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Acquisition and subsequent transmission of tobacco rattle virus isolates by Paratrichodorus and Trichodorus nematode species

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Abstract

Isolates of the PRN serotype of tobacco rattle virus (TRV) were transmitted with different efficiencies by the nematode vector *Paratrichodorus pachydermus*. Virus isolates which belonged to other serotypes were not acquired and/or transmitted by this vector, nor were PRN serotype isolates which had been obtained from naturally infected potato plants and maintained by mechanical transmission in the glasshouse for several years. PRN serotype TRV isolates from the Netherlands or from Scotland were equally well transmitted by initially virus-free *P. pachydermus* populations from either country. Allowing a naturally viruliferous nematode population access for 3 weeks to uninfected or TRV-infected roots resulted in an increased proportion of the trichodorid population transmitting TRV.

Additional keywords: Paratrichodorus pachydermus vector TRV serotype PRN.

Introduction

Tobacco rattle virus (TRV), a tobravirus, is transmitted by species of *Trichodorus* and *Paratrichodorus* nematodes (trichodorids). Harrison (1966, 1967) and Van Hoof (1968) suggested that there are specific associations between trichodorid vector species and TRV isolates. Van Hoof (1968) reported that initially virus-free *P. pachydermus* successfully acquired and transmitted only one TRV isolate from several serologically similar isolates tested, and that this isolate, in contrast to the others, originated from the same locality as the nematodes. He concluded that 'TRV will not readily be spread by infected plant material, since there is only a small chance that the TRV strain will suit the *Trichodorus* population in the new habitat'. Harrison (1966) found that virus-free *T. primitivus* acquired and transmitted a British isolate of another tobravirus, pea early-browning virus (PEBV), obtained from the same area as the nematodes, but not a serologically distinct Dutch PEBV isolate. Harrison (1967) also reported that two serologically related British isolates of PEBV were transmitted by *P. anemones*, but that a Dutch PEBV isolate was not.

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More recently, Brown et al. (1989c) and Ploeg et al. (1991, 1992) used bait tests in which trichodorid nematodes were individually tested for their ability to transmit virus to demonstrate that trichodorid vector species are naturally associated with particular serotypes of TRV. In their tests, however, they used naturally viruliferous trichodorid populations, and their results are therefore merely observations that particular species naturally transmit TRV isolates belonging to a particular serotype. It remained unproven whether true specificity occurs, i.e. whether a particular vector species is capable of transmitting only those isolates that belong to the serotype(s) with which this species is naturally associated.

In the experiments reported here, we sought to determine whether initially virus-free trichodorids given access to plants infected with various TRV isolates were subsequently able to transmit these isolates. Also, the effect of allowing naturally viruliferous trichodorids access to TRV-infected or uninfected plant roots on the proportion of the nematodes transmitting virus was studied.

Materials and methods

Nematode populations

Nematodes used in acquisition and transmission tests were extracted according to a modified decanting and sieving technique (Brown and Boag, 1988) from field soil. Samples were collected from Barry and Woodhill, Scotland, and from Wageningen, the Netherlands. Fifty adult trichodorids from each sample were picked from the suspension, heat-killed over a flame from a spirit burner and identified to species level using a high power Zeiss microscope at 650× magnification. The numbers of each species observed were: Barry, 39 *P. pachydermus*, 11 *T. cylindricus*; Woodhill, 32 *P. pachydermus*, 14 *T. primitivus*, 4 *T. cylindricus*; Wageningen, 50 *P. pachydermus*. Nematodes from Woodhill and Wageningen populations had been intensively tested for virus transmission (Ploeg et al., 1991) and were found not to be naturally viruliferous (results not shown).

TRV isolates and strains

TRV isolates and strains used in transmission tests are shown in Table 1. For simplicity, all are subsequently referred to as isolates. Serology of isolates was tested by $F(ab')_2$-ELISA as described by Ploeg et al. (1992).

Acquisition and transmission by groups of nematodes

Acquisition and transmission experiments were carried out as described by Brown et al. (1989a). The design of the different experiments is described below.

Experiment 1. Groups of nematodes, containing ca 45 trichodorids, extracted from Woodhill soil were given access to ca 6-week-old *Petunia hybrida* plants. Six plants inoculated on their leaves with each of TRV isolates PPB1, PPK20, PPM1, PPS1, PPK9, PAY8, PTW1, TCB2 or PRN, as well as twelve virus-free control plants were used. One week after inoculation, and prior to adding the nematodes, the presence of virus in the roots of these source plants was assessed by inoculation of a comminuted part of each root system onto a *Chenopodium amaranticolor* indicator plant. Nematodes were allowed to feed for 3 weeks to acquire virus, as described by Brown

Table 1. Origin and serotypes of TRV isolates and strains used in transmission tests.

Isolate	Serotype	Origin	Reference
PPK9	SYM	*P. pachydermus* (Scotland)	Ploeg et al., 1992
ORY	ORE	potato (Oregon, USA)	Lister and Bracker, 1969
PTW1	ORE	*P. teres* (Netherlands)	Ploeg et al., 1992
PRN	PRN	potato (Scotland)	Cadman and Harrison, 1959
PLB	PRN	potato (Netherlands)	Angenent et al., 1989
90-1	PRN	potato (Denmark)	B. Engsbro, pers. comm.
PPK20	PRN	*P. pachydermus* (Scotland)	Ploeg et al., 1992
PPB1	PRN	*P. pachydermus* (Scotland)	Ploeg et al., 1992
PPW1	PRN	*P. pachydermus* (Netherlands)	Ploeg et al., 1992
PPS1	PRN	*P. pachydermus* (Sweden)	Ploeg et al., 1992
PPM1	PRN	*P. pachydermus* (Scotland)	Ploeg et al., 1992
TPO3	RQ	*T. primitivus* (England)	Ploeg et al., 1992
TPE1	RQ	*T. primitivus* (Scotland)	Ploeg et al., 1992
TVC47	RQ	*T. viruliferus* (Netherlands)	Ploeg et al., 1991
TCB2	TCB2	*T. cylindricus* (Scotland)	Ploeg et al., 1992
PAY8	unknown	*P. anemones* (England)	Ploeg et al., 1992

et al. (1989a), after which they were extracted from each pot and the trichodorids recovered were counted. The complete root system of each source and control plant was again tested for presence of virus as described above. The nematodes recovered from each pot were subsequently added to a pot containing a virus-free *P. hybrida* bait plant. Three weeks later the root system of each bait plant was tested for the presence of TRV by inoculation of the comminuted root system onto a *C. amaranticolor* plant. Isolates obtained from bait plant roots were propagated in *Nicotiana clevelandii* and tested in $F(ab')_2$-ELISA as described by Ploeg et al. (1992).

Experiment 2. As *P. hybrida* appeared not to be equally suitable as a source plant with all TRV isolates, *N. tabacum* 'White Burley' and *P. hybrida* were compared as source or bait plants. Groups of nematodes, containing ca 50 trichodorids, extracted from Woodhill soil, were given access for 3 weeks to virus source plants inoculated with TRV isolates PPK20, PPB1, PPM1, PPS1, PPW1, 90-1, PRN or PLB as described above. All these isolates are serologically similar in $F(ab')_2$-ELISA, reacting with the PRN antiserum. Each isolate was inoculated onto twelve 6-week-old *P. hybrida* and twelve 6-week-old *N. tabacum* 'White Burley' plants which were used as virus source plants. Twelve *P. hybrida* and *N. tabacum* 'White Burley' plants which remained uninoculated were used as controls. Presence of virus in the roots before and after the acquisition period was assessed as described for Experiment 1. Nematodes were transferred from source plants to virus-free *P. hybrida* or *N. tabacum* 'White Burley' bait plants to give six replicates for each combination of source and bait plant species.

Acquisition by a virus-free nematode population and transmission by single trichodorids

Individual nematodes from Woodhill (*P. pachydermus, T. primitivus, T. cylindricus*) and Wageningen (*P. pachydermus*) were tested following access to virus source plants.

P. hybrida was used as source and bait plant, and TRV isolates ORY, PLB, PPK20, PPK9, PPB1, PPW1, TVC47 and TPE1 were used. Before adding nematodes to the source plants, the presence of virus in the source plant roots was confirmed by inoculation of a part of each root system onto a *C. amaranticolor* indicator plant. After a 3-week acquisition period, the nematodes were extracted from the pots containing the source plants and trichodorids were individually bait tested for 10 days as described by Brown et al. (1989a). The isolates obtained from bait plant roots were propagated in *N. clevelandii* and tested in $F(ab')_2$-ELISA as described by Ploeg et al. (1992).

Acquisition by a viruliferous nematode population and transmission by single trichodorids

Nematodes from Barry (*P. pachydermus, T. cylindricus*) were used to determine if the transmission frequency of a naturally viruliferous trichodorid population could be increased by giving them access to plants prior to testing them for virus transmission. The nematodes were allowed access for 3 weeks to virus-free or virus-inoculated *P. hybrida* plants as described by Brown et al. (1989a) prior to testing single trichodorids for virus transmission. TRV isolate PPB1 was used and single trichodorids were tested as described by Brown et al. (1989a).

Results

Acquisition and transmission of TRV by a virus-free nematode population

TRV isolates differed in the frequency and rate at which they moved into the roots of *P. hybrida* source plants. Furthermore, the experiment with a virus-free nematode population from Woodhill (Experiment 1; Table 2) showed that PRN-serotype isolates, which were originally obtained from transmissions by single *P. pachydermus* (isolates PPB1, PPK20, PPM1 and PPS1), were all successfully acquired and transmitted by the virus-free population from Woodhill. However, isolate PRN itself, which had been maintained in the glasshouse at SCRI by mechanical inoculation for many

Table 2. Transmission of TRV to *Petunia hybrida* bait plants by a nematode population from Woodhill, Scotland, which had been given access to manually inoculated *P. hybrida* source plants.

	TRV source isolates									
	PPB1	PPK20	PPM1	PPS1	PPK9	PAY8	PTW1	TCB2	PRN	TPO3
P. hybrida source plants										
TRV in roots										
before acquisition	2/6[a]	6/6	1/6	6/6	5/6	0/6	2/6	6/6	1/6	6/6
after acquisition	2/6	6/6	3/6	6/6	6/6	6/6	6/6	6/6	5/6	6/6
P. hybrida bait plants										
Transmissions	2/6	6/6	2/6	3/6	0/6	0/6	0/6	0/6	0/6	0/6

[a] Numerator is number of *P. hybrida* plants from which virus was detected from the roots, denominator is number of *P. hybrida* plants set up.

years, and non-PRN-serotype isolates (PPK9, PAY8, PTW1, TCB2 and TPO3) that had been obtained from transmissions by individual trichodorid nematodes, were not transmitted. All isolates recovered from the bait plant roots reacted with the PRN-antiserum in $F(ab')_2$-ELISA.

No virus was detected in the roots of twelve virus-free *P. hybrida* 'source' plants after the acquisition period, nor did the nematodes transferred from those plants to virus-free *P. hybrida* bait plants transmit virus to the roots of these bait plants. This indicates that the population was not naturally viruliferous. The numbers of trichodorids present in the suspensions extracted from the source plants and transferred to the bait plants ranged between 12 and 34 but were not affected by the virus source isolate.

Similarly, in Experiment 2, no virus was detected from the roots of the twelve virus-free *P. hybrida* or twelve virus-free 'White Burley' plants on which nematodes had been allowed to feed for 3 weeks. Numbers of trichodorids extracted from the source plants and added to the bait plants in this experiment ranged from 17 to 48 but were not affected by virus isolate or bait plant/source plant combination.

When amalgamating the transmission data for the eight TRV isolates in Experiment 2 (Table 3), the number of bait plants which became infected was not significantly different for the four source plant/bait plant combinations ($\chi^2 = 1.15$, N.S.). Also, using *P. hybrida* or 'White Burley' tobacco as bait or source plants did not significantly affect the acquisition and transmission of isolates PPB1, PPK20, PPS1 or PPW1. Isolates PRN, PLB and 90-1 were not recovered from bait plant roots. However, when 'White Burley' tobacco were used as virus source and *P. hybrida* as virus bait plants for isolate PPM1, significantly more bait plants became infected by the nematodes than when using 'White Burley' as both a source and bait plant for the virus ($\chi^2 = 6.00$, $P \leq 0.05$). *P. hybrida* was unsatisfactory as a source plant with this isolate, and with isolates PRN, PLB and 90-1, because infection of roots was relatively infrequent. Overall, isolate PPK20 was most efficiently transmitted although not significantly better than isolate PPW1 (Table 4). Transmission of isolate PPS1 was significantly less efficient than that of isolate PPK20, but not than that of isolate PPW1. Transmission of isolates PPB1 and PPM1 was less efficient than that of isolates PPK20, PPW1 and PPS1. Isolates 90-1, PLB and PRN were not recovered from bait plants in any of these tests.

Acquisition by a virus-free nematode population and transmission by single trichodorids

Bait tests with individual trichodorids from Woodhill or Wageningen showed that *P. pachydermus* from either population, after having had access to TRV-infected source plants, transmitted PRN-serotype isolates PPK20 and PPW1 but failed to transmit PRN-serotype isolate PLB (Table 5). *P. pachydermus* from Woodhill also transmitted PRN-serotype isolate PPB1 but failed to transmit TRV isolates belonging to other serotypes (PPK9, TVC47, TPE1, ORY). *T. primitivus* from Woodhill did not transmit any of the isolates, including TPE1. All isolates obtained from bait plant roots reacted with the PRN-antiserum in $F(ab')_2$-ELISA.

P. pachydermus from Woodhill transmitted isolates PPK20 and PPW1 equally efficiently ($\chi^2 = 1.81$, N.S.), but *P. pachydermus* from Wageningen transmitted isolate PPK20 more efficiently than isolate PPW1 ($\chi^2 = 9.35$, $P \leq 0.01$). The transmission of isolates PPK20 and PPW1 was not significantly different between the two popul-

Table 3. Transmission of TRV to *Petunia hybrida* or *Nicotiana tabacum* 'White Burley' bait plants by a nematode population from Woodhill, Scotland, which had been given access to manually inoculated *P. hybrida* or *N. tabacum* 'White Burley' source plants.

Bait/source combination	TRV source isolates							
	PPB1	PPK20	PPM1	PPS1	PPW1	PRN	PLB	90-1
COMBINATION 1								
P. hybrida source plants								
Virus in roots								
before acquisition	6/6[a]	6/6	3/6	6/6	6/6	2/6	3/6	3/6
after acquisition	6/6	6/6	5/6	6/6	6/6	4/6	4/6	5/6
P. hybrida bait plants								
Transmissions	3/6	6/6	1/6	4/6	4/6	0/6	0/6	0/6
COMBINATION 2								
P. hybrida source plants								
Virus in roots								
before acquisition	6/6	6/6	2/6	6/6	6/6	2/6	3/6	2/6
after acquisition	6/6	6/6	3/6	6/6	6/6	3/6	3/6	3/6
N. tabacum bait plants								
Transmissions	3/6	6/6	1/6	4/6	5/6	0/6	0/6	0/6
COMBINATION 3								
N. tabacum source plants								
Virus in roots								
before acquisition	6/6	6/6	5/6	6/6	6/6	6/6	6/6	5/6
after acquisition	6/6	6/6	5/6	6/6	5/5[b]	6/6	6/6	5/5[b]
P. hybrida bait plants								
Transmissions	2/6	6/6	4/6	5/6	5/5	0/6	0/6	0/5
COMBINATION 4								
N. tabacum source plants								
Virus in roots								
before acquisition	6/6	6/6	6/6	6/6	6/6	5/6	5/6	6/6
after acquisition	6/6	6/6	6/6	6/6	6/6	5/5[b]	5/6	5/5[b]
N. tabacum bait plants								
Transmissions	2/6	6/6	0/6	5/6	6/6	0/6	0/6	0/5
Total transmissions	10/24	24/24	6/24	18/24	21/23	0/23	0/24	0/22

[a] Numerator is number of plants from which virus was detected from the roots, denominator is number of plants set up.
[b] One virus source plant died during acquisition period.

Table 4. Pairwise comparisons of the transmission rates of PRN serotype TRV isolates after allowing initially virus-free nematodes to acquire and transmit. Data from Table 3.

	PPK20	PPW1	PPS1	PPB1	PPM1	90-1	PRN
PPW1	N.S.						
PPS1	**	N.S.					
PPB1	***	***	*				
PPM1	***	***	***	N.S.			
90-1	***	***	***	***	*		
PRN	***	***	***	***	*	–	
PLB	***	***	***	***	***	–	–

χ^2-test; *** significant at $P \leq 0.001$, ** significant at $P \leq 0.01$, * significant at $P \leq 0.05$, N.S. not significant, – test not valid.

Table 5. Transmission of TRV to *Petunia hybrida* by individual trichodorids from Woodhill, Scotland, and Wageningen, the Netherlands, which had been given access to manually inoculated *Petunia hybrida* source plants.

TRV source isolates	Trichodorid population[a]	
	Woodhill	Wageningen
PPK20	24/46 Pp (52%) 0/6 Tp (< 17%)	18/30 Pp (60%)
PPK9	0/75 Pp (< 2%) 0/6 Tp (< 17%)	n.d[b]
PPB1	14/78 Pp (18%) 0/6 Tp (< 17%)	n.d
PPW1	18/47 Pp (38%) 0/3 Tp (< 33%)	7/32 Pp (22%)
PLB	0/54 Pp (< 2%) 0/4 Tp (< 25%)	0/48 Pp (< 2%)
TVC47	0/48 Pp (< 2%) 0/6 Tp (< 17%)	n.d
TPE1	0/36 Pp (< 3%) 0/20 Tp (< 5%)	n.d
ORY	0/41 Pp (< 2%) 0/36 Tp (< 3%)	n.d

[a] Numerator is number of nematodes transmitting, denominator is number of nematodes tested. Pp: *P. pachydermus*, Tp: *T. primitivus*.
[b] n.d: not done.

ations (PPK20 Woodhill–Wageningen χ^2 = 0.45, N.S.; PPW1 Woodhill–Wageningen χ^2 = 2.37, N.S.). In our tests no transmission of isolate PLB was found.

Acquisition by a viruliferous nematode population and transmission by single trichodorids

Bait tests using single *P. pachydermus* from Barry which had been allowed access for 3 weeks to virus-free *P. hybrida* plants resulted in 65 out of 96 (69%) nematodes transmitting virus. When the nematodes were allowed access to *P. hybrida* source plants inoculated with TRV isolate PPB1, which originated from transmission by a single *P. pachydermus* from this site, 38 out of 69 (55%) of *P. pachydermus* transmitted. The difference in the transmission frequencies recorded here was not significant (χ^2 = 2.73, N.S.).

Discussion

The hypothesis that the transmissibility of TRV by trichodorid nematodes is largely dependent on the combination of vector species and TRV serotype (Brown et al., 1989a; Ploeg et al., 1992) is, at least for *P. pachydermus*, supported by the results obtained from our work. Thus, virus-free *P. pachydermus* only acquired and transmitted TRV isolates belonging to the PRN serotype, which confirms results by Ploeg et al. (1991, 1992) who found this vector species naturally associated uniquely with the PRN serotype. Successful acquisition and transmission was not related to obvious geographical factors, because virus-free *P. pachydermus* from Woodhill, Scotland, acquired and transmitted TRV isolates PPS1 and PPW1, which were originally obtained from transmissions by naturally viruliferous *P. pachydermus* from Sweden and the Netherlands respectively, and because *P. pachydermus* from Wageningen, the Netherlands, acquired and transmitted isolate PPK20 from Scotland. Therefore, the conclusion by Van Hoof (1968) that a trichodorid nematode population is unlikely to transmit a newly introduced TRV isolate cannot be accepted as a general rule. A population may efficiently acquire and transmit a newly introduced TRV isolate when the vector species and the TRV serotype are compatible.

Significant differences were found in the efficiencies with which different PRN-serotype TRV isolates were acquired and transmitted by *P. pachydermus* individuals or by populations containing *P. pachydermus*. TRV isolates PPK20, PPS1 and PPW1 were transmitted efficiently, PPB1 and PPM1 less efficiently and isolates PLB, PRN and 90-1 were not transmitted in our tests. Comparable results were obtained by Brown et al. (1989b), who found that significant differences occurred in the frequency with which serologically indistinguishable tomato black ring nepovirus (TBRV) isolates were transmitted by the vector nematode *Longidorus attenuatus*. Brown et al. (1989b) concluded that differences existed between the TBRV isolates, which were not detected in serological tests, and that such differences affected their transmissibility. Such differences may also explain the results obtained in the acquisition and transmission tests with *P. pachydermus* and the PRN-serotype TRV isolates. Whatever these differences are, they appear to affect the transmissibility of isolates irrespective of the *P. pachydermus* vector population. Isolate PPK20 was efficiently transmitted, isolate PPW1 had moderate transmissibility and isolate PLB was not transmitted by *P. pachydermus* from either the Woodhill or the Wageningen population. Whether the observed differences in transmissibility of these serologically similar isolates is a

reflection of differences in the ability of the vector nematodes to acquire, retain or release these isolates is unknown. Trudgill et al. (1981) attributed low transmission rates of raspberry ringspot nepovirus by *L. elongatus* and by *L. macrosoma* to different mechanisms. In electron microscopy, many particles were observed retained within the odontostyle lumen of *L. macrosoma,* but in *L. elongatus* virus particles were only rarely found. Trudgill et al. (1981) therefore concluded that *L. macrosoma* was an inefficient vector because of poor virus release, whereas with *L. elongatus* poor retention of particles caused the low transmission rates. A similar approach may reveal whether the differences in transmissibility of TRV isolates PPK20, PPW1 and PLB are caused by differential acquisition, retention or release of particles by *P. pachydermus*.

Transmissibility of a PRN-serotype isolate by *P. pachydermus* was correlated with the origin of the isolate: isolates PLB, PRN and 90-1 were originally isolated from naturally infected potato plants, had been maintained by mechanical transmission for years, and were not transmitted. The isolates which were acquired and transmitted were all originally obtained quite recently from transmissions by single *P. pachydermus* to bait plants. This correlation suggests that TRV isolates might lose vector transmissibility upon passage through potato plants, or upon repeated non-vector transmission. There are several examples of insect-transmitted viruses that have lost vector transmissibility, either during maintenance in a vegetatively propagated host (e.g. Black, 1969), or after repeated mechanical transmission (e.g. Tsai & Bath, 1974).

The failure of *P. pachydermus* to acquire and transmit non-PRN-serotype TRV isolates corresponds with the conclusion by Ploeg et al. (1992) that *P. pachydermus* is associated with PRN-serotype isolates only. Surprisingly, isolates TPO3, originally obtained from a transmission by a single *T. primitivus*, and TCB2, originally obtained from a transmission by a single *T. cylindricus*, were not transmitted by batches of nematodes from Woodhill containing *T. primitivus* and *T. cylindricus*. Also, individual *T. primitivus* from Woodhill failed to transmit isolate TPE1. The reason for this is unknown, but a possible explanation may be that conditions in the acquisition and transmission system itself are not optimal for all trichodorid species.

When viruliferous nematodes from Barry were allowed to feed for 3 weeks on uninoculated *P. hybrida* or on *P. hybrida* inoculated with PRN serotype isolate PPB1, 69% and 55% of *P. pachydermus*, respectively, transmitted virus in a subsequent bait test. When individual trichodorids from this population were bait tested immediately after extraction of the nematodes from soil, without allowing them to feed on *P. hybrida*, between 1% and 15% (average over three experiments = 7%) of *P. pachydermus* transmitted TRV (Ploeg, 1992; Ploeg et al., 1992). Thus, the proportion of viruliferous trichodorids within a population may be substantially increased by allowing the nematodes access to virus-free or virus-infected roots. Presumably, the roots of initially virus-free plants rapidly became infected under the conditions of these experiments and subsequently served as sources for virus acquisition. These results therefore suggest that in nature the proportion of viruliferous trichodorids is likely to increase where they have access to roots of virus-susceptible plant species. However, it is likely that factors that affect nematode activity (e.g. soil texture, soil moisture) and the host status of virus-susceptible plant species for the individual *Trichodorus* or *Paratrichodorus* vector species also greatly influence the proportion of viruliferous trichodorids within a population.

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