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Tobacco rattle virus serotypes and associated nematode vector species of Trichodoridae in the bulb-growing areas in the Netherlands

A.T. PLOEG[1], C.J. ASJES[2] and D.J.F. BROWN[1]

[1] Department of Zoology, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK
[2] Bulb Research Centre, Vennestraat 22, Postbus 85, 2160 AB Lisse, the Netherlands

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Abstract

Soil samples from the coastal bulb-growing areas in the provinces of North- and South-Holland and the North-East Polder in the Netherlands were examined for trichodorid nematodes and tobacco rattle virus (TRV) serotypes. At least one of a total of eight species of Trichodoridae, of which *Paratrichodorus pachydermus* was most prevalent, was found in 93% of the samples from the provinces of North- and South-Holland and TRV, including four serotypes, was obtained from 49% of these samples. In the North-East Polder one of three species of trichodorids, of which *P. teres* occurred most frequently, was present in 72% of the samples, and TRV of one serotype was obtained from 28% of these samples. The TRV isolates recovered from these samples reacted serologically with one of four antisera to strains of TRV. Virus transmitted by *P. pachydermus* reacted to the PRN-, by *Trichodorus viruliferus* to the RQ-, by *P. teres* to the N5- and by *T. similis*, to the TS-antiserum, respectively.

Additional keywords: Trichodorus, *Paratrichodorus*, transmission, specificity.

Introduction

Tobacco rattle virus (TRV) transmitted by *Trichodorus* and *Paratrichodorus* nematode species (trichodorids) can cause problems in bulbous ornamental crops, e.g. tulip, gladiolus, hyacinth and narcissus (Asjes, 1974; 1989). Problems are most severe on sandy or light loamy soils, which are the preferred habitats of the trichodorid nematodes. TRV isolates which differ in serological, symptomatological or morphological characteristics are referred to as TRV strains. Within TRV a lot of strains occur (Robinson and Harrison, 1989). Isolates which are similar in their reaction with an antiserum, belong to the same serotype. Several TRV serotypes have been identified. Until recently, studies on the association between TRV strains and trichodorid species were hampered by a lack of appropriate methodology. Recently, Brown et al. (1989a) described a method for bait testing individual trichodorid nematodes for virus transmission. By the use of this method, Brown et al. (1989b) and Ploeg et al. (1989) indicated that TRV isolates belonging to distinct TRV serotypes were transmitted by different species of *Trichodorus* and *Paratrichodorus*. In this paper results will be reported on the occurrence of *Paratrichodorus* and *Trichodorus* species and associated TRV serotypes in bulb-growing areas in the Netherlands.

Material and methods

Soil sampling and nematode extraction. Forty-seven point samples of about 5 kg soil were collected with a spade to a depth of approximately 35 cm from bulb fields with outbreaks of TRV in the past, field verges and roadsides in the vicinity of bulb fields in May 1989 and 1990. Field verges and roadsides were sampled to increase the chance to find trichodorid nematodes as agricultural practices, e.g. soil fumigation and cultivation may have strongly decreased trichodorid populations in the fields. The geographical location of the sample points is shown in Fig. 1.

Samples were collected in plastic bags and transported by car and ferry to Scotland. During the travel (24 hours) samples were not cooled but immediately upon arrival they were stored at 3 °C. Within three weeks after collection a sub-sample was taken by carefully mixing the soil by hand and, after the removal of stones and plant debris, a plastic container was filled with 250 g soil using a tablespoon. Nematodes were extracted from the sub-samples by the modified decanting and sieving technique (Brown and Boag, 1988). After 16 h extraction on a Bearmann funnel the number of trichodorid nematodes was counted at 50 times magnification. Up to 35 handpicked adult trichodorid nematodes per sample were killed, fixed and processed to anhydrous

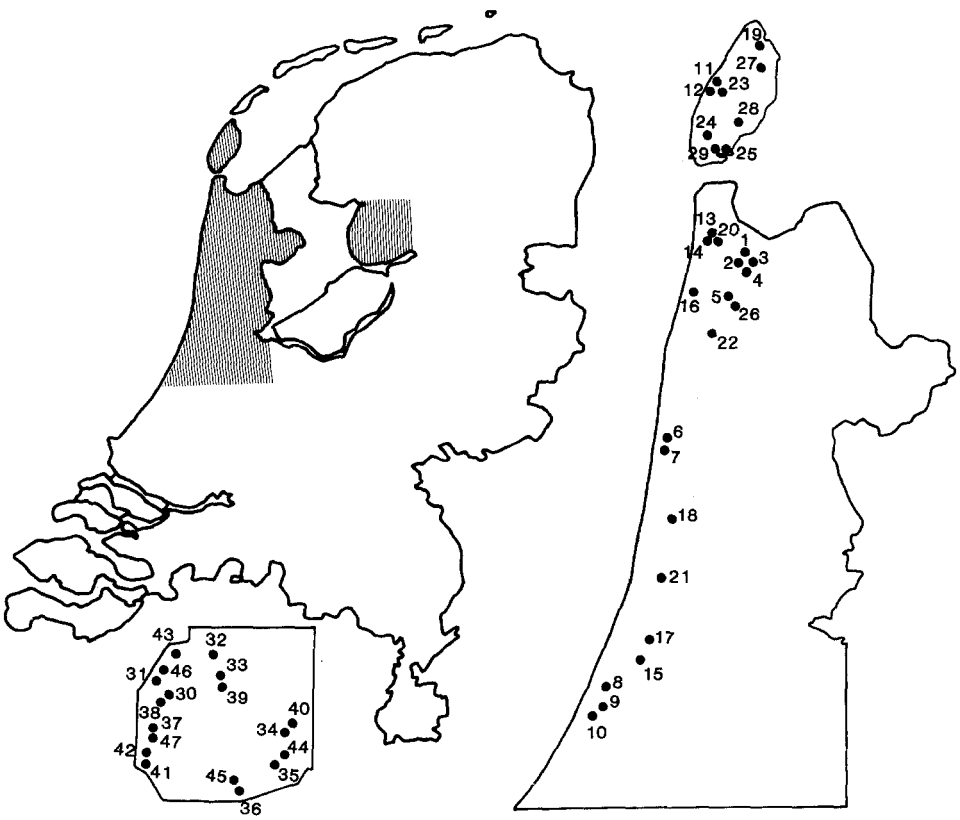

Fig. 1. Location of soil samples taken in the bulb growing areas in the Netherlands. Numbers refer to Table 1 and Table 2.

glycerol (s'Jacob and Van Bezooijen, 1984). The species were identified at 650 times magnification.

Virus bait-testing. - Pot bait-test. Soil of each sample, carefully mixed, was used to fill a 400 ml, 10 cm diameter plastic pot in which two, 3 week-old *Petunia hybrida* Vilm. and two 3 week-old *Nicotiana tabacum* cv. White Burley seedlings were planted. The pots were placed in a temperature-controlled box (Taylor and Brown, 1974) in which a soil temperature of 17 °C was maintained. Tops were grown in daylight glasshouse conditions. After 4 weeks the root system of each plant was washed free of soil, while the tops of the bait-plants were observed for virus symptoms. Virus infection of the bait-plant roots was tested by separate inoculation of the commuted root systems of the two *N. tabacum* cv. White Burley and the two *P. hybrida* bait-plants onto carborundum-dusted leaves of *Chenopodium amaranticolor* Coste and Reyn indicator plants. Where local lesions appeared on the leaves, usually within 3-4 days, sap of these leaves was manually inoculated onto a *C. quinoa* from which the virus was subsequently inoculated onto a *N. clevelandii* plant. The *C. quinoa* plant was used as an intermediate host to avoid problems caused by the presence of virus inhibitors in *C. amaranticolor* sap (Robinson, 1973). The *N. clevelandii* plants were used in ELISA tests. If 14 days after inoculation no local lesions had developed on the leaves of the *C. amaranticolor* plants, the inoculum was considered not to have contained TRV.

Individual nematode-bait-test. The transmission of PRN-serotype TRV isolates by *P. pachydermus* has been demonstrated by Brown et al. (1989b). To establish new associations between vector species and TRV-serotypes, samples were selected for individual nematode-bait-tests which contained viruliferous trichodorid populations, with species other than *P. pachydermus* present, and/or which transmitted non-PRN-serotype TRV isolates (see Table 1 and Table 2). Individual trichodorid nematodes of sample nrs. 5, 14, 19 and 21 were bait tested for virus transmission by allowing the nematodes to feed for 10 days on a 2 week-old *P. hybrida* seedling. Nematodes were subsequently identified and all virus isolates obtained from the *P. hybrida* roots were serologically tested (Brown et al., 1989a).

Serology. Virus isolates from the *P. hybrida* pot bait test plants and the isolates obtained in the individual trichodorid-transmission system were tested in $F(ab')_2$-ELISA (Barbara and Clark, 1982). Plates were coated with the $F(ab')_2$ fraction of antisera prepared against TRV strains PRN, RQ, N5 and TS, of which concentrations of 1, 1, 1, and 2 μg/ml, respectively were used. γ-Globulin concentrations used were 1 μg/ml for the PRN-, RQ- and N5-antiserum and 2 μg/ml for the TS-antiserum. Protein A-alkaline phosphatase conjugate was used at 1:2000 and the enzyme substrate was p-nitrophenylphosphate. The PRN strain was isolated from potato cv. Kerr's Pink in Scotland (Cadman and Harrison, 1959), the RQ strain from a cucumber bait-plant grown in field soil from Scotland (Robinson and Harrison, 1985) and the N5 strain from narcissus cv. Golden Harvest in Scotland (Harrison et al., 1983). The latter strain is considered to be an atypical TRV strain since it is serologically closely related to the Dutch strain of pea early-browning virus (PEBV-D), but has the RNA1 sequence of TRV (Robinson et al., 1987). The TS strain, transmitted by *T. similis*, was obtained in this study from a *P. hybrida* bait-plant.

Results

The data on the occurrence of trichodorid nematodes and TRV serotypes in the bulb-growing regions of the provinces North- and South-Holland are shown in Table 1. Trichodorid nematodes were present in 27 of the 29 samples taken from the provinces of North- and South-Holland. *P pachydermus* was the most common species occurring in 22 of these samples. *T. similis*, *T. viruliferus*, *P. nanus* and *T. cylindricus* were

Table 1. Occurrence of trichodorid nematodes and tobacco rattle virus serotypes in soil samples from the coastal bulb-growing area of the provinces of North- and South-Holland.

Sample	Location	Crop	Number of trichodorid nematodes /250 g soil	Species*	TRV serotype**
1	Breezand	grass	30	Pp	–
2	Breezand	grass	0	–	–
3	Breezand	grass	74	Pp Ts	TS
4	Breezand	grass	11	Pp	–
5	't Zand	grass	200	Ts	TS
6	Egmond	grass	48	Pp Tc	–
7	Egmond	grass	201	Pp Tc Ts	TS
8	Noordwijkerhout	grass	0	–	–
9	Noordwijk	grass	27	Pp	–
10	Noordwijk	grass	57	Pa	–
11	De Koog	grass	147	Pp Tv	PRN
12	De Koog	grass	185	Pn Pp Tv	–
13	Julianadorp	grass	150	Pp Tv	PRN
14	Julianadorp	grass	81	Pt Ts	N5
15	De Zilk	grass	13	Pp	–
16	Callantsoog	grass	57	Pp Tc	–
17	Vogelenzang	grass	45	Pp Ts	–
18	Heemskerk	grass	7	Pn Pp	–
19	Cocksdorp	grass	51	Pn Pp Tv	PRN
20	Julianadorp	tulip	21	Pp	PRN
21	Santpoort	tulip	246	Pp Tsp	PRN
22	Schagerbrug	tulip	4	juv.	–
23	De Koog	narcissus	25	Pn Pp	PRN
24	Den Hoorn	narcissus	190	Pp Ts	PRN
25	Horntje	narcissus	44	Pp Ts	PRN
26	'tZand	crocus	12	Ts	–
27	Eierland	crocus	11	Pn Pp Tv	PRN
28	Den Burg	freesia	40	Pp	PRN
29	Horntje	wheat	35	Pp	–

* Pa: *P. anemones*, Pn: *P. nanus*, Pp: *P. pachydermus*, Pt: *P. teres*, Tc: *T. cylindricus*, Ts: *T. similis*, Tsp: *T. sparsus*, Tv: *T. viruliferus*.

** TRV isolates were tested in $F(ab')_2$-Elisa with antisera against TRV strains PRN, RQ, N5 and TS. – = no virus detected.

found in 8, 5, 5 and 3 samples, respectively. *P. anemones*, *T. sparsus* and *P. teres* were each found in one sample only. TRV was detected in 14 samples, all of which also contained trichodorid nematodes. So, 52% of the trichodorid populations were viruliferous. The virus isolates were classified as belonging to TRV serotypes PRN, RQ, N5 or TS.

The data on the occurrence of trichodorid nematodes and TRV serotypes in the bulb-growing areas of the North-East Polder are shown in Table 2. Trichodorid nematodes were found in 13 of 18 samples collected from the North-East Polder. *P. teres* was the most prevalent species occurring in 11 samples. *T. primitivus* and *T. similis* were each found in only one sample. From 5 samples with trichodorid nematodes, TRV, belonging to the N5 serotype, was detected. So, 39% of the trichodorid populations were viruliferous.

Population densities of trichodorids were significantly larger in North- and South-Holland than in the North-East Polder (t-test, $P \leq 0.05$), and more than one species was detected in 16 out of the 27 samples from the coastal provinces of Holland whereas all samples from the polder contained single species populations.

Where virus recovered from the *P. hybrida* bait plants reacted to PRN- antiserum in $F(ab')_2$-ELISA, *P. pachydermus* was present in the corresponding soil sample. *P.*

Table 2. Occurrence of trichodorid nematodes and tobacco rattle virus serotypes in soil samples from the bulb-growing area of the North-East Polder.

Sample	Location	Crop	Number of trichodorid nematodes /250 g soil	Species* serotype**	TRV
30	Creil	grass	41	Pt	N5
31	Rutten	grass	84	Pt	N5
32	Bant	grass	4	Pt	–
33	Bant	grass	1	Pt	–
34	Blokzijl	grass	0	–	–
35	Kraggenburg	grass	5	Ts	–
36	Ens	grass	19	Pt	N5
37	Espel	grass	3	Pt	–
38	Creil	fallow	66	Pt	N5
39	Bant	potato	0	–	–
40	Blokzijl	potato	0	–	–
41	Tollebeek	potato	3	Pt	–
42	Tollebeek	potato	0	–	–
43	Rutten	tulip	0	–	–
44	Kraggenburg	tulip	134	Tp	–
45	Ens	strawberry	5	Pt	N5
46	Rutten	carrot	4	Pt	–
47	Espel	eremurus	1	Pt	–

* Pt: *P. teres*, Tp: *T. primitivus*, Ts: *T. similis*.

** TRV isolates were tested in $F(ab')_2$-Elisa with antisera against TRV strains PRN, RQ, N5 and TS. – = no virus detected.

Table 3. Transmission of TRV by individual trichodorid nematodes in bait tests to *P. hybrida* and serotypes detected.

		Transmission (%)		Serotype
Sample nr. 5:	*T. similis*	10/65*	(15)	TS
Sample nr. 14:	*P. teres*	14/49	(29)	N5
	T. similis	0/3	(0)	–
Sample nr. 19:	*P. pachydermus*	8/14	(57)	PRN
	T. viruliferus	6/26	(23)	RQ
	P. nanus	0 /39	(0)	–
Sample nr. 21:	*P. pachydermus*	5/9	(56)	PRN
	T. sparsus	0/32	(0)	–

* Numerator is the number of nematodes transmitting, denominator is the number tested.

teres was found when the reaction was to N5-antiserum, and a reaction to TS-antiserum coincided with the presence of *T. similis*.

Results from bait-tests with individual trichodorid nematodes transmitting TRV to *P. hybrida* are shown in Table 3. The association between TRV serotypes PRN, N5 and TS and the vector species *P. pachydermus*, *P. teres* and *T. similis* was confirmed by bait tests using individual trichodorid nematodes from samples 5, 14, 19, and 21. It was also shown that *T. viruliferus* was a vector of TRV isolates belonging to TRV serotype RQ.

Pinpoint local lesions typical of TRV infection, developed in two days after manual inoculation of four isolates transmitted by *P. teres* from sample nr. 14 onto leaves of *Phaseolus vulgaris*. This indicated that these isolates should be classified as TRV rather than PEBV (Robinson and Harrison, 1989).

Systemic symptoms characteristic for TRV were observed in the leaves of *N. tabacum* cv. White Burley bait plants which had been grown in soil from samples nrs. 11, 13 and 23 but none of the *P. hybrida* bait plants showed symptoms in the aerial parts.

Discussion

All nine species found in this study are known vectors of TRV (Brown et al., 1989a; Van Hoof, 1968; 1970). *P. pachydermus* and *T. similis* were widespread in North- and South-Holland and *P. teres* was the predominant species in the light marine sandy soils of the North-East Polder. This corresponds with the trichodorid distribution maps by Seinhorst and Van Hoof (1982). Trichodorids and TRV were widespread in the coastal bulb areas of the Netherlands, which confirms results by Van Hoof (1973a), who found trichodorids in 77% and TRV in 30% of samples from these areas. The greater species diversity of trichodorid populations in the coastal provinces of the Netherlands as compared to that in the North-East Polder was reflected in the serological variation of TRV isolates. Four TRV serotypes were present in the former and one sero-

type in the latter area. The close association between trichodorid species and TRV serotypes supports previous observations by Harrison (1966), Brown et al. (1989b) and Ploeg et al. (1989). Our results are like those of Brown et al. (1989b), who reported *P. pachydermus* from Scotland, the Netherlands and Sweden as a vector of PRN serotype TRV isolates. However, Brown et al. (1989b) reported *T. primitivus* from England as a vector for TRV isolates belonging to the RQ serotype, which in our study were transmitted by *T. viruliferus*. This indicates that at least two trichodorid species can transmit serologically closely related isolates of TRV. Also, Brown et al. (1989b) and Jensen et al. (1974) reported transmission by *P. teres* from the Netherlands and the USA of TRV isolates belonging to the ORE serotype. The ORE strain of TRV was isolated from potato in Oregon, USA (Lister and Bracker, 1969). In our study, however, *P. teres* transmitted TRV isolates reacting with the N5-antiserum. Since the N5 strain of TRV and the ORE strain of TRV are serologically distinct (Harrison et al., 1983), this indicates that one vector species may transmit at least two serologically distinct isolates. This possibility is sustained by Van Hoof (1962; 1964) who reported transmission of TRV and of the Dutch strain of pea early-browning virus (PEBV-D) by *P. teres*. Since the N5 strain of TRV is similar in serology to PEBV-D (Robinson et al., 1987), our results and those of Brown et al. (1989b), Jensen et al. (1974) and Van Hoof (1962; 1964) suggest that *P. teres* can transmit TRV isolates serologically closely related to the ORE strain of TRV and to the Dutch strain of PEBV.

The isolates naturally transmitted by *T. similis* were serologically distinct from TRV strains RQ, N5 and PRN. In further tests, particles of an isolate transmitted by a single male *T. similis* from sample nr. 5, were decorated with antiserum JG6, prepared against a TRV isolate transmitted by *T. similis* causing notched leaf in Gladiolus (Cremer and Schenk, 1967). This indicated that the two isolates transmitted by *T. similis* were serologically closely related. In our study, *T. sparsus*, *T. primitivus*, *P. anemones* and *P. nanus* did not transmit virus, although Van Hoof (1973b) reported *P. nanus* to be an efficient vector of TRV in Dutch bulb fields.

Dale and Solomon (1988) suggested that systemic TRV symptoms in *N. tabacum* cv. White Burley bait plants provided a reliable method for the detection of viruliferous trichodorids in field soil. In our study, however, systemic symptoms were observed in only 3 of 18 *N. tabacum* cv. White Burley bait plants from which TRV, of PRN serotype, was obtained from the roots. Our results correspond with those by Sol (1960; 1962), who also demonstrated TRV in the roots of symptomless *N. tabacum* cv. White Burley bait-plants, indicating that relying on TRV symptoms in the aerial parts of *N. tabacum* cv. White Burley bait-plants is not recommendable for assessing the presence of nematode transmitted TRV in field soils.

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