Use of Trifoliate Orange as a Comparative Standard for Assessing the Resistance of Citrus Rootstocks to Citrus Nematode

R. K. Niles and **D. W. Freckman,** Department of Nematology, and **M. L. Roose,** Department of Botany and Plant Sciences, University of California, Riverside 92521

ABSTRACT

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Twenty-one citrus rootstocks were screened in a greenhouse for resistance to *Tylenchulus semipenetrans*. Parasitism by *T. semipenetrans* was confirmed by verifying the host suitabilities of rootstocks expected under California conditions to be resistant (C-35 citrange, Swingle citrumelo, and *Poncirus trifoliata* cv. Rubidoux) or susceptible (*Citrus sinensis* cv. Argentina and Carrizo citrange) to *T. semipenetrans*. *P. trifoliata* cv. Rubidoux was the resistant standard for comparing the host suitabilities of individual rootstocks. Five previously unscreened rootstocks expressed greater resistance than *P. trifoliata*: 1452 citrumelo, Siamese pummelo × *P. trifoliata*, and three selections from crosses of *C. sunki* and *P. trifoliata* (C-54, C-57, and C-146). Rank correlation showed the relative host suitabilities of individual rootstocks were consistent between two experiments.

Rootstocks contribute to citrus sustainability by providing disease resistance and long-term horticultural benefits for citrus production (12). Citrus breeding programs charged with rootstock improvement address the regional needs of producers and frequently aim to combine improved horticultural characteristics with resistance to the widely distributed citrus nematode, *Tylenchulus semipenetrans* Cobb (6,22,43).

Interactions between citrus and *T. semipenetrans* are accompanied by biotypic variation among nematode populations (3,29). Most populations of *T. semipenetrans* reproduce on citrus rootstocks and cause trees to decline in vigor and yield (16,17,23,47). On susceptible rootstocks, female second-stage juveniles migrate though the rhizosphere and feed ectoparasitically on the epidermal and hypodermal cells of fibrous roots. When they find a suitable site, they penetrate the root cortex, feed on parenchyma cells, induce the formation of nurse cells around a permanent feeding site, and mature to adults (13,32,50). On resistant rootstocks, establishment of the permanent feeding site is impeded by plant defenses such as the hypersensitive reaction and formation of wound periderm (32,34,50).

Because trifoliate orange, *Poncirus trifoliata* (L.) Raf., and some of its hybrids express a high level of resistance to many populations of *T. semipenetrans* (2,7,15,-38,40,42) coupled with commercial acceptability (33), hybrids with *P. trifoliata* parentage are promising candidates for resistance screening. Frequent recovery of resistance in the first generation hybrids of *P. trifoliata* (8) suggests resistance is a dominant character controlled by one or two genes (26). Propagation of such rootstocks is commonly from the clonal seedlings that develop from apomictic embryos (10).

To identify citrus rootstocks expressing resistance to *T. semipenetrans*, we defined the standard of resistance as the abundance of female nematodes on the fibrous roots of *P. trifoliata*, and we compared this standard of resistance with the host suitabilities of selected rootstocks. Our comparative method was like using Carrizo citrange as the standard of resistance when testing citrus rootstocks for susceptibility to Phytophthora root rot (26). We also investigated the effect of rootstocks on the second-stage juveniles (J2) of *T. semipenetrans* and the effect of *T. semipenetrans* on plant growth.

MATERIALS AND METHODS

Rootstock selections. In addition to our resistant standard, *P. trifoliata* cv. Rubidoux (RUBT-R), we included rootstocks expected under California conditions to be resistant (C-35 citrange [C35-R] and Swingle citrumelo [SWIN-R]) or susceptible (Argentina sweet orange [AGSW-S] and Carrizo citrange [CARR-S]) to *T. semipenetrans* (48). These regional standards were included to confirm parasitism by the nematode inoculum (33).

Rootstocks selected for screening possessed either improved horticultural characteristics or resistance to *Phytophthora* spp., or they shared parentage with rootstocks known to be resistant to *T. semipenetrans*. We screened one accession of uncertain *Citrus* parentage, two interspecific hybrids of *Citrus* spp., and 13 intergeneric hybrids of *Citrus* and *Poncirus* (Table 1).

Screening experiments. When the germinated seedlings of individual rootstocks were at least 8 cm tall, true-to-type plants of uniform size were transplanted separately into 1,925-cm^3 molded fiber pots (pot experiment) or 60-cm^3 plastic cones (Cone-Tainers, Stuewe and Sons, Inc., Corvallis, OR) (cone experiment) containing steam-sterilized potting medium (perlite, bark humus, plaster sand, and peat moss mixed in volumetric parts equaling 6:7:7:8, plus macro- and micronutrients). A total of 21 different rootstocks was screened in the study, but due to a limited number of seedlings, 20 rootstocks were screened in the pot experiment and 18 were screened in the cone experiment. All plants were grown in the greenhouse at temperatures near 30°C during the day and 26°C at night. At transplanting, timed-release fertilizer (17-6-10 N-P-K, plus different concentrations of micronutrients than those in the potting medium) was supplemented with water-soluble fertilizer (15-30-15 N-P-K). A second application of water-soluble fertilizer was made 5 weeks after transplanting. Plants were irrigated daily with deionized water.

Forty-five days after transplanting, 15 plants of each rootstock in the pot experiment received an initial infestation of *T. semipenetrans*. Nematodes were collected from a naturally infested 25-year-old citrus grove (block 10B, University of California Agricultural Experiment Station and Citrus Research Center, Riverside) that was planted to Troyer citrange rootstock, which is grown widely in California and is a standard for judging the performance of other rootstocks (20). Field-collected roots were washed and placed in a mist chamber for nematode extraction (1). Nematodes were collected daily for 5 days and processed by a modified Baermann tray method (25) to concentrate free-living stages for inoculum. On each of 3 days over a 17-day period, 5 ml of nematode suspension was placed by syringe in the rooting zones of individual plants, resulting in an inoculum level of approximately 26 nematodes per cm^3 of soil. The same

Current address of R. K. Niles and D. W. Freckman: Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523.

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procedure was used 55 days after transplanting to infest each of five plants for each rootstock in the cone experiment with an inoculum level of approximately 353 nematodes per cm^3 of soil.

In the pot experiment, five plants of each rootstock served as controls for comparing the growth of plants in the presence and absence of *T. semipenetrans*. Control plants received a root-wash suspension that was screened through a 25-µm-pore sieve until the nematode could not be detected in a 1-ml subsample. The pot experiment was arranged in randomized complete blocks, with five greenhouse benches serving as blocks. Plants in the cone experiment were completely randomized in a tray that held the cones.

Plants in the cone experiment were harvested 59 days after soil infestation, and plants in the pot experiment were harvested 91 days after final infestation. Roots were gently rinsed of soil and weighed. Fibrous roots (<2 mm diameter) were cut into 2-cm segments, and 2-g samples were preserved in 5% formalin (4). Preserved roots were washed on a 25-

Table 1. The useful characteristics and reported host suitability for *Tylenchulus semipenetrans* of citrus rootstocks screened for *T. semipenetrans* resistance

Species Code	Rootstock[a]	Characteristic(s) (references)	Host suitability (reference)
Rutaceous species			
AGSW-S	Argentina sweet orange (*Citrus sinensis* (L.) Osbeck), CRC 2802, USDA PI 539598	Sweet orange rootstocks are important historically because they were more commonly grown in the past (20,48)	Susceptible (38,48)
RUBT-R	Rubidoux trifoliate orange (*Poncirus trifoliata* (L.) Raf.), CRC 3888, USDA PI 539791	*Phytophthora* and tristeza tolerant (10,52); potential for close-spaced planting (5); cold tolerant in San Joaquin Valley, CA (20)	Resistant (35,42) or moderately resistant (27,36); recommended in California as tolerant (48)
Interspecific hybrid			
SRCA	Sauvage sour orange × Cleopatra mandarin (*C. aurantium* L. × *C. reshni* Hort. ex Tan.), Indio code 63-191-22	Dwarfing potential[b]	Unknown, but Cleopatra mandarin is susceptible to some populations (28,38) and resistant to others (32)
SRCB	Sauvage sour orange × Cleopatra mandarin, Indio code 63-191-79	Dwarfing potential[b]	Unknown, but see comments about SRCA's suitability
Uncertain parentage			
NICL	Nicaragua lemon, CRC 3841, USDA PI 539209	*Phytophthora* resistant[c]	Unknown
Intergeneric hybrid			
BENT	Benton citrange (*C. sinensis* × *P. trifoliata*), CRC 3908, USDA PI 539819	*Phytophthora* resistant and compatible with Eureka lemon (37)	Unknown
C35-R	C-35 citrange, CRC 3912, USDA PI 539821	Greater *Phytophthora* tolerance than other citranges (20); dwarfing potential (45)	Resistant (38); recommended in California as tolerant (48)
CARR-S	Carrizo citrange, CRC 2863, USDA PI 150916	*Phytophthora* and tristeza tolerant (10,52); *Radopholus similis* resistant (21)	Susceptible (24,28,38,39); moderately infected (36); resistant (42); expected in California to be less tolerant (48)
1452	1452 citrumelo (*P. trifoliata* × *C. paradisi* Macf.), CRC 1452, USDA PI 539822	Vigorous growth resulting in good yield and fruit size (30)	Unknown
SACA	Sacaton citrumelo, CRC 3337, USDA PI 539823	Female parent of hybrid used in mapping citrus genome (31)	Susceptible (27)
SWIN-R	Swingle citrumelo, CRC 3771, USDA PI 539828	*Phytophthora* and tristeza tolerant (10,52); potential for close-spaced planting (11)	Resistant (34,42); resistant to three of five populations (39); recommended in California as tolerant (48)
ASRT	African Shaddock × Rubidoux trifoliate orange (*C. grandis* (L.) Osbeck × *P. trifoliata*), Indio code 58-172-501	*Phytophthora* resistant[c]; satisfactory tristeza tolerance (44)	Possibly more resistant than Troyer citrange (44)
SPXT	Siamese pummelo × trifoliate orange, (*C. grandis* × *P. trifoliata*), 6A-25 11[d]	*Phytophthora* resistant[c]	Unknown
C22	Sunki mandarin × trifoliate orange C-22 (*C. sunki* Hort. ex Tan. × *P. trifoliata*), Indio code 61-227	*Phytophthora* resistant[c]; dwarfing potential (9)	Unknown
C54	Sunki mandarin × trifoliate orange C-54, Indio code 62-214	*Phytophthora* resistant[c]; dwarfing potential (9)	Unknown
C57	Sunki mandarin × trifoliate orange C-57, Indio code 62-217	*Phytophthora* resistant[c]; dwarfing potential (9)	Unknown
C146	Sunki mandarin × trifoliate orange C-146, Indio code 63-313	*Phytophthora* resistant[c]; dwarfing potential (9)	Unknown
X639	Cleopatra mandarin × trifoliate orange X639 (*C. reshni* × *P. trifoliata*), CRC 3957, USDA PI 539847	South African import with good yield and fruit quality (41)	Unknown, but see comments about SRCA's suitability
MNXT	Minneola tangelo × trifoliate orange ([*C. paradisi* × *C. reticulata* Blanco] × *P. trifoliata*), CRC 3954, USDA PI 539846	South African import with good yield and fruit quality (41)	Unknown
TWTA	Taiwanica sour orange × trifoliate orange A (*C. taiwanica* Tan. & Shim. × *P. trifoliata*), 6A-26 2[d]	*Phytophthora* resistant[c]	Unknown
TWTB	Taiwanica sour orange × trifoliate orange B, 6A-26 5[d]	*Phytophthora* resistant[c]	Unknown

[a] CRC code refers to accessions in the University of California, Riverside, Citrus Research Center Citrus Variety Collection. Other accessions are unreleased hybrids from the citrus breeding program at the University of California, Riverside, and a terminated USDA breeding program in Indio, California.
[b] M. L. Roose, *unpublished data.*
[c] M. L. Roose and J. A. Menge, *unpublished data.*
[d] Tree at the University of California Agricultural Experiment Station and Citrus Research Center, Riverside from which fruit was collected for seed.

µm-pore sieve, resuspended in 10% NaOCl, and macerated in a blender for 30 s. The macerated material was washed on a 149-µm-pore sieve nested on a 44-µm-pore sieve. The nematodes retained on the 44-µm-pore sieve were suspended in 50 ml of water and stained with acid fuchsin, acetic acid substituting for lactophenol (4).

Population abundances of *T. semipenetrans* were determined by enumerating the nematode life stages in two 1-ml subsamples per nematode sample in the pot experiment and three 1-ml subsamples per nematode sample in the cone experiment. Identification of life stages was based on reproductive maturity, body shape, and body size (14,49) as follows: J2 were vermiform in shape; immature females (third-stage and fourth-stage juveniles) were slightly swollen; and adult females were sausage- to lemon-shaped. Host suitability was expressed as total female abundance (immature females + mature females) per gram of fresh root. Plant growth was expressed as the total dry weight of roots plus shoots.

Data analysis. Replicate plants of individual rootstocks varied in host suitability for *T. semipenetrans*, as seen in the analysis of similar studies (7,32,39). For this reason, nematode abundance variances were homogenized through the logarithmic transformation (log[x + 1]) of data from both experiments. Central tendencies of the relationship between nematodes and rootstocks were expressed as the geometric, back-transformed (46) mean abundances of nematodes on roots. Geometric means were used to estimate the magnitude of the percent difference between the host suitabilities of California's resistant and susceptible standards, and between RUBT-R and individual rootstocks.

Analysis of variance in total females and J2 (with the interaction term, rootstock × bench, as the error mean square in the pot experiment) was followed, when rootstocks were a source of variation, by a preplanned contrast of the host suitabilities of resistant and susceptible standards. Host suitabilities of individual rootstocks were compared with that of RUBT-R by Dunnett's *t* tests (multiple comparison procedure that compares all treatments to a single control) (19).

Ranked abundances of total females were compared between experiments with Spearman's coefficient of rank correlation (r_s) (46), which was computed on the transformed data from rootstock selections screened in both experiments (n = 17). Total female abundances, as such, were compared between experiments with an unpaired *t* test, also computed on transformed data. Within experiments, transformed abundances of J2 and total females were subjected to product-moment correlation (*r*) (46). Growth of nematode-infested and noninfested plants was compared with *t* tests.

RESULTS

Host suitability of rootstocks. Females of *T. semipenetrans* varied in abundance on rootstocks in both experiments (Table 2). In the pot experiment, female abundance was also influenced by the location of the benches in the greenhouse (Table 2). As anticipated, contrast comparisons showed that fewer females developed on the rootstocks expected to be resistant than on those expected to be susceptible, with resistant rootstocks supporting 87 and 84% fewer females in the pot and cone experiments, respectively (Table 3).

In the pot experiment, rootstock C35-R (expected to be resistant) supported 91% fewer females than RUBT-R (Table 3). Five other rootstocks supported in pots 80 to 85% fewer females than RUBT-R: 1452 citrumelo (1452), three selections from

Table 2. Analysis of variance in abundances of *Tylenchulus semipenetrans* per gram of fresh root of citrus rootstocks growing in pots and cones in two greenhouse experiments[a]

Source	df	Total females MS	Total females $P > F$	Second-stage juveniles MS	Second-stage juveniles $P > F$
Pot experiment[b]					
Rootstock	19	3.7006	0.0001	0.9782	0.0001
Greenhouse bench	4	2.8573	0.0001	0.8443	0.0129
Rootstock × bench	76	0.2787		0.2481	
Error	199	0.2486		0.2054	
Cone experiment					
Rootstock	17	1.0796	0.0034	0.3365	0.3320
Error	70	0.4251		0.2939	

[a] Analyses were performed on log-transformed (log[x + 1]) nematode abundances.
[b] The interaction term, rootstock × bench, was used as the error mean square to compute *F*.

Table 3. The abundance of second-stage juvenile and female *Tylenchulus semipenetrans* per gram of fresh root of citrus rootstocks in two greenhouse experiments[a]

Rootstock[b]	Pot experiment: Total females	Pot experiment: Second-stage juveniles	Cone experiment: Total females	Cone experiment: Second-stage juveniles
California standard rootstocks				
R vs. S[c]	2.4 vs. 18.6***[d]	1.4 vs. 3.0 NS	12.4 vs. 75.4**	24.5 vs. 41.1[e]
Individual rootstock selections				
RUBT-R[f]	7.5	3.1	22.4	8.5
C35-R	0.7**	0.9	19.1	42.8
C57	1.1*	0.6	4.7	32.5
SPXT	1.2*	1.1	...[g]	...
C54	1.3*	0.8	8.5	33.3
C146	1.4*	1.1	11.3	51.3
1452	1.5*	0.3*	4.9	7.5
SWIN-R	1.7	0.7	4.1	34.1
TWTA	3.3	2.3	17.8	31.3
TWTB	4.7	2.5	...	...
NICL	...	...	24.7	23.5
MNXT	9.4	1.5	42.4	34.4
X639	12.2	4.1	83.5	93.0
ASRT	13.8	3.1	25.7	30.2
AGSW-S	14.1	2.5	43.4	38.9
SACA	18.0	3.8	...	...
C22	20.8	3.1	22.6	36.7
SRCA	24.1	8.4	73.1	33.8
CARR-S	24.5	3.5	130.5	43.3
BENT	29.7	3.4	136.7	30.4
SRCB	84.6	11.8	144.5	103.5
Dunnett's *t* value(s), lower-tailed test	$2.687_{76,.05}$ $3.306_{76,.01}$	$2.687_{76,.05}$	$2.664_{70,.05}$, NS	...[e]

[a] Analyses were performed on log-transformed (log[x + 1]) nematode abundances, and thus the back-transformed, geometric means are reported. The interaction term, rootstock × bench, was used as the error mean square in all tests.
[b] See Table 1 for the interpretation of rootstock codes.
[c] Preplanned contrast of nematode abundances on rootstocks expected under California conditions (48) to be resistant (R: C35-R, RUBT-R, and SWIN-R) or susceptible (S: AGSW-S and CARR-S) hosts of *T. semipenetrans*.
[d] *, **, and *** indicate a significant difference between the compared means at $P \le 0.05$, $P \le 0.01$, and $P \le 0.001$, respectively. NS indicates no significant difference.
[e] Test not performed because analysis of variance failed to detect a treatment effect.
[f] RUBT-R was the resistant standard for comparing the suitability of each rootstock selection for Dunnett's test.
[g] Insufficient plant material to include this rootstock in the experiment.

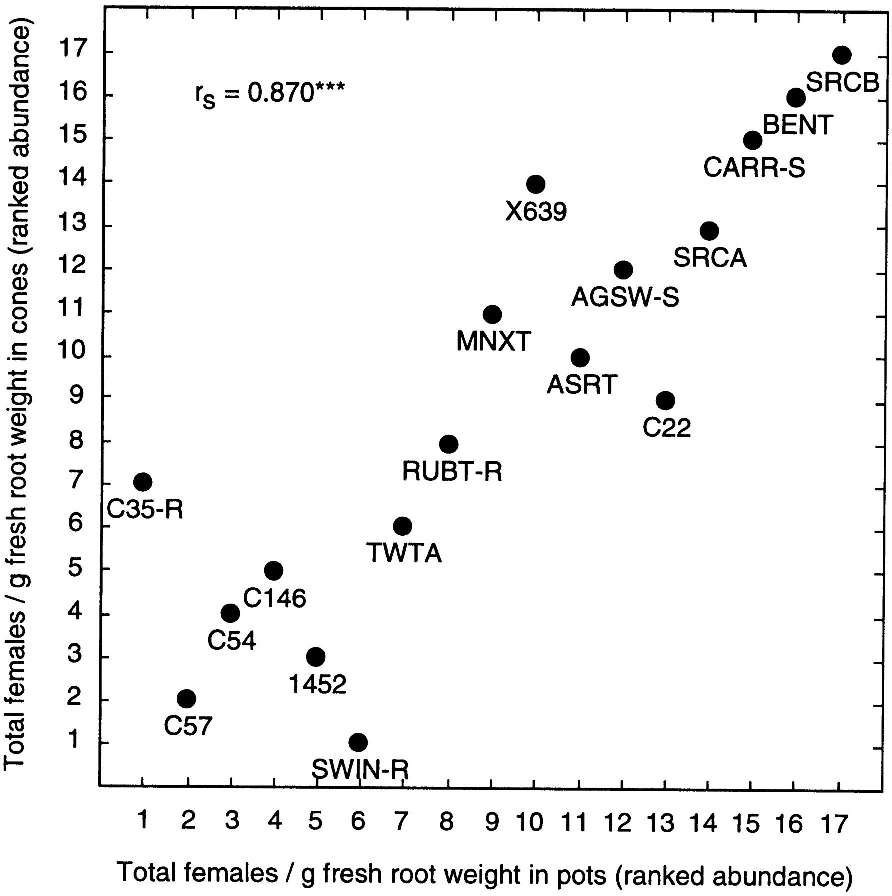

Fig. 1. Ranked abundance of the total females on rootstocks growing in pots and cones in two greenhouse experiments. (Ranks based on log[x + 1] transformation of total females per gram of fresh root weight. ***, Spearman's coefficient of rank correlation significant at $P < 0.0001$. See Table 1 for the interpretation of rootstock codes.)

Table 4. The effect of *Tylenchulus semipenetrans* on the growth of citrus rootstocks grown in pots in a greenhouse for 91 days after soil infestation

	Plant dry weight (mean g ± 1 SE)		Probability that dry weight of infested plants equals 100% of dry weight of control plants			
Rootstock[a]	Uninfested plants	Infested plants	% Control (mean)	df	*t*	*P*
Resistant[b]	42.0 ± 4.3	42.0 ± 2.3	100.1	44	0.03	0.980
Susceptible[b]	37.7 ± 4.3	40.0 ± 2.6	106.0	29	0.87	0.393
1452	62.8 ± 4.8	65.1 ± 4.1	103.8	14	0.57	0.575
AGSW-S	39.5 ± 7.1	48.1 ± 3.8	122.0	14	2.27	0.040
ASRT	35.6 ± 4.7	31.5 ± 1.8	88.6	14	−2.22	0.044
BENT	41.4 ± 4.4	36.1 ± 3.2	87.2	14	−1.63	0.125
C22	41.3 ± 6.5	32.9 ± 2.7	79.6	14	−3.12	0.008
C35-R	51.3 ± 4.8	47.7 ± 2.3	93.0	14	−1.55	0.143
C54	57.6 ± 5.5	61.1 ± 4.1	106.0	14	0.84	0.418
C57	46.5 ± 5.5	43.4 ± 3.9	93.3	14	−0.80	0.435
C146	45.4 ± 6.7	49.6 ± 3.1	112.3	14	1.59	0.135
CARR-S	36.0 ± 5.7	31.8 ± 2.0	88.5	14	−2.07	0.058
MNXT	72.7 ± 9.5	69.4 ± 5.9	95.5	14	−0.55	0.588
RUBT-R	27.2 ± 4.0	25.1 ± 1.5	92.4	14	−1.40	0.183
SACA	31.5 ± 11.7	25.3 ± 4.5	80.4	13	−1.36	0.198
SPXT	… [c]	40.4 ± 5.2	…	…	…	…
SRCA	41.4 ± 8.8	35.0 ± 2.2	84.5	14	−2.91	0.012
SRCB	24.8 ± 2.1	26.6 ± 1.9	107.2	14	0.94	0.365
SWIN-R	47.4 ± 8.6	53.3 ± 3.3	112.3	14	1.77	0.099
TWTA	31.2 ± 3.6	31.2 ± 1.6	100.0	13	−0.01	0.994
TWTB	26.8 ± 14.3	29.2 ± 4.9	108.8	13	0.48	0.640
X639	54.1 ± 5.0	50.1 ± 3.3	92.6	14	−1.21	0.248

[a] See Table 1 for the interpretation of rootstock codes.
[b] Rootstocks expected under California conditions (48) to be resistant (C35-R, SWIN-R, and RUBT-R) or susceptible (AGSW-S and CARR-S) hosts of *T. semipenetrans*.
[c] Insufficient plant material to include an uninfested control.

crosses of *C. sunki* and *P. trifoliata* (C-54, C-57, and C-146), and Siamese pummelo × *P. trifoliata* (SPXT) (Table 3). Rootstock SWIN-R (expected to be resistant) supported a number of females similar to that on RUBT-R (Table 3). The rootstocks Benton citrange (BENT) and Sauvage sour orange × Cleopatra mandarin (SRCB) supported more females than RUBT-R (the upper-tailed Dunnett's *t* test having the same specifications as the lower-tailed test [Table 3], $P \leq 0.05$).

More females of *T. semipenetrans* developed on plants in the cone experiment than on plants in the pot experiment (26.5 vs. 7.0 total females per g of fresh root in cones and pots, respectively; 385 df, $t = 6.108$, $P < 0.0001$). Ranked total female abundances on the rootstocks screened in both experiments were positively correlated (Fig. 1). However, in contrast with results from the pot experiment, none of the rootstocks in the cone experiment supported fewer females than RUBT-R (Table 3).

Second-stage juveniles. Analysis of variance showed J2 abundances in the pot experiment differed among rootstocks and among greenhouse benches (Table 2). In the pot experiment, equivalent J2 abundances were supported by rootstocks expected to be resistant and susceptible (Table 3). Among the individual rootstocks in the pot experiment, only rootstock 1452 supported fewer J2 than RUBT-R (Table 3), while SRCB supported more J2 (upper-tailed Dunnett's *t* test, $P \leq 0.05$). In both experiments, J2 abundances correlated positively with female abundances (pot experiment: $n = 299$, $r = 0.611$, $P < 0.0001$; cone experiment: $n = 88$, $r = 0.505$, $P < 0.0001$), but in the cone experiment, J2 abundances did not differ among rootstocks (Table 2).

Plant growth. The total dry weight of most rootstocks was not affected by nematode infection (Table 4). Three rootstocks grew less when the nematode was present (ASRT, C-22, and SRCA), and rootstock AGSW-S grew more.

DISCUSSION

We designed this study to support inferences about the resistance of selected citrus rootstocks to *T. semipenetrans*. Nematode inoculum was obtained from trees on Troyer citrange, a rootstock that is not tolerant to the nematode but is less susceptible than other commercial selections (48); thus, the inoculum likely possessed similar parasitic capability to that of many nematode populations in the state. Once we confirmed the host suitabilities of rootstocks expected under California conditions to be resistant or susceptible to *T. semipenetrans*, we tested the hypothesis that the host suitability of individual rootstocks would not differ from that of *P. trifoliata*. By conducting the lower-tailed Dunnett's test, we focused the analysis on identifying rootstocks that warranted further evaluation because their resistance to *T. semipenetrans* was greater than that of *P. trifoliata,* an original source of resistance (15).

In both pot and cone experiments, the rootstocks recommended in California as being resistant and susceptible to *T. semipenetrans* showed the expected host suitabilities, thereby demonstrating the parasitic capability of the inoculum. In addition, in the pot experiment, the comparative reactions between RUBT-R and the rootstocks expected to be resistant and susceptible agree with the results of McCarty et al. (38), who infested citrus rootstocks with an isolate of *T. semipenetrans* they reported as biotype 3 (*Poncirus* biotype [29]). McCarty et al. (38) showed that the recommended rootstocks, RUBT-R, C35-R, and SWIN-R, were resistant to the nematode, and RUBT-R possessed greater resistance than the susceptible standard, CARR-S. As found in our study, McCarty et al. (38) also reported that the number of females on RUBT-R was not different from the number on the second susceptible standard, AGSW-S. We conclude that the results from our pot experiment reliably indicate the suitabilities of individual rootstocks to the population of *T. semipenetrans* we tested.

In the pot experiment, hybrids of *P. trifoliata* represented diverse rootstock genotypes and expressed a range of host suitabilities for *T. semipenetrans,* similar to the range reported by Hutchison and O'Bannon (27). In pots, five hybrids of *P. trifoliata* supported fewer total females than *P. trifoliata* and thus expressed heightened resistance to the nematode. These five hybrids (previously unscreened) possess other characteristics that could benefit citrus production, and they deserve additional evaluation to verify their resistance and determine their commercial potential in California and elsewhere.

In assessing nematode resistance, the Dunnett's test controlled the experimentwise error rate at $\alpha = 0.05$ or higher, a more rigorous approach than computing the least significant difference and controlling only the comparisonwise error rate at a given α level. Nonetheless, in addition to being a source of nematode resistance, *P. trifoliata* (comparative standard in the Dunnett's test) is grown commercially in California, and it is not suited to calcareous or alkaline soils (48). This commercial limitation to using *P. trifoliata* in all plantings shows multiple criteria must be considered to balance a rootstock's nematode susceptibility with other characteristics. For that reason, we expect pairwise comparisons between rootstocks, in addition to those including *P. trifoliata*, will be important for the further evaluation of rootstocks screened in this study. Such comparisons are possible by transforming the geometric means in Table 3 ($10^x - 1$) back to the log-transformed means of the original data and then comparing the log-transformed means by least significant differences (pot experiment: LSD = 0.3596 and 0.3269 for total females and J2, respectively; cone experiment: LSD = 0.8338 for total females; $\alpha = 0.05$) to determine the relative host suitabilities of any two rootstocks (46).

Rank correlation showed the relative host suitabilities of individual rootstocks were consistent between the pot and cone experiments, concurring with our expectation for the reproducible expression of host suitability; however, in the pot experiment some rootstocks expressed greater resistance to *T. semipenetrans* than *P. trifoliata.* One striking difference between the pot and cone experiments was the greater abundance of *T. semipenetrans* on the plants in cones. Inoculum levels in both experiments corresponded to high infestations in the field (48), but plants in cones were infested with nearly 14 times more nematodes than plants in pots, and at the end of the experiments, an average of 3.8 times more females were present on the plants in cones. This result suggests a positive relationship between the initial J2 abundance in inoculum and the final abundance of total females on roots. Notwithstanding inoculum level, the preplanned contrast of resistant and susceptible rootstocks showed in the cone experiment, as in the pot experiment, less suitable rootstocks supported fewer females than more suitable rootstocks, supporting our conclusion that rootstock suitabilities were consistent between experiments.

Low female abundances, such as those occurring on resistant or less suitable rootstocks, imply that plant defenses previously constrained feeding site establishment by juveniles. As found in this study and others (27,42), the final abundances of J2 and females on roots are often related (32,34), but several factors determine the number of J2 on roots. Varying numbers of J2 migrate to different rootstocks, as influenced by the differential attractiveness of rootstocks to the nematode (32). Alternatively, numbers of J2 may reflect the relative extent of reproduction (33). For whichever reason, rootstocks supporting fewer J2, like rootstock 1452 in the pot experiment, are candidates for further screening (27,32).

Identifying new citrus rootstocks possessing resistance to *T. semipenetrans* represents a necessary, but labor-intensive, enterprise that aids the continued management of the nematode. Citrus production needs new rootstocks to replace formerly resistant rootstocks as they become susceptible (18) and to implement changes in production practice. The shift to high-density planting is made possible partially by rootstocks that combine dwarfing characteristics with nematode resistance, a combination possessed by three of the rootstocks that in this study expressed heightened levels of nematode resistance. That the nematode only rarely affected the growth of plants in these experiments agrees with the results of McCarty et al. (38) and suggests reduced plant growth may be a long-term effect of *T. semipenetrans* on susceptible rootstocks (51).

The logistical and time constraints involved in screening rootstocks have lead some investigators to suggest improved methods for conducting the studies (27,32,51). Our study required 1,130 work-hours to complete over an 11-month period, plus the time spent in planning and analysis. However, rootstocks in our cone experiment were exposed to nematodes for 2 months and rootstocks in our pot experiment for 3 months, lengths of exposure that represent noteworthy reductions in the 6 to 12 months typically invested in screening rootstocks for nematode resistance (27,33,38,42,51). We think further evaluation is needed before cones can be employed routinely in resistance screening, but cones allow more efficient use of bench space in the greenhouse than pots.

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