

TRICHODORUS PACHYDERMUS AND *T. TERES*,
VECTORS OF THE EARLY BROWNING VIRUS OF PEAS¹

Met een samenvatting:

Trichodorus pachydermus en *T. teres*, vectoren van het vroege-verbruiningsvirus
bij de erwten

BY

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INTRODUCTION

The virus causing early browning of peas (called hereafter EBV) is a typical soil-borne virus. It occurs in the coastal provinces of The Netherlands, exclusively on very light sandy soil (VAN DER WANT & BOS, 1959). This suggests that the distribution of the vector is limited to these soils.

Morphologically and biologically the virus has some similarities to rattle virus (BOS & VAN DER WANT, 1962) which is transmitted by the nematode *Trichodorus pachydermus* Seinhorst (SOL & SEINHORST, 1961). The occurrence of *Trichodorus* species in EBV-infested soils and their ability to transmit this virus were therefore investigated.

MATERIALS AND METHODS

Soil for most of the pot experiments reported below was collected on 27 March 1962 at Middenmeer (Wieringermeer), in a field infected with EBV. By means of a 10 cm wide thin-walled metal tube, cores 20 cm deep (2 kg of soil) were taken from different depths. In the literature (VAN DER WANT, 1958) it has been suggested that failures in earlier transmission experiments were due to the soil having been disturbed when collected in the field. Therefore some of the cores were placed intact in separate polythene bags for transport. Samples were also taken at Rilland-Bath (Zeeland), in the Haarlemmermeerpolder and at Wageningen.

In all experiments peas were used to demonstrate the presence of EBV in the soil. As pea plants showing no leaf symptoms may still contain virus, cotyledons of cucumber seedlings were inoculated with sap from the roots and stems of the pea plants to be investigated. Presence of virus in the pea sap resulted in local lesions on the cucumber cotyledons. Nematodes were collected from the soils by elutriation and sieving (SEINHORST, 1956).

All transmission experiments were done with a light sandy soil which had been partially sterilized about six months before use. The soil was placed in 300 ml plastic beakers and planted with three pea seedlings per beaker. Nematodes were then added by transferring them in drops of water to small holes near the seedlings.

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EXPERIMENTS

Experiment with soil from different depths at different temperatures

Six or seven cores of undisturbed soil from each of the depths 0–20 cm, 20–40 cm and 40–60 cm, taken as described above from a field infected with EBV, were placed under each of the following conditions: at 20°C (in a glasshouse), in a constant temperature room at 15°C and outdoors. Each of the cores was planted with three pea seedlings. The development of the plants was best in the glasshouse at 20°C and poorest in the constant temperature room. The plants placed outdoors developed slowly because of the low temperature.

The first disease symptoms appeared three weeks after planting. The roots and stems of the peas were then tested by inoculating cucumber seedlings with the expressed sap. The occurrence of EBV in stems and roots in the different treatments is given in table 1.

TABLE 1. Occurrence of EBV in stems and roots of pea plants three weeks after planting in soil from different depths in the field and kept at different temperatures during the experiment.

Aanwezigheid van het VV-virus in wortels en stengels van erwten, geplant in grond van verschillende diepte en opgekweekt bij verschillende temperaturen.

Depth of sampling <i>Diepte van het monster</i>	Treatment/Behandeling							
	Glasshouse 20°C <i>Kas 20°C</i>		Constant temperature room 15°C <i>Klimaatkas 15°C</i>		Outdoors <i>Buiten</i>		Totals <i>Totaal</i>	
	Roots <i>Wortels</i>	Stems <i>Stengels</i>	Roots <i>Wortels</i>	Stems <i>Stengels</i>	Roots <i>Wortels</i>	Stems <i>Stengels</i>	Roots <i>Wortels</i>	Stems <i>Stengels</i>
0–20 cm	2 (7) ¹	0 (7)	1 (6)	0 (6)	2 (7)	1 (7)	5 (20)	1 (20)
20–40 cm	4 (7)	3 (7)	2 (6)	1 (6)	5 (7)	2 (7)	11 (20)	6 (20)
40–60 cm	7 (7)	5 (7)	5 (6)	4 (6)	6 (7)	5 (7)	18 (20)	14 (20)
Totals/ <i>Totaal</i>	13 (21)	8 (21)	8 (18)	5 (18)	13 (21)	8 (21)	34 (60)	21 (60)

¹ The figures indicate that in 2 out of a total of 7 cores one or more plants infected with EBV were found.

¹ *De getallen geven aan, dat bij 2 van de 7 grondmonsters een of meer erwteplanten met het VV-virus waren besmet.*

Infection of the roots (34 out of 60) was much more frequent than infection of the stems (21 out of 60). A number of pea plants which did not show leaf symptoms were found nevertheless to contain virus in the stems. Leaf symptoms were most pronounced at 20°C.

Infectivity of the soil apparently increased markedly with depth. Only a few plants grown in soil from the 0–20 cm layer were infected, whereas diseased plants were found on almost all cores from the 40–60 cm layer.

Occurrence of Trichodorus teres in samples from different depths

In order to investigate the presence of a possible virus vector, nematodes were collected from 500 g of each of the cores kept outdoors in the experiment described above. In at least some of the cores from each depth the nematode species *Trichodorus teres* Hooper was found, but no other *Trichodorus* species

were found. Table 2 gives the population density of *T. teres* in these cores and the degree of infection with EBV of the pea plants which were growing on them in the experiment described above (see also table 1).

TABLE 2. Relation between the presence of *Trichodorus teres* in soil from different depths and infection of peas with EBV.

Verband tussen de aanwezigheid van Trichodorus teres in grondlagen van verschillende diepte en de besmetting van de erwteplanten met het VV-virus.

0-20 cm			20-40 cm			40-60 cm		
Number of <i>T. teres</i> per 500 g of soil <i>Aantal</i> <i>T. teres</i> per 500 g grond	Virus in:		Number of <i>T. teres</i> per 500 g of soil <i>Aantal</i> <i>T. teres</i> per 500 g grond	Virus in:		Number of <i>T. teres</i> per 500 g of soil <i>Aantal</i> <i>T. teres</i> per 500 g grond	Virus in:	
	Roots <i>Wortels</i>	Stems <i>Stengels</i>		Roots <i>Wortels</i>	Stems <i>Stengels</i>		Roots <i>Wortels</i>	Stems <i>Stengels</i>
2	¹ +	+	23	+++	—	34	++	—
1	+	—	10	+++	+++	18	+++	+
0	—	—	4	—	—	15	++	+
0	—	—	0	+++	—	9	+++	+++
0	—	—	0	+++	—	5	+++	+
0	—	—	0	+	+	1	++	+
0	—	—	0	—	—	0	—	—

¹ +++, ++, +, — represent, respectively, many, a moderate number, some, and no local lesions on the cotyledons of cucumber.

¹ +++, ++, +, — geven respectievelijk veel, vrij veel, enkele en geen lokale vlekken op de zaadlobben van komkommerplanten weer.

It will be seen that the peas had been infected in ten out of eleven pots in which *T. teres* was still present after the experiment, but only in three out of ten pots in which *T. teres* was not found. As only a fourth of the soil used in the pot experiment was examined, small populations of this nematode may not have been detected. Moreover the presence of these nematodes was determined after the experiment and *Trichodorus* may have died out after having transmitted virus. The correlation between presence of *Trichodorus* and infection of the pea plant with EBV is therefore strong.

Transmission experiments with T. teres

The nematodes collected from the soil samples to determine the population density of *T. teres* in the experiment described above were used to inoculate nematode-free soil. A number of pots were inoculated with *T. teres*, others with the suspensions (containing other nematodes) remaining after *T. teres* had been taken out. Peas grown in 11 pots which were not inoculated with nematodes remained healthy.

It is clear from table 3 that *T. teres* is a vector of EBV. That the suspensions remaining after *T. teres* had been taken out were still infective may be due to the fact that they were not carefully enough searched for the last *Trichodorus* specimen. The nematodes from the 40-60 cm layer seemed to be less efficient in transmitting the virus than those from the 0-40 cm layers. To investigate this, each of ten pots containing nematode-free soil and three pea seedlings per pot were inoculated with five *T. teres* specimens from the 20-40 cm deep soil layer

TABLE 3. Ability of *T. teres* from different depths in the soil to transmit EBV to peas.
Het aantal exemplaren van T. teres in de grondlagen en hun vermogen tot overdracht van VV-virus naar erwten.

Depth of collection of nematodes <i>Diepte van het monster</i>	Species placed in the pots <i>In de grond gebrachte nematoden</i>	Number per pot <i>Aantal per pot</i>	Presence of virus in pea <i>Voorkomen van virus in erwten</i>	
			Roots <i>Wortels</i>	Stems <i>Stengels</i>
0-20 cm	<i>T. teres</i>	1 (1) ¹	++ ²	—
	Remaining nematodes <i>Restant nematoden</i>	(1)	—	—
	<i>T. teres</i>	1 (1)	+++	+++
	Remaining nematodes <i>Restant nematoden</i>	(1)	+	—
20-40 cm	<i>T. teres</i>	12 (1)	+++	+++
	Remaining nematodes <i>Restant nematoden</i>	(1)	+++	+++
	<i>T. teres</i>	4 (1)	—	—
	Remaining nematodes <i>Restant nematoden</i>	(1)	+++	+++
40-60 cm	<i>T. teres</i>	20 (1)	—	—
		20 (1)	—	—
	Remaining nematodes <i>Restant nematoden</i>	(1)	—	—
	<i>T. teres</i>	16 (1)	++	—
	Remaining nematodes <i>Restant nematoden</i>	(1)	++	—

¹ Between brackets: number of pots inoculated.

Tussen haakjes het aantal geïnoculeerde potten.

² For meaning of symbol +++ etc. see footnote to table 2.

Voor de betekenis van de tekens zie noot bij tabel 2.

and another 24 pots each with five *T. teres* specimens from the 40-60 cm layer. (By the time this experiment was set up *Trichodorus* was no longer present in the available soil from the 0-20 cm deep layer.) In three out of the ten pots with nematodes from the 20-40 cm deep layer the pea plants were found to be infected with EBV 30 days after the inoculation and also in six of the 24 pots with nematodes from the 40-60 cm deep layer. The experiment thus revealed no difference in infectivity of the nematodes from the different layers. However, the number of pots with diseased plants were too small to disclose anything but very large differences.

T. teres in fields infested with EBV

In almost all soil samples from fields known to be infested with the EBV, *T. teres* was found. In one field the disease occurred where the soil was sandy but not where it was more loamy. In a 500 g sample from the sandy part of this field eleven specimens of *T. teres* and one male of *T. pachydermus* were found. A sample from the loamy part of the field contained only one juvenile *Trichodorus* specimen. Only one exception to this was noted. An experimental field near Wageningen which had recently become infected with EBV through the use of infected seed did not contain *T. teres*, but two other species: *T. pachydermus* Seinhorst and *T. similis* Seinhorst.

Transmission experiments with T. pachydermus and T. similis

Females and juveniles of *T. pachydermus* and of *T. similis* cannot be distinguished from each other under the dissecting microscope but males of the two species can be distinguished easily at 50 × magnification. An inoculation was therefore made using males of each of these species, collected from soil from the field infected with EBV at Wageningen. Eight pots with three pea plants each were inoculated with about 15 males of *T. pachydermus* per pot and 12 pots with about 16 males of *T. similis*. In six of the eight pots with *T. pachydermus* peas became infected with EBV but in none of the 12 pots with *T. similis*. *T. pachydermus* is therefore a vector of EBV whereas *T. similis* most probably is not.

CLOSING REMARKS

It is evident that EBV can be transmitted by two *Trichodorus* species. This accords with the hypothesis of HARRISON et al. (1961) that rod-shaped soil-borne viruses are transmitted by species of the nematode genus *Trichodorus*, whereas globular viruses are transmitted by *Xiphinema* and *Longidorus* species. The hypothesis of these authors that a certain virus strain would be transmitted only by one nematode species appears not to be true. The EBV can be transmitted by at least two *Trichodorus* species. Furthermore *T. pachydermus* can transmit at least two serologically unrelated viruses namely EBV and rattle virus (SOL et al., 1961). *Longidorus elongatus*, the vector of Tomato Black Ring Virus (HARRISON et al., 1961) also transmits Raspberry Ringspot Virus (TAYLOR, 1962) which is serologically unrelated to the former.

Trichodorus teres is a rather rare species of nematode, which is perhaps the reason why the EBV of peas is not generally spread in pea growing areas. *T. pachydermus* is a common species on sandy soils. Although so far very little early browning of peas has been found on soils where the latter species occurs commonly it could become an important vector of the EBV once these soils become infected.

SUMMARY

The early browning virus (EBV) of peas is transmitted by *Trichodorus teres* Hooper and *T. pachydermus* Seinhorst. *T. teres* is a rather rare nematode species, until now found only in marine sandy soils. *T. pachydermus* is common in many sandy soils. In most fields infected with EBV only *T. teres* was found.

In an infected experimental field in Wageningen, however, *T. teres* did not occur but *T. pachydermus* and *T. similis* Seinhorst did. In an experiment the latter species did not transmit EBV.

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SAMENVATTING

Het virus dat vroege verbruining bij erwten veroorzaakt (in de tabellen als

VV-virus aangeduid), is een typisch grondvirus. Het komt in de kustprovincies van Nederland voor en wel uitsluitend op zeer lichte gronden. Dit doet de veronderstelling rijzen, dat bepaalde – in het bijzonder op zeer lichte gronden voorkomende – nematoden dit virus kunnen overbrengen. Om dit te onderzoeken werden op 27 maart 1962 grondmonsters genomen van een besmet veld in de Wieringermeer en wel op diepten van 0 tot 60 cm. De hierop gezaaide erwten werden niet alle even sterk met het virus besmet (tabel 1).

Opvallend is, dat de toetsplanten, gekweekt in de grondlaag van 0–20 cm, vrijwel niet besmet werden. In de laag van 20–40 cm, doch vooral in die van 40–60 cm, kwam een hoog percentage besmette planten voor. Verschil in temperatuur tijdens de groei van de toetsplanten bleek niet van invloed te zijn op de besmetting. De verklaring voor de verschillen in besmetting in de diverse bodemlagen geven de tabellen 2 en 3. Deze tonen aan, dat de nematode *Trichodorus teres* Hooper in de bovenste lagen vrijwel niet, in de diepere lagen in toenemende mate voorkomt. Er werd speciaal gelet op de aanwezigheid van nematoden van het geslacht *Trichodorus*, omdat HARRISON et al. (1961) veronderstellen, dat staafvormige virussen door vertegenwoordigers van dit geslacht worden overgebracht. De resultaten in tabel 2 bevestigen deze veronderstelling. Reeds een enkel aaltje is nl. in staat tot virusoverdracht. De correlatie tussen de besmetting en het voorkomen van *T. teres* volgt verder uit tabel 3.

Op een besmet praktijkperceel bleek de grond op de niet besmette delen veel zwaarder te zijn en ook zeer veel minder nematoden te bevatten. In de drie onderzochte proefvelden in de kustprovincies werd *T. teres* steeds gevonden waar vroege verbruining optrad.

Op een proefveld te Wageningen, waar de grond ook besmet was, kwamen zowel *T. pachydermus* Seinhorst als *T. similis* Seinhorst voor. Van deze beide bleek echter alleen *T. pachydermus* het virus te kunnen overbrengen.

De veronderstelling van HARRISON et al., dat verschillende stammen van een bepaald virus door verschillende soorten van hetzelfde nematodengeslacht worden overgebracht, blijkt derhalve niet juist te zijn, want eenzelfde virus wordt hier zowel door *Trichodorus teres* als door *T. pachydermus* overgebracht.

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