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Reactions of Grape Rootstocks to *Pratylenchus vulnus* and *Meloidogyne* spp.[1]

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Abstract: Five grape rootstocks were inoculated with 0, 100, 1,000, and 10,000 *Pratylenchus vulnus*. Dogridge and Saltcreek supported low average total numbers of *P. vulnus*, 136–705/pot, at 12 months after inoculation. Growth of both rootstocks was not affected. Harmony, Couderc 1613, and Ganzin 1 supported high average total numbers, 6–856 times the inoculum levels. Numbers in Harmony continued to increase at all levels but reduced root weight only at the 10,000 level after 12 months. Numbers in Couderc 1613 decreased by 15–30% after 12 months, and root weight was reduced at the 10,000 level. In Ganzin 1, total nematode numbers diminished after 12 months but were still at high levels; growth reduction was proportional to numbers of nematodes added. *Meloidogyne incognita, M. javanica,* and *M. arenaria* produced galls and egg masses in Harmony and Couderc 1613 only at 36 C. Galling in Ganzin 1 increased with increasing temperature. Galls in Ganzin 1 at 18 C supported mature females after 90 days. Harmony was resistant to *M. incognita* in single and concomitant inoculations of *P. vulnus* and *M. incognita.* At 250 days after inoculation, total numbers of *P. vulnus* increased above the inoculum level and the 150-day values; increase was greatest in *P. vulnus* added singly. Neither nematode species affected growth of Harmony.
Key words: inoculum levels, temperature, Harmony, Couderc 1613, Ganzin 1, Saltcreek, Dogridge, *M. incognita, M. javanica, M. arenaria.*

Pratylenchus vulnus, Meloidogyne incognita, M. javanica, and *M. arenaria* are commonly distributed in California vineyards seriously affecting grape yield (6,8–10). The proper choice of rootstocks is important for effective control of these nematodes.

The present study using five commercial grape rootstocks was conducted in three trials. The trials were studies on 1) effects of different inoculum levels of *P. vulnus* on five rootstocks, 2) effects of soil temperature on parasitism of *Meloidogyne* spp. on three rootstocks, and 3) effects of *P. vulnus* and *M. incognita* on the growth of Harmony rootstock.

MATERIALS AND METHODS

The populations of *P. vulnus* were obtained from carrot tissue cultures propagated according to the method of Moody et al. (7).

Second-stage juveniles of *M. incognita* and *M. javanica* were obtained from tomato, *Lycopersicon esculentum* cv. Rutgers, raised in a University of California, Davis (UCD) greenhouse. *Meloidogyne arenaria* was obtained from the University of California, Riverside (UCR) from a population maintained on tomato plants in the greenhouse. The population was isolated originally from soil and roots of 'Thompson Seedless' grape in a vineyard near Selma, California. This culture was increased on Rutgers at UCD. Confirmation of species by perineal patterns was not conclusive, suggesting some contamination might have complicated the culture. However, as differences in reactions to their hosts were noted between the two species of *Meloidogyne* used in Trial II, the UCR selection is included as *M. arenaria.*

Five commercial grape rootstocks used in California—namely, Harmony, Couderc 1613, Ganzin 1, Dogridge, and Saltcreek—were chosen. These were propagated in a greenhouse, maintained at 20–23 C, from two-bud cuttings in sterile sand beds for 6 weeks before transplant.

Trial I: Effect of different inoculum levels of *P. vulnus* on five rootstocks.

Harmony, Ganzin 1, and Couderc 1613 rootings were transplanted in March to 15-cm-d sterile clay pots containing sandy-loam soil. Dogridge and Saltcreek, being slower to root, were transplanted in April. Six days after transplanting, the soil was inoculated with 100, 1,000 or 10,000 *P. vulnus.* Control plants received an equal volume of distilled water only. Each treatment was replicated 10 times for each rootstock in 10 randomized blocks. Five blocks were harvested after 6 months, the remaining five after 12 months.

The 12-month period required dormancy. Those plants were transferred in mid-

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November from the greenhouse outside to a lathhouse and returned to the greenhouse in January. Shoots were cut back and their lengths recorded. At 6 and 12 months, shoot length and root weights were recorded. Total shoot lengths at 12 months were recorded as the sum of the lengths of shoots excised at dormancy and lengths of new shoot growth after dormancy.

Nematode populations were determined by soil extraction and root incubation. The soil from each pot was mixed with water and passed through sieves with 0.833-mm and 0.043-mm openings. The residue on the finer sieve was placed on a Baerman funnel under intermittent mist for 4 days. The roots were analyzed by washing and cutting into lengths of 3–4 mm, which were thoroughly mixed; then a 5-g sample was placed on a Baerman funnel under intermittent mist for 4 days. Counts of nematodes were made from a 1-ml aliquot of this extract, and total numbers of *P. vulnus* present in soil and roots were calculated.

Trial II: Effect of soil temperature on parasitism of *Meloidogyne* spp. on three rootstocks.

Harmony, Couderc 1613, and Ganzin 1 rootings were transplanted to 474-ml clear plastic cups containing sterilized sandy-loam soil, and these were embedded in sterilized sand in 2-liter crocks. The crocks then were placed in constant-temperature water tanks maintained at 10, 18, 26, and 36 C. Five replicates at each temperature were randomized within each tank. Groups of rootstocks were inoculated with 1,000 second-stage juveniles of *M. incognita* in August 1981, *M. javanica* in November 1981, or *M. arenaria* in April 1982. Each set was grown for 45 days after inoculation. Due to low incidence of galling by the nematode species on Harmony and Couderc 1613 at 10, 18, and 26 C, and in Ganzin 1 at 10 and 18 C after 45 days, the experiments were repeated at those temperatures for 90 days. After 45 and 90 days, soil from each pot was processed, as in the previous trial, and placed under intermittent mist for 2 days. The extract was examined for larvae and males. Roots were washed and observed for galls and egg masses. Galls without egg masses were dissected to detect the nematode.

Trial III: Effects of *P. vulnus* and *M. incognita* on Harmony rootstock.

Harmony rootings were transplanted in July to 15-cm-d pots containing sandy-loam soil. After 6 days, they were inoculated with *P. vulnus* and/or *M. incognita*. Treatments were replicated 10 times and included (a) uninoculated control (0 nematodes), (b) 1,000 *P. vulnus*, (c) 5,000 *M. incognita* second-stage juveniles, (d) 1,000 *P. vulnus* followed 1 month later by 5,000 *M. incognita*, and (e) 5,000 *M. incognita* followed 1 month later by 1,000 *P. vulnus*. One series of treatments was harvested in December, 150 days after inoculation, and the other in March after 250 days. The second series required dormancy.

At harvest, data of shoot and root growth and nematode counts were taken as for the previous trials. Soil, 250 ml from each pot, was processed and kept under mist for 2 days. Roots were washed, observed for galls and egg masses, cut into 3–4-mm-long pieces, and kept under mist for 4 days.

Results and Discussion

Trial I: Two distinct reactions are clearly evident from the nematode counts shown in Table 1. In one case, Dogridge and Saltcreek reacted similarly, supporting low populations of *P. vulnus*. Six months after inoculation, a significant number survived in the soil, especially at the highest inoculation level of 10,000 nematodes where over 30% survived. After 12 months, however, the average total numbers for all inoculum levels had stabilized at 136–705/pot. For both rootstocks the predominant population was in the roots.

Harmony, Couderc 1613, and Ganzin 1, on the other hand, supported high average total numbers of *P. vulnus*, with multiplications ranging from 6 to 856 times the inoculum levels. Yet each of these rootstocks exhibited different reactions.

Harmony supported up to 96,553 nematodes per pot by 6 months, and these numbers increased even more at all levels by 12 months. Ganzin 1 produced even more *P. vulnus* per pot than Harmony after 6 months, but these numbers diminished after 12 months, although they were still at high levels. The numbers of *P. vulnus* on Couderc 1613 were the lowest of the three rootstocks after 6 months and had decreased by 15–63% after 12 months.

The root populations for Harmony and Ganzin 1 had a pattern similar to the total

TABLE 1. Number of *Pratylenchus vulnus* from soil and roots of five grape rootstocks 6 and 12 months after inoculation.

	No. of *P. vulnus* added							
	6 months				12 months			
	0	100	1,000	10,000	0	100	1,000	10,000
Soil and roots								
Saltcreek	0	33 b*	208 b	4,072 a	0	686 b	298 b	705 b
Dogridge	0	54 b	243 b	4,116 a	0	136 b	692 b	566 b
Harmony	0	12,357 c	22,727 c	96,553 b	0	19,002 c	98,924 b	128,875 a
Couderc 1613	0	5,039 d	14,463 c	60,138 a	0	3,500 d	12,311 c	22,033 b
Ganzin 1	0	85,634 ab	134,179 a	103,615 ab	0	47,512 bc	98,997 ab	65,733 b
Roots								
Saltcreek	0	14 b	35 b	556 a	0	476 a	174 b	137 b
Dogridge	0	37 d	172 cd	650 a	0	129 d	614 ab	393 bc
Harmony	0	8,243 cd	9,798 cd	18,984 bc	0	16,555 bc	24,908 b	42,006 a
Couderc 1613	0	4,441 cd	9,653 b	14,760 a	0	3,252 cd	7,598 bc	6,763 bc
Ganzin 1	0	10,174 ab	12,292 ab	4,676 bc	0	11,116 ab	16,719 a	12,115 ab

* Each figure represents the mean of five replicates. Values in each row followed by same letter do not differ statistically ($P = 0.05$, Duncan's multiple-range test).

populations, with increases at 12 months ranging from 10 to 200% of the root populations at 6 months. Couderc 1613 had diminishing numbers of *P. vulnus* at all levels but still held 3,252–7,598 per plant at 12 months.

Growth response to *P. vulnus* varied with rootstock. The nematode had no detrimental effect on growth of Saltcreek or Dogridge plants when compared with the control. At the 10,000 level of inoculum, Dogridge plants increased significantly in shoot length by 44.3 cm above control plants (110.9 cm) in 6 months time but not at 12 months. Because they supported low numbers of nematodes that did not damage the plants, both rootstocks could be classified as resistant to *P. vulnus*.

Growth of Harmony and Couderc 1613 plants was not affected by the nematode at most levels. Despite a reduction in root weight at the highest inoculum level at 12 months, Harmony supported an increase of *P. vulnus* (Table 2). Couderc 1613, however, was less tolerant than Harmony and supported lower numbers that reduced root weight at the 10,000 level after 6 and 12 months.

Nematode pathogenicity to Ganzin 1 (Table 2) increased with the numbers of *P. vulnus* added. This rootstock was susceptible at all levels of nematode inoculum.

TABLE 2. Reactions of Harmony, Couderc 1613, and Ganzin 1 to different inoculum levels of *Pratylenchus vulnus*.

	Harmony		Couderc 1613				Ganzin 1		
	12 months		6 months		12 months		12 months		
Inoculum level	Root weight (g)	*P. vulnus*/g root (No.)	Root weight (g)	*P. vulnus*/g root (No.)	Root weight (g)	*P. vulnus*/g root (No.)	Shoot length (cm)	Root weight (g)	*P. vulnus*/g root (No.)
0	123.0 a*	0.0 a	105.6 a	0.0 a	120.0 a	0.0 a	437.4 a	63.7 a	0.0 a
100	101.0 a	168.2 b	103.2 a	43.0 ab	95.2 a	34.6 b	172.9 bc	47.1 ab	254.2 ab
1,000	105.4 a	230.0 b	92.2 ab	104.2 b	91.6 a	86.6 c	225.1 b	33.4 bc	520.4 b
10,000	60.8 b	860.8 c	69.1 b	216.4 c	70.4 b	97.4 c	129.8 c	20.7 c	331.2 b

* Each figure represents the mean of five replicates. Values in each column followed by same letter do not differ statistically ($P = 0.05$, Duncan's multiple-range test).

The results confirm earlier reports in the literature on reactions of the test rootstocks (except Harmony, where this information is new) to *P. vulnus* (8,9) as well as provide new information on the stability of these reactions (including Harmony) to different inoculum levels of the nematode in the first year of plant growth.

Trial II: All three *Meloidogyne* species produced galls with eggs in Harmony and Couderc 1613 only at 36 C suggesting a breakdown in the resistance mechanisms that existed at the lower temperatures. One or two galls produced by the nematodes at 26 C in Couderc 1613 roots were with juveniles. A longer growth period, 90 vs. 45 days, had no effect on further development of juveniles in the galls or on gall production at 10, 18, and 26 C. No galling occurred in 45 days in Ganzin 1 at 10 C, but galling occurred at 18 C and increased between 45 and 90 days for *M. incognita* (4 and 111 galls, respectively), *M. javanica* (3 and 25 galls), and *M. arenaria* (14 and 21 galls). At 18 C, the nematodes did not mature in 45 days but did after 90 days. A few *M. arenaria* juveniles penetrated Ganzin 1 roots at 10 C and formed galls after 90 days, but the nematodes did not develop to maturity. All species produced galls with viable eggs at 26 and 36 C.

After 45 days at 36 C, *M. arenaria* was more infective on Harmony in producing a greater number of galls (364) than *M. javanica* (118) or *M. incognita* (30). *Meloidogyne javanica* (210 galls) and *M. arenaria* (133 galls) were more infective than *M. incognita* (13 galls) on Couderc 1613 at 36 C. After 45 days at 26 and 36 C, *M. arenaria* (199 and 516 galls, respectively) was more infective on Ganzin 1 than were *M. javanica* (63 and 224 galls) or *M. incognita* (60 and 13 galls). At 90 days, however, *M. incognita* produced significantly more galls (111) than did the other species.

Average numbers of juveniles recovered per pot after 90 days at temperatures where few or no galls were formed showed that less than 5% of the inoculum of each species survived in Harmony and Couderc 1613 soil at 18 and 26 C. However, at 10 C, where no plant growth occurred, *M. incognita* and *M. javanica* each died at a significantly lower rate than at 18 and 26 C. *Meloidogyne javanica* decreased to 10–30% at 45 days and to less than 1% at 90 days, while 20–28% of the *M. incognita* survived for 90 days. Both species produced galls when inoculated to tomato seedlings in a greenhouse. At 10 C, no *M. arenaria* was recovered from soil of any rootstocks after 45 or 90 days indicating its high sensitivity to this low temperature.

The implications of these results are important in nematode control. We have shown that soil temperature influenced grape rootstock response to these nematodes. Ganzin 1 seems to have a low temperature resistance-breaking threshold. Resistance of Harmony and Couderc 1613 to *Meloidogyne* spp. is broken at high soil temperature. Harmony and Couderc 1613 have been reported in the literature as being resistant to *M. incognita* (1,5), *M. javanica* (3), and *M. arenaria* (2,4). However, this study shows that these may not be reliable stable root-knot nematode resistant rootstocks.

Trial III: Galls and juveniles of *M. incognita* were not observed in roots of Harmony plants, nor did the nematode survive in the soil at greenhouse temperature, 20–23 C. The results support those of Trial II where no galling occurred at 18 or 26 C.

At 150 days, total numbers of *P. vulnus* diminished to a consistent level where *P. vulnus* was added singly, *P. vulnus* was added with *M. incognita,* or *P. vulnus* was added 1 month before *M. incognita* (Table 3). When *P. vulnus* was added 1 month after *M. incognita,* the total numbers of *P. vulnus* diminished to a level lower than in the other treatments. Time of inoculation and effect of the resistance mechanism released by the host, due to the presence of the nematode, may account for the initial reduction in numbers. In most treatments, half of the total population was in the root system.

At 250 days after inoculation, numbers of *P. vulnus* had increased above the inoculated levels, with averages ranging from 1,057 to 6,635 per pot. In cases where *P. vulnus* was added singly and 1 month before *M. incognita,* the respective rates of increase were 1.5–6 and 1.2–4 times the numbers of nematodes in the other treatments. In all treatments with *P. vulnus,* the predominant population was in the roots.

The numbers of nematodes recovered from Harmony treated with 1,000 *P. vulnus* singly are at variance with those numbers reported in Trial I. While the con-

TABLE 3. Numbers of *Pratylenchus vulnus* recovered after different harvest dates from Harmony plants inoculated with *P. vulnus* and *Meloidogyne incognita* singly and in combination.

	150 days		250 days	
Treatments	*P. vulnus* in roots	*P. vulnus* in roots and soil	*P. vulnus* in roots	*P. vulnus* in roots and soil
Uninoculated control	0 b*	0 b	0 c	0 c
P. vulnus	298 a	523 a	5,262 a	6,635 a
Pv + Mi†	148 ab	344 a	2,703 b	3,327 b
Pv then Mi‡	204 a	587 a	3,111 ab	4,174 ab
Mi then Pv§	23 b	30 b	886 b	1,057 b

* Each figure represents the mean of five replicates. Values in each column followed by the same letter do not differ statistically ($P = 0.05$, Duncan's multiple-range test).

† *P. vulnus* (1,000) and *M. incognita* (5,000) added at the same time.

‡ *M. incognita* added 1 month after *P. vulnus*.

§ *P. vulnus* added 1 month after *M. incognita*.

ditions for both experiments were similar, the dates of inoculation and harvest differed. The different durations of the experiments may account for some of the difference, and the seasons in which inoculations and harvests were done may have also affected the numbers supported by Harmony.

Despite the high populations supported by Harmony, *P. vulnus* had no detrimental effect on shoot or root growth.

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