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Frequency of transmission of artichoke Italian latent nepovirus by *Longidorus fasciatus* (Nematoda: Longidoridae) from artichoke fields in the Iria and Kandia areas of Argolis in northeast Peloponnesus, Greece

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Abstract

Artichoke Italian latent nepovirus (AILV) transmitted by *Longidorus fasciatus* is a causative agent of artichoke patchy chlorotic stunting (APCS) in northeast Peloponnesus in southern Greece. Populations of *L. fasciatus* collected from the Iria and Kandia areas of Argolis in northeast Peloponnesus were used in laboratory experiments to determine the frequency of transmission of the virus by its natural vector. One tenth to almost one half of the *L. fasciatus* specimens recovered from soil collected in two artichoke fields showing APCS transmitted AILV. Allowing nematodes access for 4 wk to *Nicotiana clevelandii* mechanically infected with AILV did not increase the number of individual specimens able to transmit virus. The total number of specimens transmitting virus in an experiment did not exceed fifty percent of the individuals tested. Virus-like particles were only observed adsorbed to the inner surface of the odontostyle and it is suggested that the high frequency of transmission of AILV by *L. fasciatus* is a result of efficient dissociation of virus particles from the specific sites of retention in the vector.

Introduction

An economically important 'yellowing' disease of artichoke plants growing in the Argolis area of northeast Peloponnesus in southern Greece was described as artichoke patchy chlorotic stunting (APCS) by Kyriakopoulou (1985). The disease affects the local thornless artichoke cultivar Prassini Argous, which is exclusively grown in the area. Diseased plants occur in patches and are characterized by general chlorosis and stunting. Affected crops are unproductive and the cultivation period is considerably shorter than the average of five years for artichoke grown in the region. Patches of plants showing APCS extend slowly each year, indicating a soil-borne mode of transmission.

A strain of artichoke Italian latent nepovirus (AILV), serologically distinguishable from the Italian type strain which is transmitted by *Longidorus apulus* in southeastern Italy, was isolated from artichoke showing symptoms of APCS (Rana and Kyriakopoulou, 1982). Soil samples from the rhizosphere of the diseased plants contained large numbers of *L. fasciatus* Roca and Lamberti, 1981. In laboratory experiments AILV was recovered from 3 of 20 *Nicotiana tabacum* White Burley plants to which hand-picked groups of 15 *L. fasciatus*, recovered from soil collected from around the roots of diseased artichoke plants, had been given access for 4 to 5 wk (Roca et al., 1982).

An investigation of the aetiology of APCS disease in artichoke fields in the Iria and Kandia regions of Argolis in northeast Peloponnesus, Greece, revealed that the disease was associated with AILV transmitted by *L. fasciatus* (Kyriakopoulou, 1996). However, frequency of transmission of AILV by its natural vector was not investigated (Roca et al., 1982). In the present study *L. fasciatus*, collected from fields in Iria and Kandia in which artichoke plants were showing typical APCS disease symptoms, were used in laboratory experiments to determine the frequency of trans-

mission of AILV by this vector and these studies are reported in this paper.

Materials and methods

Sampling, nematode extraction and vector transmission tests

Bulk samples of soil were collected at 10 to 50 cm depth from patches of artichoke plants showing typical symptoms of APCS disease at Iria and Kandia southern Greece. The samples were transported by air to the Scottish Crop Research Institute, Scotland and upon arrival placed in a cold room at 4 °C. *Longidorus fasciatus* were extracted by a decanting and sieving method (Brown and Boag, 1988) and bait-tested, following the procedure described by Trudgill et al. (1983). Hand-picked groups of 5 adult females and of 20 juveniles (mainly 1st and 2nd developmental stages) were added to 25 cm^3 plastic pots containing an air-dried, sieved sand and soil mixture, with a particle size $<2000\mu$m and $>500\mu$m. A single *Nicotiana clevelandii* seedling was planted in each pot and the pots were then placed in a temperature-controlled cabinet, operating at 20 °C, for 4 wk. The nematodes were recovered from the pots and counted. The roots of the *N. clevelandii* bait-plants were washed thoroughly and root tip galls, evidence of nematode feeding, were counted.

In addition, single females were bait-tested following the procedure of Brown et al. (1989) with individual nematodes placed in 0.5cm^3 plastic capsules containing a *N. clevelandii* bait seedling. After 10 d the contents of each capsule were washed into a counting dish, the nematode was recovered and root-tip galls counted. The bait seedling was then placed in a compost block and grown for a further 4 wk.

The roots of the bait-plants from both bait-test procedures were comminuted using a mortar and pestle and the suspension rubbed by finger on the leaves of *Chenopodium amaranticolor* and *C. quinoa* virus assay plants that had been lightly dusted with corundum abrasive powder. After 14 d, the assay plants were examined for the presence of virus infection *viz.* local lesions on their inoculated leaves and evidence of systemic infection. The leaves from a random selection of *C. quinoa* plants showing systemic symptoms of virus infection were harvested and used in Ouchterlony gel-diffusion serological tests with an antiserum prepared against the Greek strain of AILV (AILV-G) to confirm the identity of the virus.

In a separate experiment groups of 40 nematodes recovered from the bulk of soil from Kandia were added to 25 cm^3 pots each containing a *N. clevelandii* plantlet, the leaves of which 2 d previously had been mechanically inoculated with AILV-G, originally recovered from the roots of a White Burley *N. tabacum* bait-plant growing in soil from Kandia. The nematodes were allowed access to the virus infected plants for 4 wk, recovered from the pots and groups of 5 individuals were bait tested as described above.

Electron microscopy

Hand-picked groups of 25 females recovered from soil from Kandia were added to each of three 25cm^3 pots containing *N. clevelandii* plantlets, the leaves of which 2 d previously had been mechanically inoculated with AILV-G. After 4 wk the nematodes were recovered from the pots and individuals were examined by electron microscopy to determine the sites of retention of virus particles within the nematodes feeding apparatus. The remaining nematodes were individually assessed for their ability to transmit virus to *N. clevelandii* bait-plants using the procedure described above.

The specimens were fixed in 3% glutaraldehyde and the oesophageal region severed with a transverse cut, made with a scalpel blade, just posterior to the oesophageal/intestinal junction. The oesophageal region was then post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol series, followed by infiltration in Emix resin, which was polymerised at 70 °C, by the method of Robertson and Henry (1986). Specimens were sectioned transversely at approximately 60nm thick using a Reichart Ultracut. The sections were stained with alcoholic uranyl acetate, followed by lead citrate, by the method of Robertson and Roberts (1972), and examined in a JEOL 200 electron microscope at 80kV.

Results

Frequency of transmission by nematodes recovered from field soils

Longidorus fasciatus was the only longidorid species present in bulks of soil collected from Iria and Kandia. Nematodes from Kandia appeared better fed having dark body contents as compared with those from Iria

Table 1. Mean numbers of root tip galls and nematodes recovered from bait-plants and frequency of transmission of a Greek strain of artichoke Italian latent nepovirus (AILV) by *Longidorus fasciatus* recovered from soil samples collected from the rhizosphere of AILV infected plants growing at Iria and Kandia in Greece

Nematode pops.	Bait-plants				
	Nematodes added per plant	recov'd per plant	Galls formed per plant	Virus[1]	P[2]
Iria	1	0.85	1.7	21/65[3]	0.32
Iria	5	3.9	6.6	8/15[4]	0.14
Iria	20	4.2	4.8	0/13	<0.12
Kandia	1	0.72	2.3	28/64[3]	0.44
Kandia	5	4.6	6.5	28/29[4]	0.49

[1] Numerator, number of source and bait-plants from which virus was recovered; denominator, total number of source and bait plants.
[2] The estimated proportions of nematodes transmitting virus, calculated using the equation of Gibbs and Gower (1960).
[3] Proportions of nematodes from the two sites transmitting virus not significantly different (Chi-squared test).
[4] Proportions of nematodes from the two sites transmitting virus significantly different at P = 0.001 level (Chi-squared test).

where many specimens had translucent bodies. Adult females recovered from both locations were natural vectors of isolates of AILV-G, with significantly more nematodes from Kandia transmitting virus than those from Iria (Table 1). Virus was not recovered from 13 bait-plants to which groups of 20 juvenile *L. fasciatus*, mainly 1st and 2nd developmental stages, had been given access (Table 1).

Frequency of transmission by nematodes having had access to AILV-G infected N. clevelandii *plantlets*

After being given access to virus source-plants, mechanically infected with AILV-G, groups of five *L. fasciatus* transmitted virus to 19 of 20 *N. clevelandii* bait-plants (Table 2). The proportion of nematodes estimated to have transmitted virus (P = 0.45) was similar to that obtained with nematodes naturally associated with AILV. The precision of this test for determining the proportion of nematodes capable of transmitting virus was much reduced as a result of the large proportion of bait-plants infected with virus (Gibbs and Gower, 1960). However, in a separate experiment with single females the proportion of individuals transmitting virus was similar (P = 0.49) to that obtained in the other tests in which nematodes from Kandia were used (Table 1).

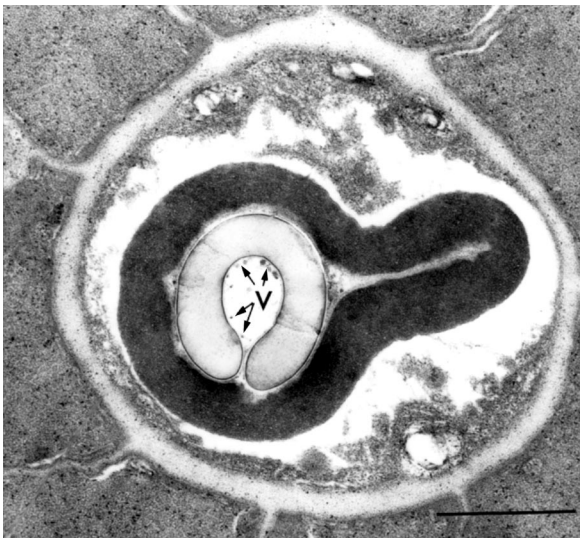

Figure 1. A transverse section through the odontostyle region of a female *Longidorus fasciatus* showing particles of artichoke Italian latent nepovirus (V) specifically adsorbed to the inner surface of the odontostyle (Bar, 1μm).

Site of virus retention within the vector

Examination of transverse sections of female *L. fasciatus* from the population from Kandia recovered from AILV-infected *N. clevelandii* plants revealed virus-like particles apparently adsorbed to the inner surface of the odontostyle (Figure 1). Only relatively few particles were present in specimens and were retained as a single layer in the odontostyle. Particles were not observed trapped in the longitudinal anterior fold of the guiding sheath and were not observed in specimens which had fed on healthy *N. clevelandii* plants.

Discussion

Roca et al. (1982) recovered AILV from 3 of 20 White Burley *N. tabacum* bait-plants to which groups of 15 *L. fasciatus* from the rhizosphere of AILV infected artichoke plants growing in the Argolis area of Peloponnesus, Greece, had been given access. The proportion of nematodes transmitting virus in this original test (P = 0.01) was substantially smaller than that in the experiments reported here (P = 0.12 to 0.49). Root galls, evidence of nematode feeding, were not observed on the White Burley plants used by Roca et al. (1982), whereas many such galls were present on *N. clevelandii* bait-plants used in our experiments. Therefore, the low

Table 2. Mean numbers of root tip galls and nematodes recovered from virus source and bait-plants and frequency of transmission of a Greek strain of artichoke Italian latent nepovirus (AILV) by *Longidorus fasciatus* recovered from soil samples collected from the rhizosphere of AILV infected plants growing at two sites in Greece

	Source-plants				Bait-plants				
Nematode pops.	Nematodes added recov'd per plant		Galls formed per plant	Virus[1]	Nematodes added recov'd per plant		Galls formed per plant	Virus[1]	p[2]
Experiment One (Nematodes given access to virus source-plants mechanically inoculated with AILV)									
Kandia	40	31.5	>20	8/8	5	4.3	7.5	19/20	0.45
Experiment Two (Nematodes given access to virus source-plants mechanically inoculated with AILV and 15 specimens used to determine the site of virus retention within the vector)									
Kandia	25	16	>20	3/3	1	0.85	1.6	16/33	0.49

[1] Numerator, number of source and bait-plants from which virus was recovered; denominator, total number of source and bait plants.
[2] The estimated proportions of nematodes transmitting virus, calculated using the equation of Gibbs and Gower (1960).

frequency of transmission in the original test may have resulted from *N. tabacum* being less suitable than *N. clevelandii* as a host for *L. fasciatus* and/or AILV-G. Also, the proportion of nematodes transmitting virus may reflect their previous access to virus infected roots in the field. In our experiments nematodes from Kandia were obtained from an old abandoned, uncultivated, artichoke field in which many weed species also were growing. The nematodes from this site appeared better fed than those from Iria and a much larger proportion of them transmitted virus. It is possible that the nematodes used in the tests by Roca et al. (1982) had less natural access to AILV infected roots upon which to feed, and thus acquire virus, than did those from the site at Kandia used in our experiments. The apparent inability of *L. fasciatus* juvenile developmental stages to transmit virus may have resulted from these nematodes having moulted. During this process juvenile nematodes shed their feeding apparatus, including any specifically retained virus particles (Taylor and Brown, 1997).

Trudgill et al. (1981) when examining the effectiveness of longidorid virus-vector species in Britain to transmit their associated virus compared three *Longidorus* species: *L. attenuatus* with tomato black ring nepovirus (TBRV) and *L. elongatus* and *L. macrosoma*, each with raspberry ringspot nepovirus (RRSV). Only a small proportion of both *L. elongatus* (P = 0.08 to 0.17) and *L. macrosoma* (P = 0.05) transmitted RRSV as compared with *L. attenuatus* transmitting TBRV (P = 0.47). Brown et al. (1989) reported that several isolates of TBRV from England were efficiently transmitted by *L. attenuatus* from England (average P = 0.42) whereas isolates from Germany were inefficiently transmitted (P = 0.04). Only a small proportion (P = 0.01 to 0.16) of *L. arthensis*, the vector of cherry rosette nepovirus (CRV) in Switzerland, transmitted virus and Brown et al. (1995) concluded that *Longidorus* species are relatively inefficient vectors. However, the results obtained with *L. fasciatus* from Kandia were similar to those obtained with *L. attenuatus* (Trudgill et al., 1981) and with *L. attenuatus* transmitting English isolates of TBRV (Brown et al., 1989) which suggests that *Longidorus* virus-vector species and their naturally associated nepoviruses form two groups *viz.* those vector species which can frequently transmit their associated viruses (P = *c.*0.45) and those which can transmit their associated viruses only relatively infrequently (P = *c.*0.08). The relative frequencies of transmission of the viruses by their natural vectors may be the result of different natural host ranges exploited by the viruses, *viz.* the Scottish serotypes of RRSV and TBRV transmitted by *L. elongatus*, the English serotype of RRSV transmitted by *L. macrosoma*,

German isolates of TBRV transmitted by *L. attenuatus* and CRV transmitted by *L. arthensis* may have evolved in association with long-term perennial hosts, whereas English isolates of TBRV transmitted by *L. attenuatus* and AILV transmitted by *L. fasciatus* may have evolved in association with annual or short-term natural hosts. Under natural conditions frequent transmission may be less important than efficient transmission of a virus by its vector where long-term perennial plants are the virus host. Conversely, frequent transmission may be a more effective strategy when the virus hosts are short-term perennial or even annual plant species.

In common with several other virus-vector *Longidorus* species (Brown et al., 1995) the specific site of retention of AILV in *L. fasciatus* is the inner surface of the odontostyle. However, our observations do not exclude the possibility that virus particles may also line the guiding sheath. Relatively few particles were present in *L. fasciatus* specimens as compared with *L. apulus*, the vector of the Italian strain of AILV. In *L. apulus* virus particles were numerous within the odontostyle and guide sheath regions, in some specimens forming crystalline arrays at both sites (Taylor et al., 1976).

It has been suggested that infrequent virus transmission by *Longidorus* species is not associated with a lack of retention, but may be due to lack of dissociation of virus particles from the specific sites of retention (Trudgill and Brown, 1978). Our results and those of several previous studies collectively provide evidence which appears to support this suggestion: vectors in which few particles are observed at the specific sites of retention e.g. *L. attenuatus* with TBRV (Brown et al., 1995; Taylor and Brown, 1997) and *L. fasciatus* with AILV, transmit virus frequently whereas those found to retain many particles are infrequent vectors, e.g. *L. macrosoma* with RRV and *L. apulus* with AILV. However, to provide further credence to the suggestion of Trudgill and Brown (1978) a study directly comparable with ours, of the frequency of transmission of an Italian isolate of AILV by *L. apulus*, should be done. Also, data on the respective abilities of these two vectors of serologically distinguishable strains of AILV to transmit the reciprocal viruses, is required.

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