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Penetration and Development of *Meloidogyne arenaria* on Two New Grape Rootstocks

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Abstract: Penetration, development, and reproduction of a virulent 'Harmony' population of *Meloidogyne arenaria* was studied on two nematode-resistant grape rootstocks 10-17A and 6-19B. 'Cabernet Sauvignon' was used as a susceptible control for comparison. Plants were inoculated with 100 freshly hatched second-stage juveniles (J2) of *M. arenaria*. Greater numbers of J2 penetrated roots of 'Cabernet' than 10-17A, and none penetrated roots of 6-19B 4 days after inoculation (DAI). At 7 DAI, vermiform J2 advanced to sausage-shaped J2 in roots of 'Cabernet,' penetrated roots of 6-19B, and had egressed from roots of 10-17A. Resistant rootstocks expressed hypersensitive responses to penetrating J2 along the root epidermis, among the cortical cells, and along the differentiating vascular bundles. At 13 DAI, 68% of the J2 had attained globose stage in roots of 'Cabernet,' whereas there was no development of vermiform J2 in roots of the other two rootstocks. The nematodes reproduced only in roots of 'Cabernet.' Lack of development of J2 in roots of the two resistant grape rootstocks might be the result of a hypersensitive response to J2 feeding.

Key words: development, grape rootstocks, hypersensitive reaction, *Meloidogyne arenaria* population, penetration, reproduction, resistance, *Vitis* spp.

The emergence of virulent populations of root-knot nematode on previously nematode-resistant 'Harmony' and 'Freedom' grape rootstocks has increased the need for new rootstocks (Cain et al., 1984; Walker et al., 1994). Grape rootstocks 10-17A (*Vitis simpsoni* × *V. muscadinia*) and 6-19B (*V. champinii* × GA-3, 4, 5) were selected from among 520 *Vitis* sources for their breadth of resistance to field populations of *Meloidogyne incognita* race 3, *M. chitwoodi,* mixed *Meloidogyne* spp., *Meloidogyne* sp. Ramsey population, and two virulent populations of *M. arenaria* (Anwar et al., 2002). The objective of this study was to investigate the host-parasite interaction of 10-17A and 6-19B grape rootstocks to a population virulent to 'Harmony,' thereafter referred to as the 'Harmony' population. 'Cabernet Sauvignon' (*V. vinifera*) was included as a susceptible control.

Materials and Methods

Rooted grape cuttings were grown from shoot tips 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 mist of 30-second duration every 9 minutes within a greenhouse. Plants of uniform root and shoot size were selected and transplanted into 5 cm × 25 cm-long propagating tubes (Stewe and Sons, Corvallis, OR) filled with autoclaved sand. The tubes were watered with Hoagland's solution, and plants were allowed 7 days to recover from transplanting before nematodes were added. Each tube was inoculated with 100 freshly hatched *M. arenaria* second-stage juveniles (J2), derived from a 'Harmony' field population. Treatments consisted of three rootstocks with five replications and six sampling dates. The tubes were arranged in a completely randomized design on a greenhouse bench with a temperature range of 13 to 21 °C.

Nematode penetration, development, and reproduction were assessed at 4, 7, 13, 21, 35, and 46 days after inoculation (DAI). Roots of five inoculated plants of each rootstock were evaluated during each sampling period. The roots were washed free of soil, blotted dry with paper, and stained with acid fuschin (Byrd et al., 1983). Each root system was spread in a film of glycerin between two glass plates, and nematode numbers and development stages were determined using a dissecting microscope. Nematodes were classified into four developmental stages (Anwar and McKenry, 2000; Syndenham et al., 1996): vermiform, non-swollen J2; swollen, sausage-shaped J2; globose juveniles with spiked tail; and females. Appearance of clear areas in the intestine indicated starvation. Severe reductions in body diameter and coiling indicated shriveling and death of the nematode (Van Gundy et al., 1967). Host cell necrosis was recorded when several deformed and brown cells appeared close to the nematode head.

The effect of nematode penetration and development was determined with analysis of variance (SAS Institute, Cary, NC). A separate analysis was conducted for each sampling date. Means were separated with Duncan's multiple-range test ($P \leq 0.05$).

Results

Penetration and subsequent development of the 'Harmony' population of *M. arenaria* varied among grape rootstocks. At 4 DAI, greater ($P \leq 0.05$) numbers of J2 had penetrated roots of 'Cabernet' than roots of 10-17A and none had penetrated roots of 6-19B (Fig. 1A). Vermiform J2 were clustered in the root tips of 10-17A but had migrated distally into the developing vascular cylinder of susceptible 'Cabernet.' By 7 DAI, sausage-shaped J2 were present in roots of 'Cabernet.' Vermiform J2 had penetrated roots of 6-19B but were not present in roots of 10-27A (Fig. 1B). By 13 DAI, 68% of the juveniles had attained globose stage in roots

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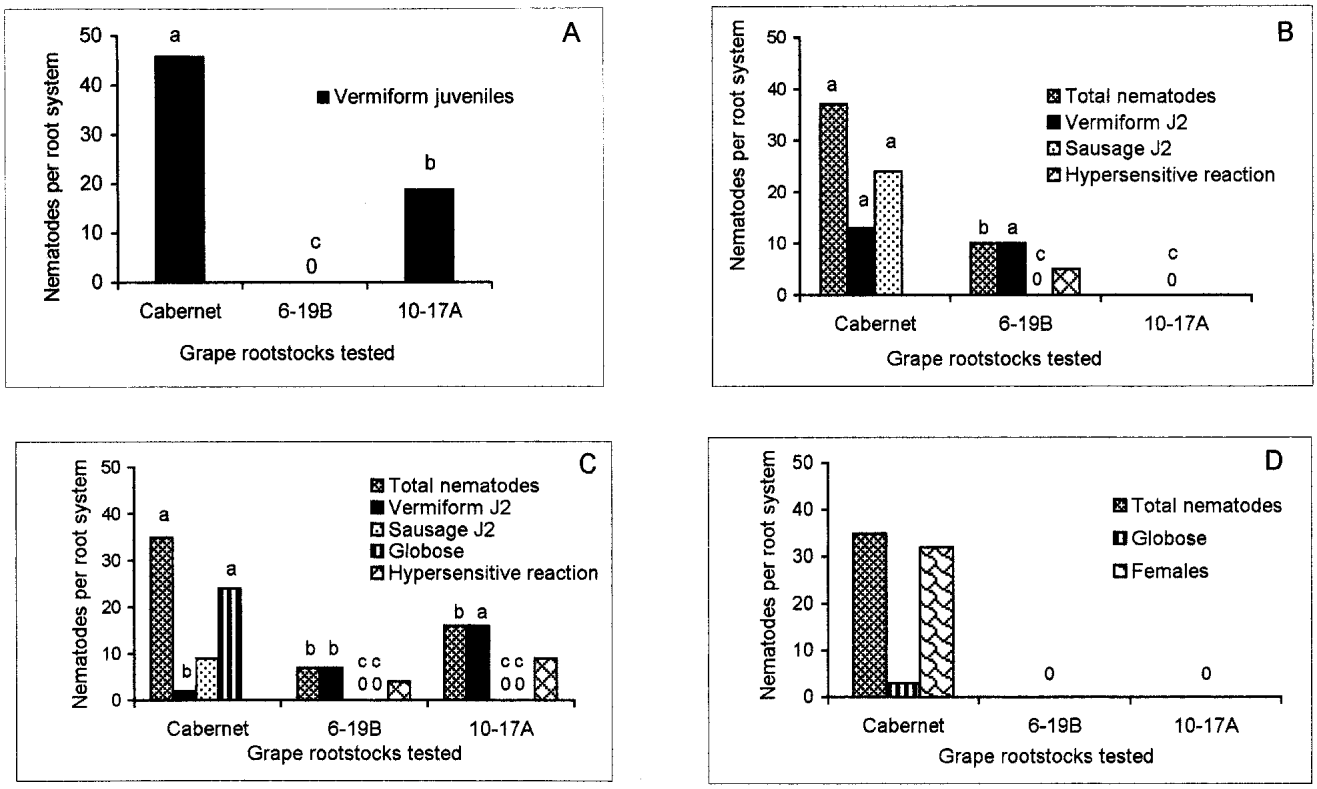

FIG. 1. Number of developmental life stages of *Meloidogyne arenaria* in roots of 'Cabernet,' 10-17A, and 6-19B grape rootstocks, 4 to 46 days after inoculation with second-stage juveniles. Data are means of five replications. Bars representing a developmental stage with a common letter are not significantly different according to Duncan's multiple-range test ($P > 0.05$). A) Penetration 4 days after inoculation. B) Penetration and development 7 days after inoculation. C) Development 13 days after inoculation. D) Development 46 days after inoculation.

of 'Cabernet.' In roots of the other two rootstocks, there was no development of vermiform J2 (Fig. 1C). Most of the juveniles were within the root tips of 10-17A and 6-19B; however, a few had migrated to the vascular cylinder. In 'Cabernet,' females without egg masses were present 21 DAI (Fig. 1D), they had begun to produce eggs 35 DAI, and most had large egg masses 45 DAI. No nematodes were found within roots of the two resistant rootstocks 21 DAI or thereafter (data not shown). The longer-than-usual life cycle was most likely due to relatively low greenhouse temperatures throughout the experiment.

Resistance response: Necrotic cells were observed in the epidermis associated with 50% of penetrating J2 in root tissues of 6-19B by 7 DAI. The entombed J2 were starved and shriveled. By 13 DAI, 57% and 56% of the total nematodes exhibited epidermal, cortical, or vascular hypersensitive reactions within roots of 6-19B and 10-17A, respectively (Fig. 1C). These necrotic tissues always surrounded the vermiform J2 and prevented their further development to J3.

DISCUSSION

Resistance of these two new grape rootstocks is associated with reduced penetration and (or) increased egression, and prevention of development. Similarly, J2 of the 'Harmony' population of *M. arenaria* also exhibited reduced penetration of three other grape rootstocks including RS-3, 10-23B (Anwar and McKenry, 2000), and RS-9 (Anwar and McKenry, 2001). These resistant plants expressed a pre-infectional resistance at the root surface. This resistance might be due to root epidermal barriers or secretions of biochemicals repellent to invading nematodes (Huang, 1985). The egression of J2 from roots of resistant rootstocks also might be related to absence of essential nutrients required for nematode development (Huang, 1985).

Post-infectional mechanisms also exist and include induction of a hypersensitive response to nematode infection, which limit further nematode development (Anwar and McKenry, 2000, 2001; Huang, 1985; Kaplan, 1981). Appearance of hypersensitive reactions coincided with limited nematode development in roots of resistant rootstocks and the disappearance of fat globules from the intestines of the nematode (Anwar and McKenry, 2000; Van Gundy et al., 1967).

The rootstock 6-19B has supported low levels of reproduction of *M. incognita* race 3, *M. chitwoodi,* mixed *Meloidogyne* spp., *Meloidogyne* sp. Ramsey population, and two virulent populations of *M. arenaria* in the field (Anwar et al., 2002). The absence of reproduction in the current study might be related to low inoculum level. Lack of reproduction by the 'Harmony' population of *M. arenaria* on 10-17A was also observed during 2-year field evaluations (Anwar et al., 2002).

These findings indicate that resistance in these two

rootstocks is expressed at the root tip against invading J2 resulting in delayed or reduced nematode entry. Defense mechanisms along the vascular bundle, expressed as a hypersensitive reaction, prevented development of J2 that attempted to establish a feeding site. These data, coupled with previous studies (Anwar and McKenry, 2000, 2001; Anwar et al., 2002), indicate that nematode resistance in four different *Vitis* spp. (rootstocks 10-17A, 6-19B, 10-23B, and RS-3) was associated with similar expressions of resistance. This does not necessarily suggest that the same gene is involved because hypersensitive responses are associated with a number of different nematode-resistant genes.

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