Ingestion, Retention, and Transmission of two Strains of Raspberry Ringspot Virus by Longidorus macrosoma

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Abstract: The transmission of two strains of raspberry ringspot virus (RRV) by small numbers of nematodes was compared. A strain of RRV from Scotland (RRV-S), originally found in the field associated with *Longidorus elongatus*, was transmitted frequently by *L. elongatus* but only once by *L. macrosoma*. A strain from England (RRV-E) associated with *L. macrosoma* in the field was transmitted infrequently by each species of nematode. The reasons why *L. macrosoma* infected only a small proportion of bait plants with virus were investigated, and it was found that most of the nematodes tested had fed on the source plants and many had ingested virus. Most nematodes exposed to RRV-E or RRV-S had fed on the roots of the bait plants and, when thin sections were examined by electron microscope, had retained particles (thought to be those of the virus) in the region of the anterior odontostyle. Thus, most nematodes seem to have had ample opportunity to transmit virus, and the low frequency of transmission may have been due to a failure of the virus particles to be released from the site of retention or to a lack of infectivity of the virus when *L. macrosoma* was the vector and *Petunia hybrida* was the host.
Key Words: Longidorus elongatus, gall formation, *Stellaria media*, *Petunia hybrida*, retention of virus.

A strain of raspberry ringspot virus (RRV-E) from blackberry in England differs serologically from a strain (RRV-S) from Scotland (1). *Longidorus elongatus* de Man from Scotland transmitted both strains (9) whereas *Longidorus macrosoma* Hooper, associated with RRV-E in the field (2, 4), transmitted RRV-S rarely or not at all. The geographical distribution of the two strains of RRV can be related to that of their specific nematode vector. In an extensive survey of the British Isles, Taylor and Brown (8) recovered *L. macrosoma* only from central and southern England, whereas *L. elongatus*, although widespread in England, was more frequent in soil samples from Scotland. Surprisingly, no naturally occurring populations of *L. macrosoma* and only a few populations of *L. elongatus*, all from Scotland, were found to be carrying virus. Those findings prompted us to conduct a more detailed investigation of the efficiency of transmission of RRV-E and RRV-S by *L. macrosoma*. Frequency of transmission was compared with opportunity to transmit by assessing feeding on the infector and bait plants, the proportions of nematodes ingesting virus, and the numbers retaining virus on the odontostyle.

MATERIALS AND METHODS

The viruses used were a culture of raspberry ringspot virus from blackberry from England (RRV-E) (1) and a culture from raspberry in Scotland (RRV-S). Both were maintained by manually inoculating plants in the glasshouse.

The viruses were detected in bait plant roots by inoculating extracts to *Chenopodium quinoa* Willd. assay plants, and were differentiated by symptoms produced in *Petunia hybrida* Vilm. (4) or by serology. Contamination of bait plant roots by virus in nematode bodies or in their faeces (5) was minimised by washing the roots and examining them under a binocular microscope before making root extracts and inoculating them to the assay plants. The tops of the bait plants were usually not tested since RRV-E takes several weeks to become systemically translocated from roots to leaves within *P. hybrida* (McNamara, personal communication).

Plants to be used as acquisition sources were inoculated with virus 2 days before being transplanted to pots containing virus-free nematodes except in the final experiment, in which they were inoculated one month before use. *P. hybrida* seedlings or, in one experiment, *Stellaria media* (L) Vill. were used as both source and bait plants.

Two populations of *L. macrosoma* were tested initially, one from raspberry at Bury St Edmunds, Suffolk, and the other from Harwell, Essex. The *L. elongatus* used as a comparison in the final experiment were from grassland at Ballo Hill, Perthshire. All populations, were maintained in the glass-

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house for 2 to 3 years, were bait-tested and found to be free of virus. In addition nematodes from the Harwell population were shown to be initially free of virus by grinding-up and inoculating groups of 5 or 20 nematodes directly to *C. quinoa* (slash-testing) and by examining sections through the odontostyle region of 4 nematodes with the electron microscope.

Groups of 30-40 nematodes were exposed for 1 month to virus-infected source plants growing in a 3:1 mixture of sieved sand and sterile loam in 25-ml plastic pots without drainage holes. The pots were partially sunk in moist sand in bins, covered with a plate-glass sheet to minimise transpiration, and maintained at 18 C in a temperature-controlled cabinet (7) in which natural light was supplemented by mercury-vapour lamps to extend the day to 16 h. Only nematodes from source plants known to be infected with virus were used in the subsequent tests.

Groups of hand-picked nematodes from the source plants were exposed for 1 month to virus-free bait plants under conditions similar to those for the source plants. The proportion of these nematodes that transmitted virus was estimated with precision by bait-testing replicated groups of 2, 5, and 20 nematodes.

Nematode feeding on both source and bait plants, a prerequisite for acquisition and transmission of virus, was assessed by counting the numbers of nematode-induced root-tip galls. Also determined were the numbers of nematodes surviving each part of the experiment.

In two experiments the proportion of nematodes ingesting virus from the infector plants was assessed by grinding groups of 2 or 5 nematodes between ground-glass slides in a drop of phosphate buffer (pH 7.0) and inoculating the extract to *C. quinoa* assay plant leaves (slash-testing) (6). When slash-tests were done, nematodes were taken concurrently from the same group of source plants for slash-testing and for testing on bait plants. In the final experiment the heads of 20 *L. macrosoma* exposed to each virus, whose bodies had been individually slash-tested, were retained for sectioning and study with the electron microscope by procedures described by Taylor and Robertson (10).

EXPERIMENTS AND RESULTS

Preliminary results suggested that *L. macrosoma* transmitted RRV-E only infrequently and RRV-S not at all. The frequency of transmission of RRV-E was similar for the two populations of *L. macrosoma* tested and was not increased when *S. media* was the source and bait plant instead of *P. hybrida*.

The low frequency of transmission of RRV-E in these experiments did not seem to be due to a lack of feeding on the source and bait plants, for the numbers of root-tip galls almost equalled the numbers of nematodes recovered. Survival of the *L. macrosoma* on the bait plants was poor, however.

Direct evidence was lacking that the *L. macrosoma* used had adequate access to the virus in the source plants. Therefore, the frequency of transmission of RRV-E by groups of 2 or 5 *L. macrosoma* was compared with the frequency with which virus was recovered when comparable groups of nematodes were slash-tested (Table 1). In this experiment the numbers of galls induced on the roots of the bait plants suggested that most of the nematodes tested had fed, yet, again, virus was transmitted only infrequently. Even though slash-testing is a rather insensitive technique (see Table 4), virus was recovered more frequently in the slash-test than in the bait-test.

These results suggest that the low frequency of transmission was due either to a lack of retention of virus at the specific site

TABLE 1. A comparison of the frequency with which RRV-E was recovered when small groups of *Longidorus macrosoma* (Harwell population) were slash- and bait-tested.

	Slash-test		Bait-test	
Number of nematodes in each test	2	5	2[a]	5[b]
Number of replicates	20	15	20	15
Number from which virus was recovered	5	7	0	3

[a]A mean of 1.0 *L. macrosoma* per plant was recovered at the end of the bait-test, and a mean of 2.6 galls per plant had been induced.

[b]A mean of 3.0 *L. macrosoma* per plant was recovered at the end of the bait-test, and a mean of 6.3 galls per plant had been induced.

of retention on the odontostyle (11) or to an inability of the nematodes to infect the bait plants with that virus. These possibilities were investigated in a further experiment (results in Tables 2-5). In this experiment the proportion of bait plants that were infected with RRV-E or RRV-S by small numbers of well-fed *L. macrosoma* (Harwell population) was again compared with the proportion of *C. quinoa* assay plants that were infected when comparable groups of nematodes were slash-tested. In addition, virus retention within the feeding apparatus of the nematodes was determined by sectioning and examining, by electron microscope, the odontostyle regions of eight *L. macrosoma* exposed to each virus.

Most of the *L. macrosoma* survived on the source plants, but fewer root-tip galls were formed than in previous experiments (Table 2). Even so, many nematodes had ingested virus, for virus was detected in 80-85% of groups of 5 *L. macrosoma* slash-tested (Table 3). When 20 individual nematodes, their heads first having been removed and preserved, were slash-tested, RRV-E was detected in 7 nematodes, and RRV-S in 4 (Table 4). To determine the proportion of nematodes that had retained virus, some of the heads from these nematodes were sectioned and examined by electron microscope. All heads whose bodies were positive for virus in the slash-test were found to have retained many particles in the region of the odontostyle, thought to be particles of the virus (Fig. 1). In addition, when nematodes were examined which had been negative for virus in the slash-test, virus-like particles were found to be retained in 1 of 3 nematodes exposed to RRV-E and in 3 of 4 nematodes exposed to RRV-S (Table 4).

TABLE 2. Survival of *Longidorus macrosoma* (Harwell population) and gall formation on the virus source plant *(Petunia hybrida)*.

Virus	Number of nematodes added per plant	Mean number of nematodes recovered per plant	Mean number of galls formed per plant
RRV-E	30	24	5
RRV-S	40	34	9

TABLE 3. Ingestion of RRV-E and RRV-S by *Longidorus macrosoma* (Harwell population) as determined by slash-testing.

	RRV-E	RRV-S
Number of nematodes in each slash-test	5	5
Number of replicates	15	15
Number which contained virus	12	13

Table 5 presents results from bait-testing groups of *L. macrosoma* that are directly comparable with results from the slash-tests. The nematodes survived well on the *P. hybrida* bait plants and induced many root-tip galls. Nevertheless, only a small proportion of the bait plants became infected with virus. With groups of 5 *L. macrosoma* per bait plant, only 2 of 15 bait plants were infected with RRV-E, and 1 of 15 with RRV-S. In contrast, when groups of 5 *L. elongatus* previously exposed to RRV-S were bait-tested to provide a comparison, 17 of 20 bait plants were infected with the

TABLE 4. Electron-microscope examination of the odontostyle region of individual *Longidorus macrosoma* following slash-testing of their bodies.

Virus	Slash-test, bodies of single nematodes		Proportion of nematodes with virus particles retained on odontostyle. (E.M.)	
	Positive	Negative	Body positive for virus in slash-test	Body negative for virus in slash-test
RRV-E	7/20[a]	13/20	4/4[b]	1/3
RRV-S	4/20	16/20	4/4	3/4

[a]The fraction shows the number of nematodes from whose bodies virus was recovered, over the number tested.

[b]The fraction shows the number of nematodes with virus-like particles retained in the region of the odontostyle, over the number examined by electron microscope.

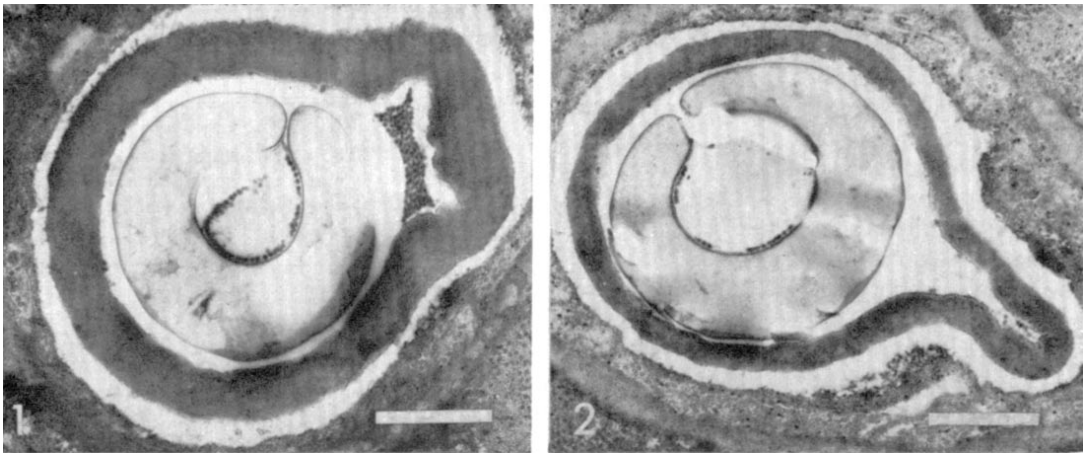

FIG. 1-2. Electronmicrographs of cross-sections of *Longidorus macrosoma* showing virus-like particles retained within the lumen of the odontostyle and between the odontostyle and guide-sheath. Nematodes exposed to: *1)* raspberry ringspot virus English strain (RRV-E); *2)* raspberry ringspot virus, Scottish strain (RRV-S). The bars represent 0.5 μm.

virus but only 2 of 20 bait plants were infected by nematodes previously exposed to RRV-E.

DISCUSSION

The techniques used were designed to minimise contamination of bait plant roots and to provide as much information as possible on the progress of each stage of the test. They assessed the numbers of nematodes surviving, the numbers of galls induced on the source and bait plants, and the ingestion, retention, and transmission of virus.

With these techniques our results showed that the *L. macrosoma* tested had adequate opportunity to transmit virus but transmitted RRV-E only infrequently and, as found by Harrison (4), RRV-S only rarely. Our results also confirmed observations of Taylor and Robertson (11) that *L. macrosoma* exposed to RRV-E and RRV-S retained particles of each virus equally well in the odontostyle lumen and between the odontostyle and the guiding sheath.

The probability of a nematode's being infective in the slash or bait test is given by the equation $P = 1 - \sqrt[n]{Q}$, where Q is the proportion of plants not infected with virus when exposed to n nematodes per test. With this equation it was calculated from the results shown in Table 5 that fewer

TABLE 5. Survival, gall formation, and transmission of RRV-E and RRV-S by groups of *Longidorus macrosoma* (Harwell population) and *Longidorus elongatus* on *Petunia hybrida* bait plants.

Species and virus	Number of nematodes tested	Mean number of nematodes recovered	Mean number of galls formed	Virus transmission
Longidorus macrosoma				
	2	1.3	1.8	2/20[b]
RRV-E	5	4.0	5.7	2/15
	20	12.8[a]	15.0	6/10
RRV-S	5	3.9	5.4	1/15
	20	8.7[a]	12.1	0/10
Longidorus elongatus				
RRV-E	5	3.1	6.1	2/20
RRV-S	5	2.9	7.0	17/20

[a]These groups of nematodes were slash-tested at the end of the bait-test. 10/10 were infective for RRV-E, 1/10 was infective for RRV-S.

[b]Fraction shows the number of bait plants infected, over number exposed.

than 5% of the *L. macrosoma* tested were able to infect a bait plant with RRV-E. Even if allowance is made for nematodes that died during the bait-test, fewer than 8% were infective. For *L. macrosoma* carrying RRV-S the proportion of nematodes able to infect bait plants with virus was even less, with perhaps as few as 1 in 300 being infective. In contrast, the results obtained from slash-testing and examining thin sections through the odontostyle region of *L. macrosoma* (Tables 3, 4) showed that many of the nematodes exposed to virus-infected source plants had ingested and retained virus. From the slash-test results (Table 3) it was calculated that about 30% of the *L. macrosoma* had ingested virus from the source plants, while the results with the electron microscope (Table 4) were consistent with a retention of both RRV-E and RRV-S by more than 50% of the *L. macrosoma*.

These results, taken together, suggest that many *L. macrosoma* with virus particles retained on their odontostyle failed to infect bait plants with virus. The relative ease with which virus was recovered from *L. macrosoma* in the slash-tests and the efficiency with which *L. elongatus* transmitted RRV-S to *P. hybrida* suggests that a lack of release of virus particles from the sites of retention may be one of the factors decreasing the frequency of transmission of RRV-E and RRV-S by *L. macrosoma*. Direct evidence is lacking, however, and other factors may be involved, such as the apparent poor host status of *P. hybrida* for *L. macrosoma*.

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