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Transmission of a Strain of Tomato Black Ring Virus by *Longidorus elongatus* (Nematoda)

B. D. HARRISON

Rothamsted Experimental Station, Harpenden, Hertfordshire, England

AND W. P. MOWAT AND C. E. TAYLOR

Scottish Horticultural Research Institute, Invergowrie, by Dundee, Scotland

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Longidorus elongatus (de Man) was always found in soil from patches in raspberry and strawberry plantations where the plants were infected with the beet ringspot (Scottish) strain of tomato black ring virus but rarely elsewhere in the same plantations. *L. elongatus* hand-picked from virus-containing field soil transmitted the virus to sugar beet, turnip, and spinach seedlings, but did so less often than might have been expected from the infectivity of the whole soil from which the nematodes came. Noninfective *L. elongatus* acquired and subsequently transmitted the virus from infected cucumber and potato plants. When hand-picked adult and larval *L. elongatus* were used separately, only larvae transmitted. Also, when infective soil was fractionated by sieving through meshes of successively smaller size, the infectivity of the fraction retained on each sieve, relative to the number of *L. elongatus* it contained, was greatest for samples containing the smallest larvae. A previously undescribed species of *Longidorus*, closely related to *L. elongatus*, was associated with an outbreak of the lettuce ringspot (English) strain of tomato black ring virus.

INTRODUCTION

Tomato black ring virus is one of a group of soil-borne viruses which are serologically unrelated but have many properties in common. They cause ringspot symptoms in several of their hosts, have a wide host range, resemble one another in their *in vitro* properties, and have polyhedral particles about 30 mμ in diameter (Harrison and Nixon, 1960). Three strains of tomato black ring virus, distinguished by their antigenic constitution, occur, respectively, in England, Germany, and Scotland (Harrison, 1958a). Three kinds of evidence suggest that a soil-inhabiting organism plays a part in transmitting the beet ringspot (Scottish) strain (Cadman and Harrison, 1960): (1) susceptible seedlings did not become infected when grown in autoclaved soil to which infective sap or washed infected roots were added; (2) the infectivity of field soils was abolished by chemicals which did not inactivate the virus *in vitro*; and (3) only some virus-free field soils became infective when cropped with artificially infected plants, and these did not become infective after autoclaving. In experiments made to get evidence on the nature of this organism, Cadman and Harrison (1960) obtained a few infections in seedlings grown in autoclaved sand to which crude suspensions of unidentified nematodes from naturally infective soil had been added, but they later failed to obtain infections with nematode preparations free from soil particles. We now describe observations and experiments which indicate that the nematode *Longidorus elongatus* (de Man) is a vector of the beet ringspot strain of tomato black ring virus.

METHODS

Soils were collected in eastern Scotland from fields where outbreaks of diseases

caused by the beet ringspot strain of tomato black ring virus (TBRV) occurred in raspberry and strawberry crops. Soils from Glendevon, Kinross, and Elgin, Morayshire, were used as the sources of nematodes. *L. elongatus* was extracted from soils by the sieving method used by Harrison and Cadman (1959) for the nematode *Xiphinema diversicaudatum* (Micoletzky), which is of similar size. Suspensions of soil in water were first passed through a sieve of 2-mm pore size to remove large pieces of debris, and then through a 250-μ sieve, which retained most of the *L. elongatus*. *L. elongatus* were picked out of a suspension of the material in the sieve extracts, transferred to distilled water, and then added to pots containing sedge peat or steamed potting soil (John Innes potting compost no. 2); in each pot 5–10 seedlings of a species susceptible to the virus were grown. When the incidence of infection among these bait seedlings was determined 4–6 weeks later, the roots of all bait plants in a pot were combined to give one sap extract for inoculating leaves of *Chenopodium amaranticolor* Coste & Reyn., which showed local lesions in 5 or 6 days and systemic symptoms within 2 weeks. In some experiments similar extracts were also made from hypocotyls. All transmission experiments were made in insect-proof glasshouses kept at a temperature of about 20°.

RESULTS

Distribution of Nematodes at Virus Outbreaks

Although TBRV infects many different crop and weed species when these are grown in naturally infective soil, disease outbreaks of economic importance have been reported only in raspberry and strawberry (Harrison, 1958b; Lister, 1960). A patchy distribution of diseased plants is characteristic of outbreaks where infection has been contracted from the soil. Nematode suspensions were prepared from soil in a patch carrying diseased plants and from soil in an adjacent area carrying healthy plants. Only the soil carrying diseased plants contained *L. elongatus*, so the distribution of this species in relation to that of patches of TBRV-infected plants was then examined in several other widely separated raspberry and strawberry plantations. *L. elongatus* was usually numerous in soil collected near infected plants but was not found or was rare in soil from parts of the plantations where the plants appeared healthy (Table 1). The soil samples most infective for turnip seedlings were also those that contained most *L. elongatus*. *L. elongatus* was also found in all the other TBRV-containing soils examined and in soil in which TBRV became established as a result of cropping it with artificially infected plants.

TABLE 1

ASSOCIATION OF *Longidorus elongatus* WITH OUTBREAKS OF DISEASES CAUSED BY TOMATO BLACK RING VIRUS

Locality	Crop	Area affected by TBRV		Healthy area	
		L. elongatus in 250 ml. soil	Infectivity of soil[a]	*L. elongatus* in 250 ml soil	Infectivity of soil[a]
Glendevon, Kinross	Strawberry	120	10/10	0	0/10
Blairgowrie, Perthshire	Strawberry	84	10/10	0	0/10
Coupar Angus A, Perthshire	Strawberry	51	10/10	1	0/10
Barry, Angus	Raspberry	21	4/10	0	0/10
Coupar Angus B, Perthshire	Raspberry	2	2/10	3	0/10

[a] Turnip bait plants were grown in ten 250-ml portions of each soil: numerator is the number of lots of plants that became infected with TBRV; denominator is the total number of lots exposed to infection.

Experiments with Hand-picked Nematodes

Transfer of L. elongatus from infective soil to bait plants. Batches of *L. elongatus* were hand-picked from 250-μ sieve extracts of TBRV-containing Glendevon soil and added to pots of peat or potting soil. The residues of the sieve extracts after the *L. elongatus* were removed were added to peat or potting soil in other pots. Bait seedlings were grown in the pots and tested for the presence of virus 4–6 weeks later. Table 2 shows that TBRV was transmitted in several of the pots with *L. elongatus* but in none of those with the extract residues. Several of the isolates of virus from the bait seedlings were subcultured by inoculation of sap from the *C. amaranticolor* assay plants to tobacco, *Petunia hybrida*, cucumber, *Phaseolus vulgaris*, and tomato, in all of which the symptoms produced were typical of tomato black ring virus (Smith, 1946; Harrison, 1957). Also, partially purified preparations (Harrison and Nixon, 1960) of these viruses reacted specifically with antiserum to the beet ringspot strain of the virus in precipitin tests made in tubes.

TABLE 2

TRANSMISSION OF TOMATO BLACK RING VIRUS BY HAND-PICKED *Longidorus elongatus* FROM NATURALLY INFECTIVE SOIL[a]

Expt. no.	Material added to peat or potting soil	Total number *L. elongatus* added	Infection of bait plants[b]
1	*L. elongatus*	280	4/7
	Residues of extracts	—	0/4
	None	0	0/7
	Undiluted Glendevon soil	450[c]	3/3
2	*L. elongatus* larvae	405	4/11
	L. elongatus adults	81	0/6
	Residues of extracts	—	0/11
3	*L. elongatus*	1480	3/20
	None	0	0/15
	Undiluted Glendevon soil	2400[c]	16/16
	Glendevon soil diluted with 3 parts sand	525[c]	12/14

[a] Soil from Glendevon.

[b] Bait plants (spinach in experiment 1, sugar beet in experiment 2, and turnip in experiment 3) were grown in 300-ml portions of peat or soil. Numerator is the number of lots of bait plants that became infected with TBRV; denominator is the total number of lots exposed.

[c] Numbers estimated by counts of nematodes from duplicate aliquots of soil.

Most batches of Glendevon soil contained about 150 *L. elongatus* per 300 ml and were highly infective; some bait seedlings usually became infected in each 300-ml aliquot of soil; diluting the soil with three parts of sand had little effect on its infectivity. Hence, in several experiments it seemed clear that fewer transmissions were obtained with hand-picked *L. elongatus* than could be expected if it was the only vector species in the soil (e.g. Expt. 3, Table 2). However, attempts to transmit TBRV with other species of nematodes from naturally infective soils failed, and the rate of transmission by hand-picked *L. elongatus* was not increased by adding to pots the roots of systemically infected plants.

L. elongatus which were hand-picked from Baermann-funnel extracts of 250-μ-sieve extracts, instead of from the sieve extracts themselves as in the experiments summarized in Table 2, failed to infect bait seedlings when 28 pots were each infested with 60–80 *L. elongatus*, although a very few seedlings became infected when complete Baermann-funnel extracts were used. Evidently the transmitting ability of infective *L. elongatus* is decreased or abolished by the Baermann-funnel extraction process.

Use of infector plants. Lots of 60 *L. elongatus* from Baermann-funnel extracts of Elgin or Glendevon soils were added to potting soil containing cucumber seedlings, of which those in half the pots had been inoculated with TBRV 3 weeks previously. Four weeks after adding the nematodes, the cucumber shoots were cut off at the junction of stem and root, and turnip seed planted. Four weeks after sowing, the incidence of infection among the turnip bait plants was determined, using hypocotyls only. Hypocotyls were infected in four of the eight pots containing both infected cucumber plants and *L. elongatus* from Glendevon soil, but in none of the pots of the other treatments.

This shows that these nematodes can acquire and transmit the virus within 8 weeks, and suggests that the conditions needed for the transmission of TBRV may vary with different populations of *L. elongatus*.

In another experiment, Elgin soil was cropped with TBRV-infected potato plants and *L. elongatus* extracted at weekly intervals, beginning 3 weeks after planting the potatoes. Initially, the soil had little infectivity, but each 400 ml contained about 60 *L. elongatus*, the intestines of most of which appeared almost empty. After 3 weeks on potato, many of the *L. elongatus* had more in their intestines than initially, indicating that they had fed on potato. TBRV was not transmitted to spinach bait plants by the *L. elongatus* transferred after 3 weeks, but was by those transferred after each subsequent week (Table 3). Two transmissions occurred in pots to which the extract residues were added, but undetected *L. elongatus* may have been responsible for these, because the large amount of organic debris in the extracts made it difficult to find and remove every specimen. Table 3 also shows that, although the larval *L. elongatus*, except the lot transferred after 3 weeks on potato, transmitted the virus, the 176 adults failed to transmit (most of the adults used were females, the sex ratio among adults being about 1 male: 15 females). By the formula

$$P = 1 - \sqrt[n]{Q}$$

where P is the probability that a single nematode transmitted and Q is the proportion of batches of bait plants that did not become infected when infested with n nematodes per batch, it is calculated that an average of 1 in 37 larvae transmitted. This difference in behavior between larvae and adults contrasts with the results obtained with grapevine fanleaf virus and *Xiphinema index*, adults and larvae of which transmit with equal facility (Raski and Hewitt, 1960), and with arabis mosaic virus and *X. diversicaudatum*, adults of which transmit at least as well as larvae (Harrison and Cadman, 1959; Harrison, unpublished).

Fractionation of Infective Soil

When infective Glendevon soil was fractionated by elutriation and sieving through meshes of successively smaller size, all the fractions collected were infective, although the great majority of *L. elongatus* were retained on the 250-μ sieve (Table 4). A notable feature of the results is the relatively high infectivity of the material retained on the 44-μ sieve; this contained only a few small larval *L. elongatus*, which again suggests that, if *L. elongatus* is the only vector in the soil, small larvae transmit TBRV very efficiently.

TABLE 3

TRANSMISSION OF TOMATO BLACK RING VIRUS BY HAND-PICKED *Longidorus elongatus* FROM SOIL CROPPED FOR VARIOUS PERIODS WITH INFECTED POTATO PLANTS[a]

Weeks between planting potato tubers and extracting nematodes	Material added to peat, and transmissions obtained				Volume of soil used (ml)
	L. elongatus			Residues of extracts	
	Adults		Larvae		
3	0/3	(42)[b]	0/4 (73)	—	800
4	0/3	(36)	2/3 (56)	0/4	800
5	0/2	(19)	1/2 (36)	0/2	400
6	0/5	(59)	2/4 (91)	1/5	800
7	0/1	(20)	1/1 (30)	1/2	400
Total	0/14	(176)	6/14 (286)	2/13	3200

[a] *Solanum tuberosum* L. var. Kerr's Pink.

[b] Numerator is the number of lots of spinach bait plants infected; denominator is the total number of lots exposed. Figures in parentheses are the total numbers of *L. elongatus* added on each occasion.

TABLE 4

INFECTIVITY AND *Longidorus elongatus* CONTENT OF SOIL FRACTIONS OBTAINED BY SIEVING NATURALLY INFECTIVE SOIL[a]

Sieve on which retained (pore size in μ)	Infectivity of fraction[b]	Average number *L. elongatus* per sample (adults: larvae)
250	14/14	23:165
74	12/14	2:31
44	6/10	0:2

[a] Samples (500 ml) of Glendevon soil were fractionated.

[b] Numerator is the number of samples in which turnip bait plants became infected; denominator is the total number of lots exposed to infection.

DISCUSSION

Because nematodes of the genus *Xiphinema* transmit grape fanleaf, arabis mosaic, and peach yellow bud mosaic viruses (Hewitt *et al.*, 1958; Jha and Posnette, 1959; Harrison and Cadman, 1959; Breece and Hart, 1959), all of which resemble TBRV in their general properties, a nematode vector of TBRV is not unexpected. *Longidorus elongatus* is the first member of its genus to be implicated in virus transmission, but species of *Longidorus* have many similarities with *Xiphinema* spp. and are grouped with them in the subfamily Tylencholaiminae (Clark, 1961). Our conclusion that *L. elongatus* is a vector of TBRV fits well with previous work on the mode of transmission of the virus (Harrison, 1958b; Cadman and Harrison, 1960), and with the fact that *L. elongatus* was numerous only in those parts of fruit plantations where the plants were infected with TBRV, a parallel with the distribution of arabis mosaic virus and *Xiphinema diversicaudatum*. *Xiphinema* spp. were not found in any of the TBRV-containing soils.

The relatively low rates of transmission by hand-picked *L. elongatus* can of course be readily explained by postulating an additional vector, but we have failed to obtain any direct evidence in favor of this idea. It seems more likely that the procedures involved in extracting and handling the nematodes impair their activity or infectivity. Extraction in Baermann funnels seems particularly detrimental. Adult *L. elongatus* may have failed to transmit TBRV in our experiments because they were more affected than larvae by the experimental procedures. Equally, however, the difference found between the transmitting ability of larvae and adults may reflect their behavior in undisturbed soil.

Recent observations show that soil from Norwich, Norfolk, containing the lettuce ringspot (English) strain of tomato black ring virus (Smith and Short, 1959) contains

TABLE 5

NEMATODE SPECIES IMPLICATED AS VECTORS OF PLANT VIRUSES

Virus	Vector	Reference
Polyhedron-shaped viruses		
Arabis mosaic, grapevine fanleaf strain	*Xiphinema index*	Hewitt *et al.* (1958)
Arabis mosaic, type strain	*Xiphinema diversicaudatum*	Jha and Posnette (1959); Harrison and Cadman (1959)
Tomato ringspot, peach yellow bud mosaic strain	*Xiphinema americanum*	Breece and Hart (1959)
Tomato black ring, Scottish (beet ringspot) strain	*Longidorus elongatus*	Present work
Tomato black ring, English (lettuce ringspot) strain	Probably *Longidorus* n. sp.	Present work
Rod-shaped virus		
Tobacco rattle, Dutch culture	*Trichodorus pachydermus*	Sol *et al.* (1960)
Tobacco rattle, English culture	*Trichodorus primitivus*	Harrison, unpublished

a previously undescribed species of *Longidorus*, which is closely related to *L. elongatus* and will be described elsewhere by D. J. Hooper. Thus, a distinct nematode is associated with a distinct form of the virus and is probably its vector. Indeed, when the range of species of nematodes is considered in relation to the viruses they transmit, it is seen that the degree of similarity between different viruses resembles the degree of systematic relationship between their vectors, that is, the more distantly related are the viruses, the more distantly related also are their vectors (Table 5).

All the nematodes yet implicated as vectors belong to the Order Enoplida, but they fall into two major groups, which correspond to the two major groups of viruses they transmit. The viruses with polyhedral particles cause symptoms of the ringspot type, have many other properties in common, and have vectors belonging to two closely related genera in the superfamily Dorylaimoidea, whereas the rod-shaped tobacco rattle virus, which differs in many properties from the polyhedron-shaped ringspot viruses, is transmitted by *Trichodorus* spp. in the superfamily Diphtherophoroidea. Within the group of viruses with polyhedral particles, the grapevine fanleaf and "type" strains of arabis mosaic virus, which have some of their antigens in common (Cadman *et al.*, 1960), have closely related species of *Xiphinema* as vectors, whereas the more distantly related *X. americanum* transmits one distinct virus and species of *Longidorus* transmit another. Observations on crops and soils, too, support the idea that the spread of each major antigenic variant of these viruses depends on the presence of a specific nematode, but this apparent specificity still needs confirmation by experiment.

ADDENDUM

D. J. Hooper is describing the *Longidorus* sp. from Norwich as *L. attenuatus* n.sp. (*Nematologica* **6,** in press); we have now shown that this species is a vector of the lettuce ringspot (English) strain of tomato black ring virus.

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