Journal of Nematology 32(3):265–270. 2000.

Reproductive Variability of Field Populations of *Meloidogyne* spp. on Grape Rootstocks

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Abstract: Variability in penetration, development, and reproduction of two resistance-breaking field pathotypes (pt.) of *Meloidogyne arenaria, M. incognita,* and a population of mixed *Meloidogyne* spp. virulent to grape hosts were compared on two resistant *Vitis* rootstocks 'Freedom' and 'Harmony' in separate tests. 'Cabernet Sauvignon' was included as a susceptible host to all four nematode populations. Second-stage juveniles (J2) of the mixed population failed to penetrate Freedom roots. By contrast, 6% of J2 in the *M. incognita* population penetrated Freedom roots but did not develop beyond the swollen J2 stage. The two resistance-breaking populations of *M. arenaria* differed in their virulence except on susceptible roots of Cabernet Sauvignon. More J2 of *M. arenaria* pt. Freedom penetrated Freedom roots and reached adult stage than did *M. arenaria* pt. Harmony. Later life stages of *M. arenaria* pt. Freedom occurred earlier and in greater numbers in Harmony roots than did *M. arenaria* pt. Harmony. Reproduction of *M. arenaria* pt. Freedom was greater in Freedom and Harmony roots than *M. arenaria* pt. Harmony. Thus, one population of *M. arenaria* is highly virulent and the other is moderately virulent.

Key words: development, grape rootstocks, *Meloidogyne arenaria, Meloidogyne incognita,* mixed *Meloidogyne* spp., nematode, pathotype, reproduction, variability, virulent.

Vineyards commonly are infested with four root-knot nematode species: *Meloidogyne incognita, M. arenaria, M. javanica,* and *M. hapla*. Resistant grape rootstocks are used to limit damage caused by root-knot nematode (*Meloidogyne* spp.) in vineyards grown on sandy soil in California (Lider, 1954; Snyder, 1936). The extent of damage inflicted depends upon the rootstock-nematode combination, the duration of this combination related to age of the planting, and plant stress factors, including edaphic soil factors. Except for the use of nematicides, the most successful management practice against root-knot nematodes in vineyards is use of resistant rootstocks. However, long-term deployment of resistant rootstocks to manage root-knot nematodes can place strong selection pressure on the population to reproduce and rapidly adapt to the roots of rootstocks, thus forming a new pathotype (McKenry, 1987). The emergence of resistance-breaking root-knot populations, such as *M. arenaria* pathotype (pt.) Freedom and *M. arenaria* pt. Harmony, has occurred due to monoculture for successive decades (Cain et al., 1984). These populations are capable of overcoming resistance in currently used rootstocks.

Several studies have demonstrated that resistance-breaking populations of *Meloidogyne* spp. can arise after continual exposure to selection pressure on resistant plants in relatively few generations (Bost and Triantaphyllou, 1982; Carpenter and Lewis, 1991; McKenry, 1987; Netscher, 1977; Noe, 1992; Prot, 1984). Noe (1992) established 13 populations of *M. arenaria* infecting and reproducing variably on peanut, soybean, and tomato. Four other populations of *M. arenaria* were differentiated on the basis of reproduction and pathology on six soybean cultivars (Carpenter and Lewis, 1991).

Field populations of *Meloidogyne arenaria* pt. Freedom and *M. arenaria* pt. Harmony are geographically isolated from each other and confined to Freedom and Harmony vineyards grown in sandy soil. Freedom and Harmony rootstocks are selections of 1613C and Dog Ridge resistant parentage. These two field populations may vary in reproduction on grape rootstocks. Reproduction variability is important in design of nematode management protocols, particularly for breeding and selecting rootstocks resistant to root-knot nematodes. The purpose of this study was to assess the variability in reproduction of the two Freedom and Harmony pathotypes of *M. arenaria* on the grape root-

Received for publication 10 December 1999.
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This paper was edited by L. D. Young.

stocks from which they were initially selected. Both Freedom and Harmony rootstocks resist root penetration by juveniles of *M. incognita* and the ''aggressive'' mixed *Meloidogyne* spp., so these two populations were included as standard controls for comparison.

Materials and Methods

Fecundity and virulence of two *M. arenaria* pathotypes on two resistant rootstocks were assessed in two experiments. 'Cabernet Sauvignon' was included as a susceptible host to all four nematode populations. *Meloidogyne arenaria* pt. Harmony population was obtained from a 25-year-old vineyard planted on Harmony rootstock, located near Livingston, California. This planting followed a vineyard on root-knot nematode-resistant 1613C-grape rootstock. An *M. arenaria* pt. Freedom population was collected from an 8-year-old planting of Freedom rootstock located 2 km from the Harmony vineyard and also was preceded by a planting on 1613C resistant rootstock. An *M. incognita* population was collected from a 25-year-old kiwifruit planting that had been preceded by a planting of susceptible Thompson Seedless grapes located at the University of California, Kearney Agricultural Center, Parlier, California. The mixed *Meloidogyne* spp. population included species of *M. incognita, M. javanica, M. arenaria,* and *M. hapla* which came from an 8-year-old kiwifruit planting located near Clovis, California. This population is damaging to grape and kiwifruit. A 50-year planting of Thompson Seedless grape had preceded this kiwifruit planting. Hatched second-stage juveniles (J2) were extracted from roots collected from these four field sites using Baermann funnels placed in a mist chamber for 5 days. Suspensions of J2 in tap water were prepared to enable the desired inoculum density to be added in 10 cm^3 of water per plant.

Freedom rootstock trial: One-year-old rooted cuttings of Freedom rootstock were supplied by Duarte Nursery (Ceres, CA). Feeder roots were clipped to encourage the development of new roots of the same age and equal size. Plants of uniform shoot size were transplanted into 5-cm × 25-cm-long deepots (Stewe and Sons Corvallis, OR) filled with autoclaved sand (80% sand, 10% clay, and 10% silt). The deepots were watered immediately with Hoagland's solution, and plants were allowed 3 weeks to initiate roots before nematode inoculation. Plants were fertilized with Hoagland's solution every other week. Plants were inoculated with 500 J2 by injecting on two sides of the plant. All plants were placed on a greenhouse bench at 30 ± 4 °C in a completely randomized design with five replications on each of five-root harvest dates.

Nematode density, juvenile growth, and reproduction were assessed 4, 13, 21, 38, and 46 days after inoculation. Five inoculated plants of each rootstock were harvested on each sampling date. Roots were washed free of soil, blotted onto paper to damp dry, and weighed. The whole root system of each plant at each harvest was stained with acid fuschin (Byrd et al., 1983). Each root system was spread in a film of glycerin between two glass plates, and nematode penetration and development within the roots were determined under dissecting microscope. The growth (= width) at the center of each developing juvenile was measured at each root harvest by using an ocular micrometer at ×100. At 46 days after inoculation a total of 10 plants of each cultivar were harvested with five receiving stains and the other five incubated in a mist chamber for 5 days to hatch the eggs. The numbers of J2 per root system were determined.

Harmony rootstock trial: Variability in reproduction of the two *M. arenaria* populations were compared on Harmony rootstock and susceptible Cabernet Sauvignon. Plants of Harmony and Cabernet Sauvignon were grown 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. Beds were irrigated by 30-second water mists every 9 minutes within a greenhouse maintained at 30 ± 4 °C. Plants of uniform root and shoot size were selected and transplanted into the deepots, then watered immediately

with Hoagland's solution. The plants were inoculated 7 days after transplant. The nematode inoculation level, staining procedure, and other experimental conditions were similar to those of the previously described experiment. The plants were arranged on a greenhouse bench in a completely randomized design with five replications on each of five root harvest dates.

The number of nematodes in each stained root system was recorded at each sampling date. Nematodes were classified into four development stages (Jenkins et al., 1995; Syndenham et al., 1996): Vermiform, non-swollen J2; swollen, sausage-shaped J2; globose juveniles with spiked tail; and female.

Eggs were extracted from galled roots placed in 800-ml sealed, Mason glass jar with 2% NaOCl (Hussey and Barker, 1973) and shaken for 4 minutes at 200 rpm on a rotary shaker (Eberbach, Ann Arbor, MI). Extracted eggs were rinsed thoroughly in tap water and eggs counted at ×40. Number of eggs per gram of root was calculated to determine the reproduction of each nematode population on each rootstock.

Data means were separated with analysis of variance using SAS (SAS Institute, Cary, NC). A separate analysis was conducted for each sampling date. Significant differences in means of nematode reproduction were separated with Duncan's multiple range test ($P \leq 0.05$).

Results

Freedom rootstock trial: Root population densities of *M. arenaria* pt. Freedom were significantly greater ($P = 0.05$) than those of *M. arenaria* pt. Harmony on all five root observation dates (Table 1). Although low population densities of *M. incognita* were observed in Freedom root tissues on the first three sampling dates, none were present on the last two sampling dates. Individuals from the mixed population of *Meloidogyne* spp. were not observed in root tissues at any of the sampling dates. Significantly greater numbers of second-stage juveniles of *M. arenaria* pt. Freedom were recovered from root tissues 46 days after inculcation than *M. arenaria* pt. Harmony. Juvenile body width of *M. arenaria* pt. Freedom was significantly greater ($P = 0.05$) than that of *M. arenaria* pt. *Harmony* and *M. incognita* on all observation dates (Fig. 1).

Harmony rootstock trial: Root population densities of *M. arenaria* pt. Freedom were significantly greater ($P = 0.05$) than *M. arenaria* pt. Harmony on Freedom rootstock during the first three observation dates, but not on the last observation dates (Table 2). No such difference was observed between two pathotypes associated with the nematode-susceptible Cabernet rootstock. The number of *Meloidogyne* eggs recovered from root tissues differed significantly ($P = 0.05$) and was greatest for *M. arenaria* pt. Freedom on Harmony rootstock and least for *M. arenaria* pt. Harmony on Harmony rootstock. Although there were significant ($P = 0.05$) differences in the rate of development among the nematode populations and rootstocks during the first three observations, no differences were observed on the last two observations (Table 3).

Table 1. Root population density, and reproduction of four *Meloidogyne* spp. populations on Freedom grape rootstock at five intervals after inoculation.

	Days after inoculation					Reproduction
Nematode populations	4	13	21	38	46	Hatched[a] J2
M. arenaria pt. Freedom	304 a	261 a	261 a	251 a	228 a	2,240 a
M. arenaria pt. Harmony	119 b	80 b	65 b	53 b	44 b	184 b
M. incognita	29 c	17 c	12 c	0 c	0 c	0
Mixed[b] *Meloidogyne* spp.	0 c	0 c	0 c	0 c	0 c	0

Data are means of 5 replicates. Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

[a] Number of juveniles hatched after roots collected at 46 days after inoculation were placed in a mist chamber for 5 days.

[b] Mixed *Meloidogyne* spp. included *M. incognita, M. javanica, M. arenaria,* and *M. hapla.*

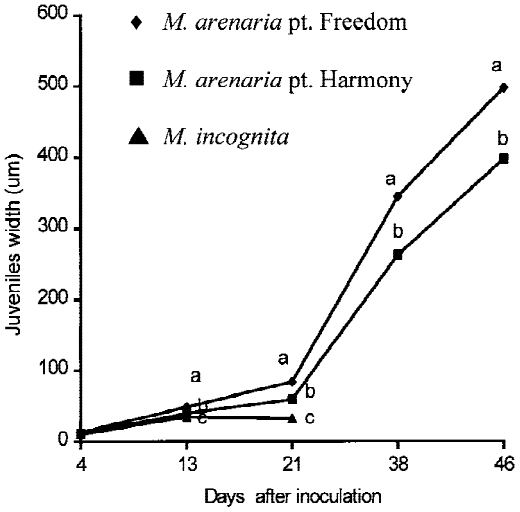

FIG. 1. Width of juveniles of three *Meloidogyne* populations in Freedom grape rootstock roots 4 to 46 days after inoculation. Data are means of 20 replications. Means on a given day after inoculation followed by the same letter are not different ($P \leq 0.05$).

DISCUSSION

These *Meloidogyne* populations are separated into two groups, depending on their development in roots of three rootstocks. The first group includes two virulent populations of *M. arenaria* that infect and complete their life cycle in roots of Freedom rootstock. The second group includes avirulent populations of *M. incognita* and mixed *Meloidogyne* spp. that do not complete their life cycle in roots of Freedom rootstocks. Juveniles of all the populations were able to develop on susceptible Cabernet as expected (unpubl.). The two virulent nematode populations used in this experiment had experienced greater selection pressure against resistant grape rootstocks than the two avirulent populations emanating more recently from kiwifruit plantings. Similar observations have been reported with higher rates of reproduction by virulent populations of root-knot nematodes on resistant host plants compared to no reproduction or suppressed reproduction by avirulent populations (Bakker et al., 1993; Roberts, 1995).

High reproduction rates of *M. arenaria* pt. Freedom on Freedom, Harmony, and Cabernet confirm that it is a virulent population. Since the two populations of *M. arenaria* reproduced equally on Cabernet but *M. arenaria* pt. Harmony reproduction was lower on Freedom and Harmony grape rootstocks, the *M. arenaria* pt. Freedom is considered more virulent than *M. arenaria* pt. Harmony.

Variability in the rate of reproduction among different *M. arenaria* populations on various crop hosts has previously been found (Carpenter and Lewis, 1991; Noe, 1992). In our study *M. arenaria* pt. Harmony exhibited limited penetration, fewer adult females, and suppressed reproduction in Freedom roots, suggesting the existence of root barriers and biochemical changes that prevent J2 entry and subsequent development. By contrast, *M. arenaria* pt. Freedom has acquired an ability to overcome the Freedom resistance, so high numbers of J2 were able to enter roots, mature to adult females, and produce eggs. These observations suggest the involvement of two different mechanisms in these two populations.

TABLE 2. Root population and egg production of two *Meloidogyne arenaria* pathotypes in roots of Harmony and Cabernet grape rootstocks, 4 to 35 days after inoculation.

	Nematodes in roots, DAI[a]					Eggs per g root[b]
Grape rootstock, *M. arenaria* pathotype	4	13	21	27	35	35 DAI
Harmony + *M. arenaria* pt. Freedom	287 a	274 a	266 a	209 a	167 a	1,652 a
Harmony + *M. arenaria* pt. Harmony	193 b	226 b	201 b	183 a	160 a	179 c
Cabernet + *M. arenaria* pt. Freedom	289 a	300 a	248 ab	215 a	184 a	731 b
Cabernet + *M. arenaria* pt. Harmony	296 a	297 a	273 a	225 a	189 a	403 cb

Data are means of 5 replicates. Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Duncan's multiple range test.

[a] DAI = days after inoculation.

[b] = Statistical analysis based on Log_{10} transformed data. Untransformed means are shown.

TABLE 3. Developmental stages of two pathotypes of *Meloidogyne arenaria* on Harmony and Cabernet grape rootstocks, 4 to 35 days after inoculation.

	Nematode developmental stages at days after inoculation									
	Vermiform J2		Swollen J2		Globose	Female	Globose	Female	Globuse	Female
Rootstock/pathotype	4	13	13	21	21	21	27	27	35	35
Harmony/*M. arenaria* pt. Freedom	287 a	14 b	262 a	56 b	156 a	50 a	44 a	165 a	5 a	162 a
Harmony/*M. arenaria* pt. Harmony	193 b	48 a	178 b	139 a	53 b	9 b	38 a	145 a	5 a	155 a
Cabernet/*M. arenaria* pt. Freedom	289 a	15 b	285 a	39 b	157 a	52 a	41 a	174 a	12 a	172 a
Cabernet/*M. arenaria* pt. Harmony	296 a	44 a	253 a	67 b	182 a	23 b	46 a	179 a	11 a	178 a

Data are means of 5 replicates. Means within a column followed by the same letter are not different ($P < 0.05$) according to Duncan's multiple range test.

Differences in mechanism seem to be related to genetic changes associated with two different grapes but related rootstocks. The farm management practices where these two pathotypes developed were uniform. These differences are therefore not comparable to the situation where two populations of *M. incognita* differed in their virulence when reared under different environmental conditions (Castagnone-Sereno et al., 1994).

The larger-sized and higher number of females of *M. arenaria* pt. Freedom in Freedom roots compared to *M. arenaria* pt. Harmony demonstrates that populations of the same species differed quantitatively and qualitatively with regard to their ability to produce females on Freedom roots. This indicates that these two populations may differ with regard to the genetic factors controlling parasitism, and with regard to the frequency of such factors in each population. Such quantitative differences can be attributed to differences in gene frequency for genes regulating the host-parasite interaction (Triantaphyllou, 1975).

Development to mature females by *M. arenaria* pt. Harmony was delayed in roots of Harmony compared to *M. arenaria* pt. Freedom. This agrees with our observations from the resistant 10-23B and RS-3 grape rootstocks, which delayed development of juveniles of *M. arenaria* pt. Harmony at vermiform J2 and swollen J2 stage (Anwar and McKenry, 2000). The delay in development of *M. arenaria* pt. Harmony is comparable to the delay in development of *M. incognita* on resistant cotton (Jenkins et al., 1995) and resistant Nemasnap cultivar of common bean (Syndenham et al., 1996).

Our findings reveal that Freedom and Harmony possess genetic systems capable of generating variation in the nematode genome leading to the creation of new resistance-breaking populations. It seems that the gene-for-gene matching system between host resistance genes and nematode avirulent genes may be operating in this interaction, as suggested for other phytonematodes (Jones et al., 1981). A nematode population has overcome preinfectional and postinfectional mechanisms that operate successfully in Freedom roots against avirulent populations such as the *M. incognita* and mixed *Meloidogyne* spp. These results have important implications for breeders wishing to develop durable plant resistance and underscore the importance of evaluating breeding material against single, characterized populations instead of mixed populations. Furthermore, the importance of changing rootstock parentage or mechanisms of resistance to avoid or minimize the selection of resistance-breaking nematode populations must be a consideration when replanting vineyards.

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