Journal of Nematology 45(1):49–57. 2013.

Soil Temperature Effects on the Interaction of Grape Rootstocks and Plant-parasitic Nematodes

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Abstract: Resistance to *Meloidogyne* spp. in commonly used resistant grape rootstocks is slightly compromised at soil temperatures above 27°C. Newly released UCD-GRN series rootstocks, which have broad nematode resistance, exhibit trace infections by *Meloidogyne* spp. at elevated temperature. Pathotypes of *M. incognita* and *M. arenaria* that are virulent on 'Harmony' rootstock, as well as *M. incognita* Race 3, which is avirulent on 'Harmony', failed to produce egg masses on the UCD-GRN series rootstocks and other resistant selections at 24°C. At 27°C and above, there was increased nematode galling and egg mass production; at 30°C, egg mass production levels of *M. incognita* Race 3 on 'Harmony' were up to 12% of that on susceptible 'Colombard' while reproduction of the virulent pathotypes on the UCD-GRN series was less than 5% of that on 'Colombard'. Resistance of several of the parental genotypes of the UCD-GRN rootstock series was slightly compromised at soil temperatures of 30°C and above; however, others maintained their resistance to even the virulent *M. arenaria* pathotype A at high temperatures. Effects of high temperature on resistance to *Xiphinema index* could not be assessed because of temperature sensitivity of the nematodes while resistance to *Mesocriconema xenoplax* was not compromised at high soil temperature. Resistance to *Meloidogyne* spp. in the UCD-GRN series rootstocks was not compromised when plants and nematodes were subjected to cyclical high and low temperature conditions, indicating that once initiated, the resistance mechanism is not reversed.

Key words: Broad resistance, durable resistance, host status, temperature effects.

A wide diversity of plant-parasitic nematodes occurs in grape vineyards throughout the world (McKenry, 1992; Brown et al., 1993; Nicol et al., 1999; Anwar et al., 2002). Many nematode species appear to have been distributed concomitantly with planting material. Besides several species *Meloidogyne* (root-knot nematodes), two or more species of *Xiphinema* (dagger nematodes), *Pratylenchus vulnus* (root-lesion nematode), *Mesocriconema xenoplax* (ring nematode), *Tylenchulus semipenetrans* (citrus nematode), and *Paratylenchus* spp. (pin nematodes) occur commonly (Raski and Lider, 1959; Siddiqui et al., 1973; Pinochet et al., 1976; Ferris and McKenry, 1976; Harris, 1983; Pinkerton et al., 2005).

Rootstocks have been used in viticulture to protect against soil pests for 150 years (Reisch et al., 2012). In California, the selection pressure resulting from wide usage of 'Harmony' rootstock, resistant to *Meloidogyne* spp., has resulted in the emergence of virulent pathotypes of *M. incognita* and *M. arenaria* (Cain et al., 1984; McKenry, 1992; Anwar and McKenry, 2002; Esmenjaud and Bouquet, 2009). Similar virulent pathotypes have been selected by the closely-related 'Freedom' rootstock (Anwar et al., 1999). Over a 15-yr period, we have screened and tested species of *Vitis*, and the progeny of crosses among *Vitis* and *Muscadinia* species to determine their suitability in providing grape rootstocks with broad and durable resistance to important plant-parasitic nematode pests of grapevines. Those studies resulted in the release to the industry of five rootstocks ('UCD-GRN1-5') that have different combinations of resistance to plant-parasitic nematodes. All have resistance to *Meloidogyne incognita* Race 3, and to pathotypes of *M. incognita* and *M. arenaria* that are virulent on the widely used root-knot resistant 'Harmony' rootstock. Additionally, the rootstocks are resistant to *Xiphinema index* and 'UCD-GRN1' has resistance to *Mesocriconema xenoplax*. Some of the rootstocks are moderately resistant to *Pratylenchus vulnus*, *Tylenchulus semipenetrans*, or *Paratylenchus hamatus* (Ferris et al., 2012).

The resistance characteristics of the 'UCD-GRN' rootstocks are maintained when the rootstocks are challenged with different combinations and population levels of nematodes (Zheng et al., 2010, 2011; Ferris et al., 2012). Resistance to root-knot nematodes in some plant species, including resistant cultivars of tomato, cotton, and other crops, is sensitive to temperature. Soil temperature affects the relationships between host plants and their parasites in three main ways: (i) rates of physiological processes, growth, and responses to infection of the host plants are temperature regulated; (ii) physiological processes, activity and population increase rates of nematodes are temperature regulated; and (iii) expression of genes that regulate the interaction of host and parasite may be temperature sensitive (Griffin, 1969; Jatala and Russell, 1972; Carter, 1982; Thies and Fery, 1998). Since the range of soil temperatures experienced geographically and seasonally in California is likely to exceed those of the climate-controlled greenhouse conditions under which the 'UCD-GRN1-5' rootstocks were selected, we tested the durability of their resistance at different temperatures.

Because soil temperatures fluctuate with ambient conditions, we tested the hypothesis that amplitude of the temperature fluctuation cycle would determine

Received for publication June 29, 2012.
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We thank XuYun Yang, Kurt Kabica, Geoffrey Dervishian, Nina Romero, and Jeffrey Wheeler for technical assistance. Nathan Cannell provided invaluable assistance in design and development of equipment. The research and rootstock development has been supported by grants to Ferris and Walker from American Vineyard Foundation, California Grape Rootstock Improvement Commission, California Grape Rootstock Research Foundation, California Raisin Marketing Board, California Table Grape Commission, California Competitive Grant Program in Viticulture and Enology, and the Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board.
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This paper was edited by Andreas Westphal.

TABLE 1. Parent species and cultivars, progeny of intermediate crosses, and genealogy of nematode-resistant rootstocks.

Species or designation	Cultivars and parent crosses
Vitis riparia	'Gloire'
V. champinii	'Ramsey'
V. champinii	'Dog Ridge'
V. rupestris	'A. de Serres'
V. champinii	'c9021'
V. rufotomentosa	'1416'
Muscadinia rotundifolia	'Cowart'
'L91-64'	*V. riparia* 'Gloire' × *V. candicans* '3642'
'L6-1'	(*V. riparia* 'Gloire' × *V. champinii* 'Ramsey') × (*V. riparia* 'Gloire' × *V. champinii* 'Ramsey')
'L514-30'	*V. rufotomentosa* × (*V. riparia* 'Gloire' × *V. champinii* 'Dog Ridge')
'L514-20'	*V. rufotomentosa* × (*V. riparia* 'Gloire' × *V. champinii* 'Dog Ridge')
'L514-10'	*V. rufotomentosa* × (*V. riparia* 'Gloire' × *V. champinii* 'Dog Ridge')
'L513-4'	*V. rufotomentosa* × *V. riparia* 'Gloire'
'L25-19'	*V. champinii* '3639' × (*V. riparia* 'Gloire' × *V. champinii* 'Ramsey')
'UCD-GRN1'	*V. rupestris* 'A. de Serres' and *M. rotundifolia* 'Cowart'
'UCD-GRN2'	'L514-30' × *V. riparia* 'Gloire'
'UCD-GRN3'	'L514-10' × *V. champinii* c9038
'UCD-GRN4'	'L514-10' × *V. champinii* c9038
'UCD-GRN5'	'L6-1' × *V. champinii* 'c9021'

whether resistance was compromised. We reasoned that if resistance mechanisms were associated with initial phases of the infection process, 7-d exposure to a resistance-compromised 30°C would allow sufficient time for root penetration and feeding-site initiation and that development would continue during a subsequent 7 days at 24°C. Alternatively, under a temperature cycle of shorter amplitude, the effective resistance would not be compromised during 3.5-d exposure at 30°C exposure due to insufficient time for feeding-site initiation before return to 24°C.

MATERIALS AND METHODS

General procedures: Populations of all nematode species used in these experiments were maintained on grapevines (*Vitis rupestris* 'St. George') except for *Meloidogyne* spp., which were maintained on tomato (*Solanum lycopersicum* 'UC82'). *Meloidogyne incognita* pathotype Harmony C and *M. arenaria* pathotype Harmony A were transferred from tomato to 'Harmony' rootstock for 3 mon each year to ensure their continued virulence to its mechanisms of *Meloidogyne*-resistance. Five rootstocks, 'UCD-GRN1-5', with resistance to *M. incognita* Race 3, *M. arenaria* pathotype Harmony A, *M. incognita* pathotype Harmony C, and *Xiphinema index* were released to nurseries after a screening program involving successive challenges with the different nematodes (Ferris et al., 2012). The rootstocks were maintained as mother vines in the greenhouse and were available for propagation via herbaceous cuttings.

TABLE 2. Durability of resistance to reproduction of *Meloidogyne incognita* Race 3 under constant and fluctuating (7-d and 3.5-d amplitude) soil temperature conditions on a susceptible and resistant grape cultivars.

Temperature profile	Egg masses/g root			
	'Colombard'	'UCD-GRN4'	'Harmony'	'UCD GRN1'
24°C constant	26.8 b	0.0 b	0.0 b	0.0 b
24°-30°, 7 d	58.9 b	0.0 b	0.0 b	0.0 b
24°-30°, 3.5 d	68.8 ab	0.0 b	0.0 b	0.0 b
30°C constant	134.6 a	13.7 a	11.5 a	3.0 a
30°-24°, 7 d	47.1 b	0.0 b	0.0 b	0.0 b
30°-24°, 3.5 d	38.9 b	0.0 b	0.0 b	0.0 b

Statistical analyses were conducted with the analysis of variance (ANOVA) routines in the General Linear Models component of the Advanced Linear/Nonlinear Models package of Statistica 8 (Statsoft). Some regression coefficients were calculated via the data analysis component of Microsoft Excel.

Effect of temperature on durability of resistance of the rootstocks: Herbaceous cuttings from greenhouse-maintained mother vines of the resistant 'UCD-GRN' series rootstocks and of susceptible genotypes (*V. vinifera* 'Colombard' and *V. rupestris* 'St. George') were rooted in a mist bed for 6 wk and then transferred to 6-cm-wide × 24-cm-deep Cone-tainers. After 1 wk to allow root development at ambient greenhouse temperatures 25 ± 2°C), Cone-tainers were suspended in Conviron air bath–controlled soil temperature tanks that allowed drainage of excess water from the base of the Cone-tainers. Air bath temperatures were adjusted so that the soil temperature in the Cone-tainers stabilized at 24, 27, 30, and 32 (± 1.5)°C.

After temperature stabilization, soil in the Cone-tainers was inoculated with the test nematodes, 1,500 freshly hatched juveniles (*Meloidogyne*) or 200 individuals freshly extracted from soil (*X. index*). There were four replications of each temperature treatment. Plants were watered with dilute Hoagland's nutrient solution through a drip irrigation system. After 8 wk, plants were removed from the Cone-tainers, roots stained with erioglaucine ($0.1 gL^{-1}$; Omwega et al., 1988) and numbers of egg masses (*Meloidogyne*) or root tip galls (*X. index*) were counted. Roots were dried at 85°C for 24 hr and weighed so that nematode levels and symptom ratings could be adjusted per g root to normalize for differences in root mass and vigor across cultivars and temperatures.

As in earlier screening trials (Ferris et al., 2012), rooted cuttings of genetically different material sometimes developed at different rates so that all of the selections were not available at a time coincident with the availability of nematode inoculum. Consequently, each soil temperature test was repeated up to three or four

times to accommodate the availability of individual genotypes at the start of the test. The inclusion of susceptible and resistant controls in every test allowed us to rate nematode reproduction on each rootstock selection relative to that on the susceptible controls and thus to compare resistance and susceptibility across different iterations of the test.

Effect of temperature on durability of resistance of the rootstock parents: We assessed the effect of soil temperature on the susceptibility of original and intermediate parents of the 'UCD-GRN' series rootstocks to *M. incognita* Race 3 and pathotype Harmony C, and to *M. arenaria* pathotype Harmony A to determine sources of durability, or lack thereof, to temperature stress. The parentage of the 'UCD-GRN' series rootstocks (Table 1) is provided in greater detail in Ferris et al. (2012). Some of the *Vitis* species and cultivars were included in the rootstock breeding program because they are sources of resistance to *Meloidogyne*, some because they provide resistance to *X. index*. Others were included to improve rooting and grafting capabilities of their rootstock progeny.

Effect of soil temperature on penetration and development of M. incognita Race 3 in resistant rootstocks: In short-term experiments, we studied the penetration and development of *M. incognita* Race 3 at different temperatures in three resistant genotypes ('UCD-GRN1', 'UCD-GRN4', and 'Harmony') with 'Colombard' as a susceptible control. Rooted cuttings were established in 6×24-cm Cone-tainers containing sand and, after establishment, suspended in Conviron air baths at 24, 27, 30, and 32 (± 1.5)°C. After temperature stabilization, each Cone-tainers was inoculated with 100 freshly hatched juveniles of *M. incognita* Race 3. There were seven time interval treatments at each temperature. The Cone-tainers were irrigated daily with dilute Hoagland's solution. At 3-d intervals, roots were gently washed from the soil of one time interval treatment of each cultivar at each temperature and stained with lactic acid-acid fuchsin (Byrd et al., 1983). They were spread between two glass plates and examined for juvenile penetration of roots and for evidence of nematode feeding and development.

In another series of experiments, three replications of fresh excised roots of 'Harmony', 'UCD-GRN1', 'UCD-GRN4' (resistant) and 'Colombard' (susceptible) were established on pluronic gel medium (Wang et al., 2009) on 10-cm-diam. petri dishes The cultures were inoculated with 500 freshly hatched juveniles of *M. incognita* Race 3 and maintained at 24°C. After 1 hr, 1 d, and 2 d, the number of juveniles within 1 mm of the root surface was counted. After 2 d, the roots were removed from the tissue culture, stained with lactic acid-acid-fuchsin, pressed between glass slides, and the number of juveniles inside the root tissue was counted.

Effects of fluctuating temperature on resistance to Meloidogyne incognita Race 3: In several series of experiments in soil temperature air baths, *M. incognita* Race 3 and the Harmony A and Harmony C pathotypes virulent on the resistant 'Harmony' rootstock failed to produce egg masses on the 'UCD-GRN' series rootstocks and other resistant selections at constant temperatures below 25°C (see Results section). However, at soil temperatures above 25°C, we observed small increases in prevalence and abundance of nematode galling and egg mass production. To test the effects of fluctuating soil temperature, we exposed plants inoculated with *M. incognita* Race 3 to the following treatments: 24 and 30°C constant temperatures, initial 24°C followed by cycling to 30°C every 7 d, or cycling at 3.5-d intervals, and the converse of initial 30°C and then cycling to 24°C with either 7-d or 3.5-d amplitude.

Effects of soil temperature on resistance to Mesocriconema xenoplax: In plant growth chambers at the University of California Davis Controlled Environment Facility, we determined the effect of soil temperature on the reproduction and survival of ring nematode on seven grape rootstock selections: three were susceptible controls and four were rootstocks known to have resistance to *M. xenoplax* through their *Muscadinia rotundifolia* heritage (Ferris et al., 2012). Plants were established in Cone-tainers and inoculated with 1,500 *M. xenoplax*. They were incubated in growth chambers at 24, 27, 30, and 33°C and watered daily with dilute Hoagland's solution. After 3 mon, nematodes were extracted by an elutriation and sugar-centrifugation process and counted (Ferris et al., 2004).

Effects of soil temperature on nematode development and life course phenology: Test plants established in Cone-tainers suspended in air baths adjusted to maintain soil temperature of 24, 27, 30, and 33°C were each inoculated with 100 juveniles of *M. incognita* Race 3 that were allowed 7 d to penetrate and establish infection sites. Then soil was gently washed from the roots of each plant and they were suspended in light-proof cylinders of a hydroponic system, modeled on that developed by Ferris et al. (1984). In that system, each cylinder is attached to a funnel and roots are irrigated by a spray nozzle for 1 of every 10 min. Thirty-six such funnels were set up in a walk-in growth chamber maintained at 30°C. Each funnel was suspended over an Erlenmeyer flask to catch juveniles emerging from egg masses that developed on the roots. There were three replications of each cultivar/penetration temperature combination.

Each day, washings from the roots collected in the Erlenmeyer flasks and were passed through a 500-mesh sieve to collect any hatched *Meloidogyne* juveniles. After 30 d of collecting nematodes, we terminated the experiment and stained the roots to determine the number of females that had been producing the eggs. The constant temperature profile allowed calculation of the number of degree-days required for development from root penetration to development through reproductive maturity to first egg hatch, including the degree-days for penetration. Using the assumption that

eggs develop at a constant rate at constant temperature, the rate of egg hatch and juvenile collection is inferred as the rate of egg production per female per degree-day on each cultivar (Ferris et al., 1984).

In a second experiment, we tested the effect of inoculum level on development and egg production. Four cultivars were inoculated with 100, 300, and 500 juveniles of *M. incognita* Race 3 and maintained at soil temperature of 30°C for 4 d before transfer to the mist spray apparatus at 30°C. Harvest procedures were as for the penetration temperature test.

Results

Effect of temperature on durability of resistance of the rootstocks: Across a series of experiments with both susceptible and resistant rootstocks, reproduction of *Meloidogyne* spp. was significantly greater at either 30 or 32°C than at lower temperatures. Symptoms due to *X. index* were greatest at 27°C and decreased to 20% of their maximum at 32°C (Fig. 1). Nematode reproduction or symptoms of feeding were expressed relative to those on the susceptible cultivar ('Colombard' or 'St. George') at each temperature to normalize for temperature effects on the nematodes (Fig. 2). In several series of experiments in soil air baths, pathotypes of *M. incognita* and *M. arenaria* that are virulent on 'Harmony' rootstock, as well as *M. incognita* Race 3, avirulent on 'Harmony', failed to produce egg masses on the 'UCD-GRN' series rootstocks and other resistant selections at 24°C. At 27°C and above, we observed increases in prevalence and abundance of nematode galling and egg mass production with temperature (Fig. 2A–C). At 30°C, egg mass production levels of Race 3 on 'UCD-GRN4' and 'Harmony' were 6% and 12%, respectively, of that on 'Colombard' (Fig. 2A). Reproduction of the virulent pathotypes on the 'UCD-GRN' series was less than 5% of that on 'Colombard' at 24 and 27°C (Fig. 2B,C). Virulence of *M. arenaria* pathotype Harmony A was greater than that of *M. incognita* pathotype Harmony C

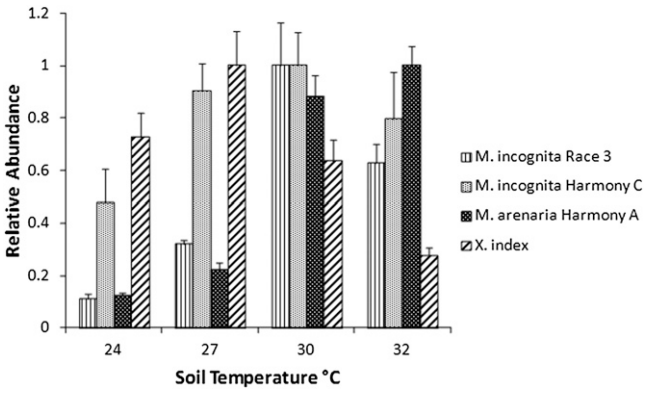

Fig. 1. Relative nematode reproduction or symptom expression per g root across soil temperatures on susceptible cultivars – 'Colombard' for *Meloidogyne* spp., 'St. George' for *Xiphinema index*. Bars represent 1-one standard error.

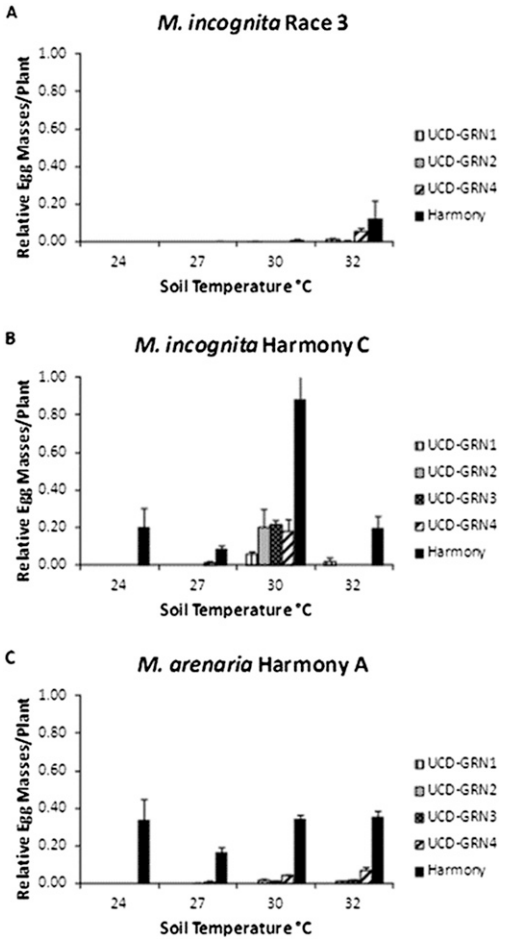

Fig. 2. Effect of soil temperature on reproduction of three *Meloidogyne* pathotypes (A-C) on resistant rootstocks expressed relative to reproduction on susceptible 'Colombard' at each temperature. Bars represent 1-one standard error.

on all rootstock cultivars at 30°C but declined at 32°C (Fig. 2B,C).

We also investigated the effect of soil temperature on resistance to *X. index*. The feeding strategy of this nematode is quite different to that of *Meloidogyne* spp., and it is likely that the nature of resistance is also different. There was no breakdown of resistance to *X. index* with increase of temperature. Although higher temperatures were less favorable to *X. index* (Fig. 1), the nematode at least persisted sufficiently to initiate feeding symptoms on the susceptible cultivars 'Colombard' and 'St. George' but not on any of the resistant selections at any temperature (data not shown).

Effect of temperature on durability of resistance of the rootstock parents: Parents of the 'UCD-GRN' series rootstocks were resistant to the three *Meloidogyne* pathotypes at temperatures below 30°C (data not shown). Two of the intermediate genotypes in rootstock development ('L91-64'and 'L25-19') were only moderately resistant at 32°C with 35% to 40% of number of egg masses on the susceptible 'Colombard' control (Fig. 3). Highest population levels on the susceptible controls occurred at soil temperatures most favorable to reproduction of the

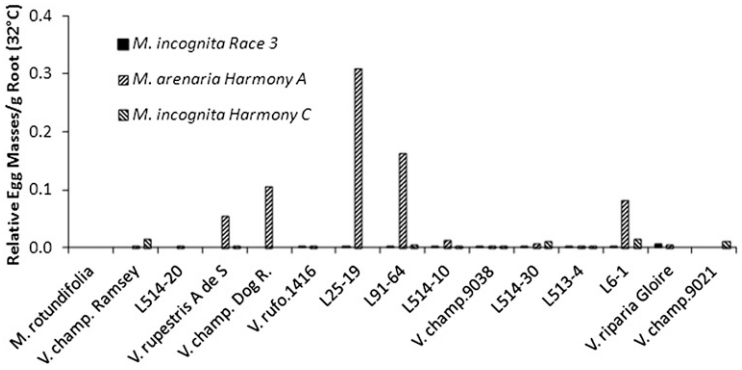

FIG. 3. Numbers of egg masses produced by three *Meloidogyne* pathotypes on parent genotypes of the 'UCD-GRN' series rootstocks at 32°C, expressed relative to the number of egg masses for each nematode on 'Colombard' at the same temperature. For cultivar designations see Table 1.

nematode (see Fig. 1). Resistance of several of the parental genotypes was slightly compromised at soil temperatures of 30°C and above but not below 27°C. However, some of the rootstock parents maintained resistance to even the virulent *M. arenaria* pathotype A at high temperatures, indicating that there is durability of resistance in relation to temperature among the parents.

Effect of soil temperature on penetration and development of M. incognita Race 3 in resistant rootstocks: After staining, it was possible to observe nematodes within the roots, development of the feeding site and enlargement of the juveniles as they commenced feeding. The following records are observational rather than the results of repeated experiments. At 24°C, 2 d after inoculation, a few juveniles were visible within the roots of the resistant cultivars ('Harmony', 'UCD-GRN1', 'UCD-GRN4') but none were seen at day 6 and beyond. On the susceptible control, development proceeded to the J4 stage by the end of the 21-d experiment. At 27°C, a few more juveniles successfully penetrated the roots of the resistant genotypes and were visible for up to 15 d. However, there was no development of giant-cell feeding sites. At that temperature, nematodes within roots of the susceptible cultivar were young adults by day 21. At 30°C, the few juveniles that penetrated the root survived, developed feeding sites and were at the J3 stage by day 21, while those in the susceptible roots were at the J4 stage by day 18. At 32°C, the development was similar but a little faster; nematodes in the susceptible cultivar reached the young adult stage by day 21, while they were still at the J4 stage in the resistant cultivars.

On pluronic gel medium at 24°C, *M. incognita* Race 3 juveniles were equally attracted to roots of both resistant and susceptible cultivars. Juveniles entered, and were visible within, the roots of the four cultivars. The juveniles began to establish feeding sites within 2 d in the susceptible 'Colombard'; but in the resistant cultivars, there was no evidence of feeding site development or swelling of the nematodes. We conclude that juveniles were unable to establish feeding sites in the resistant cultivars at 24°C.

Effects of fluctuating temperature on resistance to Meloidogyne incognita Race 3: There was greater nematode reproduction on 'Colombard' at a constant temperature of 30°C than at 24°C, consistent with our previous studies (Fig. 1). Also consistent with previous results (Fig. 2), some nematode reproduction of *M. incognita* Race 3 occurred on the 'UCD-GRN' and 'Harmony' resistant rootstocks at a constant 30°C. However, at slow (7-d) or rapid (3.5-d) temperature cycling, whether initiated at 24 or at 30°C, there was no nematode reproduction (Table 2).

Effects of soil temperature on resistance to Mesocriconema xenoplax: Reproduction of *M. xenoplax* on susceptible cultivars was significantly ($P < 0.05$) suppressed at 30°C and was extremely low at 33°C. On the other hand, the resistant selections maintained their resistance at higher temperatures, even at 30°C where the nematode was still biologically active (Fig. 4). At this time, the primary source of resistance identified

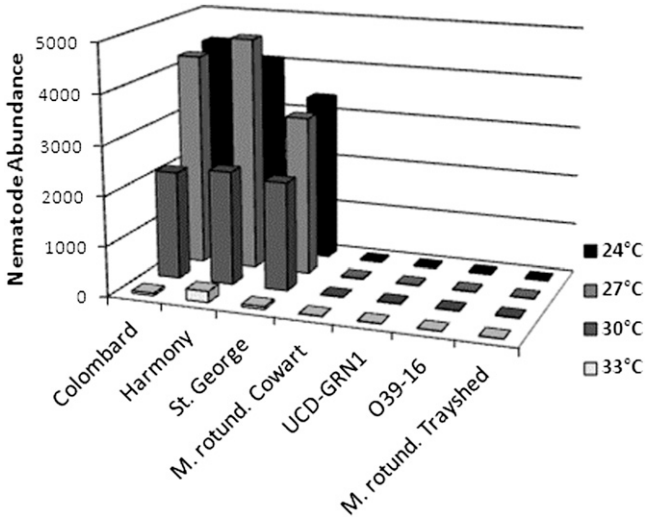

FIG. 4. Reproduction and survival of ring nematode (*Mesocriconema xenoplax*) on seven grape rootstocks at four different temperatures over 3 mon of exposure.

for both *M. xenoplax* and *X. index* is *M. rotundifolia* (Ferris et al. 2012). The four resistant selections in this study were either pure *M. rotundifolia* ('Cowart' and Trayshed) or had *M. rotundifolia* in their parentage ('UCD-GRN1' and 'VR O39-16'). Reproduction and survival of *M. xenoplax* on 'VR O39-16' and 'UCD-GRN1' was significantly less than that on 'Colombard', 'Harmony', and *V. rupestris* 'St. George'; at the end of the experiment, across all plant genotypes, there were fewer nematodes at 30°C than at 24 and 27°C, with the fewest at 32°C.

Effects of soil temperature on nematode development and life course phenology: When nematodes were inoculated and provided time for infection site development at 24, 27, 30, and 33°C before transfer to a constant temperature of 30°C in suspended root mist chambers, egg production on 'Colombard' commenced at around 550 DD_{10} and proceeded at slightly higher rates on plants inoculated at 27 and 30°C (0.64 and 0.69 eggs per female per DD_{10}; R^2 0.92 and 0.97, respectively) than on those inoculated at 24 and 33°C (0.50 and 0.45 eggs per female per DD_{10}; R^2 0.95 and 0.85, respectively) (Fig. 5A). However, on the resistant 'Harmony', feeding site establishment and nematode development occurred only at 30 and 33°C and only by very few juveniles. Development of those juveniles to maturity was much slower than in the susceptible 'Colombard' (Fig. 5B).

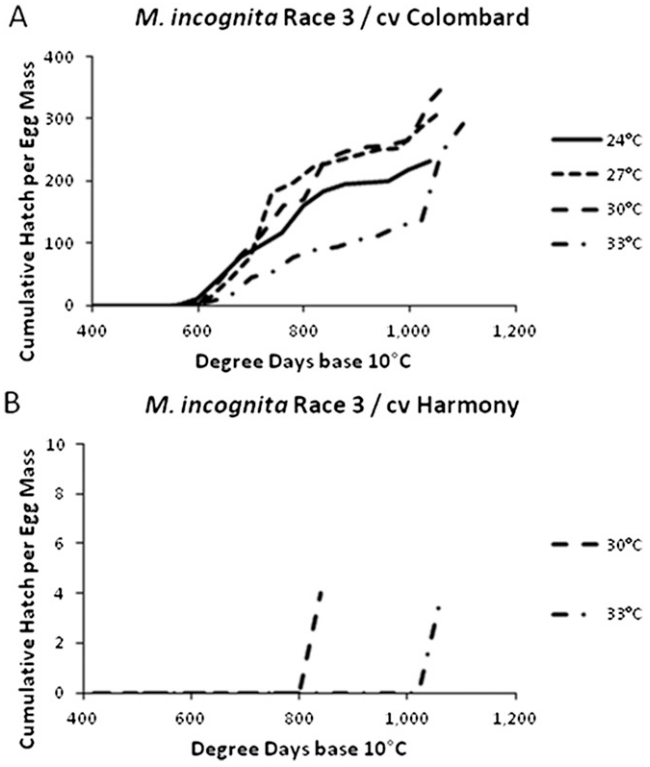

FIG. 5. Rate of development and egg production per female, inferred from rate of egg hatch when 'Colombard' (A) and 'Harmony' (B) were inoculated with 100 juveniles of *M. incognita* Race 3 at 24, 27, 30, and 33°C, allowed to establish infection sites, and then incubated in a growth chamber at 30°C.

When plants were inoculated at 30°C with different numbers of nematodes to test the effect of inoculum potential on life course phenology, development of *M. incognita* Race 3 was slower in 'Harmony', 'UCD-GRN1', and 'UCD-GRN4' than in the susceptible 'Colombard' (Fig 6A–D). In addition, the adult females were smaller-bodied and produced smaller egg masses in the resistant than in the susceptible cultivars. The number of eggs per egg mass did not differ with inoculum level but was significantly lower in the resistant than in the susceptible cultivars. In addition, the body sizes of the adult females were smaller in the resistant cultivars.

Egg production of *M. incognita* Race 3 at 30°C on 'Colombard', when averaged across infection temperatures and inoculum levels was 0.6 eggs per female per DD_{10} (Fig. 7A). Egg production rates across inoculum levels at 30°C, were more variable and much slower on the resistant rootstocks when resistance was compromised by high soil temperature, averaging 0.03 eggs per female per DD_{10} (Fig. 7B).

DISCUSSION

Resistance to *M. incognita* Race 3, *M. incognita* pathotype Harmony C and *M. arenaria* pathotype Harmony A is durable at soil temperatures up to 27°C, but we observed a small amount of reproduction after 3 mon at 30 and 32°C in the 'UCD-GRN' rootstocks (Fig. 2). Breakdown of resistance at higher temperature is reported for bacterial wilt of plants (Krausz and Thurston, 1975) and rust diseases of wheat (Dyck and Johnson, 1983). It is frequently reported with *Meloidogyne* spp. in solanaceous plants, e.g., pepper (Thies and Fery, 1998), tomatoes, particularly those deriving resistance from *Solanum peruvianum* (Veremis et al., 1999), alfalfa, sweet potato, and cotton (Griffin, 1969; Jatala and Russell, 1972; Carter, 1982). In resistance to *Meloidogyne* conferred by the temperature-sensitive Mi gene of tomato, it appears that there is a reduction of the hypersensitive reaction and a correlation with reduced peroxidase and lignin levels (Zacheo et al. 1995). We do not know whether the resistant reaction in grape is one of hypersensitivity or of failure to respond to signals for giant cell development. However, the phenomenon of apparent partial breakdown of resistance gene expression is interesting in that similar phenomena are observed in plants genetically very different than *Vitis*.

The susceptibility of their parents responded in a similar manner to temperature as the resistant rootstock selections. In all cases except 'L91-64'and 'L25-19', durability of resistance at high temperature of the parental genotypes was in the same range as that of the 'UCD-GRN' progeny. Interestingly, 'L91-64'is unique as the only selection with a *V. candicans* parent while 'L25-19' has a different source of *V. champinii* from those known to have strong nematode resistance to *Meloidogyne* spp. (*V. champinii* 'Ramsey' and 'Dog Ridge').

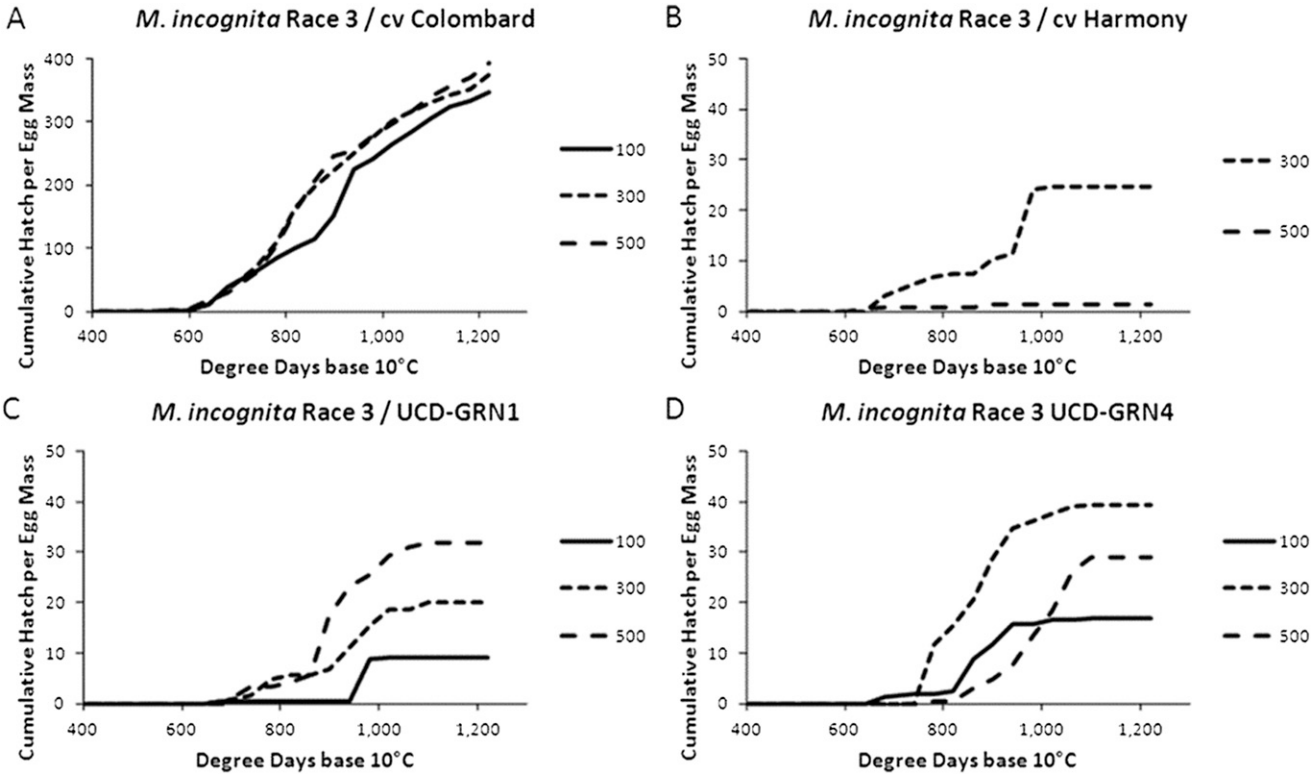

FIG. 6. Rate of development and egg production per female, inferred from rate of egg hatch when 'Colombard', and rootstocks 'Harmony', 'UCD-GRN1', and 'UCD-GRN4' were inoculated at 30°C with 100, 300, or 500 juveniles of *M. incognita* Race 3, allowed to establish infection sites, and then incubated in a growth chamber at 30°C.

Regarding the mechanism of resistance, we have observed that *Meloidogyne* juveniles are attracted to the roots of resistant cultivars in numbers similar to those attracted by susceptible cultivars. However, fewer juveniles are able to enter the root, and at temperatures below 27°C, none of them establish feeding sites or develop. At temperatures above 27°C, some of the juveniles that enter the root develop feeding sites but the feeding sites are apparently of poorer quality than those established in susceptible cultivars because the nematodes develop more slowly.

The rate of egg production by *M. incognita* Race 3 at 30°C was consistent with that observed for *M. arenaria* at 25, 27, and 30°C in roots of different grape cultivars; it ranged from 0.48 to 1.0 eggs per female per DD_{10} in cultivars of differing susceptibility levels (Ferris et al., 1984). In that study, eggs were produced at a rate of 0.81 per female per DD on 'Colombard', similar to the 0.6 measured in the present study for *M. incognita* Race 3 on the same cultivar. Egg production rates across inoculum levels at 30°C, when resistance is compromised, were slower on resistant cultivars (less than 20% of that on the susceptible cultivar). In both this and the earlier study, the development time from root penetration to first egg hatch was around 550 DD_{10} on the susceptible cultivars and 650 DD_{10} on the resistant.

Muscadinia rotundifolia is an important source of resistance to *M. xenoplax* (Ferris et al., 2012). We determined the effect of soil temperature on the reproduction and survival of the ring nematode, *M. xenoplax*, on rootstock selections with and without *M. rotundifolia* in their parentage. Although ring nematode reproduction was suppressed by 50% at 30°C on susceptible cultivars, pure *M. rotundifolia* and the resistant selections ('UCD-GRN1' and 'VR O39-16') maintained their resistance at higher temperature and there was almost no survival or reproduction of the nematode after 3 mon of exposure at soil temperatures between 24 and 33°C.

The resistance to *X. index* of the 'UCD-GRN' rootstocks was durable across temperature. Our population of *X. index*, originally from the Napa Valley, CA, was adapted to cooler temperature conditions. However, populations of *X. index*, apparently adapted to higher temperatures, flourish in warmer regions of the San Joaquin Valley, CA (McKenry et al., 2004) and also where they have been introduced on planting stock and thrive in desert regions of northern Chile (M. V. McKenry, personal communication). We believe that resistance to *X. index* in the 'UCD-GRN' rootstocks will be durable across similar ranges of climatic conditions.

Soil temperatures of 30°C and above are unlikely to be experienced frequently in vineyard soils. The resistance mechanism of 'UCD-GRN' rootstocks to *Meloidogyne* pathotypes were not permanently compromised if feeding sites were established at 30°C because development

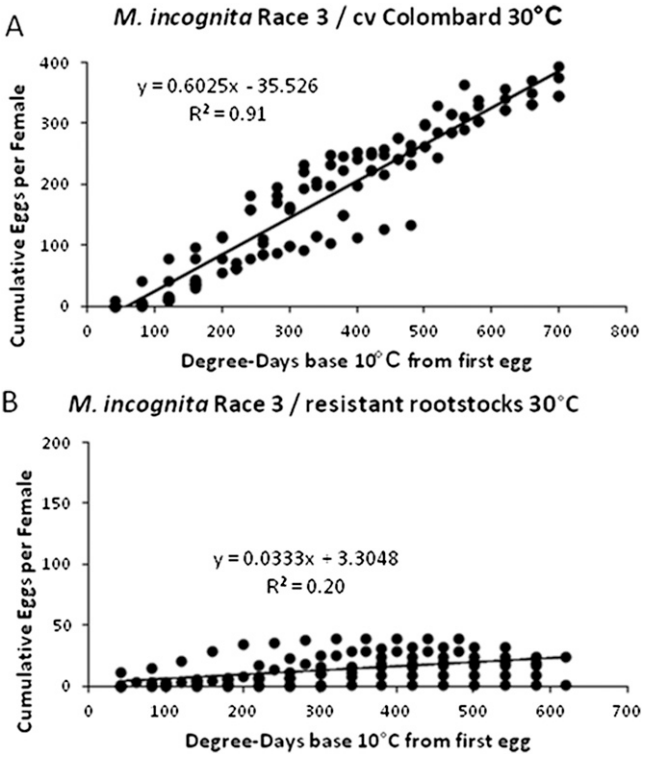

FIG. 7. Cumulative egg production by *Meloidogyne incognita* Race 3 from the initiation of the first egg, as indicated by the appearance of the first juvenile in susceptible (A) and resistant (B) grape rootstocks at 30°C.

did not continue when the temperature cycled between 30 and 24°C. We conclude that once initiated, the resistance mechanisms are not compromised by exposure to higher temperatures and that nematode resistance involves mechanisms that are continue beyond the penetration and feeding site establishment phases. We conclude that the resistance of these rootstocks will be durable in relation to temperature.

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