

# INFLUENCE OF TEMPERATURE ON THE EXPRESSION OF RESISTANCE IN SIX *PRUNUS* ROOTSTOCKS INFECTED WITH *MELOIDOGYNE INCOGNITA*

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## ABSTRACT

Fernández, C., J. Pinochet, and A. Felipe. 1993. Influence of temperature on the expression of resistance in six *Prunus* rootstocks infected with *Meloidogyne incognita*. *Nematropica* 23:195–202.

The influence of temperature on galling and nematode reproduction was determined for one susceptible and five resistant *Prunus* rootstocks inoculated with *Meloidogyne incognita* in two experiments conducted under greenhouse conditions in heated and unheated sand beds. In the first experiment, GF-677 inoculated with 5 000 nematodes per plant became extensively galled and supported about twice as much reproduction at 26 as at 30 °C. The higher soil temperature did not alter the expression of resistance substantially in Nemared peach, or in the Marianna GF 8-1 and Citation plums. In the second experiment, GF-677 that had been inoculated with 4 000 nematodes per plant developed similar numbers of galls at 23 and 31 °C, but nematode reproduction was about 10 times higher at the higher temperature. On the peach-almond hybrid G × N No 22, reproduction was negligible at 23 °C, but a measurable increase in galling and reproduction occurred at 31 °C, suggesting a partial loss of resistance with temperature increase. Nemared peach and Myrobalan 29C plum maintained resistance at the higher temperature regimes.

*Key words:* *Meloidogyne incognita*, *Prunus*, resistance, rootstocks, temperature.

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## RESUMEN

Fernández, C., J. Pinochet y A. Felipe. 1993. Influencia de la temperatura sobre la expresión de resistencia en seis patrones de *Prunus* infectados con *Meloidogyne incognita*. *Nematropica* 23:195–202.

Se determinó la influencia de la temperatura sobre el agallamiento y reproducción del nematodo en un patrón de *Prunus* susceptible y cinco resistentes inoculados con *Meloidogyne incognita*. Dos experimentos se llevaron a cabo bajo condiciones de invernadero en banquetas de arena calefactada y no calefactada. En el primer ensayo, GF-677 inoculado con 5 000 nematodos por planta mostró un agallamiento extensivo y una alta reproducción del nematodo a 26 y 30 °C. La alta temperatura de suelo no afectó la expresión de resistencia en el melocotonero Nemared y en los ciruelos Marianna GF 8-1 y Citation. En el segundo ensayo inoculado con 4 000 nematodos por planta, GF-677 agalló bien, aunque el nematodo se reprodujo considerablemente más a 31 °C que a 23 °C. El nematodo apenas se reprodujo en el híbrido de melocotonero × almendro G × N No 22 a los 23 °C. Sin embargo, este genotipo mostró un incremento en agallamiento, población final y un mayor número de nematodos por gramo de raíz a los 31 °C, indicando una pérdida parcial de la resistencia con el aumento de la temperatura. Nemared y el ciruelo Myrobalan 29C mantuvieron la resistencia en ambas temperaturas.

*Palabras clave:* *Meloidogyne incognita*, patrones, *Prunus*, resistencia, temperatura.

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## INTRODUCTION

Various factors, including temperature, nutritional status, soil pH, salinity, and plant age can affect the expression of resistance in many crop species (2,9, 11,12). Temperature has been shown to

alter resistance expression in several annual crops (1,4,8), as well as in some *Prunus* rootstocks. Wehunt (14) found that Nemaguard peach inoculated with *Meloidogyne incognita* (Kofoid & White) Chitwood developed more galled tissue

at 30 °C than at 25, 35 or 40 °C. More recently, Canals *et al.* (3) reported a partial loss of resistance at 32 °C in an experimental peach-almond hybrid that previously had shown a high level of resistance at lower temperatures. They concluded that a more rigorous evaluation considering high as well as moderate temperatures is necessary to assure that nematode resistant germplasm will maintain the trait at the high soil temperatures (> 30 °C) that prevail in warm Mediterranean environments. The purpose of this research was to study the influence of soil temperature on the expression of resistance to *M. incognita* in six *Prunus* rootstocks. Most of these materials are recent introductions into the Spanish stone fruit market.

#### MATERIALS AND METHODS

General information on the plant material tested is given in Table 1. The experimental peach-almond hybrid, G × N No 22, was supplied by the Programa de Fruticultura of the Servicio de Investigación Agraria (SIA) of the Diputación General de Aragón in Zaragoza, Spain.

Nemared peach, the peach-almond hybrid GF-677, and the plums Marianna GF 8-1, Citation, and Myrobalan 29 C were provided by private sources.

Two experiments were conducted. The first (May–July 1992) evaluated GF-677, Nemared, Marianna GF-81, and Citation and the second (April–June 1993) evaluated GF-677, Nemared, G × N No 22, and Myrobalan 29 C. In both experiments, seeds of Nemared were stratified at 4 °C for 60 days in trays filled with perlite. Seed trays were then moved to a greenhouse to induce germination. G × N No 22 was propagated from herbaceous cuttings and Citation from hardwood cuttings. Cuttings were treated for 5 seconds with a 50% ethanol solution that contained 1 000 ppm indolebutyric acid, then rooted in small 200-cm<sup>3</sup> pots containing a 3:1 (v:v) mixture of pasteurized quartz sand and peat. The remaining genotypes, GF-677, Marianna GF 8-1, and Myrobalan 29 C were micropropagated. The resulting *in vitro* plantlets were transferred from agar to 50-cm<sup>3</sup> minipots containing peat and then climatized in a high humidity chamber for 24 days. Plants with uni-

Table 1. General information on six *Prunus* rootstocks whose resistance to *Meloidogyne incognita* was evaluated under two temperature regimes.

Rootstock	Species or selection	Origin <sup>z</sup>
GF-677	Natural peach-almond hybrid ( <i>Prunus persica</i> × <i>P. amygdalus</i> )	INRA, France
G × N No 22	Nemared peach × Garfi almond ( <i>P. persica</i> × <i>P. amygdalus</i> )	SIA, Zaragoza, Spain
Nemared	Peach ( <i>P. persica</i> )	USDA, California, U.S.A.
Marianna GF 8-1	Plum ( <i>P. cerasifera</i> × <i>P. munsoniana</i> )	INRA, France
Myrobalan 29 C	Plum ( <i>P. cerasifera</i> )	Univ. California, U.S.A.
Citation	Plum ( <i>P. persica</i> × <i>P. belsiana</i> )	Zaiger, California, U.S.A.

<sup>z</sup>INRA = Institut National de la Recherche Agronomique; SIA = Servicio de Investigación Agraria; USDA = United States Department of Agriculture.

form growth were transferred to 3-L plastic pots that contained a pasteurized sandy loam soil (90% sand, 8% silt, 2% clay; pH 7.44; less than 2% organic matter; cation exchange capacity < 10 meq/100 g soil). Plants were kept in the greenhouse for 8 weeks before inoculation.

A different isolate of *M. incognita* was used in each experiment. The first was collected from the peach-almond hybrid Adafuel, in Zaragoza, and the second from GF-677 in Villaverde del Río, Seville. Both isolates were increased on tomato (*Lycopersicon esculentum* Mill.) cv. 'Redondo Liso Ace' from single egg mass cultures. Nematode inoculum was prepared by macerating infested tomato roots in a blender for 15 seconds in a 0.12–0.15% NaOCl solution (6). Eggs were collected in a 0.025-mm sieve (500 mesh) and rinsed with tap water before inoculation.

In the first experiment, inoculum was adjusted to deliver a suspension of 5 000 eggs of *M. incognita* per plant through five holes 3–4 cm deep, 3 cm from the stem. Half of the inoculated pots were placed in sand beds and subjected to normal fluctuations in ambient greenhouse temperature and humidity. The other half were placed in a heated sand bed and maintained at a mean soil temperature (measured at the center of the pot) of 30 °C. Maximum and minimum soil temperatures were measured daily in both heated and unheated sand beds. In both experiments, plants were watered as needed and fertilized weekly with full strength Hoagland's solution (5).

The number of root galls, total nematode population per plant, numbers of second-stage juveniles (J2) in soil, and number of J2 + eggs were assessed 97 days after inoculation. Nematodes in soil were obtained by removing the soil from each container and placing it 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<sup>3</sup> subsample of the slurry was obtained and nematodes extracted by differential sieving and sugar flotation (7). Nematodes were extracted from roots by the same technique used for preparing inoculum, but the blending time was increased to 30 seconds to free eggs and J2 embedded in lignified root tissue. Nematodes were separated from root debris using nested sieves with pore sizes of 0.150, 0.074 and 0.025 mm. Numbers of nematodes from soil and roots (including eggs) were added to derive an estimate of the total nematode population per pot.

An overall resistance rating was given to each cultivar according to the scale suggested by Taylor and Sasser (13), 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 initial, incipient galling); MR = moderately resistant (final population equal or slightly higher than initial, galling scarce, although noticeable); S = susceptible (nematode reproduces well in a short period with abundant galling and egg masses in the roots).

The protocol followed in the second experiment differed from that of the first only in the use of 4 000 (rather than 5 000) eggs per plant as inoculum and termination of the experiment 84 rather than 97 days after inoculation.

In the first and second experiment, each material was replicated nine and eight times, respectively, in a completely randomized design. Data were analyzed by a one way analysis of variance. Data on total number of galls, total nematode

population per pot, and amount of nematodes per gram of root were  $\log_{10}(x+1)$  transformed. Means were compared Tukey's test ( $P = 0.05$ ).

## RESULTS

*First experiment:* In heated sand beds, daily temperatures fluctuated between 27 and 33 °C and averaged 30 °C. In unheated beds, soil temperatures fluctuated between 22 and 30 °C and averaged 26 °C (Fig. 1). Thus, the mean difference between pot temperatures in heated and unheated sand beds was 4 °C.

The susceptible control (peach-almond hybrid GF-677) was extensively galled at both temperatures and the number of galls per plant at the two temperatures did not differ. However, the total population per pot and the number of nematodes per gram of roots was about twice as great

at 30 °C as at 26 °C (Table 2). Nemared peach developed virtually no galling at either temperature; the number of nematodes in the soil in the heated treatment, however, was about 10 times that at 26 °C, indicating that at 30 °C some additional reproduction occurred. Marianna GF 8-1 maintained its resistance at both temperatures, with only occasional galling; the final population detected was no more than 2% of the inoculum level at both temperatures. In Citation, neither galls nor nematodes were found in root systems and negligible numbers of J2 were recovered from soil.

*Second experiment:* In heated sand beds, daily soil temperatures fluctuated between 29 and 34 °C and averaged of 31 °C. In unheated beds, soil temperatures fluctuated between 21 and 24 °C and averaged of 23 °C (Fig. 2). Thus, the mean difference between temperatures in heated and unheated sand beds was 8 °C.

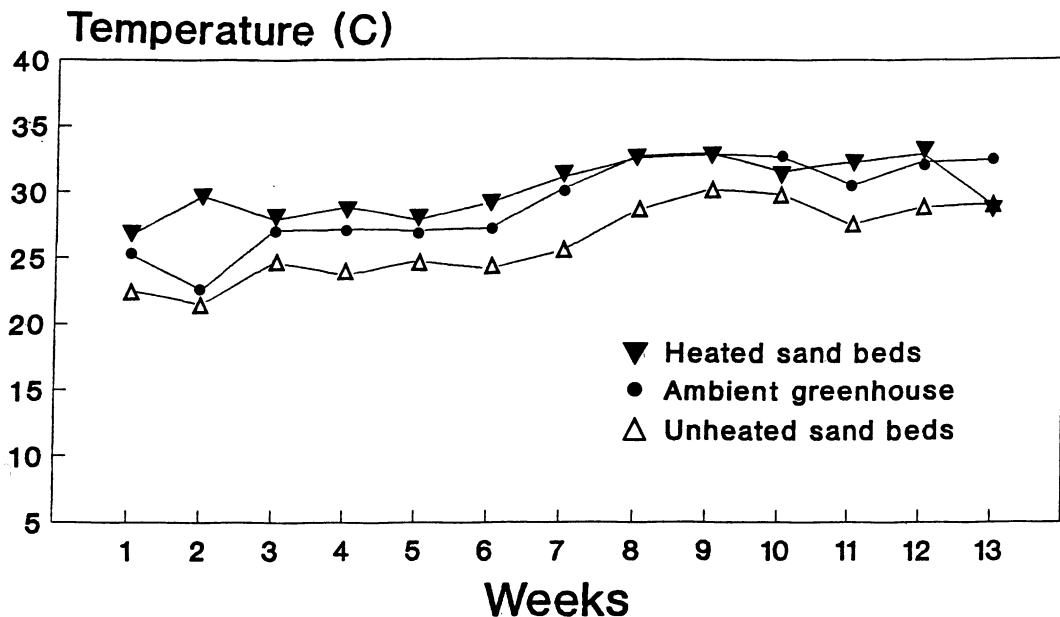


Fig. 1. Mean soil temperature (heated and unheated) and ambient temperature in the first greenhouse experiment.

Table 2. Influence of soil temperature on root galling and on reproduction of *Meloidogyne incognita* on four *Prunus* rootstocks 3 months after inoculation with 5 000 nematodes per plant.\*

Rootstock	Average soil temperature (°C)	Number of galls per plant	Final nematode population (roots and soil) <sup>y</sup>	Nematodes per gram of root	Resistance rating <sup>z</sup>
GF-677	26	165 a	22 750 a	5 070 a	S
	30	197 a	40 705 b	10 670 b	S
Nemared	26	1 a	216 a	18 a	R
	30	2 a	1 323 b	10 a	R
Marianna GF 8-1	26	1 a	45 a	0 a	HR
	30	6 b	125 a	11 a	R
Citation	26	0 a	149 a	0 a	HR
	30	0 a	70 a	0 a	HR

\*Data are means of nine replications. Arithmetic means are presented, but data were transformed to log<sub>10</sub> (x + 1) for analysis. Means in columns for each rootstock followed by the same letter do not differ according to Tukey's test (P = 0.05).

<sup>y</sup>Total number of nematodes per plant.

<sup>z</sup>S = susceptible; R = resistant; HR = highly resistant.

GF-677 was extensively galled as in the first experiment at both temperatures and had a significantly higher total nematode population per pot as well as more

nematodes per gram of roots at the higher temperature (Table 3). The peach-almond hybrid G × N No 22 was resistant to *M. incognita* at 23 °C, but at 31 °C had

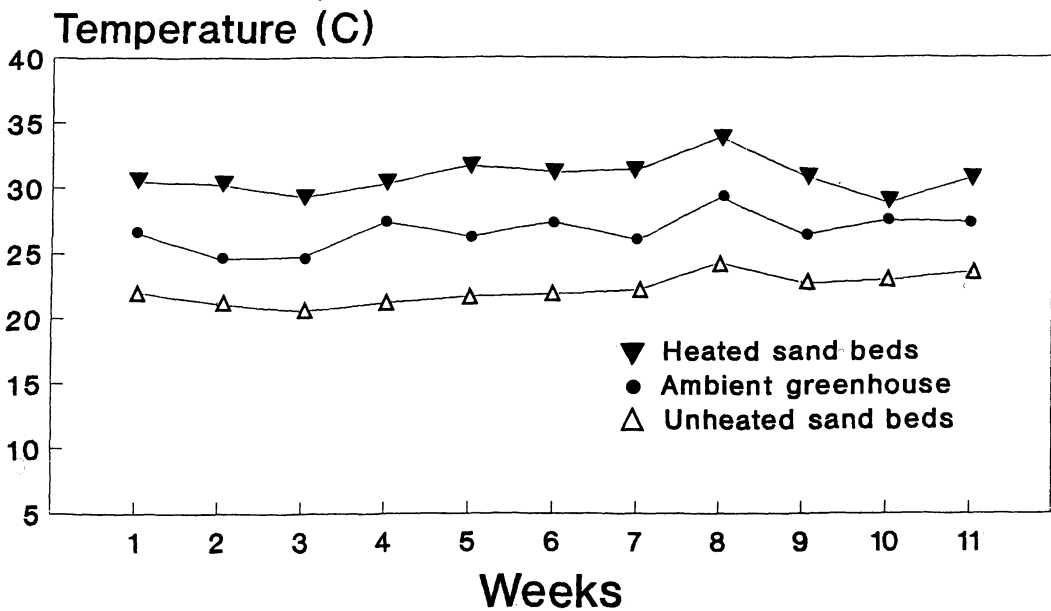


Fig. 2. Mean soil temperature (heated and unheated) and ambient temperature in the second greenhouse experiment.

Table 3. Influence of soil temperature on root galling and on reproduction of *Meloidogyne incognita* on four *Prunus* rootstocks 3 months after inoculation with 4 000 nematodes per plant.<sup>x</sup>

Rootstock	Average soil temperature (°C)	Number of galls per plant	Final nematode population (roots and soil) <sup>y</sup>	Nematodes per gram of root	Resistance rating <sup>z</sup>
GF-677	23	166 a	13 750 a	2 650 a	S
	31	170 a	161 206 b	13 900 b	S
Nemared	23	2 a	129 a	6 a	R
	31	6 a	186 a	31 b	R
G × N No 22	23	2 a	272 a	50 a	R
	31	27 b	5 710 b	950 b	MR-S
Myrobalan 29 C	23	0 a	0 a	0 a	HR
	31	1 a	9 a	13 a	HR

<sup>x</sup>Data are means of eight replications. Arithmetic means are presented, but data were transformed to  $\log_{10}(x + 1)$  for analysis. Means in columns for each rootstock followed by the same letter do not differ according to Tukey's test ( $P = 0.05$ ).

<sup>y</sup>Total number of nematodes per plant.

<sup>z</sup>S = susceptible; MR = moderately resistant; R = resistant; HR = highly resistant.

significantly more galls (27), a greater total population (5 710), and more nematodes per gram of roots (950) than at 23 °C. The high levels of resistance in Nemared peach and Myrobalan 29 C were not affected by temperature.

#### DISCUSSION

The selection G × N No 22 is an experimental peach-almond hybrid derived from a cross between the root-knot nematode resistant peach Nemared (male parent) (10) and the almond Garfi (female parent). Besides having good vigor, high compatibility with almond varieties, and desired nematode resistant features, G × N No 22 has shown a high level of tolerance to calcareous soils. In our experiments, its resistance to *M. incognita* was less at 31 °C than at 23 °C, while this did not occur with its nematode-resistant parent, Nemared peach. Nemared maintained a similar level of resistance under both temperature regimes. The partial loss of resistance in G × N No 22 should

be put into perspective by comparing it with a susceptible rootstock, such as GF-677. In relative terms, galling and nematode reproduction for G × N No 22 even at 30–31 °C were low, and likely would not affect field performance appreciably, since root systems were vigorous and well formed, and galls (2–4 mm in diameter) were detectible only after thorough examination. It is noteworthy that the high temperature regimes also affected the susceptible peach almond hybrid GF-677, considerably increasing its susceptibility to the nematode in both experiments.

The two experiments differed somewhat with regard to inoculum rates, duration and temperatures tested. In spite of these differences, the responses of GF-677 and Nemared were generally consistent, supporting the interpretation that the loss of resistance observed in GF-677 and G × N No 22 at high temperature is an inherent trait that will be observed in additional tests. This interpretation is further supported by similar results obtained

with another G × N selection (G × N No 9, now discarded) tested 2 years ago with a population of *M. javanica* (Treub) Chitwood in which a partial loss of resistance was found at 32 °C (3).

Soil temperature appears to be an important factor affecting resistance expression in some *Prunus* rootstocks that are resistant to root-knot nematodes. In general, the three plums that were tested (Marianna GF 8-1, Myrobalan 29 C, and Citation) and to a lesser extent Nemared peach, appear to maintain their good level of resistance as soil temperature increases (up to 31 °C). In contrast, the peach-almond hybrid G × N No 22 showed a partial loss of resistance, a limitation that should be taken into account in the warmer regions where stone fruit crops are grown, such as Murcia, Andalucía, and Extremadura in the southern part of Spain. In these areas, nursery material established early in the season is frequently exposed to temperatures ranging from 30 to 35 °C during the daytime in the uppermost 15 cm of soil. Our findings confirm the importance of verifying resistance to root-knot nematodes in *Prunus* rootstocks during the selection process at temperatures above 27 °C. This temperature appears near the thermal limit for retention of normal resistance in some *Prunus* genotypes and the temperature effect may be even more critical with peach-almond hybrid rootstocks.

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