

# Inheritance of Resistance to Root-knot Nematodes (*Meloidogyne* sp.) in *Prunus* Rootstocks

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**Abstract.** Two F<sub>1</sub> hybrid *Prunus* rootstocks, K62-68 and P101-41, developed from a cross of ‘Lovell’ [susceptible to both *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treb) Chitwood] and ‘Nemared’ (resistant to both root-knot nematode species), were selfed to produce two F<sub>2</sub> seedling populations. Vegetative propagation by herbaceous stem cuttings was used to produce four or eight self-rooted plants of each F<sub>2</sub> seedling for treatment replications. Eggs of *M. incognita* and *M. javanica* were inoculated into the potted media where plants were transplanted, and plants were harvested and roots examined for signs and symptoms associated with root-knot nematode infection ≈120 days later. Segregation ratios in both F<sub>2</sub> families suggested that resistance to *M. incognita* in ‘Nemared’ is controlled by two dominant genes (*Mi* and *Mij*) and that to *M. javanica* by a single dominant gene (*Mij*). Thus, *Mij* conveys resistance to both *M. incognita* and *M. javanica*.

Differences in resistance among peach [*Prunus persica* (L.) Batsch] rootstocks to root-knot nematodes (*Meloidogyne* sp.) were noted in the early 1920s, and variations were attributed to the presence of different *Meloidogyne* sp. (Tufts, 1929; Tufts and Day, 1934). *Meloidogyne incognita* and *M. javanica* are the most common species occurring in the warmer peach-growing regions in the United States (Nyczepir, 1991). ‘Shalil’ and ‘Yunnan’, two peach seedlings introduced from Asia, were initially selected for their resistance to *M. incognita*, but were found to be susceptible to *M. javanica* (Hansen et al., 1956; Weinberger et al., 1943). ‘Okinawa’ and ‘Nemaguard’ were reportedly resistant to both *M. incognita* and *M. javanica* (Okie et al., 1985; Sharpe et al., 1969). Later, other peach rootstocks, such as ‘Nemared’, ‘Flordaguard’, and ‘Guardian’ (BY520-9), were developed with resistance to root-knot nematodes (Okie et al., 1994; Ramming and Tanner, 1983; Sherman et al., 1991).

Weinberger et al. (1943) crossed susceptible peach cultivars with the resistant rootstocks ‘Shalil’ or ‘Yunnan’ and observed that resistance to root-knot nematodes was dominant. Later, Lownsbery and Thomason (1959) found that resistance to *M. incognita* was controlled by a single dominant allele. After crossing ‘Okinawa’ and testing its F<sub>1</sub> hybrids and F<sub>2</sub> progenies, Sharpe et al. (1969) reported that the resistance to *M. incognita* involves simple dominant inheritance, whereas the resistance to *M. javanica* involves duplicate, independent, dominant factors. Because the crosses made in previous studies were not strictly self-pollinated, and sample sizes of the segregating populations were limited, the results need to be confirmed. In addition, nematode races were not considered or identified in the earlier tests. The objective of our study was to further determine the genetic basis of resistance to *M. incognita* and *M. javanica* in peach rootstocks by improving sample size, experimental design, and treatment replication.

## Materials and Methods

Two peach rootstocks, ‘Lovell’ and ‘Nemared’, were used as parents in this inheritance study. ‘Lovell’ is a chance peach seedling that originated from California in 1882. It is widely used in the United States and is

homozygous-susceptible to *M. incognita* and *M. javanica* (Layne, 1987). ‘Nemared’ originated from F<sub>3</sub> seedlings of ‘Nemaguard’ crossed with a red-leaved selection. It is a new peach rootstock that is resistant to both species of root-knot nematode (Ramming and Tanner, 1983). The F<sub>1</sub> progenies of ‘Lovell’ × ‘Nemared’ were developed and evaluated at the U.S. Dept. of Agriculture–Agricultural Research Service (USDA–ARS), Horticultural Crops Research Laboratory (Fresno, Calif.), in the 1970s and 1980s, and two F<sub>1</sub> hybrids (K62-68 and P101-41) were selected for their superior resistance to *Meloidogyne* sp.

Two F<sub>2</sub> self-pollinated populations were developed from K62-68 and P101-41 at the USDA–ARS Southeastern Fruit and Tree Nut Research Laboratory (Byron, Ga.) in Spring 1995. Each F<sub>2</sub> population was maintained and studied separately. The F<sub>2</sub> seeds were stratified under moist conditions at 4 °C for 2 months, then germinated in the greenhouse (20 to 30 °C, natural sunlight). The F<sub>2</sub> seedlings were planted into 12-cm-diameter plastic pots filled with ≈1200 cm<sup>3</sup> of 50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight) for cuttings. Seeds from self-pollinated parents (‘Lovell’ and ‘Nemared’) were also collected and germinated to provide susceptible and resistant control seedlings, respectively.

A total of 55 F<sub>2</sub> seedlings from K62-68 and 176 F<sub>2</sub> seedlings from P101-41 were obtained after germination. Vegetative propagation of each F<sub>2</sub> seedling by herbaceous stem cuttings (Okie, 1984) was used to produce four or eight self-rooted plants for treatment. The plants were transplanted into 12-cm-diameter plastic pots filled with ≈1200 cm<sup>3</sup> of 50 sand : 50 vermiculite media (by volume) and grown in the greenhouse (20 to 30 °C, natural sunlight). The F<sub>1</sub> hybrids (K62-68 and P101-41), ‘Lovell’, and ‘Nemared’ were propagated from 8- to 10-cm-long hardwood cuttings by dipping the cuttings into 2000 mg·L<sup>-1</sup> IBA solution (50 ethanol : 50 water, by volume) for 10 s, then placing them into the sand/vermiculite medium under a mist system.

*Meloidogyne incognita* was originally isolated from a peach orchard in Georgia, and *M. javanica* from tobacco (*Nicotiana tabacum* L.) in North Carolina. These two isolates were separately maintained in pure greenhouse cultures on ‘Rutgers’ tomato (*Lycopersicon esculentum* Mill.) at Clemson Univ. Eggs of *M. incognita* and *M. javanica* were collected from the galled tomato roots using the NaOCl method described by Hussey and Barker (1973). The 50 sand : 50 vermiculite medium in each pot was infested with water suspensions of 6000 eggs of either *M. incognita* or *M. javanica* ≈30 d after transplanting. Osmocote fertilizer (14N–14P–14K, Scotts-Sierra Horticultural Products Co., Ohio) was supplied once at the beginning of the tests, and pots were watered daily or when necessary.

The experiment was conducted as a completely randomized design on benches in the greenhouse (20 to 30 °C, natural sunlight). The following groups of plants (four replicate plants per treatment) were inoculated with

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suspensions of eggs of each nematode species: 1) plants vegetatively propagated from each F<sub>2</sub> seedling of the F<sub>1</sub> hybrids K62-68 and P101-41; 2) plants vegetatively propagated from seedlings obtained by self-pollination of the hybrid's parents, 'Lovell' and 'Nemared'; and 3) rooted semi-hardwood cuttings of the F<sub>1</sub> hybrids K62-68 and P101-41. The same population of propagules of 55 K62-68 seedlings was used for both nematodes, but two populations of seedlings of P101-41 were used, 76 for *M. incognita*, and 100 for *M. javanica*.

About 120 d after infestation, seedlings were harvested, and the number of galls and egg masses per root system were recorded as described by Holbrook et al. (1983). The F<sub>2</sub> seedlings were evaluated for resistance or susceptibility by comparing their response to inoculation with those of their parents and both 'Lovell' and 'Nemared'. The rating for each genotype was determined by assigning a galling and an egg mass value to each root system based on the following index: R = resistant ( $\leq 10$  galls per root system, no egg masses) and S = susceptible ( $> 10$  galls per root system, with egg masses). Data were analyzed by chi square (SAS Institute, Cary, N.C.).

## Results

**Inheritance of resistance to *M. incognita*.** 'Nemared' and the two F<sub>1</sub> hybrids exhibited resistance ( $\leq 10$  galls, no egg masses) to *M. incognita* in the greenhouse tests, whereas 'Lovell' was susceptible ( $30 \pm 18.65$  galls and  $18.8 \pm 13.43$  egg masses per plant), indicating that resistance to *M. incognita* is dominant in 'Nemared'. The F<sub>2</sub> progenies derived from the K62-68 hybrid contained 49 resistant (R) : 6 susceptible (S) seedlings, supporting a segregation ratio of 15 R : 1 S with a chi square value of 2.03 ( $0.10 < P < 0.20$ ) (Table 1); the ratio among P101-41 seedlings was 69:7, again supporting the segregation ratio of 15 R : 1 S with a chi square value of 1.14 ( $0.20 < P < 0.50$ ) (Table 1). The ratios in both F<sub>2</sub> families suggested that resistance to *M. incognita* in 'Nemared' is controlled by two dominant genes.

**Inheritance of resistance to *M. javanica*.** 'Nemared' and the two F<sub>1</sub> hybrids exhibited resistance to *M. javanica* ( $\leq 10$  galls, no egg masses) in the greenhouse tests, whereas 'Lovell' was susceptible ( $26.0 \pm 15.28$  galls;  $12.3 \pm 10.16$  egg masses), indicating that resistance to *M. javanica* also was dominant in 'Nemared'. The F<sub>2</sub> progenies derived from the K62-68 hybrid contained 41 resistant: 14 sus-

ceptible seedlings, supporting a segregation ratio of 3 R : 1 S with a chi square value of 0.05 ( $0.80 < P < 0.90$ ) (Table 1); the ratio among the P101-41 seedlings was 83:17, again fitting the segregation ratio of 3 R : 1 S with a chi square value of 3.41 ( $0.05 < P < 0.10$ ) (Table 1). These ratios suggest that resistance to *M. javanica* in 'Nemared' is inherited as a single dominant gene.

**Relationship between resistance to *M. incognita* and to *M. javanica*.** In a total of 55 F<sub>2</sub> progenies of the K62-68 family, the actual segregation ratios for resistance to *M. incognita* and *M. javanica* were 49 R : 6 S and 41 R : 14 S, respectively. All six seedlings susceptible to *M. incognita* were also susceptible to *M. javanica*, but about half of those susceptible to *M. javanica* were susceptible to *M. incognita* (data not shown). Such segregation linkage of F<sub>2</sub> genotypes susceptible to both root-knot nematode species would be highly unlikely ( $P \leq 0.001$ ) if resistance to *M. incognita* and *M. javanica* were inherited independently. Our aforementioned results indicated that resistance to *M. incognita* and *M. javanica* in 'Nemared' was inherited as two dominant genes and a single dominant gene, respectively. Therefore, the dominant gene responsible for resistance to *M. javanica* also expressed resistance to *M. incognita*.

## Discussion

Lownsbery and Thomason (1959) and Sharpe et al. (1969) reported that resistance to *M. incognita* in peach rootstocks is controlled by a single dominant gene, whereas resistance to *M. javanica* is controlled by two independent dominant genes. However, we observed that resistance to *M. incognita* (Georgia isolate) and *M. javanica* were controlled by two dominant genes and a single dominant gene, respectively. These conflicting results may be explained by one or more variables in the three studies, such as differences in rootstock genotypes, isolates of root-knot nematodes, F<sub>2</sub> population sizes, or evaluation criteria (e.g., eggs vs. galls) used.

'Okinawa' and 'Nemaguard' are two major sources for *Prunus* rootstocks resistant to *Meloidogyne* sp. in the United States (Sharpe et al., 1969). 'Okinawa' originated from chance seedlings of *P. persica* and was introduced from Japan to Florida (Sharpe, 1957), and used as the breeding germplasm for root-knot nematode resistance in most previous studies (Sharpe et al., 1969). In contrast, 'Nemaguard' originated from a commercial seedlot labeled

*P. davidiana* (Okie et al., 1985), but may be either *P. persica* or a hybrid of *P. persica* and *P. davidiana*. 'Nemared', an F<sub>3</sub> selection derived from a 'Nemaguard' cross (Ramming and Tanner, 1983), was used in our study as the resistant parent. Therefore, the different inheritance models for resistance to *Meloidogyne* sp. in 'Okinawa' and 'Nemaguard' could have resulted from their different genotypes. Moreover, additional studies with molecular marker systems have confirmed that the genetic nature for resistance to root-knot nematodes differs between 'Okinawa' and 'Nemaguard' (Lu et al., 1999).

The concept of races in *Meloidogyne* sp. is an important issue in root-knot nematode management. Different races of *Meloidogyne* sp. induce different pathogenic responses in the same host plant (Nyczepir, 1991). For example, 'Nemaguard' and 'Nemared' were reported to be resistant to *M. incognita* race 1, but susceptible to *M. incognita* race 3 (Florida isolate) (Sherman et al., 1991). The *M. incognita* from our study is classified as race 3 (Georgia isolate) (Nyczepir, personal communication), which was reared from a single egg mass isolated from the root system of a stunted peach tree in Georgia. This Georgia isolate aggressively reproduced on peach rootstocks such as 'Lovell', but did not produce egg masses on roots of 'Nemaguard' and 'Nemared'; therefore, it is similar to *M. incognita* race 1 but different from the Florida isolate. Moreover, the esterase phenotype pattern of the Georgia isolate was similar to *M. incognita* race 1 and different from the Florida isolate (Nyczepir, personal communication). Janati et al. (1982) indicated that the esterase pattern of *M. incognita* race 3 (Florida isolate) differed from the standard *M. incognita* pattern and concluded that the isolate should be designated as a different species. Additional studies are currently underway to clarify the relationship between the Florida isolate and other *M. incognita* races.

The variability encountered when evaluating the degree of root-knot nematode infection on *Prunus* root systems has been noted by many investigators (Esmenjaud et al., 1997; Marull et al., 1994; Sharpe et al., 1969), and only the number of root-knot nematode galls has been the primary criterion in determining a resistant or susceptible rootstock in previous genetic studies (Minz and Cohn, 1962; Sharpe et al., 1969). Our study showed that *M. incognita* (Georgia isolate) and *M. javanica* could produce galls on root systems of the resistant parent 'Nemared', but no egg masses were

Table 1. Segregation for resistance to *Meloidogyne incognita* and *M. javanica* in F<sub>2</sub> families from the cross of 'Lovell' x 'Nemared' peach rootstock cultivars.

Species	F <sub>2</sub> families	Total no. seedlings <sup>a</sup>	Observed no.		Expected no.		$\chi^2$	P
			Resistant	Susceptible	Resistant	Susceptible		
<i>M. incognita</i> <sup>b</sup>	K62-68	55	49	6	51.6	3.4	2.03	0.10–0.20
	P101-41	76	69	7	71.3	4.7	1.13	0.20–0.50
<i>M. javanica</i> <sup>c</sup>	K62-68	55	41	14	41.2	13.8	0.05	0.80–0.90
	P101-41	100	83	17	75.0	25.0	3.41	0.05–0.10

<sup>a</sup>Four single-plant replications of each F<sub>2</sub> genotype were inoculated with each nematode. In the K62-68 family, the same 55 F<sub>2</sub> genotypes were used for both *M. incognita* and *M. javanica* treatments. In the P101-41 family, 76 F<sub>2</sub> genotypes inoculated with *M. incognita* were different from the 100 F<sub>2</sub> genotypes inoculated with *M. javanica*.

<sup>b</sup>Chi square test for 15:1 segregation ratio (two genes segregated independently).

<sup>c</sup>Chi square test for 3:1 segregation ratio (one gene segregated only).

observed. Therefore, both of these root-knot nematodes can infect and partially develop in resistant root systems, but can't complete their life cycles. The difference between resistant and susceptible *Prunus* rootstocks depends mainly on the success of *Meloidogyne* sp. in completing their life cycle in host root systems (Malo, 1967; Marull et al., 1994; Sharpe et al., 1969). To overcome the limitation of infection evaluations based on gall number, we observed and analyzed both the number of galls and the number of egg masses to determine the resistance or susceptibility to *M. incognita* (Georgia isolate) and *M. javanica*. This was a more reliable and consistent approach.

Resistance to *M. incognita* (Georgia isolate) and *M. javanica* are inherited dependently in our study. If the symbols *Mi* and *Mij* represent two genes responsible for resistance to *M. incognita*, and *Mij* for the gene resistant to *M. javanica*, the genotypes of the parents, F<sub>1</sub> hybrids, and F<sub>2</sub> progenies in our study could be interpreted as follows:

Parents:	<i>mijmijmimi</i> (Lovell)	x	<i>MijMijMiMi</i> (Nemared)
F <sub>1</sub> hybrids:	<i>MijmijMimi</i> (K62-68; P101-41)		
	<i>MijmijMimi</i> (for <i>M. incognita</i> )		<i>Mijmij</i> (for <i>M. javanica</i> )
	↓ ⊗		↓ ⊗
F <sub>2</sub> progenies:	15 R : 1 S		3 R : 1 S

Future studies with different peach F<sub>2</sub> populations segregating for resistance to root-knot nematodes are warranted to validate or refute this proposed hypothesis.

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