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Reproduction and Development of *Meloidogyne incognita* and *M. javanica* on Guardian Peach Rootstock

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Abstract: Guardian peach rootstock was evaluated for susceptibility to *Meloidogyne incognita* race 3 (Georgia-peach isolate) and *M. javanica* in the greenhouse. Both commercial Guardian seed sources produced plants that were poor hosts of *M. incognita* and *M. javanica.* Reproduction as measured by number of egg masses and eggs per plant, eggs per egg mass, and eggs per gram of root were a better measure of host resistance than number of root galls per plant. Penetration, development, and reproduction of *M. incognita* in Guardian (resistant) and Lovell (susceptible) peach were also studied in the greenhouse. Differences in susceptibility were not attributed to differential penetration by the infective-stage juveniles (J2) or the number of root galls per plant. Results indicated that *M. incognita* J2 penetrated Guardian roots and formed galls, but that the majority of the nematodes failed to mature and reproduce.

Key words: host-parasitic relationship, *Meloidogyne incognita, Meloidogyne javanica,* nematode, peach, *Prunus persica,* resistance, root-knot nematode, rootstock.

Root-knot nematodes (*Meloidogyne spp.*) are important pests of peach (*Prunus persica* (L.) Batsch) in the United States and other parts of the world (Nyczepir and Becker, 1998). Four major *Meloidogyne* spp. have been reported to cause damage to stone fruits throughout the world, but *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood are the predominant species found on peach and plum. In a recent survey of South Carolina peach orchards, *M. incognita* and *M. javanica* were detected in 95% and 5% of the orchards sampled, respectively (Nyczepir et al., 1997). The damage associated with *Meloidogyne* spp. on peach includes stunted growth, loss of vigor, and early defoliation. Damage is most severe when newly transplanted trees suffer rapid infection by the infective second-stage juveniles (J2). Gradual infection that does not alter growth rate may appear severe based on root galling, but stress symptoms may be mild or not seen at all.

Preplant chemical treatment with either 1,3-dichloropropene (1,3-D) or methyl bromide (bromomethane) provides effective control of *Meloidogyne* spp. in the Southeast. These fumigant nematicides lower nematode populations enough to prevent major root damage during tree establishment, thus allowing the tree to have a healthy start (Copes et al., 1997; Nyczepir, 1991). In recent years nematode management research has focused on alternatives to conventional nematicide applications. Emphasis on nonchemical control is the result of four factors: (i) the difficulty and cost of achieving a long-term nematode population density reduction with a single preplant fumigant application, (ii) suspended registration of several preplant fumigant nematicides (Nesmith and Dowler, 1975), (iii) the general movement away from chemical pest control wherever possible, and (iv) poor nematode control by nonfumigants.

Resistance to *Meloidogyne* spp. in peach rootstocks (e.g., Nemaguard and Nemared) is available (Nyczepir and Becker, 1998). Concomitant populations of *Mesocriconema xenoplax* (Raski) Loof & de Grisse (=*Criconemella xenoplax* (Raski) Luc & Raski) and *Meloidogyne* spp. in peach orchards throughout the major peach-producing states in the Southeast are common (Nyczepir et al., 1985). *Mesocriconema xenoplax* is associated with the predisposition of peach trees to cold injury and bacterial canker (*Pseudomonas syringae* pv. *syringae* van Hall), the two causal factors directly identified with tree

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death in the peach-tree-short-life (PTSL) disease complex (Brittain and Miller, 1978; Nyczepir et al., 1983). Unfortunately, trees grafted to *Meloidogyne*-resistant rootstocks are more readily predisposed to PTSL and suffer much higher losses than trees budded to Lovell rootstock, even though Lovell is susceptible to root-knot nematodes (Sharpe et al., 1989). Finding a rootstock superior to Lovell that survives on PTSL sites and is resistant to *Meloidogyne* spp. would be of great value to the peach industry throughout the Southeast. Such a multipurpose rootstock would provide an alternative to chemical control of nematodes.

A potential multipurpose rootstock, BY520-9 (Guardian), which provides greater PTSL tree survival than Lovell, was identified in unbudded trials in Georgia and South Carolina planted in 1983. In these trials, tree survival was greater for Guardian than for Lovell at the two different PTSL field sites through 8 years of evaluation (Okie et al., 1994b). These results were substantiated in a follow-up budded trial on PTSL sites in South Carolina (Reighard et al., 1997). Furthermore, in greenhouse experiments Guardian rootstock showed some resistance to *Meloidogyne* spp. (Okie et al., 1994a). Grower demand resulted in commercial release of Guardian before root-knot nematode evaluation had been completed. Currently, Clemson University in South Carolina and the USDA facility at Byron, Georgia, are providing commercial nurseries with bulk seed of Guardian selections from surviving seedlings of the mother tree, which was lost. However, the host suitability for root-knot nematodes, *M. incognita* and *M. javanica,* of commercially available Guardian seed is unknown. The objectives of this research were to (i) compare the susceptibility of Guardian, Lovell, and Nemaguard rootstocks to *M. incognita* and *M. javanica;* and (ii) compare the penetration, development, and reproduction of *M. incognita* in Guardian and Lovell rootstocks.

Materials and Methods

Nematode inocula: A population of *M. incognita* race 3 isolated from peach in Georgia and a population of *M. javanica* from tobacco in North Carolina were maintained on tomato (*Lycopersicum esculentum* Mill. cv. 'Rutgers') in the greenhouse. Root-knot nematode egg inoculum was extracted from tomato roots using NaOCl solution (Hussey and Barker, 1973).

Potting media: Rooted Lovell cuttings were established in 6.5-cm-diam. plastic pots (24 pots per growing tray) containing approximately 169 cm^3 of one of three potting media types. Media treatments tested included sand-vermiculite (50:50 v/v), Metro-Mix 360, or Metro-Mix 200. Established cuttings received Peter's (20-20-20) soluble fertilizer prior to nematode inoculation. Approximately 56 days later, the medium in each pot was infested with 2,000 eggs of *Meloidogyne incognita* race 3 in 20 ml of water poured onto the medium surface that had been previously tilled to a depth of 0.5 cm. An additional 40 to 50 ml of water was applied to each pot following inoculation. Nematode eggs were extracted from the roots of Rutgers tomato with an NaOCl solution as mentioned above. Media treatments were replicated 16 times in a completely randomized design on benches in a greenhouse. An additional four replications of Rutgers tomato were planted into 15-cm-diam. plastic pots containing pasteurized field soil (86% sand, 10% silt, 4% clay; 0.54% organic matter) and infested with *M. incognita* eggs to assess nematode inoculum viability. Plants were fertilized one time with Osmocote (14-14-14) after inoculation and watered daily as needed. Greenhouse temperatures ranged from 21 to 35 °C. The experiment was terminated 64 days after inoculation. Plants were removed from the pots and roots washed free of medium. The number of root galls and egg masses per root system and dry root weight (dried at 70 °C in aluminum foil until no more loss in weight occurred) were recorded. The experiment was repeated one time with minor modifications, which included inoculating 73-day-old established cuttings and terminating the study 60 days after inoculation.

Host susceptibility: Ten-day-old Guardian-USDA seed source, Guardian-Clemson seed

source, and Lovell (known susceptible) peach seedlings were planted in 10-cm-diam. plastic pots containing approximately 450 cm^3 sand-vermiculite medium (50:50 v/v). Five days later, pots were inoculated with either 4,000 *M. incognita* race 3 or *M. javanica* eggs/450 cm^3 medium. The eggs were pipetted directly onto the medium surface, which had previously been tilled to a depth of 1 cm. Additional water was applied to wash the eggs into the medium. Treatments were replicated 10 times in a randomized complete block with a split-plot design on benches in a greenhouse. The whole-plot factor was nematode treatment, with rootstock as the sub-plot factor. Two replications each of Rutgers tomato were inoculated with either *M. incognita* or *M. javanica* eggs to determine inoculum viability. Peach seedlings were watered daily and fertilized as needed with Osmocote (14-14-14). Greenhouse temperatures ranged from 21 to 35 °C. The study was terminated after 110 days, and the following data were collected: number of egg masses per root system, number of eggs per root system, and dry root weight. Root systems were also rated for number of egg masses produced (Taylor and Sasser, 1978). The egg mass index consisted of a 0-to-5 scale, with 0 = no egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses, and 5 = >100 egg masses. Host susceptibility was determined according to the rating system as follows: 0 = immune, 1–2 = a poor host (resistant), and ≥3 = a good host (susceptible). The test was repeated one time. In the second test, modifications included inoculation of 17-day-old seedlings and the addition of Nemaguard (known resistant) peach rootstock. Root systems were rated for galling (Taylor and Sasser, 1978) as well as number of egg masses per root system.

Nematode penetration and development: Penetration of Guardian and Lovell peach seedling roots by *M. incognita* race 3 was investigated in a greenhouse with ambient temperatures ranging from 21 to 35 °C. Eggs were collected from Rutgers tomato roots infected with *M. incognita* race 3 as described previously. Second-stage juveniles (J2) hatching during the first 24 hours were discarded. The J2 that hatched over the next 72-hour period were used as inoculum.

Single 16-day-old peach seedlings of Lovell or Guardian were planted into individual 190-cm^3 styrofoam cups containing 150 cm^3 sand-vermiculite medium. After 4 days, each seedling was inoculated with a 2-ml suspension of ca. 1,000 J2/cup near the base of the seedling. The nematode suspension was pipetted directly onto the medium surface on either side of the seedling, which had previously been tilled to 1 cm. Additional water was applied to each cup to further wash the nematodes into the sand-vermiculite. Two days later the entire seedling was removed from the cup, and the root system was washed free of the potting medium. The 2-day infection period allowed synchronous nematode development. Each seedling was then transplanted into 10-cm-diam. plastic pots containing approximately 450 cm^3 sand-vermiculite medium. Seedlings were harvested at 3, 6, 12, and 24 days after transplanting (DAT). Root systems were washed free of medium and stained with acid fuchsin (Byrd et al., 1983). The root systems were evaluated for numbers of J2 in roots and stage of nematode development on each harvest date with the aid of a stereo microscope. Treatment combinations were replicated five times in a randomized complete block with a split-plot design. The whole-plot factor was the date of harvest, with rootstock as the sub-plot factor. The experiment was repeated one time. Changes in the second test included: (i) transplanting an 11-day-old seedling (vs. a 16-day-old seedling) into the styrofoam cups, (ii) extending the infection period from 2 to 4 days before transplanting seedlings into 10-cm-diam. plastic pots, and (iii) adding a 48-DAT harvest date.

Statistical analysis: Data from each experiment were analyzed separately. All data were subjected to analysis of variance with the general linear model (GLM) procedure of SAS (SAS Institute, Cary, NC). In the potting media and host suitability studies, treatment means were separated with Fisher's

protected least significant difference (LSD) test following a significant *F*-test.

Results and Discussion

Potting media: The sand-vermiculite potting medium provided better reproduction (i.e., number of egg masses per plant and egg masses per gram dry root weight) by *M. incognita* on Lovell peach seedling (known susceptible) roots than Metro-Mix 200 or Metro-Mix 360 (Table 1). One explanation for sand-vermiculite medium advantage for *M. incognita* infection is the percentage of sand present. Sand-vermiculite medium consisted of 50% sand, whereas the other two media had less sand and more organic matter. All three media had vermiculite, but only Metro-Mix 200 and Metro-Mix 360 had sphagnum peat moss and perlite and only Metro-Mix 360 had processed bark ash. Typically, *Meloidogyne* spp. do more damage to plants (i.e., peach) in sandy soils than to plants in fine-textured soils (Stirling, 1975).

Host susceptibility: The interaction between nematode and rootstock was significant for all parameters, except number of egg masses per plant in Test 1 and eggs per gram dry root weight in Test 2; therefore, data for individual *Meloidogyne* spp. were analyzed separately. Both Guardian commercial seed sources (USDA and Clemson) were poor hosts to the populations of *M. incognita* and *M. javanica* tested. Reproduction by *M. incognita* and *M. javanica,* as indicated by number of egg masses per plant, number of eggs per plant, number of eggs per egg mass, and number of eggs per gram of dry root, was less ($P \leq 0.05$) on both sources of Guardian and Nemaguard (known resistant) than on Lovell (known susceptible) (Table 2). There were no differences in reproduction between Nemaguard and either Guardian seed source or between the two Guardian sources. Reproduction on tomato by *M. incognita* and *M. javanica,* as measured by eggs per plant, was 80,000 and 205,000 (Test 1) and 47,500 and 46,250 (Test 2), respectively, indicating that the nematode inoculum was viable. In these tests, Guardian would be rated resistant to both *M. incognita* and *M. javanica* infection based on the number of egg masses recovered (<1). One explanation for *M. incognita* and *M. javanica* resistance in Guardian is the presence of Nemaguard in its pedigree (Okie et al., 1994a). Nemaguard is resistant to both of these *Meloidogyne* spp. (Brooks and Olmo, 1961). However, Brooks and Olmo (1961) also noted that 25% of Nemaguard seedlings exhibited some root galling by *M. incognita,* whereas no root galling was detected with the isolate of *M. javanica* tested. In our study, root galling occurred on all rootstocks tested with either *M. incognita* or *M. javanica.* Furthermore, the number of root galls on Guardian was related to nematode species. In the presence of *M. incognita,* root galling was just as abundant on Guardian as on Lovell, with fewer ($P \leq 0.05$) galls detected on Nemaguard. However, the majority of galls on

Table 1. Effect of three different potting media on reproduction and root-gall formation of *Meloidogyne incognita* race 3 on Lovell peach seedlings grown in the greenhouse.[a]

	Egg masses per plant[b]		Egg masses per gram dry root		Galls per plant		Dry root weight (g)	
	Test 1[c]	Test 2[d]	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Sand-Vermiculite	5 a	67 a	12 a	84 a	89 a	90 a	0.5 a	0.9 a
Metro-Mix 200	2 b	17 b	3 b	23 b	74 a	61 b	0.8 b	1.2 a
Metro-Mix 360	1 b	9 b	2 b	8 b	75 a	52 b	0.8 b	1.3 a

Data are means of 16 replicates. Means within a column followed by the same letter are not different ($P \leq 0.05$) according to LSD.

[a] Initial population density of *Meloidogyne incognita* race 3 was 2,000 eggs/169 cm^3 soil.

[b] Mean number of egg masses per plant on tomato was 87 for Test 1 and 100 for Test 2.

[c] Test 1 peach seedlings were 56 days old when the media was infested with *Meloidogyne incognita* race 3 and terminated 64 days after inoculation.

[d] Test 2 peach seedlings were 73 days old when the media was infested with *Meloidogyne incognita* race 3 and terminated 60 days after inoculation.

TABLE 2. Susceptibility of two Guardian sources (USDA and Clemson), Lovell, and Nemaguard peach seedlings to *Meloidogyne incognita* race 3 and *Meloidogyne javanica* grown in the greenhouse for 110 days.[a]

Rootstock	Egg masses per plant		Eggs per plant		Eggs per egg mass		Eggs per gram of root		Galls per plant	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
					M. incognita[b]					
Lovell	92 a	13 a	218,290 a	15,640 a	2,175 a	1,543 a	72,800 a	6,999 a	—[c]	101 a
Guardian-USDA	<1 b	<1 b	20 b	20 b	15 b	5 b	7 b	13 b	—	93 a
Guardian-Clemson	<1 b	<1 b	60 b	30 b	33 b	10 b	23 b	11 b	—	101 a
Nemaguard	—	0 b	—	17 b	—	0 b	—	5 b	—	19 b
					M. javanica[b]					
Lovell	86 a	48 a	26,750 a	10,250 a	276 a	202 a	9,329 a	3,063 a	—	93 a
Guardian (USDA)	<1 b	<1 b	5 b	0 b	0 b	0 b	2 b	0 b	—	14 b
Guardian (Clemson)	<1 b	<1 b	5 b	0 b	0 b	0 b	2 b	0 b	—	26 b
Nemaguard	—	<1 b	—	0 b	—	0 b	—	0 b	—	15 b

Data are means of 10 replicates, except for Nemaguard rootstock, which had 6 replicates. The interaction between nematode and rootstock was not significant for number of egg masses per plant in Test 1 or for number of eggs per plant and eggs per gram dry root weight in Test 2. Means within a column followed by the same letter for a particular *Meloidogyne* sp. are not different ($P \leq 0.05$) according to LSD.

[a] Initial population density of *M. incognita* and *M. javanica* was 4,000 eggs/450 cm^3 sand-vermiculite medium.

[b] Number of eggs per plant by *M. incognita* and *M. javanica* on tomato was 80,000 and 205,000 (Test 1) and 47,500 and 46,250 (Test 2), respectively.

[c] — = not included.

Lovell were associated with egg masses (reproductive galls), whereas many of the galls produced on Guardian were not associated with egg masses (non-reproductive galls). Furthermore, no egg masses were observed to be embedded in the galls on Guardian roots. In the presence of *M. javanica,* fewer galls were found on Guardian and Nemaguard roots as compared to Lovell, but there were no differences in number of galls between Guardian and Nemaguard.

These data illustrate the importance of using several criteria for evaluating peach rootstocks for resistance to *Meloidogyne* spp. If numbers of root galls had been the sole criterion for classifying resistance to *M. incognita,* Guardian would have been classified susceptible. Numbers of egg masses per plant, eggs per plant, eggs per egg mass, and eggs per gram of root should be used in addition to numbers of root galls per plant when evaluating peach rootstocks for resistance to *Meloidogyne* spp. However, the effect of large numbers of galls on Guardian rootstock growth needs to be further investigated. In our host susceptibility studies, reduction in seedling growth was not detected between nematode inoculated and uninoculated treatments. One explanation might be the length of the experiments (110 days). Under field conditions, *Meloidogyne* spp. are generally associated with reduced tree growth during the first 2 years after planting (Nyczepir et al., 1993). Long-term evaluation (1 to 2 years) of Guardian rootstock infected with *M. incognita* needs to be examined to determine if (i) tree growth is reduced as a result of gall formation, and (ii) it may still be necessary to use preplant management for such *Meloidogyne* spp.

Nematode penetration and development: Comparable numbers of *M. incognita* J2 were detected in Guardian and Lovell roots 3 and 6 DAT in both tests (Table 3). The J2 stage of *M. incognita* was not detected in roots of either peach rootstock on or after 12 DAT. It appears that the mechanism of Guardian resistance is not due to differential degrees in root penetration by the number of J2. Similar results have been reported in other studies of *Meloidogyne* resistance, e.g., in resistant/susceptible alfalfa (Potenza et al., 1996), soybean (Pedrosa et al., 1996), and peach (Malo, 1967).

Various swollen stages of *M. incognita* parasitic juveniles, as illustrated by Taylor and Sasser (1978), were detected in roots of both Lovell and Guardian peach seedlings.

TABLE 3. Penetration and early development of *Meloidogyne incognita* race 3 in root systems of Lovell and Guardian peach rootstocks in the greenhouse 3, 6, 12, and 24 days after transplanting (DAT).

		Nematodes per root system					
		Vermiform[a]		Swollen[a]		Galls per plant	
DAT	Rootstock	Test 1	Test 2[b]	Test 1	Test 2	Test 1	Test 2
3	Guardian	8	21	0	32*	0	4
	Lovell	5	24	0	4	0	1
6	Guardian	2	7	19	7	26	7
	Lovell	1	2	7	20	15	17
12	Guardian	0	0	4	2	14	12
	Lovell	0	0	8	7	27	16
24	Guardian	0	0	9	0*	33	21
	Lovell	0	0	14	9[c]	48	44

Data are means of five replicates each inoculated with 1,000 *Meloidogyne incognita* J2. * = $P \leq 0.05$ on a particular date according to ANOVA.

[a] Developmental stages: vermiform = preparasitic, infective second-stage juvenile (not swollen); swollen = parasitic J2 (slightly swollen), late second-stage female (partially globose) and mature female (globose).

[b] Infection periods were 2 days for Test 1 and 4 days for Test 2.

[c] Sixty-six percent of these nematodes had an egg mass associated with their gall. Mature females with associated egg masses were detected only in Lovell roots Test 2 on Day 24.

In Test 1, swollen parasitic J2 were not detected until 6 DAT for both rootstocks. In Test 2, swollen parasitic J2 were detected as early as 3 DAT, with a greater ($P \leq 0.05$) number of nematodes occurring in Guardian roots than in Lovell. Observing swollen juveniles as early as 3 DAT in Test 2 may have resulted from extending the amount of time allowed for the J2 to penetrate roots before seedlings were transplanted into 10-cm-diam. pots. In Test 1, J2 were allowed 2 days to infect roots before seedlings were transplanted, compared to 4 days in Test 2. This would also explain why a few root galls were observed 3 DAT in Test 2 as compared to no root galls in Test 1 (Table 3). The number of swollen-stage juveniles did not differ between rootstocks on 6 or 12 DAT. However, it was observed that juvenile development was slower (i.e., rate of swelling arrested with time) in Guardian roots compared to Lovell. Furthermore, on 6 DAT 10 swollen parasitic J2s in Guardian roots (8 nematodes in Test 1; 2 nematodes in Test 2) appeared to be distorted in shape (i.e., shriveled)—a phenomenon not observed in Lovell. Egg production was observed only in Test 2 on 24 and 48 DAT. More ($P \leq 0.05$) mature females were detected in Lovell roots than in Guardian 24 DAT (Table 3). Sixty-six percent of these females in Lovell roots had an egg mass associated with their gall, whereas no nematodes or egg masses were observed in Guardian. Number of eggs per plant was also greater ($P \leq 0.05$) for Lovell (2,175) than Guardian (0). In Test 1, although egg mass formation or eggs were not detected in Lovell or Guardian roots, more early adult-stage females were observed in Lovell compared to a less swollen or mature J2 stage in Guardian 24 DAT. Root tissue at 48 DAT was more woody than on previous sampling dates, making it difficult to effectively stain and record the number of swollen stages of *M. incognita* between the two rootstocks. Nevertheless, a greater ($P \leq 0.05$) number of eggs per plant was associated with Lovell (1,540) than Guardian (20). Egg production by *M. incognita* on Guardian was practically negligible 48 DAT (ca. two complete life cycles). The nature of resistance in Guardian appeared to be inhibition of nematode development and failure to complete the life cycle. A similar phenomenon was reported for *M. javanica* on Nemaguard peach (Meyer, 1977, 1978).

Differences in numbers of galls per plant were not detected between rootstocks on any sampling date in both tests (Table 3). Galls associated with Guardian roots were generally smaller and more elliptical than those found on Lovell roots, which were

larger and globose. Occasionally, necrotic tissue was detected in the center of root galls associated with Guardian, but not Lovell. The root penetration and development studies showed that *M. incognita* J2 penetrate Guardian roots and gall formation occurs, but the majority of the nematodes do not complete their life cycle.

In these studies, both of the commercial Guardian rootstock sources were effective in suppressing the reproduction of *M. incognita* race 3 (GA-isolate) and *M. javanica* (NC-isolate). Furthermore, evaluation of *Prunus* rootstocks for resistance to *Meloidogyne* spp. should not be based on root galling alone. Additional long-term studies are needed to evaluate different isolates of *M. incognita* and *M. javanica* and other *Meloidogyne* spp. (Sharpe and Perry, 1967) to determine how broad and effective Guardian's resistance is.

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