Fundam. appl. Nematol., 1998, **21** (6), 673-677

Differential transmission of cherry rosette nepovirus by populations of *Longidorus arthensis* (Nematoda: Longidoridae) with a description of the association of the virus with the odontostyle of its vector

Derek J.F. BROWN*, Paul KUNZ**, Jurg GRUNDER** and Walter M. ROBERTSON*

**Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, U.K., and **Nematology Section, Swiss Federal Research Station, 8820 Wadenswil, Switzerland.*

Accepted for publication 16 January 1998.

Summary - *Longidorus arthensis* from the rhizosphere of cherry trees at Maiberg, Baselland, after access to cherry rosette nepovirus (CRV) did not transmit the virus whereas specimens from the rhizosphere of cherry trees infected with CRV at Talmatte, Arth, and a mixture of specimens from Talmatte, Arth, and Rossberg, Steinerberg, did transmit CRV. Minor morphometric differences were recorded between nematodes from Baselland and those from the Arth region but were considered to represent intra- and not interspecific differences. Electron microscope examination of thin sections of female specimens which had transmitted CRV revealed the presence of only a few virus particles adsorbed to the lining of the lumen of the odontostyle whereas many particles were observed adsorbed to the lining of the guiding sheath, frequently present as multiple layers. It is suggested that the effective sites of retention of nepoviruses transmitted by *Longidorus* species should be considered as both the odontostyle lumen and the space between the guiding sheath and the odontostyle. © Orstom/Elsevier, Paris

Résumé - ***Différences dans la transmission du nepovirus, agent de la rosette du cerisier, par des populations de* Longidorus arthensis *(Nematoda: Longidoridae) et description de l'association du virus avec l'odontostyle de son vecteur*** - *Longidorus arthensis* provenant de la rhizosphère de cerisiers à Maiberg, Baselland, après contact avec le nepovirus de la rosette du cerisier (CRV) ne transmet pas le virus, tandis que des spécimens provenant de la rhizosphère de cerisiers infestés par le CRV à Talmatte, Arth et à Rossberg, Steinerberg, le transmettent. De faibles différences morphométriques ont été relevées entre les nématodes du Baselland et ceux de la région du Arth, mais elles sont considérées comme intraspécifiques et non interspécifiques. L'examen en microscopie électronique de fines sections de spécimens femelles ayant transmis le CRV révèle la présence de quelques particules de virus seulement adsorbées à la surface de la lumière de l'odontostyle tandis que nombre de particules sont adsorbées, fréquemment en plusieurs couches, à la surface du fourreau-guide. Il est donc suggéré que les sites actifs de rétention des nepovirus transmis par les espèces de *Longidorus* soient considérés comme étant la lumière de l'odontostyle et l'espace situé entre ce dernier et le fourreau-guide de l'odontostyle. © Orstom/Elsevier, Paris

Keywords : *Longidorus*, Nematoda, nepovirus, sites of retention, transmission, virus-vector.

A rosetting disease occurring in cherry trees growing at Erlihof and Sonnenberg in the Arth region of Switzerland has been shown to be associated with cherry rosette nepovirus (CRV) transmitted by *Longidorus arthensis* Brown *et al.*, 1994. The disease was subsequently identified from trees in orchards at Talmatte in the Arth region, and at Rossberg in the Steinerberg region, near Arth, in association with its nematode vector (Brown *et al.*, 1994). A similar problem, referred to as Pfeffingen disease, affects cherry trees in Baselland, but is caused by raspberry ringspot nepovirus (RRSV) transmitted by *Longidorus macrosoma* (Klingler *et al.*, 1985*a*; Buser, 1990). *Longidorus* specimens from two sites in Baselland (Sutter and Wittensburg) were identified by Brown *et al.* (1994) as representing *L. arthensis*, and these authors proposed that earlier references to "*Longidorus* sp. (*goodeyi* ?)" from Switzerland (Klingler *et al.*, 1983; 1985*b*) be considered to refer to *L. arthensis*.

A limited survey was conducted in Baselland to determine if *L. arthensis* was present in the region in association with CRV. *Longidorus* nematodes, here reported as representing *L. arthensis*, were recovered from Maiberg, but CRV was not present in orchard trees (A. Buser and T. Hasler, unpubl.). *L. arthensis* populations from this site, and from Talmatte, Arth, and from Rossberg, Steinerberg, were used in virus transmission experiments and the results are presented here. Also, the sites of retention of virus particles in the feeding apparatus of *L. arthensis*, which were shown pictorially but not described in Taylor and Brown (1997), are reported here.

Materials and methods

Nematode morphology and morphometrics

Soil samples containing *Longidorus* specimens were collected at a depth of 50 to 70 cm from the rhizosphere of cherry trees growing at Maiberg, Baselland, and from trees showing symptoms of cherry rosette disease at Talmatte, Arth, and at Rossberg, Steinerberg, near Arth. Nematodes were extracted by a modified centrifugation method (Brown *et al.*, 1994) or by a decanting and sieving method (Brown & Boag, 1988). Specimens from the *Longidorus* population from Maiberg, Baselland, were heat killed, fixed in formalin, processed and mounted in anhydrous glycerol on microscope slides for morphological examination.

Virus transmission experiments

Fifty bait tests each containing 50 *L. arthensis* from Maiberg, Baselland, were established with *Nicotiana tabacum* cv. Xanthii bait-plants. After four weeks the roots of the bait plants were thoroughly washed and comminuted with a mortar and pestle and the suspension rubbed by finger onto leaves of *Chenopodium quinoa* virus assay plants which had been lightly dusted with corundum abrasive. After 14 days the assay plants were examined for the presence of virus symptoms, *i.e.*, local lesions on the inoculated leaves and symptoms of systemic infection. For the virus acquisition and transmission experiments *Longidorus* specimens extracted from soil samples were placed as hand-picked groups of 35 specimens in small plastic pots (25 cm^3) containing a sterilized, sand and soil mixture with a particle size <2000 µm and >500 µm, and a *C. quinoa* or *N. tabacum* cv. Xanthii plantlet, which two days previously had been mechanically inoculated with cherry rosette nepovirus (CRV). As relatively few *Longidorus* nematodes were recovered from soil from Rossberg, these were combined with an equal number of specimens from Talmatte. After four weeks the nematodes were recovered and individually bait tested following the method described by Brown *et al.* (1994). Single nematodes were added to small plastic capsules (0.5 cm^3) containing sterilized sand and a small, healthy *N. tabacum* cv. Xanthii seedling. After 10 days the contents of each capsule were washed into a counting dish, the nematode was recovered and root-tip galls, caused by nematode feeding, counted. The bait seedlings were then placed in compost blocks and grown for a further three weeks to provide sufficient root for virus testing as described above. The leaves of a random selection of *C. quinoa* plants showing systemic symptoms of virus infection were harvested and used in serological tests with an antiserum prepared against CRV to confirm the identity of the virus.

Electron microscopy

Single nematodes from Talmatte recovered from the bait-tests described above were individually placed into a small capsule (0.5 cm^3) one-third filled with water and hot (80°C) formalin/glycerol mixture (1/1% in water) was added to heat-kill and fix the specimen. The nematodes were stored in the capsules until the results of the bait-test were known and adult specimens which had transmitted the virus were examined by electron microscopy to identify the presence and location of virus particles specifically retained at sites of retention within their feeding apparatus.

The adult specimens were removed from the capsules and placed in 3% gluteraldehyde in phosphate buffer, pH 7.2, post-fixed in 1% osmium tetroxide in buffer and dehydrated in ethanol, followed by infiltration in Emix resin which was polymerised at 70°C. Ultrathin sections were cut on a microtome, stained with uranyl acetate and lead citrate, and examined in a JOEL electron microscope of 80 kV.

Results

Longidorus specimens from Maiberg, Baselland, were morphologically identical to *L. arthensis* specimens from the Arth region, but had several minor morphometrical differences as compared to type specimens (Table 1). The Baselland nematodes were somewhat larger than those from the Arth region but all morphometric measurements overlapped between specimens from the two regions. An exception was the size of the male spicules, which were longer in the Baselland specimens and their size did not overlap those of the type specimens.

Transmission of CRV to *N. tabacum* cv. Xanthii bait-plants did not occur in laboratory tests with 50 replicates each containing 50 specimens of *L. arthensis* recovered from orchard soil from Maiberg, Baselland (results not presented). Also, virus transmission was not detected with any of 245 individual nematodes from this population after the nematodes had been given access to *C. quinoa* plants infected with CRV. Galling present on the roots of the bait plants confirmed that the nematodes had fed on these plants. Therefore, absence of vector transmission of virus did not result from the nematodes not having fed (Table 2). Six of 28 (21%) *L. arthensis* from a population from Talmatte, Arth, transmitted CRV to *N. tabacum* bait plants after access to the virus in *N. tabacum* virus source-plants. Also, 8 of 47 (17%) *L. arthensis* specimens, from a mixture of nematodes from Talmatte, Arth, and Rossberg, Steinerberg, transmitted the virus after access to roots of *C. quinoa* plants mechanically inoculated with CRV (Table 2).

Examination of individual adult specimens from the Talmatte population which had transmitted CRV

Table 1. *Morphometrics (mean + one standard deviation, minimum and maximum) of* Longidorus arthensis *from Maiberg, Baselland and Arth Switzerland (All measurements in µm, except L in mm).*

	Baselland		Arth	
	Females	Males	Females	Males
n	9	5	17	10
L	6.79 ± 0.59 (5.80-7.50)	6.93 ± 0.64 (6.05-7.55)	5.92 ± 0.50 (5.14-6.74)	5.67 ± 0.66 (4.61-6.99)
a	91 ± 10 (76-104)	103 ± 7 (92-111)	89 ± 9 (75-110)	94 ± 4 (88-100)
b	15 ± 1.7 (13-18)	14 ± 2.0 (11-16)	13 ± 1.0 (12-15)	13 ± 1.4 (11-15)
c	185 ± 19 (155-219)	163 ± 23 (141-198)	149 ± 15 (123-174)	134 ± 19 (117-174)
c'	0.8 ± 0.04 (0.7-0.9)	0.9 ± 0.08 (0.8-1.1)	0.9 ± 0.07 (0.8-1.1)	1.0 ± 0.07 (0.9-1.1)
d*	2.3 ± 0.1 (2.1-2.4)	2.2 ± 0.2 (2.0-2.4)	2.3 ± 0.2 (1.9-2.7)	2.1 ± 0.2 (1.8-2.3)
d'*	1.8 ± 0.05 (1.8-1.9)	1.7 ± 0.08 (1.6-1.8)	1.7 ± 0.08 (1.6-1.9)	1.6 ± 0.10 (1.5-1.8)
V / T	52 ± 2 (51-57)	47 ± 6 (41-57)	51 ± 1 (50-53)	45 ± 3 (39-48)
Od. style	109 ± 5 (104-117)	109 ± 5 (101-114)	108 ± 3 (102-111)	106 ± 4 (102-116)
Od. phore	72 ± 2 (68-75)	72 ± 2 (70-75)	70 ± 2 (67-75)	68 ± 4 (64-76)
Spear	182 ± 6 (172-192)	180 ± 6 (171-188)	177 ± 4 (170-183)	175 ± 5 (170-185)
Guide ring	35 ± 2 (32-38)	38 ± 2 (36-40)	35 ± 2 (30-38)	35 ± 2 (33-39)
Ant. to oesoph./ intest. junct.	446 ± 44 (378-526)	497 ± 40 (460-563)	441 ± 30 (391-499)	438 ± 33 (395-489)
Tail	37 ± 3 (34-44)	43 ± 3 (38-47)	40 ± 3 (36-46)	43 ± 3 (37-45)
Body diam.				
- at labial reg.	16 ± 0.4 (15-16)	17 ± 0.8 (16-18)	16 ± 0.8 (14-17)	17 ± 0.8 (16-19)
- at guide ring	28 ± 0.7 (27-29)	28 ± 1.5 (26-29)	27 ± 1 (25-30)	27 ± 1 (25-29)
- at anus	46 ± 3 (42-52)	46 ± 2 (44-49)	42 ± 2 (39-46)	42 ± 3 (37-45)
Greatest body diam.	75 ± 3 (70-80)	67 ± 4 (61-72)	67 ± 5 (58-77)	60 ± 6 (48-69)
Spicules	-	70 ± 2 (67-73)	-	64 ± 3 (60-66)

* d = dist. ant. end to guide ring/ labial diam.; d' = body diam. at guide ring/ labial diam.

(Table 2) revealed the presence of only a few virus particles adsorbed to the lining of the lumen of the odontostyle. However, numerous virus particles were observed adsorbed to the lining of the guide sheath and frequently were present as multiple layers (Fig. 1).

Table 2. *Transmission of cherry rosette nepovirus to* Nicotiana tabacum *cv. Xanthii bait-plants by individual* Longidorus arthensis *recovered from different populations.*

Nematode population	Source plant	Bait plant		
		Root galls	Transmission*	Proportion**
Maiberg	*C. quinoa*	na***	0/110	< 0.010
Maiberg	*C. quinoa*	1.18	0/135	< 0.010
Talmatte + Rossberg (1:1)	*C. quinoa*	0.60	8/47	0.170
Talmatte	*N. tabacum* cv. *Xanthii*	1.32	6/28****	0.214

* Numerator, number of bait-plants from which virus was recovered; denominator, total number of bait-plants.
** Proportion virus positive plants.
*** Data not available.
**** Specimens which transmitted virus used to determine the site of virus retention within the vector.

Discussion

Longidorus nematodes recovered from the Maiberg, Baselland, site were morphologically identical with type specimens of *L. arthensis* from the Arth region. The minor size differences between specimens from the two regions are considered to represent intra- and not inter-specific variation.

Whereas *L. arthensis* are considered to occur at several sites in Baselland, CRV has not been detected in orchard trees growing at these sites. Therefore, at present CRV is known to occur only in a limited area in the Arth and nearby Steinerberg regions. Nematodes from Maiberg, Baselland which had been given access to CRV from Arth did not transmit the virus in the laboratory tests, whereas, nematodes from Talmatte, Arth and from a mixture of specimens from Talmatte, Arth and Rossberg, Steinerberg, did transmit the virus. Similar examples of differential transmission of nematode transmitted viruses by populations of their respective vector species have previously been reported, *e.g.*, serologically distinguishable strains of arabis mosaic and strawberry latent ringspot nepoviruses were differentially transmitted by populations of their associated vector nematode, *Xiphinema diversicaudatum* (Brown & Taylor, 1981; Brown & Trudgill, 1983; Brown, 1985, 1986; Taylor & Brown, 1997). The apparent lack of transmission of CRV by the Maiberg population of *L. arthensis* in our tests may reflect a very small frequency of transmission (Brown & Weischer, 1998) by this particular population, *i.e.*, a frequency less than the level of detection afforded in our experiments. Such small frequencies of transmission apparently occur with a strain of RRSV transmitted by *Paralongidorus maximus* and affecting grapevines in Germany (Jones *et al.*, 1994). Where long-term perennial plants are the virus hosts, *e.g.*, cherry trees with a life-span of 60-100 years, frequent transmission may be less important than efficient transmission. Thus, only a small proportion of the vector population need be capable of transmitting its associated virus. Alternatively, the *L. arthensis* populations in Baselland may have minor genetic differences from those in the Arth and Steinerberg regions which prevent them from acting as vectors of CRV.

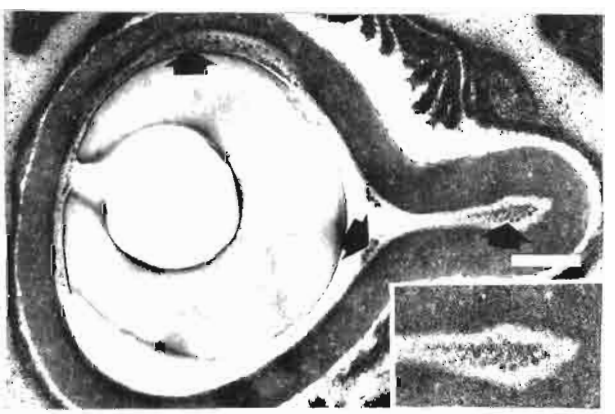

Fig. 1. *Transverse section through the anterior region of the odontostyle of a female Longidorus arthensis showing particles of cherry rosette nepovirus (V) specifically adsorbed to the food canal within the odontostyle, and, as multiple layers, adsorbed to the lining of the guiding sheath (Scale bar = 200nm; inset showing region of the guiding sheath at higher magnification; reproduced from Taylor and Brown, 1997).*

Only a few virus particles were observed adsorbed to the lining of the odontostyle in *L. arthensis*, whereas, numerous particles, frequently as multiple layers, were observed adsorbed to the lining of the guiding sheath. The specific sites of retention of nepoviruses in their associated *Longidorus* vectors, *e.g.*, artichoke Italian latent (AILV) in *L. apulus* and *L. fasciatus*, tomato black ring (TBRV) in *L. attenuatus* and *L. elongatus*, and raspberry ringspot (RRSV) in

L. elongatus and *L. macrosoma*, in each case are considered to be the inner surface of the vector nematode odontostyle (Taylor & Robertson, 1969, 1975; Harrison *et al.*, 1974; Taylor *et al.*, 1976; Brown *et al.*, 1997). However, in early studies particles of RRSV and TBRV were observed in *L. elongatus* in the space between the odontostyle and the guiding sheath (Taylor & Robertson, 1969) and initially this was considered the effective site of retention of these viruses. Particles of AILV in *L. apulus* (Taylor *et al.*, 1976), and RRSV in *L. macrosoma* (Harrison *et al.*, 1974), have also been observed in the space between the odontostyle and the guiding sheath. Therefore, it may be concluded that the effective sites of retention of nepoviruses transmitted by *Longidorus* species should be considered as both the odontostyle lumen and the space between the guiding sheath and the odontostyle.

Acknowledgements

Research at the Scottish Crop Research Institute is grant-aided from the Scottish Office of Agriculture, Environment and Fisheries Department (SOAEFD). Non-indigenous populations of nematodes and virus isolates were held under licence from SOAEFD.

References

BROWN, D.J.F. (1985). The transmission of two strains of strawberry latent ringspot virus by populations of *Xiphinema diversicaudatum* (Nematoda: Dorylaimida). *Nematol. medit.*, 13: 217-223.

BROWN, D.J.F. (1986). The transmission of two strains of arabis mosaic virus from England by populations of *Xiphinema diversicaudatum* (Nematoda: Dorylaimida) from ten countries. *Revue Nématol.*, 9: 83-87.

BROWN, D.J.F. & BOAG, B. (1988). An examination of methods used to extract virus-vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. *Nematol. medit.*, 16: 93-99.

BROWN, D.J.F., GRUNDER, J., HOOPER, D.J., KLINGLER, J. & KUNZ, P. (1994). *Longidorus arthensis* sp. n. (Nematoda: Longidoridae) a vector of cherry rosette disease caused by a new nepovirus in cherry trees in Switzerland. *Nematologica*, 40: 133-149.

BROWN, D.J.F., KYRIAKOPOULOU, P.E. & ROBERTSON, W.M. (1997). Frequency of transmission of artichoke Italian latent nepovirus by *Longidorus fasciatus* (Nematoda: Longidoridae) from artichoke fields in the Agrolis and Iria regions of Peloponnesus, Greece. Europe. *J. Pl. Path.*, 103: 501-506.

BROWN, D.J.F. & TAYLOR, C.E. (1981). Variazoni nella trasmissione di virus tra popolazioni di nematodi vettori Longidoridae. *Atti Soc. ital. Nematol. Giorn. Nematol. Firenze, 28-29 novembre 1979*: 191-204.

BROWN, D.J.F. & TRUDGILL, D.L. (1983). Differential transmissibility of arabis mosaic and strawberry latent ringspot viruses by three populations of *Xiphinema diversicaudatum* from Scotland, Italy and France. *Revue Nématol.*, 6: 229-238.

BROWN, D.J.F. & WEISCHER, B. (1998). Specificity, exclusivity and complementarity in the transmission of plant viruses by plant parasitic nematodes: an annotated terminology. *Fundam. appl. Nematol.*, 21: 1-11.

BUSER, A. (1990). *Untersuchungen über die Pfeffingerkrankheit der Süsskirsche und deren Vektor* Longidorus macrosoma *(Nematoda : Longidoridae)*. Eidgn. Techn. Hochschule Zurich, Switzerland, Dissertation No 9194, 205p.

HARRISON, B.D., ROBERTSON, W.M. & TAYLOR, C.E. (1974). Specificity of retention and transmission of viruses by nematodes. *J. Nematol.*, 6: 155-164.

JONES, A.T., BROWN, D.J.F., MCGAVIN, W.J., RUDEL, M. & ALTMAYAR, B. (1994). Properties of an unusual isolate of raspberry ringspot virus from grapevine in Germany and evidence for its possible transmission by *Paralongidorus maximus*. *Ann. appl. Biol.*, 124: 283-300.

KLINGLER, J., GUENTZEL, O. & KUNZ, P. (1983). *Xiphinema-* und *Longidorus-arthensis* (Nematoda) im Schweizerischen Mittelland. *VierteSchrift naturforsch. Gesells. Zürich*, 128: 89-114.

KLINGLER, J., KUNZ, P. & HAURI, H.P. (1985*a*). Die Verbreitung des Vektornematoden der Pfeffingerkrankheit in Kerschenanbaugebiet der Nordwestschweiz. *Schweiz. Z. Obst-Weinb.*, 121: 782-789.

KLINGLER, J., VALLOTTON, R. & GUENTZEL, O. (1985*b*). Distribution of Longidoridae and Xiphinemidae. *In*: Alphey, T.J.W. (Ed.). *Atlas of plant parasitic nematodes of Switzerland.* Dundee, UK, Scottish Crop Research Institute: 1-25.

TAYLOR, C.E. & BROWN, D.J.F. (1997). *Nematode vectors of plant viruses.* Wallingford, UK, CAB International, xii + 286 p.

TAYLOR, C.E. & ROBERTSON, W.M. (1969). The location of raspberry ringspot and tomato black ring viruses in the nematode vector, *Longidorus elongatus*. *Ann. appl. Biol.*, 64: 233-237.

TAYLOR, C.E. & ROBERTSON, W.M. (1975). Acquisition, retention and transmission of viruses by nematodes. *In*: Lamberti, F., Taylor, C.E. & Seinhorst, J.W. (Eds). *Nematode vectors of plant viruses.* London & New York, Plenum Press: 253-276.

TAYLOR, C.E., ROBERTSON, W.M. & ROCA, F. (1976). Specific association of artichoke Italian latent virus with the odontostyle of its vector, *Longidorus attenuatus*. *Nematol. medit.*, 4: 23-30.