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Effects of *Meloidogyne* spp. and *Rhizoctonia solani* on the Growth of Grapevine Rootings

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Abstract: A disease complex involving *Meloidogyne incognita* and *Rhizoctonia solani* was associated with stunting of grapevines in a field nursery. Nematode reproduction was occurring on both susceptible and resistant cultivars, and pot experiments were conducted to determine the virulence of this *M. incognita* population, and of *M. javanica* and *M. hapla* populations, to *V. vinifera* cv. Colombard (susceptible) and to *V. champinii* cv. Ramsey (regarded locally as highly resistant). The virulence of *R. solani* isolates obtained from roots of diseased grapevines also was determined both alone and in combination with *M. incognita*. Ramsey was susceptible to *M. incognita* (reproduction ratio 9.8 to 18.4 in a shadehouse and heated glasshouse, respectively) but was resistant to *M. javanica* and *M. hapla*. Colombard was susceptible to *M. incognita* (reproduction ratio 24.3 and 41.3, respectively) and *M. javanica*. Shoot growth was suppressed (by 35%) by *M. incognita* and, to a lesser extent, by *M. hapla*. Colombard roots were more severely galled than Ramsey roots by all three species, and nematode reproduction was higher on Colombard. Isolates of *R. solani* assigned to putative anastomosis groups 2-1 and 4, and an unidentified isolate, colonized and induced rotting of grapevine roots. Ramsey was more susceptible to root rotting than Colombard. Shoot growth was inhibited by up to 15% by several AG 4 isolates and by 20% by the AG 2-1 isolate. AG 4 isolates varied in their virulence. Root rotting was higher when grapevines were inoculated with both *M. incognita* and *R. solani* and was highest when nematode inoculation preceded the fungus. Shoot weights were lower when vines were inoculated with the nematode 13 days before the fungus compared with inoculation with both the nematode and the fungus on the same day. It was concluded that both the *M. incognita* population and some *R. solani* isolates were virulent against both Colombard and Ramsey, and that measures to prevent spread in nursery stock were therefore important.

Key words: disease complex, fungus, grapevine, interaction, *Meloidogyne hapla, Meloidogyne incognita, Meloidogyne javanica,* nematode, *Rhizoctonia solani,* root-knot nematode, root rot, *Vitis champinii, Vitis vinifera.*

Root-knot nematodes mainly *Meloidogyne javanica, M. incognita, M. hapla,* and *M. arenaria,* are the most widespread and economically important plant-parasitic nematodes on grapevines (*Vitis vinifera* L.) in South Australia (Stirling and Cirami, 1984). The most widely used control method in infested soils is to replant with nematode-resistant rootstocks (Hardie and Cirami, 1988). *Vitis champinii* Planchon cv. Ramsey is regarded as being highly resistant (Hardie and Cirami, 1988; Stirling and Cirami, 1984) and has been widely used as a rootstock on coarse soils of low fertility (Stirling and Cirami, 1984).

During an investigation of growth problems in a grapevine field nursery associated with the root-rotting fungus *Rhizoctonia solani* Kühn (Walker, 1992), *M. incognita* was detected on roots of various rootstocks including some classed as highly resistant. The objectives of this study were to characterize the virulence of this *M. incognita* population, in comparison with local populations of *M. hapla* and *M. javanica,* and of isolates of *R. solani* to grapevine rootings. Two cultivars were used: Ramsey and the highly susceptible Colombard (*V. vinifera*). Preliminary tests on field-grown rootings suggested that galled roots infected with *M. incognita* were more frequently infected with *R. solani* and more severely rotted than ungalled roots. Since interactions between *M. incognita* and *R. solani* are known in other plant hosts (Batten and Powell, 1970; Golden and Van Gundy, 1975), they were tested on grapevines singly and in combination.

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Materials and Methods

General procedures—Grapevines: Grapevine rootings (*V. vinifera* cv. Colombard and *V. champinii* cv. Ramsey) were propagated by taking dormant cuttings in winter and strik-

ing them in a heated sand bed. In early December rootlings were transplanted to 10-cm pots containing Barmera sand (95% sand, <2% organic matter) that had been fumigated with methyl bromide. Four-month-old rootings with uniform growth were selected for use in experiments. Pots were watered as required every 1 to 2 days and fertilized weekly with a complete soluble fertilizer. They were arranged on benches in a completely randomized split-plot design with grapevine cultivars as main plots and treatments as subplots. Data were subjected to analysis of variance ($P < 0.05$) and were first transformed using natural logarithms before analysis where departures from normality, homogeneity, or nonadditivity were found to occur. The number of plants in each root-gall or root-rot index category was compared by contingency table analysis ($P < 0.05$).

Nematodes and R. solani: The population of *M. incognita* and isolates of *R. solani* were obtained from roots of grapevines from a Riverland, South Australia, field nursery. The *M. incognita* population was identified as race 1 (Taylor and Sasser, 1978), and the local populations of *M. javanica* and *M. hapla* were identified by perineal patterns. These nematodes were increased on tomato (*Lycopersicon esculentum* Mill. cv. Grosse Lisse), and eggs were extracted from tomato roots by agitating in 1% NaOCl (Hussey and Barker, 1973). Vines were inoculated by adding egg suspensions to four equidistant holes made in potting soil near the base of each vine. Nematode-free filtrates of suspensions were added similarly to uninoculated pots.

Isolates of *R. solani* cultured from rotting roots of grapevines were maintained on corn-meal agar slants under mineral oil. Cultures were re-started on potato-dextrose agar (PDA), and discs from the growing margins were used to inoculate moistened autoclaved Japanese millet (*Echinochloa utilis* Ohwi & Yabuno) seed. Colonized seeds from cultures incubated for 7 weeks at 25 °C in the dark were used as inoculum in pot experiments. Four equidistant holes 1 cm in diameter were made 2 to 3 cm deep in soil at the perimeter of each pot using a soil tube, and 0.7 to 0.9 g of inoculum was added and covered with soil. Uninoculated pots were treated similarly using uninoculated autoclaved millet seed. *R. solani* isolates were assigned to putative anastomosis groups (AG) by S. Neate (CSIRO Division of Soils, Adelaide) by comparing extracellular pectic enzyme patterns on polyacrylamide gel electrophoresis (Sweetingham et al., 1986) with those from isolates of known anastomosis groups.

Shadehouse experiments—Experiment 1: The resistance of the two grapevine cultivars to the three *Meloidogyne* spp. was evaluated in an unheated shadehouse at Loxton Research Centre. Two successive inoculations were used: 500 eggs per pot in December (1 week after transplanting) and 5,000 eggs per pot (3 weeks later). Plants were harvested 162 days after initial inoculation. Monthly mean maximum-minimum daily air temperatures recorded at Loxton Research Centre over this period were—December (27.6–14.2 °C), January (31.2–14.4 °C), February (30.8–14.3 °C), March (28.1–12.9 °C), and April (25.8–7.3 °C). Oven-dry shoot weights and fresh root weights were measured and root-gall index was rated on a scale of 0–5 (0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, and 5 = 100% of roots galled) for each of the 15 replicate plants for each nematode isolate-grapevine cultivar combination plus uninoculated controls. To measure nematode reproduction, a subsample of 3.33 g fresh weight of roots was combined from each of three plants giving five replications per treatment group. Nematodes were extracted from the resulting 10-g samples by incubating them for 72 hours at 23 °C in plastic bags with 10 ml of 3% H_2O_2 (Tarjan, 1972).

Experiment 2: The resistance of the two grapevine cultivars to six *R. solani* isolates [four of which were assigned to AG 4, one to AG 2-1, and the last one classified as an unknown AG (AG ?)] was evaluated in an unheated shadehouse. In early January, 4 weeks after transplanting, pots were inoculated with either infested millet seed or, in the case of control pots, with uninfested

seed. A second uninoculated group was treated also with five soil drenches of pentachloronitrobenzene (41.2 mg PCNB per pot suspended in 100 ml water) at monthly intervals, starting 1 day after addition of millet, to inhibit growth of *R. solani* should accidental contamination have occurred. Plants were harvested 135 days after inoculation; mean maximum and minimum daily temperatures during this period were 27.1 and 6.9 °C, respectively. Oven-dry shoot weights and fresh root weights were measured and root-rot index was rated on a 0–5 scale analogous to that used for the root-gall index. Root colonization by *R. solani* was measured by plating two 1-cm lengths of root per plant on half-strength PDA amended with 200 mg streptomycin sulphate per liter and by incubating at 25 °C for 5 days. Fifteen replicate plants per treatment group were used.

Glasshouse experiment: The interaction between *M. incognita* race 1 and *R. solani* AG 4 isolate 3 was investigated in the two grapevine cultivars in a heated glasshouse maintained at 25 ± 7 °C. Pots were inoculated with either the nematode alone (5,000 eggs per pot), the fungus alone, or both together on the same day 6 weeks after transplanting. Another group inoculated with the fungus 6 weeks after transplanting was, 13 days earlier, inoculated with the nematode (5,000 eggs per pot). Uninfested millet and nematode-free filtrate were added to uninoculated pots 6 weeks after transplanting. Plants were harvested 92 days after inoculation with the fungus. Oven-dry shoot weights and fresh root weights were measured, and root-gall and root-rot indices were rated on 0–5 scales for each of the 15 replicate plants per treatment group. To measure nematode reproduction, a sub-sample of 2 g fresh weight of roots was combined from each of five plants, giving three replications per treatment group. Nematodes were extracted from the resulting 10-g samples by incubation in 3% H_2O_2 (Tarjan, 1972). Root colonization by *R. solani* was estimated by plating on half-strength PDA as in the shadehouse experiment.

Results

Shadehouse experiments—Experiment 1: The two grapevine cultivars differed in both root and shoot weights and in their reactions to

Table 1. Effects of *Meloidogyne* spp. on root galling, nematode reproduction, shoot and root weights of two grapevine cultivars 162 days after initial inoculation in the shadehouse.

Parameter	Root-gall index[a]	Juveniles per g roots[b]	Reproduction ratio (Pf/Pi)	Shoot dry weight (g)	Root fresh weight (g)
		Cultivar			
Colombard	2.0 a	302 (4.6)	7.3	2.1	18.3
Ramsey	1.1 b	198 (2.5)	2.2	2.9	8.1
LSD ($P = 0.05$)	—	(0.7)	—	0.2	1.8
		Treatment			
Uninoculated control	—	—	—	2.9	14.3
M. hapla	0.8 b	9 (1.5)	0.3	2.5	12.6
M. javanica	1.0 b	73 (2.9)	2.4	2.8	12.3
M. incognita	2.8 a	667 (6.2)	16.4	1.9	13.5
LSD ($P = 0.05$)	—	(0.8)	—	0.3	1.3
		Cultivar × treatment			
P	—	0.0001	—	NS	NS

Values for root and shoot weights, and for root-gall index, are means of 60 replications for cultivars and 30 replications for treatments. Values for nematode counts are means of 20 replications for cultivars and 10 replications for treatments. Analyses of variance conducted with cultivars as main plots and treatments as sub-plots.

[a] Galling rated on a scale of 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, and 5 = 100% of roots galled. The number of plants in each gall index category was compared with contingency table analysis. Means within a parameter-group followed by the same letter are not significantly different ($P < 0.05$).

[b] Numbers of *Meloidogyne* second-stage juveniles per gram fresh-weight of roots at harvest were transformed as $\log_e (n + 1)$ before analysis (in parentheses).

Meloidogyne spp. (Table 1). Colombard roots were more severely galled and nematode reproduction (number of juveniles) per g of root was higher ($P = 0.05$) in this highly susceptible cultivar. Root-gall index was higher ($P = 0.05$) in vines inoculated with *M. incognita* than with either *M. javanica* or *M. hapla* (Table 1). Reproduction (number of juveniles per g of root) of *M. incognita* also was higher than the other two species, and reproduction of *M. hapla* was lower than either *M. incognita* or *M. javanica* ($P = 0.05$). Root weight of vines inoculated with *M. hapla* or *M. javanica* was lower than that of uninoculated vines ($P = 0.05$), but root weight of vines inoculated with *M. incognita* was not significantly different from either uninoculated vines or those inoculated with other *Meloidogyne* spp. Shoot growth was suppressed by *M. incognita* and by *M. hapla* but not by *M. javanica* ($P = 0.05$) (Table 1). Shoot weight was lower in vines inoculated with *M. incognita* than in vines inoculated with the other *Meloidogyne* spp. ($P = 0.05$).

For each *Meloidogyne* spp., root-gall index was always higher in Colombard than in Ramsey ($P = 0.05$) (Table 2). Reproduction ratios indicated that Colombard was susceptible to *M. incognita* and *M. javanica*, whereas Ramsey was susceptible to *M. incognita* only (Table 2). Numbers of *M. incognita* juveniles per g of root did not differ between the two cultivars ($P = 0.05$) (Table 2).

Experiment 2: The two grapevine cultivars differed in both root and shoot weights and in their reactions to *R. solani* isolates (Table 3): root-rot index was higher in Ramsey than in Colombard ($P = 0.05$). Root-rot index was consistently higher in inoculated vines than in uninoculated vines, and *R. solani* was isolated only from the former ($P = 0.05$). All isolates except AG 4 isolates 1 and 4 limited shoot growth below that of the uninoculated control treatment ($P = 0.05$) (Table 3). All isolates except AG 4 isolate 1 restricted root growth below that of uninoculated, PCNB-treated vines; AG 4 isolate 2 and the unidentified isolate also restricted root growth below that of uninoculated, untreated vines ($P = 0.05$) (Table 3). Shoot growth but not root biomass of uninoculated vines were suppressed by treatment with PCNB ($P = 0.05$) (Table 3).

For a particular *R. solani* isolate, the root-rot index was consistently higher ($P = 0.05$) in Ramsey than in Colombard (Table 4). For AG 4 isolates 1, 3, and 4 the frequency of isolation of *R. solani* was higher in Ramsey than in Colombard ($P = 0.05$) (Table 4). *R. solani* isolates differed in the extent to which they colonized root systems: AG 2-1 was isolated from 83% and 100% of root lengths

Table 2. Effects of *Meloidogyne* spp. on root-gall index and nematode reproduction in two grapevine cultivars 162 days after initial inoculation in the shadehouse.

Cultivar-treatment	Root-gall index[a]	Juveniles per g roots[b]	Reproduction ratio (Pf/Pi)
		Colombard	
M. hapla	1.1 c	16 (2.5)	0.5
M. javanica	1.5 c	145 (4.9)	4.8
M. incognita	3.4 a	744 (6.2)	24.3
		Ramsey	
M. hapla	0.5 d	1 (0.6)	0.01
M. javanica	0.5 d	2 (0.9)	0.02
M. incognita	2.3 b	590 (6.2)	9.8
LSD1 ($P = 0.05$)	—	(0.3)	—
LSD2 ($P = 0.05$)	—	(1.6)	—

Values for root-gall index are means of 15 replications. Values for nematode counts are means of five replications.

[a] Galling rated on a scale of 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, and 5 = 100% of roots galled. The number of plants in each gall index category was compared with contingency table analysis. Means followed by the same letter are not significantly different ($P < 0.05$).

[b] Analysis of variance conducted with cultivars as main plots and treatments as sub-plots. LSD1 is for comparing treatments within a cultivar, and LSD2 is for comparing treatments in different cultivars. Nematode counts were transformed with $\log_e (n + 1)$ (in parentheses) before analysis.

TABLE 3. Effects of inoculation with six isolates of *Rhizoctonia solani* belonging to different anastomosis groups (AG) on shoot and root weights of two grapevine cultivars, and on frequency of isolation of *R. solani* from roots, and root rotting, 135 days after inoculation in the shadehouse.

Parameter	Shoot dry weight (g)	Root fresh weight (g)[a]	Frequency of isolation (%)[b]	Root-rot index[c]
		Cultivar		
Colombard	2.9	3.0	59	0.9 b
Ramsey	3.5	2.2	78	1.6 a
LSD ($P = 0.05$)	0.2	0.1	11	—
		Treatment		
Uninoculated control	3.61	2.69	—	0.3 b
Uninoculated + PCNB	2.72	2.71	—	0.3 b
R. solani—inoculated				
AG 4				
Isolate 1	3.59	2.74	45	1.4 a
Isolate 2	3.07	2.56	97	1.6 a
Isolate 3	3.07	2.59	25	1.4 a
Isolate 4	3.33	2.58	63	1.5 a
AG 2-1	2.90	2.58	92	1.8 a
AG ?	3.16	2.56	93	1.5 a
LSD ($P = 0.05$)	0.37	0.11	13	—
		Cultivar × treatment		
P	NS	NS	0.01	—

Values for cultivars are means of 120 replications, and values for treatments are means of 30 replications. Analyses of variance were conducted with cultivars as main plots and treatments as sub-plots.

[a] Root fresh weights (g) were transformed with $\log_e$ (weight) before analysis.

[b] Frequency of isolation (%) of *R. solani* from two root lengths per grapevine plated on PDA.

[c] Rotting rated on a scale of 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, 5 = 100% of roots rotted. The number of plants in each root-rot index category was compared using contingency table analysis. Means within a parameter-group followed by the same letter are not significantly different ($P < 0.05$).

tested from Colombard and Ramsey, respectively, and the unidentified isolate from 93% for both cultivars, whereas AG 4 isolates were isolated at frequencies varying from 3% to 97%, and 47% to 97%, respectively (Table 4).

Glasshouse experiment: As also was observed in the shadehouse experiments, shoot weight of Ramsey was higher than Colombard, whereas root weight was higher in Colombard ($P = 0.05$) (Table 5). Root-gall index was higher in Colombard than in Ramsey ($P = 0.05$). Shoot growth was limited by all treatments compared with the uninoculated control except for inoculation with *R. solani* alone ($P = 0.05$). Shoot weight of vines inoculated with *M. incognita* 13 days before inoculation with *R. solani* was lower than for all other treatments ($P = 0.05$). Root growth was restricted by all treatments compared with the uninoculated control and was lower in the case of vines inoculated with *M. incognita* 13 days before inoculation with *R. solani* than all other treatments ($P = 0.05$). Root-rot index was higher in inoculated than uninoculated vines ($P = 0.05$) (Table 5) and was higher in vines inoculated with *M. incognita* 13 days before inoculation with *R. solani* than all other treatment groups including vines inoculated with the nematode and fungus on the same day. Root-rot index of vines inoculated with *R. solani* alone was low and was only 36% of that observed with this isolate in the shadehouse (Tables 3,5). Overall frequency of isolation of *R. solani* from inoculated vines was low in the glasshouse experiment (Table 5) compared with the shadehouse experiment (Table 3, AG 4 isolate 3) although the cultivar × treatment frequencies (Table 4) indicated that this was in fact true only in the case of Ramsey. Conversely, the fast-growing fungi *Rhizopus* and *Trichoderma* spp. were commonly isolated in the glasshouse experiment but were much less commonly encountered in the shadehouse experiment. Root-gall index and nematode reproduction were similar in all treatment groups inoculated with *M. incog-*

TABLE 4. Effects of inoculation with six isolates of *Rhizoctonia solani* belonging to different anastomosis groups (AG) on frequency of isolation of *R. solani* from roots and root-rot index of two grapevine cultivars 135 days after inoculation in the shadehouse.

Cultivar-treatment	*R. solani* Frequency of isolation (%)[a]	Root-rot index[b]
	Colombard	
Uninoculated control	—	0.1 d
Uninoculated + PCNB	—	0.2 cd
R. solani—inoculated		
AG 4		
Isolate 1	33	1.0 b
Isolate 2	97	1.2 b
Isolate 3	3	1.0 b
Isolate 4	47	1.0 b
AG 2-1	83	1.3 b
AG ?	93	1.1 b
	Ramsey	
Uninoculated control	—	0.5 c
Uninoculated + PCNB	—	0.5 c
R. solani—inoculated		
AG 4		
Isolate 1	57	1.8 a
Isolate 2	97	2.0 a
Isolate 3	47	1.8 a
Isolate 4	80	2.1 a
AG 2-1	100	2.4 a
AG ?	93	1.8 a
LSD1 ($P = 0.05$)	19	—
LSD2 ($P = 0.05$)	20	—

Values are means of 15 replications.

[a] Frequency of isolation of *R. solani* from two root lengths per grapevine plated on PDA. Analysis of variance conducted with cultivars as main plots and treatments as sub-plots. LSD1 is for comparing treatments within a cultivar, and LSD2 is for comparing treatments in different cultivars.

[b] Rotting rated on a scale of 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, 5 = 100% of roots rotted. The number of plants in each root rot index category was compared using contingency table analysis. Means followed by the same letter are not significantly different ($P < 0.05$).

nita (Table 5). Reproduction ratios for *M. incognita* were 41.3 and 18.4 for Colombard and Ramsey, respectively.

Inoculation with *R. solani* alone did not suppress shoot growth in either cultivar (Table 6), and inoculation with *M. incognita* alone reduced shoot weight only in the case of Colombard ($P = 0.05$). Shoot weight was lowest when vines were inoculated with *M. incognita* 13 days before inoculation with *R. solani* and was lower than when the nematode and fungus were inoculated on the same day in both cultivars ($P = 0.05$). Shoot weight of vines inoculated with both the nematode and fungus on the same day was not different from that of vines inoculated with the nematode alone in either cultivar ($P = 0.05$). However, shoot weight was lower in vines inoculated with *M. incognita* 13 days before inoculation with *R. solani* than for any other treatment in the case of Colombard ($P = 0.05$).

Root biomass was restricted by all treatments compared with uninoculated vines in the case of Colombard ($P = 0.05$) (Table 6) and was lower in vines inoculated with *M. incognita* 13 days before inoculation with *R. solani* than with all other treatment groups. Root growth of Ramsey was suppressed only in vines inoculated with *M. incognita* 13 days before inoculation with *R. solani* ($P = 0.05$); root weights of other treatment groups were not different from either this group or uninoculated vines.

For a particular treatment, root-rot index did not differ between cultivars but was higher in vines inoculated with *M. incognita* 13 days before inoculation with *R. solani* than all other treatment groups ($P = 0.05$) (Table 6). Root-gall index was higher in Colombard than in Ramsey for a particular treatment ($P = 0.05$) (Table 6), but nematode reproduction was higher in Colombard only in vines inoculated with either *M. incognita* alone or the nematode and fungus on the same day. Nematode reproduction did not differ between cultivars when the nematode was inoculated 13 days before the fungus.

DISCUSSION

Ramsey was resistant to local populations of *M. javanica* and *M. hapla* but was susceptible to the population of *M. incognita* from the field nursery. Colombard was susceptible to both *M. incognita* and *M. javanica*, but galling was more severe and reproduction higher with *M. incognita*. Shoot growth was suppressed strongly by *M. incognita* and to a lesser extent by *M. hapla*, suggesting a lack of tolerance to the latter species. In South Africa *M. incognita* and *M. javanica* were found to be more virulent to grapevines than *M. hapla* (Loubser, 1988). In this

TABLE 5. Effects of inoculation with *Meloidogyne incognita* and *Rhizoctonia solani* AG 4 isolate 3 alone and together on the shoot and root weights of two grapevine cultivars and on root-rot and root-gall indices, frequency of isolation of *Rhizoctonia solani* from roots and root populations of *M. incognita* in the glasshouse at 25 °C.

Parameter[a]	Shoot dry weight (g)[b]	Root fresh weight (g)	Frequency of isolation (%)[c]	Root-rot index[d]	Root-gall index[d]	Juveniles /g root[b]
			Cultivar			
Colombard	1.3	24.3	1.7	1.2 a	3.2 a	850 (6.7)
Ramsey	1.7	22.4	3.9	1.2 a	1.8 b	411 (5.8)
LSD ($P = 0.05$)	0.1	1.8	NS	—	—	NS
			Treatment			
Uninoculated control	1.7	28.7	—	0.1 e	—	—
M. incognita (Mi)	1.4	22.7	—	1.1 c	2.5 a	570 (6.1)
R. solani (Rs)	1.7	24.7	5.8	0.5 d	—	—
Mi + Rs	1.4	22.2	2.5	1.6 b	2.4 a	669 (6.1)
Mi 13 days before Rs	1.2	18.3	0.0	2.8 a	2.7 a	652 (6.5)
LSD ($P = 0.05$)	0.1	3.3	3.6	—	—	NS
			Cultivar × treatment			
P	0.01	0.03	NS	—	—	0.01

Values for cultivars are means of 75 replications, and values for treatments are means of 30 replications except in the case of nematode counts, which are means of 15 and 6 replications for cultivars and treatments, respectively. Analyses of variance was conducted with cultivars as main plots and treatments as subplots.

[a] *M. incognita* (Mi) and *R. solani* (Rs) added to pots 92 days before harvest when inoculated either alone or together (Mi + Rs); Mi 13 days before Rs = *M. incognita* added to pots 105 days before harvest followed by *R. solani* 92 days before harvest.

[b] Transformed with $\log_e$ (shoot weight) or $\log_e$ (nematode count + 1) before analysis. Transformed nematode counts are given in parentheses.

[c] Frequency of isolation (%) of *R. solani* from two root lengths per plant plated PDA.

[d] Galling and rotting rated on a scale of 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, and 5 = 100% of roots galled or rotted, respectively. The number of plants in each gall or rot index category was compared using contingency table analysis. Column means within a parameter group followed by the same letter are not significantly different ($P < 0.05$).

study, however, both cultivars were intolerant of *M. incognita,* with shoot growth of both being reduced in the shadehouse experiment.

Stirling and Cirami (1984) found that Ramsey was highly resistant to a wide range of *Meloidogyne* populations collected from major viticultural regions of South Australia. A South African population of *M. incognita* did not reproduce on Ramsey in a pot experiment (Loubser, 1988), but limited reproduction was observed in the field (Loubser and Meyer, 1987). However, a pathotype of *M. incognita* able to severely gall and reproduce on resistant rootstocks has been reported from California (Cain et al., 1984).

Observations at the field nursery suggested that *M. incognita* was acting in concert with *R. solani* as part of a disease complex as has been reported in other crops (Batten and Powell, 1970; Golden and Van Gundy, 1975). Root rotting was more severe when both the nematode and fungus were present and was further exacerbated when nematode infection preceded inoculation with the fungus in this study. This effect occurred despite the use of an *R. solani* isolate, which was a comparatively weak colonizer of roots, and under environmental conditions (in the glasshouse) that did not favor expression of its pathogenicity. A similar effect has been observed in tobacco (Batten and Powell, 1970), and Powell (1968) first suggested that *Meloidogyne* spp. can interact with even minor, facultative parasitic fungi to cause severe root rot.

Rhizoctonia isolates assigned to putative anastomosis groups 2-1 and 4, and an unidentified isolate, colonized grapevine roots and caused root rotting. AG 4 isolates varied in the extent to which they colonized roots. Ramsey was more susceptible than Colombard to root rotting caused by *R. solani.* Shoot growth was suppressed by several AG 4 isolates and by the AG 2-1 isolate. Most previous records of AG 2-1 have been from the *Cruciferae* and AG 4 from the *Chenopodiaceae, Leguminosae,* and *Solanaceae* (Ogoshi, 1987). Grapevine root rotting caused by an AG 4 isolate and frequency of isolation of

TABLE 6. Effects of inoculation with *Meloidogyne incognita* and *Rhizoctonia solani* AG 4 isolate 3 together and alone on the root and shoot weights of two grapevine cultivars and on root-rot and root-gall indices and root populations of *M. incognita* at 25 °C in the glasshouse.

Cultivar-treatment[a]	Shoot dry weight (g)[b]	Root fresh weight (g)	Root-rot index[c]	Root-gall index[c]	Juveniles/g root[b]
		Colombard			
Uninoculated control	1.6	32.1	0.1 d	—	—
M. incognita (Mi)	1.2	24.3	1.1 bc	3.3 a	719 (6.6)
R. solani (Rs)	1.6	24.9	0.5 c	—	—
Mi + Rs	1.2	23.5	1.5 b	3.2 a	1,163 (7.0)
Mi 13 days before Rs	0.9	16.4	2.9 a	3.3 a	667 (6.5)
		Ramsey			
Uninoculated control	1.8	25.2	0.1 d	—	—
M. incognita (Mi)	1.7	21.1	1.2 b	1.7 b	421 (5.7)
R. solani (Rs)	1.9	24.4	0.6 c	—	—
Mi + Rs	1.7	20.9	1.6 b	1.7 b	174 (5.2)
Mi 13 days before Rs	1.5	20.2	2.6 a	2.1 b	637 (6.4)
LSD1 ($P = 0.05$)	0.2	4.6	—	—	(0.5)
LSD2 ($P = 0.05$)	0.2	4.5	—	—	(0.7)

Values are means of 15 replications except for nematode counts, which are means of three replications. Analyses of variance conducted with cultivars as main plots and treatments as sub-plots. LSD1 is for comparing treatments within a cultivar, and LSD2 is for comparing treatments in different cultivars.

[a] *M. incognita* (Mi) and *R. solani* (Rs) added to pots 92 days before harvest when inoculated either alone or together (Mi + Rs); Mi 13 days before Rs = *M. incognita* added to pots 105 days before harvest followed by *R. solani* 92 days before harvest.

[b] Transformed with $\log_e$ (shoot weight) or $\log_e$ (nematode count + 1) before analysis. Transformed nematode counts are given in parentheses.

[c] Galling and rotting rated on a scale of 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, and 5 = 100% of roots galled or rotted, respectively. The number of plants in each gall or rot index category was compared using contingency table analysis. Within-column means for root-rot and root-gall indices followed by the same letter are not significantly different ($P < 0.05$).

the pathogen from Ramsey roots were limited in a heated glasshouse compared with those in a shadehouse subject to generally lower ambient temperatures. Suppression of disease development caused by *R. solani* at higher temperatures has been reported in various crops including potato (Richards, 1921) and cereals (Garrett, 1970); however, limited soil moisture may also have been a factor in the glasshouse owing to increased rates of evaporation and transpiration. Also, vines were exposed to the pathogen for a longer period (135 days) in the shadehouse compared with the glasshouse (92 days), and conditions in the glasshouse evidently favored fast-growing saprophytic fungi (*Rhizopus* and the mycoparasitic fungus *Trichoderma*), which may have interfered with isolation of *R. solani*. This study confirms that *R. solani* is pathogenic to grapevines and that *V. champinii* is more susceptible than *V. vinifera* (Walker, 1992); the discovery of a race of *M. incognita* with the ability to attack and reproduce on rootstocks regarded as being highly resistant poses a significant threat to Australian viticulture. Special efforts will be required to reduce further spread of grapevine-attacking strains of *R. solani* and *M. incognita* in infested nursery stocks. Hygiene practices including fallowing and fumigation of soil and hot-water treatment of dormant rootings (Lear and Lider, 1959) are being used to restrict spread. In South Africa *R. solani* has been associated with declining vines on Ramsey rootstocks (Marais, 1979). This pathogen may therefore be important in established vineyards as well as field nurseries.

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