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SURVIVAL OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) LARVAE ON GREEN BUTTONWOOD TREES IN FLOODED MARL SOIL AND POTTING MEDIUM

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ABSTRACT

Survival of Diaprepes root weevil *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) larvae was assessed in flooded marl soil and a flooded nursery potting medium with green buttonwood (*Conocarpus erectus* L., Combretaceae) as a food source for the larvae. Root-zone flooding may be a viable control option for flood-tolerant ornamental plants including buttonwood. Significantly more larvae survived after 38 d in non-flooded than in flooded marl soil. Similarly, more larvae survived in non-flooded than in flooded potting medium; no larvae were recovered from flooded potting medium. Larval survival rates were significantly higher in flooded marl soil than in flooded potting medium, but there was no difference in survival between non-flooded marl soil and non-flooded potting medium. Larvae recovered from flooded marl soil had significantly smaller head capsule widths and probably were at least 1 instar younger than larvae recovered from non-flooded marl soil or non-flooded potting medium. In summary, flooding marl soil or potting medium reduced survival, and in marl soil flooding slows the growth of *D. abbreviatus* larvae.

Key Words: *Diaprepes abbreviatus*, buttonwood, flooding, marl, potting medium

RESUMEN

Las supervivencias de larvas del picudo de las raiz, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) fueron investigado en suelo de marl inundado o en medio de tiesto inundado y usado por criaderos con buttonwood verde (*Conocarpus erectus* L., Combretaceae) como fuente de comida para larvas. Inundando los raiz puede ser un opción de control en plantas ornamentales y tolerente de inundación incluyendo buttonwood. En suelo marl, significativamente menos larvas sobreviviron después de 38 dias en condiciónes inundados comparados a no inundados. Semejantemente, mas larvas sobreviviron en medio de tiesto no inundado comparado al medio de tiesto inundado, desde que no larvas fueron recuperadas. Tasas de supervivencias para larvas fueron significativamente mas alta en el suelo de marl inundado comparado al medio de tiesto inundado, pero no fueron diferencias en supervivencia entre suelo marl no inundado y medio de tiesto no inundado. Larvas recuperadas de suelo marl inundado fueron significativamente mas pequeños en anchos de cabezas, y por lo tanto, fueron a menos de un etapa mas pequeño comparado a larvas recuperadas de suelo marl no inundado o de medio de tiesto no inundado. En resumen, inundando suelo marl o medio de tiesto redujo la supervivencia, y en suelo marl, la augmentación por larvas de *D. abbreviatus*.

Translation provided by the authors.

Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), is an abundant and serious pest of citrus and sugarcane in its home range of Puerto Rico (Woodruff 1964). In Florida, about 100,000 ac (40,469 ha) of citrus are infested (Weissling et al. 2004), and control costs and losses have exceeded $1,200 per ac ($2,965 per ha) (Stanley 1996). The weevil has caused about $70 million in damage annually (crops not specified) (Weissling et al. 2004). Inadequate management and the wide host range sustain *D. abbreviatus* as a threat to numerous crops including many ornamental plants. Transport of the weevils to regions where it is not established on infested plants has become a recent concern. Florida is most likely the source of *D. abbreviatus* recently found in Texas (Knapp et al. 2001; Skaria & French 2001) and California (Klunk 2005). The need to reduce the introduction of *D. abbreviatus* into other states and countries has resulted in regulation, inspection, and pesticide application to nursery stock and other commodities transported from areas where the weevil is established.

Tropical agriculture in southern Florida tends to occur in low-lying areas with high water tables, which are prone to periodic flooding (Schaffer 1998). Changing water delivery practices in Everglades National Park have caused raising of water tables in these areas (Schaffer 1998). This has increased the severity, duration, and extent of flooding in regions that produce tropical fruit and

ornamental plants. Elevation of the water table above the root zone typically depletes soil O_2 levels (Kozlowski 1997). The effects of flooding on the physiology and growth of a woody perennial plant species can vary among soil types and is partly based on the rates of soil O_2 depletion and other factors such as soil pH (Schaffer et al. 1992). In southern Florida plant nurseries, woody ornamental plants are grown either in pots containing standard potting medium or in the field in marl soils. The marl type agricultural soil in southern Florida is classified as Biscayne soil (loamy, carbonatic, hyperthermic, shallow, and Typic Fluvaquent) (Nobel et al. 1996; Li 2001). These marl soils are derived from Miami limestone in areas with several months of flooding (hydroperiod) combined with several months of non-flooded conditions per year. The resulting "calcite mud" soil is high in calcium, has pH of 7.4-8.4, and poor drainage (Li 2001).

Diaprepes abbreviatus is a problematic pest due to its very large host range, which includes 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). Although some plants support only 1 stage of the insect, many plants support all stages of *D. abbreviatus*, including green buttonwood, *Conocarpus erectus* L. (Simpson et al. 1996). Green buttonwood is widely grown as an ornamental tree or shrub in southern Florida and is native to the tidal swamps of central and southern Florida (Watkins & Sheehan 1975; Wunderlin 1998). As suggested from its native range, it is very tolerant of flooding, although it thrives in non-flooded, moderately moist soil, which is common for landscape plants. Flooding has been suggested as a method for control of *D. abbreviatus* larvae in sugarcane (Shapiro et al. 1997). Flooding also may be a viable control option for flood-tolerant ornamental plants including buttonwood, but flooding of the root zone may exacerbate the effects of root feeding by *D. abbreviatus* larvae. The objective of this study was to determine the survival of *D. abbreviatus* larvae in flooded marl soils and in a flooded nursery potting medium, with green buttonwood serving as a food source.

Materials and Methods

The experiment was conducted in the winter and spring of 2007 in Homestead, FL, with *D. abbreviatus* infested green buttonwood trees in 4-L containers filled with either marl soil or potting medium in an outdoor, open site. Plants were obtained from a commercial nursery in Dec 2006 and replanted 12 Jan 2007. At the time treatments were initiated, buttonwood plants were approximately 6-12 months old.

Each plant was repotted into a 4-L plastic container, with half the plants in a nursery potting medium (40% Florida peat, 30% pine bark, 20% cypress sawdust, and 10% sand) and the other half in marl soil. The marl soil was obtained from a fallow agricultural field (Homestead, FL). Plants were fertilized (13 Feb 2007) 10 d before beginning the experiment with liquid fertilizer (Miracle-Gro 15-30-15, Stern's Miracle-Gro Products, Port Washington, NY) at the manufacturers recommended rate. Insect pests other than *D. abbreviatus* were removed manually. A total of 24 plants were used in this study with 2 soil treatments (potting medium and marl soil) and 2 flood treatments (flooded and non-flooded) arranged in a 2 × 2 factorial design with 6 single-plant replications per treatment. An additional 8 "monitoring" plants (4 flooded plants in each soil type) were used for periodic destructive harvest to assess larval survival on 1 plant in each soil type at each assessment time. The monitoring plants were used to determine when to remove test plants from flooding and when to harvest and evaluate the test plants. Data from the monitoring plants were not included in the statistical analysis.

Larval Infestation

Six weeks after repotting (23 Feb 2007), each container was infested with 15 *D. abbreviatus* larvae raised on an artificial diet and supplied by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. Head capsule widths of larvae used to infest plants were 1.15 ± 0.21 mm, indicating that they were fourth through sixth instars (mean fifth instar) (Quintela et al. 1998). These sample larvae were raised from eggs oviposited on the same day and reared from the same artificial diet and other laboratory conditions as infesting larvae, but they were not used for infestation. Larvae were placed individually into each of 15 holes in the soil, 3-5 cm deep, 4-8 cm from the stem, and 2.5 cm apart, which were then recapped with soil. All containers remained non-flooded for 16 d to allow larvae to become established.

Flooding

Sixteen days after infestation (11 Mar 2007), 6 plants in each soil type (marl soil or potting medium) were flooded by placing each plant container into a larger 19-L plastic container filled with tap water with the water level maintained 10 cm above the soil surface (24 cm total depth). The remaining 6 plants from each soil type were used as the control plants (non-flooded). One flooded monitoring plant in each soil type was evaluated after 3, 6, 9, and 23 d to determine when to evaluate the test plants based on the number and size of live larvae found in the soil. Test plants in each treatment were harvested

when less than 30% of the 15 larvae originally added per monitoring plant were found alive in both soil types (after 38 d of flooding). Here, 30% survival was arbitrarily chosen to represent a likely significant reduction compared to 100% survival when initially infested. Once the treatment combinations had below 30% survival because of flooding, soil type, or other reasons, they could be effectively compared to determine if the mortality was due to differences in treatment (flooding or soil type) or other factors. After this "30% date" was reached, an additional 2 weeks were allowed to further ensure any significant differences between flooded and non-flooded treatments. Non-flooded plants were irrigated by overhead sprinkler 30 min once a day until 23 Mar, when irrigation times were changed to 30 min twice a day. Flooded plants were irrigated before flooding began or after it ended, but not during the flood period.

Data Collection

Data collected included soil temperature, numbers of live and dead larvae recovered per plant, and larval head capsule widths. Soil temperature for non-flooded plants was recorded at 1 h intervals throughout the experiment with sensors (StowAway® Tidbit® temploggers, Onset Co., Pocasset, MA). Soil temperature of flooded plants was not recorded because it was believed to not differ significantly from that of non-flooded plants. The sensors were placed in the soil of 3 non-flooded plants not included in the experiment but held under the same experimental conditions. Sensors were located at a soil depth of 6 cm two-thirds the distance from the center to the outer edge of the pot.

When flooded plants were unflooded and all plants harvested, roots were removed from the soil, which was placed into bins and carefully inspected for larvae. The number of live and dead larvae were then determined for each plant container and preserved in separate vials of 75% ethanol. Head capsule widths were measured in the laboratory with a microscope micrometer with 50 micrometer units per mm and 80 × magnification. All data for percentage of larvae found alive were based on live/total ratios.

Statistical Analyses

A two-way analysis of variance (ANOVA) was used to determine flooding and soil type interaction in a factorial design for percentages of larvae found alive. However, because no larvae were recovered from 1 treatment combination (flooded potting medium), data for head capsule widths were analyzed with a one-way ANOVA with 3 treatments followed by a Duncan-Waller K-ratio test. For percentage of larvae surviving, proportional data based on ratios of live/total were arcsine transformed before analyses by standard *t*-tests. All statistical analyses were performed with SAS Statistical Software Version 9.1 (SAS Institute, Cary, North Carolina).

Results and Discussion

For plants used for monitoring only, mean percentages of live larvae found in flooded marl soil were 47, 87, 60, and 27 on flood days 4, 7, 10, and 24, respectively, and for flooded potting medium they were 20, 7, 7, and 0, respectively, on the same flood days. The sample on flood d 24 was the first with larval survival less than 30% in both soil types. As noted, to ensure significant differences in survival between flooded and non-flooded treatments, all flooded test plants were harvested 2 weeks after that date, or 38 d after flooding treatments commenced. No pupae or adults were found in either monitoring or test plants.

For test plants, there was no significant interaction between effects of flooding and soil type on the numbers of live/total larvae ($F = 3.98$; $df = 3, 23$; $P = 0.06$). However, because this interaction was nearly significant, data were not pooled for determination of the percentages of larvae that survived (Fig. 1). Flooding significantly reduced the mean percentage of larvae surviving compared with non-flooded conditions in marl soil ($t = -5.45$, $df = 9.3$, $P = 0.0004$) (Fig. 1a) and in potting medium ($t = -6.36$, $df = 5$, $P = 0.0014$) (Fig. 1b). The mean percentage survival out of 15 original larvae per container was significantly lower in flooded potting medium than in flooded marl soil ($t = 4.58$, $df = 5$, $P = 0.006$) (Fig. 1c). In non-flooded soil, there were no significant effects of soil type on percentages of live larvae recovered (Fig. 1d). There were significant differences in head capsule width among 3 treatments for which data were available (flooded marl soil, non-flooded marl soil, and non-flooded potting medium) ($F = 37.3$; $df = 2, 17$; $P < 0.0001$). Mean head capsule widths were significantly smaller for larvae in flooded marl soil than for larvae in non-flooded marl soil or non-flooded potting medium (Fig. 2). Larval head capsule widths from non-flooded marl and non-flooded potting medium were statistically the same and averaged eighth instar, whereas flooded marl larvae averaged sixth to seventh instar.

Lapointe (2000) examined the effects of constant, 22, 26, and 30°C temperatures on *Diaprepes abbreviatus* larval survival and rates of development on an artificial diet. The highest survival rates occurred at 22 and 26°C with lowest survival at 30°C, and the highest development rate was at 26°C with slower rates at 22 and 30°C (Lapointe 2000). Mean daily soil temperatures during the treatment period of the present study ranged from 16 to 25°C with monthly averages

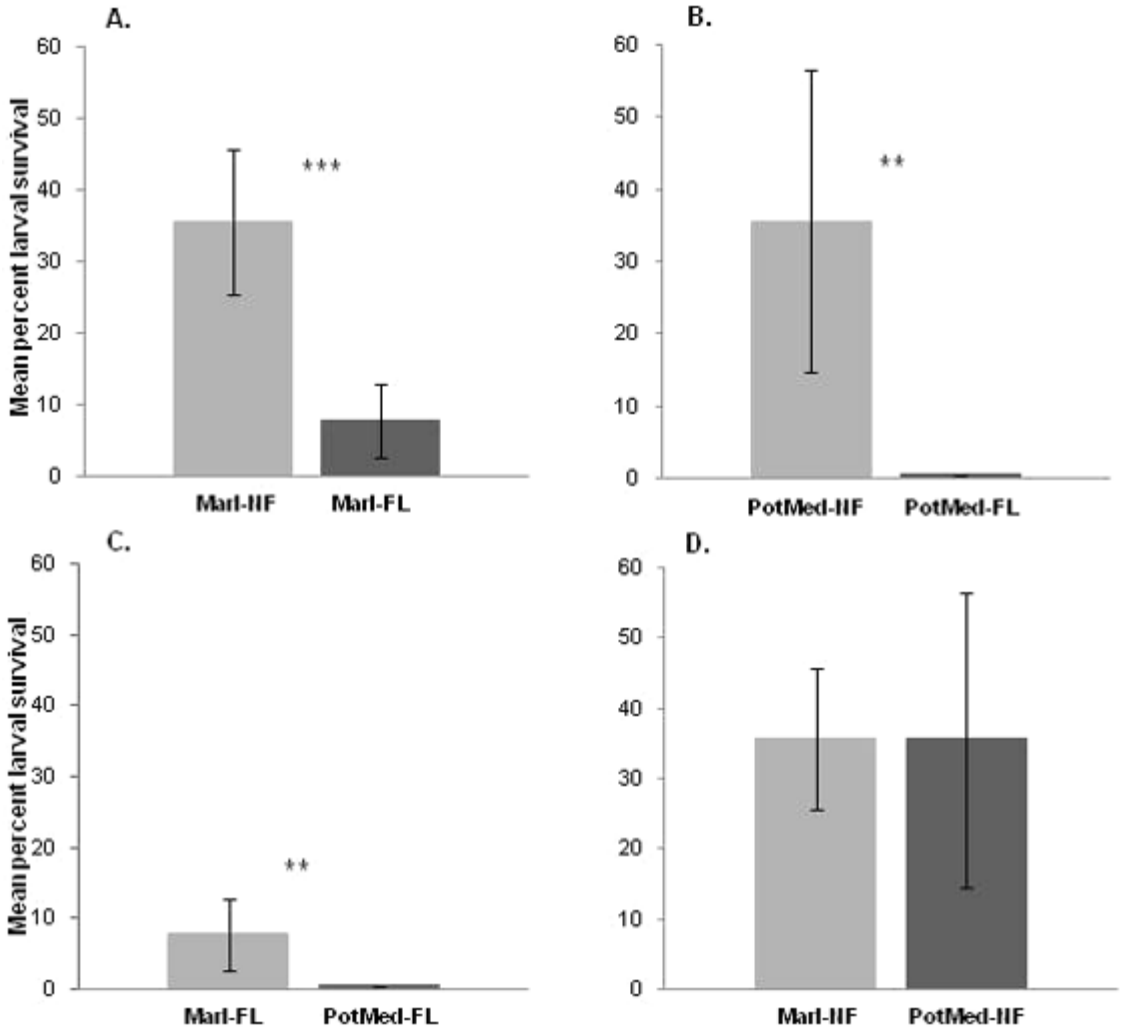

Fig. 1. Effects of flooding and soil type on percentage of 15 larvae added to each container that were found live at harvest based on ratios of live/total. (A) Non-flooded marl soil (Marl-NF) versus flooded marl soil (Marl-FL). (B) Non-flooded potting medium (PotMed-NF) versus flooded potting medium (PotMed-FL). (C) Flooded marl soil (Marl-FL) versus flooded potting medium (PotMed-FL). (D) Non-flooded marl soil (Marl-NF) versus non-flooded potting medium (PotMed-NF). Bars represent means ± SD. Asterisks indicate significant differences between treatments at * $P \leq 0.05$, ** $P < 0.01$, and *** $P < 0.001$ according to a standard t-test.

17.9 to 21.7°C (Fig. 3). Thus, average monthly soil temperatures for the present study were 4.3-8.1°C less than 26°C (ideal developmental temperature) and 0.3-4.1°C less than 22°C (ideal survival temperature). Although rates of larval survival may have been close to their maximum rates in the present study, larval development rates were probably slower than their maximum.

The present study focused on larval growth and survival and did not examine flooding and herbivory effects on biomass such as fresh and dry root, stem, and leaf weights, stem diameter, and plant height. However, larval herbivory tends to significantly reduce biomass and gas exchange of buttonwood in potting medium (Diaz 2005; Diaz et al. 2006; Martin et al. 2009). In addition, flooding buttonwoods in potting medium significantly reduced photosynthesis, transpiration, and stomatal conductance beginning 1 wk after flooding (Diaz 2005). However, flooding buttonwood plants did not significantly affect fresh or dry root, stem, or leaf weights (Diaz 2005). In another study with buttonwood, flooding did not cause significant differences in photosynthesis, stomatal conductance, or dry weight of plants grown in potting medium (Martin et al. unpublished data). However when grown in marl soil, flooding significantly reduced photosynthesis,

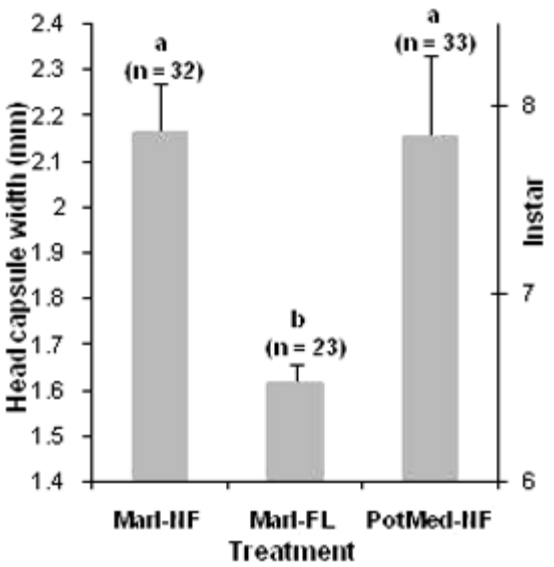

Fig. 2. Mean head capsule widths and instars (±SD) of larvae found at harvest. Values for live and dead larvae were pooled. N is the total number of larvae found in each treatment. X-axis symbols are Marl-NF (non-flooded marl soil), Marl-FL (flooded marl soil), and Pot-Med-NF (non-flooded potting medium).

stomatal conductance, and leaf dry weight compared with non-flooded plants. The native tidal-swamp habitat of buttonwood is frequently flooded and has marl soil, which is the environment where buttonwood evolved and should be best adapted.

Live/total ratios may better represented larval survival than live/found or found/total ratios because larvae not found were presumed dead and decomposed (although some larvae were found dead), and the objective was to determine survival of *D. abbreviatus* larvae. Hence, all data for percentage of larvae found alive were based on live/total ratios, and not live/found or found/total ratios, although all 3 ratios seem to be plausible definitions of survival. Comparing *D. abbreviatus* larval survival in flooded marl soil with flooded potting medium was difficult because of the high proportion of larvae not recovered. However, this was not surprising because larvae quickly decompose when they die. Survival of *D. abbreviatus* larvae in flooded marl soil was much higher than its survival in flooded potting medium. In fields of marl soil with mixed nursery stock including flood-sensitive and flood tolerant plants, flooding is probably not a good means to control this pest because of possible harm to flood-sensitive plants like Surinam cherry (*Eugenia uniflora* L., Myrtaceae). For plants grown in a potting medium similar to ours, results of our study suggest root-zone flooding of at least 3 d will help control *D. abbreviatus* larvae in flood-tolerant to moderately flood-sensitive plant species. This regime is especially suggested for plants tolerant or moderately tolerant to flooding, however, flooding containerized plants may not always be practical.

When not flooded, soil type such as marl soil or potting medium did not affect larval survival or growth of *D. abbreviatus* during this 54-d experiment. However when flooded, soil type did significantly affect percent larval survival. In addition, larvae recovered from flooded marl soil had significantly smaller head capsule widths, which indicates they averaged at least one instar smaller than larvae from non-flooded marl or non-flooded potting medium. Reduced oxygen concentration in flooded soil may have reduced larval respiration and decreased survival and size of larvae from flooded compared with non-flooded soil of either soil type. Larval survival and growth seem to be more affected by flooding than by soil type, although both treatment effects may cause significant differences in larval survival.

Flooding is sometimes used in southern Florida sugarcane fields to control pests such as grubs *Tomarus subtropicus* (Blatchley) (Coleoptera: Scarabaeidae) (Cherry 1984) and wireworm larvae *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae) (Hall & Cherry 1993). In those studies, wireworm larvae (*M. communis*) had 80% mortality after 6 wk of submergence at 27°C, whereas scarab grubs (*T. subtropicus*) had 100% mortality after ~1 wk (5-10 d) of submergence. 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). Shapiro et al. (1997) exposed *D. abbreviatus* larvae to flooding to test the effects of varying temperature (18, 21, 24, and 27°C) and flood periods (0, 1, 2, 3, 4, or 5 weeks) on larval mortality. They found that mean mortality exceeded 90% by 3 weeks at 24 and 27°C and by 5 weeks at 21°C, but was only 46% after 5 weeks at 18°C. In addition, soil pH increased significantly with time and mortality (Shapiro et al. 1997). Li et al. (2004, 2007) found that pH increased with increasing flood period, which is related to reduced oxygen content of flooded soil and not necessarily effects of *D. abbreviatus* larval infestation, such as respiration. Reduced oxygen in flooded soil is caused by many factors including replacement of soil oxygen with water and microbial and chemical conversion of oxygen to other substances. Flooding may be useful for controlling *D. abbreviatus* larvae in sugarcane fields, although only in the summer and fall when floodwater temperatures are close to maximum (27°C) (Hall & Cherry 1993; Shapiro et al. 1997).

Lapointe & Shapiro (1999) tried to determine levels of soil moisture that optimized production

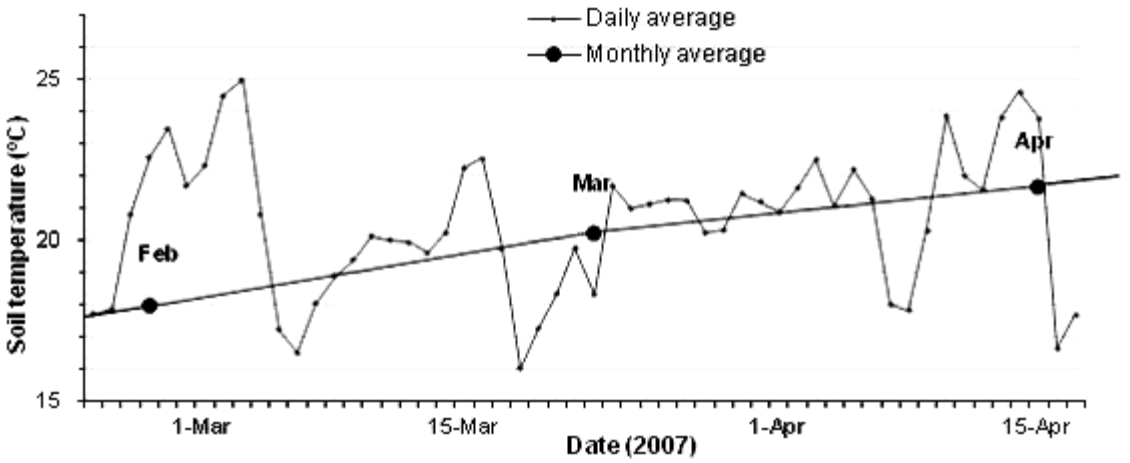

Fig. 3. Soil temperature during the experiment. Each point is the average of three temperature sensors each buried 6 cm below the soil surface in potted plants not used in the experiment but held under the same environmental conditions and with the same media as in the experiment. For Feb, the monthly average is for the whole month; however, because plants were infested on Feb 23, daily averages are shown only for Feb 23-28. Similarly, the Apr monthly average is for the entire month, but daily averages are for Apr 1-17 because plants were harvested and unflooded Apr 17. All of Mar was in the experimental period, hence, the monthly average and all daily averages are shown for the entire month.

of *D. abbreviatus* adults in the laboratory. Optimal survival to pupation occurred at 30-70% soil moisture, and about 60-65% of larvae survived to pupation under these ideal moisture conditions. The poorest survival of larvae occurred in low (20%) and in high (80%) soil moisture levels (Lapointe & Shapiro 1999). Thus, poorest larval survival would be expected under flooded conditions, which presumably have over 80% moisture levels, whereas un-flooded plants may have soil moisture levels more favorable to larval survival, 30-70%.

In the present study and the earlier study by Shapiro et al. (1997), larvae of *D. abbreviatus* were exposed directly to flooding. Several studies examined the interaction of flooding and larval feeding by *D. abbreviatus* (Li et al. 2003; Diaz 2005) or the interaction of flooding and soil type or pH on larval survival or growth (Li et al. 2006, 2007). However in these latter experiments, larvae in flooded treatments were added to previously flooded plants and were not exposed directly to flooding.

Overall, plant gas exchange and plant weights observed by Diaz (2005) seemed to have been more affected (decreased) by flooding than by larval infestation in buttonwood or live oak. However with larval recovery, there were no significant differences between previously flooded and non-flooded buttonwoods or live oaks (Diaz 2005). This lack of difference may reflect similar soil moisture contents between previously flooded and non-flooded plants during larval infestation. For Li et al. (2003), survival of Diaprepes larvae was significantly higher in previously flooded soil than in non-flooded soil, and flood-damaged seedlings were more susceptible to larval feeding injury than non-flooded seedlings. Similarly, Li et al. (2006) found that plants flooded for at least 20 d were more stressed and more prone to feeding injury from Diaprepes larvae after removal of plants from flooding than non-flooded control plants. Li et al. (2006) also found that larval survival averaged 25% higher in sandy soil than in loam soil in plants previously flooded for 20 d. Their results suggest that avoidance of flooding and early control of Diaprepes larvae may help protect young plants.

Soil type affects larval growth and survival rates, and the effects of soil type on larval survival may be chiefly based on physical characteristics of the soil, which affect soil moisture and oxygen levels (Rogers et al. 2000). Soil pH also increases with flood duration and could adversely affect larval survival (Shapiro et al. 1997; Li et al. 2006). 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). Li et al. (2007) found that when not limed, flooding increased the average soil pH up to 0.3 units for citrus seedlings flooded for 40 d, which also had the lowest larval weights and survival rates compared with seedlings flooded for shorter flood durations; this may reflect the higher soil pH at longer flood durations. Larval survival and growth were significantly decreased by pre-applied flooding (Li et al. 2007). When the soil was limed to pH 4.8-5.7, larval survival was highest at pH 5.0 for non-flooded plants. Larval survival and weight gain were sig-

nificantly correlated with pH; increasing pH from 4.8 to 5.7 decreased larval survival and increasing pH from 5.1 to 5.7 significantly decreased larval weights (Li et al. 2007).

Other factors such as soil compaction, bulk density, and water content may also influence larval survival and growth (Riis & Esbjerg 1998; Rogers et al. 2000; Li et al. 2007). Increasing the soil pH by at least 1 unit in acidic soils was recommended for optimum citrus growth, which occurs at pH 6.0-6.5, and to help control *D. abbreviatus* (Li et al. 2007). Flooding was also recommended as a possible control method in citrus (Li et al. 2007). Flooding may hence reduce larval survival while plants are flooded. However, depending on soil pH, water-stressed plants may be more susceptible to *D. abbreviatus* larval feeding when unflooded than non-stressed plants that were either never flooded or flood-tolerant and previously flooded.

As noted, marl soil native to south Florida has pH of 7.4-8.4 (Li 2001), whereas the potting medium in the present study had a pH of 6.0. As suggested above by Li et al. (2007), increasing the soil pH from 4.8 to 5.7 decreases larval survival and/or weight. Thus, a pH of 6.0 would appear less favorable than 5.0 for *D. abbreviatus* survival. Soil pH in the range of marl soil was not investigated in the foregoing studies. Hence, marl soil may offer a pH range more favorable to larval survival and growth than our potting medium, which is suggested by our higher larval survival rates in flooded marl soil than in flooded potting medium. However, this difference in survival was not present between non-flooded marl soil and non-flooded potting medium. There is a need to investigate possible survival advantages to *D. abbreviatus* larvae in the pH range of marl soil (7.4-8.4) compared with their survival in lower pH (4.8-6.0) of potting medium in the present study and of Florida sandy loam soil used by Li et al. (2007).

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