EFFECTS OF CHEMICALS AND CULTIVAR ON NEMATODES AND FUNGAL PATHOGENS OF CITRUS ROOTS

G.E. Walker

South Australian Research and Development Institute, Plant Research Centre, GPO Box 397, Adelaide, SA 5001, Australia

Summary. Citrus rootstocks sweet orange (SO) and carrizo citrange (CC) were grown in pots in two naturally infested Renmark citrus soils (A, infected with *Tylenchulus semipenetrans*, *Paratrichodorus lobatus, Xiphinema americanum* s.l., and *Pythium ultimum*; B, infected with all of these pathogens, except for *Xiphinema* sp., and with *Phytophthora citrophthora*). After planting, soil A was either drenched with water (as a control), solutions of furfural, or acetone-extracts of eugenol or camphor, or granules containing cadusafos were spread on the soil surface and then irrigated with water. After planting, soil B was drenched with water, with solutions of oryzalin, or with thiophanate/etridiazole +/– cadusafos granules, or a foliar spray was applied of phosphonate +/– cadusafos granules. The drenches containing thiophanate/etridiazole and oryzalin were re-applied after 12 weeks. After 24 weeks of growth, shoot and root growth were greater in CC compared with SO in both soils. Densities of *T. semipenetrans* females were lower on CC compared with SO roots in soil A, but not soil B, indicating that these populations differed in virulence. Soil densities of the other nematodes did not differ between cultivars. Isolation frequency of *Phytophthora citrophthora*, but not *Pythium ultimum*, was lower on CC roots, and root rotting was more severe in SO. Eugenol stimulated shoot growth, but furfural inhibited both shoot and root growth. In soil A, only cadusafos reduced root density of *T. semipenetrans* and soil density of *P. lobatus*, but all treatments reduced soil density of *Xiphinema* sp. Oryzalin, and thiophanate/etridiazole + cadusafos, stimulated root growth of both cultivars, and the latter treatment stimulated citrange shoot growth. Oryzalin, and either thiophanate/etridiazole or phosphonate + cadusafos, reduced the severity of root rot. However, phosphonate inhibited root growth of both cultivars and citrange shoot growth, indicating a sensitivity in young seedlings. In soil B, only cadusafos treatments reduced root density of *T. semipenetrans*. These treatments also reduced soil density of *P. lobatus* but oryzalin increased soil density of *P. lobatus*. Isolation frequency of *Pythium ultimum* from roots was higher from trees treated with thiophanate/etridiazole +/– cadusafos, and isolation frequency of *Phytophthora citrophthora* was higher from roots of trees treated with thiophanate/etridiazole + cadusafos. Eugenol shows promise as a growth stimulant for citrus but eugenol, camphor and furfural were much less effective as nematicides than cadusafos.

Key words: Cadusafos, eugenol, fungicides, oryzalin, *Phytophthora citrophthora*, *Tylenchulus semipenetrans.*

Tylenchulus semipenetrans Cobb occurs in all Australian citrus-producing areas, and on grapevines (Meagher, 1969). Riverland populations reproduce on olives (G. Walker, unpublished), indicating the presence of the citrus biotype (Verdejo-Lucas and McKenry, 2004). It is estimated to cause overall citrus production losses of 7% (Stirling *et al.,* 1992). Citrange rootstocks (*Citrus sinensis* L. Osb. × *Poncirus trifoliata* L. Raf.) have been widely used to replace the highly susceptible rootstocks rough lemon (*C. jambhiri* Lush.) and sweet orange (*C. sinensis*) and, although regarded as moderately resistant to *T. semipenetrans*, they perform less well in calcareous, saline soils. Root and collar rots of citrus caused by *Phytophthora citrophthora* (Sm. *et* Sm.) Leonian and *P. nicotianae* Dastur (Waterh.) have also caused damage in Australian production areas, and citrange rootstocks have been widely used as resistant rootstocks (Broadbent, 1977), but the importance of *Pythium* spp. remains unclear (Thompson *et al.,* 1995).

Use of rootstocks with some resistance to *T. semipenetrans* (and *Phytophthora* spp.) has reduced but not eliminated losses, and a range of control practices is needed, including use of chemicals (Verdejo-Lucas and McKenry, 2004). The nematicides aldicarb, fenamiphos and cadusafos, and phosphonate fungicides have been used in Australia for control of these pathogens on citrus. The herbicide oryzalin stimulated citrus seedling growth in soil infected with *Phytophthora nicotianae* and *Pythium ultimum* Trow (Walker and Morey, 1999). Furfural has shown potential as a nematicide (Rodriguez-Kabana *et al*., 1993). Eugenol (Obeng-Ofori and Reichmuth, 1997) and camphor (Obeng-Ofori *et al.*, 1998) have insecticidal activity, but have not been evaluated against nematodes. The broad-spectrum fungicide thiophanate/etridiazole (Banrot®) stimulated grapevine growth in pot experiments (Walker, 2001), but has not been evaluated against citrus pathogens in Australia.

Two greenhouse experiments using two citrus soils, naturally infested with citrus nematode (*Tylenchulus semipenetrans*), stubby-root nematode (*Paratrichodorus lobatus* [Colbran] Siddiqi), +/– dagger nematode (*Xiphinema americanum* s.l. Cobb), and with *Pythium ultimum* +/– *Phytophthora citrophthora*, were conducted to test the effects of these chemicals, in comparison with the registered nematicide cadusafos and fungicide phosphonate, on growth of sweet orange and carrizo citrange seedlings.

MATERIALS AND METHODS

Naturally infested soil was collected from under the canopies of trees in two citrus orchards located at Renmark West, South Australia (34.17 S 140.75 E): A – declining 40-year-old valencia orange trees (*C. sinensis* on rough lemon rootstock) on sandy loam soil, pH 7.0; B – declining twelve-year-old Imperial mandarin (*C. reticulata* Blanco) trees on sweet orange rootstock with root and collar rot, growing on light sandy clay loam soil, pH 7.4.

Each soil was mixed thoroughly before use. Nematodes were extracted from four 400 g samples of each soil over 5 days of incubation in extraction trays (Whitehead and Hemming, 1965), and five 100 g samples of each soil were baited with green pears to detect *Phytophthora* and *Pythium* spp. (McIntosh, 1964). Nematode population densities per 400 g of soils A and B respectively were: 5,489 and 12,394 *T. semipenetrans*; 42 and 14 *P. lobatus* and, in soil A only, 48 *Xiphinema* sp. *Pythium ultimum* was detected in both soils but *Phytophthora citrophthora* was detected only in soil B. Aliquots (1800 g) of both types of soil were dispensed into 150 mm diameter pots (ten replicate pots per treatment combination) and each pot was planted with one 11-month-old seedling of sweet orange (cv. Symons in soil A and cv. Parramatta in soil B) or carrizo citrange, which had been propagated from seed the previous winter. Sweet orange was chosen because it is highly susceptible to both *T. semipenetrans* and *P. citrophthora.* Treatments were applied (once only except where indicated) within 24 h of planting as follows:

Soil A (Experiment 1)

1. Control; each pot was drenched with 400 ml of water.
2. Eugenol (Sigma E5504); 180 µl of eugenol was dissolved in 10 ml of acetone, with 4 ml of Tergitol NP-10 non-ionic detergent (Sigma), and mixed with 3,986 ml of deionised water. Each pot was drenched with 400 ml of the emulsion containing 18 µl of eugenol.
3. Camphor (Sigma C9380); 1.8 g of camphor was dissolved in 10 ml of acetone, with 4 ml of Tergitol NP-10, and mixed with 3,986 ml of deionised water. Each pot was drenched with 400 ml of the emulsion containing 180 mg of camphor.
4. Furfural (90% furfuraldehyde by weight, Orica); each pot was drenched with either 0.2 ml or 2 ml of furfural mixed in 400 ml water (equivalent to 100 and 1000 l/ha respectively).
5. Rugby 100 G® (100 g cadusafos/kg, FMC); 0.28 g of granules (equivalent to 150 kg/ha) was sprinkled over the surface of each pot and incorporated with 400 ml of water.

Soil B (Experiment 2)

1. Control; each pot was drenched with 400 ml of water.
2. Surflan 500 Flowable® (500 g oryzalin/l; DowElanco); each pot was drenched with 400 ml of water containing 12.4 µl of Surflan 500 Flowable (equivalent to 6.8 l/ha). This treatment was re-applied after 12 weeks.
3. Foli-R-Fos 200® (200 g phosphonic acid/l, present as mono- and di-potassium salts; UIM); seedlings were sprayed to the point of leaf wetness with a solution of 10 ml product/l of water plus 0.1 ml Citowett® spreader (AgrEvo). All other seedlings were similarly sprayed with water.
4. Foli-R-Fos 200® foliar spray plus soil-applied Rugby 100 G® as described above.
5. Banrot 40% WP® (15% etridiazole and 25% thiophanate methyl, Scotts-Sierra Crop Protection; U.S. formulation); 100 ml of a suspension containing 0.9 g of Banrot 40% WP® in 1 l of water was applied to each pot and then drenched into the soil with 400 ml of water. This treatment was re-applied after 12 weeks.
6. Banrot 40% WP® plus soil-applied Rugby 100 G® as described above. The fungicide was re-applied after twelve weeks.

Seedlings were arranged in a randomised block (cultivar × treatment factorial) design for each soil type and were maintained in a greenhouse at 13-27 °C (mean 21 °C). The pots were drenched fortnightly with a complete fertiliser (Aquasol® containing N.P.K in the ratio of 23:4:18 and trace elements; applied at a rate of 1.6 g/l of water) and watered as required. After 24 weeks (except for plants treated with 1000 l furfural/ha, which were excluded because of phytotoxicity resulting in plant death), plants were harvested, and fresh and dry (72 h at 70 °C) shoot weights were recorded. Just before harvest, foliage was scored from 0-5 for severity of chlorosis (where 0 = 0%; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-99%, and 5 = 100% of yellowed leaves). Fresh root weight was also measured, and root systems were scored from 1 to 10 for severity of browning/decay (where 1 = 1-10%; 2 = 11-20%; 3 = 21-30%; ….. 10 = 91-100% of roots discoloured).

Roots were cut into 2 cm lengths and 5 g sub-samples were transferred to plastic bags for extraction of nematodes by incubation in 10 ml of 3% H_2O_2 at 22 °C for five days; roots were then vigorously washed over nested 850- and 25-µm-aperture sieves and nematodes collected in the 25-µm-aperture sieve were counted (McSorley *et al.,* 1984). The soil in each pot was weighed and nematodes were extracted from a 400 g sample of soil per pot as described above. Four browned root sections (1 cm lengths) per plant per agar medium were incubated on two selective agar media (Jeffers and Martin, 1986; Thompson *et al.,* 1995): for *Phytophthora* spp. (PARPH, containing 340 µg/ml ampicillin, 100 µg/ml hymexazol, 200 µg/ml PCNB, 20 µg/ml pimaricin, and 20 µg/ml rifampicin in corn meal agar) and for *Pythium* spp. (PARP, using the same ingredients as for PARPH except for hymexazol). Colonies of *Pythium* and *Phy-*

tophthora spp. were counted after two and five days' growth, respectively, at 26 °C in the dark.

Statistical analysis. Seedling growth, leaf chlorosis and root rot ratings, and nematode and fungal abundance data were subjected to analysis of variance (ANOVA) or contingency tests as appropriate ($P < 0.05$). Before statistical analysis, nematode counts were transformed as ln (count + 1) if plots of residuals or tests for non-additivity and normality indicated that this was required (Snedecor and Cochran, 1980). ANOVA was conducted using Statistix Version 7, Analytical Software, Tallahassee, Florida.

RESULTS

Symptoms of phytotoxicity followed by plant death were observed in plants treated with the high rate (1000 l/ha) of furfural on the day the treatment was applied, and these plants were excluded from the experiment. Soil densities of nematodes in these pots 4 weeks after treatment were: 2,614 and 1,295 *T. semipenetrans* in sweet orange and carrizo citrange respectively, 4 and 3 *P. lobatus* respectively, and 2 *X. americanum* s.l. in both cultivars, per 400 g of soil. Apart from stunted growth in the plants treated with 100 l/ha of furfural (Table I), no other symptoms of phytotoxicity were observed, and leaf chlorosis ratings did not differ significantly between cultivars or treatments in either experiment, but were higher in soil B (data not shown).

In both soils, 'stubby' root tips were observed in some plants. Browning and decortication of feeder roots, blackening of the stele and, in some plants, rotting off of the taproot were observed in soil B, especially in untreated sweet orange plants. Symptoms of rotting were less severe in citrange plants, and rotting off of the taproot was not observed.

Shoot and root growth were significantly greater in carrizo citrange compared with sweet orange in both soils (Tables I and II). In soil A, but not soil B, density of *T. semipenetrans* on roots was significantly greater on sweet orange compared with carrizo citrange (Table I). In soil B, the root rot rating and the frequency of isolation of *Phytophthora citrophthora* were significantly higher on sweet orange compared with carrizo citrange roots (Table II).

In soil A, only eugenol stimulated citrus shoot growth (Table I) and in soil B, thiophanate/etridiazole + cadusafos stimulated citrange shoot growth (Table II). In soil B, oryzalin, and thiophanate/etridiazole + cadusafos stimulated citrus root growth (Table II). However, phosphonate foliar spray inhibited citrus root growth and citrange shoot growth (Table II).

Cadusafos reduced densities of *T. semipenetrans* on citrus roots (Tables I and II), and in soil (data not shown). Cadusafos reduced densities of *P. lobatus* in both soils, but eugenol, camphor and furfural, as well as cadusafos, reduced densities of *X. americanum* s.l. (Tables I and II). *Paratrichodorus lobatus* density was also reduced by thiophanate/etridiazole, but was increased after oryzalin (Table II). *Tylenchulus semipenetrans* density was higher on citrange roots after soil treatment with thiophanate/etridiazole (Table II).

Oryzalin, thiophanate/etridiazole + cadusafos, and phosphonate + cadusafos reduced the root rot rating in soil B (Table II). However, thiophanate/etridiazole +/– cadusafos increased the frequency of isolation of *Pythium ultimum*, and thiophanate/etridiazole + cadusafos increased the frequency of isolation of *Phytophthora citrophthora* on citrus roots (Table II).

DISCUSSION

Initial densities of *T. semipenetrans* in both soils would be regarded as medium but sufficient to cause growth inhibition in highly susceptible rootstocks in pots (Deka *et al*., 2003); final densities of females on roots of untreated sweet orange (means of 51 and 90/g of roots respectively in soils A and B; range 5-299) were low to medium (University of California Statewide IPM Project, 1991). Final densities of *P. lobatus* exceeded those previously reported to reduce growth of citrus roots (Stirling, 1976) in soil A, and *X. americanum* s.l. also possibly contributed to damage. Carrizo citrange was not resistant to either *P. lobatus* or *X. americanum* s.l.

Carrizo citrange grew more vigorously in both soils than sweet orange, but densities of *T. semipenetrans* on roots were significantly lower on citrange roots only in soil A. These soils came from citrus orchards only one km apart, yet carrizo citrange varied in its reaction to these two populations of this nematode, indicating a difference in virulence. Population and/or biotype differences could explain the variation in reproductive potential between nematodes from these two soils.

In Spain, reproductive potentials varied greatly among different *T. semipenetrans* populations of one biotype, and reproduction of populations collected from citrange were higher than those collected from sour orange (Verdejo-Lucas *et al.,* 1997). Soil A came from an orchard planted to rough lemon rootstock but soil B, although collected only from sweet orange roots, came from an orchard inter-planted with sweet orange, citrange and trifoliate. Inter-planting moderately resistant with susceptible rootstocks can reduce their relative resistance level to *T. semipenetrans* (Verdejo-Lucas *et al*., 2003).

The increased *T. semipenetrans* density on citrange roots after soil treatment with thiophanate/etridiazole could be a consequence of a greater number of root sites for nematode multiplication, but this treatment appeared to suppress *P. lobatus*. Alternatively, the fungicide could have suppressed a fungal parasite or antagonist of *T. semipenetrans*. Oryzalin increased root growth, and this may have been responsible for the increased

Table I. Effect of chemical treatments on shoot and root growth, root rot rating, numbers of *Tylenchulus semipenetrans*, *Paratrichodorus lobatus* and *Xiphinema americanum* s.l., and on frequency of isolation of *Phytophthora citrophthora* and *Pythium ultimum* from roots of Symons sweet orange and carrizo citrange seedlings grown in Renmark citrus soil A[1] (Experiment 1).

Cultivar/soil treatment	Shoot dry weight (g)	Root fresh weight (g)	Root rot rating (0-10)*	Number of nematodes/dry weight roots or soil			Frequency of isolation from roots (%)	
				T. semipenetrans Adult female/g roots	*P. lobatus* /kg soil	*Xiphinema* /kg soil	*Phytophthora*	*Pythium*
Cultivar								
Sweet orange	9.9 b	13.1 b	0.8	55 (3.6) a	1137	11	0	2.5
Carrizo citrange	15.3 a	16.6 a	0.9	5 (1.3) b	904	28	0	6.2
LSD ($P = 0.05$)	1.2	1.7	—	(0.3)	—	—	—	—
Soil treatment								
Untreated	12.0 b	15.1 a	1.2	28 (2.6) a	1084 (5.9) a	60 (3.5) a	0	5.6
Eugenol	14.0 a	16.5 a	0.8	40 (2.9) a	964 (5.2) a	7 (0.7) c	0	5.0
Camphor	13.7 ab	16.6 a	0.8	42 (2.7) a	1153 (6.1) a	2 (0.4) c	0	1.2
Furfural	10.1 c	12.1 b	0.8	26 (2.5) a	881 (6.1) a	24 (2.3) b	0	3.7
Cadusafos	13.4 ab	14.0 ab	0.8	14 (1.8) b	0 (0) b	4 (0.5) c	0	6.2
LSD ($P = 0.05$)	1.9	2.7	—	(0.6)	(1.0)	(0.8)	—	—
CV (%)	33.5	32.6	54.2	166	144	204	—	258

* Treatments compared using contingency test ($P < 0.05$).

[1]For each parameter, within-column means for cultivars, or treatments, followed by the same letter are not significantly different ($P<0.05$); means in parentheses are for ln-transformed data; CV given for untransformed data.

Table II. Effect of chemical treatments on shoot and root growth, root rot rating, numbers of *Tylenchulus semipenetrans* and *Paratrichodorus lobatus*, and on frequency of isolation of *Phytophthora citrophthora* and *Pythium ultimum* from roots of Parramatta sweet orange (So) and carrizo citrange (Cc) seedlings in Renmark citrus soil B[1] (Experiment 2).

Cultivar/treatment	Shoot dry weight (g)*		Root fresh weight (g)	Root rot rating (0-10)**	Number of nematodes/dry weight roots or soil			Frequency of isolation from roots (%)	
					T. semipenetrans Adult female/g roots*		*P. lobatus* /kg soil	*Phytophthora*	*Pythium*
Cultivar									
Sweet orange	8.1 b		9.3 b	4.0 a	47		83	50 a	41
Carrizo citrange	11.1 a		10.9 a	3.3 b	52		78	10 b	39
LSD ($P = 0.05$)	1.1		1.3	—	—		—	10	—
Treatment									
	So	Cc			So	Cc			
Untreated	8.0 defg	10.1 bcd	8.6 c	4.5 a	90 (4.0) b	56 (3.7) bc	22 (2.1) b	29 bc	28 cd
Oryzalin	8.7 def	11.8 bc	13.0 a	2.9 b	77 (3.8) bc	86 (4.4) ab	211 (4.9) a	15 c	35 bcd
Phosphonate	6.3 fg	5.9 g	6.4 d	4.6 a	66 (3.9) bc	31 (3.1) c	8 (1.1) b	19 c	24 d
Phosphonate + cadusafos	6.8 efg	12.3 b	9.1 c	3.3 b	4 (1.2) de	4 (1.6) d	0 (0) c	24 bc	45 abc
Thiophanate/ Etridiazole	9.1 de	10.5 bcd	10.7 bc	3.8 a	46 (3.6) bc	134 (4.8) a	0 (0) c	39 ab	55 a
Thiophanate/ etridiazole + cadusafos	9.5 cde	15.9 a	12.7 ab	2.7 b	1 (0.1) f	2 (0.7) ef	0 (0) c	52 a	53 ab
LSD ($P = 0.05$)	2.6		2.1	—	(0.8)		(1.0)	17	19
CV (%)	41.6		42.4	41.1	129		306	124	77

*Cultivar × treatment interaction significant ($P<0.01$).

**Cultivars and treatments compared using contingency test ($P<0.05$).

[1]For each parameter, within-column means for cultivars, or treatments, followed by the same letter are not significantly different ($P<0.05$); means in parentheses are for ln-transformed data; CV given for untransformed data.

density of *P. lobatus* following this treatment. The cause for the increased frequency of isolation from roots (although without increased severity of root rotting), following treatment with thiophanate/etridiazole, of both *Pythium ultimum* and, in the case of the combined treatment with cadusafos, *Phytophthora citrophthora*, is not known. It could involve suppression of fungal antagonists/competitors; infection by *T. semipenetrans* reduces root infection by *Phytophthora nicotianae* (El-Borai *et al*., 2002), and cadusafos could perhaps have promoted infection by *Phytophthora citrophthora* except where phosphonate prevented this.

Root symptoms consistent with infection by root rot fungi were observed in soil B, especially in sweet orange plants, and a high incidence of both *Phytophthora citrophthora* and *Pythium ultimum* was demonstrated on roots. Carrizo citrange was more resistant than sweet orange to root infection by *Phytophthora citrophthora*, but not to *Pythium ultimum*, and rotting off of the taproot was observed only in sweet orange growing in soil infected with *Phytophthora citrophthora*. Taproot rotting was similarly associated with *Phytophthora nicotianae* in previous experiments (Walker and Morey, 1999), and these two species are generally associated with foot rot of citrus (Broadbent, 1977; Timmer, 1977). Root rotting was less severe in Carrizo citrange than sweet orange in soil infected with *Phytophthora citrophthora*, confirming the previously reported resistance of the former rootstock (Broadbent, 1977).

Furfural was phytotoxic at both 100 and 1000 l/ha rates, inhibiting growth at the lower rate and killing plants at the higher rate. Its efficacy against nematodes was also poor, even at the higher rate. Although densities of all three parasitic nematodes detected in soil treated with the higher rate may have been reduced, significant densities, especially of *T. semipenetrans*, remained. Cadusafos, already registered for nematode control in citrus, was the only effective chemical against *T. semipenetrans.*

Phosphonate foliar spray, although registered for the control of *Phytophthora citrophthora* in citrus, was ineffective for this purpose at the rate used, and inhibited the growth of citrus seedlings. Phytotoxicity caused by this chemical has previously been reported (Walker, 1989), and the youth of the plants may have contributed to their sensitivity. Nurseries may need to be cautious, or reduce rates, when applying this chemical to young citrus seedlings. In contrast, the herbicide oryzalin, previously reported to stimulate citrus growth in soil infected with *Phytophthora nicotianae* (Walker and Morey, 1999), and to prevent crown rot of potted cherry seedlings caused by *Phytophthora* spp. (Wilcox, 1996), reduced the level of root rot and stimulated root growth. Oryzalin is primarily used in citrus as a pre-emergent herbicide for grasses. Thiophanate/etridiazole did not stimulate citrus growth, except when it was combined with cadusafos, and it did not reduce the incidence of *Phytophthora citrophthora* or *Pythium ultimum* on roots.

Eugenol may have potential for use as a growth stimulant in citrus; its mode of action did not appear to depend mainly on its effects on nematodes as it reduced the density only of *X. americanum* s.l. Eugenol has both antifungal and antibacterial activity (Burt, 2004; Oxenham *et al*., 2005), but it did not affect the incidence of *Pythium ultimum* on roots.

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