A review of reported associations between *Trichodorus* and *Paratrichodorus* species (Nematoda : Trichodoridae) and tobraviruses with a description of laboratory methods for examining virus transmission by trichodorids

Derek J. F. BROWN, Antoon T. PLOEG and David J. ROBINSON

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland.

SUMMARY

A review of the published literature showed that adequate laboratory evidence for transmission of viruses by trichodorid nematodes is available for only twelve of the 40 reported associations. Many of the remaining associations are based on circumstantial evidence from the field of a close association between virus and trichodorid nematodes and some doubt must attach to these results. However, provided that only a single species was present it is probable that several of these reports are correct. A laboratory system using single nematodes was developed which overcomes many of the earlier difficulties involved in determining transmission of virus by trichodorid nematodes in the laboratory. The system permits relationships between viruses and their associated vectors to be studied with mixed populations of trichodorids as each nematode transmitting virus is specifically identified. It was found that *Paratrichodorus pachydermus* from three field sites in eastern Scotland and *Trichodorus cylindricus, T. similis* and *T. viruliferus,* each only present at one of these sites, transmitted different strains of tobacco rattle virus (TRV). It was also demonstrated that non-viruliferous *P. pachydermus* could acquire and transmit a field isolate of TRV originally isolated from an individual *P. pachydermus.*

RÉSUMÉ

Revue des associations signalées entre espèces de Trichodorus *et de* Paratrichodorus *(Nematoda : Trichodoridae) et TOBRA virus; description de méthodes de laboratoire pour tester la transmission des virus par les Trichodorides*

La revue de la littérature concernant la transmission des virus par les Trichodorides montre que les expériences de laboratoire reportées ne sont valables que pour douze seulement des 40 associations signalées. Une bonne partie des signalisations sont uniquement fondées sur l'observation circonstantielle au champ d'une association étroite entre virus et nématode Trichodoride; de tels résultats doivent donc être considérés avec un certain doute. Cependant, à la condition qu'une seule espèce du nématode soit présente, il est probable que plusieurs de ces signalisations sont correctes. Une méthode de laboratoire a été mise au point qui, en n'utilisant que les seuls nématodes, permet de surmonter la plupart des difficultés rencontrées auparavant pour déterminer au laboratoire la transmission des virus par les Trichodorides. Cette méthode permet l'étude des relations entre les virus et leurs vecteurs sur des populations mélangées de Trichodorides, car chaque nématode transmettant le virus est identifié au niveau de l'espèce. Il a été ainsi observé que *Paratrichodorus pachydermus* provenant de trois champs de l'est de l'Écosse, de même que *Trichodorus cylindricus, T. similis, T. viruliferus* — chacun présent dans un seul de ces trois sites — transmettent des souches différentes du tobacco rattle virus (TRV). Il a été également démontré que des individus de *P. pachydermus* normalement non-vecteurs peuvent acquérir et transmettre un isolat de TRV provenant originellement d'un individu de *P. pachydermus.*

Members of the genera *Trichodorus* and *Paratrichodorus* have been reported from most parts of the world and are widely distributed in Europe and North and South America. They damage a wide range of cultivated and wild plant species either by their direct feeding or by transmitting plant viruses (Winfield & Cooke, 1975; Taylor & Brown, 1981).

Forty different associations have been reported between species of *Trichodorus* and *Paratrichodorus* and the tobraviruses, pepper ringspot, tobacco rattle (TRV) and pea early-browning (PEBV) viruses (Tab. 1). Many of these reports are based upon a field association between a particular nematode species and an isolate of tobravirus but usually few details are given of any laboratory experiments done to confirm the nematodes' vectoring ability. More than forty associations between longidorid nematodes and nepoviruses have been reported, but Trudgill, Brown and McNamara (1983) con-

Table 1

An annotated bibliography of associations between trichodorid nematodes and tobraviruses

Nematode	Virus	Isolate or serotype	Authority	Reason**
	A - REPORTS WHICH FULFIL CRITERIA* FOR ASSESSING NEMATODE TRANSMISSION OF VIRUS			
Paratrichodorus				
allius	Tobacco rattle	(Californian)	Ayala & Allen (1968)	
minor	Tobacco rattle	(Florida)	Walkinshaw, Griffin & Larson (1961)	
minor	Tobacco rattle	(Japanese)	Komuro, Yoshino & Ichinohe (1970)	
minor	Pepper ringspot	(Artichoke yellow band)	Salomao (1973)	
nanus	Tobacco rattle	(Scottish; PRN)	Cooper & Thomas (1970)	
pachydermus	Tobacco rattle	(Dutch)	Cremer & Schenk (1967)	
pachydermus	Tobacco rattle	(English; PRN)	Gibbs & Harrison (1964*b*)	
tunisiensis	Tobacco rattle	(Italian)	Roca & Rana (1981)	
teres	Tobacco rattle	(Dutch)	Van Hoof (1964*b*)	
Trichodorus				
viruliferus	Tobacco rattle	(Italian)***	Van Hoof, Matt & Seinhorst (1966)	
viruliferus	Pea early-browning	(English)	Gibbs & Harrison (1964*a*)	
similis	Tobacco rattle	(Dutch)	Cremer & Schenk (1967)	
	B - OTHER REPORTS OF SOME TRICHODORIDS LISTED ABOVE WHICH DO NOT FULFIL THE CRITERIA			
Paratrichodorus				
allius	Tobacco rattle	(Oregon)	Jensen & Allen (1964)	1, 2, 3, 4
allius	Tobacco rattle	(Oregon)	Jensen & Allen (1979)	1, 2, 3
minor	Tobacco rattle	(Californian)	Ayala & Allen (1966, 1968)	1, 2, 3, 4
nanus	Tobacco rattle	(Dutch)	Van Hoof (1968)	1, 4
pachydermus	Tobacco rattle	(Dutch)	Van Hoof (1968)	1
pachydermus	Tobacco rattle	(Dutch)	Cremer & Kooistra (1964)	4
pachydermus	Tobacco rattle	(German)	Sänger (1961)	4
pachydermus	Tobacco rattle	(Dutch)	Sol & Seinhorst (1961)	1, 3
pachydermus	Tobacco rattle	(Dutch)	Van Hoof (1964*a*)	1
teres	Tobacco rattle	(Oregon)	Jensen, Koepsell & Allen (1974)	1, 2, 3
teres	Tobacco rattle	(Dutch)	Van Hoof (1968)	1, 4
Trichodorus				
viruliferus	Tobacco rattle	(Dutch)	Van Hoof (1968)	1, 4
	C - ORIGINAL REPORTS OF OTHER TRICHODORIDS TRANSMITTING VIRUS WHICH DO NOT FULFIL THE CRITERIA			
Paratrichodorus				
anemones	Pea early-browning	(English)	Harrison (1967)	4
anemones	Tobacco rattle	(Dutch)	Van Hoof (1968)	1, 4
pachydermus	Pea early-browning	(Dutch)	Van Hoof (1962)	1
pachydermus	Tobacco	(Dutch)	Sol, Heuven & Seinhorst (1960)	
porosus	Tobacco rattle	(Californian)	Ayala & Allen (1966, 1968)	1, 2, 3, 4
teres	Pea early-browning	(Dutch)	Van Hoof (1962)	1
Trichodorus				
cylindricus	Tobacco rattle	(Dutch)	Van Hoof (1968)	1, 4
hooperi	Tobacco rattle	(English)	Alphey (1974)	4
primitivus	Tobacco rattle	(English)	Harrison (1961)	4
primitivus	Tobacco rattle	(German)	Sänger (1961)	4
primitivus	Tobacco rattle	(Scottish)	Mowat & Taylor (1962)	4
primitivus	Tobacco rattle	(Dutch)	Van Hoof (1968)	1
primitivus	Tobacco rattle	(English, SYM)	Kurppa *et al.* (1981)	2
primitivus	Pea early-browning	(English)	Gibbs & Harrison (1964*a*)	4
similis	Tobacco rattle	(Dutch, GNL)	Cremer & Kooistra (1964)	4
similis	Tobacco rattle	(Dutch)	Van Hoof (1968)	1, 4

* Criteria as described fully in Trudgill, Brown and McNamara (1983), see also Reasons below.

** Reasons 1 : Virus recovered from bait plants not unequivocally identified.
2 : Possibility of transmission by alternative nematode or other biological vector.
3 : Inadequate methodology i.e. possibility of contamination of bait plants, etc.
4 : Inadequate description of methods.

*** Three isolates, including one (No 6) originally identified as pea early-browning virus which subsequently was reclassified as an atypical isolate of tobacco rattle virus (Robinson *et al.*, 1987).

sidered that many were unacceptable because the published reports provided inadequate evidence to prove the nematodes to be vectors of the viruses. Trudgill, Brown and McNamara (1983) proposed criteria which should be fulfilled to establish transmission of a nepovirus by a longidorid nematode. *« 1) The virus and nematode must be fully characterised and correctly identified, 2) Bait plant tissue must be shown to be infected with the virus under test and 3) The nematode under test must be shown to be the only possible vector in the experiment. »* These criteria also are applicable to trichodorid nematodes and tobraviruses and when applied only 12 of the 40 reported associations were supported by adequate evidence (Tab. 1).

Methods were also described by Trudgill, Brown and McNamara (1983) for assessing the ability of longidorid nematodes to transmit nepoviruses. No similar methodology or techniques have been developed for use with trichodorids and consequently it is not possible to compare the results from virus transmission experiments where different methods have been employed. Similarly, it has not been possible to determine if specific associations exist between species of trichodorid nematodes and isolates of tobraviruses as have been found with nepoviruses and their vectors (Brown, 1985, 1986; Brown & Trudgill, 1985). We therefore include in Table 1 our assessment of all published reports of individual nematode species transmitting isolates of tobraviruses. Also the efficiency of transmission of different field isolates of tobraviruses by different populations or species of trichodorid nematodes have not been accurately determined.

We describe a method for studying the effectiveness and specificity with which trichodorid nematodes transmit field isolates of TRV and results are presented from experiments in which the method was used. Results also are presented from an experiment in which trichodorids were shown to acquire and transmit a field isolate of TRV.

Materials and methods

Virus transmission system

Plastic Beem capsules, 0.5 cm^3 capacity (Size 00, TAAB Laboratories, Reading, England) were one third filled with air-dried sand with a particle size < 1 500 µm and > 250 µm delivered from a 10 cm^3 conical plastic centrifuge tube (BDH, Dagenham, England) with a 2 mm diam. hole made in the base (Fig. 1 A). Water was added to half fill each capsule. Trichodorid nematodes were extracted by the method of Brown and Boag (1988) from bulks of soil collected in eastern Scotland during April 1987 from fields known to contain tobacco rattle virus at Morton and Kinshaldy, both in Fife and at Barry, Angus (Brown, Boag & Topham, 1989). Individual adult nematodes were hand-picked with a fine needle under a binocular microscope at × 50 magnification and floated-off in the water above the sand in the capsules. A single one week-old *Petunia hybrida* Vilm. seedling with a washed root system was placed in each capsule and further sand was then added to fill each capsule. To maintain a high humidity to decrease the frequency with which the seedlings needed watering the capsules were partly plunged into wet sand contained in a plastic tray (Fig. 1 B) and placed in a seedling growth chamber (see chamber in Fig. 1 D). When necessary individual capsules were watered by means of a Pasteur pipette.

The chambers were placed in a controlled environment room at 20° with 18 h, 2 000 lux lighting for seven days, after which the contents of each capsule were washed into a 25 cm^3 plastic polypot (Fig. 1 A) by means of a Pasteur pipette using a maximum of 5 cm^3 of water. To ensure the nematode did not remain entrapped in the *P. hybrida* root system the plant roots were vigorously shaken in the water in the polypot. The contents of each polypot were gently mixed into suspension and after a few seconds the supernatant fluid poured into a 5 cm × 5 cm × 1 cm nematode counting dish. The supernatant fluid was examined under a binocular microscope and the nematode if found was transferred to a temporary water-mount on a microscope slide, heat-killed and the species identified with the aid of a high power compound microscope.

Each *P. hybrida* plant was transferred to a seed tray containing 40 individual compartments, each filled with a standard potting compost (Fig. 1 C) and allowed to grow for a further three weeks in a glasshouse at 20° in natural daylight. This allowed any virus that had been transmitted to the plants to replicate sufficiently for it to be readily detected.

After three weeks the root system of each *P. hybrida* plant was washed free from adhering compost, separately comminuted with a mortar and pestle and the resultant suspension rubbed on to leaves of a *Chenopodium amaranticolor* Coste & Reyn. indicator plant. Local lesions appeared on the leaves of the *C. amaranticolor* plants about five days after inoculation with extracts from *P. hybrida* roots infected with TRV. Virus isolates obtained in this way were propagated in *C. quinoa* and *Nicotiana clevelandii* Gray plants. The serotypes of selected isolates were determined by $F(ab')_2$ — ELISA (Barbara & Clark, 1982), using $F(ab')_2$ fragments and detecting IgG preparations from antisera to TRV strains PRN (Cadman & Harrison, 1959), SYM (Kurppa *et al.*, 1981) and N5 (Robinson *et al.*, 1987).

Virus acquisition system

Plastic polypots, 25 cm^3, were one third filled with an air-dried sand and steam-sterilised loam mixture in the ratio 3 : 1 with a particle and soil aggregate size < 1 500 µm and > 250 µm which was then wetted. A

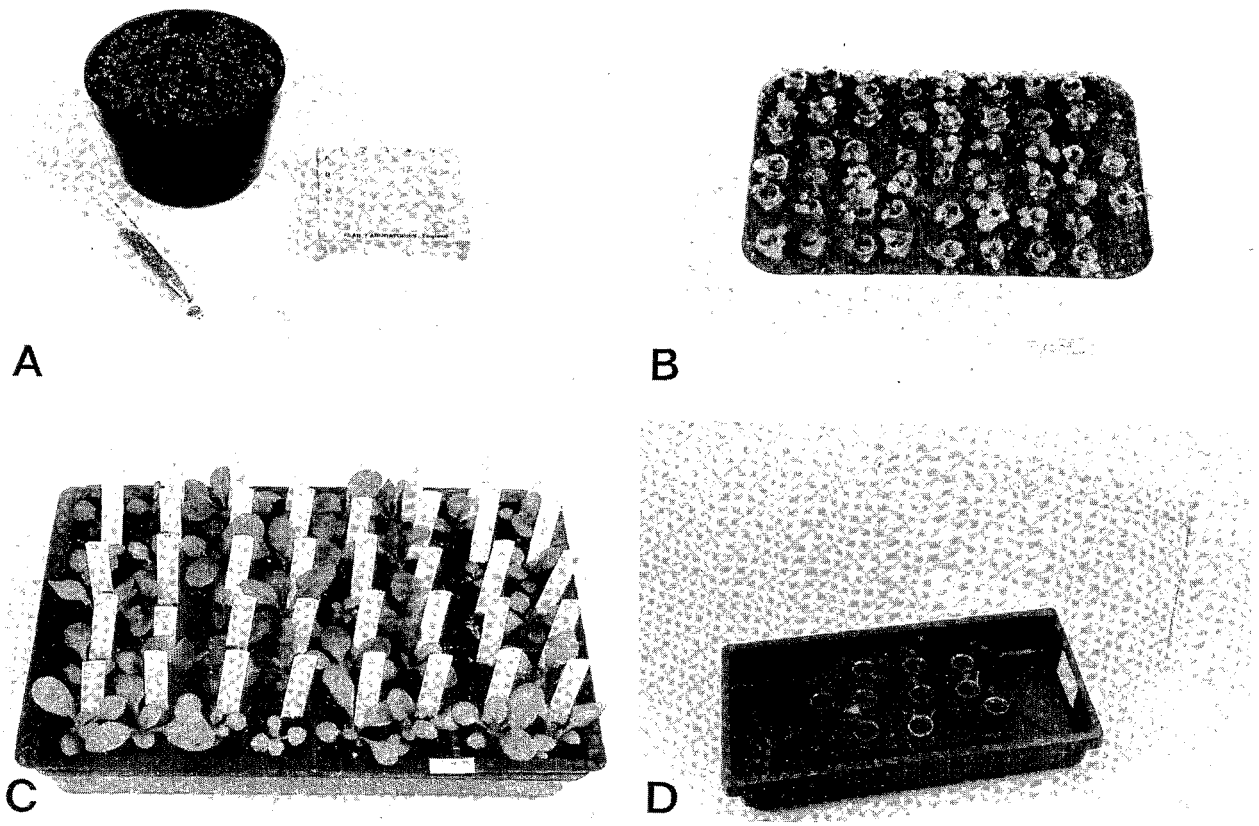

Fig 1. Components of the virus acquisition and transmission system for use with trichodorid nematodes. A : a, pan 1 week old *Petunia hybrida* seedlings; b, Beem capsule holder; c, 0.5 cm³ Beem capsules; d, 25 cm³ plastic polypot and e, 10 cm³ conical centrifuge tube containing dry sand, with a 2 mm dia. hole made in the base to deliver sand into Beem capsules. B : the individual nematode bait test system — Beem capsules after being loaded with a trichodorid nematode and a one week old *P. hybrida* seedling. C : bait plants removed from the Beem capsules and allowed to grow for a further three weeks in seedling trays containing forty individual compartments. D : the virus acquisition system — *c.* 50 trichodorids are added to 25 cm³ plastic polypots in which are growing 2 week old *P. hybrida* seedlings previously manually infected with virus — a, polypots loaded with nematodes and plants and plunged in wet sand contained in a tray; b, seedling growth chamber.

two week-old *P. hybrida* seedling was planted in each pot. The leaves were dusted with abrasive (225 grade silicon carbide and aloxite, Sohio Electro Minerals Co., Manchester, England) and virus in infective sap from a *N. clevelandii* plant inoculated by rubbing virus infective sap on to the leaves of each seedling. The virus isolate used was obtained from a bait plant infected by a *Paratrichodorus pachydermus* from Barry, Angus. The pots were placed in a seedling growth chamber in a controlled environment cabinet (see Virus Transmission for details). The next day trichodorid nematodes from a field site at Carnoustie, Angus known to be free of tobraviruses (virus was not recovered in bait tests from 130 samples each of 600 g soil collected from a 1 ha area; Brown, Boag & Topham, 1988) were extracted (Brown & Boag, 1988) and added to each pot as aliquots of *c.* 50 nematodes in 5 cm³ of water. Before adding the aliquots about half of the sand and soil mixture was removed from the polypots which were then refilled with dry fresh sand and soil mixture. The pots were replaced in the chamber in the controlled environment cabinet.

After four weeks the nematodes were extracted and their ability to transmit virus was tested in the virus transmission system as described. The roots of the virus source plants were tested for the presence of virus.

An alternative virus acquisition system to that described above also was tested. Plastic cylinders 25 cm³, 2.5 cm dia. with bases made of 5.0 µm aperture bolting nylon were used and loaded with soil mixture, nematodes and plants in the same way as the polypots. However, the cylinders were plunged into wet sand which enabled them to be self-watering by capillary action.

Results

Virus transmission

TRV was transmitted by individual *Paratrichodorus* and *Trichodorus* nematodes collected from three field sites at which TRV occurred naturally. *P. pachydermus* were present at each of the three field sites. The numbers of individuals of this species that transmitted TRV were 6 of 24 tested from Barry, 8 of 31 from Kinshaldy and 5 of 13 from Morton. TRV was also transmitted by *Trichodorus* spp. present at these sites, the numbers being 3 of 12 *T. cylindricus* from Barry, 2 of 4 *T. similis* from Kinshaldy and 2 of 5 *T. viruliferus* from Morton.

Virus acquisition

The ability of virus-free trichodorids from a field site at Carnoustie, Angus to acquire and transmit TRV which previously had been transmitted by a *P. pachydermus* from the nearby Barry field site was examined. The virus was acquired and transmitted by 17 of 93 individual *P. pachydermus* tested but not by any of 13 *T. cylindricus* tested. Seven of the seventeen isolates obtained from these transmissions, as well as the isolate used as inoculum in the acquisition procedure, were tested by ELISA. All reacted with antiserum to TRV strain PRN, but not with antisera to strains SYM or N5, confirming that the virus transmitted was of the same serotype as that inoculated into the plants used for virus acquisition.

A further experiment with trichodorids naturally associated with TRV from the Barry, Angus field site was done to determine if the proportion of nematodes transmitting virus could be increased. Nematodes extracted from field soil were given access either in polypots or in plastic cylinders for four weeks to *P. hybrida* plants infected with the PRN serotype isolate of TRV which previously had been transmitted by a *P. pachydermus* from the Barry field site. Fifteen percent of the *P. pachydermus* recovered directly from field soil transmitted TRV. However, following access to viruliferous plants in the polypots 41 % of the *P. pachydermus* subsequently transmitted virus but only 15 % from the cylinders transmitted virus. None of the *T. cylindricus* recovered from the cylinders or polypots transmitted virus but 7 % from the field soil did so (Tab. 2). The virus transmitted by *T. cylindricus* did not react with any of the antisera available and probably represents an uncharacterised strain of TRV.

Discussion

Reports of the transmission of TRV by trichodorid nematodes (Tab. 1) mostly deal with the vectoring of virus by nematodes naturally associated with virus under field conditions. Unfortunately many reports contain insufficient and/or inadequate details to offer irrefutable evidence of the nematodes ability to transmit virus. Other reported associations require verification as they appear only in abstracts in which it is stated that a particular nematode species transmits virus. Furthermore, in several reports the methods described permit several interpretations of the results obtained i.e. contamination of bait-plant root systems with root debris from virus infected plants and, in many reports, virus identification was made only on symptomatology in test

Table 2

A comparison of two systems used to examine the acquisition and transmission of a field isolate of tobacco rattle virus previously transmitted by *Paratrichodorus pachydermus* by individual *Trichodorus cylindricus* and *P. pachydermus* from a field naturally infected with tobacco rattle virus

SYSTEM		NEMATODES			
Acquisition	*Transmission*	*T. cylindricus*	P	*P. pachydermus*	P*
25 cm³ cylinders with*** 5 µm mesh base	0.5 cm³ " Beem " capsules	0/9	< 0.09	5/40**	0.13
25 cm³ plastic pot***	0.5 cm³ " Beem " capsules	0/4	< 0.04	32/79	0.41
Nematodes obtained directly from field soil	0.5 cm³ " Beem " capsules	2/30	0.07	9/60	0.15

* Proportion of nematodes transmitting virus.
** Numerator is the number of bait plants infected, denominator is the number tested.
*** With virus infected *P. hybrida*.

plants, without verification by serology or electron microscope identification of virus particles (see Tab. 1). Also, many reports describe diseases in crops apparently caused by tobraviruses transmitted by trichodorids where the nematode species were not identified other than to generic level.

Trudgill, Brown and McNamara (1983) proposed a set of criteria which must be fully satisfied to demonstrate that a particular nepovirus was transmitted by a given species of longidorid nematode. The methods described by Trudgill, Brown and McNamara (1983) for examining the transmission of nepoviruses by longidorid nematodes were not applicable to tobraviruses and trichodorid nematodes. However, the virus transmission system described here now permits the researcher to fulfil these criteria when working with trichodorid nematodes and tobraviruses.

There is considerable serological and symptomatological variation within the tobraviruses, especially within TRV (Harrison, 1970; Harrison & Robinson, 1981, 1986). Moreover, it has been shown that considerable specificity exists between nepoviruses and populations and species of longidorid nematodes (Harrison, 1964; Brown & Taylor, 1981; Taylor & Brown, 1981; Brown & Trudgill, 1985; Brown, 1985, 1986). Van Hoof (1968) reported that in the Netherlands virus free populations of *P. anemones, P. nanus, P. pachydermus, P. teres, T. cylindricus* and *T. viruliferus* apparently acquired and transmitted uncharacterised field isolates of TRV when the nematode species and viruses originated from the same locality. Conversely, Kurppa *et al.* (1981) reported that a Scottish population of trichodorids, which included *T. primitivus,* acquired and transmitted spinach yellow mottle, an isolate of TRV obtained from spinach plants growing in southern England. Progress on studying specificity between trichodorid nematodes and tobraviruses has been hampered by problems of culturing the nematodes and the lack of an appropriate laboratory system for studying virus and vector associations (Brown & Boag, 1987). The virus transmission system described here for use with trichodorids overcomes many of the problems encountered by earlier workers studying the specificity between tobraviruses and their vector trichodorids.

The laboratory system described here also offers the advantage that populations of trichodorids can be used which comprise several species — as is frequently the case (Alphey, 1985) — because each individual nematode transmitting virus is specifically identified. In this respect the system is somewhat similar to that of van Hoof (1964*a*) who demonstrated serial transmission of TRV by individual male *P. pachydermus.* Our results also indicate that where TRV and a population of trichodorids, comprising several species, occur naturally together, two or more of the nematode species may transmit virus. It may be speculated that, as tobacco rattle virus occurs as many serologically distinct variants (Harrison, 1970), the possibility exists that at a field site where mixed populations of trichodorids occur each species may be transmitting a different serological variant of TRV. De Pelsmaeker and Samyn (1986) suggested that variability in TRV symptomatology and degree of infection in crops may be the result of different TRV strains being specifically transmitted by different species of trichodorids. Further work, however, is required to determine the extent and nature of the specificity between the virus isolates and their associated vectors.

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