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

Zhen-Xiang Lu
Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, T1J 4B1, Canada

Gregory L. Reighard
Department of Horticulture, Clemson University, Clemson, SC 29634-0375

Andrew P. Nyczepir and Thomas G. Beckman
U.S. Department of Agriculture, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008

David W. Ramming
U.S. Department of Agriculture, Horticultural Crops Research Laboratory, Fresno, CA 93727

Additional index words. Prunus persica, Meloidogyne incognita, Meloidogyne javanica

Abstract. Two F1 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 (Treub) Chitwood] and ‘Nemared’ (resistant to both root-knot nematode species), were selfed to produce two F2, seedling populations. Vegetative propagation by herbaceous stem cuttings was used to produce four or eight self-rooted plants of each F2, 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 F2 families suggested that resistance to M. incognita in ‘Nemared’ is controlled by two dominant genes (Mi and Mj) and that to M. javanica by a single dominant gene (Mj). 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 F1 hybrids and F2 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 F1, 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 F1 progenies of ‘Lovell’ x ‘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 F2 hybrids (K62-68 and P101-41) were selected for their superior resistance to Meloidogyne sp.

Two F2 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 F2 population was maintained and studied separately. The F2 seeds were stratified under moist conditions at 4 °C for 2 months, then germinated in the greenhouse (20 to 30 °C, natural sunlight). The F2 seedlings were planted into 12-cm-diameter plastic pots filled with ~1200 cm3 of 50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight) for 2 months. The F2 seedlings were inoculated with 6000 eggs of either M. incognita or M. javanica and resistant control seedlings, respectively. The 5 F2 seedlings from each F2 population were grown in 2000 mg·L–1 IBA solution (50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight). The F2 seedlings were inoculated with 6000 eggs of either M. incognita or M. javanica and resistant control seedlings, respectively. The 5 F2 seedlings from each F2 population were grown in 2000 mg·L–1 IBA solution (50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight). The F2 seedlings were inoculated with 6000 eggs of either M. incognita or M. javanica and resistant control seedlings, respectively. The 5 F2 seedlings from each F2 population were grown in 2000 mg·L–1 IBA solution (50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight). The F2 seedlings were inoculated with 6000 eggs of either M. incognita or M. javanica and resistant control seedlings, respectively. The 5 F2 seedlings from each F2 population were grown in 2000 mg·L–1 IBA solution (50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight). The F2 seedlings were inoculated with 6000 eggs of either M. incognita or M. javanica and resistant control seedlings, respectively. The 5 F2 seedlings from each F2 population were grown in 2000 mg·L–1 IBA solution (50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight). The F2 seedlings were inoculated with 6000 eggs of either M. incognita or M. javanica and resistant control seedlings, respectively. The 5 F2 seedlings from each F2 population were grown in 2000 mg·L–1 IBA solution (50 sand : 50 vermiculite media (by volume), and grown in the greenhouse (20 to 30 °C, natural sunlight). The F2 seedlings were inoculated with 6000 eggs of either M. incog...
Nemared'. The F2 progenies derived from the 3) rooted semi-hardwood cuttings of the F1 seedlings obtained by self-pollination of the M. javanica square (SAS Institute, Cary, N.C.).

The results from the analysis of the F2 progeny of the F1 hybrids K62-68 and P101-41 for resistance to M. incognita and M. javanica are presented in Table 1. The data were analyzed by chi square test for 3:1 segregation ratio (one gene segregated only). The chi square value for the segregation ratio of 3 R : 1 S with a chi square value of 0.05 (0.05 < P < 0.10) (Table 1) suggests that resistance to M. javanica in 'Nemared' is inherited as a single dominant gene.

Results

Inheritance of resistance to M. incognita. ‘Nemared’ and the two F1 hybrids exhibited resistance (≤10 galls, no egg masses) to M. incognita in the greenhouse tests, whereas 'Lovell' was susceptible (30 ± 18.65 galls and 18.8 ± 13.43 egg masses per plant), indicating that resistance to M. incognita is dominant in ‘Nemared’. The F2 progenies derived from the K62-68 hybrid contained resistant (R) : 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, indicating that resistance to M. incognita and M. javanica was inherited independently. The ratio among the F2 genotypes susceptible to both root-knot nematode species was highly unlikely (P < 0.001) if resistance to M. incognita and M. javanica were inherited independently. Our 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.

Table 1. Segregation for resistance to Meloidogyne incognita and M. javanica in F2 families from the cross of ‘Lovell’ x ‘Nemared’ peach rootstock cultivars.

| Species | Total no. | Observed no. | Expected no. |
|---------|-----------|--------------|--------------|
|         | seedlings | Resist. | Suscept. | Resist. | Suscept. | χ² | P |
| M. incognita | 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 |
| M. javanica | 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 |

Footnotes:
- Four single-plant replications of each F2 genotype were inoculated with each nematode. In the K62-68 family, the same 55 F2 genotypes were used for both M. incognita and M. javanica treatments. In the P101-41 family, 76 F2 genotypes inoculated with M. incognita were different from the 100 F2 genotypes inoculated with M. javanica.
- Chi square test for 15:1 segregation ratio (two genes segregated independently).
- Chi square test for 3:1 segregation ratio (one gene segregated only).

Discussion

Lowenshby 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 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, F1 population sizes, or evaluation criteria (e.g., galls 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 a single F1 hybrid containing a resistant rootstock in a single egg mass from a root system of a grafted 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, if resistance to M. incognita race 1 and 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 (Eschenjaud et al., 1997; Marulis et al., 1994; Sharpe et al., 1969), and only the number of root-knot nematode galls has been the primary criterion in determining resistance 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...
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 Meloidogyne and the number of egg masses to determine the resistance or susceptibility to Meloidogyne species and correlative genetic implications. J. Nematol. 29:370–380.

Hansen, C.J., B.F. Lownsbury, and C.O. Hesse. 1956. Nematode resistance in peaches. Calif. Agr. 10(9):5–11.

Holbrook, C.C., D.A. Knauf, and D.W. Dickson. 1983. A technique for screening peanut for resistance to Meloidogyne arenaria. Plant Dis. 67:957–958.

Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. Plant Dis. Rptr. 57(12):1025–1028.

Janati, A., J.B. BERGE, A.C. Triantaphylliou, and A. Dalmasso. 1982. New data on isoesterase polymorphism in Meloidogyne spp.: Application to the taxonomy of some species of this genus. Revue Nematol. 5(1):147–154.

Janati, A., J.B. BERGE, A.C. Triantaphylliou, and A. Dalmasso. 1982. New data on isoesterase polymorphism in Meloidogyne spp.: Application to the taxonomy of some species of this genus. Revue Nematol. 5(1):147–154.

Laive, R.E.C. 1987. Peach rootstocks, p. 185–216. In: R.C. Rom and R.F. Carlson (eds.). Rootstocks for fruit crops. Wiley, New York.

Lowensbury, B.F. and J.J. Thomson. 1959. Progress in nematology related to horticulture. Proc. Amer. Soc. Hort. Sci. 74:730–746.

Lu, Z.-X., K. Sossey-Alaoui, G.L. Reighard, W.V. Baird, and A.G. Abbott. 1999. Development and characterization of a codominant marker linked to root-knot nematode resistance, and its application to peach rootstock breeding. Theor. Appl. Genet. 99(1/2):115–122.

Malo, S.E. 1967. Nature of resistance of ‘Okinawa’ and ‘Nemaguard’ peach to the root-knot nematode Meloidogyne javanica. Proc. Amer. Soc. Hort. Sci. 90:39–46.

Marull, J., J. Pinochet, A. Felipe, and L.L. Cenis. 1994. Resistance verification in Prunus selections to a mixture of thirteen Meloidogyne isolates and resistance mechanisms of a peach almond hybrid to M. javanica. Fund. Appl. Nematol. 17(1):85–92.

Minz, G. and E. Cohn. 1962. Susceptibility of peach rootstocks to root-knot nematodes. Plant Dis. Rptr. 46(7):531–534.

Nyczepir, A.P. 1991. Nematode management strategies in stone fruits in the United States. J. Nematol. 23(3):334–341.

Okie, W.R. 1984. Rapid multiplication of peach seedlings by herbaceous stem cuttings. HortScience 19:249–151.

Okie, W.R., T.G. Beckman, A.P. Nyczepir, G.L. Reighard, W.C. Newall, Jr., and E.I. Zehr. 1994. BY520-9, a peach rootstock for the southeastern United States that increases scion longevity. HortScience 29:705–706.

Okie, W.R., D.W. Ramming, and R. Scorza. 1985. Peach, nectarine, and other fruit breeding by the USDA in the last two decades. HortScience 20:633–641.

Ramming, D.W. and O. Tanner. 1983. ‘Nemared’ peach rootstock. HortScience 18:376.

Sharpe, R.H. 1957. ‘Okinawa’ peach resists root-knot nematodes. Florida Agr. Res. Rpt. 1957(1):8.

Sharpe, R.H., C.O. Hesse, B.F. Lownsbury, V.G. Perry, and C.J. Hansen. 1969. Breeding peaches for root-knot nematode resistance. J. Amer. Soc. Hort. Sci. 94:209–212.

Sherman, W.B., P.M. Lyrene, and R.H. Sharpe. 1991. ‘Flordaguard’ peach rootstocks. HortScience 26:427–428.

Tufts, W.P. 1929. Nematode resistance of certain peach seedlings. Proc. Amer. Soc. Hort. Sci. 26:98–110.

Tufts, W.P. and L.H. Day. 1934. Nematode resistance of certain deciduous fruit tree seedlings. Proc. Amer. Hort. Sci. 31:(suppl.)75–82.

Weinberger, J.H., P.C. Marth, and D.H. Scott. 1943. Inheritance study of root-knot nematode resistance in certain peach varieties. Proc. Amer. Hort. Sci. 42:321–325.

Future studies with different peach F2 populations segregating for resistance to root-knot nematodes are warranted to validate or refute this proposed hypothesis.

| Parents: | mirmirmimi X MijMijMiMi (Lovell) ↓ (Nemared) |
|---------|-----------------------------------------------|
| F1 hybrids: | MijMijMimi (K62-68; P101-41) |
| MijmijMimi (for M. incognita) | Mijmij (for M. javanica) ↓ ⊗ ↓ ⊗ |
| F2 progenies: | 15 R : 1 S | 3 R : 1 S |

Differences to a mixture of thirteen Meloidogyne isolates and resistance mechanisms of a peach-almond hybrid to M. javanica. Fund. Appl. Nematol. 17(1):85–92.

Minz, G. and E. Cohn. 1962. Susceptibility of peach rootstocks to root-knot nematodes. Plant Dis. Rptr. 46(7):531–534.

Nyczepir, A.P. 1991. Nematode management strategies in stone fruits in the United States. J. Nematol. 23(3):334–341.

Okie, W.R. 1984. Rapid multiplication of peach seedlings by herbaceous stem cuttings. HortScience 19:249–151.

Okie, W.R., T.G. Beckman, A.P. Nyczepir, G.L. Reighard, W.C. Newall, Jr., and E.I. Zehr. 1994. BY520-9, a peach rootstock for the southeastern United States that increases scion longevity. HortScience 29:705–706.

Okie, W.R., D.W. Ramming, and R. Scorza. 1985. Peach, nectarine, and other fruit breeding by the USDA in the last two decades. HortScience 20:633–641.

Ramming, D.W. and O. Tanner. 1983. ‘Nemared’ peach rootstock. HortScience 18:376.

Sharpe, R.H. 1957. ‘Okinawa’ peach resists root-knot nematodes. Florida Agr. Res. Rpt. 1957(1):8.

Sharpe, R.H., C.O. Hesse, B.F. Lownsbury, V.G. Perry, and C.J. Hansen. 1969. Breeding peaches for root-knot nematode resistance. J. Amer. Soc. Hort. Sci. 94:209–212.

Sherman, W.B., P.M. Lyrene, and R.H. Sharpe. 1991. ‘Flordaguard’ peach rootstocks. HortScience 26:427–428.