Table 6. Spray burn intensities on oranges or grapefruit sprayed with urea products and mixes containing urea. All sprays mixed at rate of 125 gal/acre except for undiluted urea products as noted.

Cultivar/timing	Product, lb/acre	Additions/acre	Leaf burn	Fruit burn
	N-SURE, 20-30	Aliette, 5	Slight	None
	Spray grade urea, 10-30	Aliette, 5	None	None
Grapefruit/fall	Unocal, 10-30	—	None	None
	Unocal, 10	Aliette, 5 + buffer	None	None
	Unocal, 20-30	Aliette, 5 + buffer	None	Slight
	N-SURE, 10-30	—	None	None
	N-SURE, 10-30	Aliette, 5 + buffer	None	None
	Trisert KS, 10-30	—	None	None
	Trisert KS, 10-20	Aliette, 5 + buffer	None	None
	Trisert KS, 30	Aliette, 5 + buffer	None	Moderate
	Spray grade urea, 10-30	—	None	None
	Spray grade urea, 10-30	Aliette, 5 + buffer	None	None
	—	Comite, 2.7	None	Slight
	All products, 20	Comite, 2.7	None	Moderate-Severe

methods would indicate phytotoxicity potential. We did not try to prevent burn. New emphasis should be on safe mix formulation. The possible need for dilute spraying with the more phytotoxic chemicals, pH adjustment and combinations that reduce burn potential should be examined more critically. Unfortunately from a spray burn standpoint, there is increased interest in the addition ofurea as a foliar nutritional. This material enhances absorption (Yamada et al., 1965). It can be phytotoxic on its own or if combined with other nutritionals or citrus leafminer spray materials such as oil and Agrimek (Table 6). Leaf burn was more likely to occur on spring, unhardened leaves. In the fall, Trisert KS at 30 lbs N/ac with Aliette caused a moderate fruit burn, and urea products aggravated the tendency of Comite to burn fruit.

Adjuvants of the penetrant grade, intended for enhancing the absorption of herbicides, will increase the likelihood of spray burn if the other spray ingredients can be phytotoxic with sufficient absorption. A given adjuvant may be more or less harmful in the spray mix depending on the specific chemical being absorbed and how it interacts with the surfactant. The real need for a surfactant and determining safe surfactants for standard tank mixes should be evaluated carefully by the grower. Any questionable mixture can be examined rather easily by the limb spray method. The peel disk method is very subjective and requires the most exact tissue culture

skills. Suspension culture test is the most precise for evaluating phytotoxicity of a given chemical at the cellular level but required technical equipment and personnel. The techniques using peel infusion or droplet deposit over small punctures on young fruit may be satisfactory alternatives that are fast and easy for anyone to use.

Literature Cited

Albrigo, L. G. 1978. Occurrence and identification of preharvest fruit blemishes in Florida citrus groves. Proc. Fla. State Hort. Soc. 91:78-81.

Grosser, J. W. and F. G. Gmitter, Jr. 1990. Protoplast fusion and citrus improvement. Plant Breed. Rev. 8:339-374.

Maas, E. V. 1993. Salinity and citriculture. Tree Physiol. 12:195-216.

Oberbacher, M. F. and G. E. Brown. 1968. Use of benzimidazoles for control of fungi in peel cultures of citrus fruits. HortScience 3:286-287.

Sonoda, R. M., M. Vathakos and R. R. Pelosi. 1989. Interaction of fosetyl-aluminum fungicide and copper fungicide on citrus fruit and foliage. Proc. Fla. State Hort. Soc. 102:10-13.

Vakis, N., W. Grierson, J. Soule and L. G. Albrigo. 1970. A tissue culture technique for studying chilling injury of tropical and subtropical fruits. HortScience 5:472-473.

Yamada, Y., W. H. Jyung, S. H. Wittver and M. J. Bukovac. 1965. The effects of urea on ion penetration through isolated cuticular membranes and ion uptake by leaf cells. J. Amer. Soc. Hort. Sci. 87:429-432.

INSECT-PLANT PATHOGEN INTERACTIONS: PRELIMINARY STUDIES OF *DIAPREPES* ROOT WEEVIL INJURIES AND *PHYTOPHTHORA* INFECTIONS

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Abstract. **It has been speculated that larval injuries by *Diaprepes abbreviatus* L. serve as preferred infection courts for root rot diseases caused by soil pathogens such as *Phytophthora* spp. A completely randomized block experiment was performed in the greenhouse where roots of young Cleopatra mandarin (*C. reticulata* Blanco) and trifoliate orange (*Poncirus trifoliata* L.) were first exposed to *D. abbreviatus* larvae of known density and then later inoculated with *Phytophthora nicotianae* Breda de Haan. Results suggest that the incidence and severity of root rot disease on *Phytophthora*-susceptible seedlings generally increased in relation to the amount of root damage caused by *D. abbreviatus* larvae. *Phytophthora*-tolerant seedlings, however, showed little or no increase in disease severity with increasing insect injury. Infection of root tips dis-**

tal to *D. abbreviatus* damaged roots suggest that root wound exudates serve as nutritional substrates for developing *P. nicotianae* populations. This paper presents the first study describing an interaction between soil insect injuries and the subsequent infection by a plant pathogen in the citrus rhizosphere.

Diaprepes abbreviatus L., the root weevil attacking *Citrus* spp. and other agricultural crops, was introduced into Florida from Puerto Rico around 1964 (Woodruff, 1964). Since then, it has spread to 19 other counties in the state infesting about 152,960 acres of commercial agriculture, including approximately 25,000 acres of citrus (McCoy et al. 1995). Due to a lack of early detection methods and the availability of effective management options, much of the *Diaprepes*-infested acreage is in severe decline or out of production (McCoy et al. 1996). Freezes during the 1980's likely played a role in temporarily reducing its dissemination rate; however, many trees in the areas of infestation have since recovered from the freezes and aggressive replanting in the early 1990's has resulted in an increasing amount of acreage infested with *Diaprepes*. The adult weevil causes a characteristic notched leaf feeding injury, while the subterreanean larval stages inflict severe cortex injury by carving a feeding pathway into root tissues up to 1 cm wide (McCoy et al. 1996).

Phytophthora spp. also cause economic losses to citrus by attacking susceptible roots. Symptoms caused by this plant pathogen most often are root and crown rots in nurseries, fibrous root rot, foot rot of the trunk and brown rot of fruits in bearing citrus groves (Graham and Timmer, 1992). Fibrous root rot caused by *Phytophthora nicotianae* Breda de Haan occurs primarily on susceptible rootstocks in nurseries, but may also occur in bearing citrus groves where damage can lead to tree decline and loss of production. This soil borne pathogen first infects the root cortex, causing it to become soft and discolored. Roots may also appear water-soaked. *Phytophthora*-infected roots often slough their cortex, leaving only the white, thread-like stele. If many roots are infected, the root system can have a stringy appearance (Graham and Timmer, 1992).

Many plant diseases are exacerbated by mechanical injury, by the environment, by arthropods or other stresses that compromise the integrity of the epidermis. Wounds create sites for invasion by numerous microbial pathogens (Manners, 1993). It has been speculated that root injuries caused by citrus root weevils or other subterranean insects serve as preferred infection courts for *Phytophthora* spp. Citrus production managers frequently report that trees in *P. nicotianae*-infested areas are among the first to show canopy decline symptoms of weevil injury. However, no research has been conducted on the interactions between insect feeding patterns and the associated infections caused by this pathogen on *Phytophthora*-sensitive and *Phytophthora*-tolerant trees. Since the cost of managing both *D. abbreviatus* and *P. nicotianae* can be significant, it is critical to understand the relationships between damage caused by root weevils and root rot disease. The objective of this research was to evaluate differences in root rot disease between *Phytophthora*-susceptible and *Phytophthora*-tolerant seedlings following *D. abbreviatus* injury.

Materials and Methods

Approximately 100, 6 to 12 month old, greenhouse-grown citrus seedlings were used in each of two experiments. Trifoliate orange (*Poncirus trifoliata* L.) and Cleopatra mandarin (*C. reticulata* Blanco) rootstocks were grown from registered seeds obtained from the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Seeds were sown in 125 cm³ Cone-

tainers containing Metromix potting mixture. Seedlings were fertilized weekly with Peter's 20-20-20 and watered regularly. Plants used in the experiments were selected for uniformity of shoot and canopy size, within varieties, by visual inspection.

Experiment 1. First instar neonate larvae of *D. abbreviatus*, less than 48 h old, were obtained from eggs laid by field-collected adult females confined to screen cages in a greenhouse at $27 \pm 2°C$ (McCoy et al. 1995). Neonate larvae (0, 3 or 5 per treatment) were counted into Eppendorf tubes and then distributed on the soil surface within each Conetainer containing Cleopatra mandarin seedlings. Larval infestation periods were varied over 5 wk to generate different levels of root injury. The plants were then removed from the Conetainers and the soil was washed from the roots. Weevils recovered from the soil were counted and a visual assessment of feeding injury was performed. Roots were rated according to a scale ranging from 1 to 4, where 1 = no visible weevil injury and 4 = few to no visible healthy roots.

The isolate of *P. nicotianae* (R-1) was obtained from the rhizosphere soil of a commercial Hamlin orange (*C. sinensis* L.) on sour orange (*C. aurantium* L.) grove located near Fort Pierce, FL. Chlamydospores were produced by the method of Tsao (1971), and mixed with a small aliquot of moistened (5% w/w), unfertilized, autoclaved Candler fine sand soil (Typic quartzipsamments, 96.5% sand, 2% silt, 1.5% clay) with a pH of 6.8, organic matter content of 1% and extractable P of 3.5 ug/g soil. The moistened and inoculated soil was incubated for 7 d. The inoculum density was then determined by dilution plating on pimaricin-ampicillin-rifampicin-pentachloronitrobenzene (PARP) selective medium using 125 mg/l instead of 250 mg/l of ampicillin and adding 25 mg/l of hymexazol (Graham, 1990). The soil inoculum was then thoroughly mixed in a sterilized concrete mixer with known volumes of autoclaved Candler fine sand to render the desired inoculum density.

Weevil-injured citrus seedlings were transplanted into Conetainers in Candler fine sand containing chlamydospore densities of 0 or 5 propagules of *P. nicotianae* per cm³ of prepared soil. Treatments were assigned at random to the seedlings according to a completely randomized block design. After transplanting, the seedlings were fertilized weekly with Peter's 20-20-20 liquid fertilizer and watered regularly.

Approximately 6 wk after *P. nicotianae* inoculation, seedlings were removed from the Conetainers and the soil was washed from the roots. Root rot ratings were obtained in a manner similar to that used to obtain larval injury ratings, i.e., root systems were inspected for visual indications of fibrous root sloughing. Root systems were rated according to a scale of 1 to 5, where 1 = no visible root rot disease and 5 = few to no visible healthy roots. Propagule densities of *P. nicotianae* per cm3 were obtained by dilution plating the potting medium samples on PARP medium as described above. The percentage of infected root tips was determined by plating 20 root tips from each plant onto PARP medium and counting the number positive for *P. nicotianae*. When fewer than 20 root tips were found on a plant, all available tips were plated on PARP medium. Fibrous root, tap root and shoot weights were obtained by cutting the root systems into sections, oven drying and then weighing.

Experiment 2. Cleopatra mandarin and trifoliate orange seedlings were grown, inoculated with *D. abbreviatus* and *P. nicotianae* and rated for root rot disease as in Experiment 1. A broader range of weevil injuries appeared in Experiment 2, so the weevil injury rating scale ranged from 1 to 5 where 1 = no visible larval

injury and 5 = few to no healthy roots (Fig. 1). From 3 to 18 plants

Figure 1. Rating system used for evaluating *Diaprepes abbreviatus* larval feeding injury to Cleopatra mandarin and trifoliate orange rootstocks. From left to right: 1 = healthy root system; 2 = light feeder root damage with some cortical injury; 3 = moderate feeder root damage and cortical injury; 4 = extensive cortical injury and feeder root damage, and; 5 = extensive cortical injury and feeder root damage, girdling and few or no healthy roots. The *Phytophthora nicotianae* root rot disease rating system was similarly categorized, except the primary observation variable was fibrous root sloughing.

Table 1. The development of *Phytophthora nicotianae* on Cleopatra mandarin (*P. nicotianae*-sensitive) following individual and combined applications of chlamydospores of *P. nicotianae* and neonate larvae of *Diaprepes abbreviatus*[1].

Treatment (No. of plants)	*Phytophthora* rating[2]	Propagules per cm³ of soil	Infected root tips (%)	Fibrous root weight (g)	Tap root weight (g)	Shoot weight (g)
Untreated (7)	2.00a	0.00a	0.00a	0.82a	1.19a	3.42a
Weevil (19)	2.14a	0.00a	0.00a	0.71ab	1.16a	3.70a
Fungus (9)	3.11b	116b	61b	0.65b	1.02a	3.34a
Weevil + Fungus (9)	3.90c	157b	65b	0.62b	1.09a	3.56a

[1]Numbers not followed by the same letter are significantly different at P = 0.10 according to Fisher's Protected LSD procedure.
[2]*P. nicotianae* rating system ranges from 1 to 5, where 1 = healthy plant and 5 = dead plant or few or no healthy roots.

from each injury category were used for exposure to chlamydospores of *P. nicotinana*.

Statistical analyses. The relationships between larval injury and root rot were determined by subjecting the data to analyses of variance (P = 0.10) to test the effects of weevil injury on root sloughing, populations of soil *P. nicotianae* and the incidence of root rot disease.

Results

Experiment 1. Feeding injury by larvae ranged from mild to severe, but little or no root cortical sloughing symptomatic of *P. nicotianae* root rot was observed in the absence of *P. nicotianae* (Table 1). However, some symptoms of root rot were found on noninoculated root systems, resulting in disease ratings of 2.0 or less. These symptoms on the noninoculated roots were later attributed to the fungal pathogen, *Thielaviopsis basicola*, based on dilution plating of soil and root samples and the fact that no *P. nicotianae* propagules were present in those samples (Graham and Timmer, 1991). When plants were inoculated with *P. nicotianae* only, the level of disease increased over that of the untreated and weevil only treatments. However, when plants were challenged with the combination of insect injury and *P. nicotianae* inocula-

tion, the level of root rot disease was greater than any other treatment (Table 1).

No propagules of *P. nicotianae* could be detected in control plants or plants exposed only to weevil larvae. When plants were inoculated with *P. nicotianae* only, propagule density was high, typical of young seedlings grown under conditions conducive for disease (Table 1). When plants were exposed to both *D. abbreviatus* and *P. nicotianae*, the density of propagules was higher than in the *P. nicotianae* alone. Although this difference was not statistically significant, the trend is consistent with the ratings of disease severity and suggest that larval feeding injuries predispose young citrus trees to *P. nicotianae*-induced root rot disease.

When seedlings were inoculated with *P. nicotianae* only, the percentage of infected root tips increased over that of the untreated and the weevil only treatment. No *P. nicotianae*-infected root tips were found in control plants or plants exposed only to weevil larvae (Table 1). However, when plants were exposed to both organisms, the percentage of *P. nicotianae*-infected root tips was greater than for the other treatments (Table 1), but not significantly greater than for the *P. nicotianae* alone treatment.

Generally, the greater the incidence of disease, the greater was the loss in fibrous root weight for *P. nicotianae*-treated seedlings of Cleopatra mandarin. Control plants and plants exposed to weevils only exhibited the highest fibrous root weights, but they were

not significantly different from each other. When plants were inoculated with *P. nicotianae* only, the loss of fibrous roots increased over that of the other two treatments. When plants were exposed to both organisms, the loss of fibrous root weight was greater, but not significantly greater than root loss from *P. nicotianae* alone (Table 1). No statistically significant differences in tap root and shoot weights were detected between any of the root weevil/fungal treatments.

Generally, the greater the larval feeding injury, the greater the *P. nicotianae* disease rating (Table 2). The amount of root rot disease increased as feeding injury increased for the *P. nicotianae*-sensitive Cleopatra mandarin rootstock. The increase in root rot disease appeared to stabilize or drop somewhat at the highest larval injury ratings, suggesting that declining resource substrates may have governed root rot disease development. Further, *P. nicotianae* propagules per cm³ were significantly higher on roots showing increasing amounts of weevil feeding injury (Table 3) and the per-

Table 2. The development of *Phytophthora nicotianae* root rot disease on Cleopatra mandarin (*P. nicotianae*-sensitive) and trifoliate orange (*P. nicotianae*-tolerant) following combined applications of chlamydospores of *P. nicotianae* and neonate larvae of *Diaprepes abbreviatus*.

Weevil Rating[1]		Cleopatra mandarin			Trifoliate orange		
		No. of seedlings[2]	Mean root rot rating[1]	S.E.	No. of seedlings[2]	Mean root rot rating[1]	S.E.
1	(Exp. 1)	18	2.56	0.17	—	—	—
	(Exp. 2)	6	1.00	0.00	12	1.33	0.14
2	(Exp. 1)	14	3.07	0.36	—	—	—
	(Exp. 2)	7	1.71	0.36	8	1.00	0.00
3	(Exp. 1)	6	3.83	0.60	—	—	—
	(Exp. 2)	11	1.91	0.25	9	1.00	0.00
4	(Exp. 1)	6	3.83	0.31	—	—	—
	(Exp. 2)	10	2.50	0.37	16	1.31	0.20
5	(Exp. 1)	—	—	—	—	—	—
	(Exp. 2)	9	1.89	0.39	21	1.10	0.07

[1]*D. abbreviatus* and *P. nicotianae* rating systems range from 1 to 5, where 1 = healthy plant and 5 = dead plant or few or no healthy roots.
[2]— = measurement not included in that experiment.

centage of infected root tips of Cleopatra mandarin also increased as the level of larval feeding injury increased (Table 4).

Experiment 2. In Experiment 2, there was a wider distribution of larval feeding damage that developed on the seedling root systems, so an insufficient number of weevil-damaged Cleopatra mandarin root systems were obtained to split into the four treatments described in Table 1. Thus, the combined treatments of larval injury and *P. nicotianae* infection were examined to characterize root rot disease as related to the amount of insect injury. Generally, increased larval feeding injury resulted in increased *P. nicotianae* disease ratings (Table 2). These results are consistent with those obtained in Experiment 1. The amount of disease did not increase as dramatically for the *P. nicotianae*-tolerant trifoliate orange rootstock (Table 2), however. As in Experiment 1, the increase in root rot disease appeared to stabilize or drop somewhat at the higher larval injury ratings. Although there were significant differences between root rot on Cleopatra mandarin as the severity of insect injury increased, no such differences were observed on trifoliate orange (Table 2).

In contrast to Experiment 1, *P. nicotianae* propagules per cm³ were not consistently higher on Cleopatra mandarin roots showing increasing amounts of weevil feeding injury (Table 3). On trifoliate orange, however, fungal propagules per cm³ were nondetectable until the level of feeding injury was very high (Table 3). When weevil injury to trifoliate orange approached that of completely consuming the roots, *P. nicotianae* propagules per cm³ dropped to near nondetectable levels. The percentage of infected root tips also was not consistently greater as root weevil feeding injury increased (Table 4). These soil propagule and root tip measurements on Cleopatra mandarin were more variable than those of Experiment 1, due possibly to the smaller number of replicate seedlings in the samples. Further, it was difficult to control the degree of injury generated during larval feeding, so it is possible that the amount of root cambium exposed was quite variable within given injury rat-

ings. These variations may have affected the precision of the propagule density and root tip measurements.

Although root rot disease on the *Phytophthora*-susceptible and -tolerant seedlings differed (Table 2), weight and volume measurements between the two root systems as related to increasing larval feeding were comparable (Fig. 2-A and 2-B). Separate studies have shown that larval feeding injuries do not differ between the two rootstocks of (Grosser and McCoy, 1996; Shapiro and Gottwald, 1995), so the effects of root rot disease on total root biomass likely were negligible compared to the weevil injury. Total root weight decreased by approximately 66% for both the *P. nicotianae*-tolerant and *P. nicotianae*-sensitive rootstocks between replicates showing the least and the most severe larval injury. There were significant decreases in total root weights as the level of feeding injury within a given rootstock increased, but differences between rootstocks at given levels of larval feeding injury were not as significant. Declining trends in total shoot weight for both seedlings were also observed (Fig. 2-C), but the shoot weight difference between the seedling cultivars was a result of inherent size differences in the seedling canopies.

Root rot disease as measured by disease ratings, propagules per cm³ of soil, percentage infected roottips and fibrous root weight loss, was generally greater in the combined *P. nicotianae*/*D. abbreviatus* inoculations than in the untreated plants or the plants inoculated with only *P. nicotianae* or *D. abbreviatus* (Tables 1 and 2). Experiments 1 and 2 data suggest that the incidence and severity of *P. nicotianae* root rot on Cleopatra mandarin (*P. nicotianae*-sensitive) rootstock increases in relation to the severity of larval feeding injury. Increased root rot disease occurred most often on the *P. nicotianae*-sensitive Cleopatra mandarin rootstock (Table 2), whereas only high levels of weevil feeding injury were sufficient to induce low levels of root rot disease on the *P. nicotianae*-tolerant trifoliate orange (Table 2). In no case, however, did the highest levels of root rot disease on trifoliate orange compare to the

Table 3. Propagule densities of *Phytophthora nicotianae* on Cleopatra mandarin (*P. nicotianae*-sensitive) and trifoliate orange (*P. nicotianae*-tolerant) following combined applications of chlamydospores of *P. nicotianae* and neonate larvae of *Diaprepes abbreviatus*.

Weevil Rating[1]	Cleopatra mandarin			Trifoliate orange		
	No. of seedlings[2]	Mean propagules/cm³	S.E.	No. of seedlings[2]	Mean propagules/cm³	S.E.
1 (Exp. 1)	18	58.11	17.02	—	—	—
(Exp. 2)	0	—	—	6	0.00	0.00
2 (Exp. 1)	14	74.43	24.36	—	—	—
(Exp. 2)	3	132.68	89.09	5	0.00	0.00
3 (Exp. 1)	6	120.67	39.36	—	—	—
(Exp. 2)	5	79.60	40.20	3	0.00	0.00
4 (Exp. 1)	6	203.67	54.73	—	—	—
(Exp. 2)	6	75.33	38.67	9	17.33	11.23
5 (Exp. 1)	—	—	—	—	—	—
(Exp. 2)	7	109.71	52.34	11	0.18	0.18

[1]*D. abbreviatus* rating system ranges from 1 to 5, where 1 = healthy plant and 5 = dead plant or few or no healthy roots.

[2]— = measurement not included in that experiment.

Table 4. Percentage of root tips infected with *Phytophthora nicotianae* on Cleopatra mandarin (*P. nicotianae*-sensitive) and trifoliate orange (*P. nicotianae*-tolerant) following combined applications of chlamydospores of *P. nicotianae* and neonate larvae of *Diaprepes abbreviatus*.

Weevil Rating[1]	Cleopatra mandarin			Trifoliate orange		
	No. of seedlings[2]	Infected root tips (%)	S.E.	No. of seedlings[2]	Infected root tips (%)	S.E.
1 (Exp. 1)	18	30.56	7.90	—	—	—
(Exp. 2)	—	—	—	5	0.00	0.00
2 (Exp. 1)	14	34.29	10.20	—	—	—
(Exp. 2)	3	15.00	7.64	5	0.00	0.00
3 (Exp. 1)	6	61.67	11.95	—	—	—
(Exp. 2)	5	30.67	17.16	3	0.00	0.00
4 (Exp. 1)	6	65.00	10.57	—	—	—
(Exp. 2)	6	10.00	4.28	9	6.11	4.39
5 (Exp. 1)	—	—	—	—	—	—
(Exp. 2)	3	23.33	13.01	10	1.00	0.67

[1]*D. abbreviatus* rating system ranges from 1 to 5, where 1 = healthy plant and 5 = dead plant or few or no healthy roots.

[2]— = measurement not included in that experiment.

highest levels of root rot disease on trifoliate orange compare to the lowest levels of root rot disease observed on Cleopatra mandarin. Weevil feeding injury did not differ significantly between the Cleopatra mandarin and the trifoliate orange (Fig. 2). These results are consistent with those of Shapiro and Gottwald (1995) and Grosser and McCoy (1996). Thus, larval preference for these root systems does not appear to be affected by *Phytophthora* susceptibility or tolerance in these two rootstocks and there is no known reason to suspect that the results of similar comparisons between any other two citrus rootstocks would be different. However, the responses of the two rootstocks to *P. nicotianae* and its associated disease were dramatically different, consistent with those reported by Graham (1995). In all cases, root rot disease and populations of *P. nicotianae* were higher on Cleopatra mandarin rootstock than on trifoliate orange. Thus, although the levels of larval feeding injury were comparable, the levels of root rot disease were substantially different between the two rootstocks. These results suggest that the defense of trifoliate orange roots against *P. nicotianae* does not require an intact root epidermis or cortex, and that there is some other varietal difference accounting for the observed resistance (Graham, 1995).

Weevil larvae were absent from roots when *P. nicotianae* was inoculated, so the increase in root rot resulted from weevil feeding injury and not from larvae spreading infectious propagules through the soil. Although a vector-pathogen interaction between the neonate larvae and *P. nicotianae* cannot be excluded, its effects are likely secondary compared to the effect of feeding injury on pre-disposing citrus roots to *P. nicotianae* infection. Most experiments involving soil-borne insect plant pathogen interactions, however, have been co-inoculations of the insect with the pathogen (Latin et al. 1985; Leath, K. T. and A. A. Hower 1993). Our data suggest that increased root rot disease results directly from increased root wounding, so it is likely that other root-feeding insects, such as subterreanean termites (*Reticulitermes flavipes*) (Stansly, 1993; Stansly et al. 1992) or fire ants (*Solenopis invicti*) might also contribute to the development of *Phytophthora* root rot on susceptible root tissues.

The advanced larval stages prefer larger roots, such as the tap root, possibly because such roots do not become limiting food resources for their rapid growth. Further, weevil feeding injuries were not visually apparent on plated root tips. However, the percentage of root tips infected with *P. nicotianae* increased as a result of the weevil feeding injury in Experiment 1. This development of disease distal from the locus of weevil injury suggests a possible role of root wound exudates contributing to the development of *P. nicotianae* populations in the rhizosphere, which in turn can increase the probability of *Phytophthora* infection (Graham and Graham, 1994; Olunloyo, 1978; Zeinab et al. 1986). For example, Duncan et al. (1993) found that *P. nicotianae* propagule densities were correlated with increased levels of ketone and reducing sugars and it is probable that such substrates are released into the rhizosphere upon injury by the feeding *D. abbreviatus* neonates. Such nutritional resources would then become available to devel-

oping *P. nicotianae* populations, increasing the probability of infection at root locations distal from the insect feeding locations.

Root rot disease indicators increase in the presence of weevil injuries; thus, increased emphasis should be placed on the proper dual management of root rot when subterranean insect injuries are likely on susceptible rootstocks. Presently, however, there are no clearly defined tactics for managing *P. nicotianae* in such plantings. Further, it is possible that other, less virulent microbial pathogens may become of concern due to the weakening of root defense systems by larval feeding. The preliminary results of this study suggest that *P. nicotianae* management considerations should be more actively integrated into IPM programs on *P. nicotianae*-sensitive and -tolerant rootstocks in *D. abbreviatus*-infested groves.

Acknowledgments

Literature Cited

1. Duncan, L. W., J. H. Graham and L. W. Timmer. 1993. Seasonal patterns associated with *Tylenchulus semipenetrans* and *Phytophthora parasitica* in the citrus rhizosphere. Phytopathol. 83:573-581.
2. Graham, J. H. 1990. Evaluation of tolerance of citrus rootstocks to *Phytophthora* root rot in chlamydospore-infested soil. Plant Dis. 74:743-746.
3. Graham, J. H. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. Phytopathol. 85:111-117.
4. Graham, J. H. and L. W. Timmer. 1992. *Phytophthora* diseases of citrus, p. 250-269. In: Plant Diseases of International Importance: Volume III: Diseases of Fruit Crops. J. Kumar, H. S. Chaube, U. S. Singh and A. N. Mukhopadhyay (eds.). Prentice Hall, Englewood Cliffs, New Jersey.
5. Graham, J. H. and N. H. Timmer. 1991. Peat-based media as a source of *Thielaviopsis basicola* causing black root rot on citrus seedlings. Plant Dis. 75:1246-1249.
6. Graham, M. Y. and T. L. Graham. 1994. Wound-associated competency factors are required for the proximal cell responses of soybean to the *Phytophthora sojae* wall glucan elicitor. Plant Physiol. 105:571-578.
7. Grosser, J. and C. W. McCoy. 1996. Feeding response of first instar larvae of *Diaprepes abbreviatus* to different novel intergeneric citrus somatic hybrids. Proc. Fla. State Hort. Soc. 109:62-66.
8. Latin, R. X. and G. L. Reed. 1985. Effect of root feeding by striped cucumber beetle larvae on the incidence and severity of *Fusarium* wilt of muskmelon. Phytopathol. 75:209-212.
9. Leath, K. T. and A. A. Hower. 1993. Interaction of *Fusarium oxysporum* f. sp. *medicaginis* with feeding activity of clover root curculio larvae in alfalfa. Plant Dis. 77:799-802.
10. Manners, J. G. 1993. Principles of Plant Pathology, p. 76-78. University Press, Cambridge, United Kingdom.
11. McCoy, C. W., L. W. Duncan, E. D. Quintela, R. F. Mizell, J. Fojtek, S. Rogers and S. R. Krueger. 1996. An update on a developing IPM strategy for the control of citrus root weevil larvae in soil. Citrus Ind. 76:9-11.
12. McCoy, C. W., E. D. Quintela, S. E. Simpson and J. Fojtik. 1995. Effect of surface-applied and soil-incorporated insecticides for the control of neonate larvae of *Diaprepes abbreviatus* in container-grown citrus. Proc. Fla. St. Hort. Soc. 108:130-136.
13. Olunloyo, O. A. 1978. The relation of sugary exudate and insects to fungal infection of developing cashew nuts (*Anacardium occidentale*) in the plantation. Plant Dis. Rep. 62:416-420.
14. Shapiro, J. P. and T. R. Gottwald. 1995. Resistance of eight cultivars of citrus rootstock to a larval root weevil, *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae). J. Econ. Entomol. 88:148-154.
15. Stansly, P. A. 1993. Causes and prevention of injury to young citrus by termites. Citrus Ind. 74:98-100.
16. Stansly, P. A., R. E. Rouse and S. B. Davenport. 1992. Protection of young citrus trees from damage by subterranean termites. Proc. Fla. St. Hort. Soc. 105:7-10.
17. Tsao, P. H. 1971. Chlamydospore formation in sporangium-free liquid cultures of *Phytophthora parasitica*. Phytopathol. 61:1412-1413.
18. Woodruff, R. E. 1964. A Puerto Rican weevil new to the United States (Coleoptera: Curculionidae). Fla. Dept. Agric. Div. Plant Ind. Entomol. Circ. 30:1-2.
19. Zeinab, A., Z. A. El-Hamalawi and D. C. Erwin. 1986. Components in alfalfa root extract and root exudate that increase oospore germination of *Phytophthora megasperma* f. sp. *medicaginis*. Phytopathol. 76:508-513.

FEEDING RESPONSE OF FIRST INSTAR LARVAE OF *DIAPREPES ABBREVIATUS* TO DIFFERENT NOVEL INTERGENERIC CITRUS SOMATIC HYBRIDS

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Abstract. **The larvae of the citrus root weevil, *Diaprepes abbreviatus* (L.) will inflict severe injury to young tree roots of all rootstocks grown commercially in Florida. In an attempt to find germplasm with resistance to larval feeding, the following intergeneric somatic citrus hybrids were screened in the greenhouse using first instar larvae as an inoculum source: 'Succari' sweet orange + *Citropsis gilletiana*, 'Succari' + *Microcitrus papuana*, 'Succari' + 'Meiwa' kumquat, 'Succari' + *Atalantia ceylanica*, 'Nova' tangelo + *Citropsis gilletiana*, and 'Nova' + *Citrus ichangensis* (an interspecific hybrid). Sour orange and Swingle citrumelo rootstocks served as standards. Cohen citrange (a triploid) and 'Limon Gigante' (provided by W. S. Castle) were also included in the study. Somatic hybrids, other test plants and controls planted in plastic containers in Fafard Citrus B soil mix were exposed to larvae for 45-70 days in two completely randomized experiments. Larval weight and survival, root biomass consumption, and gross root injury ratings were per-**