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Reproduction of Plant-parasitic Nematodes on Winter Rapeseed (*Brassica napus* ssp. *oleifera*)

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Abstract: The reproduction of isolates of five plant-parasitic nematode species on the winter rapeseed cultivars Bridger, Gorzanski, H-47, Lindora, and Viking was evaluated. Each cultivar was a good host for *Helicotylenchus pseudorobustus, Meloidogyne hapla,* and *M. incognita.* All rapeseed cultivars were poor hosts for *Pratylenchus scribneri,* in comparison with a susceptible reference host. *Heterodera glycines* females rarely developed on any cultivar, but low numbers of juveniles invaded roots and males occasionally reached maturity.

Key words: Brassica napus, canola, *Helicotylenchus pseudorobustus, Heterodera glycines,* host range, *Meloidogyne hapla, Meloidogyne incognita,* nematode, population dynamics, *Pratylenchus scribneri,* rapeseed, susceptibility, winter annual.

Winter rapeseed, or oilseed rape (*Brassica napus* L. ssp. *oleifera* (Metzg.) Sinsk. var. *biennis*), has potential in the southern United States as a contracted specialty crop (6). Winter rapeseed is grown for oil extracted from the seed, which is high in erucic acid (>40%) and widely used for industrial purposes (1). Oil from low erucic acid seed (<2%) is suitable for human consumption. The term "canola" refers to rapeseed low in both erucic acid and glucosinolates (<30 μmol/g). Commercial and agricultural genetic research firms are interested in developing new industrial rapeseed cultivars and creating a larger market for their exploitation (6).

Our knowledge of nematode–rapeseed interactions in the United States is very limited, but they have been examined more extensively in Europe, primarily with cyst nematodes. Densities of both *Heterodera schachtii* Schmidt and *H. cruciferae* Franklin increase substantially on oil rapeseed (4,5,8,18), but only *H. cruciferae* is considered a yield-limiting pest (5,8). A distinctive yellow isolate of *H. trifolii* Goffart parasitized and reproduced on rapeseed (11). Of six *Pratylenchus* spp., only *P. fallax* Seinhorst and *P. penetrans* (Cobb) Filipjev & Schuurmans Stekhoven reproduced on rapeseed (15,18). *Pratylenchus convallariae* Seinhorst, *P. crenatus* Loof, *P. thornei* Sher & Allen, and *P. vulnus* Allen & Jensen did not increase on this host. In field surveys in France (16), *P. neglectus* (Rensch) Filipjev & Schuurmans Stekhoven was considered the most important parasite of rapeseed. *Pratylenchus fallax* induced necrosis on excised rapeseed roots but did not increase in numbers significantly at either of two initial population densities.

The objective of this research was to determine the ability of some plant-parasitic nematodes commonly found in Tennessee to parasitize and reproduce on the roots of selected winter rapeseed cultivars.

Materials and Methods

All experiments were conducted in a greenhouse environment with a temperature of 26 ± 2 C. Stock cultures of nematodes were maintained as follows: *Helicotylenchus pseudorobustus* (Steiner) Golden on sunflower (*Helianthus annuus* L. 'Mammoth'); *Heterodera glycines* Ichinohe on soybean (*Glycine max* (L.) Merr. 'Essex'); *Meloidogyne hapla* Chitwood on peanut (*Arachis hypogaea* L. 'Florunner'); *M. incognita* (Kofoid & White) Chitwood on tomato (*Lycopersicon esculentum* Mill. 'Rutgers'); and *Pratylenchus scribneri* on corn root explant cultures (*Zea mays* L. 'Seneca Chief').

Three industrial rapeseed cultivars (Bridger, Gorzanski, H-47) and two canola cultivars (Lindora, Viking) were used in all

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experiments. Seeds of each cultivar were broadcast in a flat with a peat–sand mix. Seedlings were grown to the two-true-leaf stage and then were transplanted singly into 10-cm-d pots filled with a steam-sterilized loamy sand mix. Two weeks later, five holes were punched into the rhizosphere with a glass rod, and nematode inoculum was added. Each nematode species isolate was tested in a separate experiment. Pots were arranged in a randomized block design with susceptible reference hosts for monitoring inoculum viability and reproduction of each isolate. Each treatment was replicated five times, and each experiment was repeated once. Initial inoculum (Pi), duration of experiment, and reference host for each nematode were as follows: *H. pseudorobustus,* 1,000 adults + juveniles/pot, 6 weeks (experiment 1) or 1,500/pot, 8 weeks (experiment 2), 'Mammoth' sunflower; *H. glycines,* 3,500 eggs/pot, 7 weeks, 'Essex' soybean (both experiments); *M. hapla,* 5,000 eggs/pot, 8 weeks, 'Rutgers' tomato (both experiments); *M. incognita,* 1,000 newly hatched second-stage juveniles (J2), 8 weeks, 'Mammoth' sunflower (both experiments); *P. scribneri,* 1,000 adults + juveniles/pot, 10 weeks, *Phaseolus vulgaris* L. 'Kentucky Wonder' (both experiments). At the conclusion of each experiment, reproduction was measured. For *H. pseudorobustus,* soil in each pot was thoroughly mixed, a 100-cm^3 portion was selected, and nematodes were extracted with a sugar flotation–centrifugation method (9). Numbers per pot were extrapolated from the actual counts. Females and cysts of *H. glycines* were gathered on a sieve with 180-μm pores by repeated decanting of a water suspension of the soil in each pot. In addition, roots were examined for adhering females and cysts and were then stained (3) to visualize juveniles within the roots. For *M. hapla* and *M. incognita,* egg mass production was rated on a 0–5 scale, where 0 = no egg masses, 1 = 1–2, 3 = 3–10, 4 = 11–30, and 5 = more than 100 egg masses/plant (19). For *P. scribneri,* roots were stained (3) and all nematodes in each root system were counted. Data from each experiment were analyzed with analysis of variance and means were separated with Duncan's multiple-range test. Because results were statistically identical for each pair of experiments, data from both experiments were combined for each nematode except *H. pseudorobustus,* for which there were large differences in Pi and final numbers between experiments.

Results

All rapeseed cultivars were suitable hosts for *H. pseudorobustus, M. hapla,* and *M. incognita* (Table 1), but reproduction of *H. pseudorobustus* on rapeseed was not as high as on sunflower, the susceptible host. Galls of *M. hapla* and *M. incognita* were very small and their external morphologies were typical of the two species (Figs. 1,2). Adventitious roots grew from galls of *M. hapla* (Fig. 1), whereas galls of *M. incognita* were fusiform and smooth (Fig. 2). Egg masses were numerous, were well-developed, and contained many eggs each.

All of the rapeseed cultivars were very poor hosts of *H. glycines.* Some J2 (<20) were observed in roots of Bridger, H-47, and Lindora, but only a very few reached the female stage on H-47 and Lindora (Table 1). None of the extracted females contained eggs or were associated with egg masses. Rapeseed was also a poor host for *P. scribneri,* which was unable to maintain itself on any cultivar (Table 1). Total *P. scribneri* per root system were higher on the susceptible reference host, pole bean, than on rapeseed.

Discussion

The potential influence of plant-parasitic nematodes on the growth and yield of commercial rapeseed is difficult to estimate. In most of the United States, rapeseed is grown as a winter annual. Seed is planted in autumn and young plants grow as a rosette through autumn, winter, and early spring, after which a raceme elongates and seeds are produced. Because plants grow mostly during cooler pe-

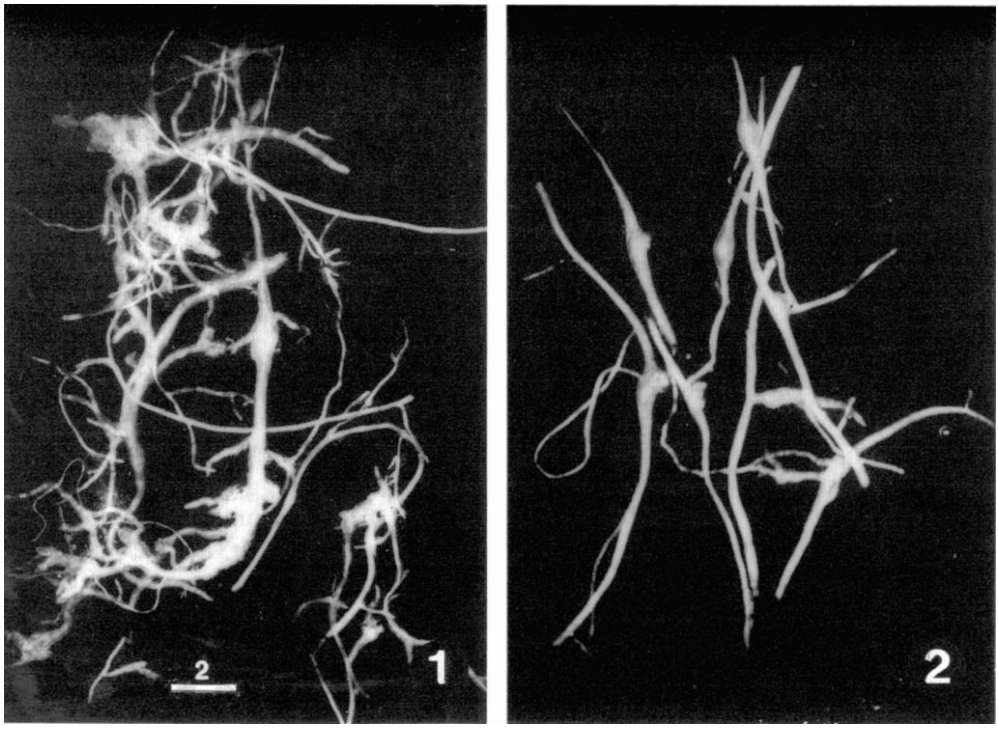
TABLE 1. Development and/or reproduction of *Helicotylenchus pseudorobustus* (Hp), *Heterodera glycines* (Hg), *Meloidogyne hapla* (Mh), *Meloidogyne incognita* (Mi), and *Pratylenchus scribneri* (Ps) on rapeseed cultivars and reference hosts.

Rapeseed or reference host	Hp per pot		Hg females per root system	Egg mass index†		Ps per root system
	Expt. 1	Expt. 2		Mh	Mi	
Bridger	2,912 a	7,168 a	0 a	4.4 a	3.5 a	271 a
Gorzanski	2,336 a	13,200 a	0 a	4.4 a	3.5 a	189 a
H-47	5,120 a	9,664 a	0.4 a	4.4 a	3.5 a	385 a
Lindora	1,792 a	12,224 a	0.2 a	4.2 a	4.0 a	191 a
Viking	832 a	8,384 a	0 a	4.4 a	4.0 a	165 a
'Mammoth' sunflower	11,420 b	19,360 b	—	—	5.0 a	—
'Essex' soybean	—	—	59 b	—	—	—
'Rutgers' tomato	—	—	—	5.0 a	—	—
'Kentucky Wonder' pole bean	—	—	—	—	—	929 b
Pi per pot	1,000	1,500	3,500 eggs	5,000 eggs	1,000 J2	1,000

Each value is the mean of five replicates (Hp), or the mean of two experiments of five replicates each. Column means followed by a common letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

† Egg mass index: 0 = no egg masses, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = more than 100 egg masses per root system.

riods of the year, nematodes are unlikely to be active enough to cause noticeable damage during most of the growing season. However, if rapeseed followed a summer crop heavily infested with root-knot nematodes, subsequent invasion of rapeseed seedlings by large numbers of hatching J2 could lead to increased plant stress or damage in winter. In west Tennessee, rapeseed is often double-cropped with



FIGS. 1–2. Root knots on roots of winter rapeseed cv. Viking. 1) *Meloidogyne hapla.* 2) *Meloidogyne incognita.* White scale bar = 2 mm.

corn, which is not a good host of *M. incognita* in Tennessee (Bernard, unpubl.). Provided that *Meloidogyne* spp. were absent, rapeseed would be a suitable rotation crop with soybean, because *H. glycines* does not appear to successfully reproduce in rapeseed roots.

Reproduction of *M. incognita* on rapeseed is variable. In our study, all rapeseed cultivars were suitable hosts of *M. incognita,* but Johnson et al. (10) reported very little galling of rapeseed crop rotations in Georgia. These differences may be due to different growing conditions, particularly temperature. Johnson et al. (10) conducted field rotation experiments with rapeseed planted as a winter crop, whereas the present study, designed to determine the intrinsic suitability of the cultivars as hosts for the nematode, was performed in the greenhouse. Johnson et al. (10) planted rapeseed in October or November, when soil temperatures and nematode activity were decreasing. In Tennessee, the highest rapeseed yields were obtained from September plantings (6), when soil temperatures were high and nematodes were likely more active; November plantings were unsatisfactory due to winter damage of young plants. Thus, we hypothesize that in the southern United States, later-planted rapeseed may avoid nematode invasion due to temperature effects. Other factors may be involved, because the Georgia study was conducted near Tifton, a much warmer and more southerly location than Tennessee. Among these are cultivar differences, timing of nematode seasonal dormancy, or possible nematode metapopulation (isolate) differences. In our study, we used only one isolate of each nematode species. Geographically distinct isolates of a root-knot nematode species may have widely varying development and reproduction on the same host cultivar (2,17). The only common cultivar in the two studies was Bridger, which was moderately susceptible in this study but was a poor host in others (10). Bridger was a very suitable host for *M. hapla* in our studies and for *M. hapla* and *M. chitwoodi* Golden et al. in others (13).

Our results concerning *P. scribneri* are in agreement with most other investigations of lesion nematodes, for which rapeseed is a poor host (16,21). The case for *P. fallax,* however, is equivocal. This nematode reproduced well on rapeseed plants when assayed 2 months after planting (16), but barely maintained itself on excised roots when assayed 1 month after infestation (21). The results may be due to the difference in times between infestation and assay, or to fundamental differences in the physiology of roots detached from shoots.

The potential of rapeseed as a green-manure crop for nematode control should be investigated further. Mojtahedi et al. (13) found that the incorporation of 2-month-old shoot tissue reduced *M. chitwoodi* densities more than did chopped wheat shoots, corn shoots, or fallow, and suggested that degradation products of rapeseed glucosinolates, principally isothiocyanate, were responsible for the suppressive effect. Methyl isothiocyanate is an active component of some commercial nematicides (7). Johnson et al. (10), however, observed no effect of 6-month-old rapeseed incorporation into soil on *Criconemella ornata* (Raski) Luc & Raski, *M. incognita,* or *M. javanica* (Treub) Chitwood. The differences between the two reports may be due to quantity and age of the green manure used in each study. Johnson et al. (10) speculated that their incorporated 6-month-old plants contained lower levels of glucosinolates than did incorporated plants grown for 2 months (13). Glucosinolate concentration declines with plant age (20). The results of Johnson et al. (10), however, suggest that plant age may have an influence on root suitability, since they observed very little galling on 6-month-old field grown plants. This suggestion is confounded by the plants having been grown as a winter-cover crop. The nematicidal effects of rapeseed grown for seed production, then incorporated after harvest, is unknown.

The nematicidal substances postulated to occur in rapeseed shoots (13) may not occur in the roots, because root-knot and other nematodes can invade, grow, develop, and reproduce in rapeseed roots (13). All parts of rapeseed plants contain glucosinolate compounds (14). When plant tissue is injured, some, but not all, glucosinolates are hydrolyzed by the enzyme myrosinase to form isothiocyanates (12,14). Several of the 10 glucosinolates found in rapeseed by Sang et al. (14) were localized in the shoots or roots, but not in both. Even if some glucosinolates are the precursors for effective nematicidal compounds (13), they may not be the same glucosinolates found in the roots.

Should rapeseed become an important winter rotation or cover crop in the southern United States, additional research will be needed to resolve the question of the timing of root-knot nematode invasion of roots, to determine optimal rapeseed planting dates. The importance of rapeseed roots as overwintering sites, and thus as reservoir hosts, for root-knot nematodes should also be investigated. Screening of germplasm for effective glucosinolates or other putative nematicides in roots may be useful for avoiding nematode invasion in early-planted rapeseed.

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