Journal of Nematology 39(1):50–54. 2007.

Virulence of *Meloidogyne* spp. and Induced Resistance in Grape Rootstocks

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Abstract: Harmony grape rootstock displays resistance to several *Meloidogyne* spp. but that resistance is not durable in commercial vineyard settings. A 2-year experiment in a microplot setting revealed host specificities of two virulent populations of *Meloidogyne arenaria* and an avirulent population of *Meloidogyne incognita*. In a subsequent split-root experiment, the avirulent nematode population was demonstrated to induce resistance to the virulent nematode population. To quantify the level of resistance, reproduction of the virulent nematode population was determined 63 days after being challenged by an avirulent nematode population using a range of inoculum densities and timeframes. Induction of resistance became apparent when the virulent nematode population was inoculated 7 days after the avirulent nematode population and increased thereafter. The level of induced resistance increased with increased inoculum levels of the avirulent nematode population. Root systems of perennial crops are commonly fed upon simultaneously by multiple nematode species. These two studies indicate that field populations can become preferentially virulent upon one or multiple rootstocks and that co-inhabiting populations may induce existing resistance mechanisms. In perennial crops, it is common for numerous nematode species besides *Meloidogyne* spp. to be present, including some that feed without causing apparent damage.

Key words: systemic acquired resistance, rootstocks, split-root, virulence.

Resistant plants possess diverse physiological and anatomical defense mechanisms for use against invading pathogens. These include the ability to induce localized hypersensitive reactions upon pathogen ingress and also the ability to initiate defenses in a systemic manner (Anwar and McKenry, 2001, 2002a, 2002b). Van Loon (1997) defined induced resistance as a phenomenon, once appropriately triggered, that provides qualitative and quantitative enhancement of plant defense mechanisms. From the perspective of nematode population dynamics, induced resistance is any change in a plant triggered by biological or chemical agents that reduces development and reproduction of nematodes (Ross, 1964; Glazer and Orion, 1985; Kuc, 1990; Kessmann et al., 1994; Crute and Pink, 1996; Sticher et al., 1997).

Emergence of virulent and avirulent populations of *Meloidogyne* spp. on crops has been reported (Carpenter and Lewis, 1991; Castagnone-Sereno et al., 1994; Anwar et al., 2000). Populations of *M. incognita* differ in their reproductive capability and the amount of disease they cause on tomato, soybean, cowpea, cotton and tobacco (Riggs and Winstead, 1959; Golden and Birchfield, 1978). Anwar et al. (2000) compared the reproductive variability of four field populations of *Meloidogyne* spp. on three grape rootstocks. Two populations of *M. arenaria* were found to be virulent and another two, including *M. incognita* and mixed *Meloidogyne* spp., avirulent. Resistant Freedom and Harmony rootstocks of grape are normally non-hosts to avirulent nematode populations and respond by developing necrotic lesions in response to penetrating J2.

Our objectives were prompted by the faster and higher developmental rates of a particular *M. arenaria* population on various grape rootstocks and the associated development of plant hypersensitive responses (HR) on resistant grape rootstocks. We hypothesized that HR associated with grape rootstocks resistant to avirulent *Meloidogyne* spp. might induce protection against virulent nematode populations. We evaluated our hypothesis by splitting the root of Harmony rootstock into two pots with an avirulent nematode population that stimulates HR on one-half the root systems and a virulent nematode population that produces minimal HR on the other half of the root system (Anwar et al., 2000).

Materials and Methods

Virulence and avirulence of five populations of Meloidogyne spp.: A microplot experiment was conducted from 1996 to 1998 at the UC Kearney Agricultural Center, Parlier, CA. Microplots were established by drilling a 75-cm-diam., 150-cm-deep hole with a truck-mounted auger. A 61-cm-diam., 122-cm-long corrugated polyethylene resin tube was installed into each hole, and the native soil returned to each microplot. These open-bottomed microplots each had a 10-cm lip aboveground after soil settling. From center to center, microplots were 145 cm apart within the row and 175 cm between rows. A drip irrigation system was installed for uniform water delivery. Weeds were controlled by hand hoeing.

Five grape rootstocks exhibiting variable nematode resistance (Anwar et al., 2000) were exposed to five different populations of *Meloidogyne* spp. In spring 1997, Duarte Nursery (Ceres, CA) supplied 1-yr-old rootings of rootstocks including Ramsey, Teleki 5C, Harmony, Freedom and Cabernet Sauvignon. Cabernet Sauvignon was included as the control highly susceptible to all nematode populations screened. Freedom and Harmony served as susceptible control to two populations of *M. arenaria*, and Ramsey was the suscep-

Received for publication: 6 October 2006.
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This paper was edited by James LaMondia.

tible control to *Meloidogyne* spp. population Ramsey. Teleki 5C was included because we had observed *Meloidogyne* resistance on its older root tissues (McKenry, unpublished). Vines were planted randomly throughout the microplots, with 1 vine/microplot and three replicates for each rootstock/nematode combination.

Microplots were inoculated individually with soil containing five different populations of *Meloidogyne* spp. Our procedure was to insert 1 kg of soil into three locations dug around each root system. Inoculum had been collected from field soils at specific locations in California and inoculated into the microplots in July 1997. The Harmony population of *M. arenaria* was obtained from a 25-yr-old vineyard planted on Harmony rootstock, located near Livingston, CA. One kilogram soil (560 J2/250 cm^3) was added to the appropriate microplots. The *M. arenaria* population of Freedom was collected from an 8-yr-old vineyard located near Livingston, CA, and 1 kg soil (852 J2/250 cm^3) was added to the appropriate microplots. The *Meloidogyne* spp. population Ramsey was a mixed population of approximately half *M. incognita* and half *M. arenaria*, which was collected from King City, CA, and 1 kg soil (211 J2/250 cm^3) was added into each appropriate microplot. *Meloidogyne incognita* R3 originated from a cotton field near Shafter, CA, and 1 kg soil (1,500 J2/250 cm^3) was added around each root system per microplot.

Root samples were collected in April 1998 for assessment of egg density of each root-knot species. Roots were washed free of soil, blotted onto paper, damp-dried and weighed. Roots were placed in an 800-ml sealed Mason glass jar with 1% NaOCl (Hussey and Barker, 1973), shaken for 4 min at 200 cycles/min on a mechanical shaker (Eberbach Corporation, Ann Arbor, MI) and placed on a 500 mesh screen (openings of 25 µm) to remove eggs. This treatment was followed by a thorough rinse in tap water, and a sub-sample of the egg suspension was counted at ×40 magnification. Number of eggs per gram of root was calculated to determine the reproductive ability of each nematode population on each rootstock.

Induced Resistance Experiments: Plants of Harmony rootstock that had exhibited resistance to *Meloidogyne* spp. but not to Harmony and Freedom populations of *M. arenaria* (Anwar et al., 2000) were rooted from shoot-tip cuttings by placing them in a bed consisting of a 2.5-cm-thick layer of autoclaved sand layered over a 5-cm-thick layer of a peat-perlite mixture (50:50). Propagation beds were irrigated by a water mist of 30-sec duration every 9 min in a greenhouse maintained at 30°C. One-month-old plants of uniform root and shoot size were selected, and each root system divided into two equal halves. This was accomplished using a sharp knife to divide the rooting upward from its base until reaching a distance 5 cm above the total root ball. Each half was transplanted into two adjacent 9-cm-diam. pots filled with autoclaved soil (80% sand, 10% silt, 10% clay). The pots were watered immediately and then biweekly with Hoagland's solution. Plants were allowed to grow for 7 d to heal injuries before nematode inoculation. Each pot was inoculated with 5,000 eggs.

A study using timed inoculations: One-half of the split root system of Harmony plants was inoculated with 5,000 eggs of an avirulent kiwifruit population of *M. incognita* as the prior or inducer inoculum. The kiwifruit population was obtained from a 1970 planting at Kearney Agricultural Center near Parlier, CA. After that inoculation, 5,000 eggs of virulent Harmony population of *M. arenaria* were applied to the other half of the root system at intervals of 0, 7, 13, 21 and 27 d as challenge inoculum. The reproduction of these populations was assessed 63 d after the challenge inoculation by dividing the final population by the initial population (Pf/Pi). Controls consisted of Harmony plants inoculated only with *M. arenaria* or *M. incognita* at 0, 7, 13, 21 and 27 d. Each treatment was replicated five times.

A study with differing inoculation densities: Induction of resistance mechanisms by the avirulent *M. incognita* from kiwifruit was also assessed using a range of inoculum levels. Eggs were inoculated to roots of Harmony grape at 0 (control for comparison), 500, 1,000, 5,000 and 10,000 per vine 7 d before a challenge inoculation with 5,000 eggs/vine of *M. arenaria*. Each treatment was replicated five times. Vines were grown for 63 d after the challenge inoculations, at which time egg counts per pot were determined. Vines inoculated with either *M. incognita* or *M. arenaria* alone served as the comparative control.

Data analysis: Data were subjected to analysis of variance using SAS (1987). Significant differences in means of nematode reproduction were separated using Duncan's multiple range test at ($P = 0.05$).

Results

Reproduction of five populations on grape rootstocks: Populations of *M. arenaria* reproduced well on all five grape rootstocks, whereas the Shafter population of *M. incognita* reproduced poorly on resistant Freedom, Harmony, Ramsey and susceptible Teleki 5C, but reproduced well on highly susceptible Cabernet Sauvignon (Fig. 1). Reproduction of the Ramsey population of *Meloidogyne* spp. was high on susceptible Cabernet Sauvignon, intermediate on Ramsey and Teleki 5C, and very poor on Freedom and Harmony rootstocks.

Results of timed inoculation studies: Reproduction of the Harmony population of *M. arenaria* was significantly reduced ($P = 0.05$) when measured 63 d after an adjacent inoculation using the avirulent population of *M. incognita* (Fig. 2). Effectiveness of the avirulent nematode population at depressing reproduction of the virulent nematode population became evident 7 d after inoculation. Longer inoculation intervals did not result

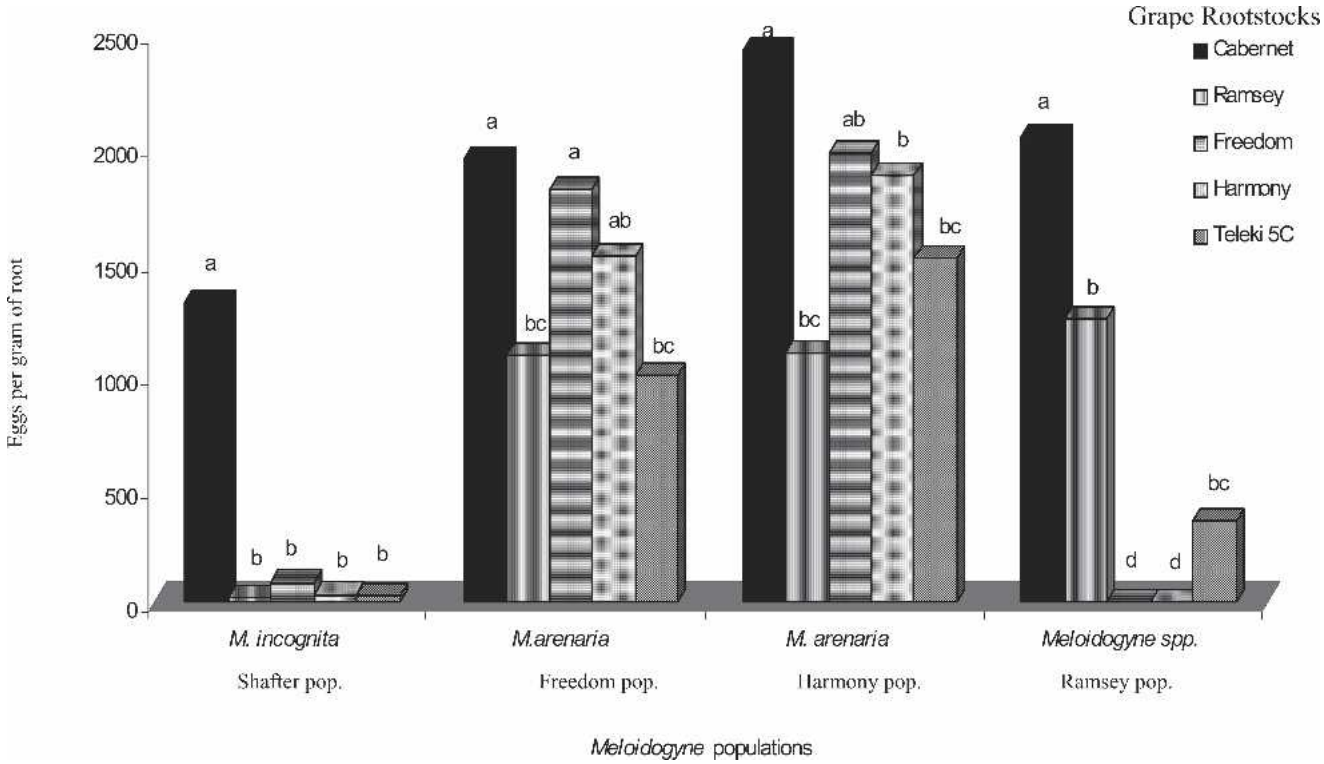

FIG. 1. Reproduction of four populations of *Meloidogyne* spp. on five grape rootstocks. Bars for a given population followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

in additional significant ($P \leq 0.05$) reductions in reproduction by the virulent nematode population. Reproduction of the avirulent population of *M. incognita* on roots of Harmony was less than 1.0 at all inoculation intervals. Meanwhile, reproduction of the virulent population of *M. arenaria* on adjacent roots of Harmony was 72, 45, 12, 7 and 6, when determined at 0, 7, 13, 21 and 27 d inoculation intervals, respectively.

Result of differing inoculum densities: In this split-root system, reproduction of the virulent *M. arenaria* population was suppressed by 9%, 28%, 76% and 81% at 500, 1,500, 5,000, and 10,000 eggs of *M. incognita* applied as inoculum, compared to the control (Fig. 3). Reproduction of the avirulent populations of *M. incognita* was less than 1.0 in every case (Fig. 3). The rate of reproduction (Pf/Pi) by the virulent nematode population on half the root system was 73, 66, 54, 18 and 14 when roots on the other half were inoculated with the avirulent nematode population at 0, 500, 1,500, 5,000 and 10,000 eggs/vine, respectively.

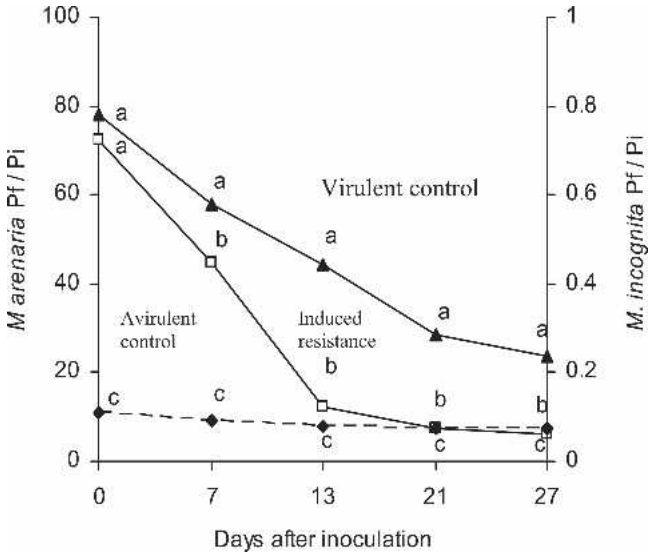

FIG. 2. Reproduction of a virulent population of *M. arenaria* on roots of Harmony grape rootstock: A. Inoculated at five intervals, either alone (check) or as challenge after avirulent *M. incognita* inoculated at d 0. Means on a given day after inoculation followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

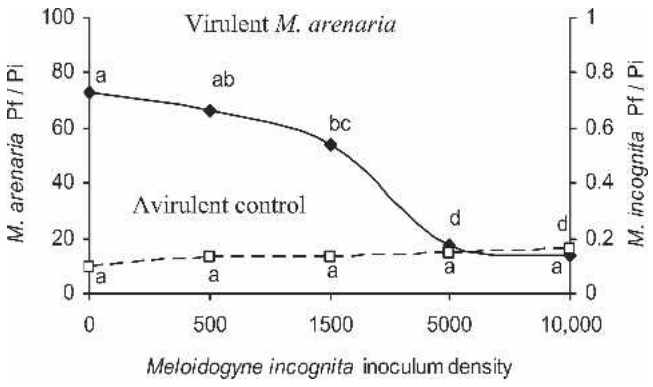

FIG. 3. Inoculated at d 7 after avirulent *M. incognita* inoculation at five inoculum densities. Means among the inoculum densities followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

Discussion

Harmony and Freedom are closely related grape rootstocks that share a portion of their parentage (*Vitis champinii*) with Ramsey rootstock. Teleki 5C is a hybrid of *V. berlandieri* with *V. riparia*, whereas Cabernet Sauvignon is *V. vinifera*. Teleki 5C and Cabernet Sauvignon rootstocks have nematode-rootstock profiles that differ from the first three including Harmony, Freedom and Ramsey (Anwar et al., 2000). The virulent *M. arenaria* population utilized in this study was originally found on Harmony rootstock, but its virulence is notable on all these grape rootstocks as well as 40 others (McKenry, unpublished). We show here that the mixed population of *Meloidogyne* spp. with virulence to Ramsey is not virulent to Harmony or Freedom rootstocks. It appears that populations with virulence to one rootstock may not be virulent to another having similar parentage. With perennials, it is common to encounter a complexity of *Meloidogyne* species in a single field site. It is equally common for virulent nematode populations to coinhabit root galls along with non-virulent *Meloidogyne* spp.

This research has established that prior infections with an avirulent *M. incognita* population on resistant Harmony grape rootstock can induce a level of resistance to virulent populations of *M. arenaria* on distant roots of that same rootstock. This phenomenon of induced resistance occurred without successful reproduction by the avirulent nematode. Our results are in agreement with reported findings of induced resistance in which avirulent nematodes suppressed population levels of virulent nematodes. Ogallo and McClure (1995, 1996) reported induced resistance in tomato and pyrethrum plants where there was significant reduction in populations of virulent *M. hapla* after infection with avirulent *M. incognita*. Similarly, Kiyohara (1986) reported that prior inoculation with an avirulent nematode population of the pine wilt nematode, *Bursaphelechus xylophilus*, contained the population buildup of an invading virulent nematode population by inducing systemic resistance in the same plants.

The mechanisms by which avirulent nematode populations induce resistance to virulent nematode populations have not been as thoroughly studied as those of viruses, fungi and bacteria (Yarwood, 1956; Ross, 1964; Kuc, 1983; Fuchs et al., 1997). The induced resistance mechanisms are active, energy-requiring systems typified by specific recognition of primary infection that ultimately leads to the production of plant defense genes, including PR-proteins (Hwang et al., 1997; Cohen et al., 1999; Anwar et al., 2003), peroxidase and lignin formation (Cohen et al., 1999), and modification of plant cell walls (Cohn and Gisi, 1994). The final result is antagonism to invaders. These phenomena have also been suggested as part of the resistance mechanisms to plant-parasitic nematodes (Kogan and Paxton, 1983; Zacheo and Bleve-Zacheo, 1995). Vasiukova et al. (2001) reported that Chitosan root applications enhanced resistance of tomato plant against root-knot nematodes by producing defense-related chemicals and enzymes.

In these experiments, induction of effective resistance became apparent about seven days after the challenge inoculations. This delay might be related to the post-infection accumulation of antimicrobial substances and is in agreement with reported observations of Zacheo et al. (1983) that accumulation of peroxidase enzymes in tomato plants resistant to *M. incognita* attained maximum level about 10 days after inoculation with the avirulent nematode population.

By varying nematode densities, we showed an increase in the magnitude of induced resistance. Our findings are similar to those of Caruso and Kuc (1979), who reported that the level of resistance induced in cucumber to *Colletotrichum langenarium*, an anthracnose fungus, was directly related to the concentration of induction inoculum.

We have demonstrated in previous studies that virulent populations of *M. arenaria* turn off defense mechanisms of Harmony and Freedom rootstocks, resulting in greater J2 penetration, development and reproduction (Anwar and McKenry, 2003). The HR response of Harmony to avirulent *M. incognita* could be a result of turning on plant defense mechanisms to which *M. arenaria* had become capable of circumventing.

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