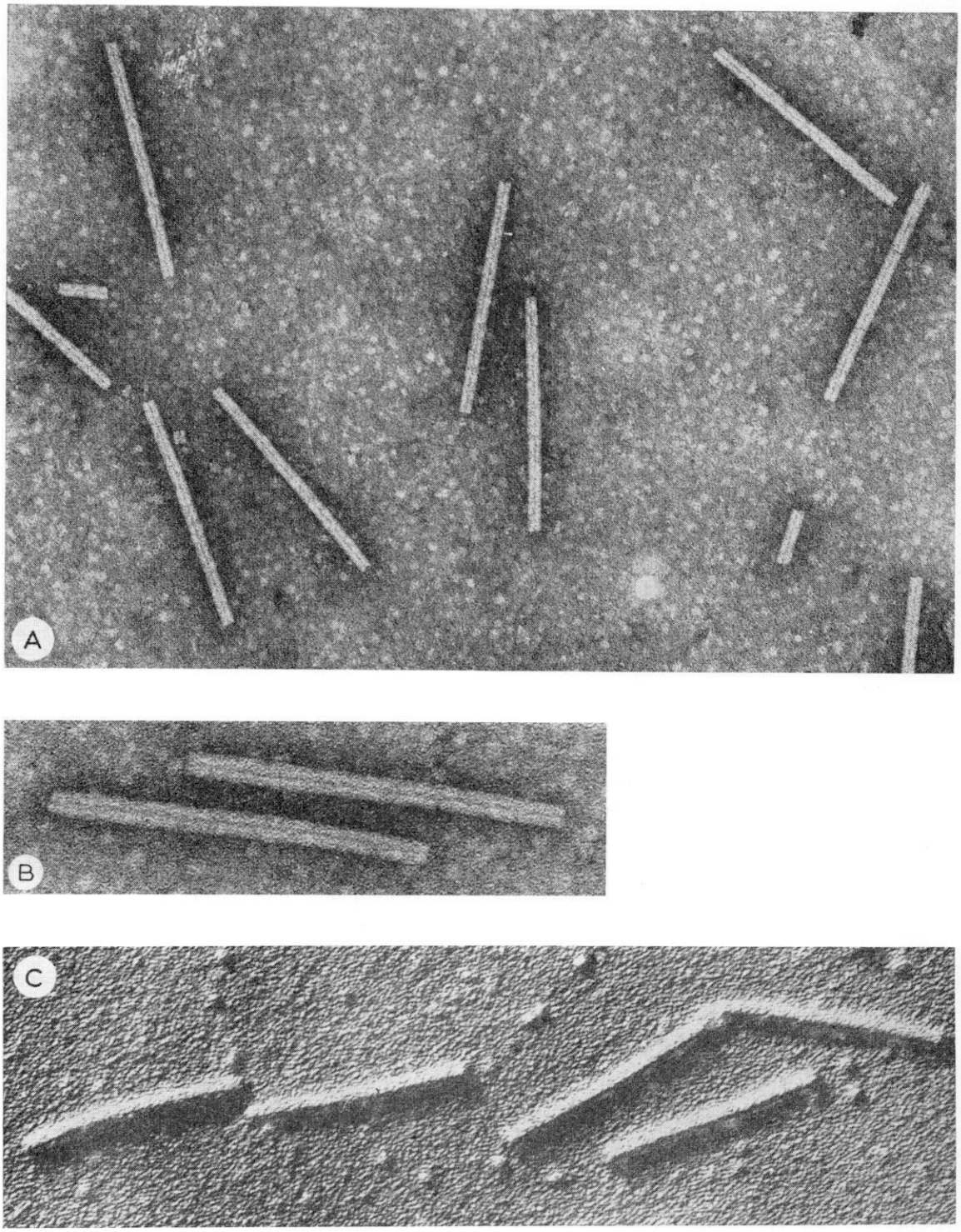


Fig. 6. CGMV, isolate K16/62; dip method; A. and B. stained with 0.5 % PTA, C. Pd-shadowed, angle 20°. A. × 130 000, B. × 210 000, C. × 130 000. Orig.

Kuva 6. CGMV, isolaatti K16/62.

Pathogenicity tests (Table 4) demonstrated that the symptoms induced by the two isolates in the cucumber test plant, Butcher's OE Spec., were quite similar, namely dark green mottling and rugosity of the leaves. The infectivity at mechanical inoculation was especially strong, since nearly all the plants became infected. The symptoms appeared about 3 weeks after inoculation, or in some exceptional cases as early as 2 or as late as 4 weeks after inoculation. The isolates also caused infection in *Citrullus vulgaris*, resulting in relatively weak systemic mottling of the leaves. No infection occurred in *Cucurbita pepo*, *Bryonia alba*, *Datura stramonium*, *Nicotiana tabacum* cv. White Burley, *Chenopodium amaranticolor* or *Vigna sinensis* cv. Black Eye. Attempts at transmission with *Myzus persicae* gave negative results in Butcher's cucumber.

Table 5. Amino acid composition of protein coat of CMV and CGMV

Taulukko 5. CMV:n ja CGMV:n proteiinikuoren aminohappokoostumus

Amino acid	Virus and isolate		
	g amino acid per 100 g protein		
	CMV	CGMV	
	K6/64	K14/62	K16/62
Alanine	3.8 ± 0.2	8.0	8.2
Arginine	7.3 ± 0.2	8.7	8.5
Aspartic acid	8.1 ± 0.5	11.5	11.9
Glutamic acid	9.0 ± 0.3	7.1	7.4
Glycine	4.1 ± 0.2	2.1	2.1
Histidine	2.3 ± 0.1	0.0	0.0
Isoleucine	4.1 ± 0.1	4.8	4.6
Leucine	6.9 ± 0.2	6.8	7.2
Lysine	6.6 ± 0.2	3.3	3.3
Phenylalanine	5.5 ± 0.0	8.3	8.2
Proline	3.6 ± 0.6	4.6	4.8
Serine	4.4 ± 0.2	10.1	10.0
Threonine	3.4 ± 0.2	5.6	5.6
Tryptophan	2.0 ± 0.0	1.1	1.3
Tyrosine	2.1 ± 0.0	3.2	3.3
Valine	4.7 ± 0.2	6.1	6.1

The results of analyses of the amino acids of the protein coats of both viruses CMV and CGMV are described together.

Preliminary qualitative analyses were made on two CMV isolates (K31/63 and K6/64) and one CGMV isolate (K16/62). For all the isolates tested, thin-layer chromatograms showed distinct, separate spots of alanine, arginine, aspartic acid, glycine, lysine, phenylalanine, proline, serine, tyrosine and valine as well as two joint spots, one of glutamic acid and threonine and the other of isoleucine and leucine.

Quantitative analyses were made on the CMV isolate K6/64 as well as on the two CGMV isolates (Table 5). The protein of CGMV consisted of 15 amino acids (Fig. 7). The various amino acids occurred in about the same proportions in both isolates. The small differences in the amounts of some amino acids are on the same order as those in the parallel determinations of the protein of the CMV isolate analysed. The protein of the latter contained histidine in addition to the acids found in CGMV, or a total of 16 amino acids (Fig. 7). Two parallel determinations were made, and they agreed well with one another. The amounts of arginine, isoleucine and leucine were about the same in CMV as in CGMV. The largest differences were found for alanine and serine, whose amounts in CMV were about one-half those in CGMV, and for glycine and lysine, which occurred about twice as abundantly in CMV as in CGMV.

Discussion

Cucumber mosaic virus has a wide range of host plants and is known to cause mosaic disease in cucumbers in many parts of the world. It is especially injurious in the U.S.A. (Doolittle 1920, Faan and Johansson 1951) and Canada (McClanahan and Guyer 1964). It is also common in Israel (Cohen and Nitzany 1962) and Japan (Komuro 1963). In Europe the first reports of cucumber mosaic virus in cucumber cultivations are apparently from England (Ainsworth 1935). In Holland (Tjallingii 1952, van Koot and van Dorst 1959) and in certain parts of Germany (Herold and Bremer 1958) this virus may cause extensive damage to field cucum-

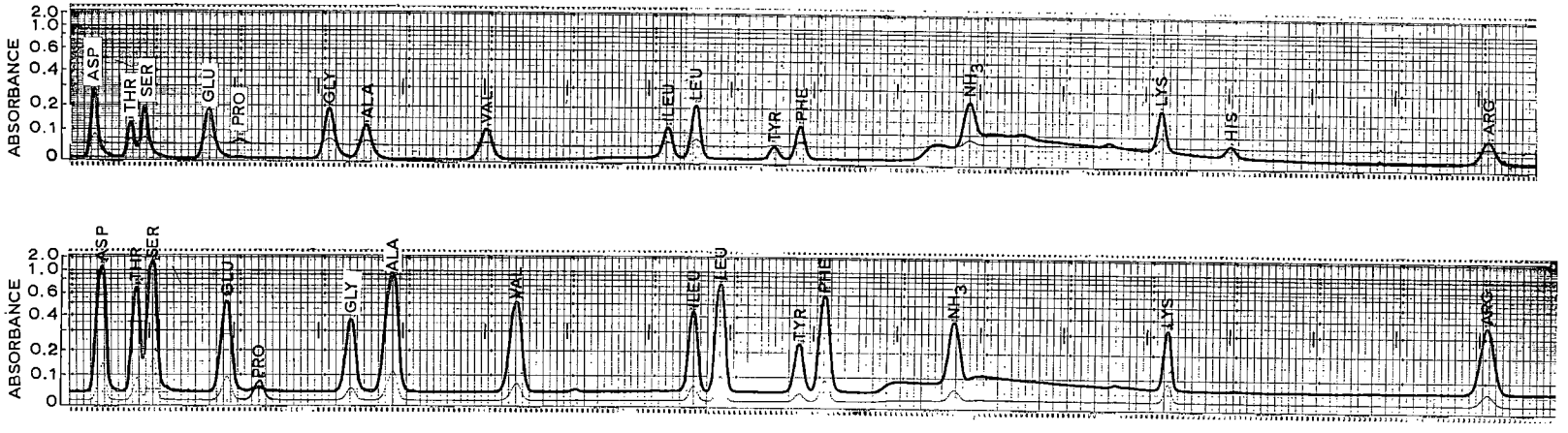


Fig. 7. Amino acid chromatograms of CMV and CGMV. Aliquot 1 ml; above CMV isolate K6/64, concentration: nitrogen 21 γ/ml; below CGMV isolate K16/62, concentration: nitrogen 67 γ/ml.

Kuva 7. CMV:n ja CGMV:n aminohappokromatogrammit.

bers in unfavourable years. The disease also occurs in cucumbers in east-European countries (SUHOV 1954, KOVACHEVSKI 1961) and in southern Europe (GRANCINI 1955).

In the Nordic countries CMV in cucumber cultivations is not economically so important as in many of the above-mentioned countries. Severe outbreaks of the disease are rare in Denmark (KRISTENSEN 1956) and are not common in Norway, either (RAMSFJELL 1952). In Sweden, LIHNELL (1951) mentions that CMV has never been encountered in cucumbers, even though it is otherwise common, for instance, in spinach. The situation in Scandinavia is thus the same as in Finland, where, according to previous reports (RAINIO 1941, JAMALAINEN 1957) and to the present investigation, CMV is found only sporadically, in both greenhouse and field cultivations of cucumber.

An abundant aphid population is held to be a prerequisite for the epidemic occurrence of cucumber mosaic disease. The following aphid species are especially important: *Myzus persicae* Sulz. (= *Myzodes persicae* Sulz.), *Aphis gossypii* Glov. (= *Cerosipha gossypii* Glov.), *A. fabae* Scop. and *Macrosiphum euphorbiae* (Thomas) (= *Macrosiphon solani* Kittel = *M. solanifolii* Ashm.) (FAAN and JOHNSSON 1951, TJALLINGII 1952, HEROLD and BREMER 1958, MCCLANAHAN and GUYER 1964). Due to the climatic conditions in Finland, the anholocyclic chief vectors of CMV, *Myzodes persicae*, *C. gossypii* and *M. solani*, are unable to overwinter outdoors. They may survive during the winter mainly in greenhouses, and consequently they seldom occur abundantly on the field. *A. fabae* is the only one of the chief CMV vectors which, being holocyclic, can overwinter in Finland and often occurs in large numbers (HEIKINHEIMO 1959, VAPPULA 1965, HEIE and HEIKINHEIMO 1966).

In the present investigation it was not possible to show that the soil had been a source of CMV infection. Indeed, this virus, which is generally rapidly destroyed in vitro (HEIN 1957, SMITH 1957), apparently does not persist actively in the soil.

Owing to the wide range of host plants of the cucumber mosaic, this virus can survive and propagate not only in vegetables and ornamentals but also in weed species. Certain plants which in Finland commonly grow as weeds, such as *Artemisia absinthium* L., *Melandrium album* (Mill.) Garcke, *Senecio vulgaris* L., *Sonchus oleraceus* L., *Stachys palustris* L., and *Stellaria media* (L.) Vill. (HIITONEN 1933, HULTÉN 1950), are natural sources of CMV infection in central Europe (TJALLINGII 1952, HEIN 1957, 1959, HEROLD and BREMER 1958, SCHWARZ 1959). This matter has not been studied in Finland; in this country the only plants which could be considered as harbouring the virus are perennial weeds with underground parts which survive in the winter. An example of this was noted at Tikkurila, from 1963 onwards, when CMV was found to be present in *Mentha gentilis* L. var. *Arrhenii* Lindb. fil. (A. Murtomaa, unpublished data) and presumably spread from it every year to the cucumbers grown in the plot. Transmission of CMV via the seeds is very rare and thus of no significance (CROWLEY 1957).

In previous studies many different strains of cucumber mosaic virus have been described, chiefly on the basis of the symptoms they produce in different plants.

The isolates tested in the present investigation were observed to be, in respect of the symptoms they induced in cucumber, similar to the green strains described in the literature (cf. SMITH 1957). Under the experimental conditions employed here, i.e. the 20—25° C temperatures prevailing in the greenhouse during the growing period, it was not possible for the top necrosis and wilting symptoms caused by certain CMV strains to appear, since according to TJALLINGII (1952), VAN DORST (1963), and HEROLD and BREMER (1958) such symptoms occur only at low temperatures.

The symptoms induced by the isolates in *N. glutinosa* resembled those produced by Porter's cucumber mosaic virus, according to the descriptions given by PRICE (1934). Such symptoms, namely green and pale green mottling as well as blistering, leaf distortion and stunting were char-

acteristic of both Porter's cucumber mosaic virus and the Finnish isolates in *N. glutinosa*.

The local lesions caused by the present isolates in *C. quinoa* were similar to those induced in this species by most of the CMV strains tested by USCHDRAWEIT (1955 a).

The isolates obtained from tomato (KT144/63, KT106/64) produced symptoms in this plant which were identical with the fern-leaf disease described in tomato by different workers (JOHNSSON 1926, MOGENDORFF 1930, VAN KOOT and CAMFFERMAN 1952, KOMURO 1963).

Many investigators have determined the thermal inactivation point, the dilution end point and the longevity *in vitro* of their strains (or isolates). The first-named property is the most useful of these as a reliable characteristic of different virus strains, even though the validity of the thermal inactivation point and the comparability of the results of different workers are dependent on the species of tests plant, the homogeny of the seed and the experimental conditions (cf. FULTON 1950, TJALLINGII 1952).

According to reports in the literature, the thermal inactivation point of CMV isolates originally obtained from cucumber and tomato range from 50 to 74° C. The inactivation point of the American strains appears to be around 70° C (PRICE 1934) and that of the European strains generally lower, in the range 50—60° C (AINSWORTH 1935, TJALLINGII 1952, KRISTENSEN 1956, LOVISOLO and BENETTI 1961). Of the twelve CMV isolates tested in the present investigation, nine had thermal inactivation points approaching that of the American strains, while only three were inactivated at around 60 ° C (cf. p. 310). In testing the thermal inactivation point, different workers have used different test plants, and in some studies no mention is made of the plant species employed. *C. quinoa*, which was used in the present investigation, is very liable to develop local lesions when infected with CMV (cf. USCHDRAWEIT 1955 a). One reason for this may be that the inactivation point of most Finnish strains is apparently higher than that reported in earlier studies, where other plant species were evidently used.

Two of the Wisconsin cucumber cultivars used in the resistance tests, SMR15 and SMR18, have a single dominant gene for resistance to the CMV strain prevalent on cucumber in Wisconsin (WASUVAT and WALKER 1961). As mentioned above, most of the Finnish strains tested had thermal inactivation points similar to those of the CMV strains studied in the U.S.A. The results of the resistance tests corresponded to the above findings in that all the isolates which had thermal inactivation points higher than 70° C caused 100 % infection in all the Wisconsin cultivars, giving rise to leaf mottling and stunting symptoms in them, just as occurred in the experiments of WASUWAT and WALKER (1961). On the other hand, the Finnish isolates with thermal inactivation points below 70° were evidently less pathogenic, since the comparison cultivar Superb OE 48, which, as far as is known, has no genetic resistance to CMV, was only partially infected, and under the same experimental conditions the Wisconsin cultivars were completely or almost completely resistant to certain of these isolates with low thermal inactivation points.

For more than a decade it has been known that CMV particles are spherical in shape (cf. FRANCKI et al. 1966). However, owing to the instability of the virus, its purification for electron microscopy was so difficult that only in recent years has it been possible to obtain reliable results with micrographs made from preparations stained with PTA or uranyl acetate. Such electron microscopic studies have shown that the average diameter of the CMV particles is 26—30 mμ. Similar dimensions were found for the two isolates obtained from cucumber in the present study and are furthermore in agreement with the figures reported for the CMV-Y strain of cucumber (BETTO et al. 1964, MURANT 1965) as well as the isolates from *Capsicum* sp. (QCMV) and lettuce (NCMV) (FRANCKI et al. 1966).

Cucumber green mottle mosaic virus differs from CMV both in terms of its restricted host range and in its limited geographical distribution. According to investigations thus far, it infects only plants of the family

Cucurbitaceae (SMITH 1957, KLINKOWSKI 1958) and apparently occurs only in Europe.

The first reports of this virus are from England in the 1930's (BEWLEY and CORBETT 1930, AINSWORTH 1935). The disease was first mentioned in Germany in 1953 (USCHDRAWEIT 1955 b), and in Denmark it first caused extensive damage to cucumbers in 1956 (KRISTENSEN 1956). It has been found in cucumber cultivations in Sweden (LIHNELL 1951), where in recent years it has been quite prevalent in greenhouses (RYDÉN 1965 a), and also in Holland (VAN KOOT and VAN DORST 1959). In the Soviet Union it has been encountered in greenhouses in some parts of the country (WLASSOW 1962).

The above reports demonstrate that CGMV occurs sporadically in certain limited regions, as has also been observed in Finland. In this connexion, it should be mentioned that the virus disease of cucumber described by RAINIO (1941) was thought by TJALLINGII (1952) to be caused by *Cucumis* virus 2 Smith, while KRISTENSEN (1956) believed that both CV1 and CV2 were responsible for the disease in question. On the basis of Rainio's description, it appears possible that CGMV was partly involved in this case (cf. JAMALAINEN 1957). On the other hand, the statement of TJALLINGII (1952, p. 12) that »Vermoedelijk moet ook het door Rainio (1941) bij kaskomkommers in Finland beschreven virus, door hem ten onrechte als *Cucumis-virus 1* beschouwd, als een stam van *Cucumis-virus 2* worden opgevat», i.e. that the disease was caused solely by CV2, seems unjustified, since as far as is known at present CGMV has not a single animal vector (SMITH 1957, KLINKOWSKI 1958, cf. HEINZE 1959 and KENNEDY et al. 1962), whereas *Aphis gossypii*, which was successfully used by Rainio to transfer the virus, is held to be — in addition to *Myzus persicae* — one of the most important vectors of cucumber mosaic virus (TJALLINGII 1952, MCCLANAHAN and GUYER 1964).

Transmission of green mottle mosaic virus via infected seed has been proved in the studies of VAN KOOT and VAN DORST (1959), where it was found that freshly harvested seed in particular may be an important source of infection. However, the virus becomes inactivated relatively quickly during storage, and consequently this reduces the danger of virus spread if the seed is stored for some time before being used, as is often the case in Finland.

An important factor limiting the spread of CGMV, especially in Finland, is the restriction of this virus to plants of the family *Cucurbitaceae*. In addition to cucumber, the only cultivated host plants of CGMV are *Cucumis melo* L. and *Citrullus vulgaris*, which are occasionally grown in greenhouses. In Finland there are no wild species of *Cucurbitaceae* (HIITONEN 1933, HULTÉN 1950).

Possible sources of danger in cucumber growing are the survival of CGMV in soil (VAN KOOT and VAN DORST 1959), the persistence of its activity in dry and frozen plant material (KRISTENSEN 1959) as well as its spread in irrigation water (RYDÉN 1965 b). In both CGMV cases examined during the present investigation, the virus appeared to have persisted in the soil of greenhouses left cold during the winter months. The danger of the spread of this virus through soil, however, is diminished in Finland through the general practice of using fresh soil or peat in the greenhouses every year.

On the basis of the s y m p t o m s produced in cucumber, both isolates in this investigation are green strains of cucumber green mottle mosaic virus, similar to the causal agents of AINSWORTH's (1935) green-mottle mosaic disease. According to AINSWORTH (1935) and USCHDRAWEIT (1955 b) this virus is inactivated by temperatures of 80—90° C, while KRISTENSEN (1956) reports a value of slightly over 90°. The thermal inactivation point of the Finnish isolates, ca. 95° C, is closer to the figure 98—99° reported by LIEM (1959).

The s i z e a n d s h a p e of CGMV particles has been determined in previous studies using different techniques of purification and metal-shadowing with subsequent electron microscopy. KNIGHT and STANLEY (1941) purified their virus material by differential centrifugation (cucumber virus 4 = CV2 A Smith), and the electron micrographs gave dimensions of 275 ×

15 mμ. A similar result, 2757 Å, was obtained by BRČÁK and HRŠEL (1961) with Au + Pd and carbon-shaded preparations made from crude leaf homogenate (CV4). LIEM (1959) employed the ammonium sulphate precipitation technique and metal shading, and his electron micrographs indicated the mean length of the rods to be 325 mμ, a measurement, which, however, appears to be too large. His method of purification causes breakage and aggregation of the particles — as can be seen from his electron micrographs — and consequently the actual length of the rods is difficult to determine.

More reliable results in measuring the dimensions of elongated virus particles are obtained when, instead of employing various methods of purification, as in the above-mentioned studies, preparations are made directly from leaf sap by the dip method. Likewise, negative staining will probably replace metal shading in electron microscopy work with plant viruses. In the present investigation, when PTA-stained preparations were made by the dip method, the CGMV rods were found to have a mean length of ca. 290 mμ, a value which is intermediate between the figures reported in the above studies.

Thus far, only a few publications have given data on the chemical composition of CMV and CGMV. Two strains of CGMV which were studied, CV3 (= CV2 Smith) and CV4, contained 5 % ribonucleic acid (KNIGHT and STANLEY 1941). Moreover, the nucleotide composition of these strains is known (KNIGHT 1954, 1963). In recent years several investigations have been made dealing with the RNA in CMV; its amount and composition have been determined in certain strains. CMV particles were found to contain about 18 % RNA (KAPER et al. 1965, FRANCKI et al. 1966). RNA analyses of the Finnish isolates are at present under way.

The first complete amino acid analysis to be published concerning the protein coat of CGMV was made by Knight on the strain CV4 and partly on CV3, in both cases using microbiological methods (KNIGHT 1947, 1963). The two strains contained the amino acids in the same proportions. Despite the different methods used in Knight's and in the present study, it is seen that in both investigation the CGMV protein was composed of the same 15 amino acids (cf. Table 5), among which no S-containing acid was included. The ratios between the different acids were also remarkably similar in the two studies.

As previously mentioned (p. 318), purification of CMV is quite difficult. Particularly for chemical analyses, it is not easy to obtain sufficient amounts of pure virus. According to the knowledge of the writer, no data have been published on analyses of the amino acid composition of the protein coat of CMV. In the present study, the CMV isolate analysed was found to differ from the CGMV isolates similarly analysed and also from the findings of Knight, since the former contained an additional amino acid, namely histidine, and furthermore it contained proportionally less alanine and serine but more glycine and lysine than the CGMV isolates. Future investigations on a larger number of isolates will probably reveal whether this amino acid composition is characteristic of the protein coat of different CMV strains.

Summary

Virus diseases of cucumber in Finland are rare and economically unimportant both in greenhouses and in the field. In the years 1962—65, inspection of 217 greenhouse cultivations, representing about 35 % of the total number of cucumber cultivations, and 46 field cultivations, representing 3—5 % of the area of commercial cultivations, revealed 10 cases of cucumber mosaic caused by cucumber mosaic virus (= *Cucumis* virus 1 Smith) and two cases of cucumber green mottle mosaic virus (= *Cucumis* virus 2 Smith).

The virus particles of the CMV isolates studied were spherical and had a mean diameter of 29 ± 3.5 mμ. The thermal inactivation point of three isolates was around 60—68° C, while that of 9 isolates (8 from cucumber, 1 from tomato) was ca.

72—76° C. All the isolates produced pale local lesions and systemic mottling in the leaves of *Cucumis sativus* L. and *Nicotiana glutinosa* L. as well as leaf deformation and blistering in the latter and local lesions in the leaves of *Chenopodium quinoa* Willd. None of the Wisconsin cultivars SMR15, SMR18 and SMR58 were resistant to all the isolates.

The particles of the CGMV isolates studied were elongated and had mean dimensions of 14 $\times$ 292 $\pm$ 11 mμ. The thermal inactivation point of the isolates was around 94—96° C. This virus caused typical dark green mottling as well as crinkling and rugosity of the leaves of *Cucumis sativus* and mild green mottling in *Citrullus vulgaris* Schrad. The isolates did not infect the test plants of the families *Solanaceae*, *Chenopodiaceae* and *Leguminosae*, nor were they capable of being transmitted by the vector *Myzus persicae* Sulz. Serological testing with CGMV antiserum gave a positive result.

The protein coat of the CGMV particles consisted of the following 15 amino acids: alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The protein of CMV comprised these acids and histidine in addition, or 16 amino acids altogether.

Acknowledgements. — In some stages of this work I have been aided by two research assistants, Mr. A. Murtomaa, M.Sc., Mrs. Sirkka Rinne, M.Sc., and a technical assistant, Mrs. Kirsti Nieminen. Their participation was made possible by a grant awarded to me by the National Research Council for Agriculture and Forestry, which I acknowledge with gratitude.

The electron microscopy was carried out at the Electron Microscope Institute, Director Prof. A. Telkkä, M.D., of the University of Helsinki, with the collaboration of Mr. M. Nyholm, M.Sc. The University Institute of Dairy Science, Director Prof. M. Antila, D. Agr. and For. placed at my disposal an auto-analyser for the quantitative amino acid analyses. The CGMV antiserum was kindly supplied by the Danish State Plant Pathology Institute.

I express my sincere thanks to all the above persons and institutes for their valuable help in the present work.

REFERENCES

Ainsworth, G. C. 1935. Mosaic diseases of the cucumber. Ann. Appl. Biol. 22: 55—67.

Antila, V., Alivaara, K. & Antila, M. 1966. The amino acid content of Finnish milk proteins. 17. Intern. Dairy Congr. B, 2: 361—363.

Betto, E., Belli, G., Conti, G. G. & Giussani, G. 1964. Sulla purificazione e sulla visulizzazione al microscopio elettronico del virus del mosaico del cetriolo. Phytopath. Z. 51: 85—89.

Bewley, W. F. & Corbett, W. 1930. The control of cucumber and tomato mosaic diseases in glasshouses by the use of clean seed. Ann. Appl. Biol. 17: 260—266.

Brandes, J. 1957. Eine elektronmikroskopische Schnellmethode zum Nachweis faden- und stäbchenförmiger Viren, insbesondere in Kartoffeldunkelkeimen. Nachr.bl. Deut. Pfl.schutzd. (Braunschweig) 9: 151—152.

Brčák, J. & Hršel, I. 1961. The electron microscopic examination of inclusions of Cucumber virus 4. Biol. Plant. 3: 132—136.

Cohen, S. & Nitzany, F. E. 1962. Identity of viruses affecting cucurbits in Israel. Phytopath. 53: 193—196.

Crowley, N. C. 1957. Studies on the seed transmission of plant virus diseases. Austr. J. Biol. Sci. 10: 449—464.

Doolittle, S. P. 1920. The mosaic disease of cucurbits. Bull. U.S. Dept. Agric. 879: 1—69.

Dorst, H. J. M. van 1963. Komkommervirus 1 in de herfstteelt van komkommer. Jaarversl. Proefst. Gr.-Fr. teelt onder Glas 1963: 147—150.

Faan, H. C. & Johnson, J. 1951. The overwintering of the cucumber mosaic virus. Phytopath. 41: 1001—1010.

Francki, R. I. B., Randles, J. W., Chambers, T. C. & Wilson S. B. 1966. Some properties of purified cucumber mosaic virus (Q strain). Virology 28: 729—741.

FULTON, J. P. 1950. Studies on strains of cucumber virus 1 from spinach. Phytopath. 40: 729—736.

GRANCINI, P. 1955. Osservazioni su alcune malattie da virus delle piante. Repr. Boll. Agric. (Milano) 89, 34, p. 32.

HEIE, O. & HEIKINHEIMO, O. 1966. Aphids collected in Finland during the 12th NJF Congress in 1963. Ann. Ent. Fenn. 32: 113—127.

HEIKINHEIMO, O. 1959. Kasviviruksia siirrostavien lehtikirvojen esiintymisestä maassamme. Summary: On the occurrence of virus vector aphids in Finland. Publ. Finn. Stat. Agr. Res. Board 178: 20—40.

HEIN, ALICE 1957. Beiträge zur Kenntnis der Viruskrankheiten an Unkräutern III. Das Gurkenmosaikvirus. Phytopath. Z. 29: 204—229.

—»— 1959. Beiträge zur Kenntnis der Viruskrankheiten an Unkräutern IV. Stachys palustris, ein Wirt des Gurkenmosaikvirus. Ibid. 35: 119—121.

HEINZE, K. 1959. Phytopathogene Viren und ihre Überträger. 290 p. Berlin.

HEROLD, F. & BREMER, H. 1958. Untersuchungen zur Epidemiologie, Ökologie und Bekämpfung des Gurkenmosaikvirus. Gartenbauwiss. 23: 254—274.

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

HULTÉN, E. 1950. Atlas över växternas utbredning i Norden. 119* + 512 p. Stockholm.

JAMALAINEN, E. A. 1957. Virustaudeista ja virustautien kaltaisista kasvitaudeista Suomessa. Summary: On plant virus diseases and viruslike diseases in Finland. Publ. Finn. Stat. Agr. Res. Board 158: 1—58.

JOHNSON, J. 1926. Mosaic diseases on differential hosts. Phytopath. 16: 141—149.

KAPER, J. M., DIENER, T. O. & SCOTT, H. A. 1965. Some physical and chemical properties of Cucumber mosaic virus (strain Y) and of its isolated ribonucleic acid. Virology 27: 54—72.

KENNEDY, J. S., DAY, M. F. & EASTOP, V. F. 1962. A conspectus of aphids as vectors of plant viruses. 114 p. London.

KLINKOWSKI, M. 1958. Pflanzliche Virologie Bd II. Die Virosen des europäischen Raumes. 393 p. Berlin.

KNIGHT, C. A. 1947. The nature of some of the chemical differences among strains of tobacco mosaic virus. J. Biol. Chem. 171: 297—308.

—»— 1954. The chemical constitution of viruses. Adv. Virus Res. 2: 153—182.

—»— 1963. Chemistry of viruses. 177 p. Wien.

—»— & STANLEY, W. M. 1941. Aromatic amino acids in strains of tobacco mosaic virus and in the related Cucumber viruses 3 and 4. J. Biol. Chem. 141:39—49.

KOMURO, Y. 1963. Studies on cucumber mosaic virus. IV. Variation in type of symptoms in different hosts and by different strains of virus. Ann. Phytopath. Soc. Japan 28: 131—138.

KOOT, IJ. van & CAMFFERMAN, J. 1952. Een ziekte bij tomaat, veroorzaakt door een combinatie van tomaten- en komkommermozaiek (Nicotiana-virus 1 + Cucumis-virus 1). Tijdschr. Pl.ziekten 58: 14—20.

KOOT, Y. van & DORST, H. J. M. van 1959. Virusziekten van de komkommer in Nederland. Ibid. 65: 257—271.

KOVACHEVSKI, I. 1961. Uvyakhvane na Krastavitsite i Pupeshite v Bulgriya. (Wilting of cucumbers and melons in Bulgaria) — (Rast. Zasht.) 9: 14—19. (Ref. Rev. Appl. Myc. 41: 424).

KRISTENSEN, H. RØNDE 1956. Virussygdomme hos agurker i Danmark. Horticultura 10: 161—172.

—»— 1959. Grønmosaiksyge-virus i tørrede og frosne plantedele fra agurk. Ibid. 13: 61—62.

LIEM, S. N. 1959. Cucumis virus 2 in Nederland. Tijdschr. Pl.Ziekten 65: 158—160.

LIHNELL, D. 1951. Några värdväxter för Cucumis-virus 1 i Sverige. Växtskyddsnot. 15: 52—56.

LINNASALMI, A. 1964. The characteristics of some isolates of tobacco mosaic virus and potato virus X causing streak on tomato. Ann. Agric. Fenn. 3: 224—234.

LOVISOLO, O. & BENETTI, M. P. 1961. Su di un ceppo del virus del mosaico del cetriolo di tipo alloiofillia isolato da pomodoro. Boll. Sta. Pat. Veg. III 19: 35—49.

MCCLANAHAN, R. J. & GUYER, G. E. 1964. The role of insects in the spread of cucumber mosaic virus. Canad. J. Pl. Sci. 44: 1—6.

MOGENDORFF, N. 1930. »Fern-leaf» of tomato. Phytopath. 20: 25—46.

MUNRO, J. 1954. Maintenance of virus X-free potatoes. Amer. Potato J. 31: 73—82.

MURANT, A. F. 1965. The morphology of cucumber mosaic virus. Virology 26: 538—544.

NYBOM, N. 1964. Amino acid analysis by means of thin-layer electrophoresis combined with chromatography. Physiol. Plant. 7: 434—442.

PRICE, W. C. 1934. Isolation and study of some yellow strains of cucumber mosaic. Phytopath. 24: 743—761.

RAINIO, A. J. 1941. Untersuchungen über *Cucumis-virus* 1, Erreger der Kräuselkrankheit auf Gurkenpflanzen. Publ. Finn. Stat. Agric. Res. Board 109: 1—24.

RAMSFJELL, T. 1952. Virussjukdommar på hagebruksplanter. Frukt og Baer 1952: 44—55.

RYDÉN, KERSTIN 1965 a. Grönmosaik hos gurkor. Växtskyddsnot. 29: 62—63.

—»— 1965 b. Kan grönmosaikvirus spridas med vatten? Ibid. 29: 68—69.

SCHWARZ, R. 1959. Epidemiologische Untersuchungen über einige Viren der Unkraut- und Ruderalflora Berlins. Phytopath. Z. 35: 238—270.

SCOTT, H. 1963. Purification of cucumber mosaic virus. Virology 20: 103—106.

SMITH, K. M. 1957. A textbook of plant virus diseases. 652 p. Boston.

SPIES, J. R. & CHAMBERS, D. C. 1949. Chemical determination of tryptophan in proteins. Anal. Chem. 21: 1249—1266.

SUHOV, K. S. 1954. Virusnye bolezni sel'skohozjajstvennyh rastenij i mery bor'by s nimi. Izv. Akad. Nauk SSSR 1954: 49—61. (Ref. Rev. Appl. Myc. 34: 277—278).

TJALLINGII, F. 1952. Onderzoekingen over de mozaïekziekte van de augurk (Cucumis sativus L.). Summary: Investigations on the mosaic disease of gherkin (Cucumis sativus L.). Meded. Inst. Pl. ziektenk. Onderzoek Wageningen 47: 1—117.

TOMLINSON, J. A., SHEPHERD, R. J. & WALKER, J. C. 1959. Purification, properties and serology of cucumber mosaic virus. Phytopath. 49: 293—299.

USCHDRAWEIT, H. A. 1955 a. Chenopodium quinoa als Testpflanze für das Gurkenmosaik. Nachr.bl. Deut. Pfl.schutzd. (Braunschweig) 7: 151—152.

—»— 1955 b. Das Grünscheckungsmosaik der Gurke. Ibid. 7: 150—151.

VAPPULA, N. A. 1965. Pests of cultivated plants in Finland. Acta Ent. Fenn. 19: 1—239.

WASUWAT, L. & WALKER, J. C. 1961. Inheritance of resistance in cucumber to cucumber mosaic virus. Phytopath. 51: 423—428.

WLASSOW, J. I. 1962. Die Bekämpfung von Viruskrankheiten bei Gemüse und Kartoffeln. Nachr.bl. Deut. Pfl.schutzd. (Berlin) 16: 31—33.

SELOSTUS

Kurkun virustaudit Suomessa ja niiden aiheuttajien kurkun mosaiikkiviruksen ja kurkun vihermosaiikkiviruksen ominaisuudet

ANNIKKI LINNASALMI

Maatalouden tutkimuskeskus, Kasvitautien tutkimuslaitos, Tikkurila

Kurkun virustaudit ovat harvinaisia ja taloudellisesti vähämerkityksisiä sekä kasvihuone- että avomaan kurkkuviljelmillä Suomessa. Vuosina 1962—65 todettiin tutkituilla 263 viljelmällä, joista 217 oli kasvihuoneviljelmiä (n. 35 % viljelmien kokonaismäärästä) ja 46 avomaanviljelmiä (3—5 % kaupallisten viljelmien pinta-alasta), yhteensä 10 kurkun mosaiikkiviroositapausta, aiheuttaja kurkun mosaiikkivirus (CMV), sekä kaksi kurkun vihermosaiikkiviroositapausta, aiheuttaja kurkun viherkirjovirus (CGMV).

Virustautitapaukset todettiin yleisesti viljellyissä lajikkeissa Arla, Bestseller, Favör ja Superb OE 48 sekä lajikkeissa Vestervang ja Rheinin rypäle. Kasvihuoneviljelmistä 65 %:ssa käytettiin kasvualustana multaa, neljäsosassa jyrsinturvetta ja 10 %:ssa mullan ja jyrsinturpeen seosta; verraten yleisesti kasvualusta vaihdettiin vuosittain (60 % viljelmistä). Tämä menettelytapa vähentää virustautien, erityisesti maassa saastutuskykyisenä säilyvän kurkun vihermosaiikin esiintymistä Suomessa.

Tutkittujen CMV-isolaattien pyöreät hiukkaset olivat keskimäärin 29 $\pm$ 3.5 mμ:n läpimittaiset. Kolmen isolaatin lämmönsietoraja oli 60—68° C:n, yhdeksän 72—76° C:n vaiheilla (8 isolaattia kurkusta, 1 tomaatista). Kaikki isolaatit aiheuttivat vaaleita paikallislaikkuja ja systeemisiä viherkirjo-oireita kurkun (*Cucumis sativus* L.) ja *Nicotiana glutinosa*n L. lehdissä, viimeksi mainitussa lisäksi lehtien epämuotoisuutta ja kupruilua. *Chenopodium quinoa*ssa Willd. ilmeni saastunta paikallislaikkuina lehdissä. Wisconsin lajikkeista SMR15, SMR18 ja SMR58 ei mikään ollut resistentti kaikkiin isolaatteihin nähden.

Tutkittujen CGMV-isolaattien sauvamaiset hiukkaset olivat kooltaan keskimäärin 14 $\times$ 292 $\pm$ 11 mμ. Isolaattien lämmönsietoraja oli 94—96° C:n vaiheilla. Ne aiheuttivat tyypillisiä voimakkaita, systeemisiä viherkirjo- ja kurttuoireita kurkun lehdissä sekä lieviä viherkirjo-oireita vesimeloonin (*Citrullus vulgaris* Schrad.) lehdissä. Isolaatit eivät saatuttaneet *Solanaceae*, *Chenopodiaceae* ja *Leguminosae*- testikasveja eivätkä olleet siirrostettavissa *Myzus persicae*n Sulz. välityksellä. Serologinen testaus CGMV-antiseerumilla antoi positiivisen tuloksen.

CGMV-hiukkasten proteiinikuori koostui seuraavista 15:sta aminohaposta: alaniini, arginiini, asparagiinihappo, glutaniinihappo, glysiini, isoleusiini, leusiini, lysiini, fenylalaniini, proliini, seriini, treoniini, tryptofaani, tyrosiini ja valiini; CMV-hiukkasten proteiinikuori sisälsi edellisten lisäksi histidiinin, siis yhteensä 16 aminohappoa.

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 324—333 (1966)

Seria PHYTOPATHOLOGIA N. 15 — Sarja KASVITAUDIT n:o 15

ASTER YELLOWS-TYPE VIRUS INFECTING GRASSES IN FINLAND

AARNO MURTOMAA

Agricultural Research Centre, Department of Plant Pathology, Tikkurila, Finland

Received October 12, 1966

The first report of the aster yellows virus (AYV) occurring in gramineous plants is from the year 1960, when BANTTARI and MOORE (1960) in the United States established that it produced symptoms in barley. Subsequently, six grasses have been reported to belong to the host range of AYV in the United States and Canada, (CHIYKOWSKI 1963, BANTTARI 1966). In addition, FREITAG (1964) succeeded in infecting a few plants of oats in one test.

In Europe, BLATTNÝ et al. (1965) have published data on »a virus disease of maize (*Zea mays* L.) indicated as rough dwarf and streak disease», which in experiments was transmitted by *Calligypona pellucida* (F.) and possibly also by other leafhoppers from AYV infected *Origanum vulgare* L., *Anagallis arvensis* L. and maize. BLATTNÝ and PROCHÁZKOVÁ (1965) concluded that this disease belongs to the European aster yellows group and found that *C. pellucida* was not able to transmit it to barley and oats.

The present paper describes a disease caused by a virus of aster yellows-type transmitted by *Macrosteles laevis* (Rib.). HEINZE and KUNZE (1955) have demonstrated that *M. laevis* is a vector of AYV. According to OSSIANNILSSON (1947), *M. laevis* is common in Sweden and causes damage to cereals. In certain years it has been found to be especially prevalent in Norway and Sweden (FJELDDALEN 1958). Information on the occurrence of *M. laevis* in Finland has been given by LINDBERG (1947). According to KONTKANEN (1954), in Finland *M. laevis* produces one generation each year.

Materials and methods

The temperature in the greenhouse where the experiments were carried out was about 18°C during the winter of 1964—65 and thereafter about 18—28°C. When short-day conditions prevailed, additional illumination was provided for the plants and leafhopper rearings by means of 400 W mercury vapour lamps. The leafhoppers were reared on cereal seedlings grown in 5-inch pots and covered by plastic cages.

The *Macrosteles* spp. specimens which were used in the experiments were collected as nymphs in September 1964 from the stubble of oats in the rural commune of Porvoo (60°25'N, 25°50'E). In the sample taken for species determination and comprising 40 leafhoppers, all the male specimens but one were *M. laevis*. During the winter rearing period of 1964—65, the leafhoppers did not reproduce in the green-

house. In the spring, females were reared individually, and identifications of the progeny of seven virus-free females showed that they were of the species *M. laevis*. The progeny of these females were combined, and this stock has subsequently been used in the experiments. Virus-free stock was reared on healthy barley, oats or *Lolium perenne*, and periodically checked, with barley as indicator plant.

The virus-free aphids and *Javesella pellucida* (F.) (*Calligypona pellucida*) were reared in the same way as *M. laevis*. In trials concerned with the ability of the aphids to transmit virus, use was made of the same kind of glass tube method as was employed by BREMER (1965) in tests on barley yellow dwarf virus.

The source of virus consisted of naturally infective leafhoppers of *Macrosteles* spp. been collected from the field. The virus was maintained in barley.

Transmission tests with the virus were carried out in the following way. After 1—8 days' acquisition feeding, the leafhoppers were caged on healthy barley seedlings for 3—7 days, and subsequently transferred to new test plants after an interval of about a week. Unless especially mentioned, the leafhoppers were used in groups of 30—300. The plants from which viruliferous leafhoppers had been taken for transfer to new test plants, were sprayed with an aqueous solution of mevinphos. The weekly treatments with mevinphos killed the later-emerging nymphs as well as any aphids which may possibly have entered the greenhouse. When the leafhoppers were transferred to the test plants, the cereal seedlings were 4—10 cm high, flax was usually at the cotyledonous stage, while the aster seedlings were 5—15 cm high.

In the summer of 1965, a field trial was conducted, in which infective *M. laevis* specimens were placed on seedlings of barley, oats and wheat. The plants had been sown on May 11, and protected by cages 100 × 90 × 100 cm in size made of cotton and terylene cloth. There were six plots of each plant species, three of which were controls. On June 10, fifteen leafhoppers were introduced into each cage. After two weeks the cages were removed and the trial area sprayed with parathion. The sprayings were repeated twice, during June and July.

Results

Trials with Macrosteles spp.

When *Macrosteles* spp. collected from the field were reared on oat seedlings, the seedlings became chlorotic and stunted and finally died. Since the first observations were made on plants which had been fed on for by many leafhoppers for quite a long time, it might have been possible that the toxic effect of the saliva had caused the above symptoms. In order to demonstrate the viral nature of the disease, the inoculation time was shortened and the number of leafhoppers on the plants reduced.

Using short inoculation times, a test was carried out in which three leafhoppers were transferred to each plant in the cages; there were 4—6 plants per cage. The results, shown below, indicate that inoculation for as little as one day was enough to produce symptoms in barley and oats.

Inoculation time, days	Infected plants / inoculated plants	
	Balder barley	Sisu oats
1	8/11	8/10
2	5/9	9/9
4	9/10	9/9
5	10/10	5/5

The ability of the individual leafhoppers to transmit virus was studied in tests in which one insect was transferred to each plant; a total of 63 leafhoppers was tested. It was found that 65 % of the leafhoppers were capable of transmitting the virus. The other plants, on which the remaining 35 % had been placed, grew normally. The incubation period on the plants was 4—5 weeks. The tests were made in January, and the leafhoppers used had previously been

Table 1. Plant species in which aster yellows symptoms appeared, and the success of transmission between different plant species. Most of the tests were made at least twice; some, particularly those with rye, oats and asters, were repeated more than ten times. + = virus transmitted, — = virus not transmitted, . = no test made.

Acquisition plant	Plant species in which AYV symptoms appeared						
	Barley	Rye	Oats	Wheat	Flax	Aster	*Poa annua*
Barley	+	+	+	+	+	+	+
Rye	+	+	+	.	+	+	.
Oats	+	+	+	.	+	+	+
Wheat	+	+	+	.	+	.	.
Flax	—	—	—	—	—	—	.
Aster	+	—	+	.	+	—	.
Poa annua	+	+	+	.	+	—	+

kept on plants, some of which had already shown symptoms. Consequently, the tests did not demonstrate the ability of the leafhoppers to transmit the virus immediately after being collected from the field.

In the winter, leafhoppers were reared on several species of plants, and symptoms were seen in the following: oats, barley, rye, wheat, as well as one aster plant.

Trials with M. laevis

After the spring of 1965, virus-free stock of *M. laevis* was available for tests (cf. p. 325). In Table 1 are shown the plant species which developed symptoms after leafhoppers had been transferred to them from diseased plants. No symptoms appeared in the control plants which had been fed on by virus-free leafhoppers.

Transmission of AYV from and to gramineous plants by *M. laevis* was readily accomplished. The incubation period in the insect was over 11 days, and 31—38 days after acquisition feeding, 73—100 % of the leafhoppers were infective (Table 2). In Table 3 data are presented on the ability of three groups of *M. laevis* to transmit the virus to certain cereals and flax; these leafhoppers had first been tested and demonstrated to be infective.

A total of ten tests, using 70—200 leafhoppers each time, were carried out on the ability of *M. laevis* to transmit the virus from asters. In order to intensity the acquisition (cf. MARAMOROSCH 1962), the green leaves of the aster plants were removed in two tests before the virus-free leafhoppers were transferred to them. In both tests the virus was successfully transmitted. In addition, successful tranmission occurred once from an aster plant which had all its leaves intact. Nineteen tests were made to

Table 2. Incubation period of AYV in *Macrosteles laevis* as well as numbers of infective leafhoppers after acquisition feeding on certain cereals. There was one leafhopper per plant.

Trial no.	Acquisition plant	Acquisition period, days	Period after beginning of acquisition feeding when leafhoppers transferred to healthy plants, days				
			2(4)—11	11—18	18—24	24—31	31—38
6532	Barley	2	[1])0/20 [2])0	1/19 5	6/14 43	7/14 50	
6602	Oats	4	[1])0/50 [2])0	1/50 2	21/48 44	30/48 63	33/45 73
6610	Oats	2	[1])0/32 [2])0	7/32 22	16/31 52	22/30 73	23/29 79
»	Rye	2	[1])0/25 [2])0	11/24 46	20/24 83	23/23 100	23/23 100

[1]) Number of infective leafhoppers/total number of living leafhoppers
[2]) Percentage of infective leafhoppers

Table 3. The ability of infective *Macrosteles laevis* to transmit AYV to certain plant species. The leafhoppers in each group were transferred four times at weekly intervals to new test plants, one leafhopper per plant.

Leafhopper group	Test plant species			
A	Flax 10/10[1])	Flax 6/9	Flax 6/6	Flax 6/6
B	Oats 9/9	Wheat 4/7	Oats 5/6	Barley 6/6
C	Rye 9/9	Rye 6/9	Rye 4/6	Rye 2/3

[1]) Number of plants showing symptoms/number of inoculated plants

study the transmission of the virus to asters. The leafhoppers had first been tested and demonstrated to transmit the virus to barley or flax. In each test many aster seedlings were inoculated, but only five plants in four different tests developed symptoms.

M. laevis effectively transmitted the virus to flax (Table 3), whereas acquisition of AYV from flax was not successful (Table 1). Nine tests were made on the transmission of the virus from flax. In three of them, the upper 3—4 cm of diseased flax plants were used for acquisition feeding (cf. FREDERIKSEN 1964), and virus-free leafhoppers (30, 42 and 100 in number) were put on these parts of the plants and kept in glass tubes. In the other tests, the leafthoppers were allowed to feed on entire growing flax plants.

In tests where one infective leafhopper was used on each plant, it was found that a feeding period of 24 hours was sufficient to cause infection of the plant. Shorter times were not tried. When leafhoppers were transferred weekly to new plants, it was demonstrated that infective insects maintained their ability to transmit the virus for their entire lifetime.

Other tests and observations

Several transmission tests with *Rhopalosiphum padi* (L.), as well as one test with *Macrosiphum avenae* (F.) and one with *M. dirhodum* (Wlk.), gave negative results. In two tests *J. pellucida* leafhoppers were placed on AY diseased barley (200 insects), in one test on diseased oats (75 insects) and in one test on rye (100 insects). When these leafhoppers were subsequently transferred to test plants which were changed after weekly intervals, the plants grew normally.

When both healthy and infected plants were grown together in the same pot, no symptoms appeared in the former. Similarly, no symptoms developed in healthy plants grown in soil with which bits of AYV infected plants had been mixed.

Mechanical transmission of the virus in sap inoculum was tested, using carborundum powder (400 mesh) as abrasive. The test plants were barley, oats, wheat, *Phleum prantense* L., *Chenopodium quinoa* Willd., *C. amaranticolor* Coste et Reyn. and *Nicotiana glutinosa* L. The results of the three tests carried out were negative.

In field trials, barley, wheat and oats became infected. The symptoms were similar to those appearing in plants which were grown in the greenhouse and which had been inoculated at late stage. The infection was quite mild, especially in oats.

As far as could be seen at the end of July 1965, the field where *Macrosteles* spp. had been collected the previous autumn contained no plants with symptoms of AY. At this time the crops were wheat and rye. The field is situated at the edge of a forest and has many open drainage ditches, so that it contained a varied plant population. Up to the present, no plants with symptoms of natural AYV infection have been found in Finland. On the other hand, infective *M. laevis* leafhoppers have been found not only in the rural commune of Porvoo but also at Nurmijärvi (July 1965).

Symptoms

Hordeum vulgare L. cultivar Balder. The first symptoms began to appear 2—5 weeks after inoculation. Occasionally, chlorotic blotches developed in the older leaves and later became brown and necrotic. At the same time the whole

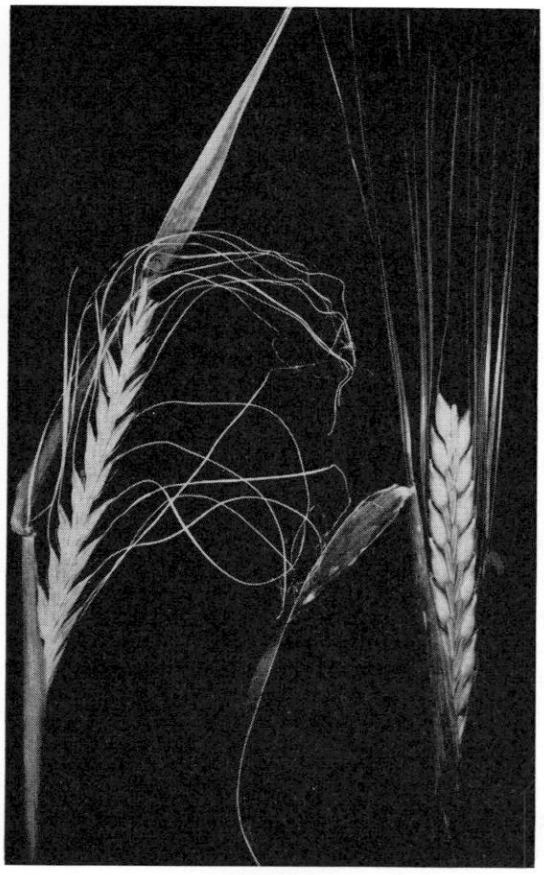


Fig. 1. AYV infected Balder barley, healthy control on the right.

Kuva 1. AKV Balder-ohrassa, oikealla terve kontrolli.

Fig. 2. AYV infected Apu wheat, healthy control on the right.

Kuva 2. AKV Apu-vehnässä, oikealla terve kontrolli.

leaf turned yellow. The young leaves which emerged were chlorotic. Such leaves were often limp and curled along their margins. Plants infected young were stunted, with numerous short tillers at their base. If an ear was produced, it was chlorotic, failed to form grain, and had twisted awns (Fig. 1).

Triticum aestivum L. cv. Apu. About 2—5 weeks after inoculation the plants were stunted, and the young leaves were chlorotic. Plants which were inoculated young, subsequently died. If ears were produced, they were chlorotic, lacked grain, and had twisted awns (Fig. 2).

Avena sativa L. cv. Sisu. A very typical feature was that about 2½—5 weeks after inoculation one of the old leaves began to turn slightly reddish. Sometimes its margins became curled, while the leaf blade remained straight and rigid. In addition to the reddish colouration, there were short, narrow, dark necrotic stripes on the leaf. The plant as a whole was stunted, and the young leaves were limp and chlorotic. When the plants were inoculated young, they often died as little as a month after inoculation. If a panicle developed, it was chlorotic and limp, and failed to produce grain (Fig. 3).

Secale cereale L. cv. Pekka. (Figs. 4 and 5). The first symptoms began to appear somewhat

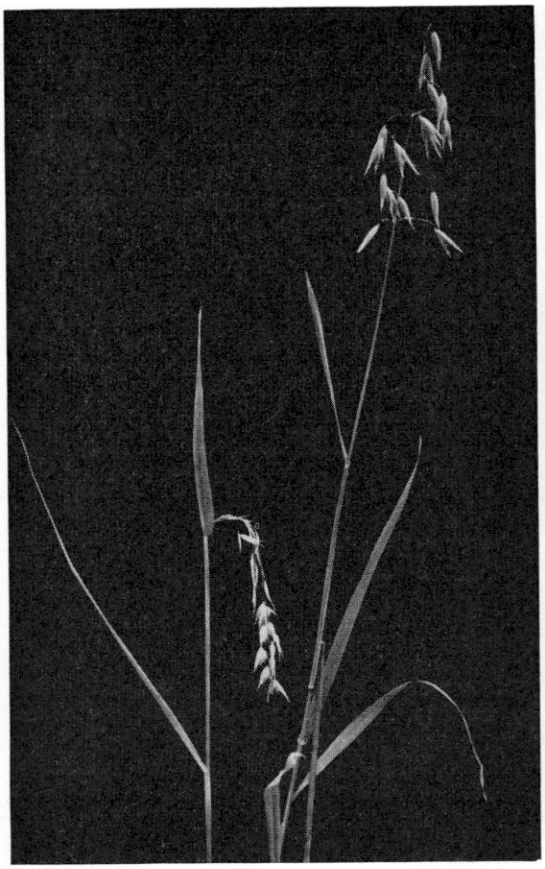


Fig. 3. AYV infected Sisu oats, healthy control on the right.

Kuva 3. AKV Sisu-kaurassa, oikealla terve kontrolli.

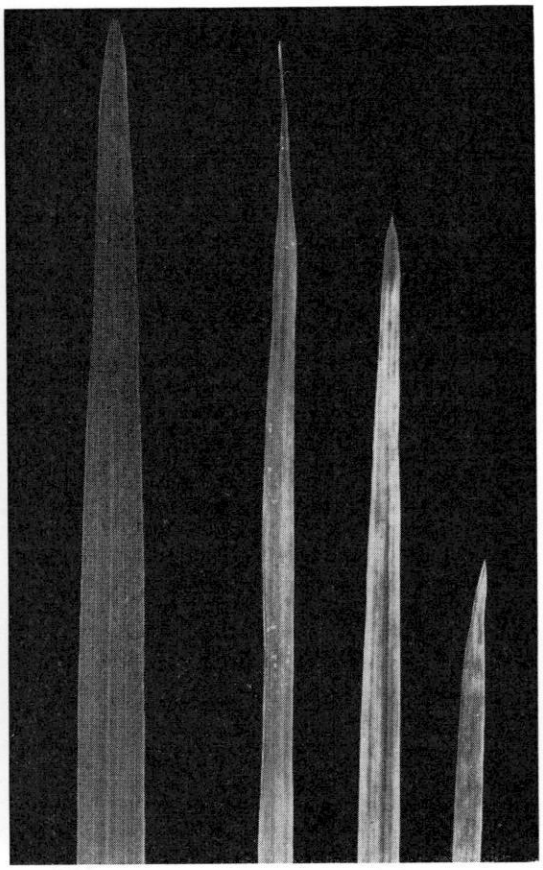


Fig. 4. Young leaves of Pekka rye infected with AYV, healthy control on the left.

Kuva 4. AKV nuorissa Pekka-rukiin lehdissä, vasemmalla terve kontrolli.

later than in barley and oats, 4—6 weeks after inoculation. The plant was stunted, and the young leaves limp, with chlorotic stripes or wholly chlorotic. Sometimes during the initial stages of the disease, short necrotic stripes appeared in the older leaves, just as in the case of oats.

Poa annua L. The plants were stunted, and the young leaves chlorotic and occasionally mildly mosaic. The panicle was distorted.

Linum usitatissimum L. cv. Wiera. About 2½—3 weeks after inoculation the young leaves and soon the entire upper part of the plant turned chlorotic. The leaves were small and distorted. Chlorotic secondary shoots developed in the leaf axils, and the growth of the whole plant was retarded (Fig. 6). Very often the main shoot grew normally, but the axillary shoots of the lower leaves turned chlorotic.

Callistephus chinesis (L.) Nees. Within 3—6 weeks after inoculation, vein clearing occurred in the young leaves, and subsequently the entire upper part of the main shoot, as well as the new axillary shoots, became very chlorotic (Fig. 7). All the plants which were infected in the trials died before reaching the flowering stage.

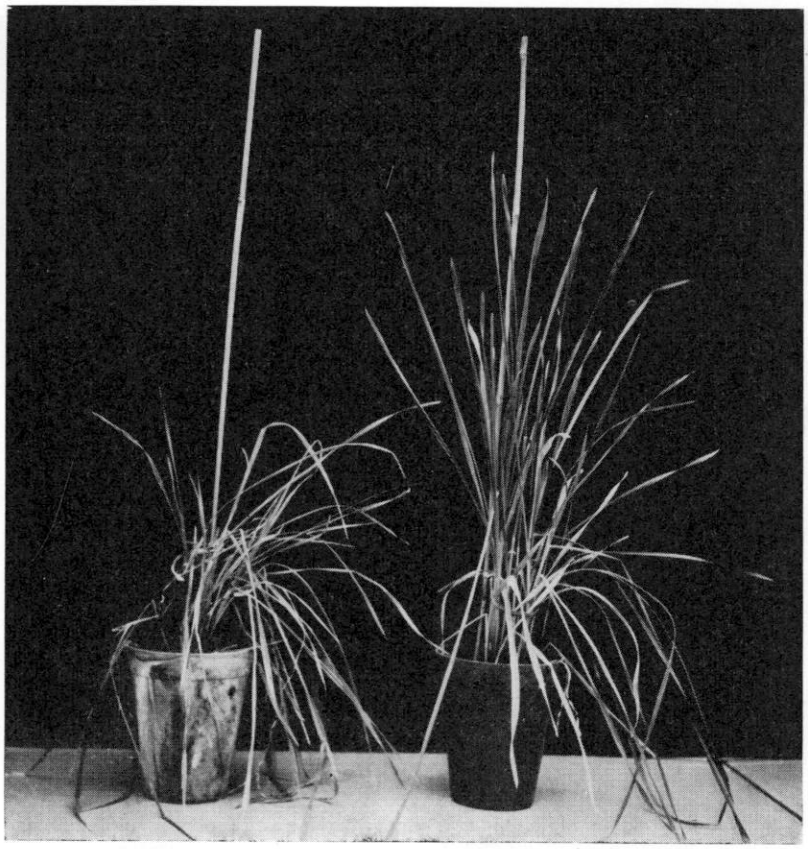


Fig. 5. AYV infected Pekka rye, healthy control on the right.
Kuva 5. AKV Pekka-rukiissa, oikealla terveitä kasveja.

Discussion

Since the above-described virus is transmitted by the same vector as the virus described by HEINZE and KUNZE (1955) and since symptoms and incubation period in asters are the same as in the studies of the latter workers, it is concluded that it belongs to the aster yellows virus group. Similarly, in comparing the characters of the virus described in this paper with those of American AYV strains, it is evident that the symptoms of the disease in barley in Finland are similar to those reported in America (BANTTARI and MOORE 1960, FREITAG 1964). When Dr. Banttari visited Finland in 1965, he observed that the symptoms, not only in barley but also in asters and flax, were the same as those of the aster yellows occurring in Minnesota. (Information by letter from E. E. Banttari, June 23, 1966.) The virus determined in Finland, however, has certain host plants which have not been infected by AYV strains in America. According to RATAJ (1958), in Czechoslovakia there have been occurrences of virus yellows of flax which were similar to virus yellows of flax in California.

The virus found in the present studies differs from the virus of to the same group which has been found to infect maize in Czechoslovakia (BLATTNÝ et al. 1965, BLATTNÝ and PROCHÁZKOVÁ 1965) at least in regard to its vector.

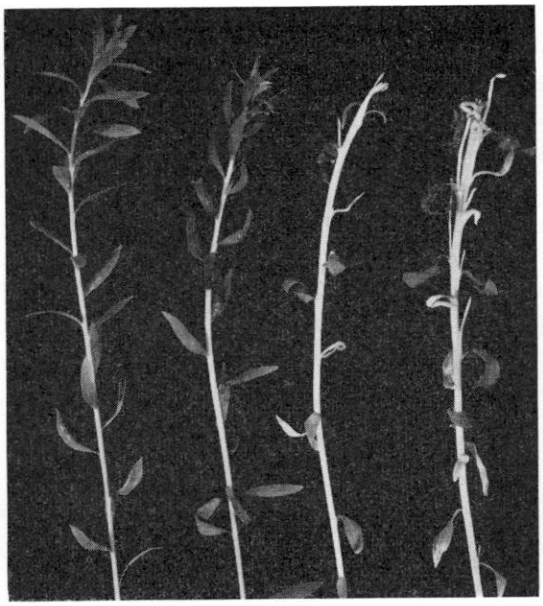


Fig. 6. AYV infected main shoots of Wiera flax, healthy control on the left.

Kuva 6. AKV Wiera-pellavan pääversoissa, vasemmalla terve kontrolli.

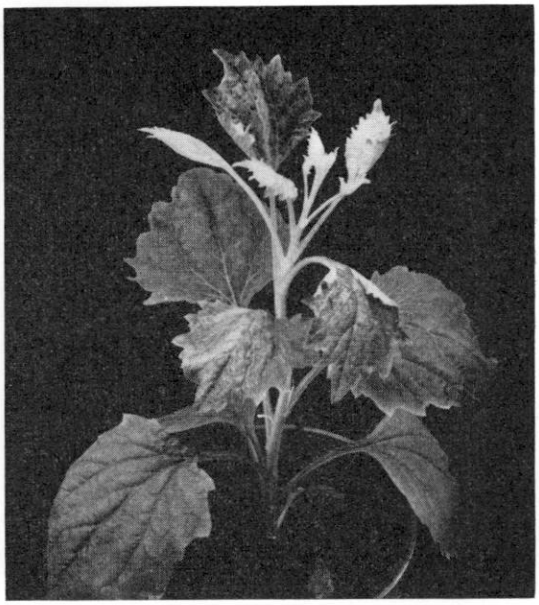


Fig. 7. Aster infected with AYV.

Kuva 7. AKV asterissa.

Furthermore, in Czechoslovakia the virus has not been transmitted to barley or oats.

According to FREDERIKSEN (1964), flax is highly susceptible to AYV, but attempts to transmit the virus from flax by the vector *Macrosteles fascifrons* (Stål) met with poor success. In the present trials carried out with the virus found in Finland, it was not transmitted at all from flax, even though this plant proved to be very susceptible to it. On the other hand, transmission of the virus from and to asters with *M. laevis* was found to be uncommon in numerous tests.

The transmission specificity of Eastern and California strains of AYV by the European vector *M. laevis* was investigated by MARAMOROSCH (1958). He found that *M. laevis* did not transmit these virus strains from asters to asters. Taking into consideration the fact that transmission of the Finnish aster yellows virus from and to asters is extremely rare, whereas the virus is readily transmitted to and from barley, it is probably justifiable to assume that if a similar transmission test were to be made with the American AYV, using barley as test plant, the result might be positive.

Summary

Macrosteles spp. leafhoppers collected from the stubble of oats caused symptoms in cereals in the greenhouse. The cause of the disease was established to be an aster yellows-type virus (AYV) and the vector was *Macrosteles laevis* (Rib.).

In tests made in the greenhouse the virus produced symptoms in the following plants: *Callistephus chinensis*, *Linum usitatissimum* cv. Wiera, *Hordeum vulgare* cv. Balder, *Triticum aestivum* cv. Apu, *Avena sativa* cv. Sisu, *Secale cereale* cv. Pekka and *Poa annua*.

The incubation period in *M. laevis* was over 11 days, while in the plants it exceeded two

weeks, varying according to species. When leafhoppers were allowed to feed on AYV infected cereals, 24—31 days after the beginning of acquisition feeding 50—100 % of the insects were infective. The virus was readily transmitted to and from gramineous plants. Flax was very receptive to the virus, but acquisition from flax did not succeed. Only a few asters became infected, and the ability of *M. laevis* to acquire the virus from asters was weak.

The virus was found not to be soil-borne or mechanically transmissible. Transmission tests with *Rhopalosiphum padi* (L.), *Macrosiphum avenae* (F.), *M. dirhodum* (Wlk.) and *Javesella pellucida* (F.) gave negative results.

In field trials AYV was transmitted to barley, oats and wheat. The infection was quite mild.

Thus far plants with visible symptoms of aster yellows have not been found growing under natural conditions in Finland.

Acknowledgements. — Sincere thanks are tendered to Assist. Professor Paavo Kontkanen, who made the species identification of the leafhoppers, and to Assist. Professor E. E. Banttari, who gave much useful advice during this investigation while working at the Department of Plant Pathology, Tikkurila. Furthermore, the skilled technical assistance of Mrs. Sigrid Kinnunen is gratefully acknowledged.

REFERENCES

Banttari, E. E. 1966. Grass hosts of aster yellows virus. Pl. Dis. Rep. 50: 17—21.

—»— & Moore, M. B. 1960. Transmission of the aster yellows virus to barley. Ibid. 44: 154.

Blattný, C., Pozděna, J. & Procházková, Z. 1965. Virusbedingte Rauhverzwergung und Streifenkrankheit bei *Zea mays* L. Phytopath. Z. 52: 105—130.

—»— & Procházková, Z. 1965. Weitere Versuche mit der Rauhverzwergung und Streifenkrankheit des Maises. Biol. Plantarum 7: 391—393.

Bremer, K. (née Ikäheimo). 1965. Characteristics of the barley yellow dwarf virus in Finland. Ann. Agric. Fenn. 4: 105—120.

Chiykowski, L. N. 1963. *Endria inimica* (Say), a new leafhopper vector of a celery-infecting strain of aster-yellows virus in barley and wheat. Can. J. Bot. 41: 669—672.

Fjelddalen, J. 1958. Sterke angrep av skadedyr på korn og gras i 1956. Tidsskr. Norske Landbr. 65: 66—83.

Frederiksen, R. A. 1964. Aster yellows in flax. Phytopath. 54: 44—48.

Freitag, J. H. 1964. Leafhopper transmission of three strains of aster yellows virus to barley. Proc. 5th Conf. Czechoslovak Pl. Virologists, Prague 1962. p. 136—142.

Heinze, K. & Kunze, L. 1955. Die europäische Asterngelbsucht und ihre Übertragung durch Zwergzikaden. Nachr.bl. Deut. Pfl.schutzd. (Braunschweig) 7: 161—164.

Kontkanen, P. 1954. Studies on insect populations I. The number of generations of some leafhopper species in Finland and Germany. Arch. Soc. Zool. Bot. Fenn. Vanamo 8: 150—156.

Lindberg, H. 1947. Verzeichnis der ostfennoskandischen *Homoptera Cicadina*. Fauna Fenn. 1: 1—81.

Maramorosch, K. 1958. Studies of aster yellows virus transmission by the leafhopper species *Macrosteles fascifrons* Stål and *M. laevis* Ribaut. Proc. 10th Intern. Congr. Ent., Montreal 1956. p. 221—227.

—»— 1962. Acquisition and transmission of aster yellows virus. [Abstr.] Phytopath. 52: 1 219.

Ossiannilsson, F. 1947. Svensk insektfauna 7. Halvvingar. *Hemiptera. Homoptera Auchenorrhyncha* 2. p. 151—270. Stockholm.

Rataj, K. 1958. Virová žloutenka lnu. [Abstr.] Preslia 30: 362—363.

SELOSTUS

Heinäkasveja saastuttava asterin keltavirusryhmään kuuluva virus Suomessa

AARNO MURTOMAA

Maatalouden tutkimuskeskus, Kasvitautien tutkimuslaitos, Tikkurila

Viljoihin, joilla oli kasvatettu Porvoon maalaiskunnasta kauran sängestä kerättyjä *Macrosteles* spp. -kaskaita, ilmestyi oireita, joiden aiheuttajaksi todettiin asterin keltavirusryhmään (AKV) kuuluva virus, vektorina *M. laevis* (Rib.). Kasvihuonekokeissa virus aiheutti oireita asterissa, pellavassa, ohrassa, vehnässä, kaurassa, rukiissa ja kylänurmikassa. Kolme viimeksi mainittua ovat uusia AKV:n isäntäkasveja.

Viruksen inkubaatioaika oli vektorissa yli 11 vuorokautta ja kasveissa yli kaksi viikkoa. Kun kaskaita oli ruokittu AKV:n saastuttamilla viljoilla, oli 24—31 vuorokauden kuluttua akvisitioruokinnan alkamisesta 50—100 % kaskaista infektoivia. AKV siirtyi helposti heinäkasveihin ja heinäkasveista. Pellava oli hyvin altis, mutta akvisitio pellavasta ei onnistunut. Vain muutamia astereita saastui, ja *M. laevis* -kaskaan kyky akvisoida virusta asterista oli heikko.

Viruksen ei todettu olevan maalevintäinen eikä mehulevintäinen. Tulokset kokeista, joissa yritettiin siirtää virusta *Rhopalosiphum padi*n (L.), *Macrosiphum avenae*n (F.) ja *M. dirhodum*in (Wlk.) samoin kuin *Javesella pellucida*n (F.) välityksellä, olivat negatiivisia.

Pellolla tehdyssä häkkikokeessa AKV siirtyi ohraan, vehnään ja kauraan. Saastunta oli melko lievää.

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 334—341 (1966)

Seria ANIMALIA NOCENTIA N. 22 — Sarja TUHOELÄIMET n:o 22

THE EFFECT OF THE ENGLISH GRAIN APHID MACROSIPHUM AVENAE (F.) (HOM., APHIDIDAE) ON THE YIELD AND QUALITY OF WHEAT

JORMA RAUTAPÄÄ

Agricultural Research Centre, Department of Pest Investigation, Tikkurila, Finland

Received November 9, 1966

The English grain aphid *Macrosiphum avenae* (F.) is one of the commonest aphid species inhabiting cereals in Finland. According to VAPPULA (1965), this species, which has been known as a pest in this country since at least 1894, occurred abundantly in 1915, 1917, 1920 (in North Finland), 1930, 1946—1948, 1952, 1956 and 1958. Its numbers were especially great in 1964 (MARKKULA 1965).

Several estimates have been made regarding the injury caused by *Macrosiphum avenae*. According to RAATIKAINEN and TINNILÄ (1961), this species did not, as far as is known, cause any considerable damage in 1959 when it evidently occurred in large numbers. The prevalent opinion seems to be that damage is seldom great, since the aphids are usually most numerous at the end of the summer, when the grain has already begun to harden (cf. e.g. VAPPULA 1965). In field trials, FORBES (1962) did not discover any significant yield reduction of oats caused by *Macrosiphum avenae*, and he concluded that »the economic injury level for aphids on oats is higher than 47 per tiller». Similarly, in the field experiments of WOOD (1965) *Macrosiphum avenae* inhabiting wheat during the preboot stage of growth did not reduce the yield.

Preliminary trials carried out at the beginning of August, 1964, at Tikkurila (16 km NE of Helsinki) showed that when *Macrosiphum avenae* were confined on heads of wheat for four weeks, the yield decreased by about 30 %. Since the loss seemed to be rather large, an investigation was carried out in 1965 in order to determine the effect of *Macrosiphum avenae* on the yield and quality of wheat.

Material and methods

The aphids belonged to the same parthenogenetic line which had been used in the studies of MARKKULA and PULLIAINEN (1965). At the beginning of May, about 50 reddish-brown apterous viviparous females were placed on seedlings of Sisu oats inside PVC cylinders. The larvae produced by these females were transferred to other oat seedlings before they could insert their stylets into the plant on which they were produced. These larvae and their progeny were reared until the beginning of the experiment on Sisu oat plants enclosed in PVC

cylinders. The purpose of this procedure was to obtain non-viruliferous aphids for the tests.

On May 7, the spring wheat variety Apu was sown in a field which had lain fallow during the two previous summers. There were twelve plots having dimensions of 50 × 50 cm. The soil type was fine sand. Before shooting occurred, cages constructed of transparent plastic sheeting and fine-meshed galvanized screening, 60 × 60 × 120 cm in size, were placed on the plots.

Four treatments were used:

A — cages 1—3. Aphids were introduced into the cages on July 12 at the beginning of heading. The aphids were allowed to propagate on the plants until harvest.

B — cages 4—6. Aphids were put in the cages on July 12. On August 14, at the beginning of ripening, the plants were sprayed with pyrethrin to kill all the aphids.

C — cages 7—9. Aphids were placed in the cages at the beginning of ripening on August 10. They were allowed to propagate on the plants until harvest.

D — cages 10—12 were controls without aphids.

One reddish-brown apterous female which had just emerged was placed on each head in all the cages of treatments A-C. The number of aphids per cage varied in accordance with the number of heads from 70 to 106.

At intervals of 4—24 days (cf. Fig. 1) all the heads were examined and the numbers of aphids on them were counted. On the basis of the aphid numbers, the so-called aphid index was calculated for each cage. The aphid index represents the sum of the aphids inhabiting one head on each day for the whole period of the experiment. The change in numbers of aphids taking place between inspections was assumed to be equally large every day, and on the basis of this assumption the numbers of aphids on the heads during the intermediate days between inspections were also calculated. The following equation was formulated by Mr. Seppo H y v ä r i n e n (Agricultural Research Centre, Department of Soil Science):

$$\text{Aphid index} = a_1 + \sum_{i=2}^{m} \sum_{k=1}^{n_i} \left[a_{i-1} + \sum^{k} \left(\frac{a_i - a_{i-1}}{n_i} \right) \right]$$

where m = number of aphid counts made,
n = length of period between counts, in days, and
a = number of aphids per head at the time of counting, ($a_1 = 1$)

The plants were harvested on September 14. The heads were dried at + 35°C in a drying oven and threshed by hand. The grain was stored at about + 20°C and 40 % relative humidity. In order to study the effect of the aphids on the yield, the grain was counted and the heads weighed.

Possible alterations in the quality of the grain were studied by determining Hagberg's falling number (Olered 1964) and the Pelshenke value (Pelshenke 1933) on selected samples. Six-gram samples were taken at random from the yield of each cage. Owing to the paucity of material, the yields of cages 1 and 2 had to be combined for determination of the falling number, and it was not possible to determine the Pelshenke value on the yields from cages 1, 2, 5 and 10. These analyses were performed at the Research Laboratory on the State Granary, Helsinki, under the direction of Mrs. Hilkka S u o m e l a, Dr. Agr. and For.

The State Seed Testing Station determined the germinative capacity of 600 grain taken at random from the yield of each cage.

Results

Numbers of aphids in heads

In the cages of treatment A, the numbers of aphids reached a maximum at the end of August and beginning of September, during the stage of yellow ripening (Fig. 1). The maximum number of aphids per head in cage 1 was 242.0 (range 166—309), in cage 2 175.1 (133—201) and in cage 3 129.0 (68—170). At the beginning of August many fungus-infected aphids were found in cage 3, and evidently for this reason there were fewer aphids in this cage than

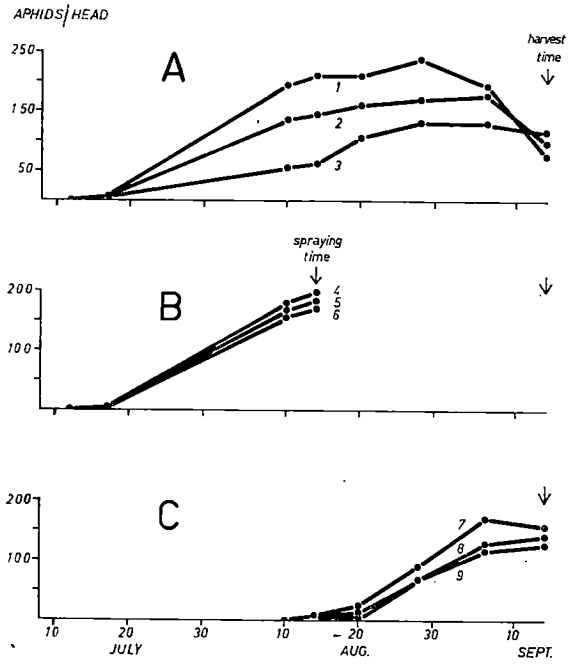


Fig. 1. Numbers of *Macrosiphum avenae* in the heads of wheat in the cages of treatments A, B and C at different times of the growing period in 1965.

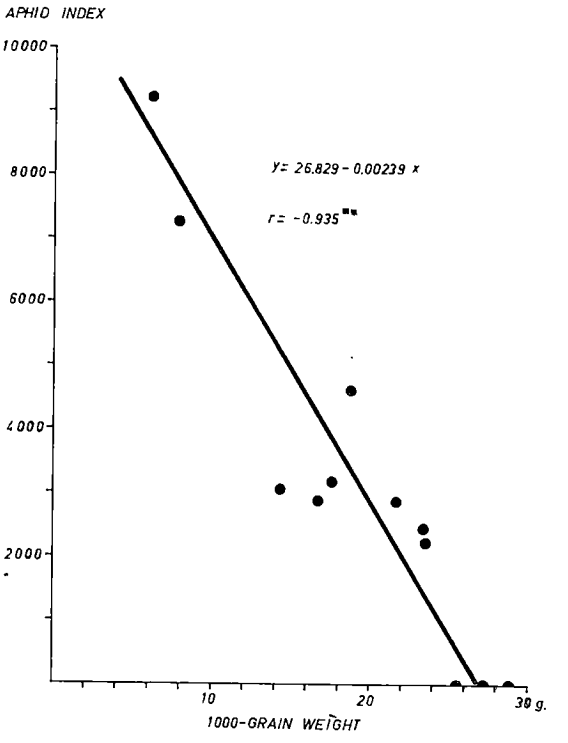


Fig. 2. Correlation between aphid index and 1 000-grain weight. The dots show averages for each cage.

in the others until nearly the end of the experiment.

The numbers of aphids in the cages of treatment B were at a maximum on August 14, at which time the pyrethrin spraying was carried out. On this date the average number of aphids per head in cage 4 was 198.0 (115—226), in cage 5 186.3 (84—217) and in cage 6 172.0 (70—245). These numbers were almost the same as in cages 1 and 2 but greater than those in cage 3.

As for treatment C, the largest numbers of aphids occurred at the beginning of September. The maximum number of aphids per head in cage 7 was 171.0 (75—190), in cage 8 140.2 (60—165) and in cage 9 134.5 (84—150). No aphids were found in the control cages of treatment D.

Up to August 20, there were at most only a few aphids inhabiting the leaves of the plants in the cages of treatment A. However, toward the end of the growing season, the numbers of aphids on the leaves increased. On September 6, counts made on all the leaves of ten plants randomly selected in each cage showed an average of 24.0 (0—135) aphids per leaf. In the cages of treatments B and C there were at most only a few individuals inhabiting the leaves of the wheat plants.

Effect of aphids on grain yield

The 1 000-grain weight of the cages was inversely proportional to the aphid index (Fig. 2). The negative correlation was highly significant ($r = -0.935$, $P < 0.01$). The smallest 1 000-grain weight was 6.08 grams (cage 2) and the largest 28.92 grams (cage 12) (Table 1). When the aphid index increased by one thousand, the 1 000-grain weight decreased on an average by 2.4 grams. The 1 000-grain weights were significantly smaller in treatments A, B and C than in the control ($P < 0.05$). The difference between treatments B and C was not significant ($P > 0.05$).

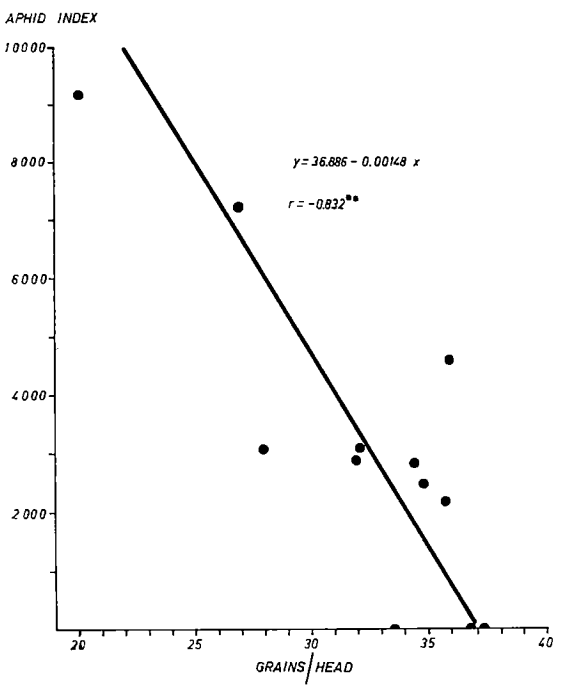


Fig. 3. Correlation between aphid index and number of grains per head. The dots show averages for each cage.

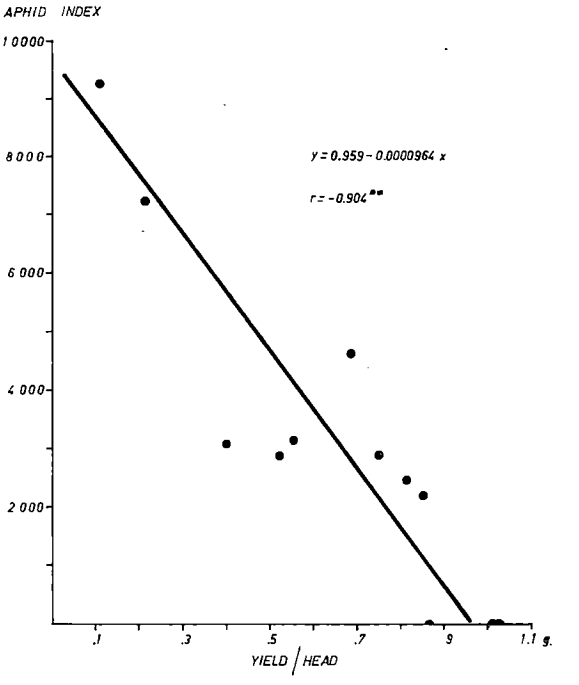


Fig. 4. Correlation between aphid index and grain yield per head. The dots show averages for each cage.

The average number of grains per head was inversely proportional to the aphid index (Fig. 3). The negative correlation was highly significant ($r = -0.832$, $P < 0.01$). The mallest average grain number per head was 20.01 (cage 2) and the largest 37.02 (cage 12) (Table 1). When the aphid index increased by one thousand, the number of grains per head decreased by 1.5. There were significantly fewer ($P < 0.05$) grains per head in treatments A and B than in the control. On the other hand, the difference between treatment C and the control, as well as between treatments B and C, was not significant ($P > 0.05$).

Completely shrivelled heads occurred only in cages 1 (7.5 %), 2 (13.9 %), 5 (3.3 %) and 8 (1.0 %). The correlation between the aphid index and the number of completely shrivelled heads was not significant ($r = +0.264$, $P > 0.05$). The correlation was calculated from transformed percentage values (arcsin $\sqrt{\text{percentage}}$, cf. SNEDECOR 1959).

There was a highly significant negative correlation ($r = -0.904$, $P < 0.01$) between the aphid index of the cages and the average yield of the heads (Fig. 4). The average yield per head in cage 2, only 0.12 grams, was about 1/9 that of the corresponding value in the control cages, 0.99 grams (Table 1). The difference between treatment B and the control, 0.42 grams, was significant ($P < 0.05$). On the contrary, the differences between treatment C and the control, 0.26 grams, and also between treatments B and C, 0.16 grams, were not significant ($P > 0.05$).

The yield loss was calculated from the average head yield, using the equation

$$X = \frac{100(A - B)}{A}$$

in which X = yield loss, A = average head yield in control and B = average head yield corresponding to the aphid index calculated from the correlation equation $y = 0.959 - 0.0000964\,x$. When the aphid index increased by one thousand, the yield loss rose by about ten percentage

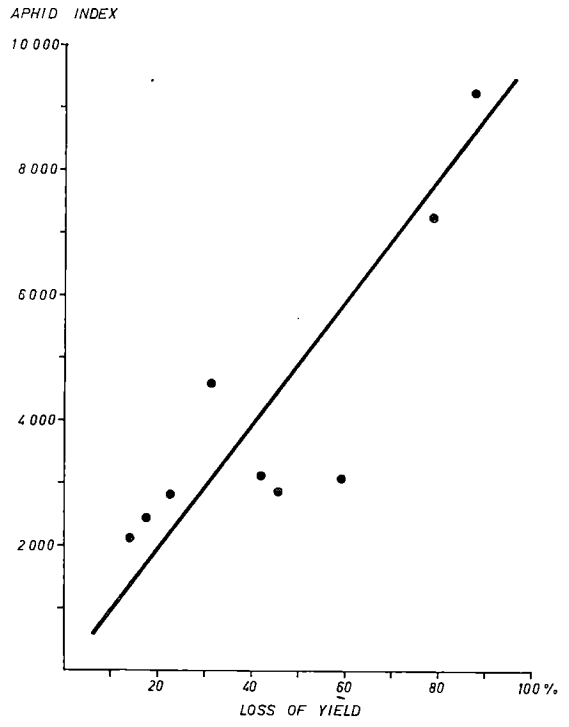


Fig. 5. Loss of yield caused by *Macrosiphum avenae* as a function of the aphid index. The dots show averages for each cage.

units. The largest yield loss was 87 % (cage 2) and the smallest 14 % (cage 9). The average yield losses of the different treatments were: A 68 %, B 41 %, and C 26 %. The difference between A and B was significant ($P<0.05$) but not between B and C ($P>0.05$). The significance was calculated from transformed percentage values.

Effect of aphids on grain quality

The falling numbers proved to be very high (Table 1). The smallest falling number was 260 (cage 10) and the largest 430 (cage 12). There was practically no correlation between falling number and aphid index ($r = -0.062$, $P>0.05$).

The lowest Pelshenke value was 28 (cage 4) and the highest 40 (cage 12) (Table 1). The negative correlation between the Pelshenke value and the aphid index was almost significant ($r = -0.600$, $P>0.05$). None of the differences between the treatments, however, were significant ($P>0.05$).

The smallest germination percentage was 97 (cages 1, 2, 5 and 9) and the largest 100 (cage 12) (Table 1). The negative correlation between germination percentage and aphid index, calculated from transformed percentage values, was nearly significant ($r = -0.525$, $P>0.05$). The differences between the treatments, however, were not significant ($P>0.05$).

Table 1. Effect of *Macrosiphum avenae* on the yield quantity, quality, and germination of Apu wheat. The method of calculating the aphid index is described in the text

Treatment	Cage	Aphid index	Number of heads	1 000-grain weight g	Average grain number per head	Average grain weight of head g	Falling number	Pelshenke value	Germination %
A	1	7 236	107	7.79	26.87	0.21	350	—	97
	2	9 292	79	6.08	20.01	0.12		—	97
	3	4 594	74	18.84	35.96	0.68	390	30	99
	$\bar{x}$	7 040	85.1	11.42	27.37	0.32	370.0	30	97.6
B	4	2 886	102	16.73	31.75	0.53	300	28	99
	5	3 063	91	14.31	27.96	0.40	300	—	97
	6	2 852	106	21.69	34.33	0.75	370	35	98
	$\bar{x}$	2 934	96.3	18.09	31.51	0.57	323.3	31.5	98.0
C	7	3 160	111	17.51	32.01	0.56	400	32	99
	8	2 495	96	23.30	34.75	0.81	410	35	99
	9	2 212	92	23.58	35.80	0.84	370	30	97
	$\bar{x}$	2 622	96.3	21.37	34.06	0.73	393.0	32.3	98.3
D	10	0	92	25.85	33.45	0.86	260	—	99
	11	0	102	27.12	36.78	1.03	410	33	99
	12	0	82	28.92	37.02	1.07	430	40	100
	$\bar{x}$	0	92.0	27.28	36.14	0.99	366.6	36.5	99.3

Discussion

The conditions for propagation of aphids in the cages were favourable, since natural enemies, for example, were almost completely lacking. Consequently, the numbers of aphids on the wheat heads were very high, at their maximum evidently as high as possible. The effect of the aphids on the wheat yield proved to be great: when the aphid index increased by one thousand, the yield loss amounted to about ten percentage units. On the basis of these results, it may be possible to estimate the destructivenes of *Macrosiphum avenae* in the field from their numbers and the duration of their infestation.

A certain number of aphids may possibly live on wheat heads for a limited period of time without affecting the yield. This is suggested by the fact that the aphids in the studies of FORBES (1962) and WOOD (1965) had no visible influence on the grain yield of oats and wheat. On the basis of the present investigation, however, it is apparently not possible to conclude how large a number of aphids may infest the heads without effecting the yield.

It cannot be decided with certainty whether the aphids inhabiting the heads in treatment B during the period of wheat flowering caused more severe damage than those of treatment C which lived on the heads during the ripening period. The grain yield in the cages of treatment B was significantly smaller than in the control cages, but nevertheless the difference between the two treatments B and C was not significant. However, these results indicate that under certain conditions, aphids infesting wheat heads during the time of flowering may reduce the ultimate grain yield more than the same number of aphids infesting the heads during the period of ripening.

An increase in the amount of enzymes causing the breakdown of strach is usually reflected in a decrease in the falling number. Similarly, as the protein-hydrolyzing enzymes increase, there is a subsequent lowering of the Pelshenke value. In the present studies, however, no significant change in the falling number was caused by the aphids. Regarding the protein-hydrolyzing enzymes, the correlation between the aphid index and the Pelshenke value was almost significant. It was not possible to determine this latter value from the most severely damaged grain lots, so that the deleterious effect of aphids on the gluten quality was not perfectly established. The Pelshenke value of the most badly injured samples may possibly have been quite small, in which case the correlation between aphid index and Pelshenke value may have become significant. The above results suggest that under certain conditions, *Macrosiphum avenae* may have a detrimental effect on the gluten of wheat. As far as is known, the only enzymes that have been identified in the saliva of *Macrosiphum avenae* are pectinases (MCALLAN and ADAMS 1961).

The germination of the grain was very good, and aphids caused no great effect, although the correlation between the aphid index and germination was almost significant. Owing to the scanty material, it was not possible to study shooting. The food reserves in the smallest grains may possibly not have been adequate for successful shooting of the seedling.

Summary

The effect of the English grain aphid *Macrosiphum avenae* (F.) (*Hom., Aphididae*) on the grain yield and quality of Apu spring wheat was studied in cage trials in 1965. Besides the controls, there were three different treatments. In two treatments, aphids were placed in the cages at the beginning of heading; in one of these treatments the aphids were allowed to propagate until harvest, in the other they were killed by pyrethrin spraying just after the end of flowering.

In the third treatment, the aphids infested the plants from the end of flowering until harvest. On the basis of the numbers of aphids living on the wheat plants during the season, the aphid index was calculated for each cage. This index represents the sum of the average daily number of aphids per head for the whole period of their infestation.

The aphids had a pronounced effect on the yield. The negative correlation between the aphid index and the 1 000-grain weight, the average grain number per head and the average grain yield per head was highly significant ($P < 0.01$). The average 1 000-grain weight in the most severely infested cages was only 6.08 grams, while in the control cages the corresponding value was 27.28 grams. When the aphid index increased by one thousand, there was an average reduction of 2.4 grams in the 1 000-grain weight. The number of grains per head in the most severely damaged samples averaged 20.01, while in the control heads it was 36.14. An increase of one thousand in the aphid index caused an average decrease of 1.5 in the grain number per head. The grain yield of the most severely damaged heads was 0.12 grams, whereas the figure for the undamaged heads was 0.99 grams.

The yield loss was calculated on the basis of the average grain yield of the heads. When the aphid index increased by one thousand, there was a subsequent increase in yield loss of ten percentage units.

The falling number was used as an indicator of starch quality and the Pelshenke value was the indicator of gluten quality. The falling number ranged from 260 to 430 and the Pelshenke value from 28 to 40. Germination varied from 97 % to 100 %. The aphids did not affect the falling number, but the negative correlations between the aphid index and both the Pelshenke value and the germination percentage were nearly significant. These findings suggest that under certain conditions *Macrosiphum avenae* may have an appreciable effect on the Pelshenke value and the germination of wheat.

Results seem to indicate that under certain conditions, aphids infesting wheat heads at flowering time may cause greater yield losses than the same number of aphids infesting the plants during the period of ripening.

Acknowledgements. — I wish to express my gratitude to Mrs. Hilkka Suomela, Dr. Agr. and For., Head of the Research Laboratory of the State Granary, for determining the falling number and the Pelshenke value, and for reading the manuscript. My best thanks are also due to Mr. Seppo Hyvärinen for formulating the equation for the aphid index.

REFERENCES

Forbes, A. R. 1962. Aphid population and their damage to oats in British Columbia. Can. J. Pl. Sci. 42: 660—666.

Markkula, M. 1965. Viljelykasvien tuhoeläimet 1964. (Summary: The pests of cultivated plants in Finland in 1964). Maatal. ja Koetoim. 19: 202—213.

—»— & Pulliainen, E. 1965. The effect of temperature on the lengths of the life periods of the English grain aphid *Macrosiphum avenae* (F.) (*Hom. Aphididae*) and on the number and colour of its progeny. Ann. Ent. Fenn. 31: 39—45.

McAllan, J. W. & Adams, J. B. 1961. The significance of pectinase in plant penetration by aphids. Can. J. Zool. 39: 305—310.

Olered, R. 1964. Falltalsmetoden. Sver. Utsädesför Tidskr. 74: 25—41.

Pelshenke, P. 1933. A short method for the determination of gluten quality of wheat. Cereal Chem. 10: 90—96.

Raatikainen, M. & Tinnilä, A. 1961. Occurrence and control of aphids causing damage to cerels in Finland in 1959. Valt. Maatal. koetoim. Julk. 183: 1—27.

Snedecor, G. W. 1959. Statistical methods. Iowa. 534 p.

Vappula, N. 1965. Pests of cultivated plants in Finland. Ann. Agric. Fenn. 4, Suppl. 1: 1—239.

Wood, E. A., Jr. 1965. Effect of foliage infestation of the English grain aphid on yield of Triumph wheat. J. Econ. Ent. 58: 778—779.

SELOSTUS

Viljakirvan vaikutus Apu-kevätvehnän satoon ja sadon laatuun

JORMA RAUTAPÄÄ
Maatalouden tutkimuskeskus, Tuhoeläintutkimuslaitos, Tikkurila

Viljakirvan *Macrosiphum avenae* (F.) (*Hom., Aphididae*) vaikutusta Apu-kevätvehnän satoon ja sadon laatuun tutkittiin häkkikokein vuonna 1965. Koejäseniä oli vertailuhäkkien lisäksi kolme. Kahden tähkiin asetettiin kirvat tähkien tullessa esiin tupesta; toisessa annettiin kirvojen lisääntyä sadonkorjuuseen saakka, toisesta kirvat hävitettiin pyretriinillä kukinnan jälkeen. Kolmannen koejäsenen tähkissä kirvat saivat lisääntyä kukinnan päättymisestä sadonkorjuuseen. Kasvukauden eri aikoina tähkissä eläneiden kirvamäärien perusteella laskettiin kullekin häkille kirvasumma. Tällä tarkoitetaan koeajan kunakin päivänä keskimäärin tähkissä eläneiden kirvojen summaa.

Kirvat vaikuttivat suuresti sadon määrään. Negatiivinen korrelaatio kirvasumman ja 1 000 jyvän painon, tähkän keskim. jyväluvun sekä tähkän keskim. jyväsadon välillä oli erittäin merkitsevä ($P < 0.01$). Eniten vioitettujen tähkien 1 000 jyvän paino oli 6.08 grammaa, kun vioittumattomien keskiarvo oli 27.28. Kirvasumman suureneminen tuhannella pienensi 1 000 jyvän painoa keskim. 2.4 grammaa.

Eniten vioitettujen tähkien jyväluku oli keskim. 20.01 ja vioittumattomien 36.14. Kirvasumman suureneminen tuhannella pienensi tähkän jyvälukua keskim. 1.5:llä.

Eniten vioitettujen tähkien jyväsato oli keskim. 0.12 grammaa, kun vioittumattomien tähkien keskiarvo oli 0.99 grammaa.

Satotappio laskettiin tähkän keskimääräisen jyväsadon perusteella. Kirvasumman suureneminen tuhannella lisäsi satotappiota kymmenellä prosenttiyksiköllä.

Sakolukua käytettiin tärkkelyksen laadun ja Pelshenken lukua sitkon laadun kuvaajana. Sakolukujen ääriarvot olivat 260 ja 430. Pienin Pelshenken luku oli 28 ja suurin 40. Jyvien itävyyden ääriarvot olivat 97 % ja 100 %. Kirvat eivät vaikuttaneet sakolukuun, mutta kirvasumman ja Pelshenken luvun sekä itävyysprosentin välinen negatiivinen korrelaatio oli lähes merkitsevä. Tulokset johdattavat päätelmään, että viljakirva saattaa tietyissä oloissa vaikuttaa merkitsevästi sitkon laatuun sekä itävyyteen.

Tulokset antoivat aiheen olettaa, että kukinnan aikana tähkissä elävät viljakirvat saattavat tietyissä oloissa aiheuttaa suuremman satotappion kuin tuleentumisen aikana tähkissä elävä yhtä suuri kirvamäärä.

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 342—344 (1966)
Seria ANIMALIA DOMESTICA N. 22 — Sarja KOTIELÄIMET n:o 22

THE PROPORTION OF SALIVA IN THE FLUID FLOWING THROUGH THE RETICULO-RUMEN OF THE COW[1])

ESKO POUTIAINEN
Agricultural Research Centre, Department of Animal Husbandry, Tikkurila, Finland

Received November 12, 1966

Since spring 1964, studies have been carried out at this department on the passage of fluid and its dissolved mineral elements through the reticulo-rumen and on the secretion of saliva in two fistulated Ayrshire cows. These studies are a continuation of previous investigations relating to the fluctuation of pH in the rumen contents (LAMPILA 1964) and the passage of fluid out of the reticulo-rumen (LAMPILA 1965). This entire series of investigations is associated with the question of neutralization of the rumen contents.

The measurement of the flow of fluid was made using polyethylene glycol (PEG) as reference substance. The rate of flow, expressed as percentage of the fluid volume per hour, has been calculated from the dilution of the reference substance. For computing the total flow, the volume of the fluid in the rumen was measured using PEG (HYDEN 1961).

The apparent contribution of saliva to the fluid flow has been calculated by substracting from the total flow the water contained in the feeds as well as the drinking water[2]). Determinations of sodium and potassium in the saliva and rumen fluid were also made.

Studies were made on the effect of three dietary factors, namely, the level of the dry-matter intake, the physical character of the ration and the dosage of sodium chloride, the passage of fluid and the proportion of saliva in it. Most of the rations comprised 50 % hay and 50 % concentrates (of the dry matter). Water was freely available, and dicalcium phosphate was given in an amount of 100 g/day. The total daily ration was given in two equal portions at 12-hour intervals. The results were calculated from the values obtained from 12-hour daytime periods.

The level of the dry-matter intake was the most effective factor influencing both the flow of fluid and the secretion of saliva. In one of the cows, the total passage of fluid through the reticulo-rumen, as calculated for 24 hours, varied from about 210 to 65 litres when the dry-matter intake varied correspondingly from 12 to 3 kilograms. The proportion of saliva amounted to about 175 and 40 litres respectively.

In the other cow, the volume of fluid passing through the reticulo-rumen was approximately 220 l and the salivary secretion 170 l when the dry-matter intake was 14 kg per day. When the daily dry matter was 6 kg, the values were about 105 and 75 litres, respectively.

[1]) Communication presented at the meeting of the Scientific Agricultural Society of Finland on November 17, 1966.
[2]) The water initially present in the feeds is not entirely available to the PEG (Hyden 1961). This fact, however, has only a small quantitative influence on the calculations of saliva flow.

The proportion of saliva in the total fluid flow averaged 72 % for all the rations, ranging from 56 to 83 %.

The effect of the physical character of the ration was studied by grinding a part of the hay (hay made up 50 % of the dry matter in the ration). When 80 % of the hay was ground, the flow through the reticulo-rumen on the 9-kg dry-matter level decreased by 30—40 % and the salivary secretion diminished by 40—50 %. In this case, the unground hay made up 10 % of the total dry matter of the ration.

Grinding of hay had no appreciable effect on the concentrations of sodium and potassium in the saliva and rumen fluid.

The effect of sodium on the flow of fluid as well as the secretion and composition of saliva was studied by offering sodium to the animals at three different dosage levels. The lowest level consisted of the ordinary diet without supplements, while the second level comprised 50 g NaCl per day and the third 100 g/day.

It was found that addition of sodium to the rations increased the flow of fluid and the secretion of saliva only if the sodium supply was insufficient to meet the requirements of the animal. The ratio between the sodium and potassium contents in the saliva was taken to indicate sodium deficiency. When the amount of sodium in the diet was inadequate, the Na values of the saliva declined from their normal level, 150—160 me/l, to 70—75 me/l. At the same time, the concentration of potassium rose 5—10 to 65—75 me/l. Similar changes were also found in the Na and K concentrations of the rumen fluid.

When one of the cows, producing about 20 kg milk daily, had subsisted without sodium supplements for 5 weeks (being in a state of sodium deficiency), the addition of 50 g NaCl per day increased the flow of fluid by 9 % and the salivary secretion by 14 %. With the dosage of 100 g NaCl, the increases were as much as 28 % and 40 % respectively.

When the sodium requirement of the animal diminished parallel with milk production, no differences were observed in the flow of fluid or in the secretion of saliva on increasing the dosage of NaCl from 50 to 100 g. When the cow was dry or giving only a few kilograms of milk per day, the amount of sodium present in the feed was apparently adequate and consequently the addition of sodium chloride had no effect on the passage of fluid through the reticulo-rumen or the proportion of saliva in it.

Acknowledgement. — This research has been financed by a grant made by the Suomen Kulttuurirahasto (Finnish Cultural Foundation).

REFERENCES

HYDEN, S. 1961. Determination of the amount of fluid in the reticulo-rumen of the sheep and its rate of passage to the omasum. Ann. Agric. Coll. Sweden 27: 51—79.

LAMPILA, M. 1964. Volatile fatty acids, pH and microbial activity in the rumen contents of the cow. Ann. Agric. Fenn. 3, Suppl. 3: 1—76.

—»— 1965. The passage of fluid, certain mineral elements, and volatile fatty acids from the reticulo-rumen of the cow. Ibid. 4: 134—144.

SELOSTUS

Syljen osuus lehmän pötsi-verkkomahan läpi tapahtuvassa nestevirtauksessa

ESKO POUTIAINEN

Maatalouden tutkimuskeskus, Kotieläinhoidon tutkimuslaitos, Tikkurila

Syljen osuus lehmän pötsi-verkkomahan läpi tapahtuvassa nestevirtauksessa on määritetty vähentämällä PEG:n avulla mitatusta nesteen kokonaisvirtauksesta juomaveden ja rehujen sisältämän veden osuus.

Nestevirtaukseen ja syljen erityksen vaikuttavista ruokinnallisista tekijöistä on tutkittu syödyn kuiva-ainemäärän, rehun fysikaalisen olomuodon ja rehuannoksen natriumsisällön merkitystä.

Voimakkaimmin vaikuttava tekijä on ollut syödyn kuiva-aineen määrä. Kun se vaihteli alueella 14—3 kg vuorokaudessa, aleni nestevirtaus vastaavasti noin 220 litrasta 65 litraan ja syljen eritys noin 175 litrasta 40 litraan vuorokaudessa. Pitkän heinän osuuden pienentäminen 50 %:sta 10 %:iin syödystä kuiva-aineesta, antamalla loppu rehuannokseen kuuluneesta heinästä jauhettuna, aiheutti nestevirtauksessa 30—40 %:n ja syljen määrässä 40—50 %:n vähenemisen. Eläimen saadessa tarpeeseen nähden niukasti natriumia oli nestevirtaus ja syljen eritys vähäisempää kuin natriumin saannin ollessa riittävän.

Syljen ja pötsinesteen natrium- ja kaliumkonsentraatioissa tapahtui tasomuutoksia vain eläimen natriumin saannin ollessa niukan. Syljen natriumarvot alenivat tällöin noin 50 %, mutta kaliumin konsentraation nousu kompensoi muutoksen lähes täydellisesti.

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 345—354 (1966)

Seria PHYTOPATHOLOGIA N. 16 — Sarja KASVITAUDIT n:o 16

VIRUS DISEASES OF TOMATO IN FINLAND

I. Occurrence and causal agents of the diseases

ANNIKKI LINNASALMI and AARNO MURTOMAA

Agricultural Research Centre, Department of Plant Pathology, Tikkurila, Finland

Received November 12, 1966

The first reports of virus diseases of tomato in Finland appeared in the 1930's, when the cultivation of this crop began to become common. Toward the end of the 1930's and in the beginning of the 1940's, mosaic and streak diseases of tomato were widespread at least in greenhouses in southern Finland; in addition, a few cases of tomato fern leaf were observed (JAMALAINEN 1943). Since those times the Department of Plant Pathology of the Agricultural Research Centre has received abundant evidence every year of the occurrence of tomato virus diseases (JAMALAINEN 1957, LINNASALMI 1963, 1964).

The cultivation of tomatoes, which, owing to the cool climate in Finland, is carried out exclusively in greenhouses, has increased from year to year and now is practised throughout the country. Large-scale commercial production, however, is concentrated in the southern and southwestern parts of Finland as well as along the west coast in some communes in the province of Vaasa. Furthermore, greenhouse tomatoes are grown in the vicinity of cities and densely populated areas in various regions of the country. In earlier days, greenhouse tomatoes were produced to some extent on farms and estates, but today this practice has virtually disappeared. Tomato growing originated as a subsidary occupation on small farms, particularly in the province of Vaasa. However, progress both in this area and in the rest of Finland has led to the situation that today tomato production takes place mainly in commercial nurseries which specialize in this crop. According to estimates made by professional horticultural organizations, there have been about 1 200 tomato growers in Finland during the period 1962—65. It was during these years that the present investigation was carried out on the occurrence of tomato virus diseases in Finland and their causal agents.

Materials and methods

The material investigated consisted of 452 tomato cultivations under glass, which comprised about 35—40 % of all such cultivations in the country during the period 1962—65. About 80 % of the greenhouses were personally inspected between April and September. The inspections were made without definite selection and thus included both large and small enterprises, which

Table 1. Indicator plant tests compared with serological tests.

Virus	Result of plant test	Result of serological test compared with plant test (%)			Samples total no.
		same	opposite	indistinct [1]	
TMV	positive	78	11	11	339
	negative	77	8	15	85
PVX	positive	86	14	0	7
	negative	87	3	10	352

[1]) normal serum agglutinated

from the standpoint of their condition also varied from well-managed greenhouses to more primitive establishments. About 95 % of the houses were heated, while the rest were unheated houses used only in summer.

A total of 777 samples, usually one per greenhouse, were taken from the top or axillary shoots of virotic or possibly virotic plants for subsequent determinations of the causal agent by tests in the laboratory or greenhouse. Data were collected at the same time on the cultivar, the numbers of flower and fruit trusses at the time of inspection, as well as the type of substrate and its disinfection. Tomato samples suspected of being virotic which were sent to the Department of Plant Pathology by growers or agricultural advisors were also included in the experimental material and comprised about 20 % of the total material. In certain instances, chiefly involving samples sent by growers, data were not obtained on the substrate, the cultivar or the developmental stage at the time of sampling.

The causal agent of the virus disease in each sample was determined by the indicator plant method; in addition, serological tests were made on about one-half of the samples.

In the indicator plant tests the following plant species were regularly used: *Nicotiana tabacum* L. cultivar White Burley, *N. glutinosa* L., *Datura stramonium* L., *Chenopodium amaranticolor* Coste et Reyn. or *C. quinoa* Willd. and *Gomphrena globosa* L. In special cases the samples were also tested by using *Capsicum annuum* L. and *Physalis floridana* Rydb., the latter especially for determining possible infection with potato virus Y (PVY), and *Tetragonia expansa* Thunb. for establishing the presence of aspermy virus, and *Cucumis sativus* L. cv. Butcher's OE Spec. and *Spinacia oleracea* L. cv. Viking in order to confirm infection with cucumber mosaic virus (CMV). There were usually two plants of each species in the tests; if the result was not conclusive, further tests were performed.

Mechanical sap inoculation was carried out as described in earlier publications (LINNASALMI 1964, 1966). These publications also give descriptions of the types of symptoms produced by the viruses in the test plants used; special symptoms will be described in a later paper on virus strains. In the other test plants employed in the present studies not mentioned in the above papers, the symptoms were as follows: tobacco mosaic virus (TMV) and potato virus X (PVX) caused necrotic local lesions and systemic stem and top necrosis in *Capsicum annuum*; aspermy virus caused local lesions and mild systemic leaf deformation in *Tetragonia expansa*; and cucumber mosaic virus produced extreme malformation of leaves and stunting in *Spinacia oleracea*.

The temperature in the greenhouses used for the tests averaged 20—25°C in the day time and about 5° lower at night, and the relative humidity was 60—70 %. Insect pests were controlled by spraying with mevinphos twice a week.

Serological tests [1]) were made by the slide agglutination method (MUNRO 1954). In 1962—63, all the samples were tested with TMV and PVX antiserum. Samples which were suspected of being infected with aspermy virus were tested with the antiserum of this virus.

As regards speed of testing, the serological method is superior to the procedure with indicator plants. However, when parallel determinations were made both with test plants and with TMV and PVX antisera, it was found that these two methods did not always agree (Table 1). Divergent results occurred more

[1]) Sera were obtained from the Laboratorium voor Bloembollenonderzoek, Te Lisse, Holland.

frequently with TMV than with PVX. For both viruses the discrepancy was usually that the indicator plant test was positive for the virus but the serological test negative. This shows that the plant test is more dependable for revealing virus infection. Moreover, in about 10 % of the serological tests, an agglutination reaction occurred with both antiserum and normal serum, and thus the test had no value whatsoever. Owing to the unreliability of the serological test method, at least with the TMV and PVX antisera employed, this procedure was abandoned and in the years 1964—65 only indicator plants were used for virus determination.

Results and discussion

Occurrence of virus diseases

Virus diseases of tomato were very common in all parts of the country (Fig. 1). The highest proportions of virotic cultivations (ca. 90 %) were found in the islands of Ahvenanmaa and in the province of Vaasa. In both regions tomato growing is a recent innovation, having been started in the region of Vaasa somewhat over twenty years ago and in Ahvenanmaa only in the last few years. In other respects, however, conditions are very different in the two regions. In Ahvenanmaa the tomato cultivations are situated far from one another; in some cases there may be only a single one on a whole island. In the province of Vaasa, on the other hand, is located the largest uninterrupted tomato-growing area in Finland, where there may be dozens of tomato greenhouses belonging to different growers within an area of a few hectares. The lowest percentages of virotic cultivations were found in the provinces of Keski-Suomi, Kuopio and Uusimaa, where nearly one-half were virus-free at the time of the inspections. Tomato growing is relatively infrequent in the first two provinces, while in Uusimaa there are many areas in the vicinity of Helsinki where numerous and long-established tomato greenhouses are concentrated. The apparently favourable situation regarding virus diseases in Uusimaa may probably be due in part to the fact that inspections in this province were begun in April and May, when most of the plants were still young and consequently were either actually virus-free at this stage or had not yet produced visible symptoms (cf. p. 350). In the other provinces, 2/3—3/4 of the cultivations inspected were virotic. This was the case both in the region round Turku in Southwest Finland, where tomato cultivation is long-established and prevalent, and also in northern Finland (Oulu, Kemi, Tornio), where this crop is only grown on a small scale.

Diseases and their causal agents

The commonest disease was tomato mosaic, caused by the tobacco mosaic virus (= *Nicotiana* virus 1 Smith); 89 % of the samples with mosaic were infected with this virus alone. No appreciable regional differences were noted in the prevalence of this disease, with the exception of its slightly lower incidence in Uusimaa (Table 2). Cases of tomato mosaic in which the causal agents were both TMV and the potato virus X (= *Solanum* virus 1 Smith) were much rarer, making up only 1 % of all virotic samples.

The commonest symptoms of tomato mosaic were green mottling of varying severity in the leaves as well as stunted growth of the plants; the fruits were apparently undamaged. Symptoms of yellow mottling were rare (4 %), and typical leaf narrowing did not occur in plants infected with TMV alone.

Two cases of ordinary tomato fern leaf were encountered, one in Mikkeli province caused jointly by TMV and the cucumber mosaic virus (= *Cucumis* virus 1 Smith), and the other in Lappi province caused solely by CMV (cf. LINNASALMI 1966).

Tomato streak was much less common than mosaic, making up only 10 % of the virotic

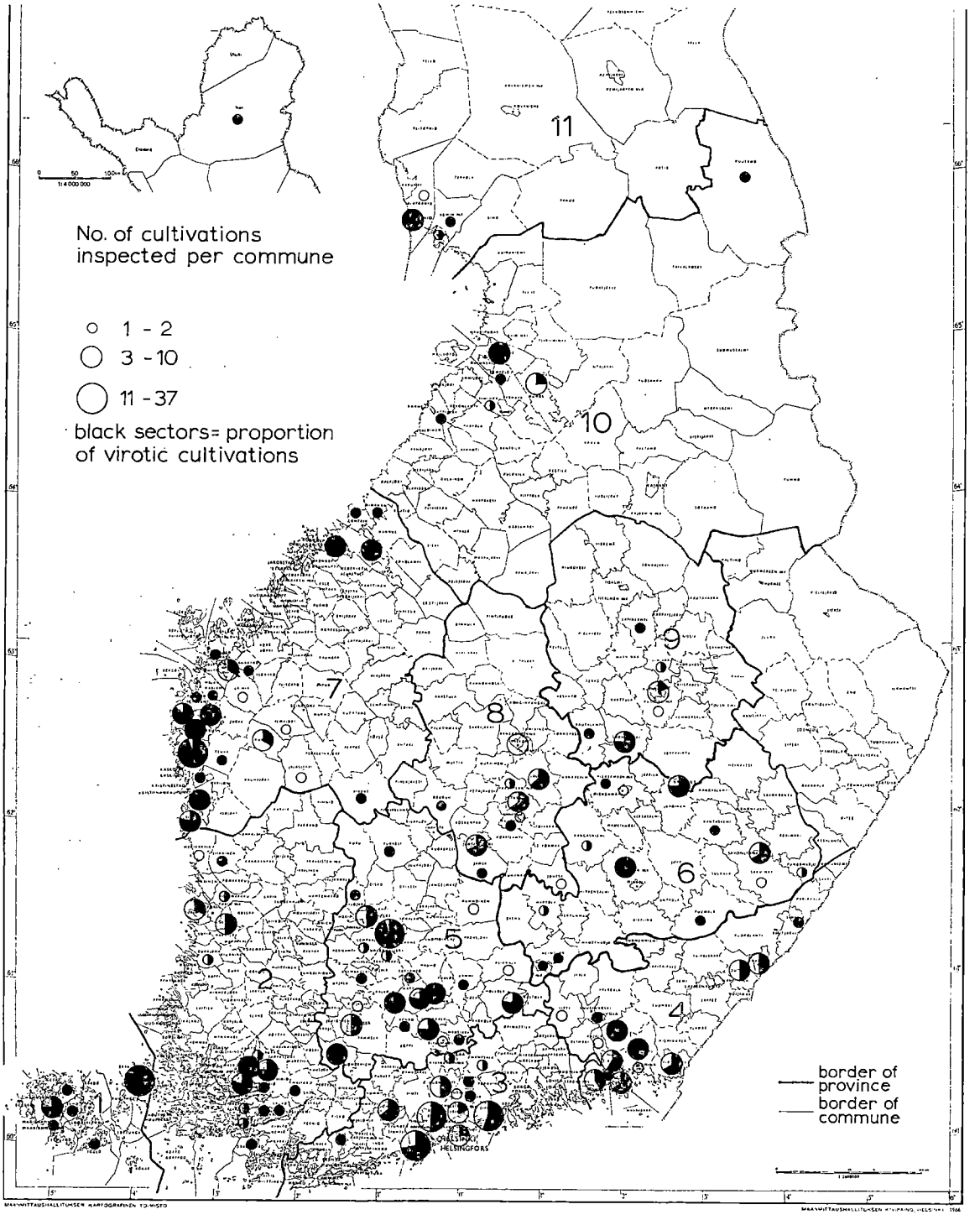


Fig. 1. Location of inspected tomato cultivations and occurrence of virus diseases.
Kuva 1. Tarkastettujen tomaattiviljelmien sijainti ja virustautisuus.

Table 2. Occurrence of virus diseases and their causal agents in tomato crops in Finland, 1962—65.

Province	Cultivations		Samples			Percentage of causal agents in virotic samples					
	total	virotic	total	virotic		tomato mosaic		tomato streak		tomato fern leaf	tomato aspermy
						TMV	TMV + PVX	TMV	TMV + PVX		aspermy virus
	no.	%	no.	no.	%					CMV	
Ahvenanmaa ..(1)[1])	24	96	39	38	97	92	0	0	8	0	0
Turku—Pori ..(2)	39	67	70	52	74	90	2	6	2	0	0
Uusimaa .....(3)	107	56	175	110	63	73	1	23	1	0	2
Kymi .....(4)	44	64	91	60	66	89	2	7	2	0	0
Häme .....(5)	71	75	130	102	78	90	0	7	3	0	0
Mikkeli .....(6)	21	67	37	28	76	92	0	0	4	4	0
Vaasa .....(7)	82	88	132	118	89	92	3	4	1	0	0
Keski-Suomi ..(8)	23	57	41	28	68	100	0	0	0	0	0
Kuopio .....(9)	19	53	35	20	57	95	0	0	5	0	0
Oulu .....(10)	14	71	19	15	79	100	0	0	0	0	0
Lappi .....(11)	8	75	8	6	75	83	0	0	0	17	0
Total	452	70	777	577	74	89	1	8	2	(0.3)	(0.3)

[1]) number of provinces in the map, Fig. 1 p. 348

samples. The causal agent was either TMV alone (8 %) or a combination of TMV and PVX (2 %). Single streak was most widespread in Uusimaa (23 %), while double streak was commonest in Ahvenanmaa (8 %).

The symptoms of tomato streak include leaf mottling, usually green mottling but sometimes yellow mottling, notably in plants infected with both TMV and PVX. Dark necrotic streaking of leaf veins, petioles and stems were characteristic of both forms of the disease; in some cases necrotic spots or lesions also occurred on the laminae or fruits. The symptoms were particularly marked in all the cases of double streak. A partial reason for the fact that tomato streak, which may even cause the death of the plant, was much rarer than mosaic is that the grower readily observes such infected plants and eliminates them in order to prevent spread of the disease. This measure, performed at an early stage, is indeed of benefit to the grower. Such cases of the disease, however, are not recorded by the investigator.

Only one case of aspermy disease of tomato was encountered in a greenhouse where chrysanthemums were being grown at the same time for cuttings. The symptoms were interveinal mottling and some leaf malformation as well as a slightly bushy appearance of the plants. A test performed with aspermy virus antiserum on the samples gave a positive result. The disease was also detected in the chrysanthemums

Table 3. Occurrence of virus diseases and their causal agents in various tomato cultivars.

Cultivar	Samples			Percentage of causal agents in virotic samples				
	total	virotic		tomato mosaic		tomato streak		tomato fern leaf
				TMV	TMV + PVX	TMV	TMV + PVX	
	no.	no.	%					CMV
Potentat	57	35	61	86	0	8	3	3
Selandia	348	242	70	88	1	9	2	(0.4)
Growers Pride	22	17	77	100	0	0	0	0
Earlymuna	41	35	85	94	0	6	0	0
Immuna	106	92	87	90	0	3	7	0
Revermun	10	10	100	90	0	10	0	0
Others	24	17	71	88	0	12	0	0
Total	608	448	74	89	1	7	2	1

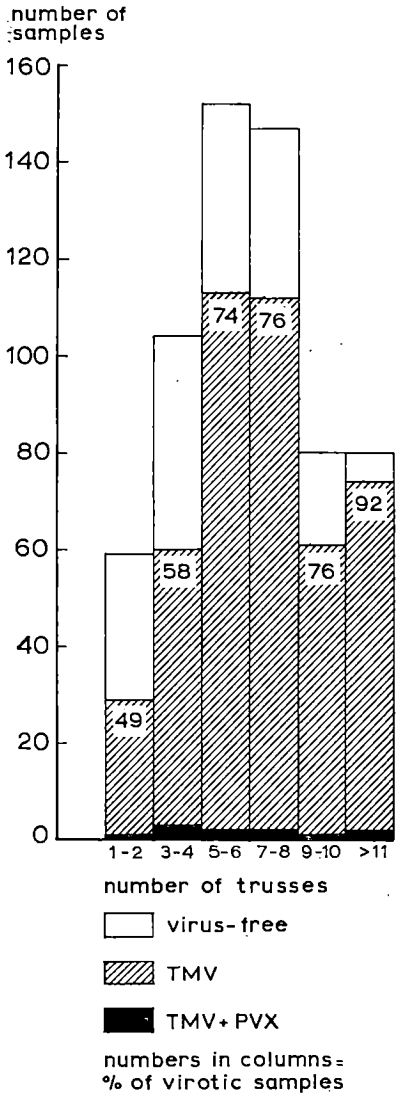


Fig. 2. Occurrence of virus diseases in various developmental stages of tomato.
Kuva 2. Virustautien esiintyminen tomaatin eri kehitysvaiheissa.

(E. Tapio, unpublished data), and the greenhouse was found to be infested with large numbers of *Myzus persicae* Sulz., a vector of the aspermy virus (cf. LINNASALMI 1963).

Occurrence of virus diseases in the various cultivars

Of the 18 tomato cultivars in the greenhouses inspected, six were more commonly grown than the others (Table 3). Three of these — Immuna, Earlymuna and Revermun — are F_1-hybrids bred for resistance to leaf mold (*Cladosporium fulvum* Cke.), and are also found to be resistant to the *C. fulvum* strains occurring in Finland (LINNASALMI 1955 and unpublished data, SALOKANGAS 1961). All three of these cultivars were more frequently infected with virus than the three cultivars Selandia, Potentat and Growers Pride, which are susceptible to leaf mold, with an average difference of 20 % in the incidence of virus infection between the two groups. It appears that when the breeders succeeded in achieving leaf mold immunity in the tomato cultivars, their susceptibility to viruses, especially TMV, increased at the same time. The cultivar Immuna seemed also to be sensitive to PVX infection; this virus, as well as CMV, was likewise encountered in Selandia and Potentat. The group of infrequently grown cultivars, in which 70 % of the samples were virotic, comprised Bonner Beste, Danimuna, Fionia, Golden Queen, Hinnonmäki, Juno, Kondine, Dansk Export, Veris and Ware Cross, as well as the leaf mold resistant cultivars Danderyd and Reverdan.

Occurrence of virus diseases in various developmental stages

During the course of these investigations the appearance of symptoms of tomato virus at different developmental stages of the plants was determined (Fig. 2).

The cultivation of tomatoes in Finland is generally started in winter. Seeding is carried out in December and January, and the seedlings are usually transplanted to heated greenhouses in February and March. When unheated houses are used, growing is not begun until late April or early May; such unheated houses, however, are steadily decreasing in numbers.

When inspection was begun in April, at which time the plants were generally small, virus diseases were rarely encountered. Over one-half of the samples taken from tomato plants at the 1- to 2-truss stage were still virus-free. At this stage of development the few virotic plants

discovered occurred singly or in small scattered groups in the greenhouse. The first infected plant groups were often observed close to the outer doors of the greenhouse, and the infection was later seen to spread to other plants, particularly on either side of the pathways.

It is quite probable that in many such cases the primary TMV infection was seed-borne, since according to recent studies (JOHN and SOVA 1955, CROWLEY 1957, HOWLES 1961, TAYLOR, GROGAN and KIMBLE 1961, BROADBENT 1964, 1965 b) seed infection is an important source of TMV. TAYLOR et al. (loc. cit.) found TMV in the endosperm in 2 % of the seed examined, and BROADBENT (1965 b) in as high a proportion as 10 %. He is of the opinion that most commercial seed is obtained from infected plants. Even if only a small proportion of the seed is actually infected with TMV, not many diseased seeds are needed before the young seedlings form sources of contamination after transplantation.

The frequency of virus diseases increased markedly as the plants attained the 5- to 6-truss stage; 75 % of the samples were virotic at this stage, and according to visual observations 40—50 % of the plants were infected. The most important reason for the increase in disease frequency was obviously the fact that during cultural operations the readily sap-transmissible viruses TMV and PVX spread in the greenhouse from plant to plant and further to other greenhouses.

Broadbent and his collaborators have presented much experimental evidence concerning the epidemiology of TMV in tomato cultivations. These workers demonstrated that besides by direct contact between the plants and hands or implements (BROADBENT 1963, 1964), infection can also be spread by means of the workers' clothing. In such infected clothing, TMV has been found to retain its activity for some weeks in daylight and over three years in darkness (BROADBENT and FLETCHER 1963). The above-mentioned tendency of the virus disease to spread along the plants bordering the pathways was probably due to contamination via the clothing of the workers. Infection may also take place through the roots, in which case, according to BROADBENT (1965 a), symptoms begin to appear in young shoots after periods varying from 3 weeks to 6 months after inoculation. However, in the present investigation it was difficult from the observations to assess the importance of this mode of infection.

The susceptibility of tomato plants to TMV was found by BROADBENT (1964) to increase with age. Similarly, in the present studies, the frequency of disease increased steadily as the plants grew. At the 11-truss stage about 90 % of the samples were virotic and 100 % of the plants in infected greenhouses were diseased.

Occurrence of virus diseases in plants grown in different substrates

At the present time, humus soil is still much more widely used as a substrate in tomato-growing than peat (Fig. 3). The use of peat began in the early 1960's, when sales of the so-called mill-peat were started, and this product has gained in popularity from year to year.

Over one-half of the growers raised tomatoes in the same soil for more than one year (usually 2—4 years) in succession. Only about 10 % of the growers sterilized the soil by steaming or chemically with chloropicrin, dazomet or metam-sodium preparations. On the other hand, renewal of soil was quite common, since about one-third of the greenhouses were using fresh soil in the year they were inspected. As for the houses with peat, this was fresh in more than half, while a few growers had steam-sterilized their peat or had disinfected it with dazomet preparation.

The frequency of virus diseases was lower in steamed soil, fresh soil and fresh peat (65—70 %) than in previously used or chemically disinfected soil and peat (80—100 %). TMV infection was common in plants grown both in soil and in peat, while of the two CMV cases one was in soil and the other in peat. PVX was not found in a single plant grown in peat.

The prevalence of virus diseases in plants

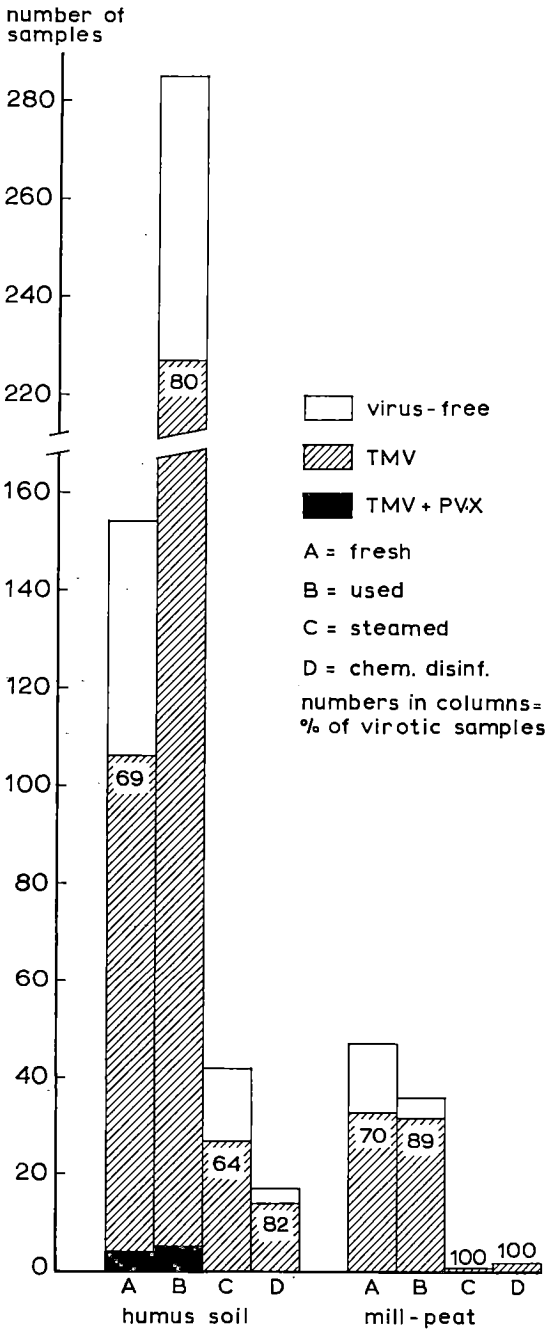


Fig. 3. Occurrence of virus diseases in tomatoes grown in different substrates.
Kuva 3. Virustautien esiintyminen eri kasvualustoilla kasvatetuissa tomaateissa.

which grew in soil or peat previously used for raising tomatoes is quite understandable, since plant debris remains in the substrate. If such debris is virus-infected, the substrate becomes a source of contamination for the next tomato crop. According to numerous investigations, TMV persists in soil, especially in root debris, for many months, sometimes even almost two years, depending on the kind of soil, its moisture and aeration, etc. The infection may be restricted to the roots only or may systemically involve the whole plant (HOGGAN and JOHNSON 1936, BERKELEY 1942, ROBERTS 1950, BROADBENT 1965 a, BROADBENT et al. 1965).

During the course of this investigation it became evident that the growers did not pay enough attention to thorough cleaning of the interior of the greenhouse and removal of all traces of plant debris when renewing the substrate. Consequently, the new soil or peat immediately became contaminated, and this obviously increased the likelihood of virus diseases appearing in the new crop, despite the renewed substrate. Mill-peat is probably originally virus-free. Similarly, soil taken from fields, provided weed control has been carried out, is presumably free of TMV at least, since none of the most important field crops grown in Finland belong to the actual host range of TMV. On the other hand, it is less certain that PVX contamination will be avoided by using field soil, since this virus is common in potatoes in Finland (AURA 1957, JAMALAINEN 1957, SEPPÄNEN 1966). It may thus persist in tubers that have remained in the soil (cf. MACNEILL 1955) and consequently be transported to greenhouses, where it may cause infection in tomatoes. This possibility, for example, was strongly suggested by a certain case in Ahvenanmaa. Early potatoes were temporarily grown in a greenhouse, and in the following year tomatoes were planted in the same soil without sterilization. At the time of inspection the entire crop was severely infected with double streak (TMV + PVX).

The lower incidence of virus diseases in tomatoes grown in steamed soil is understandable, since steaming is effective in eliminating the most resistant viruses, provided that the procedure is carried out deep enough and at a

sufficiently high temperature (cf. BROADBENT et al. 1965).

The chemical soil disinfectants used by the growers are not known to inactivate viruses. On the contrary, after soil treatment with metam-sodium and chloropicrin, TMV infection may be even more severe than before, possibly due to the fact that these chemicals destroy the microflora which ordinarily decomposes virotic plant debris (BROADBENT et al. 1965). Likewise in the present investigation, it seemed that the above chemical soil disinfectants as well as dazomet were unable to reduce the incidence of virus diseases.

Summary

During investigations carried out in 1962—65 on the occurrence of tomato virus diseases in Finland and their causal agents, material was collected from 452 greenhouse cultivations, representing 35—40 % of all such commercial cultivations in the country during those years.

Samples totalling 777, taken from plants which were virotic or suspected of being infected, were analyzed by the indicator plant method. In addition, about one-half of the samples were also tested serologically for TMV and PVX, and the samples showing aspermy disease symptoms for aspermy virus.

Virus diseases were common in all parts of the country; an average of 70 % of the cultivations inspected were found to be virotic.

The commonest form of virus disease was tomato mosaic caused by the tobacco mosaic virus (TMV), making up 89 % of the virotic samples. Tomato streak was less frequent (10 %) and occurred either as single streak caused by TMV alone or as a severe double streak caused by both TMV and the potato virus X (PVX) together. Both tomato fern leaf, caused by the cucumber mosaic virus (CMV), either alone or together with TMV, and the aspermy virus disease were quite rare.

Of the most commonly grown tomato cultivars, virus diseases were less frequent in Selandia, Potentat and Growers Pride than in the cultivars Immuna, Earlymuna and Revermun, which are resistant to leaf mold (*Cladosporium fulvum* Cke.).

The incidence of virus diseases was lowest in young seedlings up to the 1- to 2-truss stage, but increased markedly as the plants developed; 75 % of the samples at the 5- to 6-truss stage were virotic. At the 11-truss stage the disease was present in 90 % of the samples and infected crops were usually 100 % virotic by this time.

Virus diseases were more common in plants grown in humus soil or mill-peat which had previously been used for tomatoes (80—100 % of the samples were virotic) than in plants grown in fresh or steamed humus soil or fresh mill-peat (65—70 % being virotic). The use of the chemical soil disinfectants chloropicrin, dazomet or metam-sodium did not appear to be effective in reducing the incidence of virus diseases of tomatoes (82—100 % being virotic).

Acknowledgements. — The technical assistance of Mrs Kirsti Nieminen is acknowledged with gratitude.

REFERENCES

AURA, K. 1957. Suomessa viljellyn perunan virustautisuudesta. Summary: On potato virus diseases in Finland. J. Sci. Agric. Soc. Finl. 29: 103—110.

BERKELEY, G. H. 1942. Tobacco mosaic in Ontario and Quebec. Sci. Agric. 22: 265—278.

BROADBENT, L. 1963. The epidemiology of tomato mosaic. III. Cleaning virus from hands and tools. Ann. Appl. Biol. 52: 225—232.

BROADBENT, L. 1964. Tomato mosaic. N.A.A.S. Quart. Rev. 65: 13—22.

—»— 1965a. The epidemiology of tomato mosaic. VIII. Virus infection through tomato roots. Ann. Appl. Biol. 55: 57—66.

—»— 1965b. The epidemiology of tomato mosaic. XI. Seed-transmission of TMV. Ibid. 56: 177—205.

—»— & FLETCHER, J. T. 1963. The epidemiology of

tomato mosaic. IV. Persistence of virus on clothing and glasshouse structures. Ibid. 52: 233—241.

BROADBENT, L., READ, W. H. & LAST, F. T. 1965. The epidemiology of tomato mosaic. X. Persistence of TMV-infected debris in soil, and the effects of soil partial sterilization. Ibid. 55: 471—483.

CROWLEY, N. C. 1957. Studies on the seed transmission of plant virus diseases. Austr. J. Biol. Sci. 10: 449—464.

HOGGAN, I. A. & JOHNSON, J. 1936. Behaviour of the ordinary tobacco mosaic virus in the soil. J. Agric. Res. 52: 271—294.

HOWLES, R. 1961. Inactivation of tomato mosaic virus in tomato seeds. Pl. Path. 10: 160—161.

JAMALAINEN, E. A. 1943. Tomaatin virustaudeista. [Virus diseases of tomato.] Publ. Hort. Assoc. Finl. 30. Repr. 8 p.

—»— 1957. Virustaudeista ja virustautien kaltaisista kasvitaudeista Suomessa. Summary: On plant virus diseases and viruslike diseases in Finland. Publ. Finn. State Agric. Res. Board 158: 1—58.

JOHN, C. A. & SOVA, C. 1955. Incidence of tobacco mosaic virus on tomato seed. Phytopath. 45: 636—637.

LINNASALMI, A. 1955. Lehtihometta vastaan kestävistä tomaattilajikkeista. [Tomato cultivars resistant to leaf mold.] Publ. Hort. Assoc. Finl. 105. Repr. 3 p.

LINNASALMI, A. 1963. Tomaatin virustaudit. Summary: Virus diseases of the tomato in Finland. Maatal. ja Koetoim. 17: 185—191.

—»— 1964. The characteristics of some isolates of tobacco mosaic virus and potato virus X causing streak on tomato. Ann. Agric. Fenn. 3: 224—234.

—»— 1966. Virus diseases of cucumber in Finland and characteristics of their causal agents cucumber mosaic and cucumber green mottle mosaic viruses. Ibid. 5: 305—323.

MACNEILL, B. H. 1955. Some viruses of the field tomato in Ontario. Pl. Dis. Rep. 39: 191—193.

MUNRO, J. 1954. Maintenance of virus X-free potatoes. Amer. Potato J. 31: 73—82.

ROBERTS, F. M. 1950. The infection of plants by viruses through roots. Ann. Appl. Biol. 37: 385—396.

SALOKANGAS, K. 1961. Uusia lasin alla viljeltäviä tomaattilajikkeita. Summary: New varieties of forcing tomatoes. Maatal. ja Koetoim. 15: 196—204.

SEPPÄNEN, E. 1966. Virustaudit yleisiä perunaviljelyksillämme. [Virus diseases common in our potato cultivations.] Kylvösiemen 1966, 3: 22—24.

TAYLOR, R. H., GROGAN, R. G. & KIMBLE, K. A. 1961. Transmission of tobacco mosaic virus in tomato seed. Phytopath. 51: 837—842.

SELOSTUS

Tomaatin virustaudit Suomessa

I. Esiintyminen ja taudinaiheuttajat

ANNIKKI LINNASALMI ja AARNO MURTOMAA

Maatalouden tutkimuskeskus, Kasvitautien tutkimuslaitos, Tikkurila

Vuosina 1962—65 suoritetussa tutkimuksessa tomaatin virustautien ja niiden aiheuttajien levinneisyydestä Suomessa koottiin aineisto yhteensä 452 kasvihuoneviljelmältä, mikä oli 35—40 % koko maan kaupallisista tomaattiviljelmistä tutkimusvuosina.

Viroottisista tai viroottisiksi epäillyistä kasveista otettu näyteaineisto (777 näytettä) analysoitiin indikaattorikasvimenetelmällä. Noin puolet näytteistä testattiin myös serologisesti TMV- ja PVX -antiseerumilla ja martovirusnäytteet tämän viruksen antiseerumilla agglutinaatiomenetelmällä.

Virustauteja esiintyi yleisesti eri puolilla maata; keskimäärin 70 % tarkastetuista tomaattiviljelmistä todettiin viroottisiksi.

Tomaatin kirjoviroosi tupakan mosaiikkiviruksen (TMV) aiheuttamana oli yleisin taudinmuoto (89 % viroottisista näytteistä). Tomaatin viiruviroosi oli harvinaisempi (10 %) ja esiintyi joko TMV:n aiheuttamana tai TMV:n ja perunan X-viruksen (PVX) yhdessä aiheuttamana aina vaikealaatuisena kaksoisviiruviroosina. Suikaleviroosi, aiheuttajana kurkun mosaiikkivirus (CMV) yksinään tai yhdessä TMV:n kanssa samoin kuin mattoviroosi aspermiaviruksen aiheuttamana, olivat harvinaisia.

Yleisimmistä viljelylajikkeista todettiin virooseja vähemmän Selandia-, Potentat- ja Growers Pride-lajikkeissa kuin lehtihomeen *(Cladosporium fulvum* Cke.) -resistenteissä Immuna-, Earlymuna- ja Revermun -lajikkeissa.

Virustautisuus oli vähäisintä taimien 1—2 -terttuasteelle saakka, mutta yleistyi 5—6 -terttuvaiheessa voimakkaasti (näytteistä 75 % viroottisia). 11-terttuasteella tautisuus oli 90 % ja saastuneet kasvustot olivat yleensä 100-prosenttisesti viroottisia.

Virustaudit olivat yleisempiä tomaatin viljelykseen käytetyssä vanhassa mullassa tai turpeessa kasvatetuissa taimissa (80—100 % näytteistä viroottisia) kuin uudessa tai höyrytetyssä mullassa taikka turpeessa kasvatetuissa taimissa (65—70 % viroottisia näytteitä). Kemiallisella maandesinfioinnilla, käytettäessä klooripikriini-, datsometti- tai metamivalmisteita, ei näyttänyt olevan merkitystä virustautien vähentäjänä (82—100 % viroottisia näytteitä).

ANNALES AGRICULTURAE FENNIAE, VOL. 5: 355—356 (1966)
Seria PHYTOPATHOLOGIA N. 17 — Sarja KASVITAUDIT n:o 17

AMINO ACID COMPOSITION OF SOME ISOLATES OF TOBACCO MOSAIC VIRUS AND POTATO VIRUS X FROM TOMATO

ANNIKKI LINNASALMI
Agricultural Research Centre, Department of Plant Pathology, Tikkurila, Finland

Received November 18, 1966

Analyses of the amino acid composition of the protein coat of tobacco mosaic virus (TMV) and potato virus X (PVX) are being carried out at this department on virotic material collected from tomato cultivations in Finland (LINNASALMI and MURTOMAA 1966). The present paper is a preliminary report of these investigations.

Pure lines of TMV and PVX were separated by the single local lesion method. In the case of TMV, this was done by making four passages with sap inoculation in *Nicotiana glutinosa* L., while for PVX the passages were made in *Gomphrena globosa* L.

The purified TMV lines were maintained in *N. tabacum* cv. Samsun, except the line T80/62, which was grown in *Lycopersicum esculentum* Mill. cv. Bonner Beste, since it was only weakly infective to Samsun tobacco. The PVX lines were maintained in *Nicotiana glutinosa* L. The purification method was the same as that used for cucumber green mottle mosaic virus (LINNASALMI 1966), except that during the purification of PVX the first 2 or 3 high-speed cycles were done at 40 000 g, while the speeds of the following cycles were stepwise increased to 57 000 g. In order to completely separate PVX particles from cellular material, it was necessary to have more alternating low- and high-speed cycles, 7 or 8, as compared with the 4—6 cycles needed to purify TMV.

The chemical methods used for analysing the amino acids have been described in an earlier paper (LINNASALMI 1966). Both qualitative and quantitative analyses were made of each pure line. The quantitative analyses of PVX were performed with the auto-analyser equipment of Technicon Instruments Ltd., while the TMV analyses were made with the auto-analyser constructed at the Biochemical Institute, Helsinki.

Table 1 gives the amino acid contents of seven TMV and three PVX isolates. The amounts of each of the 17 amino acids in the protein coat were approximately equal in the different TMV isolates, except for isolate T20/62, which lacked methionine. The other isolates likewise contained only small amounts of this acid. Similarly, all the isolates had a low content of cysteine, less than 1 %. Aspartic and glutamic acids were the most plentiful amino acids in TMV protein, averaging around 15 %, while the following acids, in order of their abundance, were valine, leucine and arginine (about 8—10 % each).

The protein of the PVX isolates contained histidine in addition to the acids occurring in TMV, or a total of 18 amino acids. The amounts of the different acids were approximately the same in each of the three isolates, the most abundant being alanine, threonine, aspartic acid and glutamic acid (about 10 % each) and the least abundant histidine, tyrosine and cysteine

Table 1. Amino acid composition of protein coat of TMV and PVX

Taulukko 1. TMV:n ja PVX:n proteiinikuoren aminohappokoostumus

Amino acid	Virus and isolate									
	g amino acid per 100 g protein									
	TMV							PVX		
	T7/62	T17/62	T20/62	T80/62	T97/62	T115/62	T143/62	T80/62	T97/62	T115/62
Alanine	5.4	6.0	6.4	6.5	5.4	5.4	4.6	11.0	10.5	11.7
Arginine	8.2	8.3	9.5	6.5	8.4	8.2	8.5	5.7	5.4	5.9
Aspartic acid	14.0	13.1	12.9	17.0	12.8	12.3	11.9	9.1	8.4	9.3
Cysteine	0.4	0.5	0.5	0.8	0.5	0.6	0.8	0.9	0.2	0.9
Glutamic acid	15.3	15.4	11.9	18.6	15.7	15.4	13.6	8.2	8.1	8.7
Glycine	2.5	2.5	2.3	3.1	2.5	2.5	2.1	2.7	2.7	2.9
Histidine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.2	1.5
Isoleucine	4.3	3.6	5.1	5.3	4.4	4.3	3.8	4.0	4.3	4.4
Leucine	9.2	9.1	8.1	11.0	8.8	9.1	7.7	3.5	3.3	3.6
Lysine	1.7	1.7	1.6	2.1	1.7	1.6	1.8	6.1	5.7	6.2
Methionine	0.7	0.8	0.0	0.8	+	0.8	0.6	2.5	2.1	2.9
Phenylalanine	7.1	7.0	7.0	8.7	7.2	7.2	6.1	5.4	5.3	5.9
Proline	4.8	5.0	4.7	5.9	5.1	4.9	3.9	5.3	5.4	5.6
Serine	7.9	8.3	7.5	9.7	8.7	8.8	6.6	5.1	4.7	5.0
Threonine	10.1	10.8	9.4	12.0	10.8	10.9	8.1	9.7	9.3	10.8
Tryptophan	2.0	0.7	3.3	2.2	2.2	3.6	2.0	2.7	4.2	3.7
Tyrosine	4.8	4.8	3.8	5.8	5.0	5.0	4.4	1.2	1.1	1.3
Valine	9.0	9.9	7.8	11.3	9.5	9.0	7.8	3.8	4.0	4.1

(at most 1½ %). The low quantities of cysteine found in both the PVX and TMV analyses may have been due to the fact that this acid is evidently partially destroyed during hydrolysis.

As regards their biological properties, the two TMV isolates T17/62 and T20/62 differed considerably from the others, which represent the normal TMV type. All three PVX isolates were of biologically the same type.

Acknowledgements. — I express my sincere thanks to Mrs. Sirkka Rinne, M.Sc., for her collaboration in the laboratory work, as well as to Prof. A. I. Virtanen, Ph. D., Director of the Biochemical Institute, Helsinki, and to Prof. M. Antila, D. Agr. and For., Director of the Institute of Dairy Science of the University of Helsinki, for providing the use of the amino acid auto-analysers of their institutes.

REFERENCES

LINNASALMI, A. 1966. Virus diseases of cucumber in Finland and characteristics of their causal agents cucumber mosaic and cucumber green mottle mosaic viruses. Ann. Agric. Fenn. 5: 305—323.

LINNASALMI, A. & MURTOMAA, A. 1966. Virus diseases of tomato in Finland. I. Occurrence and causal agents of the diseases. Ann. Agric. Fenn. 5: 345—354.

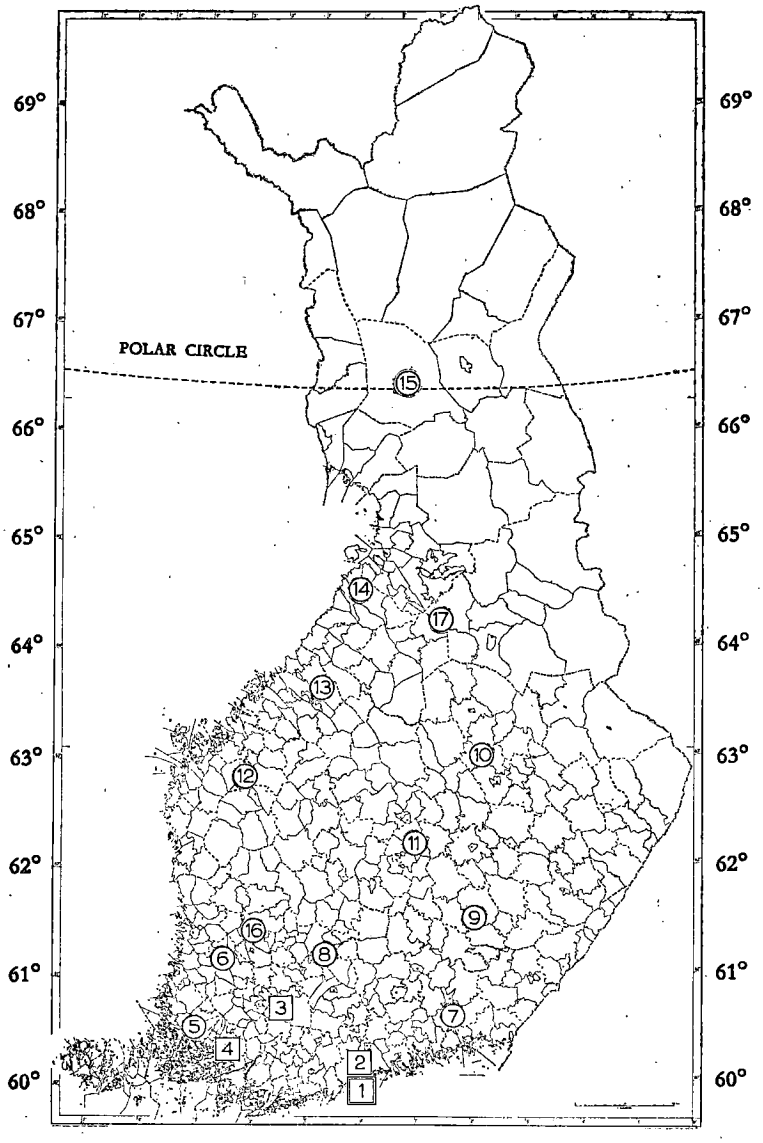
SELOSTUS

Tomaatista eristettyjen tupakan mosaiikki- ja perunan X-virusisolaattien proteiinikuoren aminohappokoostumus

ANNIKKI LINNASALMI

Maatalouden tutkimuskeskus, Kasvitautien tutkimuslaitos, Tikkurila

Tiedonannossa esitetään tomaatista eristettyjen seitsemän TMV- ja kolmen PVX-isolaatin proteiinikuoren kvantitatiivisen aminohappoanalyysin tulokset. TMV-isolaattien proteiinikuori koostui 17:sta aminohaposta, paitsi isolaatin T20/62, josta puuttui metioniini. PVX-isolaattien proteiinikuori sisälsi samat 17 aminohappoa sekä lisäksi histidiinin, yhteensä 18 aminohappoa.



DEPARTMENTS, EXPERIMENT STATIONS AND BUREAUS OF THE AGRICULTURAL RESEARCH CENTRE IN FINLAND

1. Administrative Bureau, Bureau for Local Experiments (HELSINKI) — 2. Departments of Soil Science, Agricultural Chemistry and Physics, Plant Husbandry, Plant Pathology, Pest Investigation, Animal Husbandry and Animal Breeding; Isotope Laboratory, Office for Plant Protectants, Pig Husbandry Exp. Sta. (TIKKURILA) — 3. Dept. of Plant Breeding (JOKIOINEN) — 4. Dept. of Horticulture (PIIKKIÖ) — 5. Southwest Finland Agr. Exp. Sta. (HIETAMÄKI) — 6. Satakunta Agr. Exp. Sta. (PEIPOHJA) — 7. Karelia Agr. Exp. Sta. (ANJALA) — 8. Häme Agr. Exp. Sta. (PÄLKÄNE) — 9. South Savo Agr. Exp. Sta. (Karila, MIKKELI) — 10. North Savo Agr. Exp. Sta. (MAANINKA) — 11. Central Finland Agr. Exp. Sta. (KUUSA) — 12. South Ostrobothnia Agr. Exp. Sta. (PELMA) — 13. Central Ostrobothnia Agr. Exp. Sta. (LAITALA) — 14. North Ostrobothnia Agr. Exp. Sta. (RUUKKI) — 15. Arctic Circle Agr. Exp. Sta. (ROVANIEMI) — 16. Pasture Exp. Sta. (MOUHIJÄRVI) — 17. Frost Research Sta. (PELSONSUO)

SISÄLLYS—CONTENTS

Helsinki 1967. Valtion painatuskeskus