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LEAF GAS EXCHANGE AND GROWTH RESPONSES OF GREEN BUTTONWOOD AND SWINGLE CITRUMELO TO *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) LARVAL FEEDING AND FLOODING

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ABSTRACT

Effects of flooding and herbivory by *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae) larvae on leaf gas exchange [net CO_2 assimilation (A), transpiration (E), and stomatal conductance (g_s)] and growth of green buttonwood (*Conocarpus erectus* L.) and Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.] trees were tested. Growth and survival of the larvae were also examined. For each plant species, there were 2 larval infestation treatments (infested and non-infested) and 2 flooding treatments (flooded and non-flooded). Beginning 6 d after larval infestation, plants were flooded in three 1-wk cycles each with 2 d of flooding followed by 5 d of non-flooding. For green buttonwood, E was higher for non-flooded than flooded plants on the third of 5 measurement dates and A and g_s were higher for non-flooded than flooded plants on the fifth (final) measurement date. For Swingle citrumelo, E and g_s were higher for non-infested than infested plants on the fifth (final) measurement date. Root dry weight of Swingle citrumelo was higher for flooded, infested than for non-flooded, infested plants and for non-flooded, non-infested than for non-flooded, infested plants. Larval survival rate, head capsule width, and root damage rating of Swingle citrumelo were lower for flooded than for non-flooded plants, whereas flooding did not affect larval survival or growth on green buttonwood. Thus, short-term cyclical flooding of three 2-d cycles may control *D. abbreviatus* larvae on Swingle citrumelo but did not control larval populations or reduce damage on green buttonwood.

Key Words: net CO_2 assimilation, transpiration, stomatal conductance

RESUMEN

Se evaluaron los efectos de inundación y herbivoria por larvas de *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) sobre el intercambio de gases foliar (asimilación de CO_2, transpiración, y conductancia estomatica) y el crecimiento de buttonwood verde (*Conocarpus erectus* L.) y Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.]. Tambien, se evaluaron el crecimiento y supervivencia de larvas. Para cada especie de planta, hubo dos tratamientos de infestación larval (infestado y no infestado) y dos tratamientos de inundación (inundado y no inundado). Seis dias después de la infestación larval, las plantas fueron inundadas en tres ciclos de una semana. Cada ciclo tuvo 2 dias de inundación seguido por 5 dias de no inundación. En la tercera de las cinco fechas de registro, la transpiración en buttonwood verde fué más alta en plantas no-inundadas que en las inundadas mientras que la asimilación de CO_2 y conductancia estomatica fueron más altas en el quinto (ultimo) día de registro en plantas no-inundadas que en las inundadas. Para Swingle citrumelo, el quinto (ultimo) día de registro, la transpiración y la conductancia estomatica fué más alta en plantas no-infestadas que en las infestadas. El peso seco de las raices de Swingle citrumelo fue más alto en plantas inundadas e infestadas y en plantas no-inundadas, no-infestadas que en las no-inundadas e infestadas. La tasa supervivencia de las larvas, el ancho de sus cabezas, y la tasa de daño a la raíz de Swingle citrumelo fué más baja en plantas inundadas que en las que no fueron inundadas. Sin embargo, la inundación no afectó la supervivencia ni el crecimiento de larvas en buttonwood verde. En resumen, inundaciones cíclicas de tiempo corto con tres ciclos de 2 dias cada vez, puede controlar larvas de *D. abbreviatus* en Swingle citrumelo, pero no controla las poblaciónes larvales, ni reduce el daño, en buttonwood verde.

Translation provided by the authors.

Diaprepes abbreviatus L. (Coleoptera: Curculionidae: Entiminae) commonly called *Diaprepes* root weevil is a pest of sugarcane and citrus in its native Puerto Rico (Woodruff 1964). In Florida, it infests approximately 24,281 ha (60,000 ac) of citrus, and control costs and losses have exceeded $2,965 per ha ($1,200 per ac) (Stanley 1996). Agricultural losses due to the weevil in Florida have been estimated at $70 million annually (Weissling et al. 2004). There is a continued need for improved management strategies because *D. abbreviatus* has threatened the survival of several crop plants in the past (Simpson et al. 1996) and continues to be an economic pest for both citrus and the ornamental industry. This pest has a very large host range of at least 317 varieties, 280 species, 180 genera, and 68 families of plants (Simpson et al. 1996, 2000; Knapp et al. 2000; Mannion et al. 2003; Godfrey et al. 2006). In addition to damage caused by the pest, there are regulatory concerns of spreading the weevil into non-infested areas, which are particularly important to the ornamental plant industry because plants are shipped throughout the U.S. and abroad (Mannion and Glenn 2003).

Some plant species support only 1 stage of the insect: for example, *Ardisia crenata* Sims supports only larval feeding. However, many plants including green buttonwood (*Conocarpus erectus* L.) and citrus are affected by both larval and adult feeding (Simpson et al. 1996). Mannion et al. (2003) surveyed several ornamental plant nurseries in southern Florida and found that egg masses, feeding damage, and adult weevils were common on many woody ornamental plant species. Young weevil larvae feed on small roots, but as they grow may excavate deep grooves on larger roots and consume the outer bark and cambial layers (McCoy et al. 2002). Roots may be girdled causing severe root damage or death, which reduces the ability of the plants to take up nutrients, and often kills small citrus trees (Wolcott 1936, 1948; Quintela et al. 1998; McCoy et al. 2002).

Measurements of leaf gas exchange, including net CO_2 assimilation *(A)*, transpiration *(E)*, and stomatal conductance (g_s), can help quantify insect damage to plants before visual symptoms appear. Insect herbivory can increase, decrease, or have no effect on leaf gas exchange (Andersen and Mizell 1987; Welter 1989; Schaffer and Mason 1990; Schaffer et al. 1997). How insect herbivory affects leaf gas exchange can vary with the type of feeding damage or guild (i.e., mesophyll feeders, phloem feeders, stem borers, root feeders, and direct leaf consumers) (Root 1973; Welter 1989). *Diaprepes abbreviatus* has 2 feeding guilds; larvae are in the root-feeder guild whereas adults are in the direct-leaf-consumer guild.

Agriculture in southern Florida is often in low-lying areas with high water tables which are prone to periodic flooding (Schaffer 1998). Flooding typically depletes soil oxygen which can inhibit root metabolism causing decreased plant growth and photosynthesis. Prolonged flooding can result in plant mortality (Schaffer et al. 1992; Kozlowski 1997). Green buttonwood is a popular ornamental tree or shrub in southern Florida and is native to the tidal swamps of central and southern Florida (Watkins and Sheehan 1975; Wunderlin 1998). Hence it tolerates flooding well, though it also thrives in non-flooded, moderately moist soils in which landscape plants are commonly found.

Previous research with *D. abbreviatus,* including interactions between larval infestation and soil flooding, soil type, or soil pH mainly focused on *Citrus* spp. or their intergeneric crosses with *Poncirus* spp. (Li et al. 2003, 2004, 2006, 2007). Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.] was used in several studies of *Diaprepes* and flooding interactions because it is a very common rootstock for commercial citrus trees in Florida (Auscitrus 2004; F. S. Davies, personal communication 2008). Unlike buttonwood, however, Swingle citrumelo has moderate to low flood tolerance (Auscitrus 2004). Only very young plants infested with neonates were evaluated in previous studies with citrus (Li et al. 2003, 2004, 2006, 2007). To the authors' knowledge, there is no published research on interactions between *D. abbreviatus* larval feeding and soil flooding with more mature larvae on larger citrus plants. Also, little information is available on effects of flooding on *D. abbreviatus* damage to woody ornamental plants.

Our primary objective was to investigate effects of cyclical (intermittent) soil flooding and herbivory by large (fourth to sixth instar) *D. abbreviatus* larvae and their interactions on green buttonwood and Swingle citrumelo trees. An additional objective was to compare effects of flooding on the survival and growth of *D. abbreviatus* larvae on green buttonwood with those on Swingle citrumelo, a trees species known to be sensitive to interactions between soil flooding and *D. abbreviatus* neonates (Li et al. 2003, 2006).

Materials and Methods

The experiment was conducted in fall 2008 in Homestead, Florida with green buttonwood and Swingle citrumelo plants in 11-liter plastic containers placed on ground cloth at an outdoor site exposed to full sun.

Plant Material

Green buttonwood and Swingle citrumelo trees (obtained from a commercial nursery) were approximately 2 yrs old and 1 yr old, respectively when treatments were initiated. Initial plant height 6 d before infestation was 122 ± 11 cm (mean ± SD) for green buttonwood and 132 ± 14 cm for Swingle citrumelo. In a previous study (Martin et al. 2010), no difference was observed between marl soil (typical in landscape plant nurseries in southern Florida) and standard potting medium for survival of *D. abbreviates* larvae in flooded or non-flooded conditions. In this study we used a standard medium, typical for potted ornamental plants in southern Florida ornamental plant nurseries, to avoid potential damage to root systems from repotting plants in marl soil. The potting medium for both plant species was Fafard mix 2 (70% Canadian peat, 20% perlite and 10% vermiculite).

Flooding Treatments

Plants of each species were flooded by submerging their 11-liter containers into 19-liter plastic buckets filled with tap water with water levels maintained at 10 cm above the soil surface. Control plants were not flooded. For each replication in each test, there were 2 flooded plants (1 infested and 1 non-infested) and 2 non-flooded plants (1 infested and the other non-infested). Flooded treatments were initially flooded 6-8 Nov 2008. Plants were flooded for 2 d followed by a 5-d drying period resulting in a 7-d cycle that was repeated 3 times. All plants (flooded and non-flooded) were irrigated throughout the experiment by overhead sprinkler for 30 min twice per day.

Larval Infestation

For each plant species, one-half of the plants in each flood treatment (flooded or non-flooded) were infested with *D. abbreviatus* larvae on 31 Oct 2008. Larvae were obtained from a rearing facility with the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL (see http://www.doacs.state.fl.us/pi/methods/diaprepes.html for rearing procedures). At the time of infestation, larvae were about 28 d old with an average head capsule width of 1.15 ± 0.14 mm, hence they were fourth to sixth instar or late fifth instar on average (Quintela et al. 1998). Larvae were placed individually into each of 10-20 holes made in the soil that were 3-10 cm deep, 4-8 cm from the stem, and 3 cm apart with 20 total larvae per container. The holes were then covered with soil. All containers remained non-flooded for 6 d to allow larvae to become established.

Temperature and Soil Redox Potential

Air and soil temperatures were recorded at 1-h intervals throughout the experiment with 2 air sensors and 2 soil sensors (StowAway® Tidbit® temploggers, Onset Co., Pocasset, MA). Sensors were placed in the soil (soil temperature) or canopies (air temperature) of plants not included in the experiment but in the same potting media and container type, which were located next to the test plants. Air sensors were each placed in plant canopies 66-71 cm above the soil surface and soil sensors were placed at a soil depth of 6 cm, two-thirds the distance from the center to the outer edge of the pot.

To provide an indication of soil oxygen content, soil redox potential was measured with a metallic combination electrode (Accumet Model 13-620-115, Fisher Scientific, Pittsburgh, PA) attached to a portable volt/pH meter (Accumet model AP62, Fisher Scientific, Pittsburgh, PA). Soil redox potential was measured daily during each flood period for 2 flooded, infested plants and 2 flooded, non-infested plants. The 4 measurements were averaged to calculate the mean redox potential for flooded treatments of each plant species. Soil redox potential was measured by inserting the electrode into a polyvinyl chloride (PVC) pipe inserted into the soil 2 cm from the edge of the pot. In addition, soil pH was measured for all flooded plants 2 times per flood period with a pH electrode attached to the same portable volt/pH meter used for redox measurements. For each flood cycle, the first pH measurement was made on the same day that plants were flooded and the second was 2 d later on the day they were drained. An exception was for Swingle citrumelo plants during the first flood cycle, when pH was measured 1 d after plants were flooded, and again 1 d later when plants were drained.

Plant Data Collection

Leaf gas exchange (A, E, and g_s) was measured on 2 fully expanded, recently mature leaves or leaflets per plant with a CIRAS-2 portable gas analyzer (PP Systems, Amesbury, Massachusetts). Values of the 2 leaves or leaflets were averaged and the mean value per plant (replication) was used for statistical analyses. Leaf gas exchange was initially measured 2-3 d before infesting plants with larvae and then periodically throughout the experiment. On each measurement date, measurements of all 4 treatment combinations in each replication per plant species were made within 50 min of each other. During gas exchange measurements, the photosynthetic photon flux was maintained at 1,000 µmol photons $m^{-2} s^{-1}$ with a halogen lamp attached to the leaf cuvette and the reference CO_2 concentration in the cuvette was kept constant at 375 µmol mol^{-1}. Swingle citrumelo has compound leaves with 3 leaflets per leaf, and the terminal leaflet is larger than lateral leaflets (Hutchison 1974; Wunderlin 1998). All leaf gas exchange measurements for Swingle citrumelo were made on the large terminal leaflets.

Plant height was measured from the soil surface to the apex of the highest plant part (leaf or branch), and stem diameter was measured 10 cm above the soil surface; for plants with multiple stems at this height, diameter of the largest stem was recorded. The first measurement of plant height and stem diameter was made before infestation and flooding, and the second measurement was after the final draining but before harvest. For plant height or stem diameter, final minus initial values were calculated to compare growth data among treatments. All plants were harvested 32-33 d after larval infestation, 26-27 d after initially flooding, and 10-11 d after the final draining. At harvest, stems were cut 2-3 cm above the surface of the potting medium. The roots were removed from the potting medium and the medium was placed into bins and carefully inspected for larvae. The number of live and dead larvae was determined for each plant and preserved in separate vials of 75% ethanol. Head capsule widths were measured in a laboratory with a microscope micrometer. Roots, stems, and leaves were then oven-dried for 5 d at 75°C to a constant weight and dry weights were determined. Leaf dry weight included leaf blades and petioles for green buttonwood plants and leaflets, petiolules, and petioles for Swingle citrumelo plants. Root damage was evaluated for infested Swingle citrumelo plants using a visual rating system where 0 = no visible damage, 1 = minimal visible damage, 2 = moderate visible damage, and 3 = maximum visible damage. However, root damage was not rated for green buttonwood because there were no visible signs of root damage.

Experimental Design and Statistical Analysis

For each plant species, there were 2 larval infestation treatments (infested or not infested) and 2 flooding treatments (flooded or non-flooded) arranged in a 2 × 2 factorial design with 5 single-plant replications per treatment combination. A two-way factorial analysis of variance (ANOVA) was used to test for significant interactions between infestation and flooding treatments, separately for each sampling date and plant species for leaf gas exchange variables. For plant growth data (root, stem, leaf, and total dry weights, stem diameter, and plant height), a separate ANOVA was performed for plant species. For each variable or group of variables per plant species (A, E, g_s, stem diameter, plant height, or dry weights), if there were no significant statistical interactions for any 2-way ANOVA, data were pooled and non-paired t-tests were used to compare flooded with non-flooded and infested with non-infested treatments. For percentages of larvae surviving, proportional data based on ratios of live/total larvae were arcsine transformed prior to statistical analysis. All statistical analyses were performed with SAS Statistical Software Version 9.1 (SAS Institute, Cary, North Carolina).

Results

Temperature, Soil Redox Potential and Floodwater pH

During the treatment period, mean daily soil temperatures ranged from16.8°C to 27.7°C and air temperatures ranged from 13.0°C to 24.3°C (Fig. 1). Soil redox potential for green buttonwood during the first, second and final flood periods ranged from +193 mV to +162 mV, +597 mV to +166 mV and +508 mV to +153 mV, respectively. For Swingle citrumelo, the corresponding values ranged from +378 mV to +165 mV, +498 mV to +174 mV and +523 mV to +193 mV, respectively. For each plant species in every flood cycle, the highest redox potential occurred on day 1 (when flooded) and the lowest was on day 3 (when drained) except for green buttonwood flood cycle 1, in which the highest redox potential was on day 2 and the lowest was on day 3. The pH of the floodwater during the flood period was 7.2-7.8.

Leaf Gas Exchange

There were no significant statistical interactions between flooding and larval infestation for leaf gas exchange variables on any of the 5 measurement dates for either plant species. Hence, to test responses to flooding for all leaf gas exchange variables of each plant species, infestation treatments were pooled, and to test responses to larval infestation, flooding treatments were pooled.

For green buttonwood, A (t = -2.21, df = 18, P = 0.0403) and g_s (t = -2.70, df = 18, P = 0.0146) were significantly higher for non-flooded than flooded plants on the fifth (final) measurement date (Fig. 2a and c). However, there were no significant differences between infested and non-infested green

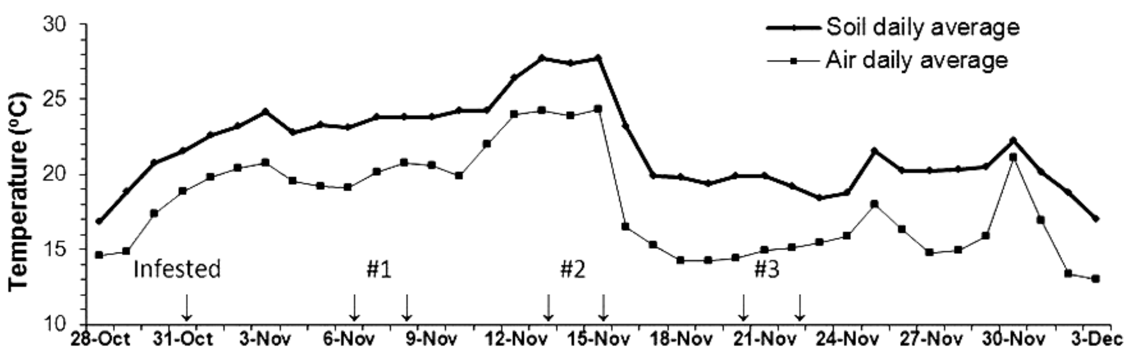

Fig. 1. Air and soil temperatures during the experiment. Each point is the average of 2 sensors. Successive flood cycles are denoted by pairs of arrows with the number of the flood cycle above the arrows.

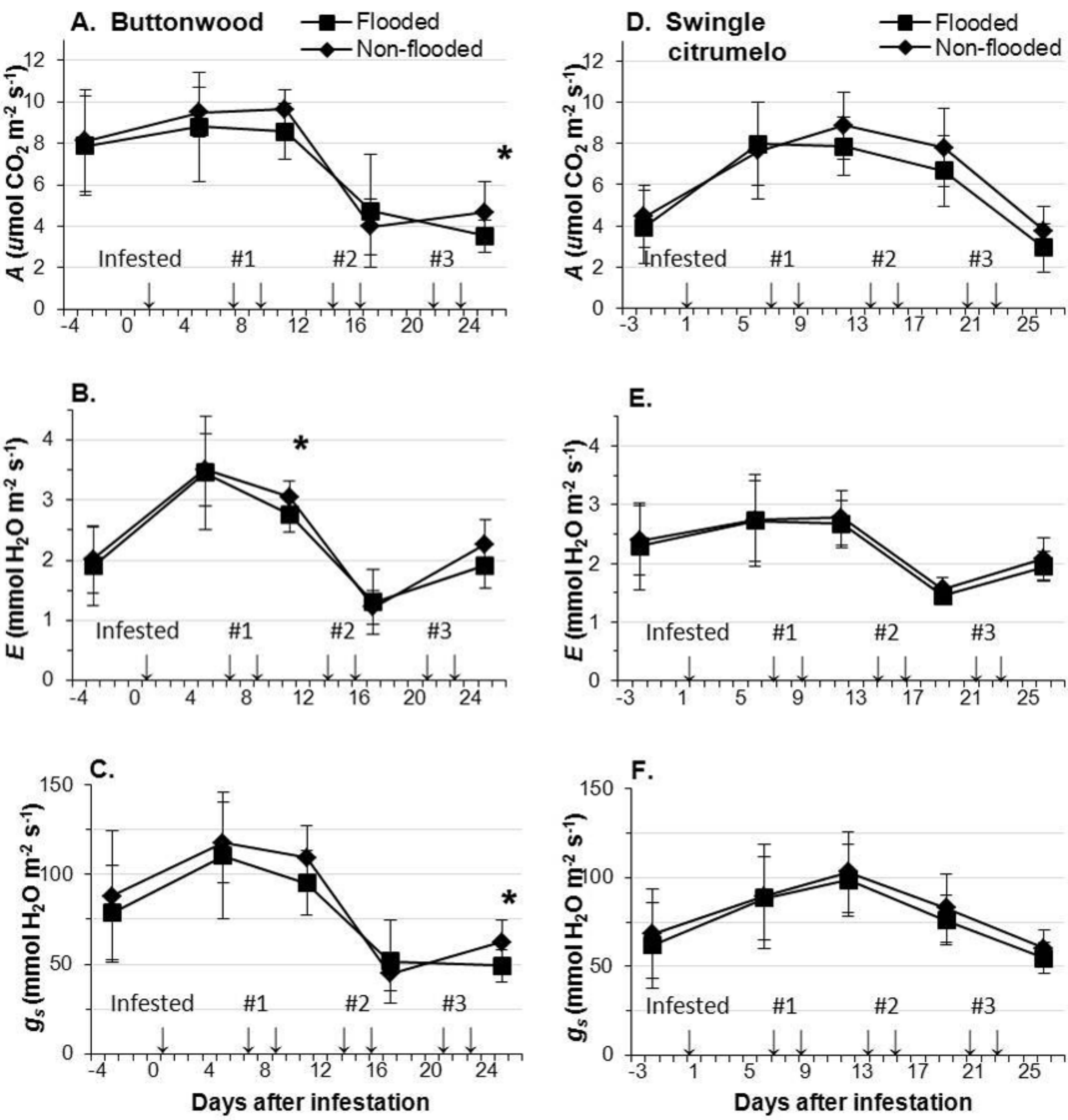

Fig. 2. Effects of flooding on A) net CO_2 assimilation (A), B) transpiration (E), and C) stomatal conductance (g_s) of green buttonwood trees and D) net CO_2 assimilation (A), E) transpiration (E), and F) stomatal conductance (g_s) of Swingle citrumelo trees. Symbols represent means ± SD. Successive flood cycles are denoted by pairs of arrows with the number of the flood cycle shown above the arrows. Asterisks indicate significant differences between treatments ($P \leq 0.05$) according to a non-paired t-test.

buttonwood plants for A or g_s (Fig. 3a and c). For green buttonwood, E was significantly higher for non-flooded than flooded plants on the third measurement date, or after infestation and the first flood cycle but before the second flood cycle (t = -2.24, df = 18, P = 0.0381) (Fig. 2b). There were no significant differences in E between infested and non-infested green buttonwood plants (Fig. 3b).

There were no significant differences in A between flooded and non-flooded or infested and non-infested Swingle citrumelo plants (Figs. 2d and 3d). For Swingle citrumelo, E (t = -2.64, df = 18, P = 0.0167) and g_s (t = -3.10, df = 18, P = 0.0061) were significantly higher for non-infested than infested plants on the fifth (final) measurement date (Fig. 3e and f). However, there were no other

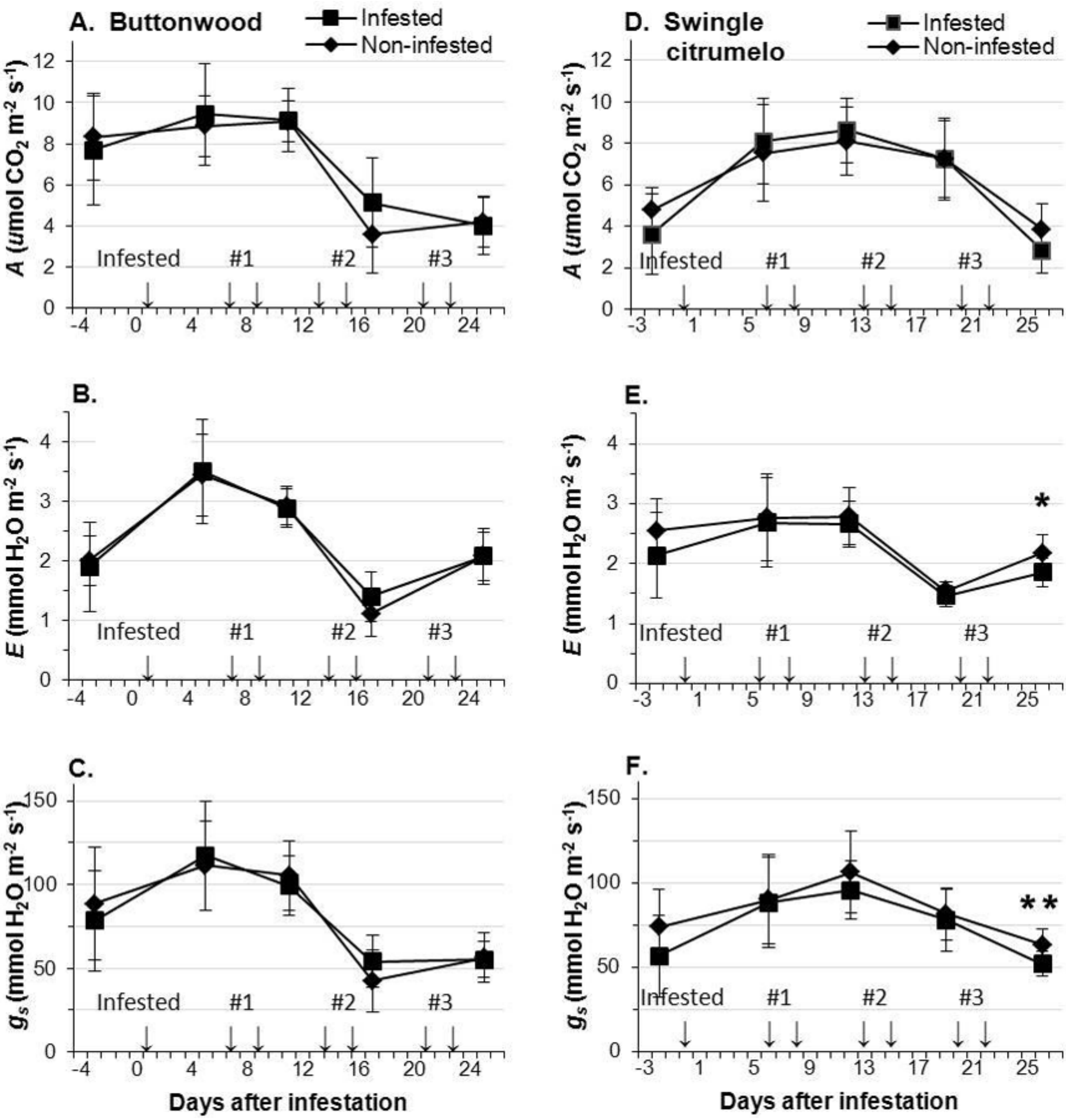

Fig. 3. Effects of larval infestation on A) net CO_2 assimilation (A), B) transpiration (E), and C) stomatal conductance (g_s) of green buttonwood trees and D) net CO_2 assimilation (A), E) transpiration (E), and F) stomatal conductance (g_s) of Swingle citrumelo trees. Symbols represent means ± SD. Successive flood cycles are denoted by pairs of arrows with the number of the flood cycle above the arrows. Asterisks indicate significant differences between treatments at * $P \leq 0.05$ or ** $P < 0.01$ according to a non-paired t-test.

significant differences in E or g_s between flooded and non-flooded or infested and non-infested Swingle citrumelo plants (Figs. 2e and f, 3e and f).

Plant Growth

There were no significant statistical interactions between larval infestation and flooding for stem diameter or plant height of either plant species. For tissue dry weights, the only significant flooding × larval infestation interaction was for root dry weight of Swingle citrumelo trees ($F = 4.87$; $df = 3$; $P = 0.0422$). Therefore, dry weights of Swingle citrumelo were not pooled for analysis, whereas for all other dry weight, stem diameter and plant height data were pooled for each plant species.

There were no significant effects of flooding or larval infestation on stem diameter or plant height for either green buttonwood or Swingle citrumelo (data not shown). There were no significant effects of larval infestation or flooding on root, stem, leaf, or total dry weights of green buttonwood (ranges for roots 49-103 g, stems 82-204 g, leaves 56-110 g, and total 193-407 g), or stem, leaf, or total dry weights of Swingle citrumelo (Table 1). However, root dry weight of Swingle citrumelo was significantly higher for flooded, infested than for non-flooded, infested plants (Table 1). Root dry weight of Swingle citrumelo was also significantly higher for non-flooded, non-infested than for non-flooded, infested plants (Table 1).

Larval Survival and Growth

For green buttonwood, there were no significant effects of flooding on percent larval survival or head capsule width of recovered larvae (Table 2). For Swingle citrumelo, however, percent survival and head capsule width were each significantly lower for flooded than non-flooded plants (Table 2). Root damage rating (Mean ± SD) for infested Swingle citrumelo was also significantly lower for flooded (0.2 ± 0.45) than for non-flooded (2 ± 1.2) plants (t = -3.09, df = 8, P = 0.0150).

Discussion

The average monthly soil temperatures during this study were 0.9 to 6.2°C below the ideal developmental temperature for *D. abbreviatus* and up to 2.2°C below the ideal survival temperatures for this weevil (Lapointe 2000). Lapointe (2000) found that the highest larval survival rates occurred at 22 and 26°C with lowest survival at 30°C and the highest developmental rate was at 26°C with slower rates at 22 and 30°C. Although larval development rates in the present study may have been slower than their maximum, larval survival rates were probably close to or slightly below their maximum levels.

Effects of flooding on physiology and growth of woody perennial plant species can vary among soil types and are partly based on rates of O_2 depletion in the soil and other factors such as soil pH (Schaffer et al. 1992). Soil redox potential provides an indication of oxygen content in the soil. Well-drained, well-oxygenated soils have redox

Table 1. Effects of flooding and *Diaprepes abbreviatus* larval infestation on dry weights of swingle citrumelo plants.

Tissue	Dry weight (g)		T	df[c]	P	Sig[d]
Infested[a]	Flooded	Non-flooded				
Roots	38 ± 2.0[b]	30 ± 5.1	3.16	8	0.0134	*
Stems	94 ± 18	84 ± 19	0.81	8	0.4425	NS
Leaves	15 ± 5.8	14 ± 3.2	0.17	8	0.8706	NS
Total	146 ± 24	129 ± 26	1.12	8	0.2970	NS
Non-infested	Flooded	Non-flooded				
Roots	39 ± 8.3	43 ± 5.7	-0.79	8	0.4513	NS
Stems	86 ± 21	93 ± 17	-0.59	8	0.5693	NS
Leaves	15 ± 5.2	13 ± 2.7	0.96	8	0.3669	NS
Total	141 ± 32	149 ± 24	-0.46	8	0.6604	NS
Flooded	Infested	Non-infested				
Roots	38 ± 2.0	39 ± 8.3	-0.32	4.48	0.7627	NS
Stems	94 ± 18	86 ± 21	0.62	8	0.5532	NS
Leaves	15 ± 5.8	15 ± 5.2	-0.20	8	0.8466	NS
Total	146 ± 24	141 ± 32	0.32	8	0.7589	NS
Non-flooded	Infested	Non-infested				
Roots	30 ± 5.1	43 ± 5.7	-3.65	8	0.0065	**
Stems	84 ± 19	93 ± 17	-0.79	8	0.4544	NS
Leaves	14 ± 3.2	13 ± 2.7	0.70	8	0.5008	NS
Total	129 ± 26	149 ± 24	-1.27	8	0.2412	NS

[a]Data were not pooled because there was a significant interaction between flooding and insect infestation ($P \leq 0.05$) with root dry weight based on a two-way analysis of variance (ANOVA).

[b]Mean ± SD in grams.

[c]Degrees of freedom. Variances were equal according to a test for equality of variances.

[d]Significance levels at * $P \leq 0.05$, ** $P < 0.01$ and NS (non-significant) were determined with a non-paired t-test; n = 5.

TABLE 2. EFFECTS OF FLOODING ON PERCENT SURVIVAL AND HEAD CAPSULE WIDTH OF *DIAPREPES ABBREVIATUS* LARVAE RECOVERED AT HARVEST.

		Treatments[a]					
Variable	Species	Flooded	Non-flooded	T	*df*[c]	*P*	Sig[c]
Percent survival[d]							
	Buttonwood	44 ± 12	54 ± 20	-0.96	8	0.3649	NS
	Swingle	24 ± 7.4	42 ± 15	-2.44	8	0.0406	*
Head capsule width (mm)							
	Buttonwood	1.79 ± 0.10	1.89 ± 0.07	-1.89	8	0.0949	NS
	Swingle	1.76 ± 0.15	2.07 ± 0.06	-4.32	8	0.0025	**

[a]Mean ± SD.
[b]Degrees of freedom. All variances within each flooded vs. non-flooded pair were equal according to a test for equality of variances.
[c]Significance levels at * $P \leq 0.05$, ** $P < 0.01$ and NS (non-significant) were determined with a non-paired t-test; n=5.
[d]Percent survival data were Arcsine transformed before statistical analysis.

potentials of +300 mV or more, whereas flooded soils have redox potentials of +200 mV or less (Ponnamperuma 1972, 1984). All mean soil redox potentials for this experiment varied from 140 to 597 mV indicating the soil was either aerobic, or moderately hypoxic (low in oxygen). In addition, for both plant species, during all 3 flood cycles (except for green buttonwood flood cycle 1), the highest redox potential occurred on day 1 (when flooded) and the lowest was on day 3 (when drained). Hence, redox potential of cyclically flooded soil in this study indicated that while there was a decline in soil O_2 content during the flooding periods, the soil did not become very depleted of oxygen. This may have resulted from the short duration of the 3, 2-d cyclical flood periods each separated by 5 d without flooding. However, longer flooding durations are uncommon in ornamental plant nurseries in southern Florida (B. Schaffer, personal observations).

The duration of flooding and larval infestation periods in the present study were relatively short compared to previous studies, such as by Diaz (2005), where green buttonwood was exposed to 21-36 d of flooding followed by 90 d infestation. For green buttonwood plants in the present study, *E* was significantly higher for non-flooded than flooded plants on the third measurement date, and *A* and g_s were each significantly higher for non-flooded than flooded plants on the fifth (final) measurement date. However, there were no significant differences between flooded and non-flooded green buttonwood plants in stem diameter and no adventitious roots were observed on flooded plants. In 2 other studies with green buttonwood (Martin 2009), flooding for longer durations (23 d or 180 d) resulted in larger stem diameters of flooded compared to non-flooded plants. Also, plant adaptations to hypoxic soil conditions, such as development of adventitious roots and hypertrophic stem lenticels (Schaffer et al. 1992), were observed on flooded plants (Martin 2009). In both these previous studies with longer flood durations, there were almost no significant differences in *A* or g_s between flooded and non-flooded green buttonwoods (Martin 2009), whereas in the present study *A* and g_s were each significantly higher for non-flooded than flooded plants on the last of 5 measurements. For flooded green buttonwood, the reduction in leaf gas exchange without a growth or developmental response in the present study was presumably due to short flooding durations, which apparently were not long enough for buttonwood to exhibit statistically significant growth changes or morphological or anatomical adaptations to flooding.

There were no significant differences in leaf gas exchange between flooded and non-flooded Swingle citrumelo plants. Based on leaf gas exchange and plant growth in the present study, green buttonwood was more susceptible to flooding than Swingle citrumelo. This seems unusual because buttonwood is relatively flood tolerant (Watkins and Sheehan 1975; Wunderlin 1998; Martin 2009), whereas Swingle citrumelo is not particularly flood tolerant (Auscitrus 2004). Although plants in the present study were subjected to repeated flood cycles, durations of flooding and larval infestation were short compared to previous studies where Swingle citrumelo was exposed to 20-40 d of flooding followed by 40-56 d of larval infestation (Li et al. 2004, 2007). In addition, Syvertsen et al. (1983) found that when rough lemon *(Citrus jambhiri* Lush.) and sour orange *(C. aurantium* L.) seedlings were flooded for at least 3 wk, there was sloughing of fibrous roots and significant reduction in g_s, shoot growth, root conductivity, and leaf water potential. Thus, unlike in studies of longer flooding duration, we observed no significant effects of flooding on leaf gas exchange for Swingle citrumelo trees despite repeated flooding cycles. The only significant effect of flooding on Swingle citrumelo growth was higher root dry weight under flooded, infested than under non-flooded, infested conditions; this may have resulted from flooded conditions preventing feeding larvae from decreasing root dry weight instead of flooding directly affecting plant growth. Although flooding durations in the present study were relative short, each intermittent flooding event was similar to the length of time that standing water is generally observed in southern Florida plant nurseries after heavy rains or tropical storms (B. Schaffer, personal observations). Durations of flooding under these conditions were apparently too short for buttonwood to develop flooding adaptations such as adventitious roots, and Swingle citrumelo may have not been flooded long enough to reduce its leaf gas exchange or growth.

Lapointe and Shapiro (1999) determined that optimal survival to pupation of *D. abbreviatus* in the laboratory occurred at 30-70% soil moisture, under which 60-65% of larvae survived to pupation (Lapointe and Shapiro 1999). The poorest survival of larvae occurred in low (20%) and in high (80%) soil moisture levels (Lapointe and Shapiro 1999). Our results support the observation that the poorest survival occurs under flooded conditions. Larval survival was significantly lower in cyclically flooded than in non-flooded soil with Swingle citrumelo. Similarly, Martin et al. (2010a) observed significantly lower *D. abbreviatus* larval survival rates on green buttonwood in flooded marl soil than in non-flooded marl soil and in flooded potting medium than in non-flooded potting medium. Also, significantly smaller head capsule widths were noted from flooded marl soil than from non-flooded marl or non-flooded potting medium (Martin et al. 2010a). Hence, the lowest survival rates of *D. abbreviatus* larvae would be expected under flooded conditions, whereas highest survival should be in non-flooded conditions without excessively low soil moisture (30-70%) (Lapointe and Shapiro 1999).

Nearly all root damage appeared to occur on roots larger than 2 mm diameter and involved gouging of the bark and presumably cambium of roots. Very few roots smaller than 2 mm in diameter showed evidence of larval damage, thus, larval damage was disproportionately biased towards the crown and larger diameter roots. A rating of 3 (maximum visible damage) corresponded to 10-15 percent removal of bark and cambium by larvae on roots at least 2 mm diameter with girdling more than half the crown circumference in at least 1 place, whereas minimum damage rating was 0 percent. Larval feeding by *D. abbreviatus* has been shown to reduce leaf gas exchange and growth in several woody ornamental plant species including green buttonwood (Diaz 2005; Diaz et al. 2006; Martin et al. 2009). In the present study, effects of flooding and insect damage on each plant species were presumably cumulative because most significant differences from flooding or insects were after the final flood cycle.

Previous results suggest that flooding plants in potting medium for at least 3 d would help control *D. abbreviatus* larvae (Martin et al. 2010a). Flooding is sometimes used in southern Florida sugarcane fields to control larvae of *Tomarus subtropicus* (Blatchley) (Coleoptera: Scarabaeidae) (Cherry 1984) and *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae) (Hall and Cherry 1993). Shapiro et al. (1997) found that mean mortality of flooded, unfed larvae of *D. abbreviatus* exceeded 90% by the third week after flooding at 24 and 27°C. Mortality may have been caused by drowning (suffocation) from a lack of oxygen and surplus carbon dioxide or by sepsis from a buildup of microbes in stagnant water and larval cadavers (Shapiro et al. 1997). Thus, flooding may be useful for controlling *D. abbreviatus* larvae infestations on green buttonwood, and flooding was recommended by Li et al. (2007) as a possible control method in citrus.

In the present study, cyclical flooding did not significantly affect larval growth (head capsule width) or survival on green buttonwood. However, Swingle citrumelo plants had significantly reduced larval growth and survival in flooded compared to non-flooded soil. Therefore, 3 periods of 2-d flooding with 5-d drying periods in-between, such as may occur in the field from heavy rain, may help control *D. abbreviatus* larvae without affecting leaf gas exchange or growth of trees on Swingle citrumelo rootstock, but these short-term flood periods would probably not benefit green buttonwoods. Additionally, root dry weight of Swingle citrumelo was significantly greater for flooded infested than for non-flooded infested plants, and when plants were not flooded, it was significantly greater for non-infested than infested plants. Reduced larval growth, survival, feeding, and root damage rating of flooded infested compared to non-flooded infested Swingle citrumelo plants may have allowed for the increased root dry weight of flooded infested plants. Thus, flooding may reduce effects of *D. abbreviatus* larval herbivory and damage to Swingle citrumelo plants.

Diaprepes abbreviatus larvae were exposed directly to flooding in the present study. However, Li et al. (2003, 2006, 2007) and Diaz (2005) drained plants before infestation with larvae. In the study by Diaz (2005), plants were drained 1 d before larval infestation so both stresses were not simultaneous. Overall, decreases in leaf gas exchange and plant dry weight observed by Diaz (2005) were attributed more to flooding than to larval infestation in green buttonwood.

Effects of flooding and *D. abbreviatus* larval infestation on plant growth and larval survival on

Swingle citrumelo and 1 other citrus rootstock were previously examined in a greenhouse (Li et al. 2003, 2006, 2007). Li et al. (2006) found that Swingle citrumelo plants flooded for at least 20 d were more stressed and more prone to *D. abbreviatus* larval feeding injury than non-flooded control plants. Their results suggested that avoidance of flooding and early control of *Diaprepes* larvae may help protect young plants. Similarly, Li et al. (2006) investigated the effects of flooding and soil type on *Diaprepes* larval survival and found that for plants previously flooded for 20 d, larval survival averaged 25% higher in sandy soil than in loam soil. Waterlogged soils are also typically denser than non-flooded soils (Saqib et al. 2004), which is a potential problem for survival of larvae in flooded soil (Li et al. 2006). Other factors such as soil type, compaction, bulk density, and soil water content may also influence larval survival and growth (Riis and Esbjerg 1998; Rogers et al. 2000; Li et al. 2007). Flooding may hence reduce larval survival while plants are flooded. However, depending on soil pH, flood-stressed plants may be more susceptible to *Diaprepes* larval feeding when un-flooded than non-stressed plants that were either never flooded or flood-tolerant and previously flooded. Hence, flooding may either increase or decrease larval survival rates based on soil moisture, pH, and plant health while soil is infested.

In summary, the following suggest that flooding reduced insect damage to Swingle citrumelo plants after three 2-d flood cycles: reduced larval growth, survival, root damage, and increased root dry weight of flooded, infested compared to non-flooded, infested plants; higher root dry weight for non-flooded, non-infested than for non-flooded, infested plants; and reduced E and g_s in infested compared to non-infested plants. However for green buttonwood plants, flooding seemed to have no effect on larval growth, survival, or insect damage. Thus, while cyclical flooding for three 2-d cycles may control *D. abbreviatus* larvae on Swingle citrumelo, short-term flooding seems unlikely to control larvae or reduce damage to green buttonwood.

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