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WATER STRESS AND ROOT INJURY FROM SIMULATED FLOODING AND *DIAPREPES ABBREVIATUS* ROOT WEEVIL LARVAL FEEDING IN CITRUS

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Environmental stress from flooding can occur simultaneously with root weevil infestation to damage plant root systems. We conducted two factorial studies of flooding duration and *Diaprepes abbreviatus* (L.) root weevil larval feeding injury on citrus in the greenhouse in 2002 and 2003. Our objectives were to investigate the effect of soil anoxia by simulated flooding on plant water stress and the impact of prior flooding on root susceptibility to subsequent larval weevil feeding. The treatments consisted of two rootstock varieties, Swingle citrumelo [SWI; *Citrus paradisi* Macfad × *Poncirus trifoliata* (L.) Raf.] and Smooth Flat Seville (SFS; *Citrus aurantium* L.), flooding durations of 0, 10, 20, 30, or 40 days, and *Diaprepes* larval infestations of 0 and 5 neonates per seedling for 40 days. We used a Candler sand with 8 replicates in Experiment I and a Floridana loam with 15 replicates in Experiment II. Treatments were arranged in a completely ramdomized design. Plants were flooded, drained for a week, and then 1-day-old neonate larvae were introduced onto the soil surface of each seedling. Flooding significantly reduced soil redox potential (E_h), leaf stomatal conductance (g_s), and shoot growth ($P < 0.05$). Soil E_h decreased from +220 to −100 mV within 1–3 days after flooding, and leaf g_s declined from 260 to 80 mmol m^{-2} s^{-1} within 20 days of flooding. Flood-injured and larval-injured roots had little growth. With equal previous flooding durations (20 days), the larval survival was on average 25% higher in sandy soil than in loamy soil. Twenty-day prior flooded roots were more water stressed and also more susceptible to *Diaprepes* larval feeding injury. It is suggested that limited soil waterlogging and early root weevil larval control would be useful for plant protection. (Soil Science 2006;171:138–151)

Key words: Citrus leaf stomatal conductance, *Diaprepes* root weevil, flooding stress, larval survival, root injury, soil redox potential.

FLOODING events and soil waterlogging can be critical stress factors for citrus trees. In Florida, citrus is often cultivated on low-lying flatwoods soils with poor drainage (Boman and Obreza, 2002). Plant growth is related to demands of oxygen, water, and nutrition from roots, leaves, and shoots (Steudle and Peterson, 1998). The influence of flooding and waterlogging on the plant–soil system has received much attention (Dat et al., 2004; Kaelke and Dawson, 2003; Li et al., 2003, 2004a; Oren et al., 2001; Pezeshki and Delaune, 1998; Ruiz-Sanchez et al., 1996; Syvertsen et al., 1983). It was established that flooding and waterlogging cause oxygen deprivation in the root system and lead to a disturbance of the soil–plant system equilibrium, damage to roots, decline in plant growth, and a long-term negative impact on

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soil–plant systems (Li et al., 2003, 2004a; Oren et al., 2001; Pezeshki and Delaune, 1998; Saqib et al., 2004; Yoo and James, 2003).

Soil oxidation–reduction (redox) potential (E_h), an indicator of the capacity of oxidation (positive E_h) or reduction (negative E_h) of the soil, was reduced by soil waterlogging that translated into a greater demand for oxygen within the soil and increased plant water stress (Li et al., 2003; Patrick et al., 1996; Pezeshki and Delaune, 1998; Syvertsen et al., 1983). Soil E_h influenced nutrient mobilizing for plant uptake during a sequence of oxic–anoxic–oxic transitions (Jayaweera and Bigger, 1996). Plant physiological responses to flooding stress were limited, as shown by a decrease in leaf stomatal conductance (g_s), leaf gas exchange, leaf water potential, and leaf dry weight (Blanke and Cooke, 2004; Kreuzwieser et al., 2004; Li et al., 2003, 2004a; Mielke et al., 2003; Oren et al., 2001). Leaf conductance and leaf turgor potential were significantly reduced in flooded 2-year-old sour orange plants (Ruiz-Sanchez et al., 1996), and flooding induced stomatal closure, one of the earliest plant responses to soil inundation, due to leaf dehydration linked to a lowered root hydraulic permeability (Pezeshki and Delaune, 1998; Steudle and Paterson, 1998). In a poorly drained citrus grove, flooded trees were more water stressed than nonflooded trees, as indicated by leaf g_s, which was significantly lower in an area flooded for 3 weeks than in a nonflooded area (Li et al., 2004a).

In citrus, plant environmental stress from flooding can occur simultaneously with root weevil infestations (Li et al., 2004a). The *Diaprepes abbreviatus* (L.) root weevil, originally from the Caribbean, has been dispersed primarily by nursery stock into citrus groves in Florida (Jones and Schroeder, 1983; McCoy et al., 2003; Quintela and McCoy, 1997; Rogers et al., 2000; Stuart et al., 2004). Injury inflicted by *Diaprepes* larvae on roots has resulted in plant decline and death (McCoy et al., 2003; Rogers et al., 2000; Stuart et al., 2004). *Diaprepes* neonate larvae invade the soil after hatching from eggs laid by adults in the citrus canopy, and larvae feed on tree roots and subsequently pupate in the soil (Rogers et al., 2000; Stuart et al., 2004). However, because of their small size, neonate larvae are virtually impossible to detect in the soil and the initial injury to roots can be difficult to quantify (Jones and Schroeder, 1983; Quintela and McCoy, 1997; Rogers et al., 2000).

The soil environment influenced plant growth and the abundance of most herbivorous insects (Orians and Fritz, 1996). Flooding increased soil pH, decreased nutrient availability and leaf dry matter yield (Yoo and James, 2003), but leaf beetle larval pupal weight was not influenced by nutrient or flooding conditions to the plant (Lower et al., 2003). In the *Diaprepes* root weevil, flooding duration and floodwater pH influenced larval survival (Li et al., 2004b; Shapiro et al., 1997), and citrus root injuries from larval feeding were associated with larval density, rootstock variety, soil type, and moisture (Rogers et al., 2000). It was reported that the ability of citrus seedlings to tolerate larval feeding differs among rootstocks and the correlation between root loss and larval weight gain were significantly positive (Li et al., 2004b). It was estimated that injury to different rootstocks growing in well-drained soil ranged from 50% to 80% by 40 days after infestation by two to five *Diaprepes* neonate larvae, and many root tissues were completely consumed after 79 days (Rogers et al., 2000). However, no information about plant growth or root injury related to larval survival was reported in the study of Shapiro et al. (1997), and plants were not subjected to flooding in the study of Rogers et al. (2000). It is not known whether the distribution patterns of *Diaprepes* root weevils were associated with flooding, or whether the responses of flooded and nonflooded trees to larval feeding differed. There is a need for information about the relative vulnerability of flood-damaged seedlings to larval feeding injury.

We hypothesized that flooded roots would be more susceptible to injury by *Diaprepes* root weevil larvae than nonflooded roots, and that the combination of flooding and larval feeding might complicate treatments for weevil control. In this study, we investigated how the combined effects of soil flooding and *Diaprepes* larval infestation influence citrus growth and root damage. Specifically, the objectives of the study were to (i) examine temporal changes in citrus soil E_h, plant growth, and leaf g_s of two rootstock varieties under different flooding durations; (ii) determine the impact of prior flooding on citrus plant root susceptibility to subsequent larval weevil feeding; and (iii) compare *Diaprepes* larval survival and seedling root injury with equal prior flooding durations in different types of soils. We expected plants and larvae to grow better in nonflooded conditions than in flooded conditions because

flooding prohibits gas exchange in the plant-soil system (Kreuzwieser et al., 2004; Mielke et al., 2003; Yoo and James, 2003) and flooded soil is compacted (Saqib et al., 2004). We attempted to quantify how long seedling plants can tolerate flooding stress. This information should contribute to our understanding of the relationship of citrus water stress to larval root injury in the field and might suggest management options for root weevil larval control.

MATERIALS AND METHODS

Citrus Cultivation

Two greenhouse studies (Experiments I and II) were conducted at the Citrus Research and Education Center, University of Florida. Two commercial citrus rootstock varieties, Swingle citrumelo [SWI; *Citrus paradisi* Macfad × *Poncirus trifoliata* (L.) Raf.] and Smooth Flat Seville (SFS; *Citrus aurantium* L.), were used for both studies. Three-month-old seedlings were obtained from a commercial nursery (Reed Bros Nursery, Dundee, Florida). The peat-moss-based soil less potting media were gently washed from the roots, and bare-root seedlings were each transplanted into a single 130-cm^3 plastic pot 2 weeks before seedling submergence. Seedlings were selected for uniformity of root density and canopy size for each variety.

A CRD was used to arrange the treatments as defined by variety and flooding duration in the greenhouse. The transplanted seedlings were randomly placed in a pot tray. In each experiment, there were three separate procedures, flooding, draining, and larval feeding (Table 1). Seedlings were flooded simultaneously by submerging the tray for the flooding test. In each experiment, the flooding procedure was completed before neonate larvae were introduced to seedlings for the root feeding injury test. The soils were not flooded during the larval feeding period during the experiments.

Experiment I

Experiment I was conducted between October and December 2002. The treatments consisted of two citrus varieties, SWI and SFS, four flooding durations (0, 10, 20, or 30 days), and two levels of *Diaprepes* larvae (0 or 5 neonates per seedling). Soil was a Candler fine sand, a typic quartzsamment containing 965 g kg^{-1} of sand, 20 g kg^{-1} of silt, 15 g kg^{-1} of clay, 10 g kg^{-1} of organic matter, and 5.5 mg kg^{-1} of extractable P. The design was as follows: 2 varieties × 4 flooding durations × 8 replicates, infested by five *Diaprepes* neonate larvae per seedling; we also added a nonflooded, nonlarvae (NF-ND) control, which were 2 varieties × 8 replicates. There were a total of 80 seedlings in Experiment I (Table 1).

On October 1, 2002, the 30-day (F30) flooding treatments were submerged in a 1.5 × 0.5 × 0.8 m plastic tub. Submergence was initiated to 2 cm above the tops of seedling pots and the shoots remained in the atmosphere. The floodwater surface was covered with polyvinyl pieces to reduce water evaporation and minimize gas exchange into the system. Floodwater temperature was on average 26/23 °C day/night. After 10 days, the 20-day (F20) flooding treatments were submerged, and 10 days later the 10-day (F10) treatments were submerged. On October 30, all flooded plants were removed from the water, and the seedlings were drained for 1 week.

TABLE 1

Experimental treatments and procedures

1. Flooding procedures (one seedling in each 130-cm^3 pot).		
Treatments	Experiment I (Candler sand)	Experiment II (Floridana loam)
Variety	SWI, SFS	SWI, SFS
Flooding (days)	0; 0, 10, 20, 30 (8 replicates)	0, 20, 40 (15 replicates)
2. Draining for 1 week for flooded seedlings after flooding termination.		
3. Larval feeding for 40 days using flooded and nonflooded seedlings after draining procedures.		
Larvae (5 neonates)	Flooding 0, 10, 20, and 30 days (8 replicates)	Flooding 0, 20, and 40 days (10 replicates)
Control (0 neonate)	Flooding 0 day (8 replicates)	Flooding 0, 20, and 40 days (5 replicates)
Total seedlings	2 × 4 × 8 = 64 (with larvae)	2 × 3 × 10 = 60 (with larvae)
	2 × 8 = 16 (nonflooded and nonlarval control)	2 × 3 × 5 = 30 (nonflooded and flooded larval control)

SWI, rootstock Swingle; SFS, rootstock Smooth Flat Seville.

For testing flooded root injury from *Diaprepes* larval feeding, *Diaprepes* neonates were obtained from eggs laid by field-collected adults confined to screen cages at a temperature of 25 ± 2 °C. One-day-old neonates were selected, just before the larval infestation, for high vigor using the light drop procedure (Quintela and McCoy, 1997). The initial neonate weights were determined using three sets of 100 one-day-old neonates. Five active neonate larvae were carefully placed in a tube and then scattered onto the soil surface of each seedling pot. The inoculated neonates moved into the moist soil quickly and exhibited positive geotaxis, as described in Jones and Schroeder (1983). There was a 1 ± 0.3 cm gap between the soil surface and the top of the pot, and as in previous studies, no further steps were taken to prevent the escape of neonates from pots before soil penetration (Rogers et al., 2000).

Experiment II

Experiment II was conducted between June and September 2003 and incorporated a different soil type, longer flooding durations, and more replicates. The treatments were SWI and SFS, three levels of flooding duration (0, 20, or 40 days), and two levels of *Diaprepes* larvae (0 or 5 larvae per seedling; Table 1). We used a Floridana loamy soil, collected at a depth of 0–0.3 m in an orange grove in Osceola County, Central Florida (28°07′40″ N, 81°21′10″ W). The grove has been damaged by *Diaprepes* root weevil over the last 10 years. The soil was classified as Siliceous, Hyperthermic, Arenic Argiaquolls Alfisols. The soil contained 527 g kg^{-1} of sand, 323 g kg^{-1} of clay, pH 4.9, 80 g kg^{-1} of organic matter, 15 Cmol kg^{-1} of cation exchangeable capacity, 35 mS m^{-1} of electrical conductivity, and 22, 114, 259, and 992 mg kg^{-1} of exchangeable P, K, Mg, and Ca. Using this loamy soil allowed a comparison of larval survival and root injury by prior flooding and current larval feeding in different soils with the 20-day flooded treatments in Experiments I and II (Table 1). The long flooding duration treatment (40 days) in Experiment II was to assess if longer flooded roots would be more vulnerable to larval feeding injury.

The design for the flooding procedure in Experiment II was as follows: 2 varieties × 3 flooding durations × 15 replicates = 90 seedlings (Table 1). The flooding procedure was done using the same method described for Experiment I. The 40-day (F40) flooding treatment was submerged 20 days before the 20-day (F20) flooding treatment. After the termination of the flooding procedure, all flooded plants were allowed to drain for 1 week.

For the *Diaprepes* larval feeding test, 10 seedlings were taken randomly per nonflooded (NF) and flooded (F) treatment, and then five 1-day-old neonate larvae were inoculated onto the soil surface for each seedling, using the method described in Experiment I (Table 1). The larval feeding (D) period was also 40 days, the same length as in Experiment I. Per nonflooded and flooded treatment, five seedlings received no larvae (ND). The full array of treatments was NF-ND, NF-D, F20-ND, F20-D, F40-ND, and F40-D.

For both experiments, plants were irrigated and fertilized every other day. The fertilizer was a commercial nutrient solution with pH 4.5 and an electrical conductivity of 250 mS m^{-1}. Per liter of nutrient solution, there were 237 mg of N, 31 mg of P, 90 mg of K, 398 mg of Ca, 18 mg of Mg, 42 mg of S, and additional recommended micronutrients of B, Cu, Zn, Mn, and Mo. About 30 mL of diluted (50%) fertilizer was applied to a seedling each time, which was sufficient to cause leaching. During the larval feeding period, infested and noninfested seedlings received the same rates of fertilization and irrigation. Air temperature and relative humidity in the greenhouse were controlled during the times of flooding and larval feeding. The greenhouse was maintained at an air temperature of 28 ± 4 °C and a relative humidity of 35 ± 5% throughout the two experiments.

Soil, Plant, Root, and Larval Measurements and Data Analysis

Floodwater temperature was measured using an Omega HH64 thermometer (Omega Engineering, Stamford, Connecticut). Soil E_h was measured using the method described in (Patrick et al., 1996), using an Orion oxidation–reduction probe (ORP, Model 290A, Orion Research Inc., Boston, Massachusetts). The electrode was calibrated using a standard solution of 240 mV. The E_h was measured in each pot at 10:00–11:00 in the morning. Measurements were taken every day during the flooding period and twice per week during the larval infestation period.

Leaf g_s was measured using a Delta-T porometer (Delta-T Devices, Cambridge, UK). For each seedling, the first fully expanded leaf, situated about 3 cm below the shoot tip, was

selected as a representative leaf for measurement. Leaf g_s was measured twice per week at 9:00–10:00 A.M., and seedling shoot length was measured using a ruler every 2 weeks throughout the experiment. Leaf area was determined at the end of each experiment using a LI-COR leaf area meter (model LI-3000, Lambda Instruments, Lincoln, Nebraska).

Soil surface gaps (distance between the soil surface to the top of the pot) were measured for each pot using a rule before and after the flooding–draining procedure. Larval survival rate, larval weight, root damage by flooding and larval feeding, and root, leaf, and shoot dry weights were evaluated at the end of the experiments. Each plant was removed from the pot and placed on a shallow examination tray. A spatula was used to gently remove the soil from around the roots. Larvae were removed from the soil and counted for each seedling. Weights of surviving larvae per seedling were determined using a Mettler AM100 balance (Mettler Instrument Crop, Hightstown, New Jersey). Root damage by flooding and larval feeding was assessed after determining larval survival. The root damage rating was visually assessed by reference to the undamaged control treatment (NF-ND) and by classifying the whole seedling root system by percentage damage as 0% (control), 0–25%, 25–50%, 50–75%, and >75% damage (Rogers et al., 2001).

The effects of treatments (variety, flooding duration, and larval infestation) on plant and soil-water stress, seedling growth, root damage, and larval survival were evaluated by analysis of variance using the general linear models procedure (SAS Institute, 1990). Homogeneity of variance of data sets was verified using the Bartlett test, and normality and residual distribution of data sets were confirmed using PROC UNIVARIATE. Comparison of treatment means was done using the LSD test (SAS Institute, 1990).

RESULTS

Soil Redox Potential

Flooding had a significant effect on soil E_h (Table 2). The E_h became negative within 1–3 days of flooding and then became positive within 1 day after the seedlings were removed from the floodwater (SWI, Experiment I; Fig. 1). There were abrupt decreases of soil E_h following submergence of the F30, F20, and F10 treatments, which showed a complete lack of oxygen was attained as quickly as 1 day after submergence. For SWI, the soil E_h varied between -8 ± 142 mV during the first 10 days of flooding and was reduced to -129 ± 43 mV within 20 days of flooding. The soil E_h increased slightly during the draining period and then became constant (216–237 mV) during larval infestation. The E_h remained high (mean 216 mV) for the nonflooded SWI seedlings in the atmosphere (Fig. 1). The difference in E_h was greater for the contrast of F10 vs. F30 than F20 vs. F30 during the flooding period (Table 2). The E_h patterns also varied with flooding for SFS (data not shown), similar to the changes in E_h in the SWI shown in Fig. 1.

Changes in E_h for the two varieties in Experiment II were caused by flooding (Table 3). The development pattern of E_h as a function of flooding in Experiment II was comparable to that of Experiment I (graph not shown). For the longer flooding period (40 days), the soil E_h was

TABLE 2

Contrast for soil redox potential (E_h), leaf stomatal conductance (g_s), shoot length, larval survival, and root rating for treatments during the flooding period and larval feeding period for all seedlings in Experiment I

Contrasts	*df*	E_h[†]	g_s[†]	Shoot length[†]	Larval survival[†]	Root rating[†]
Flooding period						
NF vs. F[††]	1	1018**	8.22**	15.6**		
F10 vs. F20[††]	1	7.96**	4.6*, ns	4.7*		
F10 vs. F30[††]	1	6395**	17.1**	7.6**		
Larval feeding period						
ND vs. D[†††]	1	45.7**	3.7, ns	8.6**		6.8**
NF-ND vs. NF-D[†††]	1	2.5, ns	0.1, ns	3.2, ns		6.0*
F10-D vs. F20-D[†††]	1	1.4, ns	5.7*	1.2, ns	26.4**	27.1**
F10-D vs. F30-D[†††]	1	3.5, ns	14.0**	2.1, ns	461.7**	41.3**

[†]*F* values. ns, nonsignificant; * and **significant at $P < 0.05$ and $P < 0.01$, respectively.

[††]F10, 10-day flooded; F20, 20-day flooded; F30, 30-day flooded; F40, 40-day flooded treatments.

[†††]ND, no *Diaprepes* larvae; NF, nonflooded treatments.

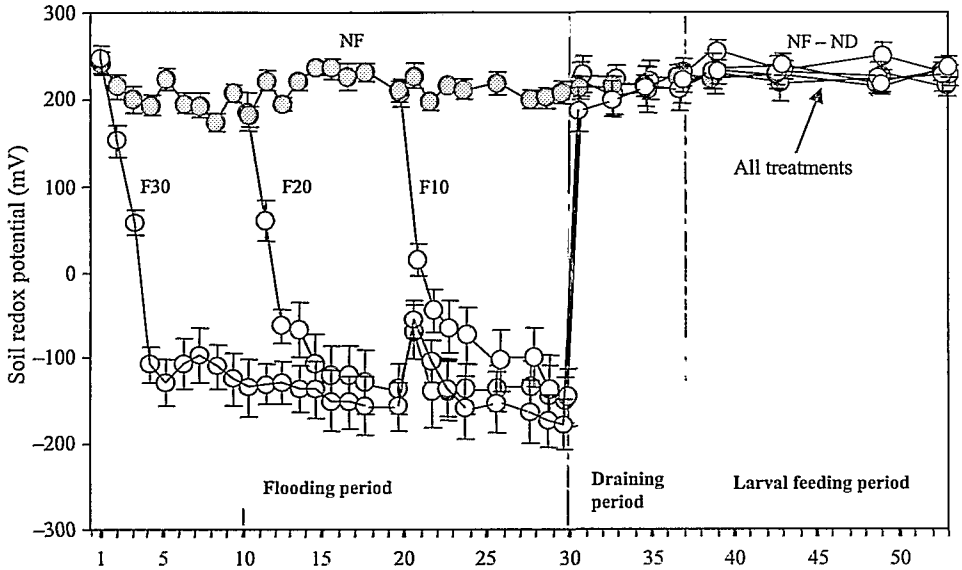

Fig. 1. Temporal patterns of soil redox potential (E_h) for Swingle citrumelo (SWI) throughout Experiment I. NF, nonflooded; F30, 30-day flooded; F20, 20-day flooded; F10, 10-day flooded treatments. Each point represents the mean and standard error of eight measurements.

lower (−230 ± 40 mV) than that measured for the 30-day flooding period in Experiment I. Soil E_h remained constant in the nonflooded treatments throughout the two experiments as shown in Fig. 1.

Leaf Stomatal Conductance

Leaf g_s varied significantly between the flooding treatments in Experiment I (Table 2) and in Experiment II (Table 3). The model root mean square errors (RMSE) for the g_s were the highest among all measured variables (Table 3). The g_s tended to decrease in all flooded treatments in Experiment I (Fig. 2). The g_s values for the F30 treatments in SWI started to decrease after 2 days of flooding and then decreased consistently with time. During recovery from flooding, leaf g_s increased and then the values decreased again following larval infestation (Fig. 2).

TABLE 3

Effects of citrus rootstock, flooding duration, *Diaprepes* larval infestation and interactions of the treatments on soil redox potential (E_h), leaf stomatal conductance (g_s), seedling shoot length growth, larval survival, root damage rating, root dry weight, and leaf area in Floridana loam in Experiment II

Sources	*df*	E_h[+]	g_s[†]	Shoot length[†]	Leaf area[†]	Root dry weight[†]	Larval survival[†]	Root rating
Variety (V)	1	ns	ns	13.8**	6.57**	14.6**	7.5**	6.33**
Flooding (F)	2	100.6**	3.67*	21.5**	20.5**	51.0**	9.1**	25.3**
Diaprepes larvae (D)	1	ns	ns	4.77*	ns	22.1**	168**	66.2**
V × F	2	ns	ns	ns	3.77*	ns	7.34**	ns
V × D	1	ns	ns	ns	ns	ns	3.75*	ns
D × F	2	ns	ns	ns	ns	ns	4.55*	6.54**
V × F × D	2	ns	ns	ns	ns	ns	3.67*	ns
Model R^2		0.80**	0.22ns	0.54**	0.55**	0.70**	0.78**	0.74**
CV		4.94	105	62.3	36.1	43.3	51.8	32.5
Mean[††]		357	46.3	3.85	78.1	0.81	1.94	2.97
RMSE[†††]		17.6	48.9	2.39	28.2	0.35	1.00	0.96

[+] *F* values. ns, nonsignificant; * and **significant at $P < 0.05$ and $P < 0.01$, respectively.

[††] Mean E_h in mV; g_s in mmol m^{-2} s^{-1}; shoot length in cm; root dry weight in g; and leaf area in cm^2.

[†††] RMSE: root mean square error.

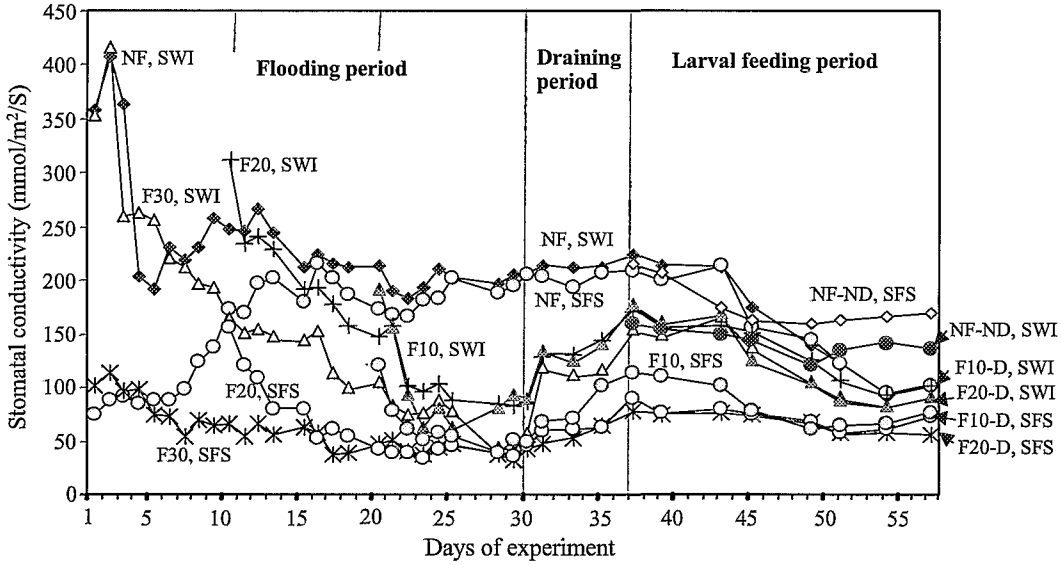

Fig. 2. Temporal patterns of leaf stomatal conductance (g_s) of Swingle citrumelo (SWI) and Smooth Flat Seville (SFS) during Experiment I. The treatments were as follows: NF, nonflooded; F30, 30-day flooded; F20, 20-day flooded; F10, 10-day flooded. NF-ND, nonflooded and nonlarvae; F30, 30-day flooded with larvae; F20, 20-day flooded with larvae; F10, 10-day flooded with larvae. Each point represents the mean of eight measurements.

During the flooding period, the variation of leaf g_s was greater in SWI than in SFS in the two experiments, as shown by their means and standard errors (Fig. 3). During the first 10 days of flooding in Experiment I, the g_s was slightly lower than the nonflooded treatments (264 ± 104 mmol m^{-2} s^{-1} in SWI, and 83 ± 37 mmol m^{-2} s^{-1} in SFS, n = 72). The conductance in the 20-day flooded treatment was lower than the nonflooded treatment but it was 2-fold higher than the 30-day flooded treatment (Fig. 3A). There was a similar decreasing trend of leaf g_s in all treatments in SFS (Fig. 3B).

In Experiment II, the leaf g_s decreased to 86 ± 34 mmol m^{-2} s^{-1} in 20 days then to 42 ± 23 mmol m^{-2} s^{-1} within 40 days of flooding, a drop of 50% in g_s for every 10 days of flooding. In general, mean g_s decreased with flooding duration in the order of F30 > F20 > F10 > NF (Experiment I; Fig. 3A). The g_s value for the flooding control (NF) decreased slightly with time (Fig. 3A) as leaf resistance (r) to water loss increased with age ($g_s = 1/r$). As a result, mean g_s was in the order of NF > F10 > F20 > F30 for the two rootstocks in Experiment I (Figs. 3A and B).

Leaf g_s increased after the plants recovered from flooding for 1 week and then decreased progressively following larval infestation (Fig. 3C). In SWI, the NF-ND treatment had the highest g_s value (177 ± 50 mmol m^{-2} s^{-1}, n = 54), followed by the nonflooded with larvae treatment (NF-D, 153 ± 62 mmol m^{-2} s^{-1}, n = 62). The longest flooded seedlings (F30-D) had the lowest g_s (120 ± 51 mmol m^{-2} s^{-1}, n = 57) (Fig. 3C). In SFS, the mean g_s was also in the order of F10-D > F20-D > F30-D (Fig. 3D).

Changes in g_s in Experiment II were also mainly due to flooding (Table 3). Mean g_s was small (46 ± 28 mmol m^{-2} s^{-1}) and decreased with flooding duration in the order of F0-D < F20-D < F40-D. There was a significant difference in g_s between SWI and SFS ($P < 0.001$). Leaf g_s was significantly higher in F10 than F30 in SWI and SFS ($P < 0.001$), and g_s was significantly greater in F10-D than F30-D (ANOVA contrasts not shown).

Shoot Growth, Leaf Area, and Root Dry Weight

Flooding affected significantly shoot growth in Experiment I (Table 2). With initial shoot lengths of 39.4 ± 3.9 cm in SWI and 27.6 ± 6.4 cm in SFS, shoot lengths grew faster in SWI (2.7 ± 1.5, 1.5 ± 1.2, and 0.3 ± 0.6 cm) than in SFS (0.3 ± 0.5, 0.4 ± 0.7, and 0.3 ± 0.4 cm) for the F10, F20, and F30 treatments, respectively, during the flooding period. However, shoot growth was greater in SFS (3.1 ± 5.1, 1.4 ± 1.8, and 0.1 ± 0.1 cm) than in SWI (0.2 ± 0.1, 0.1 ± 0.1, and 0.3 ± 0.3 cm) for the previously flooded

F10, F20, and F30 treatments, respectively, during the larval infestation period. There was a significant difference in shoot growth for larval feeding treatments compared to nonlarval feeding treatments (Table 2).

The main effect of flooding was significant on shoot growth, leaf area, and root dry weight in Experiment II (Table 3). Total leaf area was significantly higher in the nonflooded (124 cm^2 in SFS and 100 cm^2 in SWI) than in the flooded seedlings (Figs. 4A and B). Leaf area was affected significantly by the interaction of variety and larval infestation (Table 3). Maximum shoot length growth of 8.3 ± 3.1 cm occurred in the nonflooded and nonlarvae treatment in SWI (Fig. 4C), and differences in shoot growth were significant (Figs. 4C and D). Root dry weight showed similar patterns to shoot growth in all treatments (Figs. 4E and F). The greatest mean and standard deviation of root dry weight (1840 ± 430 mg) was found in NF without larval feeding in SWI. The root dry weight was in the order of NF > F20 > F40 in SWI and in SFS, and the longer flooding F40 resulted in no growth in shoots or roots (Figs. 4E and F).

Larval Survival, Larval Weight, and Root Damage of Previously Flooded Seedlings

Larval survival was significantly different between the previously flooded treatments in Experiment I (Table 2). With an initial infestation of five neonate larvae per seedling, the lowest larval survival was found in the nonflooded treatment (60 ± 22%), and the survival rate was significantly greater in the longest flooded treatments, 30-day flooded (88 ± 10%) for both varieties. However, no difference in larval survival was found between the two varieties (SFS, 82 ± 14%; SWI, 78 ± 22%).

In Experiment II, flooding and larval infestation showed significant effects on larval survival and root damage, and the RMSE were small (Table 3). In addition to the significant effect

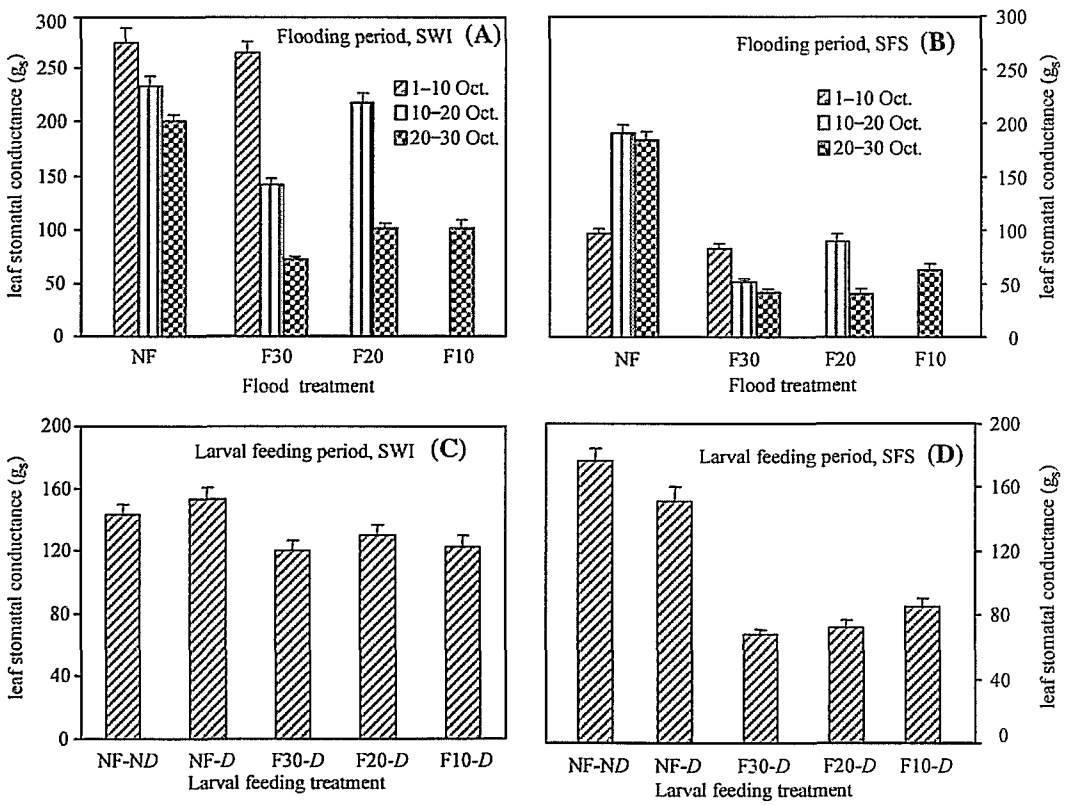

Fig. 3. Leaf stomatal conductance (g_s) of Swingle citrumelo (SWI) and Smooth Flat Seville (SFS) in Experiment I. The g_s was related to flooding duration (A–B) and *Diaprepes* larval feeding (C–D). NF, nonflooded; F30, 30-day flooded; F20, 20-day flooded; F10, 10-day flooded treatments; ND, no *Diaprepes* larval infestation; D, *Diaprepes* larval infestation. Each bar represents the mean and standard error of 120 measurements for NF, ND, and F30, 80 measurements for F20, and 40 measurements for F10.

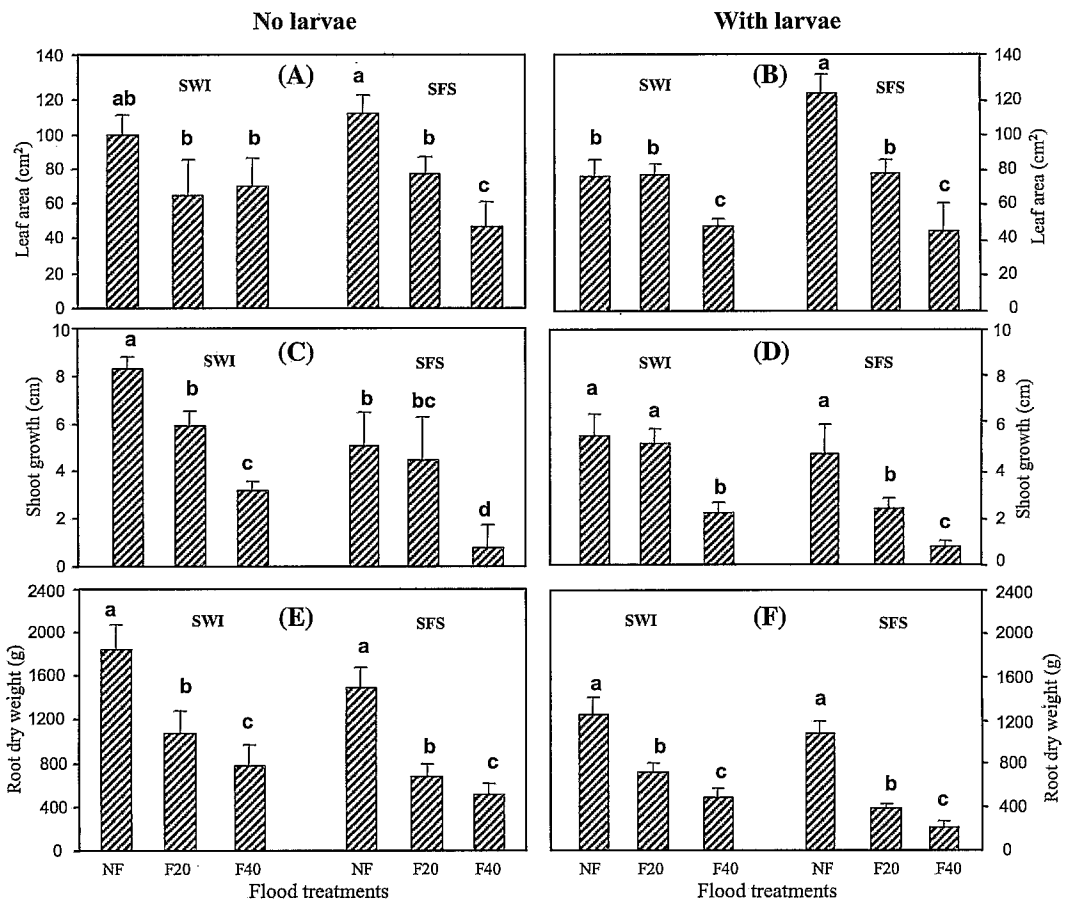

Fig. 4. Comparison of leaf area (A–B), shoot growth (C–D), and root dry weight (E–F) with and without *Diaprepes* larval feeding in Experiment II. The treatments were as follows: SWI, Swingle citrumelo; SFS, Smooth Flat Seville; NF, nonflooded; F20, 20-day flooded; F40, 40-day flooded. Each bar represents the mean and standard error of 15 measurements.

of flooding and larval infestation, the effect of variety and the interaction between all treatments were significant on larval survival (Table 3). Larval survival was significantly higher in SWI (66 ± 19%) than in SFS (50 ± 22%), and the longest flooding duration F40 had the lowest larval survival rate (16%) in SFS (Fig. 5A).

For the SWI and SFS seedlings previously flooded for 20 days, the larval survival means, standard deviations, and total percentages were significantly higher (Table 4) in the Candler sandy soil (mean 81%, n =16, Experiment I) than in the Floridana loamy soil (mean 56%, n = 20, Experiment II). The larval survival was on average 25% higher in the Candler sandy soil than in the Floridana loamy soil with the same prior flooding durations, 20 days. Combining all prior flooded treatments together, larval survival rate was up to 38% higher in the Candler sandy soil than in the Floridana loamy soil. It was to note that before larval feeding, the soil surface gaps (distance from the soil surface to the top of the pot) were significantly different between the Candler sandy soil and the Floridana loamy soil. The soil surface gap was more than two times greater in the Floridana loamy soil than in the Candler sandy soil after the flooding–draining procedures (Table 4).

Total weight of larvae varied between 16 and 169 mg per seedling (Experiment II). The initial larval weight was on average 0.45 mg (five 1-day-old neonates). Larval weight increased by 36–375 times after 38 days of feeding on seedling roots, which indicates a strong growth (or feeding) potential of neonate larvae. Larval weight decreased with the duration of

TABLE 4

Comparison of soil texture, gaps from the soil surface to the top of pots after the flooding–draining procedure, and larval survival of treatments by citrus variety (SWI and SFS). Seedlings were F20 and D for 40 days in Candler sandy soil and in Floridana loamy soil

Treatment	Sand† (g kg⁻¹)	Clay† (g kg⁻¹)	Soil-pot gap† (cm)		Larval survival†		
			Mean	S.D.	Mean	S.D.	%
Candler sandy soil (Experiment I)							
SWI-F20-D††	965[a]	15[b]	1.3[b]	0.4[b]	4.3[a]	1.02[b]	85[a]
SFS-F20-D††	965[a]	15[b]	1.4[b]	0.5[b]	3.7[ab]	0.81c	76[a]
Floridana loamy soil (Experiment II)							
SWI-F20-D††	527[b]	323[a]	3.1[a]	1.1[a]	3.2[b]	1.03[b]	64[b]
SFS-F20-D††	527[b]	323[a]	3.4[a]	1.3[a]	2.6c	1.72[a]	58[b]

†The same letters in the same column are not significantly different at $P < 0.05$.

††SWI, Swingle; SFS, Smooth Flat Seville; F20, prior flooding 20 days; D, *Diaprepes* larval infestation.

previous flooding, and the lowest larval weight was found in the longest flooded treatment F40 in SFS (Fig. 5B). The nonflooded (NF) seedlings had the highest larval weight in SWI (169 ± 90 mg) and in SFS (113 ± 76 mg), and the difference in larval weight was significant between SWI and SFS (Fig. 5B).

Root injury by larval feeding increased with the duration of previous flooding in Experiment I. The injury ranged between 0% and 3% for the nonflooded roots, 0–6% for the 10-day and 20-day flooded roots, and 3–12% for the 30-day flooded roots in SWI and SFS. In Experiment II, root injury by larval feeding was relatively low in all treatments.

Root injury was attributed to both flooding and larval infestation (Table 3). Whole seedling root damage by flooding and larval feeding increased with flooding duration (Fig. 6). About 25–50% of root damage was attributed to flooding of 20–40 days (Fig. 6A). The root damage rating of the previously flooded seedlings increased significantly to 50–75% after 40 days of larval feeding, and the highest root damage rating was found for the longest flooding duration (Fig. 6B).

DISCUSSION

Plant Water Stress and Root Injury vs. Flooding and Larval Infestation

The decrease of leaf g_s from flooding (Fig. 2) was associated with the negative soil E_h under flooding conditions (Fig. 1). Plant growth requires a free exchange of atmospheric gases, and plant roots can easily be suffocated by water saturating the root environment (Dat et al., 2004; Mielke et al., 2003; Oren et al., 2001; Steudle and Peterson, 1998; Syvertsen et al., 1983). Initially, the floodwater contained oxygen, but this was depleted within hours and the soil E_h became negative within 1 day (Fig. 1). When the aerated soil was waterlogged, it quickly became anaerobic causing plant stress. Soil E_h is directly related to soil aeration (Jayaweera and Bigger, 1996; Mielke et al.,

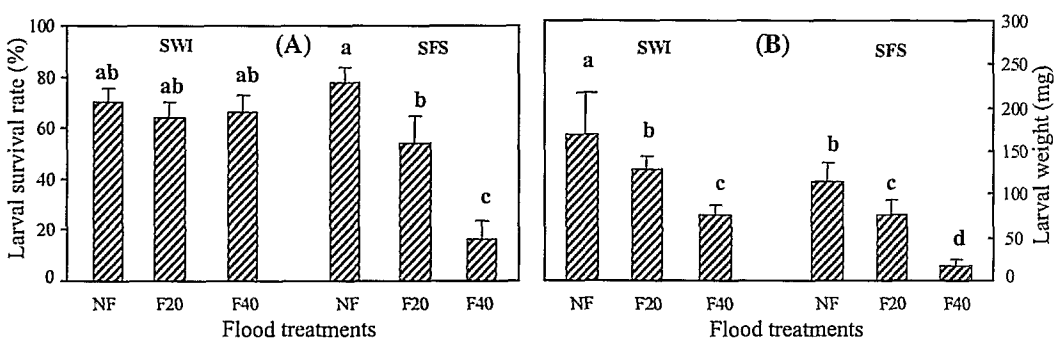

Fig. 5. *Diaprepes* larval survival (A) and larval weight (B) by flooding duration in Swingle citrumelo (SWI) and Smooth Flat Seville (SFS) in Experiment II. Each bar represents the mean and standard error of 15 measurements. Bars with the same letters in the same panel are not significantly different at $P < 0.05$ (LSD).

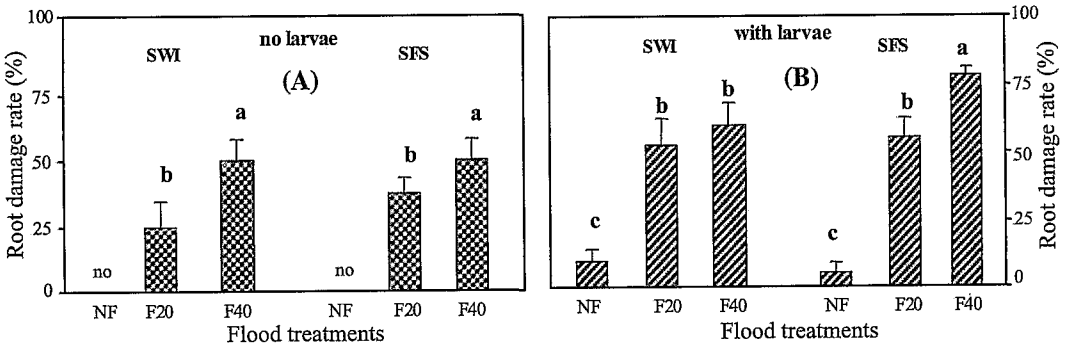

Fig. 6. Root damage percentage without larvae (A) and root damage with larvae (B) by flooding duration in Swingle citrumelo (SWI) and Smooth Flat Seville (SFS) in Experiment II. Each bar represents the mean and standard error of 15 measurements. Bars with the same letters in the same figure are not significantly different at $P < 0.05$ (LSD).

2003; Pezeshki and Delaune, 1998; Syvertsen et al., 1983). The anoxic soil conditions attained within 1–3 days of flooding (Fig. 1) differed from the findings in Syvertsen et al. (1983). It was reported that the soil E_h dropped below zero mV within a week of flooding, and that the E_h reached a minimum E_h of −250 mV after 3 weeks of flooded conditions (Syvertsen et al., 1983). The differences in these studies were probably because soil E_h varies with soil type, floodwater pH, and temperature, and consequently the E_h became stabilized within different time periods (Patrick et al., 1996; Pezeshki and Delaune, 1998; Yoo et al., 2003).

Flooding stress led to root injury, as shown by the root damage rating that was attributed to flooding (nonlarvae treatments; Fig. 6A). Anaerobic conditions inhibit almost immediately the transport of nutrient ions by roots and plant growth processes (Blanke et al., 2004; Saqib et al., 2004; Yoo et al., 2003). Seedlings damaged by long flooding experienced more water stress because of soil compaction (Saqib et al., 2004) and therefore reduced plant growth (Fig. 4), similar to that reported in Kaelke and Dawson (2003). Some Florida soils are very poorly drained and subject to flooding for periods longer than 4 months (Li et al., 2004a). It would be interesting to monitor the leaf g_s development over a longer flooding period.

Initially, 30 days of flooding reduced leaf g_s more than larval feeding injury (Fig. 3). Decreasing leaf g_s during the larval infestation period (Fig. 3B) could mean the continuous deterioration of flood-damaged roots and root injury by larval feeding. Because leaf g_s was significantly lower in flooded treatments (Fig. 3B), flood-damaged plants were more susceptible to water stress and more vulnerable to larval feeding pressure. The 20-day flooded treatment was likely a critical duration for plant flooding and larval feeding stresses because of a significant decrease of leaf g_s (Fig. 3A) and a significant increase in root damage for this flooding treatment (Fig. 6).

It is to note that day length was longer in Experiment II (June–September) than during Experiment I (October–December). Would the day length difference make any difference in citrus seedling shoot and root growth or larval survival between the two experiments? Because the air temperature and relative humidity in the greenhouse were controlled throughout the experiments, the day length difference could not greatly influence the seedling growth, root system, or larval survival under the controlled temperature conditions.

E_h depends on rate of consumption and influx of molecular oxygen and the amount of easily metabolized organic matter in the system (Mielke et al., 2003; Steudle and Peterson, 1998; Yoo and James, 2003). In our study, the floodwater surface was covered with polyvinyl pieces and the flooding system was stationary; therefore, gas exchange or influx of oxygen into the system should be minimal. However, the Candler sandy soil had little organic matter (10 g kg^{-1}) whereas the Floridana soil contained a high organic matter content (80 g kg^{-1}). It would be useful to further determine E_h and plant relations through measurements of the rates of consumption of molecular oxygen in the system and the amount of easily metabolized organic matter in the two soils.

Larval Survival vs. Soil Type and Flooding

With equal previous flooding duration (20 days), the difference in larval survival rate between Experiments I and II might be due to the difference in soil texture between the two soils (Table 4). After flooding-draining processes, the Candler soil used in Experiment I was probably more aerated because of high sand content (965 g kg^{-1}) compared to the Floridana soil used in Experiment II, which contained only 54% (527 g kg^{-1}) of the sand content in the Candler soil. As indicated, the initial soil surface gap between the soil surface and the top of the pot was 1 ± 0.3 cm for the two soils. However, the gap increased up to 3.4 ± 1.3 cm for the Floridana loamy soil compared to 1.6 ± 0.4 cm for the Candler sandy soil after flooding–draining procedures. With 21 (323 g kg^{-1} vs. 15 g kg^{-1}; Table 4) times more clay content than the Candler soil, the Floridana soil could be compacted by waterlogging.

More aeration and greater porosity in soil could be more favorable to larval survival. Soil type affected the rate of larval growth and survival, and the effect of soil type on larval survival could be primarily physical including soil moisture and oxygen levels (Rogers et al., 2000). Because we used seedling plants and small containers (130 cm^3), soil compaction and soil bulk density were not measured in this study. However, the Floridana loamy soil could have been more compacted due to the influence of the flooding–draining processes, as suggested by the significantly larger soil surface gaps (Table 4). Flooded and waterlogged soils were typically more compacted with higher bulk density than nonflooded soil (Saqib et al., 2004), which could be a problem for larval survival in flooded soil. It would be useful to further investigate the roles of soil aeration, porosity, and water content in *Diaprepes* larval survival.

It is noteworthy that the highest larval survival rate was found for the longest previously flooded treatments (flooding 30 days) in Candler sand, but that the lowest larval survival rate and larval weight gain occurred in the longest previously flooded treatments (flooding 40 days) in Floridana loamy soil (Fig. 5). In addition to soil texture, flooded soil pH could be a factor influencing larval survival. Although changes of floodwater pH and flooded soil pH were not measured in these two studies, a subsequent study conducted in the greenhouse using the same citrus varieties as in Experiment II found that the pH value of 40-day floodwater increased by 0.7 U, and the pH value of Floridana loamy soil increased by 0.1–0.3 U by the end of a 40-day flooding period (Li et al., 2004b). The higher pH of the floodwater was related to the duration of flooding because of the depletion of oxygen from the floodwater. Floodwater pH increased by 0.9 U (from 7.9 to 8.8) after 5 weeks of flooding, and larval mortality increased with floodwater pH with a correlation coefficient of 0.43 (Shapiro et al., 1997). A significant increase in soil pH was found in flooded soil (Yoo and James, 2003). It would be useful to test whether soils submerged for a longer period (30–40 days) have a higher pH that is less favorable for larval survival.

The critical 20-day flooding duration for plant water stress and root damage is in agreement with information on tree water stress obtained from a *Diaprepes*-infested citrus grove that was flooded for 3 weeks during 2002–2003 (Li et al., 2004a). Based on our data, roots could be injured by waterlogging and larval feeding. A negative soil E_h and a decrease leaf g_s could be an early indicator of plant water stress and root damage from flooding, and soil type and soil texture could also be factors affecting larval survival in the field. There is a need for more information about the associations of waterlogging, E_h, soil pH, soil texture, and larval survival for plant protection.

CONCLUSIONS

Plant environmental stress was not limited from soil anoxia by flooding only or from root weevil larval feeding only. Our results have implications for soil waterlogging management and root weevil control for plant protection. The data support the notion that flooding significantly reduced soil E_h, plant leaf g_s, and shoot growth. Temporal patterns of leaf g_s varied with rootstock, flooding duration, and *Diaprepes* root weevil larval feeding on roots. Leaf g_s and shoot growth were significantly higher and root injury was less for shorter flooding (10 days) than for longer durations (20 days or longer). Roots flooded for longer periods were more susceptible to waterlogging stress and larval feeding pressure, and these stresses were additive. A 20-day flooding period could be a critical duration for plant water stress and larval feeding tolerance because roots injured from a 20-day prior flooding were more susceptible to larval feeding injury. In addition,

soil texture could be a factor affecting larval survival, and a negative soil E_h and a decrease of leaf g_s could be indicators of plant water stress from waterlogging. Flooding can be critical for plant survival but it would be beneficial by reducing larval survival, and flooding events can be more frequent and flooding duration cannot be controlled in the field. This study focused on the responses of young seedlings to flooding and neonate larval feeding, and because of using citrus grove soil the results may be helpful for understanding root weevil larval survival, larval growth and tree decline associated with waterlogging, soil texture, and larval feeding in the field. We suggest that future work determine soil, water, and E_h relations by examining the amount of easily metabolized organic matter from soil and the rate of consumption and influx of molecular oxygen in the system. We also suggest future work assess whether soil compaction and changes in soil pH and water content influence larval survival and adult emergence in citrus groves.

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REFERENCES

Blanke, M. M., and D. T. Cooke. 2004. Effects of flooding and drought on stomatal activity, transpiration, photosynthesis, water potential and water channel activity in strawberry stolons and leaves. Plant Growth Regul. 42:153–160.

Boman, B., and T. Obreza. 2002. Water table measurement and monitoring for flatwoods citrus. Circular 1409. University of Florida, IFAS, Gainesville, Florida.

Dat, J. F., N. Capelli, H. Folzer, P. Bourgeade, and P. M. Badot. 2004. Sensing and signaling during plant flooding. Plant Physiol. Biochem. 42:273–282.

Jayaweera, G. R., and J. W. Biggar. 1996. Role of redox potential in chemical transformation of selenium in soils. Soil Sci. Soc. Am. J. 60:1056–1063.

Jones, I. F., and W. J. Schroeder. 1983. Study of first-instar *Diaprepes abbreviatus* (Coleoptera: Curculionidae) activity for control purposes. J. Econ. Entomol. 76:567–569.

Kaelke, C. M., and J. O. Dawson. 2003. Seasonal flooding regimes influence survival, nitrogen fixation, and the partitioning of nitrogen and biomass in *Alnus incana ssp rugosa*. Plant Soil. 254:167–177.

Kreuzwieser, J., E. Papadopoulou, and H. Rennenberg. 2004. Interaction of flooding with carbon metabolism of forest trees. Plant Biol. 6:299–306.

Li, H., J. P. Syvertsen, C. W. McCoy, A. W. Schumann, and J. Dunlop. 2003. Soil redox potential, and leaf stomatal conductance of two citrus rootstocks subjected to flooding and root weevil feeding. Proc. Fla. State Hort. Soc. 116:252–256.

Li, H., J. P. Syvertsen, R. J. Stuart, C. W. McCoy, A. W. Schumann, and W. S. Castle. 2004a. Soil and *Diaprepes abbreviatus* root weevil spatial variability in a poorly drained citrus grove. Soil Sci. 169:650–662.

Li, H., J. P. Syvertsen, R. J. Stuart, C. W. McCoy, A. W. Schumannt, and W. S. Castle. 2004b. Soil liming and flooding effects on *Diaprepes* root weevil larval survival and citrus seedling growth. Proc. Fla. State Hort. Soc. 117:157–162.

Lower, S. S., S. Kirshenbaum, and C. M. Orians. 2003. Preference and performance of a willow-feeding leaf beetle: Soil nutrient and flooding effects on host quality. Oecologia. 136:402–422.

McCoy, C. W., R. J. Stuart, and N. H. Nigg. 2003. Seasonal life stage abundance of *Diaprepes abbreviatus* (L.) in irrigated and nonirrigated citrus plantings in central Florida. Fla. Entomol. 86:34–42.

Mielke, M. S., A. A. F. de Almeida, F. P. Gomes, M. A. G. Aguilar, and P. A. O. Mangabeira. 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. Environ. Exp. Bot. 50:221–231.

Oren, R., J. S. Sperry, B. E. Ewers, D. E. Pataki, N. Phillips, and J. P. Megonigal. 2001. Sensitivity of mean canopy stomatal conductance to vapor pressure deficit in a flooded *Taxodium distichum* L. forest: Hydraulic and non-hydraulic effects. Oecologia. 126:21–29.

Orians, C. M., and R. S. Fritz. 1996. Genetic and soil-nutrient effects on the abundance of herbivores on willow. Oecologia. 105:388–396.

Patrick W. E., R. P. Gambrell, and S. P. Faulkner. 1996. Redox measurements of soils. *In*: Methods of soil analysis: Part 3. Chemical methods. D. Sparks (ed.). Soil Science Society of America Book Series, no. 5. Madison, Wisconsin, pp. 1255–1273.

Pezeshki, S. R., and R. D. Delaune. 1998. Responses of seedlings of selected woody species to soil oxidation-reduction conditions. Environ. Exp. Bot. 40:123–133.

Rogers, S., J. H. Graham, J. H., and C. W. McCoy. 2000. Larval growth of *Diaprepes abbreviatus* (L.) and resulting injury to three citrus varieties in two soil types. J. Econ. Entomol. 93:380–387.

Ruiz-Sanchez, M. C., R. D. Domingo, D. Morales, and A. Torrecillas. 1996. Water relations of Fino lemon plants on two rootstocks under flooded conditions. Plant Sci. 120:119–125.

Quintela, E., and C. W. McCoy. 1997. Effects of

imidacloprid on development, mobility, and survival of first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). J. Econ. Entomol. 90:988–995.

Saqib, M., J. Akhtar, and R. H. Qureshi. 2004. Pot study on wheat growth in saline and waterlogged compacted soil II. Root growth and leaf ionic relations. Soil Tillage Res. 77:179–187.

SAS Institute. 1990. SAS/STAT user's guide: Vol. 2. GLM-VARCOMP. Version 6, 4th ed. SAS Inst. Cary, North Carolina.

Shapiro, J. P., D. G. Hall, and R. P. Niedz. 1997. Mortality of the larval root weevil *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in simulated flooding. Fla. Entomol. 80:277–285.

Steudle, E., and C. A. Peterson. 1998. How does water get through roots? J. Exp. Botany. 49:775–788.

Stuart, R. J., D. I. Shapiro-Ilan, R. R. James, R. R., K. B. Nguyen, and C. W. McCoy. 2004. Virulence of new and mixed strains of the entomopathogenic nematode *Steinernema riobrave* to larvae of the citrus root weevil *Diaprepes abbreviatus*. Biol. Control. 30:439–445.

Syvertsen, J. P., R. M. Zablotowicz, and M. L. Smith, Jr. 1983. Soil temperature and flooding effects on two species of citrus. 1. Plant growth and hydraulic conductivity. Plant Soil. 72:3–12.

Yoo, M. S., and B. R. James. 2003. Zinc extractability and plant uptake in flooded, organic waste-amended soils. Soil Sci. 168:686–698.