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SOME DATA ON THE OCCURRENCE OF RATTLE VIRUS AT VARIOUS DEPTHS IN THE SOIL AND ON ITS TRANSMISSION[1]

Enkele gegevens over het voorkomen op verschillende diepte en de overdracht van ratelvirus in de grond

BY

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INTRODUCTION

Rattle virus, belonging to the group of soil-borne viruses, has a very wide host range (SCHMELZER, 1957). Besides a number of agricultural crops, including tobacco, potatoes and tulips (ROZENDAAL & VAN DER WANT, 1948; VAN SLOGTEREN, 1958), many weeds may be infected (NOORDAM, 1956). Nematodes play an important part in the transmission of the virus (SOL, VAN HEUVEN & SEINHORST, 1960; SOL & SEINHORST, 1961). In this article some observations will be described on the occurrence of the virus at various depths and on its transmission in the soil.

OCCURRENCE OF RATTLE VIRUS AT VARIOUS DEPTHS

The virus is mainly found in sandy soils. From the investigations carried out by VAN DER WANT, THUNG & ROZENDAAL (1959) it appeared that in such soils the virus even occurs as deep as 50 cm below the surface. EIBNER (1959) mentions a similar observation. The experiment described below indicates that the virus may be present in even deeper layers.

From a field at Wageningen, infested with rattle virus, soil samples were taken at depths of 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–80, 80–100, 100–120, 120–140 and 140–160 cm.

The field had earlier been proved to be infested with rattle virus with the aid of the spinach test (SOL & SEINHORST, 1961). The soil concerned was a well-drained sandy soil with a top layer of black earth to a depth of 35 cm, beneath which were yellowish-brown (35–80 cm) and yellow (80–160 cm) sandy layers. Sampling was done extremely carefully, in order to avoid mixing of the soil from the various sampling depths. The samples were tested for the presence of virus in the following way. Three series, A, B and C, of Mitscherlich pots were filled with soil from each of the sampling depths mentioned above. In the pots of series A tobacco plants, *Nicotiana tabacum* L. var. 'White Burley', were planted, while spinach was sown in the pots of series B and C. The pots of series C had previously been sterilized at 120 °C for two hours. Whether or not the plants had been infected was ascertained in two ways: firstly from the presence or absence of symptoms in the above-ground parts, and secondly by

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testing the underground parts for the presence of virus by means of back inoculation to five healthy tobacco plants. The roots of the spinach plants were tested one month after sowing, those of the tobacco plants two months after planting. The results are shown in table 1.

TABLE 1. Infection of tobacco and spinach plants with rattle virus in soil samples taken at different depths from an infested field at Wageningen.
Infectie van tabaks- en spinazieplanten in grondmonsters van verschillende diepte, genomen van een besmet perceel te Wageningen.

Series / *Serie*	A		B		C	
Crop *Gewas*	Tobacco *Tabak*		Spinach *Spinazie*		Spinach *Spinazie*	
Treatment of soil *Behandeling van de grond*	–		–		sterilized	
Depth in cm *Diepte in cm*	Leaves *Bladeren*	Roots *Wortels*	Leaves *Bladeren*	Roots *Wortels*	Leaves *Bladeren*	Roots *Wortels*
0– 10	–	+	–	+	–	–
10– 20	–	+	–	+	–	–
20– 30	–	+	–	+	–	–
30– 40	–	+	–	+	–	–
40– 50	–	+	–	+	–	–
50– 60	+	+	–	+	–	–
60– 80	–	+	–	+	–	–
80–100	–	+	–	–	–	–
100–120	–	–	–	–	–	–
120–140	–	–	–	–	–	–
140–160	–	–	–	–	–	–

+ and –: presence and absence of rattle virus
+ *en* –: *aanwezigheid en afwezigheid van ratelvirus*

Of the tobacco plants of series A only one showed leaf symptoms of rattle virus. However, testing the roots for the presence of rattle virus proved many more plants to be infected with the virus. Rattle virus was even found to be present in the pot with soil from the layer at a depth of 80–100 cm. Testing of the roots of the spinach plants of series B gave similar results. Sterilization of the soil abolishes its infectivity which was proved by testing the roots of series C. This experiment clearly demonstrates that the virus may be present at considerable depths. In this case it was present in the layer of black earth, in the yellowish-brown sandy layer, and in the upper regions of the yellow sandy layer. Analysis of the soil samples proved various nematode species to occur in the deeper sampling layers. Springtails and mites were found only in the samples taken to a depth of 35 cm. Thrips were not found. It was therefore concluded that in the deeper layers the virus might be present in and transmitted by nematodes.

In a second experiment samples were taken from a completely different type of sandy soil at Lisse. From observations by VAN SLOGTEREN (1958) this soil was known to be infested with rattle virus. Samples were taken at depths of 0–10, 10–20, 20–30, 30–40, 40–50 and 50–60 cm. The high level of the soil-

TABLE 2. Extent of infection with rattle virus of the roots of test tobacco plants grown in soil samples taken at different depths from an infested field at Lisse.
Mate van infectie van wortels van tabaksplanten met ratelvirus in grondmonsters van verschillende diepte, genomen van een besmet perceel te Lisse.

Depth in cm *Diepte in cm*	0–10	10–20	20–30	30–40	40–50	50–60
	7/9[1]	9/9	7/9	4/9	2/9	0/9

[1] Numerator = number of infected plants
Teller = aantal geïnfecteerde planten

Denominator = total number of plants
Noemer = totaal aantal planten

water, which averaged 55 cm below ground level, made it impossible to take samples of deeper layers, as had been done in the preceding experiment. Nine pots, 10 cm in diameter, were filled with soil from each sampling depth. Tobacco plants were planted in these pots. No leaf-symptoms of rattle virus developed in any of the plants. Therefore the roots were tested for the presence of the virus three weeks after planting. The results are shown in table 2. Many of the plants grown in soil from the sampling depths of 0–10, 10–20, and 20–30 cm were found to be infected; somewhat fewer infections occurred in the plants grown in soil from the 30–40 and 40–50 cm layers; while no infection was found in the plants grown in soil from the 50–60 cm layer. The vector of the virus, the nematode species *Trichodorus pachydermus* Seinhorst (see below), was not found in the samples of this layer, whereas it was found in those of the other layers. So in this experiment there was a positive correlation between the presence of vector and virus.

TRANSMISSION OF RATTLE VIRUS BY *TRICHODORUS PACHYDERMUS*

Rattle virus is transmitted to healthy tobacco plants by the nematode species *Trichodorus pachydermus* (SOL & SEINHORST, 1961). Continued research has shown that this may be done by both male and female nematodes. Young nematodes also transmit the virus. This nematode species transmits the virus not only to tobacco plants, but also to potato plants, as appears from the results of the following experiment. In this experiment nematodes of the species *Trichodorus pachydermus* were isolated according to the "SEINHORST method" (SEINHORST, 1956) from soil in which potato crops frequently showed stem mottle. Pieces of healthy potato tubers (var. 'Sientje'), each with one eye, were placed in plastic cups filled with soil which had previously been autoclaved and washed with boiling water. After the eyes had sprouted and the plants had formed enough roots, twenty of the above mentioned nematodes were added to each cup. A fortnight after the nematodes had been added, plants and soil were transferred to big flower-pots with sterilized potting compost, and were then left for three months. As no stem mottle symptoms were observed in the above-ground parts or in the tubers, sap was expressed from the roots and was then rubbed on the leaves of healthy 'White Burley' tobacco plants. In this way the presence of rattle virus was detected in five plants out of the seventeen to which nematodes had been added. No rattle virus was found in any of the seventeen

TABLE 3. Transmission of rattle virus by *Trichodorus pachydermus* to tobacco, after starvation periods of different lengths.
De overdracht van ratelvirus door Trichodorus pachydermus *op tabak na een hongerperiode van verschillende duur.*

Starvation period in days / *Hongerperiode in dagen*	1	4	8	15	20	32	36
Experiment / *Proef*							
A		6/8[1]	3/6	1/5	1/3	1/3	
B	5/8	4/7	8/10		2/6		1/3
C							2/4
Total / *Totaal*	5/8	10/15	11/16	1/5	3/9	1/3	3/7

[1] Numerator = number of infected plants
Teller = aantal geïnfecteerde planten

Denominator = total number of plants
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control plants. This indicates that rattle virus can be transmitted to potato plants by *Trichodorus pachydermus*.

In order to investigate whether the rattle virus is capable of surviving in the nematodes for some time, experiments were carried out with *Trichodorus pachydermus* from soil infected with rattle virus. The nematodes were transferred to plastic bags filled with sandy soil which had previously been sterilized and washed with boiling water. In these bags the nematodes were kept for different lengths of time. After various periods of starvation the nematodes were transferred to plastic bags with sterilized soil, in which a small tobacco plant var. 'White Burley' was planted. After some time these plants were tested for infection with the virus. In those cases where the above-ground portions of the plants did not show any symptoms, the roots were tested for the presence of the virus in the way already described. In table 3 the results of three such experiments are shown. Even after the longest starvation period of 36 days, the nematodes proved to be still capable of transmitting the virus and it seems possible that they may also be capable of doing this after even longer starvation periods.

TRANSMISSION OF RATTLE VIRUS BY ROOT CONTACT

As already stated, nematodes are important for the transmission of rattle virus. However, under certain circumstances the virus may get from one plant to another in the absence of these organisms. This appeared from experiments in which two tobacco plants were grown together in one pot of sterilized soil. The leaves of one of the plants were inoculated with rattle virus. A plastic screen, placed between the above-ground parts, prevented infection of the non-inoculated plant via these parts.

No symptoms of rattle virus were found on the leaves of the plants which had not been inoculated. In order to ascertain whether or not infection of the plants had taken place, the customary procedure is to test the roots for the presence of the virus. However, as it was difficult to separate the roots of two plants growing in the same pot, the test was performed using the lower part of the

stem of the non-inoculated plant. The sap was expressed from these parts of the stems and was rubbed with carborundum on to the leaves of healthy tobacco plants. In this way it was possible to demonstrate the presence of rattle virus in non-inoculated plants (table 4).

TABLE 4. The transmission of rattle virus by root contact.
De overgang van ratelvirus door wortelcontact.

Plants in soil *Planten in grond*	5/8[1]	5/7	9/10	8/10	4/10
Plants on nutrient solution *Planten op voedingsoplossing*	0/5	0/10			

[1] Numerator = number of infected plants
Teller = aantal geïnfecteerde planten

Denominator = total number of plants
Noemer = totaal aantal planten

The same experiment was also carried out with tobacco plants which were not growing in soil, but in a Hoagland nutrient solution. In this case it was not possible to prove transmission of rattle virus via the roots from the inoculated to the non-inoculated plants (table 4). When sap from the roots of the inoculated plants was rubbed on the leaves of tobacco plants, it proved to contain a high amount of rattle virus. The roots of the two plants had intertwined considerably, so there seemed every opportunity for transmission by root contact. In no case, however, were the roots of the two plants observed to have grown together.

In any case it appears that transmission of the virus by root contact will not occur if the root system is not intact. This was found in the following experiment. The leaves of ten tobacco plants in flower-pots (10 cm in diameter) with sterilized soil, were inoculated with rattle virus. After one week the leaves were cut off. The roots were sampled and cut into large pieces, which were then again mixed with the soil in the pots. Healthy tobacco plants were then planted in the pots. No leaf symptoms being observed, the roots of these plants were tested for the presence of rattle virus at the end of four weeks in the way described above. None of the plants appeared to be infected with rattle virus. A repetition of the experiment gave the same result.

DISCUSSION

The greatest depth at which rattle virus may occur in the soil depends on local soil conditions. The latter may influence the presence or absence of the vector, the nematode species *Trichodorus pachydermus*. In one of the experiments with soil from various depths infection was found to occur only in the presence of the vector. No large numbers of the vector are required, for the experiments of SOL & SEINHORST (1961) proved one single nematode to be capable of transmitting the virus to a healthy tobacco plant. The presence of virus-infected, living root material is not essential, as the experiments described have clearly demonstrated the ability of the virus to survive in the nematodes for a considerable period of time.

From the foregoing data it is clear, that in controling diseases caused by rattle virus with the aid of nematicides, the possibility of the upper layers becoming reinfected with the virus from the lower layers, should be taken into account.

In the transmission of rattle virus, the presence of a vector is not always imperative. The experiments with a diseased and a healthy plant growing together in one pot of sterilized soil proved that the virus can get from the infected to the healthy plant via the roots in the absence of nematodes. In these experiments the roots of the two plants were not observed to have grown together. Whether this is essential for the virus to pass from one plant to another should be further investigated. There is also a possibility that the virus gets from the roots of the diseased plants to those of the healthy ones via small injuries if roots of the two plants grow closely past each other in the soil. The fact that healthy plants were not infected when grown in a nutrient solution instead of in soil may be significant in this respect. At any rate intact root systems are essential for this mode of virus transmission, for no infection occurred when parts of the roots of infected tobacco plants were added to the soil in which healthy tobacco plants were growing.

Summary

Rattle virus can be present in the soil at great depth. In one of the experiments it was possible to detect the virus in a soil sample taken at 80–100 cm below the surface. In the experiments with field soils, infection occurred only in the presence of the vector, the nematode species *Trichodorus pachydermus*.

After a starvation period of 36 days the nematodes were still capable of transmitting the virus to healthy tobacco plants. Thus the virus may retain its activity for a considerable length of time within the vector. The virus is also transmitted to potato plants by the nematodes.

The presence of nematodes is not always essential in the transmission of rattle virus. When an infected and a healthy tobacco plant were put in one pot containing sterilized soil, the above ground portions of the two plants being kept separate, the virus could be detected in the healthy plant after some time. It is not yet known whether this mode of transmission requires a fusion of the roots of the two plants.

Samenvatting

De grootste diepte, waarop het ratelvirus nog in de grond aanwezig kan zijn, wordt bepaald door de bodemgesteldheid ter plaatse. In één van de proeven kon het virus nog worden aangetoond in een grondmonster, afkomstig van de laag 80–100 cm beneden maaiveld (tabel 1). In een tweede proef met monsters van een geheel andere type grond was het virus tot 50 cm diepte aanwezig (tabel 2). Infectie van planten in grondmonsters van verschillende diepte trad bij deze proef alleen op indien de overbrenger, de aaltjessoort *Trichodorus pachydermus*, aanwezig was.

De aaltjes waren na een hongerperiode van 36 dagen nog zeer goed in staat het virus over te brengen naar gezonde tabaksplanten (tabel 3). Dit maakt het waarschijnlijk, dat ze hiertoe ook in staat zijn na een hongerperiode van langere duur.

De wortels van gezonde aardappelplanten var. 'Sientje' werden met ratelvirus geïnfecteerd door aaltjes, afkomstig uit grondmonsters van een perceel waar veel stengelbont bij aardappelen optrad.

Niet altijd zijn aaltjes nodig voor overdracht van ratelvirus. Wanneer een ratelviruszieke en een gezonde tabaksplant samen in een pot met gesteriliseerde grond werden geplaatst waarbij de bovengrondse delen van elkaar gescheiden werden gehouden, kon het virus enige tijd later in de gezonde plant worden aangetoond (tabel 4). Daarentegen trad geen infectie van een gezonde tabaksplant op als deze tezamen met een ratelzieke plant op watercultuur werd gekweekt (tabel 4). De mogelijkheid bestaat dus, dat het virus overgaat van een wortel van een zieke plant naar die van een gezonde als deze in de grond contact met elkaar maken. Of hiervoor vergroeiingen van wortels van beide planten nodig zijn is niet bekend.

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