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Screening Citrus Rootstock Genotypes for Tolerance to the *Phytophthora–Diaprepes* Complex under Field Conditions

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Rootstock germplasm from the USDA Horticultural Research Lab breeding program was evaluated in each of four growing seasons at the Florida Research Center for Agricultural Sustainability (FLARES) in Vero Beach. The screening site is located on Winder and Manatee fine sand soils naturally infested with *Diaprepes abbreviatus,* and *Phytophthora nicotianae* and *P. palmivora*. Seedlings previously grown in conetainers were field planted into a mixture of rhizosphere soil with fibrous roots from beneath 'Sunburst' trees on Swingle rootstock adjacent to the test block supporting both *Phytophthora* spp. Adjacent trees also served as a source of egg-laying adults of *D. abbreviatus.* Seedlings were planted in May 2002 and 2003 and in Jan. 2005 and 2006. Seedlings were harvested after 6, 7, 10, and 10 months, respectively. At harvest, rhizosphere soil was taken from beneath each tree for enumeration and identification of *Phytophthora* spp. Root systems were visually rated for root rot by the fungi and feeding damage by the weevil on a scale from 1 to 5 (1 = no damage, 5 = severe root damage). When 2002 and 2003 data were combined, there was a significant positive correlation between whole-root system damage and total *Phytophthora* spp. populations. Among the genotypes, mandarins and pummelo hybrids showed greater tolerance to the *Phytophthora–Diaprepes* (PD) complex than trifoliate orange and some of its hybrids. In 2005 and 2006, screening focused on hybrids of pummelo and mandarins. In these two seasons, phytophthora populations were lower overall (<20 propagules/cm^3), and no relationship between populations and root damage was detected for these genotypes. Tolerance of genotypes tested in the third and fourth seasons was greater than for genotypes tested in the first two seasons. Findings confirm the promise of certain pummelos and mandarins as parents for hybrids with requisite *Phytophthora* resistance to develop rootstock tolerance to PD complex in the field.

Diaprepes abbreviatus L. (Coleoptera: Curculionidae) is a polyphagous root weevil introduced into Florida from the Caribbean Basin that attacks *Citrus* spp. and other agricultural crops. Since discovery of diaprepes root weevil (DRW) in Orange County in 1964, the weevil has been dispersed primarily by nursery stock and now infests more than 66,000 ha of commercial agriculture, including approximately 12,000 ha of commercial citrus (Hall, 2000). Larvae of DRW feed on all commercial rootstocks budded with *Citrus* spp. At later developmental stages, the large larvae can strip the bark from the taproot and structural roots, causing girdling and eventual death of trees.

As DRW infestations have grown in scope over the last four decades, citrus production managers noted that trees in lower-elevation, wetter areas of the orchards were the first to decline. Trees on rootstocks such as sour orange (*Citrus aurantium* L.) and 'Cleopatra' mandarin (*C. reticulata* Blanco), susceptible to the root rot pathogen, *Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica*), declined more rapidly than in adjacent groves on rootstocks more resistant to this pathogen, like 'Swingle' citrumelo [*C. paradisi* Macf. x *Poncirus trifoliata* (L). Raf] (Graham, 1995). Conversely, on east coast flatwoods in poorly drained high pH soils of high calcium carbonate content, trees on 'Swingle' citrumelo were more severely declined than those on 'Cleopatra' and sour orange (Graham, 2000). Severity of root damage by the *Phytophthora–Diaprepes* (PD; Graham et al., 1997) complex was probably not due to differences between the rootstocks in susceptibility to larval feeding since damage to 'Cleopatra' mandarin and trifoliate hybrid rootstocks, 'Swingle' citrumelo and 'Carrizo' citrange [*C. sinensis* (L.) Osbeck x *P. trifoliata*], is similar (Rogers et al., 2000).

Greenhouse studies confirmed that larval feeding predisposed fibrous roots of seedlings of 'Cleopatra' mandarin to greater infection by *P. nicotianae* and higher infection of trifoliate orange by *P. palmivora* (Graham et al., 2003; Rogers et al., 1996). More infection by these *Phytophthora* spp. resulted in greater root damage and higher populations of the pathogens in the rhizosphere. The potential importance of the PD complex in the decline of trees on different rootstocks prompted a survey of the east coast of Florida near Vero Beach (Indian River County) and Ft. Pierce (St. Lucie County) where trees were rapidly declining despite aggressive management of the weevil. More severe damage was

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encountered where *P. palmivora* (Butler) Butler was the predominant pathogen in the complex with DRW (Graham, 2000). The *P. palmivora*–Diaprepes complex was associated with fine-textured, poorly drained soils on rootstocks normally resistant or tolerant of *P. nicotianae*: 'Swingle' and 'Carrizo'.

A field trial with 'Flame' grapefruit was planted in May 2000 at the FLARES site affected by *P. nicotianae*, *P. palmivora*, and DRW (Graham, 2000). The trial contained advanced rootstock selections from the USDA Horticultural Research Lab (USHRL) breeding program, as well as 'Swingle', 'Carrizo', and 'Cleopatra'. Soil in the test area is Winder and Manatee fine sand with calcareous deposits, and trees in the adjacent beds were heavily infested by DRW. Trees in the trial were inoculated at the time of planting with visibly diseased roots from the nearby trees. After 24 months, a strong correlation was confirmed between tree size and *Phytophthora* spp. populations on roots. After 36 months, trees on US-802, US-942, US-897, and 'Cleopatra' were growing strongly, while trees on 'Swingle', 'Carrizo', and some other USHRL rootstocks were small and weak (Bowman et al., 2003). Differences among the rootstocks were related to their ability to tolerate PD conditions because the poorest-performing rootstocks supported the highest soil populations of the two *Phytophthora* spp. Thus, in this site, *Phytophthora* susceptibility was an important predictor of tree performance. The relationship of *Phytophthora* to rootstock seedling susceptibility was also evaluated in tubs of infested Winder soil from this site in the greenhouse (Bowman et al., 2002). Rootstocks with the highest levels of mortality were 'Swingle', 'Carrizo', and 'Flying Dragon' trifoliate orange, while rootstocks with the lowest levels of mortality were sour orange, 'Sun Chu Sha' mandarin, 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. Therefore, this greenhouse assay appeared 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 (Bowman et al., 2002).

New rootstocks are needed to replace stunted or declining trees on sour orange and 'Swingle' citrumelo in many east coast flatwoods sites. Although the best-performing rootstocks in the FLARES field site may be sufficiently tolerant of PD complex to support commercial production of grapefruit under the conditions of the evaluation., further rootstock selection is warranted. While greenhouse testing can aid in this process, field testing of candidates is also desirable to determine rootstock tolerance to a wide range of pest, pathogen, environmental, and soil conditions. The most important limiting factors for existing commercial rootstocks in these areas include susceptibility to *P. nicotianae* or *P. palmivora*, and intolerance of common flatwoods soils. A screening method is described to rapidly test rootstocks for response to these conditions in the field.

Materials and Methods

Rootstock germplasm from the USHRL breeding program in Table 1 was grown in containers (Stuewe & Sons, Inc., Corvallis, OR) in a greenhouse at the USHRL, Ft. Pierce. Seedling material (number of replicate seedlings for each genotype; see Table 1) was evaluated in each of four growing seasons at the FLARES in Vero Beach. The screening site was located on Winder and Manatee fine sand soil series that were naturally infested with DRW and *P. nicotianae* and *P. palmivora* (Adair et al., 2000). Previous studies showed this site and the soil types to be conducive for development of the PD complex and for distinguishing tolerance of rootstocks based on tree performance (Bowman et al., 2002, 2003; Graham, 2000). A mixture of rhizosphere soil with fibrous roots was harvested from the 0–10 cm depth of the soil profile beneath 'Sunburst' tangerine (*C. reticulata* hybrid) trees on 'Swingle' rootstock that supported moderate to high populations of both *Phytophthora* spp. To ensure that all seedlings were exposed to the two *Phytophthora* pathogens, 200 cm^3 of the root/soil mixture was placed in the bottom of each planting hole before the seedling was set. DRW exposure was due to egg-laying adults immigrating from infested older trees adjacent to the test block. Plantings were established in May 2002 and 2003 and in Jan. 2005 and 2006. Seedlings were fertilized and irrigated as needed with a microjet irrigation system.

Genotypes were harvested in Jan. 2003 (n=25) , Mar. 2004 (n=22), Mar. 2006 (n=26), and Mar. 2007 (n=33) at 6, 7, 10, and 10 months after planting in the 2002, 2003, 2005, and 2006 seasons, respectively. Trees were carefully excavated with a shovel to keep the fibrous root system intact. A handful of rhizosphere soil was removed from below the root zone during the excavation process. Soil samples from each tree were dilution plated onto semi-selective PARPH medium for enumeration and identification of *Phytophthora* spp. (Graham et al., 2003). Whole-root systems and structural roots were visually rated for root rot by the fungi and feeding damage by the weevil on a scale from 1 to 5 (1 = no damage, 5 = severe root damage).

The relationship between whole-root system damage rating and total phytophthora counts in rhizosphere soil at time of harvest from the 2002 and 2003 seasons was examined using correlation analyses (SAS Institute, Cary, NC). In 2005 and 2006, no relationship was observed between these parameters, so for clarity the data are presented separately for each season.

Results

When 2002 and 2004 data were combined, a significant correlation ($r = 0.38$, $P < 0.0001$) was found between whole-root system damage and total phytophthora populations in rhizosphere soil (Fig. 1). Among the genotypes tested, mandarins and pummelo hybrids showed greater tolerance to PD complex than trifoliate and some of its hybrids. Tolerance was judged by whether the genotypes supported fewer than 20 propagules of total phytophthora per cm^3 of soil (Fig. 1). In 2005 and 2006, screening focused on hybrids of pummelo, mandarin, and Volkamer lemon, for which the polynomial regression of root damage with populations was nonsignificant because the majority of the genotypes supported fewer than 20 propagules (Figs. 2 and 3). Overall, the tolerance of genotypes in the third and fourth trials was greater than for the genotypes tested in the first 2 years of screening.

Discussion

These findings 1) validate use of field screening of rootstock seedlings for early assessment of genotype tolerance to PD complex; and 2) confirm the promise of certain pummelo and mandarins as parents for hybrids with requisite *Phytophthora* resistance to develop rootstocks tolerant to the PD complex. Similarly, in a greenhouse evaluation of tolerance to PD complex in phytophthora-infested Winder soil, Grosser et al. (2003) concluded that mandarin + pummelo somatic hybrids used to develop "tetrazyg" rootstocks were among the most promising

Table 1. Plant material tested in this study.

Clone	Parentage	Citrus species	No. of seedlings
		2002–03 Screening	
C-35	Citrange	*P. trifoliata* x *C. sinensis* (L.) Osb.	*86*
Changsha		*C. reticulate* Blanco	*8*
Chinka		*C. reticulata*	*10*
Cleopatra		*C. reticulata*	10
Creollo		*C. reticulata*	*10*
Daidai Sour orange		*C. aurantium* L.	*9*
Heen mandarin		*C. reticulata*	*10*
Laranja Cravo		*C. reticulata*	*9*
Mandarinette		*C. reticulata*	*9*
Ninkat		*C. reticulata*	*10*
Ponkan		*C. reticulata*	*10*
Scarlett Emperor		*C. reticulata*	*10*
Shekwasha		*C. reticulata*	*8*
Sour orange #2		*C. aurantium*	*8*
Sun Chu Sha		*C. reticulata*	*10*
Sunki		*C. reticulata*	*7*
Tachibana		*C. tachibana* (Mak.) Tan.	*9*
Tien Chieh		*C. reticulata*	*10*
Trifoliate orange (TO)		*Poncirus trifoliata* (L.) Raf.	*31*
US-809	Changsha x TO	*C. reticulata* x *P. trifoliata*	*10*
US-952	Pearl x TO	(*C. reticulata* x *C. paradisi* Macf.) x *P. trifoliata*	10
US-1351	Mandarin	*C. reticulata*	*9*
US-1352	Mandarin	*C. reticulata*	*6*
US-1353	Mandarin	*C. reticulata*	*9*
US-1355	Mandarin	*C. reticulata*	*10*
		2003–04 Screening	
Benton citrange		*P. trifoliata* x *C. sinensis*	*9*
C-35		*P. trifoliata* x *C. sinensis*	*19*
Cleopatra		*C. reticulata*	*10*
Kinkoji		*C. obvoidea* Taka.	*10*
Murraya Paniculata		*Murraya paniculata* (L.) Jack.	*11*
Sour orange #2		*C. aurantium*	*11*
Swingle		*C. paradisi* x *P. trifoliata*	*9*
Trifoliate orange		*P. trifoliata*	*18*
US-1269	TO x Pummelo	*P. trifoliata* x *C. grandis* (L.) Osbeck	*11*
US-1402	Pummelo x Sweet orange	*C. grandis* x *C. sinensis*	*12*
US-1403	Pummelo x Sweet orange	*C. grandis* x *C. sinensis*	*12*
US-1404	Smooth Flat x TO	(*C. reticulata* x *C. paradisi*) x *P. trifoliata*	11
US-1405	Smooth Flat x TO	(*C. reticulata* x *C. paradisi*) x *P. trifoliata*	8
US-1406	Sun Chu Sha x Swingle	*C. reticulata* x (*C. paradisi* x *P. trifoliata*)	*11*
US-1407	Smooth Flat x Sour orange	(C. *reticulata* x *C. paradisi*) x *C. aurantium*	10
US-1408	Smooth Flat x Sour orange	(C. *reticulata* x *C. paradisi*) x *C. aurantium*	11
US-1409	Smooth Flat x Sour orange	(C. *reticulata* x *C. paradisi*) x *C. aurantium*	11
US-1410	Smooth Flat x Sour orange	(C. *reticulata* x *C. paradisi*) x *C. aurantium*	9
US-1414	Sour orange x Sweet orange	*C. aurantium* x *C. sinensis*	*5*
US-1415	Sour orange x Sweet orange	*C. aurantium* x *C. sinensis*	*10*
US-1418	Warburg x Sweet orange	*Microcitrus warburgiana* (F.M. Bail.) Tan. x *C. sinensis*	*12*
X-639		*C. reticulata* x *P. trifoliata*	*12*
		2005–06 Screening	
Changsha		*C. reticulata*	*6*
Cleopatra		*C. reticulata*	*9*
Ridge		*C. sinensis*	*8*
Sour orange #2		*C. aurantium*	*9*
US-812	Sunki x TO	*C. reticulata* x *P. trifoliata*	*9*

Table 1. Continued on next page.

Table 1. Continued from previous page.

Clone	Parentage	Citrus species	No. of seedlings
US-942	Sunki x TO	*C. reticulata* x *P. trifoliata*	*10*
US-1355	Mandarin	*C. reticulata*	*8*
US-1409	Smooth Flat x Sour orange	(*C. reticulata* x *C. paradisi*) x *C. aurantium*	*7*
US-1503	Pummelo x TO	*C. grandis* x *P. trifoliata*	*9*
US-1504	Pummelo x TO	*C. grandis* x *P. trifoliata*	*8*
US-1510	Pummelo x TO	*C. grandis* x *P. trifoliata*	*7*
US-1511	Pummelo x TO	*C. grandis* x *P. trifoliata*	*10*
US-1513	Pummelo x TO	*C. grandis* x *P. trifoliata*	*10*
US-1516	Pummelo x TO	*C. grandis* x *P. trifoliata*	*9*
US-1520	Pummelo x TO	*C. grandis* x *P. trifoliata*	*9*
US-1521	Pummelo x TO	*C. grandis* x *P. trifoliata*	*7*
US-1524	Pummelo x TO	*C. grandis* x *P. trifoliata*	*5*
US-1531	Smooth Flat x Sour orange	(*C. reticulata* x *C. paradisi*) x *C. aurantium*	*6*
US-1532	Smooth Flat x Sour orange	(*C. reticulata* x *C. paradisi*) x *C. aurantium*	*5*
US-1534	Sour orange x Sweet orange	*C. aurantium* x *C. sinensis*	*6*
US-1540	Wild grapefruit	*C. paradisi* hybrid	*5*
US-1544	Wild grapefruit	*C. paradisi* hybrid	*8*
US-1545	Grapefruit	*C. paradisi*	*9*
US-1547	Pummelo hybrid	*C. grandis* hybrid	*10*
US-1561	Mandarin	*C. reticulata*	*10*
US-1562	Mandarin	*C. reticulata*	*5*
		2006–07 screening	
Cleopatra cutting		*C. reticulata*	*6*
Cleopatra seedling		*C. reticulata*	*6*
Swingle seedling		*C. paradisi* x *P. trifoliata*	*8*
Sour orange cutting		*C. aurantium*	*5*
Sour orange seedling		*C. aurantium*	*8*
US-802	Pummelo x TO	*C. grandis* x *P. trifoliata*	*20*
US-1287	Complex hybrid	[*M. inodora* (Bail.) Swing. x *C. ichangnensis* Swing.] x (*P. trifoliata* x *C. sinensis*)	5
US-1406	Sun Chu Sha x Swingle	*C. reticulata* x (*C. paradisi* x *P. trifoliata*)	*7*
US-1460	Volkamer x Sour orange	*C. volkameriana* x *C. aurantium*	*6*
US-1467	Mandarin hybrid x Sour orange	*C. reticulata* hybrid x *C. aurantium*	*5*
US-1478	Mandarin x Sour orange	*C. reticulata* x *C. aurantium*	*6*
US-1503	Pummelo x TO	*C. grandis* x *P. trifoliata*	*8*
US-1510	Pummelo x TO	*C. grandis* x *P. trifoliata*	*8*
US-1511	Pummelo x TO	*C. grandis* x *P. trifoliata*	*7*
US-1513	Pummelo x TO	*C. grandis* x *P. trifoliata*	*8*
US-1516	Pummelo x TO	*C. grandis* x *P. trifoliata*	*8*
US-1518	Pummelo x TO	*C. grandis* x *P. trifoliata*	*8*
US-1521	Pummelo x TO	*C. grandis* x *P. trifoliata*	*8*
US-1524	Pummelo x TO	*C. grandis* x *P. trifoliata*	*6*
US-1605	Pummelo x Shekwasha	*C. grandis* x *C. reticulata*	*5*
US-1651	Pummelo x Sunki	*C. grandis* x *C. reticulata*	*5*
US-1666	Pummelo x Cleopatra	*C. grandis* x *C. reticulata*	*5*
US-1667	Pummelo x Cleopatra	*C. grandis* x *C. reticulata*	*5*
US-1668	Pummelo x Cleopatra	*C. grandis* x *C. reticulata*	*5*
US-1679	Pummelo x Tachibana	*C. grandis* x *C. tachibana*	*5*
US-1689	Pummelo x Cleopatra	*C. grandis* x *C. reticulata*	*6*
US-1696	Pummelo x Cleopatra	*C. grandis* x *C. reticulata*	*5*
US-1705	Pummelo x Shekwasha	*C. grandis* x *C. reticulata*	*6*
US-1710	Pummelo x Shekwasha	*C. grandis* x *C. reticulata*	*6*
US-1711	Pummelo x Shekwasha	*C. grandis* x *C. reticulata*	*6*
US-1743	Pummelo x Batangus	*C. grandis* x *C. reticulata*	*6*
US-1745	Pummelo x Batangus	*C. grandis* x *C. reticulata*	*6*
US-1753	Ninkat x Pummelo	*C. reticulata* x *C. grandis*	*5*

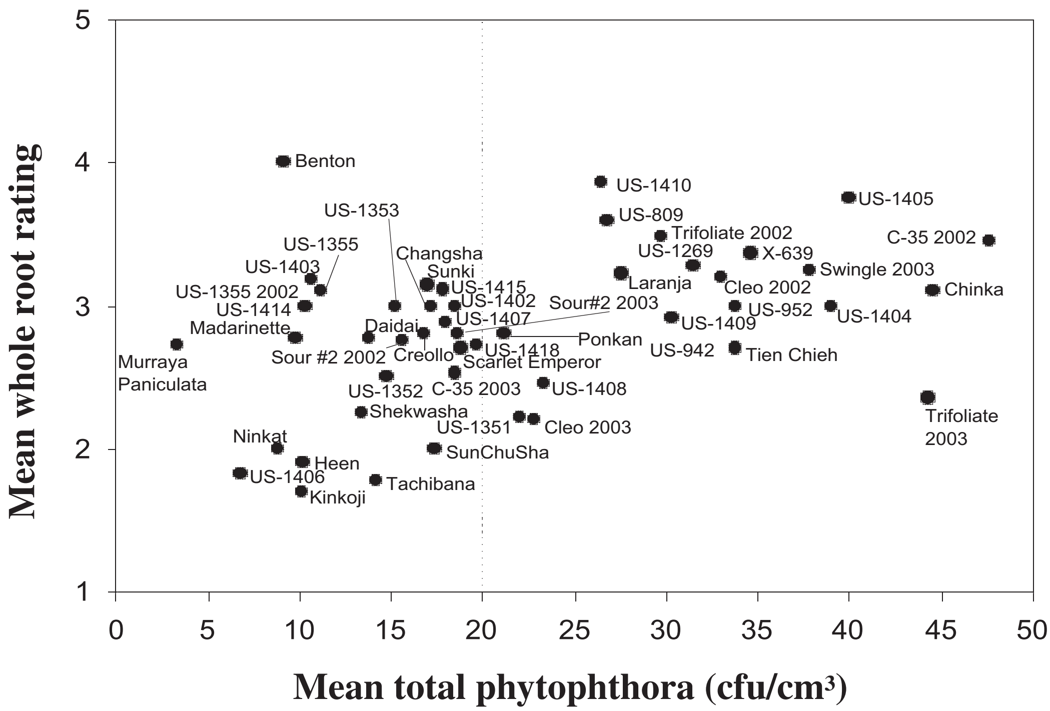

Fig. 1. Relationship between whole root system damage from *Phytophthora* spp. and *Diaprepes abbreviatus* root weevil rated on a scale from 1 to 5 (1 = no damage and 5 = severe root damage) and the combined populations of *Phytophthora nicotianae* and *P. palmivora* in rhizosphere soil at time of harvest of citrus genotypes (see Table 1) from Block K10 at FLARES in Vero Beach in the 2002–03 and 2003–04 seasons.

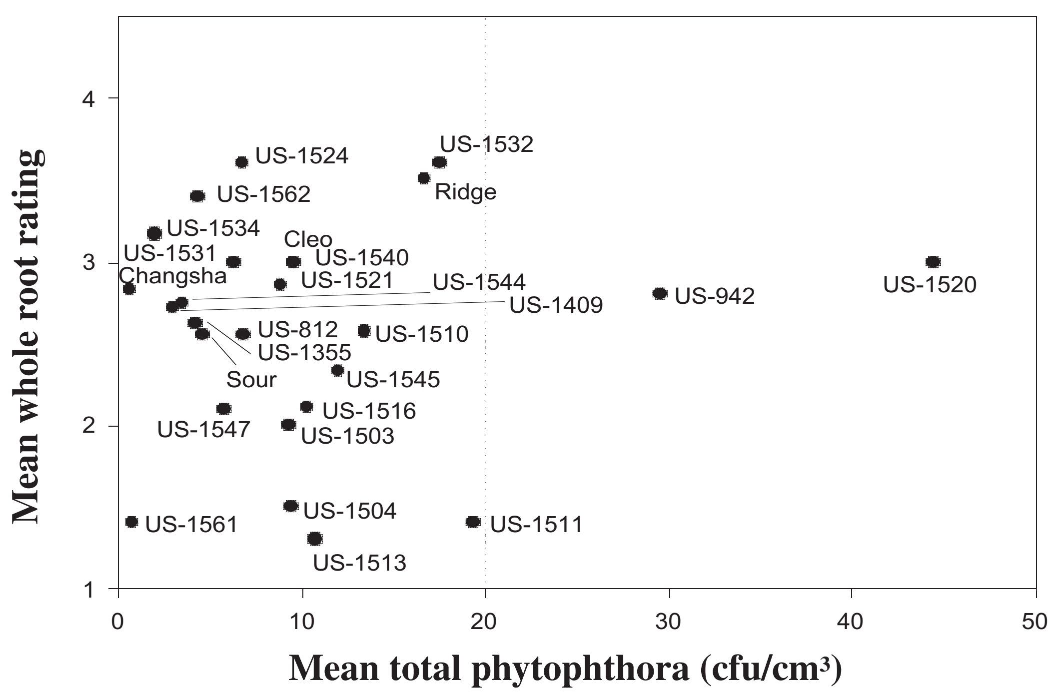

Fig. 2. Relationship between whole root system damage from *Phytophthora* spp. and *Diaprepes abbreviatus* root weevil rated on a scale from 1 to 5 (1 = no damage and 5 = severe root damage) and the combined populations of *Phytophthora nicotianae* and *P. palmivora* in the rhizosphere soil at time of harvest of citrus genotypes (see Table 1) from Block K10 at FLARES in Vero Beach in the 2005–06 season.

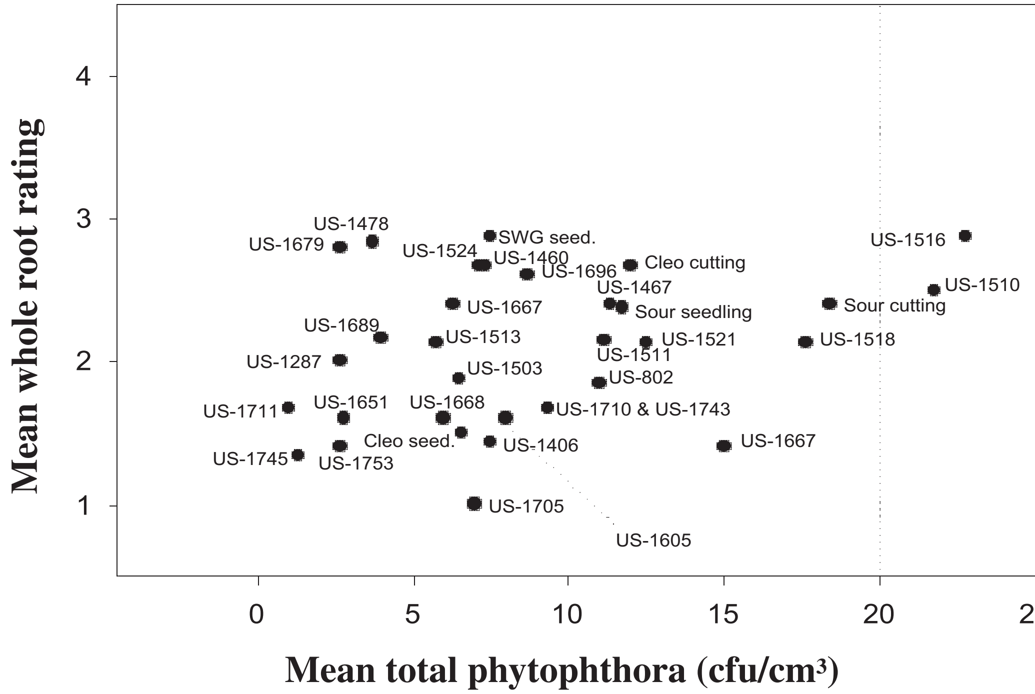

Fig. 3. Relationship between whole root system damage from *Phytophthora* spp. and *Diaprepes abbreviatus* root weevil rated on a scale from 1 to 5 (1 = no damage and 5 = severe root damage) and the combined populations of *Phytophthora nicotianae* and *P. palmivora* in the rhizosphere at time of harvest of citrus genotypes (see Table 1) from Block K10 at FLARES in Vero Beach in the 2006–07 season.

performers in their assay. Mandarin + pummelo somatic hybrids as a source of tolerance were chosen as a result of the outstanding performance of a 'Nova' mandarin + 'Hirado Buntan' pummelo seedling observed in a block affected by PD complex at the Indian River Research and Education Center (IRREC) in Ft. Pierce. Likewise, USHRL rootstocks that performed best at the FLARES site in the evaluation of performance of 'Flame' grapefruit were US 802 (pummelo x *P. trifoliata*), US-942 ('Sunki' mandarin x *P. trifoliata*), and US 897 ('Cleopatra' mandarin x *P. trifoliata*), hybrids of either pummelo or mandarins with trifoliate orange. Two of these rootstocks, US-802 and US-897, have been released recently by the USHRL for propagation by the Florida citrus nursery industry.

Interestingly, sour orange rootstock that is relatively tolerant to *Phytophthora* but susceptible to citrus tristeza virus (CTV) has been suggested to be a mandarin–pummelo hybrid (Nicolosi et al., 2000). Thus, efforts in rootstock development are being directed toward a widely adapted hybrid of mandarin and pummelo that is CTV quick-decline tolerant and has resistance to both *P. nicotianae* and *P. palmivora.*

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