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GREENHOUSE TESTING OF ROOTSTOCKS FOR RESISTANCE TO *PHYTOPHTHORA* SPECIES IN FLATWOODS SOIL

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Abstract. **New rootstocks are needed to replace stunted or declining trees on sour orange and Swingle citrumelo in many east coast flatwoods sites. Important factors that make existing rootstocks unsuitable for use in these areas include susceptibility to *Phytophthora nicotianae* or *Phytophthora palmivora*, and intolerance of common flatwoods soils. A technique is described to rapidly test rootstocks for response to these conditions. Winder soil from a field site in Indian River County was collected and used as a potting medium in the greenhouse, with and without inoculation by roots infested with *P. nicotianae* and *P. palmivora*. Seed germination, seedling growth, and survival were evaluated for 13 rootstocks. When compared with a peat/perlite potting mix, non-infested Winder soil supported good germination, good seedling growth, and did not cause significant seedling death for any of the rootstocks, including sour orange, Volkamer, Cleopatra, Carrizo citrange, and Swingle citrumelo. In contrast, there were highly significant levels of seedling death in Winder soil infested with *Phytophthora* spp. Rootstocks with the highest levels of mortality in infested Winder soil were Swingle citrumelo, Carrizo citrange, and Flying Dragon, while rootstocks with the lowest levels of mortality were sour orange, Sun Chu Sha, and US-897. The responses of different rootstocks to this rapid greenhouse test were similar to the relative field performance of these rootstocks on Winder soil in the Indian River area. This greenhouse assay appears to be valuable for rapidly screening and evaluating new rootstocks for potential adaptation to soil and pathogen conditions prior to the establishment of long-term field trials in flatwoods sites.**

Ideal citrus rootstocks are not available for many citrus production areas with poorly-drained flatwoods types of soil, especially in the Indian River Region. Soils in these areas often vary widely, sometimes producing several quite different types of root environments in a single production block. These conditions were once considered ideal for grapefruit (*Citrus paradisi* Macf.) trees on sour orange (*C. aurantium* L.) rootstock, which grew well on the wide range of soils found in the Indian River Region. Unfortunately, the spread of strains of citrus tristeza virus (CTV) that cause a decline or severe stunting of trees on susceptible rootstocks has forced the Florida industry to abandon the use of sour orange as a rootstock.

In the place of sour orange, millions of trees have been planted on alternative rootstocks that are resistant to CTV-related decline and have other desirable horticultural characteristics. Some of these, including Swingle citrumelo [*C. paradisi* Macf. × *P. trifoliata* (L.) Raf.] and Carrizo citrange [*Citrus sinensis* (L.) Osbeck × *P. trifoliata*], have proven over time to be poorly adapted to many Indian River areas. Fine-loamy sands that are poorly drained, such as Winder and Manatee soils, have proven especially problematic for Swingle citrumelo and Carrizo citrange. Several alternative rootstocks are now being commercially planted (Stover and Castle, 2002) and others are in development by researchers at the USDA (Bowman, 2001) and the University of Florida (Grosser et al., 1998). However, experience with Swingle citrumelo has shown that poor rootstock adaptation may not be revealed under natural field conditions for 10-15 yr or even longer. Development and application of methods to quickly assess potential suitability of new rootstocks for these conditions are needed to identify the most promising candidates for long-term field trials. Rapid testing methods are already employed by the USDA rootstock program to screen for other important traits (Bowman et al., 2001; Bowman and Garnsey, 2001; Lapointe and Bowman, 2002) and are needed for traits related to soil adaptation.

Phytophthora species are important pathogens of citrus rootstocks in Florida (Graham and Menge, 2000). *Phytophthora nicotianae* Breda de Haan and *Phytophthora palmivora* (Butler) Butler have been identified as particularly important citrus rootstock problems in some Florida flatwoods areas. We hypothesize that epidemics of these pathogens on roots in fine-textured, poorly drained flatwoods soils play an important role in citrus rootstock failure. Therefore, the objectives were 1) to define a method to test plant material in the greenhouse for resistance/tolerance to the combination of a problem field soil (e.g. Winder), *P. nicotianae*, and *P. palmivora*; and 2) to use this method to test a group of standard and new rootstocks of interest for use in the Indian River Region. Based on good correlation of test results with field performance for the known rootstocks, the method will then be used as one component in the preliminary selection/testing of new USDA rootstocks for the flatwoods.

Materials and Methods

To prepare for greenhouse tests, Winder type field soil was collected from a depth of 5-23 cm below the soil surface at a site without citrus trees at the Kerr Center, Vero Beach, Florida (Indian River County). The soil was carefully graded by hand to remove any roots, other plant material, rocks, or insects. The Winder soil was determined to have a pH of 6.2 by the saturated media extract method. A potting mix composed of steam-sterilized peat/perlite/vermiculite (Pro-Mix BX: Premier Horticulture Inc., Red Hill, Pa.) was also used

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Table 1. Plant material tested.

Rootstock	Parentage
Swingle citrumelo	*Citrus paradisi* × *Poncirus trifoliata*
Flying Dragon	*P. trifoliata*
Carrizo citrange	*Citrus sinensis* × *P. trifoliata*
US-942	*Citrus reticulata* Blanco 'Sunki' × *P. trifoliata*
US-812	*C. reticulata* 'Sunki' × *P. trifoliata*
US-801	*C. reticulata* 'Changsha' × *P. trifoliata*
US-896	*C. reticulata* 'Cleopatra' × *P. trifoliata*
Cleopatra	*C. reticulata*
Standard sour	*Citrus aurantium*
Volkamer	*Citrus limon* (L.) Burm. f.
US-897	*C. reticulata* 'Cleopatra' × *P. trifoliata*
Daidai sour	*C. aurantium*
Sun Chu Sha	*C. reticulata*

for comparison with the Winder soil. All experiments were conducted in drained plastic tubs measuring 63 cm × 40 cm × 22 cm with planting media at a final depth of 15 cm after seed planting.

Citrus roots were collected from rootstocks in soil (to 10 cm depth) under the canopy of citrus trees at the Kerr Center, in areas determined by survey to be severely infested with *P. nicotianae* and *P. palmivora* at ranges of 100-700 propagules per cm^3 and 80-740 propagules per cm^3, respectively. Roots were carefully washed, graded, and kept moist until use. For inoculated treatments, a mixture of roots that ranged from fine fibrous roots to 1 cm diameter was added to each tub, 2 d after root collection, in a layer at a rate of 350 g per tub. Soil in tubs was kept damp continuously after root incorporation through the duration of the experiment by irrigation three times per week.

Eight tubs for each of four treatments were prepared and tested:

1. Potting Mix - 15 cm depth of steam-sterilized Pro Mix BX.

2. Winder - 15 cm depth of Winder soil.

3. Potting Mix plus Roots - 15 cm depth of steam-sterilized Pro Mix BX plus a layer of infected roots 5 cm below surface.

4. Winder plus Roots - 15 cm depth of Winder soil plus a layer of infected roots 5 cm below surface.

Seeds of 15 rootstock genotypes were collected from the Whitmore Foundation Farm for use in the experiment (Table 1), and stored at 4 °C. Immediately before planting, seeds were washed in 70% ethyl alcohol, soaked in 0.5% sodium hypochlorite for 10 min, and finally rinsed thoroughly with water. Each tub was planted with 15 rows of seeds, one row of 12 seeds for each rootstock. Seeds were planted 2 cm below the soil surface in all treatments. Rootstocks were randomized within tubs and tubs were randomized on the greenhouse benches. The experiment was conducted from October 2000 through January 2001.

Throughout the experiment, tubs were maintained in a warm greenhouse and watered as needed, alternating between water and a water-soluble fertilizer mix, 15N-7P-14K (Peters Fertilizer Products, W. R. Grace, Fogelsville, PA) applied with a proportioner at a rate of 400 $mg^{-1}liter^{-1}$ N. No supplemental light was supplied. Maximum photosynthetic photon flux (PPF) in the greenhouse was 800 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

For the next three months, observations were made three times a week to evaluate seedling emergence and decline. Declining seedlings were removed from the tubs once death was certain. After three months of growth, plants were clipped at the soil line and weighed.

Two months into the test, samples of declining seedlings were collected from the two treatments inoculated with root material and tested for presence of *P. nicotianae* and *P. palmivora.* The seedlings were inserted into PARPH semi-selective agar medium in petri plates, incubated in the dark, and examined for colony development over 10 d as described previously (Graham, 1995). Colonies were presumptively identified to *Phytophthora* species by colony morphology and then confirmed as *P. palmivora* or *P. nicotianae* microscopically by sporangial characteristics.

The experiment included eight randomized locations of 12 seeds for each of the 15 rootstocks in each of the four soil treatments, and was analyzed as a completely randomized design. The data were tested by analysis of variance using Statistica version 6.0 (StatSoft, Tulsa, OK) and Duncan multiple range test was used for mean comparison within rows or columns when the F-test was significant at $P < 0.05$.

Table 2. Seedling emergence in potting mix and the percent of emergence in the Winder soil, potting mix plus roots, and Winder soil plus roots treatments.

Rootstock	Potting mix (PM) (number of plants)	Winder soil (% of PM)	Potting mix plus roots (% of PM)	Winder soil plus roots (% of PM)
Swingle citrumelo	121	92	107 ab[z]	45 c-e
Flying Dragon	97	70	105 ab	66 a-d
Carrizo citrange	136	90	113 a	42 de
US-942	154	97	97 a-c	87 a
US-812	103	83	82 c	54 b-e
US-801	138	76	85 c	36 e
US-896	167	83	104 ab	71 a-c
Cleopatra	117	72	97 a-c	56 b-e
Standard sour	88	92	97 a-c	62 a-e
Volkamer	103	70	96 a-c	66 a-d
US-897	157	74	93 bc	77 ab
Daidai sour	117	86	97 a-c	89 a
Sun Chu Sha	138	77	103 ab	71 a-c
Average	136	82	98	63

[z]Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

Table 3. Post-emergence seedling death (percent).

Rootstock	Pro Mix BX	Winder soil	Pro Mix BX plus roots	Winder soil plus roots
Swingle citrumelo	0	2 b[z]	40 a	82 a
Flying Dragon	0	1 b	38 a	76 ab
Carrizo citrange	0	0 b	31 ab	68 a-c
US-942	0	0 b	15 cd	67 a-c
US-812	1	3 b	30 ab	58 a-d
US-801	0	2 b	15 cd	57 a-d
US-896	0	4 b	21 bc	53 a-d
Cleopatra	0	2 b	9 cd	42 b-d
Standard sour	2	13 a	12 cd	36 cd
Volkamer	0	3 b	13 cd	35 cd
US-897	1	1 b	17 b-d	34 cd
Daidai sour	0	0 b	11 cd	33 cd
Sun Chu Sha	0	3 b	3 d	27 d
Average	0	3	20	51

[z]Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

Results and Discussion

Two rootstocks planted in the tubs were not included in the analyses for these experiments because they had very poor germination in all four treatments. For the other 13 rootstock cultivars, the 96 planted seeds produced between 88 and 167 seedlings (Table 2) in the potting mix treatment (0.92 to 1.74 seedlings per seed). Obviously, many of the seeds for many of the rootstocks produced more than one seedling. All of the cultivars used in this experiment are polyembryonic and produce a high proportion of nucellar seedlings. A small number of seedlings identified as off-types during the experiment were discarded and not included in the data summaries.

Water-holding behavior of the two media were noted to be quite different. Water infiltrated Pro Mix BX potting mix immediately, whereas Winder soil drained water very slowly. During watering of the tubs with Winder soil, some of the water ponded on the surface and then infiltrated the soil slowly over a few hr.

Seedling emergence began at about two weeks after planting for all four treatments and continued at a decreasing rate throughout the three months of the experiment. Potting mix plus roots had a minimal effect on seedling emergence (Table 2), while Winder soil reduced overall seedling emergence by about 20%. Across all rootstocks, seedling emergence was reduced by 40% on Winder soil plus roots. The rootstocks suffering the largest reduction of emergence were Swingle citrumelo, Carrizo citrange, and US-801, while those least affected were Daidai sour and US-942.

In potting mix and Winder media without root inoculum, very few plants were lost (Table 3). In contrast, post-emergence seedling decline and death were noted in the two treatments with root inoculation within the first 2 weeks after seedlings began to emerge. Frequently, seedling death was preceded by severe leaf wilt and/or necrosis on the visible stem just above the soil line. For the potting mix plus roots treatment, about 20% of plants died during the test period, while losses on the Winder soil plus roots were about 50% (Table 3). For both media types inoculated with roots, Swingle citrumelo suffered the most losses and Sun Chu Sha had the fewest.

Two months into the test, *P. nicotianae* or *P. palmivora* were identified from tissues of many of the declining seedlings in the following two treatments: potting mix plus roots and Winder soil plus roots. One or both *Phytophthora* species were recovered from 64% (196) of the sample of 308 plants collected from the two treatments. Among the declining plants positive for *Phytophthora* spp., 39% were infected with *P. nicotianae*, 59% were infected with *P. palmivora*, and only 2% were infected with both. Each of the *Phytophthora* species was identified in many plants from both of the inoculated soil treatments (Winder and potting mix). Despite the highly significant differences among rootstocks for seedling death in the root incorporation treatments, there was no clear pattern in the recovery of the two *Phytophthora* species from declining trees of the different rootstocks (Table 4).

For all treatments, Volkamer plants were the largest and Flying Dragon plants were the smallest (Table 5). Generally, rootstocks that produced relatively larger plants did so regardless of the soil treatment. There was no apparent relationship between average rootstock seedling weight at the end of the experiment and frequency of seedling death during the experiment. Rootstocks that had a higher frequency of death were no more likely to have smaller seedlings. However, it was noted that a large proportion of the recorded plant deaths for all rootstocks occurred while the seedlings were still small and could be described as post-emergence damping-off.

Table 4. Percent of each Phytophthora species recovered from *Phytophthora*-infected plants.

Rootstock	*Phytophthora nicotianae*	*Phytophthora palmivora*	Both species
Swingle citrumelo	37	63	0
Flying Dragon	35	58	7
Carrizo citrange	17	83	0
US-942	37	60	3
US-812	83	17	0
US-801	56	44	0
US-896	30	70	0
Cleopatra	17	83	0
Standard sour	60	40	0
Volkamer	33	67	0
US-897	9	82	9
Daidai sour	50	50	0
Sun Chu Sha	100	0	0

Table 5. Average shoot fresh weight of surviving seedlings.

Rootstock	Pro Mix BX	Winder soil	Pro Mix BX plus roots	Winder soil plus roots
Swingle citrumelo	1.43 ab[z]	2.20 a	0.79 b	2.17 a
Flying Dragon	0.50	0.68	0.43	0.66
Carrizo citrange	1.13	1.02	0.76	0.77
US-942	1.27	1.57	1.28	1.60
US-812	0.60 b	0.88 b	0.59 b	1.33 a
US-801	0.40	0.73	0.44	0.93
US-896	0.74	0.73	0.72	0.85
Cleopatra	0.81	1.06	1.09	1.41
Standard sour	1.60 b	2.98 a	2.09 ab	3.12 a
Volkamer	2.37	3.44	2.93	3.73
US-897	0.91	0.86	1.01	1.13
Daidai sour	1.62 b	2.48 ab	1.77 b	2.87 a
Sun Chu Sha	1.03 b	1.36 ab	1.16 b	1.70 a
Mean	1.11 b	1.54 a	1.16 b	1.71 a

[z]Mean separations for significant ANOVA were within rows by Duncan's multiple range test at $P < 0.05$.

Without root inoculation, seedlings were significantly larger when grown on Winder soil than on potting mix (Table 5). This effect was only significant ($P < 0.05$) for the individual rootstock sour orange. Seedlings were also significantly larger when grown on Winder soil than on potting mix, with root inoculum. This effect was significant for the individual rootstocks Sun Chu Sha, Daidai sour, US-812, and Swingle citrumelo.

Surviving plants on media inoculated with roots were not significantly smaller than those growing on uninoculated media of the same type. The minimal effect of root inoculum on seedling fresh weight may be primarily the result of the heavy crowding of seedlings in treatments without root inoculum. Treatments with inoculum had much lower seedling numbers and corresponding less competition for water, nutrients, and light among the surviving seedlings. Total number of surviving seedlings per tub at the end of the experiment for the potting mix treatments were 211 and 165 for those without and those with root inoculum, respectively. Total number of surviving seedlings per tub at the end of the experiment for the Winder soil treatments were 170 and 76 for those without and those with root inoculum, respectively.

Swingle citrumelo and many other hybrids of trifoliate orange were identified in early literature as highly resistant to *Phytophthora* diseases, based on field observations from well-drained soils and greenhouse testing with *P. nicotianae* (Timmer and Menge, 1988). Recent field observations, especially in some Florida east coast flatwoods sites, have noted that Swingle citrumelo and some other trifoliate hybrids fail entirely or grow well initially but then dramatically weaken after several years (Stover and Castle, 2002). It has been suggested that these rootstocks are failing because of poor soil adaptation. Experimental work has also recently indicated that Swingle citrumelo and other similar hybrids are highly susceptible to *P. palmivora*, a second species of *Phytophthora* found in flatwoods areas (Graham et al., in press). Recent field observations (Bowman, 2001) and the results reported here suggest that soil characteristics and both *Phytophthora* species may play important roles in rootstock performance in these environments.

The method of soil and pathogen testing we have described appears to successfully identify Swingle citrumelo, Carrizo citrange, and Flying Dragon as poorly adapted and Sun Chu Sha, sour orange, Volkamer, and Cleopatra as better adapted to stresses associated with heavy flatwoods soil and the two *Phytophthora* species. Winder soil alone did not appear detrimental to the rootstocks that were extremely sensitive to it when combined with root inoculation. Similar results were obtained whether potting mix or Winder soil were inoculated with roots, although the negative effect of the root inoculation was much more pronounced on Winder. The size of surviving plants seemed to be a better indication of the innate vigor of the rootstock than the tolerance of the rootstock for the test conditions. The percentage of plants that died post-emergence in the Winder soil plus roots treatment appeared to yield the clearest indication of which rootstocks could tolerate similar conditions in the field. This screening technique will be an important aid in identifying rootstocks, such as US-897, that appear to tolerate common Indian River Region growing conditions (Bowman, 2001).

Literature Cited

Bowman, K. D. 2001. Finding better rootstocks for the flatwoods. Citrus Industry 82:42-44.

Bowman, K. D. and S. M. Garnsey. 2001. A comparison of five sour orange rootstocks and their response to citrus tristeza virus. Proc. Fla. State Hort. Soc. 114:73-77.

Bowman, K. D., J. P. Shapiro, and S. L. Lapointe. 2001. Sources of resistance to Diaprepes weevil in subfamily Aurantiodeae, Rutaceae. HortScience 36:332-336.

Graham, J. H. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. Phytopathology 85:111-117.

Graham, J. H., D. B. Bright, and C. W. McCoy. *Phytophthora-Diaprepes* Weevil Complex: *Phytophthora* spp. relationship with citrus rootstocks. Plant Dis. In press.

Graham, J. H. and J. A. Menge. 2000. Phytophthora-induced diseases. p. 12-15. In Compendium of Citrus Diseases, Second Edition. L. W. Timmer, S. M. Garnsey, and J. H. Graham (eds.). APS Press, St. Paul, MN.

Grosser, J. W., J. Jiang, E. S. Louzada, J. L. Chandler, and F. G. Gmitter, Jr. 1998. Somatic hybridization, and integral component of citrus cultivar improvement. II. Rootstock improvement. HortScience 33:1060-1061.

Lapointe, S. L. and K. D. Bowman. 2002. Is there meaningful plant resistance to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in citrus rootstock germplasm? J. Economic Entomol. 95(5) In press.

Stover, E. and W. Castle. 2002. Citrus rootstock usage, characteristics, and selection in the Florida Indian River Region. HortTechnology 12:143-147.

Timmer, L. W. and J. A. Menge. 1988. *Phytophthora*-induced diseases. p. 22-24. In Compendium of Citrus Diseases, First Edition. J. O. Whiteside, S. M. Garnsey, and L. W. Timmer (eds.). APS Press, St. Paul, MN.