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Temperature and Age of Plant Affect Resistance in Peach-Almond Hybrid Rootstock Infected with *Meloidogyne javanica*

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Abstract. **The influence of temperature and age of the plant was determined on nematode reproduction on a susceptible almond *(Prunus amygdalus* Batsch.) and on a resistant peach-almond hybrid *(P. persica* Stok. x *P. amygdalus* Batsch.) rootstock inoculated with *Meloidogyne javanica* (Treub) Chitwood. Experiments were conducted under greenhouse conditions in heated and unheated sand beds. 'Garrigues' almond inoculated with 3000 nematodes per plant showed extensive galling, high final nematode population levels, and high counts of nematodes per gram of root at 27 and 32C. The hybrid G x N No. 1 showed minimal galling and reproduction at 27C but higher levels of galling and final population and nematode counts per gram of root at 32C, suggesting a partial loss of resistance with temperature increase. One-month-old and 1-year-old plants of 'Garrigues' were susceptible following inoculation with 2000 nematodes per plant, although plantlets (l-month) were significantly more affected. Plantlets of hybrid G x N No. 1 were also susceptible, but 1-year-old plants were resistant. Resistant genotypes (G x N selections) seem to require root tissue maturation before expressing full resistance.**

The expression of resistance to nematodes can be modified by environmental conditions that affect defense mechanisms in many crop species (Canto-Saenz, 1985; Rohde, 1972; Stover and Buddenhagen, 1986). Temperature, nutritional status, soil pH, and plant age are some of the major factors affecting resistance expression to nematode-infected plants (Ammati et al., 1986; Davide and Triantaphyllou, 1967; Dropkin, 1963; Jaffee and Mai, 1979; McClure et al., 1974; Sarah et al., 1991). Therefore, even without taking into account the nematode pathogenic variability, the assessment of resistance expression in plant material supposedly resistant after screening tests should consider additional stress evaluations to correlate quick and efficient tests carried out with plantlets (seedlings, in vitro or vegetatively propagated plantlets) with field performance. From the practical standpoint, a more rigorous evaluation will allow the detection of germplasm that would maintain desired nematode resistance under environmental stress,

Recent studies in Spain indicate that some experimental peach-almond hybrids (G x N) show a high level of resistance to *Meloidogyne incognita* (Kofoid and White), *M. arenaria* (Neal) Chitwood, and *M. javanica* (Marull et al., 1991; Marull and Pinochet, 1991). These G x N selections were derived from crosses between the almond (female parent) 'Garfi' and the root-knot-nematode-resistant peach (male parent) 'Nemared' (Ramming and Tanner, 1983). Besides the nematode-resistant features, most G x N selections are vigorous, have red leaves, show good compatibility with almond varieties, and are adapted to calcareous soils. In spite of these encouraging results, further testing is required to verify that the desirable root-knot-nematode-resistant features are maintained under environmental stress, such as those that may affect nursery material in Spain and other warm Mediterranean environments. The purpose of this research was to study the influence of soil temperature and age of plant tissue on the level of resistance of G x N No. 1 rootstock to root-knot nematodes.

Seeds of the almond rootstock 'Garrigues', together with hardwood and herbaceous cuttings of the peach-almond hybrid, G x N No. 1, were supplied by the Programa de Fruticultura of the Servicio de Investigación Agraria (SIA) of the Diputación General de Aragón in Zaragoza, Spain. Seeds of 'Garrigues' were treated with a solution of copper oxychloride for 24 h, rinsed with running water to eliminate traces of the fungicide, and stratified at 4C for 60 days in perlite-filled trays. These were then moved to a greenhouse to induce seed germination. The peach-almond hybrids were propagated from hardwood and herbaceous cuttings. Cuttings were treated for 5 sec with a 50% ethanol solution that contained 2000 ppm of indole butyric acid. Cuttings to be rooted were then

Table 1. Influence of soil temperature on root galling and reproduction of *Meloidogyne javanica* on 'Garrigues' almond (susceptible) and G x N No. 1 peach-almond hybrid (resistant) rootstocks 3 months after inoculation with 3000 nematodes per plant.

Rootstock[z]	Temp (°C)	No. galls/plant	Final nematode population (roots and soil)[y]	Nematodes/ g root	Resistance rating[x]
Garrigues	27	99 a	46,200 a	6,395 a	S
	32	67 a	20,050 a	1,600 b	S
G x N No. 1	27	5 a	90 b	5 b	R
	32	31 b	6,210 a	145 a	S

[z]Data are means of seven replications. Arithmetic means are presented, but data were transformed to log,, (N + 1) for analysis. Means in columns for each rootstock followed by the same letter do not differ according to Student *t* test (P = 0.05).
[y]Total number of nematodes per plant.
[x]S = susceptible; R = resistant.

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Table 2. Response of 'Garrigues' almond (susceptible) and G x N No. 1 peach-almond (resistant) rootstocks of different ages inoculated with 2000 *Meloidogyne javanica* per plant at 75 and 65 days after inoculation, respectively.

Rootstock[z]	Age of plants (months)	No. galls/plant	Final nematode population (soil and roots)[y]	Nematodes/ g root	Resistance rating[x]
Garrigues	1	45 a	14,040 a	5,370 a	S
	15	17 b	14,480 a	470 b	S
G x N No. 1	1	41 a	1,430 a	570 a	S
	13	0 b	0 b	0 b	HR

[z]Data are means of eight replications. Arithmetic means are presented, but data were transformed to $\log_{10}$ (N + 1) for analysis. Means in columns for each rootstock followed by the same letter do not differ according to Student *t* test *(P* = 0.05).

[y]Total number of nematodes per plant.

[x]S = susceptible; HR = highly resistant.

planted in 200-cm^3 pots containing a 3 quartz sand : 1 peat mixture (v/v) previously pasteurized at 80C. Germinated seeds and rooted cuttings were transplanted to 3-liter pots containing a pasteurized sandy loam textured soil (75% sand, 12% silt, 3% clay; pH 7.5; < 1% organic matter; and a cation exchange capacity <10 meq/100 g soil). Plants used for temperature studies were kept in the greenhouse for 9 weeks before inoculation, whereas hardwood cuttings and seedlings used in plant age studies had been started the previous year. One-month-old herbaceous cuttings were prepared the 2nd year.

The isolate of *M. javanica* was collected from fig *(Ficus carica* L.) in Cabrils, Barcelona, Spain. This isolate was increased on tomato *(Lycopersicum esculentum* Mill. cv. Roma) from single egg mass cultures. Nematode inoculum was prepared by macerating infested tomato roots in a blender for 15 sec in a 0.12% to 0.15% NaOCl solution (Hussey and Barker, 1973). Eggs were concentrated in a 0.025-mm sieve (500 mesh) and rinsed with tap water before inoculation.

Temperature study. Inoculum was adjusted to deliver a suspension of 3000 eggs of *M. javanica* per plant placed in six holes 3 to 4 cm deep located 3 cm from the base of the plant. The plants of 'Garrigues' (susceptible) and G x N No. 1 (resistant) rootstocks had uniform growth (10 to 15 leaves). A set of inoculated pots of 'Garrigues' and G x N No. 1 were placed in sand beds and subjected to ambient greenhouse temperature and humidity fluctuations. The other set of inoculated pots was placed in a thermoregulated sand bed and maintained at a mean soil temperature (measured at the center of the pot) of 32C throughout the study. Soil thermometers that recorded maximum and minimum temperatures were placed in four pots. Three daily readings were made on ambient and soil temperature in heated and unheat sand beds. The total number of root galls, final nematode population per plant [second-stage juveniles (J2) in soil and eggs together with J2 in roots counted separately and then added], and the number of nematodes per gram of root were assessed 90 days after inoculation (DAI). Nematodes in soil were obtained by removing soil from containers and placing contents in a large pan. Roots were washed free of soil particles in a second pan with a known volume of water. Contents of both pans were mixed and stirred for 1 min. A 250-cm^3 subsample of the slurry was obtained, and nematodes were extracted by differential sieving and sugar flotation (Jenkins, 1964).

Nematode extraction from roots was similar to that used for inoculum preparation (Marull et al., 1991). The extra time used to macerate the roots was needed to break lignification present in both rootstocks to free eggs and J2 embedded in the root tissue. Nematodes were then concentrated using 0.150, 0.074, and 0.025-mm sieves (100, 200, and 500 mesh, respectively). Root tissue and debris collected on the 0.150-mm sieve were discarded.

Plant age study. Plant material for this study was prepared in 2 years. One-month-old seedlings and 15-month-old plants of 'Garrigues' inoculated with 2000 eggs of *M. javanica* per plant were compared 75 DAI. Mean ambient temperature in the greenhouse during daytime was 27.5C (±5C) in this study. Seeds used in the first and 2nd years were from the same batch. Similarly, 1-month-old rooted cuttings and 13-month-old hardwood cuttings of G x N No. 1 with the same inoculum level were compared at 65 DAL Galling and nematode reproduction were assessed at the end of each experiment as described above. Resistance was rated according to the scale suggested by Taylor and Sasser (1978), based on galling and nematode reproduction: HR = highly resistant (nematode invades root but there is little or no reproduction, normally no galling); R = resistant (limited reproduction with final nematode population lower than initially, incipient galling); MR = moderately resistant (final population equal or slightly higher than initially, galling scarce although noticeable); S = susceptible (nematode reproduces well in a short period with abundant galling and egg masses in the roots).

In both studies, plants were watered daily, or as needed, and fertilized with full-strength Hoagland's nutrient solution once a week (Hoagland and Arnon, 1950). In the temperature and the plant age study, each material was replicated seven and eight times, respectively, in a completely randomized design. Data were analyzed by a one-way analysis of variance. Gall index, final nematode population density, and nematodes per gram of root data were $\log_{10}$-transformed (N + 1). Means were compared by Student's ***t*** test *(P = 0.05).*

Temperature study. Soil temperature recordings fluctuated between 27.7 and 33.9C (night/day), reaching an average of 31.K in heated sand beds, whereas in unheated beds, soil in pots fluctuated between 19.8 and 32.1C, averaging 27C. Mean difference between pots in heated and unheated sand beds was 4.8C. 'Garrigues' almond (susceptible) showed extensive galling and a high level of parasitism at both temperatures, although significantly more nematodes were found per gram of roots at 27C. The peach-almond hybrid G x N No. 1 expressed its high level of resistance to the root-knot nematode at 27C. However, it showed a significantly higher number of galls, and final population and number of nematodes per gram of root in pots maintained at 32C (Table 1). In spite of the visible galling and nematode reproduction at 32C on G x N No. 1, no egg masses were found in the root systems.

Plant age study. One-month-old and 15-month-old 'Garrigues' plants were susceptible, with no differences in the final nematode population (Table 2). However, there was significantly less galling and there were fewer nematodes per gram of root in 15 month-old plants than in 1-month-old plantlets; the latter had a considerably smaller root system. One-month-old plantlets of hybrid G x N No. 1 were also susceptible, but 13-month-old plants were highly resistant (Table 2). Galls of G x N No. 1 plantlets were considerably smaller (2 to 3 mm in diameter) than those of 'Garrigues' (2 to 10 mm in diameter).

Soil temperature and plant age appear to be two important factors that affect resistance expression in root-knot-nematode-resistant peach rootstocks. Wehunt (1972) reported the influence of temperature on the development of a root-knot species in a resistant *Prunus* rootstock. In that study, resistant 'Nemaguard' peach inoculated with *M. incognita* showed more galling at 30C than at 25, 35, or 40C. The author concluded that rootstock evaluation should be made at soil temperatures optimal for nematode development. Our findings with G x N No. 1 inoculated with *M. javanica* indicate similar susceptible host response at 32C in comparison to 27C (five incipient galls and no apparent reproduction). A partial loss of resistance in G x N No. 1 to the nematode resulting in larger root galls and a host response at the histological level similar to that found in the susceptible 'Garrigues' rootstock (giant cell formation and nemaiode development) was also observed (unpublished data). Practical implications at the field level could be important in that young and resistant rootstocks established in early spring can be affected in warm soils (30 to 35C during daytime in first 15 cm of top soil) infested with *Meloidogyne* spp., such as those that prevail in the peach-growing regions of Murcia, Andalucia, and Extremadura in southern Spain. These results could also explain galling observed in root-knot resistant 'Nemaguard' and 'Nemared' peach rootstocks in field plantations in Seville (C. Orero, personal communication).

However, high soil temperatures (mean of 32C) also adversely affected the number of nematodes per gram of root on the susceptible 'Garrigues' rootstock in comparison to 27C, even though there were no significant differences in the final nematode populations at the two temperature regimes. Although, in relative terms, reproduction of *M. javanica* can still be considered as high.

One-month-old G x N No. 1 responded as susceptible when inoculated with *M. javanica*. In contrast, the same plant material, when 13 months old, was highly resistant. Apparently, this resistant genotype seems to require a physiological maturation process of the root tissues before expressing full resistance against the nematode. Even in the susceptible 'Garrigues', 15-month-old plants showed less tendency to gall and had a considerably lower level of parasitism in comparison to less-lignified 1-month-old plants. These results indicate a high vulnerability of young rootstocks, which is likely to be important in material originating from seedlings, in vitro, or hardwood cuttings transplanted into infested soil during the first 3 to 6 months. 'Garrigues' and the resistant G x N No. 1 apparently can be severely attacked by root-knot nematodes in the initial stages of plant growth but would be less damaged or completely resistant after the first year of growth. It is concluded that plantlets of *Prunus* at certain stages of development might not be suitable for root-knot nematode rootstock selection procedures, since resistant genotypes could be overlooked due to the lack of full resistance expression in the early stages. The mechanisms involved in the delayed expression are unknown.

Literature Cited

Ammati, M., I.J. Thomason, and H.E. McKinney. 1986. Retention of resistance to *Meloidogyne incognita* in *Lycopersicon* genotypes at high soil temperature. J. Nematol. 18:491-495.

Canto-Saenz, M. 1985. The nature of resistance to *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, p. 225-231. In: J.N. Sasser and C.C. Carter (eds.). An advanced treatise on Meloidogyne. vol. I, Biology and control. North Carolina State Graphics, Raleigh.

Davide, R.G. and G. Triantaphyllou. 1967. Influence of the environment on development and sex differentiation of root-knot nematodes. I. Effect of infection density, age of the host plant and soil temperature. Nematologica 13:102-110.

Dropkin, V.H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne* reversed by temperature. Phytopathology 59:1632-1637.

Hoagland, D. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347. Univ. of California, Berkeley.

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. 57:1025-1028.

Jaffee, B.A. and W.F. Mai. 1979. Growth reduction of apple seedlings by *Pratylenchus penetrans* as influenced by seedling age at inoculation. J. Nematol. 11:161-165.

Jenkins, W.R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Dis. Rptr. 48:692.

Marull, J. and J. Pinochet. 1991. Host suitability of *Prunus* rootstocks to four *Meloidogyne* species and *Pratylenchus vulnus* in Spain. Nematropica 21:185-195.

Marull J., J. Pinochet, S. Verdejo, and A. Soler. 1991. Reaction of *Prunus* rootstocks to *Meloidogyne incognita* and *M. arenaria* in Spain. J. Nematol. 23(48):564-569.

McClure, M.A., K.C. Ellis, and E.L. Nigh. 1974. Resistance of cotton to the root-knot nematode *Meloidogyne incognita*. J. Nematol. 6:17-20.

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

Rohde, R.A. 1972. Expression of resistance in plants to nematodes. Annu. Rev. Phytopathol. 10:233-251.

Sarah, J.L., B. Osseni, and R. Hugon. 1991. Effect of soil pH on development of *Pratylenchus brachyurus* in pineapple roots. Nematropica 21:211-216.

Stover, R.H. and I. Buddenhagen. 1986. Banana breeding: Polyploidy, disease resistance and productivity. Fruits 41:175-191.

Taylor, A.L. and J.N. Sasser. 1978. Biology, identification and control of root-knot nematodes *(Meloidogyne* species), North Carolina State Univ. Graphics, Raleigh.

Wehunt, E.J. 1972. Influence of temperature on infection of *Meloidogyne incognita acrita* on 'Nemaguard' peach. Plant Dis. Rptr. 56:305-308.