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Host suitability of some crucifers for root-knot nematodes in southern Spain

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Summary – Six crucifer species as potential cover crops, Ethiopian mustard (*Brassica carinata* line C-101), turnip (*Brassica rapa* cv. Norfolk), radish (*Raphanus sativus*), wild rocket (*Eruca vesicaria*), wild cabbage (*Moricandia moricandioides*) and white mustard (*Sinapis alba*), were tested for susceptibility to *Meloidogyne arenaria* race 2, *M. incognita* race 1 and *M. javanica*. Experiments were conducted under glasshouse conditions at 22-28°C for 2 months after inoculation of plants with eggs and second-stage juveniles. All crucifers were infected by *Meloidogyne* spp. The nematode and crucifer species significantly influenced the severity of root galling and nematode reproduction. Among the plants tested, turnip was the most suitable host for all three *Meloidogyne* spp. as indicated by severity of root galling and nematode reproduction. The least suitable hosts were wild rocket for *M. arenaria*, radish for *M. incognita* and white mustard for *M. javanica*. The reproduction fitness of *M. javanica* was greater than that of *M. arenaria* race 2 and *M. incognita* race 1 on all plants.

Keywords – *Brassica carinata*, *B. rapa*, *Eruca vesicaria*, *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Moricandia moricandioides*, *Raphanus sativus*, *Sinapis alba*.

Over the last decade, significant research efforts have been devoted in Andalusia (southern Spain) to the use of cover crops in olive orchards (Castro, 1993; Garcia Torres, 2001). The use of a permanent orchard floor vegetation is the most common orchard soil management system used (Hogue & Neilsen, 1987). Some of the potential benefits of such cover in olive orchards in Andalusia include reduced soil erosion, improved soil structure and fertility, increased water infiltration, and reduced surface run-off and weeds (Castro, 1993; Pelegrín *et al.*, 2001). Several plant species are being tested in Andalusia, among which some crucifer species are of great interest. However, cruciferous cover crops may be hosts for plant-parasitic nematodes, such as *Meloidogyne* spp. (Gardner & Caswell-Chen, 1994; McSorley & Frederick, 1995). Therefore, since crucifers are being included as cover crop systems in olive orchards in Andalusia, their host status to the major root-knot nematodes infesting this region must be determined.

The suitability of a host for plant-parasitic nematodes is expressed as the ability of the nematode to multiply on the plant. Host suitability may be expressed objectively as the ratio of the number of nematode units recovered at the end of the test, the final nematode population density (*Pf*), to the number of nematode units used to inoculate the plant, the initial population density (*Pi*) (Lewis, 1987). For root-knot nematodes, host suitability can be assessed by measuring the severity of root galling and reproduction on plants after artificial inoculations (Hussey, 1985).

The objective of this study was to determine the host suitability of some cultivated and wild crucifers, with potential use as cover crops in Andalusian olive orchards, to the three common root-knot nematodes *M. arenaria*, *M. incognita* and *M. javanica*.

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Materials and methods

Nematode inoculum

Nematode populations used in this study were obtained from olive planting stocks collected in commercial nurseries at Córdoba province of Andalusia, southern Spain (Nico *et al.*, 2002). The host suitability of crucifers to root-knot nematodes was evaluated by assessing the severity of root galling and nematode reproduction after artificial inoculation. Three *Meloidogyne* spp. representative of those infecting olive planting stocks in Spain were chosen for the studies: *M. arenaria* race 2, *M. incognita* race 1 and *M. javanica* were isolated from infected roots of olive planting stocks (Nico *et al.*, 2002). Nematodes were identified to species and race based on features of their perineal pattern and differential host experiments (Nico *et al.*, 2003). Inocula were increased on tomato plants (*Lycopersicon esculentum* Mill. cv. Roma) starting from a single egg mass for each species. Inoculum for experiments was obtained by extracting eggs and second-stage juveniles (J2) from 2-month-old cultures using 1% sodium hypochlorite (Hussey & Barker, 1973) followed by centrifugal flotation (Coolen, 1979). The population density of eggs and J2 released was determined from ten replicates of 1 ml aliquots of the inoculum suspension.

Host suitability test

The plant material used for experiments consisted of six crucifer species, three cultivated species: Ethiopian mustard (*Brassica carinata* Brown) line C-101 (Velasco *et al.*, 1997), turnip (*Brassica rapa* L.) cv. Norfolk and radish (*Raphanus sativus* L.); and three wild species: wild rocket (*Eruca vesicaria* (L.) Cav.), wild cabbage (*Moricandia moricandioides* (Boiss.) Heywood) and white mustard (*Sinapis alba* L.). All these are potential cover crops in olive orchards in Andalusia.

Seeds of these plants were surface-disinfested with 2% NaOCl for 3 min and germinated on sterile, moistened filter paper in Petri plates at 25°C in darkness for 48 to 72 h. Germinated seeds, selected for uniformity (length of radicle = 2-3 cm), were sown into 15 cm diam. clay pots (one seed per pot) containing 0.5 dm^3 of an autoclaved (121°C for 1 h, twice on 2 consecutive days) soil potting mixture (sand: clay loam, 2:1, v/v), infested or not infested with the pathogens. For the nematode inoculation, 10 ml of sterile distilled water with nematode inoculum (10 000 eggs and J2) were added to soil at sowing. Control plants of each crucifer species were treated similarly but without nematode inoculum. Plants were maintained in a glasshouse with daily mean temperatures of 22-28°C for 2 months. Four tomato cv. Roma plants were included for each *Meloidogyne* isolate to confirm viability of inoculum. Plants in pots were watered as needed and fertilised once a week with 100 ml of a 0.1% solution of 20-5-32+micronutrients hydro-sol fertiliser (Haifa Chemicals Ltd, Haifa, Israel). Treatments were replicated ten times, each replicate consisting of a single potted plant, in a randomised complete block design and the experiment was repeated once.

At the end of the experiment, individual plants were cut at the soil level and the roots washed free of soil. Root gall severity (*RGS*) was rated on a 0-5 scale: 0 = no galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; and 5 = more than 100 galls (Taylor & Sasser, 1978). Nematodes from 100 cm^3 samples of infested soil and from 5 g samples of roots were extracted by centrifugation (Coolen, 1979), as already described for inoculum preparation. Extracted nematodes were used to estimate final nematode population densities, and reproduction factor ($Rf = Pf/Pi$) values determined.

Statistical analysis

The experiment was repeated once and similarity among experiments was tested by preliminary analyses of variance using experimental runs as blocks. This allowed combining data for analysis of variance. Data of root gall severity (*RGS*) and *Rf* were normalised before analysis by transforming them into $log_{10}(x + 1)$ (Gomez & Gomez, 1984). Analyses of variance were carried out using Statistix 7.0 (NH Analytical Software, Roseville, MN, USA). Means of *RGS* and *Rf* values were compared using Fisher's protected least significant difference test (LSD) at $P = 0.05$.

Results

In both experiments, *Meloidogyne*-infected tomato plants were heavily galled (rating = 5) and had high nematode population increase ($Rf > 100$), confirming the viability of the inoculum (Table 1, data not included in analyses). All crucifer species were infected by *M. arenaria* race 2, *M. incognita* race 1 and *M. javanica* (Table 1). Both *Meloidogyne* spp. and crucifer species, as well as their interaction, significantly ($P < 0.001$) influenced the severity of root galling and nematode reproduction (Table 1). All six plant species gave *Rf* values greater

Table 1. *Severity of root galling (*RGS*) and reproduction (*Rf*) of three species of* Meloidogyne *on six crucifer species and on tomato as susceptible control (data are means of two experiments, with ten replicates of each plant-nematode combination per experiment).*

Plant species	Common name	*M. arenaria*		*M. incognita*		*M. javanica*	
		RGS[1)]	*Rf*[2)]	*RGS*	*Rf*	*RGS*	*Rf*
Brassica carinata	Ethiopian mustard line C-101	1.40 bcB[3)]	2.42 bB	1.70 bB	4.10 bcB	4.50 abA	23.41 aA
B. rapa	turnip cv. Norfolk	2.30 aC	5.49 aC	3.10 aB	11.82 aB	5.00 aA	51.07 aA
Eruca vesicaria	wild rocket	1.20 cB	1.61 bA	1.90 bB	3.90 bcA	3.90 bA	5.84 bA
Moricandia moricandioides	wild cabbage	1.50 bcB	1.65 bB	2.75 aB	5.55 bA	4.25 bA	3.94 bA
Raphanus sativus	radish	2.00 abB	2.40 bB	1.30 bB	3.06 cB	3.90 bA	13.48 bA
Sinapis alba	white mustard	1.50 bcB	1.97 bA	1.40 bB	4.75 bcA	4.20 bA	3.49 bA
Lycopersicon esculentum[4)]	tomato cv. Roma	5.0	116.2	5.0	104.8	5.0	128.3

[1)] *RGS*: 0 = no galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 = more than 100 galls.
[2)] *Rf* = final nematode density per plant/initial nematode population density per plant.
[3)] Means within columns followed by common lower case letters and within rows by common upper case letter do not differ ($P \leqslant 0.05$) according to Fisher's protected LSD test based upon analyses of $log_{10}(x + 1)$ transformed data. Untransformed means are tabulated.
[4)] Tomato data not included in the analyses.

than 1.0, indicating that these plants are effective hosts for *Meloidogyne* spp. Nevertheless, turnip was the most suitable host for all three species: root galling and reproduction on turnip increased in the order *M. arenaria* < *M. incognita* < *M. javanica* (Table 1). The least suitable hosts were wild rocket for *M. arenaria*, radish for *M. incognita*, and white mustard for *M. javanica* (Table 1). In addition, reproduction of *M. javanica* was greater ($P < 0.001$) than that of *M. arenaria* or *M. incognita* on all plant species, except for white mustard, in which reproduction was not affected by nematode species (Table 1). Reproduction fitness of *M. incognita* was greater ($P < 0.001$) than that of *M. arenaria* only in turnip and wild cabbage (Table 1).

Discussion

The results indicated that the tested crucifers are hosts of all three *Meloidogyne* species evaluated and that several of these support moderate ($Rf > 5$) or high ($Rf > 10$) nematode reproduction. Although all three *Meloidogyne* spp. reproduced substantially on all of the crucifers tested here, reproduction was less than that on tomato, confirming that crucifers in general are less good hosts for *Meloidogyne*. Results on severity of root galling and reproduction of *M. javanica* are consistent with some previous findings on radish, turnip and white mustard (Gardner & Caswell-Chen, 1994; McSorley & Frederick, 1995). However, some minor differences with those findings on root galling and reproduction of *M. arenaria* and *M. incognita* were observed in our study. Differences in the environmental and experimental conditions, virulence of the *Meloidogyne* populations, or differences in the genotype of tested plant material could be responsible for these differences. However, our data on the response of Ethiopian mustard, wild rocket and wild cabbage to infections by *Meloidogyne* spp. are the first reports on the host suitability of these plants to those nematode species.

The fact that all three *Meloidogyne* species reproduced on all of the crucifers tested here could have serious implications regarding use of these plants as cover crops in olive orchards infected by *Meloidogyne* spp. because of the increasing of nematode populations. Nevertheless, in Andalusia, these crucifers are sowed as cover crops in autumn and managed by means of chemical mowing (herbicide application) in late winter or early spring, when soil temperatures are low (10-18°C) and do not allow nematode infection and development. Optimal developmental temperatures for root-knot nematodes are 25-30°C (Van Gundy, 1985).

On the other hand, glucosinolates (amino-acid-derived products of secondary metabolism) stored in the vacuoles of some of the tested crucifers have shown nematicidal activity against some plant-parasitic nematodes (Lazzeri *et al.*, 1993; Potter *et al.*, 1999). However, release of these toxic compounds requires disruption of cells. Cyst forming and root-lesion nematodes migrate intracellularly and damage cells as they invade roots. In contrast, *Meloidogyne* move intercellularly and cause minimal cell damage while migrating through root tip regions (where cells

are young and not vacuolated) to the vascular cylinder where they settle and grow (Wyss *et al.*, 1992). These findings may be responsible for the lack of nematicidal activity of these crucifers against *Meloidogyne* spp.

In conclusion, our results will be of general interest to other areas because although these plants were not good hosts for *Meloidogyne* spp. they support some nematode reproduction, and their use as cover crops must be limited to soils not infested or with low inoculum density of *Meloidogyne* spp. Additionally, growing the cover crops when temperatures are suboptimal for root-knot nematodes might allow their use in olive orchards without *Meloidogyne* spp. numbers increasing. Even so, additional investigations are needed to determine reproduction of *Meloidogyne* spp. on these crucifers at the lower soil temperatures typical of southern Spain during the early winter to early spring.

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