THE APPEARANCE OF SOIL-BORNE VIRUSES IN FINNISH PLANT NURSERIES

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Abstract. In studies conducted by the Department of Plant Pathology of the Agricultural Research Centre, soil-borne viruses were found in soil samples from seven nurseries out of thirteen sampled and from fields at two experimental stations in south and south-west Finland. Tobacco necrosis virus was identified for the first time in Finland in 12 isolates and in addition tobacco rattle virus in 8 isolates. All these isolates were made from 193 soil and plant samples collected in late autumn of 1970. For the first time in Finland the nematode *Trichodorus pachydermus* Seinhorst was identified from extracts of TRV-infected soil and plant samples.

In Finland serious attention has been directed in recent years to the health of nursery plants. At the Department of Plant Pathology, a nuclear stock of raspberry plants free of known viruses has been produced by heat treatment and testing. Additional plants are beeing propagated under carefully controlled conditions. In connection with this it was considered important to study also whether soil-borne viruses occur in our nurseries to determine whether they are suitable, in this respect, for growing healthy plants.

Material and methods

Late in the autumn of 1970, when the ground was partly frozen, 193 soil samples were collected from thirteen nurseries in south and south-west Finland and from the experimental fields of the Agricultural Research Centre at Tikkurila and of the Department of Horticulture at Piikkiö. Weeds were present in 43 of these samples and in 17, perennial plants with roots.

The roots of the weeds and perennials were macerated in phosphate buffer, and leaves of *Chenopodium quinoa* Willd. and *Nicotiana tabacum* L. cv. Samsun were inoculated with the resultant suspension. In addition, Samsun tobacco seedlings were planted as »bait plants» in soil samples which were put into 5' pots. After two weeks sap from their roots was used to inoculate *C. quinoa* and *N. tabacum*. The experiments were continued with all samples which caused primary and/or secondary symptoms on the above-mentioned test plants. In addition to these, the indicator plants given in Table 1 were used. The thermal end point was determined for all the virus isolates and the dilution end point and ageing in vitro for some of these.

Table 1. Symptoms produced on test plants by virus isolates examined.

	Reaction a of plants inoculated with[1]				
	TRV isolates		TNV isolates	Isolate Mn 58b	
	+L	+S	+L	+L	+S
Callistephus chinensis Nees.	2/7	7/7	0/7	0/1	1/1
Chenopodium amaranticolor Costa et Reyn.	8/8	0/7	12/12	1/1	1/1
C. quinoa Willd.	8/8	0/8	12/12	1/1	1/1
Cucumis sativus L. cv. Butcher	4/7	(1)/5	10/12	1/1	1/1
Gomphrena globosa L.	8/8	0/8	11/11	1/1	0/1
Nicotiana clevelandii A. Grey	2/8	7/8	5/11	0/1	1/1
N. glutinosa L.	4/6	4/6	1/12	0/1	0/1
N. hybridum	2/5	4/6	4/9	0/1	1/1
N. tabacum L. cv. Samsun	8/8	7/8	9/12	1/1	1/1
Phaseolus vulgaris L. cv. Bonita	0/7	0/7	10/12[2]	0/1	0/1
Petunia hybrida L.	0/8	2/8	2/12	1/1	1/1
Tetragonia expansa Thunb.	8/8	8/8	12/12	0/1	1/1

[1]) L = local lesions; S = systemic symptoms. In each column, the numerator is the number of virus isolates which infected the plant species examined, the denominator is the number of virus isolates with which the plant species was inoculated.

[2]) Two of the virus isolates caused veinlet necrosis lesions.

From all soil samples from which viruses were isolated free-living nematodes were extracted and prepared by the writer using the SEINHORST (1962) method. Later, Mr. OSMO ROIVAINEN of the Department of Pest Investigations kindly identified the nematodes in the preparations. Part of the extracted nematodes were immediately transferred to roots of healthy Samsun tobacco seedlings grown in steamed soil. Two weeks later, sap from the tobacco roots was used to inoculate test plants.

The roots of the virotic test- and bait-plants (tobacco) were also examined microscopically for *Olpidium brassicae*. Those grown in soil samples in which *O. brassicae* zoosporangia were found were quickly washed free of soil and then soaked for 15—20 minutes in fresh water in Petri dishes. Young Samsun tobacco seedlings just transplanted into steamed soil were watered with these zoospore suspensions. After two weeks, test plants were inoculated with sap from the tobacco roots.

The antisera for the soil-borne viruses were kindly sent to me by the following research workers: Dr. Mogens Christensen, State Plant Pathology Institute, Lyngby, Denmark, Dr. A. F. Murant, Scottish Horticultural Research Institute, Dundee, Scotland and Dr. D. Spaar, Institute für Phytopathologie, Aschersleben, DDR. The serological tests were carried out by the agglutination, agar-gel-diffusion and micro-precipitation methods (cf. TAPIO 1970 p. 12).

The viruses were purified by the chloroform-butanol method (STEERE 1956). The shapes and sizes of the viruses were determined at the Department of Electron Microscopy of the University of Helsinki with a Philips EM 200. The electron microscope preparations were made by BRANDES (1957) dip method and by the spraying method of BRENNER and HORNE (1958).

Results

21 viruses were isolated from samples originating from seven of the thirteen nurseries and from both experimental stations. Twelve of these isolates were indentified as tobacco necrosis virus and eight as tobacco rattle virus. In addition one isolate, which somewhat resembles tomato black ring virus, was found but is still not identified with certainly.

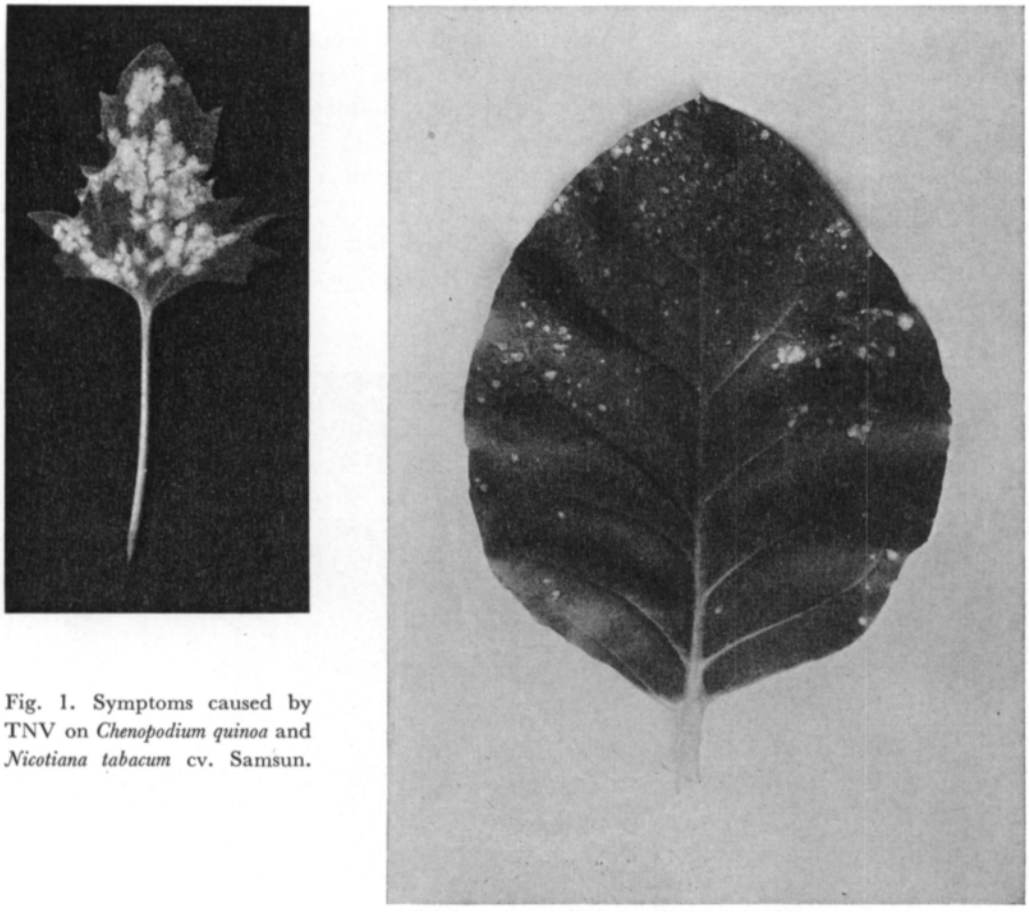


Fig. 1. Symptoms caused by TNV on *Chenopodium quinoa* and *Nicotiana tabacum* cv. Samsun.

Tobacco necrosis virus

TNV isolates caused local lesions (PRICE 1940 a) characteristic of TNV on a wide range of indicator plants (table 1). *Chenopodium quinoa*, *Gomphrena globosa* and *Tetragonia expansa* (Fig. 1) reacted most sensitively and *Cucumis sativus* rather well. Of the *Nicotiana* species examined, *N. tabacum* cv. Samsun was best suited as an indicator plant. Only one isolate (Mn 9) out of twelve caused necrotic spots on *N. glutinosa* leaves. Slight systematic symptoms were observed twice (Mn 9, Mn 31) on *N. clevelandii* and the virus was back-transmitted from these. Two TNV-isolates (Mn 6, Mn 31) caused characteristic flecks typical of serotype A on *Phaseolus vulgaris* (BABOS and KASSANIS 1963 a). These enlarged into

veinlet-necrotic spots. The others caused necrotic lesions characteristic of the D serotype. One of the isolates (Mn 77) caused a few scattered yellow necrotic spots only on leaves of *Chenopodium* species and *T. expansa*. A preparation purified from infected *C. quinoa* leaves reacted only slightly with TNV antisera.

The thermal end point varied considerably from 70 to 95° C, with different isolates (cf. PRICE 1940 b and BABOS and KASSANIS 1963 b), although attempts were made to keep the methods as uniform as possible by taking the infected sap of *T. expansa* leaves four days after inoculation and then inoculating *C. quinoa*. The experiments were mostly done simultaneously so that the test plants belonged to the same batch and their growing conditions were the same. The weak isolate Mn 77 had the lowest thermal end point, 70° C.

The dilution end point varied with the different isolates and according to the test plant species, but was most frequently between 10^{-3} and 10^{-4}. At room temperature (about +22° C) the infectivity lasted from 6 weeks to less than 6 months for all the isolates examined.

The virus particles, which were photographed and measured from preparations made from two purified TNV isolates, were polyhedral, and about 25 nm in diameter (Fig. 2).

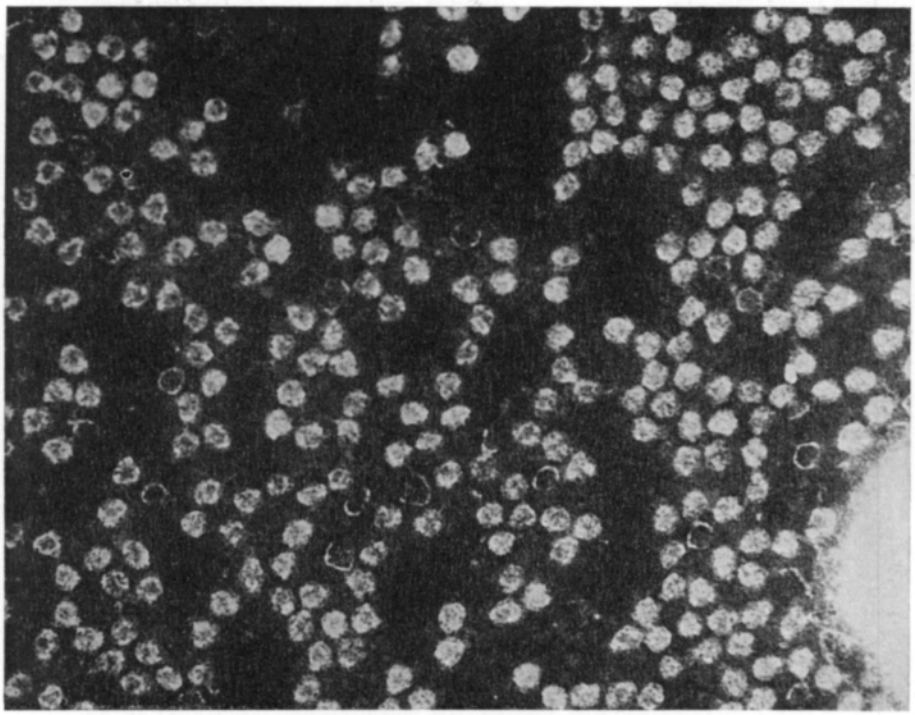


Fig. 2. TNV particles.

All TNV isolates reacted positively with the TNV antisera from Scotland and East Germany (Table 2). According to Dr. Murant, the former contained antibodies to both A and D serotypes. According to the information from Dr. Christensen the TNV isolates

which react with TNV-antisera 197b and 198b do not react with antisera 152 and 323 and vice versa. The two serotype-A, TNV isolates (Mn 6 and Mn 31) which caused veinlet necrosis on bean leaves reacted with the latter, and all others, probably of the D serotypes, with the former antisera (Table 2).

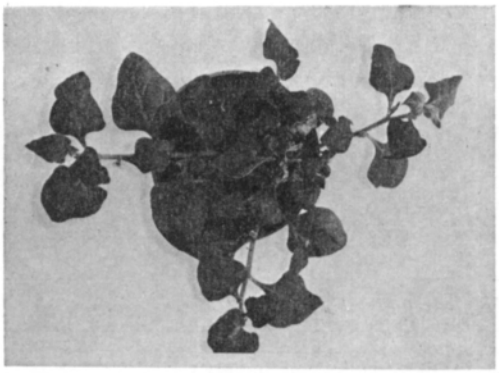
Table 2. Serological reactions of some TNV isolates to TNV antisera from various sources.

	Precipitation intensity with TNV antisera from					
Antigen	Scotland	East	Denmark			
TNV isolates	serot. A + D	Germany	197b	198b	152	323
Mn 6, Mn 31	++	++	—	—	+	+
Mn 36, 52, 78, 99a, 162	++	++	++	+++	—	—
Mn 77	+	+		+		

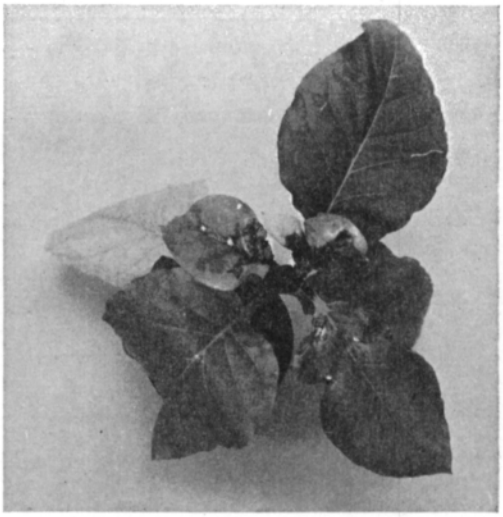
Zoosporangia of the *Olpidium brassicae* (Wor.) Dang. were observed in the roots of many tobacco plants grown in the soil samples and also in roots of *Viola arvensis* from which the TNV isolate Mn 36 was isolated. Resting spores were detected in roots of some partly withered tobacco plants. The size of the zoosporangia varied considerably, from 10—150 µ, with the round ones, however, being mostly 15—35 µ (cf. SAHTIYANER 1961). Only seven resting spores were measured; their diameter varied from 8 to 20 µ. Many one-tailed zoospores, Ø 3—4 µ in size, were observed in the water in Petri dishes in which infected roots has been soaked for 15—20 minutes after a quick washing. Young Samsun tobacco seedlings which were watered with a suspension of *Olpidium brassicae* obtained from roots infected with TNV isolate Mn 36 became infected with TNV. TEAKLE (1962) and KASSANIS and MACFARLANE (1964) have reported the same results from similar experiments as well as from more detailed experiments. For comparison, other tobacco seedlings were watered with a suspension of macerated tobacco roots from a sap-inoculated, TNV infected plant grown in steamed soil. These tobacco seedlings did not become infected.

Tobacco rattle virus

Test plants inoculated with TRV isolates had symptoms broadly similar to those described by SCHMELZER (1957) (Table 1). *Chenopodium quinoa*, *Gomphrena globosa*, *Nicotiana tabacum* and *Tetragonia expansa* reacted well, showing local lesions. Plants reacted systematically as follows: *Callistephus chinensis* with intense chlorosis or top necrosis, *N. clevelandii* with diffuse chlorosis or necrosis and slight stunting, *N. tabacum* with mosaic or necrotic spots and stripes and sometimes with strong veinbanding necrosis but later recovering, and *T. expansa* with chlorotic or necrotic spots or rings, distorted and stunted leaves and sometimes shoot necrosis (Fig. 3). Only two isolates out of eight caused systemic symptoms on *Petunia* from which a very good virus preparation was obtained by purification (Fig. 4).



a.



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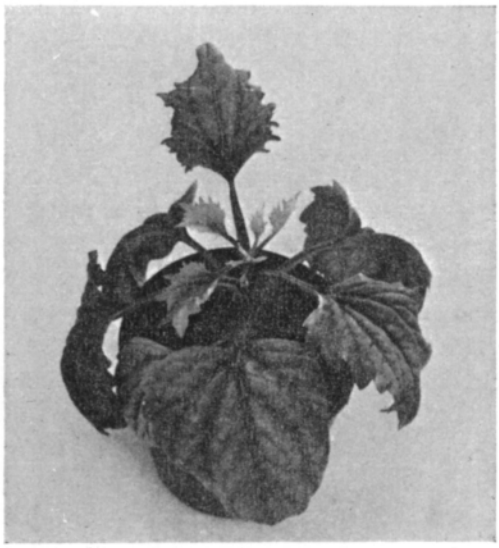

c.

Fig. 3. Symptoms caused by TRV on: a) *Tetragonia expansa*, b) *Nicotiana tabacum* cv. Samsun, and c) *Callistephus chinensis*.

Seven isolates had a thermal end point of 70° C, slightly lower than that reported by van der WANT (1952). However, it was 80° C for the isolate Mn 58a, which infected *Petunia* systematically. The dilution end point varied from 10^{-4}—10^{-5}. The single isolate kept for longer than 6 months at room temperature was still infective after this time.

All the TRV isolates were photomicrographed, but only two, Mn 58a and Mn 159, were measured. According to many other workers (e.g. PAUL and BODE 1955, HARRISON and NIXON 1959), virus particles of these two TRV isolates fall into two size groups: the shorter average 81 nm (24 nm—143 nm) and the longer 182 nm (155 nm—224 nm) (Fig. 4 and 5).

The intensity of the serological reaction in micro-precipitation experiments varied to some extent with different isolates (Table 3). The Danish antisera reacted more strongly than the East German ones.

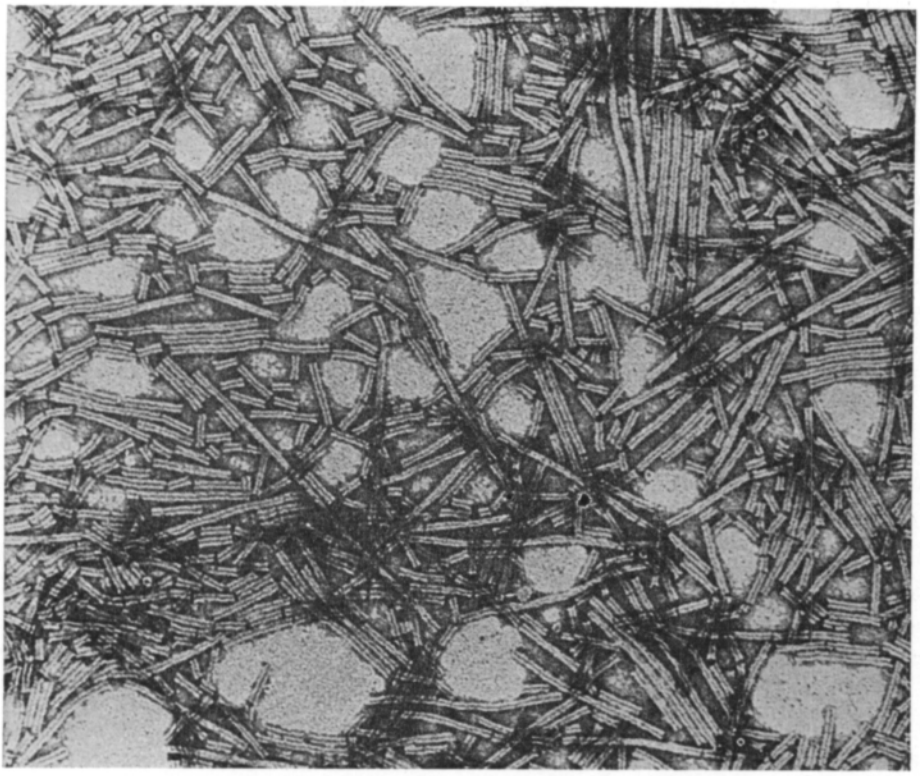

Fig. 4. TRV particles.

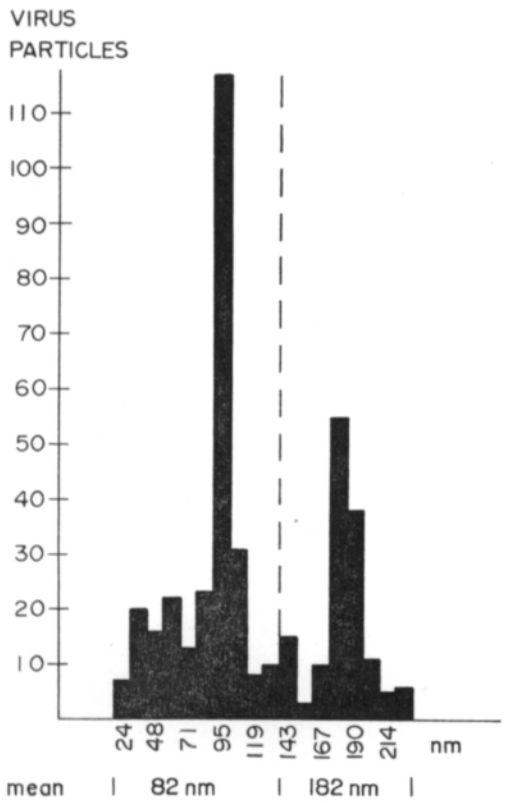

Fig. 5. The distribution of the length of TRV particles.

Table 3. Serological reactions of some TRV isolates to TRV antisera from various sources.

Antigen TRV isolates	Precipitation intensity with TRV antisera from East Germany AS/Ra	Denmark 339S	Denmark 341
Mn 22	+		
» 58a	++	—	+
» 78		++	+++
» 99b		±	+
» 159	+	++	+
» 165		++	+
» 167	+	+	+
» 193	++	+++	+++

Nematodes extracted from soil samples containing TRV when added in a suspension around the roots of healthy young tobacco plants transmitted the virus to these plants. On the basis of the work of Sol and Seinhorst (1961), it was suspected that these were *Trichodorus* sp. nematodes. In 1971, Mr. Osmo Roivainen of the Department of Pest Investigation identified the nematodes from the preparation made in 1970 as *Trichodorus pachydermus*. This was the first time this nematode was identified in Finland. Shortly thereafter he identified the same species in many new nursery soil samples collected by the writer in the autumn 1971 and in the potato soil samples from which Seppänen (1972) isolated TRV.

Fig. 6. Systemic symptoms caused of the virus isolate Mn 58 b on *Chenopodium quinoa*.

On the basis of preliminary results TRV and other soil-borne viruses are more abundant in the new samples collected from nurseries in August-September 1971 than in the material reported here. The 1971 samples were taken to a depth of 20—30 cm from unfrozen ground, whereas those made in the autumn of 1970 were taken from partly frozen ground to a depth of 5—15 cm. The nematodes had probably migrated deeper into the partly frozen ground in 1970 (cf. SOL 1963).

An unidentified spherical virus was isolated from *Phlox paniculata* cv. Spitfire (sample Mn 58) from which the tobacco rattle virus also was isolated. It partly resembles tomato black ring virus, although the ring spots it caused on *Chenopodium* species, *Gomphrena globosa* and *Petunia hybrida* were not as clear as HARRISON (1957) and RYDÉN (1965) reported but resembled more those described by SCHMELZER (1963). The *Chenopodium* species and *Petunia* were infected systemically (Table 1). It often appeared in *C. quinoa* as a top necrosis (Fig. 6). The chlorotic or green mottled symptoms in *Petunia* recovered later. The virus isolate Mn 58b did not cause local lesions characteristic of the TBRV on *Phaseolus vulgaris* or *Nicotiana*-species. Its thermal end point was 55° C and the spherical virus reacted only slightly with TBRV antisera.

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SELOSTUS

MAALEVINTEISTEN VIRUSTEN ESIINTYMINEN TAIMISTOISSA SUOMESSA

Eeva Tapio

Kasvitautien tutkimuslaitos, Maatalouden tutkimuskeskus, Tikkurila

Kasvitautien tutkimuslaitoksella suoritetuissa tutkimuksissa todettiin Etelä- ja Lounais-Suomessa esiintyvän yhdeksällä paikkakunnalla viidestätoista tutkitusta taimistoissa ja koekentillä maalevintäisiä viruksia. Syksyllä 1970 kerätyistä 193 maa- ja kasvinäytteestä eristettiin ensi kertaa Suomessa 12 tobacco necrosis virus-isolaattia sekä lisäksi 8 tobacco rattle virus-isolaattia, jota Seppänen (1972) on samanaikaisesti eristänyt perunanäytteistä. Maisteri Osmo Roivainen määritti maanäytteistä ensi kertaa Suomessa *Trichodorus pachydermus* Seinhorst-ankeroisen.