

EFFECTS OF HERBIVORY BY *DIAPREPES ABBREVIATUS* (L.) (COLEOPTERA:  
CURCULIONIDAE) AND FLOODING ON THE PHYSIOLOGY AND GROWTH OF  
SELECT ORNAMENTAL PLANT SPECIES IN SOUTH FLORIDA

By

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CURCULIONIDAE) AND FLOODING ON THE PHYSIOLOGY AND GROWTH OF  
SELECT ORNAMENTAL PLANT SPECIES IN SOUTH FLORIDA

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The objective of this research was to determine effects of herbivory by *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) and flooding on the physiology and growth of select ornamental plant species in south Florida. Susceptibility to larval feeding was greatest for green buttonwood and Surinam cherry, followed by mahogany, then pond apple. Pond apple supported *Diaprepes* larvae to pupation and represents a new host plant and family (Annonaceae). Under flooded conditions and/or planted in marl soil, pond apple performed optimally, whereas buttonwood, mahogany, and Surinam cherry had reduced leaf gas exchange and growth. Surinam cherry had the greatest “preference” of potting medium over marl soil, followed by buttonwood, then mahogany, then pond apple. Pond apple and buttonwood tolerated flooding the best, followed by mahogany, then Surinam cherry. When flooded 38 days, larval survival was lowest in flooded potting medium, followed by flooded marl soil, then non-flooded marl soil and non-flooded potting medium. Larvae from flooded marl soil had smaller head capsule widths and instars than non-flooded larvae in marl soil or potting medium. Flooding more than soil type influenced larval survival, though both factors affected it. Effects on leaf gas exchange and plant growth were cumulative for flooding in green buttonwood and for

infestation in Swingle citrumelo. Contrary to similar studies, buttonwood was not flood-adapted and was the most flood-sensitive species, possibly because of short flood periods, while Swingle citrumelo was the most sensitive species to larval feeding. Flooding with three 2-d cycles seemed more likely to control larvae in Swingle citrumelo than in green buttonwood. In an adult *Diaprepes* study, green buttonwood adapted to flooding rendering leaf gas exchange, growth, and adult preference the same under flooded and non-flooded conditions. For Surinam cherry and mahogany, leaf gas exchange, growth, and adult feeding damage in infested cages was higher for non-flooded than flooded plants. Pond apple had the lowest adult feeding damage and egg clusters per plant, hence, it seems unlikely to become infested. Surinam cherry was most susceptible to flooding, followed by mahogany, with buttonwood and pond apple least affected, whereas buttonwood and mahogany were most susceptible to adult *Diaprepes*, followed by Surinam cherry, then pond apple.

CHAPTER 1  
INTRODUCTION AND LITERATURE REVIEW

**Introduction**

*Diaprepes abbreviatus* L. (Coleoptera: Curculionidae: Entiminae) commonly called Diaprepes root weevil, was first discovered in Florida in a citrus nursery in Apopka in 1964 (Woodruff 1964). However by 1968, workers had collected hundreds of additional adults and larvae near Apopka (Woodruff 1968). Diaprepes root weevil is believed to have entered Florida from Puerto Rico in a shipment of ornamental plants (Woodruff 1985). Diaprepes root weevil is an abundant and serious pest of citrus and sugarcane in its home range of Puerto Rico (Woodruff 1964). In Florida, it infested approximately 60,000 ac (24,281 ha) of citrus, and control costs and losses have exceeded \$1,200 per ac (\$2,965 per ha) (Stanley 1996). The weevil has cost the Florida citrus industry an estimated \$72 million annually (Anonymous 1996, Stanley 1996). More recently, the weevil caused about \$70 million annually (crops not specified), and about 100,000 ac (40,469 ha) of citrus were infested (Weissling et al. 2004). Because of inadequate management strategies and its wide host range, Diaprepes root weevil has threatened the survival of several agronomic crops (Simpson et al. 1996) and nearly put many central Florida ornamental plant nurseries out of business during the 1970s (Schroeder 1994). In Florida, Diaprepes root weevil is currently found in 23 counties in the south and central parts of the state, which includes Miami-Dade, Broward, Collier, Palm Beach, Lee, Hendry, Desoto, Glades, Highlands, Martin, St. Lucie, Indian River, Manatee, Hillsborough, Osceola, Polk, Lake, Sumter, Pasco, Orange, Seminole, Volusia, and Marion (Anonymous 1996, Peña 1997, Weissling et al. 2004).

In addition to damage caused by the pest, there are regulatory concerns of spreading Diaprepes root 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). Diaprepes root weevil has also been found in Texas (Knapp et al. 2001, Skaria and French 2001) and southern California (Klunk 2005), and these weevils likely came from Florida. Hence, there is a need to continually control Diaprepes root weevil to reduce the risk of its introduction into other states and countries.

**Host range.** Diaprepes root weevil 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. 2000b, Mannion et al. 2003, Godfrey et al. 2006, C.G. Martin personal observation and unpublished data). The plant families with the largest number of varieties, species, and genera attacked by the pest include Rutaceae (95 varieties, 66 species, and 24 genera) and Fabaceae (43 varieties, 42 species, and 31 genera). Both these families are in the order Rurales (Zomlefer 1994). In addition, the four remaining families of Rurales (Meliaceae, Anacardiaceae, Sapindaceae, and Aceraceae) each have representative species on the above list of Diaprepes root weevil host plants. Besides Rurales and other dicots, monocot taxa such as Poaceae and Gymnosperm taxa such as Cupressaceae also have representative species on this list of Diaprepes root weevil host plants (Simpson et al. 1996). Some plants support only one stage of the insect; for example *Ardisia crenata* Sims (Myrsinaceae), which supports only the larval stage. However, many economically important plants support all stages of the weevil from egg to adult, such as sweet potato, *Ipomoea batatas* (L.) (Convolvulaceae) Lam., and buttonwood, *Conocarpus erectus* L. (Combretaceae) (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 Diaprepes root weevils were common on field-grown ornamental plants. In the field, highest percentages of plants with egg masses were found on live oak (*Quercus virginiana* Mill., Fagaceae), silver buttonwood (*C. erectus* L. variety

*sericeus* Fors.Ex DC), and black olive (*Bucida buceras* L.) (the latter two species Combretaceae). In addition, adult feeding damage in the field was commonly found on dahoon holly (*Ilex cassine* L., Aquifoliaceae), cocoplum (*Chrysobalanus icaco* L., Chrysobalanaceae), *Bauhinia* sp., and *Cassia* sp. (both Fabaceae). Highest adult population densities in the field were found on black olive, dahoon holly, and *Bauhinia* sp. (Mannion et al. 2003). Mannion et al. (2003) also evaluated *Diaprepes* root weevil in choice tests of caged adults. Based on comparisons of mean numbers of egg masses per plant species, silver buttonwood was among the most preferred host.

Adair et al. (1998) also compared mean numbers of *Diaprepes* root weevil egg masses oviposited on two to four different above-ground substrates to determine adult ovipositional preferences. Substrates included young and mature grapefruit leaves (*Citrus paradisi* Macf., Rutaceae), freezer paper, wax paper, transparency film, and several other materials. Based on mean numbers of egg masses per substrate, *Diaprepes* root weevil females preferred freezer paper over young or mature grapefruit leaves for oviposition (Adair et al. 1998). Freezer paper is now widely used in laboratories to collect eggs from *Diaprepes* root weevil adults.

**Biology, taxonomy, and range.** *Diaprepes* root weevil is part of a complex of problematic root weevils that are taxonomically related (Coleoptera: Curculionidae: Entiminae) and share two feeding guilds: root feeders (larvae) and direct-leaf-consumers (adults). They have very similar life cycles and behavior with the following characteristics (Syvertsen and McCoy 1985, Futch and McCoy 1993): relatively long, univoltine life cycles; broad host ranges (70 or more plant species); larvae that feed on roots starting with fibrous roots, channeling the bark, or girdling the plant as they get older; pupation periods of 2 to 4 wk; pre-pupal and post-pupal resting periods in the soil; adult emergence often triggered by heavy rains; adults that typically notch leaves starting along the edges and progressing toward midveins; eggs that are

laid in masses between two leaves; and neonates that drop to the ground upon hatching and burrow into soil. In addition to *Diaprepes* root weevil, this pest complex includes the southern blue-green citrus root weevil, *Pachnaeus litus* (Germ.), and the northern citrus root weevil, *P. opalus* (Oliv.) (Coleoptera: Curculionidae: Entiminae) (Woodruff 1981, Anderson 2002).

The Entiminae is the “broad-nosed” or “scarred snout” beetle subfamily (Wolcott 1936, Anderson 2002). Their snouts are shorter and less developed than those in typical long-nosed weevil subfamilies such as Cyclominae (Anderson 2002). Entiminid adults bear scars at the ends of the snouts marking the disappearance of “mandibular appendages”-- prominent, curved, black claws, which are deciduous and larger than the final mandibles of the adult (Wolcott 1936, Anderson 2002). Adults shed the mandibular appendages when burrowing from their pupal chambers to the ground surface (Wolcott 1936, Anderson 2002).

Within the genus *Diaprepes*, Blackwelder (1947) lists 19 species; 18 from the West Indies and one from Nicaragua. More recently, Peña (1997) noted at least 20 species (including *D. abbreviatus*) in the Caribbean Basin. However, O'Brien and Kovarik (2000) recognized 16 species in *Diaprepes*, which is restricted to the Caribbean Basin with one species from Trinidad and Venezuela and the other 15 species from the West Indies.

O'Brien and Kovarik (2000) examined 1,000 specimens of *Diaprepes* root weevil and concluded that because of the similarity of Florida and Puerto Rico populations, the weevil was originally introduced from Puerto Rico. This island is probably the center of origin of *Diaprepes* root weevil since it is the center of diversity, which is suggested by the high degree of phenotypic diversity of the weevil there (Lapointe 2000a, O'Brien and Kovarik 2000). In addition to *Diaprepes* root weevil and two *Pachnaeus* spp., other problematic root weevils to ornamentals and/or citrus in Florida include two Fuller rose beetles, *Pantomorus cervinus* (Boheman) and *Asynonychus godmani* Crotch, the little leaf notcher, *Artipus floridanus* Horn, and *Myllocerus*

*undatus* Marshall (Woodruff and Bullock 1979, Woodruff 1982, Futch and McCoy 1993, DOACS 2002). Although these weevils are frequent pest species, they are less common on citrus based on geographic distribution than *Diaprepes* root weevil or the two *Pachnaeus* spp. suggesting they may be less problematic to citrus and/or ornamentals than the latter two taxa (McCoy et al. 2004).

**Life cycle.** The *Diaprepes* root weevil life cycle in the field lasts 8 to 18 mo from egg hatching to the last egg laid by the female or death of the male (Wolcott 1936). On an artificial diet, females required an average of 381 d to develop from oviposition to pupal eclosion (adulthood), and males fully developed in 382 d, hence, 12.5 mo were needed for both males and females (Beavers 1982).

*Diaprepes* root weevils normally lay eggs in clusters between leaves held together with an adhesive (Wolcott 1936). In the field, the average female produces about 5,000 eggs in her lifetime, and 30 to 264 eggs per cluster (Wolcott 1936, 1948). On an artificial diet, Beavers (1982) found that mated females averaged 6,517 eggs per lifetime, or 69 eggs per cluster. Eggs are oval-oblong, shiny, smooth, 1.2 mm X 0.4 mm, and initially milky-white, but beginning 1 to 2 d after oviposition, they develop clear spaces at one end (Wolcott 1936). Eggs are usually laid in single layers, although egg clusters can be up to two layers thick toward the center (Wolcott 1936). Eggs hatch about 7 d after oviposition (Wolcott 1936).

*Diaprepes* root weevil neonates move across the leaf in a galloping motion and fall off the edge to the ground (Wolcott 1936). On the ground, they continue crawling until they fall into a crack and work their way down into the ground (Wolcott 1936). Here after searching for up to 2 wk, they find and start feeding on small roots (Wolcott 1936). As they grow larger, the larvae prefer larger roots, ideally large enough for them to burrow into (Wolcott 1948). Larvae

sometimes burrow into corn kernels, though normally only roots and similar underground structures, such as potato tubers, are consumed (Wolcott 1936, Knapp et al. 2001).

The rate of larval development for *Diaprepes* root weevil varies with the season(s) in which they hatch. A March hatchling reached the sixth instar in 126 d, while a September hatchling took 27 d to reach the same instar. In the field, 2 to 4 mo is typical for the entire larval stage (Wolcott 1936). On an artificial diet, however, Beavers (1982) noted average larval durations of 362 d for females and 363 d for males, or 12 mo for either sex. *Diaprepes* root weevil larvae have 6-16 molts before pupation, with an average of 8 molts (Wolcott 1936). A surplus of food seems to increase the number of instars, whereas in most other insects, excess larval molting is caused by unfavorable environmental conditions like cold or lack of food (Wolcott 1936).

*Diaprepes* root weevil larvae normally move horizontally or transversely in the soil except when ready to pupate. They form vertical pupal chambers by compacting the walls with their caudal ends and then rest in the chambers with heads up (Wolcott 1936). The two periods that vary the most in the *Diaprepes* root weevil life cycle are the prepupal and pre-emergence resting periods (2 to 13 mo combined) (Wolcott 1936). The prepupal resting period lasts 2 to 3 wk after formation of the pupal chamber and is required for *Diaprepes* root weevil to pupate (Wolcott 1936). The intervening pupal stage is 14 to 26 d (2 to 4 wk) long (Wolcott 1936). Pupation occurs throughout the year, although highest percentages of annual pupation are in March and October (Wolcott 1936). Upon eclosion from pupa, the adult resting period is 11 to 126 d (0.4 to 4.2 mo), with the average approximately 2 mo, or about half the 4-5 mo mean adult lifespan (Wolcott 1936). In the field, female adults usually live longer than males, often more than twice as long (Wolcott 1936). On an artificial diet, the mean duration of an adult *Diaprepes*

root weevil lifespan is similar to in the field--147 d (5 mo) for females and 135 d (4.5 mo) for males (Beavers 1982).

According to Wolcott (1936), adult emergence seems to not be triggered by external factors such as periods of rain. However, according to Grafton-Cardwell et al. (2004), dry, compact soil inhibits adult emergence, whereas irrigation and rainfall tend to promote it. Contrary to the above opinion by Wolcott (1936), large numbers of adult *Diaprepes* root weevil have been seen emerging after periods of heavy rain in south Florida (C. Mannion pers. comm.). Beavers and Selhime (1976) also observed that heavy rainfall usually preceded field emergence of adults. Although *Diaprepes* root weevil is univoltine, generations overlap throughout its range (Beavers and Selhime 1976, Peña 1997). In central Florida there are two peak adult emergence periods, May to June and August to September (Beavers and Selhime 1976, Peña 1997).

The majority of adult feeding is by females to meet the requirement of egg production. Feeding drops off rapidly when oviposition stops, and males eat much less than females (Wolcott 1936). *Diaprepes* root weevils typically mate in the foliage, and 3 to 7 d after females emerge from the ground, they begin oviposition (Wolcott 1936, 1948). Duration of oviposition is typically most of an adult female's lifespan and varies from 2 mo after emergence in May or June to 7 mo at other times of the year (Wolcott 1936, 1948).

*Diaprepes* root weevil adults are gregarious, and some trees may have large numbers congregated in the branches, while nearby trees have none (Wolcott 1936). Peña et al. (2007) sampled tree branches of silver buttonwood and dahoon holly trees and found that egg-cluster distribution was aggregate in the upper and middle branches where most egg clusters were found, whereas lower branches had a random distribution. This may reflect that *Diaprepes* root weevil adults are gregarious and tend to gather in the upper and middle canopies. In addition, *Diaprepes* root weevil adults prefer to oviposit on mature, expanded leaves of citrus (Schroeder

and Sutton 1977, Lapointe 2001). On silver buttonwood, mature leaves in the upper and middle canopy tend to be more flattened than the more curved, sooty-mold-laden, mature leaves in the lower canopy. Thus, leaf shapes that favor oviposition in the upper and middle canopies and the gregarious habits of adults may cause greater abundance of egg clusters and the more aggregate distribution in upper and middle branches than in lower branches (Peña et al. 2007).

**Damage.** Neonate *Diaprepes* root weevils start feeding on small, fibrous roots and work their way up to larger lateral and main roots as they mature (McCoy et al. 2002). Their feeding on the larger roots often forms deep grooves, and they consume outer bark and cambial layers (McCoy et al. 2002). Roots may be girdled causing root death or the crown may be girdled causing tree death (McCoy et al. 2002). Larval girdling often kills small citrus trees by destroying their ability to take up nutrients (Wolcott 1936, 1948; Quintela et al. 1998). Quintela et al. (1998) found that the mean rate of larval weight gain was highest for fifth through seventh instars, which presumably cause the most damage. Effects of root feeding damage from *Diaprepes* root weevil larvae have been less thoroughly examined for ornamental plants than for citrus, although they can be just as severe in either group. For example, root feeding by *Diaprepes* root weevil larvae resulted in severe damage to many field-grown ornamental plants in south Florida (C. Mannion, pers. comm.). Additionally, *Diaprepes* root weevil larval feeding and root injury often serve as infection sites for root rot diseases, which exacerbate economic losses compared to those caused by the weevils alone (McCoy et al. 2002). The principle fungi that cause severe root rot are *Phytophthora nicotinae* Breda de Haan and *P. palmivora* (Butl.) Butl. (Oomycota) (McCoy et al. 2002).

The most obvious feeding damage includes the aforementioned notching from adults feeding along margins of especially young, tender leaves (Wolcott 1936, 1948, McCoy et al. 2002). This can result in moderate to severe defoliation of host plants such as young, replanted

citrus trees (Quintela et al. 1998, McCoy et al. 2002, Mannion et al. 2003). Unlike larval root feeding, however, prolonged adult feeding does not seem to economically reduce yields of mature citrus orchards (McCoy et al. 2002). However, for ornamental plant species, excessive foliar damage makes them less attractive at the time of sale and can reduce sales (C. Mannion pers. comm.).

**Arthropod effects on photosynthesis.** Insect herbivory often affects leaf gas exchange including net CO<sub>2</sub> assimilation ( $A$ ), also called net photosynthesis ( $P_n$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and substomatal partial pressure of CO<sub>2</sub>, also called internal CO<sub>2</sub> concentration ( $C_i$ ) of host plants (Andersen and Mizell 1987, Schaffer and Mason 1990, Schaffer et al. 1997). Root (1973) and Welter (1989) classified arthropods according to their type of feeding damage or guild, such as mesophyll feeders, phloem feeders, stem borers, root feeders, and direct leaf consumers. The effects of insect herbivory on leaf gas exchange can vary according to feeding guild (Welter 1989). In a review by Welter (1989) on how arthropod herbivory affects leaf gas exchange, most studies claimed that herbivory is detrimental to this process, although insignificant to beneficial effects where herbivory increased leaf gas exchange have been noted.

Diaprepes and other root weevils such as *P. litus* and the little leaf notcher *A. floridanus* have two feeding guilds. Larvae are in the root-feeder guild, whereas adults are in the direct-leaf-consumer guild (Syvertsen and McCoy 1985). Syvertsen and McCoy (1985) studied the rates of photosynthesis, transpiration, and water use efficiencies of citrus infested with adult *A. floridanus*. They found that when weevil population densities exceeded one weevil per leaf, herbivory reduced water use efficiencies (defined as photosynthesis divided by transpiration) up to 20%. Syvertsen and McCoy (1985) also found that when adult *A. floridanus* increased consumption of citrus leaf area, photosynthesis and water use efficiency declined. Because

water use efficiency decreased more rapidly than photosynthesis, drought stress from injured leaves may have enhanced the loss of photosynthesis with increasing leaf area consumed. Hence, drought stress may increase the severity of feeding injury or vice versa (Syvertsen and McCoy 1985). Findings of Syvertsen and McCoy (1985) thus exemplify detrimental effects of herbivory on photosynthesis, which are the most common effects caused by root feeders. However according to Welter (1989), insects in the direct-leaf-consumer feeding guild usually increase photosynthesis measured on a leaf-area basis, but this effect is atypical compared to other guilds: for example, stem borers and root feeders tend to decrease photosynthesis (Welter 1989).

According to Welter (1989), effects of herbivory from the root-feeder guild have been less thoroughly documented than effects of herbivory from above ground guilds, such as phloem feeders and gall formers. In addition, studies of the effects of herbivory by nymphs and larvae on leaf gas exchange, such as those done by Schaffer et al. (1997) and Diaz et al. (2006) have been conducted less frequently than studies of the effects of herbivory by adult insects, such as those conducted by Syvertsen and McCoy (1985) and Boucher and Pfeiffer (1989).

Nigg et al. (2001a) tested the responses of seven varieties of citrus rootstock seedlings to larval feeding by *Diaprepes* root weevil neonates. They compared fresh and dry root weights, trunk diameters, and larval recovery of infested and non-infested plants of each variety to determine whether it was tolerant to *Diaprepes* root weevil larval feeding (Nigg et al. 2001a). Hence, none of the seven varieties were tolerant to larval feeding (Nigg et al. 2001a).

Infestation by another root-feeding larval pest, the western corn rootworm, *Diabrotica vergifera vergifera* Leconte (Coleoptera: Chrysomelidae) resulted in varied effects on leaf gas exchange and compensatory growth depending on severity of feeding (Riedell and Reese 1999, Urias-Lopez et al. 2000). During corn vegetative growth (48 d after planting), photosynthesis

was lower in root-damaged than in non-damaged plants, and it was lower in severely infested than in moderately infested or non-infested plants (Riedell and Reese 1999). In the tassel stage of corn (54 d after planting), feeding by rootworms significantly lowered stomatal conductance relative to non-infested plants, though photosynthesis was unchanged (Riedell and Reese 1999). In addition, adventitious roots grew as compensatory growth more often in moderately infested than in severely infested or non-infested plants (Riedell and Reese 1999). Hence, the severity of root damage and amount of compensatory growth each played important roles in mediating shoot growth and the level of photosynthesis (Riedell and Reese 1999).

The stem-borer feeding guild may be the most similar guild to root-feeders because their larvae also consume plant parts such as stems, which are not principle sites of photosynthesis. Godfrey et al. (1991) found that the stem-boring European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) significantly reduced corn photosynthesis up to 22% and stomatal conductance up to 28%. As with many foliage and root feeders, stem borers typically reduce photosynthesis of their host plants such as corn, *Zea mays* L. (Welter 1989, Godfrey et al. 1991).

For the gall-forming guild, all the papers reviewed by Welter (1989) reported that herbivory reduced photosynthesis in the galls and often in surrounding tissue. This was the case for galls created by *Phylloxera notabilis* Pergande (Homoptera: Phylloxeridae) on pecan foliage, *Carya illinoensis* (Wangenh.) K. Koch (Juglandaceae) (Andersen and Mizell 1987). Gall formers reduced photosynthesis by 27% in infested tissue, whereas the galls only occupied 6.4% of the infested area measured (Andersen and Mizell 1987). In addition to reducing photosynthesis, these gall formers significantly reduced transpiration, stomatal conductance, and chlorophyll. They also changed concentrations of some nutrients within galls (e.g., significantly decreasing N, but increasing P and K), and in surrounding tissue (e.g., significantly decreasing

N, not changing P, and increasing K), and hence altered metabolic functioning in areas affected (Andersen and Mizell 1987).

Members of another guild, phloem feeders, also tend to reduce photosynthesis according to slightly more than half the papers surveyed by Welter (1989). The lignum vitae tree *Guaiacum sanctum* L. (Zygophyllaceae) exemplifies this majority because its photosynthesis and other variables were reduced by the phloem-feeding scale insect, *Toumeyella* sp. (Hemiptera: Coccoidea) (Schaffer and Mason 1990). *Toumeyella* sp. lowered photosynthesis, transpiration, stomatal conductance, leaf area, and dry weights of the roots, stems, shoots, and leaves of lignum vitae trees (Schaffer and Mason 1990).

Leafminers exemplify another feeding guild, the mesophyll feeders. As in most feeding guilds, they usually reduce photosynthesis of the plants they feed on (Welter 1989). In a study of citrus leafminers, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), which are also herbivorous larvae, a visual estimation of leaf mining damage proved to be rapid, accurate, and practical (Schaffer et al. 1997). Mined leaves were chlorotic or necrotic, which suggested a reduction in photosynthesis that was at least partly caused by reduced chlorophyll content. In plants infested with citrus leafminers, increased leafmining duration and increased numbers of the larvae per leaf were each correlated with greater leaf area damage and photosynthesis reduction (Schaffer et al. 1997). Herbivorous mites are another group of arthropods within the mesophyll feeding guild that usually reduce photosynthesis (Welter 1989). In almond trees, spider mite herbivory caused significantly greater reduction of photosynthesis on water-stressed plants than on properly watered ones (Youngman and Barnes 1986, Welter 1989). In pecans infested with the pecan leaf scorch mite, *Eotetranychus hicoriae* (McGregor) (Acari: Actinedida: Tetranychidae), high levels of nitrogen fertilizer increased photosynthesis of mite-damaged leaves compared to undamaged leaves (Welter 1989). At moderate (optimal) nitrogen levels,

however, herbivory by mites significantly decreased photosynthesis compared to non-infested plants (Welter 1989). However, at low nitrogen levels, photosynthesis was the same between mite-damaged and undamaged leaves (Welter 1989). Hence, although mite feeding generally reduces photosynthesis, factors such as levels of water and fertilization can interact with effects of mite herbivory and greatly affect photosynthesis levels.

### **Management of Diaprepes Root Weevil**

**Cultural control.** In areas infested with *Diaprepes* root weevil, the following cultural practices help to optimize growth and maintain health of citrus and other cultivated plants (McCoy et al. 2002): regular fertilization, timely irrigation, skirt-pruning of low hanging branches to reduce adult dispersal, and weed control to remove sinks for nutrients and water, and reduce the number of alternate hosts. Borowicz et al. (2005) tested effects of nutrient supply and below-ground herbivory by *Diaprepes* root weevil larvae on citrus growth and mineral nutrient content, which suggests that tolerance to *Diaprepes* root weevil feeding is not a function of nutrient status. Borowicz et al. (2005) also found that application of excess, balanced fertilizer is not likely to offset growth reductions due to root herbivory, and they suggest supplementing specific nutrients to plants may be useful.

Li et al. (2003b) found that low soil concentrations of Mg, Ca, and H (hydrogen, high acidity), and low cation exchange capacity were correlated with increased *Diaprepes* root weevil populations. Li et al. (2003b) also suggested that cultural practices, such as adding lime to soil with an acidic or neutral pH, soil erosion by rain or floodwater, and flooding could affect field distributions of the weevil. Water applied for cultural control may lessen the incidence of *Diaprepes* root weevil in two ways, either by supplying water in needed amounts or in extremely large or small quantities (lack or surplus). Adding optimum amounts of water may control the pests indirectly, such as by improving plant turgidity, which helps in nutrient transport and

photosynthesis. As suggested in the example of spider mites on almonds, careful irrigation may control pests through increased plant development or growth to offset herbivory (Youngman and Barnes 1986, Welter 1989). Sustained flooding can be an important mortality factor for *Diaprepes* root weevil larvae and has been suggested as a possible control tactic in sugarcane fields (Shapiro et al. 1997). Hence, flooding may also be a viable control option for flood-tolerant, ornamental plants including buttonwood.

**Mechanical control.** Mechanical control of *Diaprepes* root weevil includes restricted movement of materials that spread pests from infested to non-infested areas (Knapp et al. 2000a). This material, commonly called debris, includes soil, plants, leaves, fruit, grass, branches, and stumps that may harbor the pests. In addition, shipping and other containers should be cleaned before reuse (Knapp et al. 2000a). After harvesting vegetables such as potatoes, the soil should also be disked several times to destroy foci of infestation (Peña 1997). Access to areas with known infestations should be limited; for example, only to crews moving plants within infested zones (Knapp et al. 2000a).

**Physical control.** Gould and Hallman (2004) found that irradiating materials containing adult *Diaprepes* root weevil at low enough levels not to damage commodities (~50 Gy/min) is a control option because adults are sufficiently sensitive to radiation. Because radiation tolerance increases with insect development (Hallman 2001), larvae and eggs should be more sensitive to radiation and easier to control than adults. Although irradiation would be detrimental to the growth of nursery stock and is not advisable, this method may be useful for controlling various stages of *Diaprepes* root weevil in sugar cane pieces, root crops, and similar commodities amenable to this technique (Gould and Hallman 2004). Because ionizing radiation is a viable disinfestation technique for treatment of exported agricultural commodities produced in Florida, this method may help control the spread of *Diaprepes* root weevil (Gould and Hallman 2004).

**Regulatory control.** Regulatory control of *Diaprepes* root weevil includes quarantine restrictions, which have been imposed to limit the movement of plant materials from infested to non-infested areas. However, these treatments can be expensive, labor intensive, and time consuming (Mannion et al. 2003). Sales and customers may be lost because quarantine treatments are in place, hence, plants cannot be shipped to a particular customer or location.

**Chemical control.** Numerous insecticides have been used to control both the larvae and the adults (Peña 1997, McCoy et al. 2004). Traditionally, broad spectrum insecticides with long residuals have been applied to the soil for larval control (Wolcott 1948). Since the 1980s, imidacloprid (Admire), bifenthrin (Talstar or Capture), and chlorpyrifos (Suscon Green) have been successfully used to control neonates in container-grown citrus (Peña 1997). For small larvae infesting ornamental plants in containers, imidacloprid and bifenthrin are widely used (Peña 1997, Peña et al. 2005).

McCoy et al. (2004) recommended the following for adult control in citrus: Danitol 2.4EC, Imidan 70WP, diflubenzuron (Micromite 80WGS), acephate (Orthene 97), carbaryl (Sevin 80S, Sevin 4F, and Sevin XLR). Other adulticides available since the 1980s include azinphos (Guthion), and formetanate hydrochloride (Carzol) (Peña 1997). Many of these chemicals are no longer available because their environmental toxicity has resulted in laws which ban their sale.

According to Schroeder et al. (1977) and Peña (1997), spray oils such as Florida citrus oil (FC 435-66), are used to separate leaves stuck together by egg masses. Although spray oils did not reduce *Diaprepes* root weevil fecundity or fertility according to Schroeder et al. (1977), they significantly reduced its reproductive potential due to loss in eggs. Bullock and Pelosi (2002) found that the insect growth regulator, diflubenzuron 25w (Dimilin or Micromite), reduced egg hatching rates an average 80% without oil and 98% with FC 435-66 spray oil added.

Diflubenzuron is highly effective at reducing the reproductive potential of not only *Diaprepes* root weevil, but also other citrus root weevils including *P. litus*, *P. opalus*, and *A. floridanus* (Lovestrand and Beavers 1980). As noted, McCoy et al. (2004) also lists diflubenzuron (Micromite) as a pesticide available to combat adult *Diaprepes* root weevil.

**Host plant resistance.** Host plant resistance has also been suggested to control *Diaprepes* root weevil. However, according to Lapointe and Bowman (2002), all currently used rootstocks for citrus are susceptible to the pest. Nevertheless, Bowman et al. (2003) tested resistance of citrus rootstocks to *Diaprepes* root weevil and to infections by *Phytophthora palmivora* and *P. nicotianae* under field conditions. They found large differences in tolerances of plant varieties to root rot, which was enhanced by herbivory from *Diaprepes* root weevil larvae. Based on these citrus studies, host plant resistance may protect against infections often indirectly caused by *Diaprepes* root weevil, such as by *Phytophthora* spp., but they would not protect against the weevil directly (Lapointe and Bowman 2002, Bowman et al. 2003).

**Biological control.** In southern Florida, 75% (3,750) of 5,000 eggs laid by the average *Diaprepes* root weevil female in her lifetime are killed by parasitoids (Peña et al. 2005). Of the remaining 1,250 viable eggs, only 1 in 164, or 7 individuals, will survive to adulthood (Nigg et al. 2004). Although parasitoids probably do not stop the spread of *Diaprepes* root weevil, they play a major role in controlling the weevil. Since 1997, 16 egg parasitoids have been introduced, six reared in quarantine, and three released in Florida (Peña et al. 2005). The three released species include two endoparasitoids, *Quadrastichus* [*Tetrastichus*] *haitiensis* Gahan (Hymenoptera: Eulophidae) and *Ceratogramma etiennei* Delvare (Hymenoptera: Trichogrammatidae), and an ectoparasitoid, *Aprostocetus vaquitarum* (Wolcott) (Hymenoptera: Eulophidae) (Peña et al. 2005). *Quadrastichus haitiensis* and *A. vaquitarum* are successfully established in south Florida (Amalin et al. 2004, Peña et al. 2005).

Although *Q. haitiensis* and *A. vaquitarum* are successfully established in south Florida (Amalin et al. 2004, Peña et al. 2005), they have not expanded their range into central Florida, where *Diaprepes* root weevil also abounds (Castillo et al. 2006). Lapointe et al. (2007) examined mean air and soil temperatures at the northern range limit of *Diaprepes* root weevil in Florida and compared them to corresponding temperatures for development thresholds of *Diaprepes* root weevil and these parasitoids to predict where they would establish. This method predicted that *Diaprepes* root weevil could establish in many areas in southern California, extreme southern Texas, and extreme southwest Arizona (Lapointe et al. 2007). As noted, the weevils have spread to some of these areas in southern California (Klunk 2005) and Texas (Knapp et al. 2001, Skaria and French 2001). However, Lapointe et al. (2007) predicted that the parasitoids would not establish in these new areas, hence, there would be no important biocontrol agents to help control the weevils.

Numerous studies have evaluated entomopathogenic nematodes for control of *Diaprepes* root weevil larvae which include *Steinernema [Neoaplectana] carpocapsae* (Weiser) (Mexican strain), *Heterorhabditis bacteriophora* Poinar (Rhabditida: Steinernematidae and Heterorhabditidae, respectively) (Beavers et al. 1983), and *S. riobravis* (Cabanillas, Poinar, and Raulston) (Duncan and McCoy 1996, Peña 1997). *Heterorhabditis bacteriophora* did not affect *Diaprepes* root weevil population densities (Duncan and McCoy 1996). However, Schroeder (1987, 1990) found that *S. carpocapsae* offers effective, environmentally safe control. Peña (1997) claimed that *S. riobravis* adds to the arsenal of biocontrol agents available, and control of *Diaprepes* root weevil larvae should include a fall application of the nematodes.

Mannion and Glenn (2003) tested the effects of combining nematodes (*H. bacteriophora* or *S. riobravis*), a soil drench of bifenthrin, or a combination of both to control large *Diaprepes* root weevil larvae. They found that combining soil drench and either species of nematode

worked better than nematodes or soil drench alone (Mannion and Glenn 2003). This is especially useful considering the need for efficacious treatments to regulate the spread of *Diaprepes* root weevil larvae when ornamental plants are shipped. These results also provide a method for controlling older larvae, which are traditionally difficult, and to reduce the cost and use of pesticides (Mannion and Glenn 2003).

According to Woodruff (1964), the green muscardine fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycota), takes a heavy toll on *Diaprepes* root weevil larvae. However, Wolcott (1936) claims that it sometimes kills larvae that are injured, mite-infested, or “sub-normal,” but does not attack healthy larvae in the field. In addition to *M. anisopliae*, Beavers et al. (1983) found that the following entomopathogenic fungi were present in soil and infectious to *Diaprepes* root weevil larvae: *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ascomycota), *Paecilomyces lilacinus* (Thom) Samson (Deuteromycota), and *Aspergillus ochraceous* K. Wilh. (Ascomycota). These fungi were present in nine citrus orchards throughout the year, though fungi-infested larvae were most prevalent May through July (Beavers et al. 1983).

Homestead, Florida, the study site for the research in this dissertation, has many common predators, which may affect numbers of *Diaprepes* root weevil at various stages of their life cycle. These include the Cuban tree frog (*Osteopilus septentrionalis* Duméril & Bibron), giant toad (*Bufo marinus* L.) (Amphibia: Hylidae and Bufonidae, respectively), brown anole lizard (*Anolis sagrei* Duméril & Bibron) (Reptilia: Polychrotidae) (Behler and King 1979), and spiders (many spp., Arachnida: Araneae). Other predators that attack *Diaprepes* root weevil include several ant species that prey on eggs and larvae. Examples include *Pheidole dentata* Mayr and *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Whitcomb et al. 1982, Peña 1997, Peña et al. 2005). The ants consume eggs but may have difficulty finding *Diaprepes* root weevil neonates on the ground; in addition, neonates produce chemicals that repel ants (Jaffe et al.

1990a, b). Also, lady bugs such as *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) prey upon Diaprepes root weevil eggs and neonates, although the level of control they provide is unclear (Stuart et al. 2002). Other arthropod predators of Diaprepes root weevil eggs and larvae include the earwig, *Labidura riparia* Kirby (Dermaptera: Labiduridae), and the jumping spider *Corythalia canosa* Walckenaer (Araneae: Salticidae) (Whitcomb et al. 1982, Peña et al. 2005). Another predator of Diaprepes root weevil is *Euthyrhynchus* sp. (Hemiptera: Pentatomidae) (Peña et al. 2005). Although many of the foregoing predators are present in the average citrus orchard or ornamental plant field nursery (Whitcomb et al. 1982), they tend to be most effective at controlling Diaprepes root weevil when more than one species are present simultaneously.

### **Flooding Effects on Plants**

Tropical agriculture in southern Florida, particularly between Biscayne and Everglades National Parks, 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 recently mandated the 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. For agriculture to remain viable in these areas, it is important to understand how flooding affects crop physiology, growth, and yield to help identify flood-adapted crops and production systems (Schaffer 1998). Therefore, considerable agricultural research in extreme southern Florida has focused on testing the responses of tropical and subtropical fruit trees, and more recently ornamental plants, to flooding and improving their water use efficiency (Schaffer 1998). This includes an understanding of how increased water table elevation affects pests such as Diaprepes root weevil and diseases like *Phytophthora* root rot.

Elevation of the water table above the root zone typically depletes soil O<sub>2</sub> levels (Kozlowski 1997). A measure of oxygen abundance in the soil is redox potential. Well-drained,

well oxygenated soils have redox potentials of +300 mV or more, whereas flooded soils have redox potentials of +200 mV or less (Ponnamperuma 1972, 1984). Soil redox potential varies with the soil type, pH, and temperature (Patrick et al. 1996).

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<sub>2</sub> depletion and other factors like soil pH (Schaffer et al. 1992). For example, when undamaged by *Phytophthora* root rot, flooding caused more damage to avocado trees in nursery potting medium than in calcareous, Krome gravelly loam soil (previously referred to as “Rockdale soil”), which is native to south Florida (Ploetz and Schaffer 1989). However, when infested with *Phytophthora cinnamomi* Rands, there was a stronger additive effect between flooding and *Phytophthora* root rot in Krome soil than in nursery potting medium (Ploetz and Schaffer 1989). Responses of woody, perennial plants to flooding can hence be quite different in calcareous soils with a high pH found in south Florida compared to potting mixes used by ornamental plant nurseries, or to more acidic natural soils (B. Schaffer, pers. comm.).

Gravelly loam and marl soils are collectively high in calcium, have pHs of 7.4 - 8.4, were derived from Miami limestone, and occur in Monroe (including the Florida keys), Miami-Dade, and parts of Broward Counties (Nobel et al. 1996, Li 2001). Marl soils cover low-lying terrain and formed in areas with several months of flooding (hydroperiod) combined with several months of non-flooded conditions per year, whereas rocky soils formed in non-flooded, higher terrain (1.5-6 m elevation) (Li 2001). The marl type agricultural soil in south Florida is classified as Biscayne soil (loamy, carbonatic, hyperthermic, shallow, typic, fluvaquents) (Nobel et al. 1996, Li 2001). The rocky agricultural soil in south Florida is classified as Krome very gravelly loam soil (loamy-skeletal, carbonatic, hypothermic, lithic, udorthents) (Nobel et al.

1996, Li 2001). Marl soil is a “calcite mud” with poor drainage, whereas gravelly soils have good drainage (Li 2001).

Responses of woody plants to flooding include senescence, shoot dieback, premature leaf abscission, decreased cambial growth, and suppressed formation and expansion of leaves and internodes (Schaffer et al. 1992, Kozlowski 1997). Flooding also inhibits root formation, root branching, and the growth of roots and mycorrhizae. Root mortality, loss of mycorrhizae, and suppressed root metabolism help to decrease the absorption of macronutrients. In addition, flooding reduces photosynthesis, carbohydrate transport, initiation of flower buds, anthesis, and fruit size, set, quality, and growth (Schaffer et al. 1992, Kozlowski 1997). Flooding often causes a change in the allocation of photosynthates within plants. For example, flooding suppressed height and diameter growth of flooded seedlings of *Acer platanoides* L. (Aceraceae), whereas bark growth increased, which suggests that flooding affects carbohydrate partitioning (Yamamoto and Kozlowski 1987). Thus, flooding may reduce photosynthates, which help to produce leaves and root masses that provide food for larvae and adults of *Diaprepes* root weevils. Flooding may indirectly reduce the initial food available to larvae and adults as well as subsequent products of photosynthesis used to repair feeding damage. Therefore, flooding should reduce the ability of the plant to repair insect and other kinds of damage.

Flooding also causes anaerobic decomposition of organic matter and promotes the decay of root systems (Kozlowski 1997). It worsens the severity of diseases by causing a discharge and dispersal of zoospores (Duniway 1983, Wilcox and Mircetich 1985, Schaffer et al. 1992). After zoospores are released from sporangia, their movement through soil depends on high matrix potentials of flooded soils. Flooding thus augments the infection and mortality of plants by promoting production and dispersal of inoculum (Kenerley et al. 1984). There is often an additive effect or interaction between flooding and root disease, such as between flooding and

Phytophthora root rot with avocado (Ploetz and Schaffer 1989). Flooding does not directly predispose plants to infection from root rot diseases (Kenerley et al. 1984). As noted in the foregoing discussion of *Phytophthora* spp., root injury locations, such as from Diaprepes root weevil larval feeding, often serve as infection sites for root rot diseases (McCoy et al. 2002).

Many compounds can be produced or their quantity increased in waterlogged soil, such as sulfides, CO<sub>2</sub>, soluble Fe, and Mn (Wang et al. 1967, Hook et al. 1971, Culbert and Ford 1972) and cyanogenic compounds, acetaldehyde, and ethanol (Fulton and Erickson 1964, Rowe and Catlin 1971, Ponnampereuma 1984). Furthermore, anaerobic microbes synthesize methane, ethane, propylene, fatty acids, hydroxy-, and decarboxylic acids, unsaturated acids, aldehydes, ketones, diamines, mercaptans, and heterocyclic compounds. These compounds can help to make the flooded environment a toxic one. The foregoing substances in flooded soils can contribute to the injury, reduced growth, and mortality of woody plants (Kozlowski 1997). Injury to flooded plants can be caused by products of anaerobic plant metabolism, such as aldehydes, organic acids, and ethanol (Kozlowski 1997).

Considering its effects on plants, flooding can alter or reduce the activity of several metabolic pathways (Kennedy et al. 1991). Flooding affects the production of proteins, carbohydrates, organic acids, and lipids (Kozlowski 1997). The resulting stress on metabolic pathways can decrease carbohydrate production and shoot growth (Kozlowski 1997). A lack of O<sub>2</sub>, buildup of CO<sub>2</sub>, added toxins, and other side effects of flooding contribute to the phytotoxicity and physiological dysfunctions in plants (Kozlowski 1997).

To counteract the detrimental effects of flooding, such as reduced photosynthesis, some plants have evolved the following adaptations: development of hypertrophic (swollen) stem lenticels, development of aerenchyma tissue, and production of adventitious (above-ground) roots (Kozlowski 1997). Hypertrophic lenticels benefit flooded plants in two ways: 1) They

exchange gases in flood water through breaks in the closing layers (Hook et al. 1970, Hook 1984); and 2) they release potentially toxic compounds from plants, such as acetaldehyde and ethylene (Chirkova and Gutman 1972). Aerenchyma tissue occurs in root epidermal layers and shoot cortexes of flooded highbush blueberries (*Vaccinium corymbosm* L., Ericaceae) (Abbott and Gough 1987, Crane and Davies 1989). Here, aerenchyma tissue facilitates movement of O<sub>2</sub> from shoots to submerged roots.

Based on the foregoing discussion, flooding may increase chemicals in the soil and/or plants that tend to be mildly to severely toxic to plants and animals. In addition, there is a decrease in plant matter such as edible leaves and roots to nourish Diaprepes root weevil. Flooding may therefore reduce herbivory from Diaprepes root weevil because the plants may be more toxic and less nourishing, hence, more repulsive and less attractive than non-flooded plants. However, flood-induced production of compounds such as ethanol can also attract insects. For example, Schroeder and Weslien (1994), using traps baited with ethanol and alpha pinene, attracted six phloem-feeding and four predatory insect species in significantly higher numbers than without the baited traps. The attracted insect species included beetles (Coleoptera) in the families Cerambycidae, Pythidae, Histeridae, and Nitidulidae. Because ethanol attracted beetles in these families (Schroeder and Weslien 1994), it may also lure populations of Diaprepes root weevil, which is in the family Curculionidae.

### **Plant Species Used in This Dissertation**

Green buttonwood (*C. erectus*), mahogany (*Swietenia mahagoni* Jacq., Meliaceae), and Surinam cherry (*Eugenia uniflora* L., Myrtaceae) are widely grown in south Florida as ornamental plants (Watkins and Sheehan 1975). Surinam cherry is also occasionally grown as a fruit crop. In addition, pond apple (*Annona glabra* L., Annonaceae) is a tree native to Florida potentially useful as a flood-tolerant rootstock for Annona species fruit crops (Wunderlin 1998,

Nuñez-Elisea et al. 1999). Green buttonwood is a frequent ornamental tree or shrub in south Florida and is native to tidal swamps of central and south Florida (Watkins and Sheehan 1975, Wunderlin 1998). As suggested from its native range, buttonwood is fairly tolerant of flooding, though it also thrives in non-flooded, moderately moist soil, which is common for landscape plants. In south Florida, tidal swamps frequently have marl soil, which cover low-lying terrain and originally formed in areas with several months of flooding (hydroperiod) combined with several months of non-flooded conditions per year (Li 2001). However, some tidal swamps may not be covered with marl soil because they are tidally replenished and do not have a chance to dry out for several months per year, and the soil appears much darker than marl soil. The native habitat of buttonwood frequently has flooding combined with marl soil, but is the environment where buttonwood evolved and should be best adapted. Mahogany is native to “coastal hammocks” in south Florida (Wunderlin 1998), where it is a widely planted lawn and street tree (Watkins and Sheehan 1975). Surinam cherry is found in “disturbed hammocks” of south and central Florida, is native to South America (Wunderlin 1998), and is also widely planted as a south Florida shrub, occasionally as a fruit crop. Although Surinam cherry is named after the country Suriname, its common name is Surinam cherry (Wunderlin 1998). Pond apple is a native tree in “swamps” of south and central Florida (Wunderlin 1998), parts of the Caribbean, and Central and South America (Popeno 1920, Morton 1987). Although typically not grown as a commercial fruit crop (Popeno 1920, Morton 1987), this species is very tolerant to flooded soil conditions (Schaffer 1998, Nuñez-Elisea et al. 1999, Ojeda et al. 2004). When used as a rootstock, pond apple greatly increases the flood tolerance of commercial *Annona* species (Nuñez-Elisea et al. 1999). Hence, commercial fruit crops in the Annonaceae including cherimoya (*Annona cherimoya* Mill.), ilama (*Annona diversifolia* Saff.), sugar apple (*A.*

*squamosa* L.), and atemoya (*A. squamosa* X *A. cherimoya*) (Popeno 1920, Morton 1987) may potentially be grafted onto *A. glabra* for flood tolerance.

Swingle citrumelo rootstock trees were also included for comparison with ornamental plants because most previous research on *Diaprepes* root weevil herbivory was conducted with citrus and its rootstocks (Li et al. 2003a, 2004, 2006, 2007a, b). Other reasons for choosing Swingle included its use by *Diaprepes* root weevil as a host plant (Simpson et al. 1996, Li et al. 2003a, 2004, 2006, 2007a, b) and at least half the commercial Rutaceous fruit trees in Florida are grown on this rootstock (Auscitrus 2004, F. Davies pers comm. 2008). According to Hutchison (1974) and Auscitrus (2004), another advantage of Swingle is its tolerance to root rot caused by *Phytophthora parasitica* Dast. (Oomycota) in Florida (Grimm and Hutchison, unpublished data) and in California (Carpenter and Furr 1962). Hence, Swingle may also tolerate *P. nicotinae* and *P. palmivora* associated with feeding injury from *Diaprepes* root weevil larvae. However, Swingle has moderate to low flood tolerance, though it may be more flood-tolerant than another popular rootstock, Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb., (Rutaceae) (Auscitrus 2004, Li et al. 2004). A test involving a widely planted and studied citrus rootstock like Swingle may allow the results of this dissertation to compare with earlier *Diaprepes* root weevil research.

### **Previous Studies with Rutaceae on Soil Moisture, Nutrients, and *Diaprepes* Root Weevil Herbivory**

Li et al. (2007a, 2007b) conducted studies relating abundances of *Diaprepes* root weevils to soil moisture levels and other factors. Because of slow dispersal by *Diaprepes* root weevil adults, areas with high adult populations can have high larval populations and high root-feeding damage (Nigg et al. 2001b, McCoy et al. 2003). More adults per tree would presumably increase the egg-laying rate and neonate drop compared to areas with lower adult populations. Li et al.

(2007a) conducted a field study involving two orchards of 'Hamlin' oranges, *Citrus sinensis* (L.) Osb. (Rutaceae), one in Osceola County on a poorly drained loam soil and the other in Desoto County on a poorly drained sandy soil. At the Osceola site, which was strongly acidic with a pH of 4.9, highest adult weevil population densities occurred in areas lowest in soil Mg and Ca. However at the Desoto site, which had a more neutral pH of 6.6, adult population densities were highest in areas with the highest Mg, pH, and lowest sand content (Li et al. 2007a). Weevil density was lowest at a pH of 5.7-6.2, which suggested that adjustment of acidic soil pH by liming may help control *Diaprepes* root weevil and simultaneously render pH more favorable for citrus growth and production (Li et al. 2007a). However, larger, healthier, more densely foliated trees are likely to be good food sources and attract more weevils per tree than less healthy trees (Lower et al. 2003, Li et al. 2007a). Management of *Diaprepes* root weevil should hence be coordinated on a site-specific basis (Li et al. 2007a).

#### **Previous Studies with Rutaceae on Effects of Flooding and Herbivory by *Diaprepes* Root Weevil**

Studies by Li et al. in 2004 and 2007b were conducted in greenhouses with a sandy loam soil (pH 4.8) from a central Florida citrus orchard infested with *Diaprepes* root weevil to test combined effects of flood duration and pH on survival and/or growth of *Diaprepes* root weevil larvae. In these studies, pH increased with increasing flood period, which was related to reduced oxygen content of flooded soil. Li et al. (2004, 2007b) discovered that flooding increased soil pH 0.3 units above the non-flooded average by the end of the 40-d flood period. In addition, Li et al. (2007b) found that the longest flood period (40-d) had the lowest survival rate of larvae compared to shorter flood periods, which may be related to higher soil pH at longer flood durations. Here, larval survival and growth were significantly decreased by pre-applied flooding (Li et al. 2007b). Larval survival and weight gain observed by Li et al. (2007b) were

significantly correlated with pH; increasing pH from 4.8 to 5.7 by liming decreased larval survival, and increasing pH from 5.1 to 5.7 significantly decreased larval weights. In a test when the soil was limed to pH 4.8-5.7, larval survival was highest at pH 5.0 for non-flooded plants (Li et al. 2007b). Similar to results of their 2007b study, in 2004, Li et al. found that larval survival and weights were highest when soil pH was near 5.0 for non-flooded plants, and survival tended to decrease as pH dropped below 5.0 or increased above 5.1. Larval survival and weight gain in Li et al.'s 2004 and 2007b studies were significantly correlated with pH.

In 2004, Li et al. noted higher weight gain for larvae in previously flooded than in non-flooded plants, which was different from their 2007b observations where larval survival and growth were significantly decreased by pre-applied flooding in both tests. In their 2004 study, soil moisture levels during infestation were higher for previously flooded than for non-flooded plants, and higher soil moisture levels of flooded treatments were correlated with higher weight gain observed (Li et al. 2004). Other factors such as soil type, compaction, bulk density, and water content may also influence larval survival and growth (Riis and Esbjerg 1998, Rogers et al. 2000, Li et al. 2007b).

Flooding was recommended as a possible control method for *Diaprepes* root weevil in citrus by Li et al. (2007b), and it may reduce larval survival while plants are flooded. However, depending on soil pH, water-stressed plants may be more susceptible to *Diaprepes* root weevil larval feeding when un-flooded than non-stressed plants that were either never flooded or flood-tolerant and previously flooded. Hence, pre-applied flooding may either increase or decrease larval survival based on resulting soil moisture, pH, and plant health while soil is infested. One potentially important conclusion made by Li et al. was that increasing the soil pH by at least 1 unit in acid soils is recommended for optimum citrus growth, which occurs at pH 6.0-6.5, and to help control *Diaprepes* root weevil (Li et al. 2004, 2007b).

Li et al. (2003a, 2006) examined effects of *Diaprepes* root weevil larval infestation, flooding, or soil type on plant growth, larval survival, and other characteristics in a greenhouse. Flooding occurred before larval infestation so that both stresses were not simultaneous (Li et al. 2003a, 2006). They used seedlings of Swingle citrumelo and Smooth Flat Seville citrus rootstock varieties. In both studies, flooding significantly reduced soil redox potential, which dropped from +220 to -100 mV (Li et al. 2006) or from +200 to -100 mV (Li et al. 2003a) within the first 3 d of flooding. Flooding also significantly reduced leaf stomatal conductance in both studies from 260 to 60 mmol m<sup>-2</sup> s<sup>-1</sup> in the first 30 d of flooding (Li et al. 2003a), and from 260 to 80 mmol m<sup>-2</sup> s<sup>-1</sup> in the first 20 d of flooding, while significantly reducing shoot growth (Li et al. 2006). Survival of *Diaprepes* root weevil 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 (Li et al. 2003a). Flooding plants for 30 d resulted in more serious root injury and significantly lower stomatal conductance, and therefore caused greater water stress than flooding plants just 10 d (Li et al. 2003a). Similarly, Li et al. (2006) investigated effects of flooding and soil type on larval survival. In plants previously flooded for 20 d, larval survival averaged 25% higher in sandy soil than in loam soil (Li et al. 2006). Soil pH increases with flood duration and could adversely affect larval survival (Shapiro et al. 1997, Li et al. 2006). Soil type affects larval growth and survival rates, and the effects of soil type on larval survival may be chiefly physical including soil moisture and oxygen levels (Rogers et al. 2000). Flooded and waterlogged soils are also typically denser than non-flooded soils (Saqib et al. 2004), which is a potential problem for larval survival in flooded soil (Li et al. 2006). Similar to Li et al. (2003a), Li et al. (2006) found that plants flooded for at least 20 d were more water stressed and more prone to *Diaprepes* root weevil larval feeding injury after removal of plants from flooding than non-flooded control plants. Their results suggest that

minimizing plant exposure to waterlogging, avoidance of flooding, and early control of *Diaprepes* root weevil larvae may help protect young plants (Li et al. 2006).

Lapointe and Shapiro (1999) attempted to determine soil moisture conditions that optimized production of *Diaprepes* root weevil adults. They initially raised larvae on an artificial diet and transferred them to an artificial soil with varying moisture levels (20-80%) at 25°C. Optimal survival to pupation occurred at 60% soil moisture for the 68-d larvae, and 30-70% for 180-d larvae. About 60-65% of 180-d larvae survived to pupation under these optimal moisture conditions. Both 68-d and 180-d larvae showed their poorest survival in low (20%) and in high (80%) soil moisture levels.

Li et al. (2003a, 2006), and Diaz (2005) removed plants from flooding before infesting the soil with *Diaprepes* root weevil larvae. In contrast, Shapiro et al. (1997) exposed 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 wk) on larval mortality. The larvae were large (11.5 mo old), and one larva was placed into each flooded polystyrene box (5.1 cm cube) filled with Immokalee sandy soil and no food (Shapiro et al. 1997). Mean mortality exceeded 90% by 3 wk at 24 and 27°C and by 5 wk at 21°C, but was only 46% after 5 wk at 18°C (Shapiro et al. 1997). In addition, insect mortality was correlated with pH increase over time, although no such correlation existed between mortality and oxygen level (Shapiro et al. 1997).

In other studies, wireworm larvae *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae) had 80% mortality after 6 wk of submergence at 27°C (Hall and Cherry 1993). However, sugarcane grubs *Tomarus subtropicus* (Blatchley) (Coleoptera: Scarabaeidae) had 100% mortality after only 5-10 d (1 wk) of submergence (Cherry 1984). 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).

Flooding is sometimes used in sugarcane fields of southern Florida to control pests, such as sugarcane grubs (*T. subtropicus*) (Cherry 1984) and wireworm larvae (*M. communis*) (Hall and Cherry 1993). Flooding may hence be useful for controlling Diaprepes root weevil larvae in sugarcane fields, although only in the summer and fall when floodwater temperatures are close to their maximum (27°C) (Hall and Cherry 1993, Shapiro et al. 1997).

### **Previous Studies on Effects of Diaprepes Root Weevil and Flooding on Ornamental Plants**

Although the host range of Diaprepes root weevil includes citrus, ornamental plants, and grasses (Simpson et al. 1996), the majority of previous research with this insect has focused on citrus. There have been relatively few reports on the effects of Diaprepes root weevil larval feeding on plant species other than citrus and related genera, such as ornamental plants.

Diaz (2005) tested the effects of flooding and larval infestation on photosynthesis, transpiration, stomatal conductance, and fresh and dry root weights of buttonwoods and live oaks. Also, Diaz (2005) examined effects of larval root feeding on photosynthesis, transpiration, and stomatal conductance, leaf, stem, and root fresh and dry weights, plant height and stem diameter, and recovery of larvae from green buttonwood, live oak (*Q. virginiana*), and pygmy date palm (*Phoenix roebelenii* O'Brien, Arecaceae) in 2006 (Diaz et al. 2006). Overall, the measured plant variables seemed to be more affected by flooding than by larval infestation for both buttonwoods and live oaks (Diaz 2005). There was no significant difference in mean number of larvae recovered from infested plants between previously flooded and non-flooded buttonwoods or live oaks (Diaz 2005).

In the 2006 study, root feeding by Diaprepes root weevil larvae did not significantly affect photosynthesis, transpiration, stomatal conductance, plant weights, or other variables for live oak. In contrast, fresh and dry weights for roots and stems, and dry leaf weights, were significantly lower for infested than non-infested plants for pygmy date palm (Diaz et al. 2006).

However, leaf gas exchange and other variables were not significantly different between infested and non-infested treatments of pygmy date palm (Diaz et al. 2006). For green buttonwoods, the weevil significantly reduced photosynthesis, transpiration, stomatal conductance, plant heights, stem diameters, dry root weights, and often dry stem and leaf weights (Diaz 2005, Diaz et al. 2006). Larval herbivory hence seemed to affect green buttonwoods the most, followed by pygmy date palms, and affected live oaks the least (Diaz 2005, Diaz et al. 2006).

Diaz et al. (2005) measured effects of adult *Diaprepes* root weevil feeding on photosynthesis, transpiration, stomatal conductance, root, stem, and leaf fresh and dry weights, and leaf areas of green buttonwood and live oak. Herbivory by adult *Diaprepes* root weevil caused variable results. Adult herbivory did not affect green buttonwood photosynthesis, transpiration, or stomatal conductance in one test, but in another test, all three variables were significantly higher in infested than in non-infested mature leaves after 2 mo of infestation (Diaz et al. 2005). This study suggested that as adult *Diaprepes* root weevil removed more leaf area from infested than non-infested green buttonwoods, though with insignificant differences, root, stem, and leaf weights were also reduced. Although live oaks are a host plant for adult *Diaprepes* root weevil (Simpson et al. 1996, Mannion et al. 2003), adult herbivory did not significantly affect photosynthesis, transpiration, or stomatal conductance in a test by Diaz et al. (2005), which may have been related to lack of young leaves.

### **Objectives**

**Goal.** The goal of this project was to determine effects of *Diaprepes* root weevil herbivory, flooding, and the interaction of these two stresses on the leaf gas exchange and growth of selected ornamental plant species commonly grown and sold by south Florida nurseries. To help clarify relationships between *Diaprepes* root weevil herbivory, host plant

physiology, and flooding, and to address questions not answered in previous research, the following objectives were proposed:

1. To determine effects of larval feeding by *Diaprepes* root weevil on leaf gas exchange and growth of selected woody ornamental plants commonly grown in south Florida. Hypothesis: larval herbivory reduces leaf gas exchange and growth of all plant species tested.
2. To test effects of flooding and of soil type (marl soil and a nursery potting medium) on the growth and physiology of selected woody ornamental plants, which are potential hosts of *Diaprepes* root weevil. Hypothesis: flooding lowers plant biomass and leaf gas exchange more in marl soil than in nursery potting medium.
3. To determine the survival of *Diaprepes* root weevil larvae in a flooded marl soil and in a flooded nursery potting medium with green buttonwood as a food source. Hypothesis: flooding reduces larval survival more in marl soil than in potting medium.
4. To investigate effects of intermittent and continuous flooding combined with *Diaprepes* root weevil larval feeding on plant physiology and growth. Hypothesis: survival rates of *Diaprepes* root weevil larvae and plant biomasses are lowered more by continuous than by intermittent flooding.
5. To test effects of flooding and adult herbivory by *Diaprepes* root weevil on leaf damage and physiology of selected woody ornamental plants and effects of flooding on adult host plant preference. Hypothesis: flooding reduces leaf feeding, damage, and attraction to host plants by adult *Diaprepes* root weevil, and it adversely affects leaf gas exchange of host plants.

CHAPTER 2  
EFFECTS OF HERBIVORY BY *DIAPREPES ABBREVIATUS* (L.) (COLEOPTERA:  
CURCULIONIDAE) LARVAE ON FOUR WOODY ORNAMENTAL PLANT SPECIES

**Introduction**

*Diaprepes abbreviatus* L. (Coleoptera: Curculionidae: Entiminae) commonly called Diaprepes root weevil, was first found in Florida in a citrus nursery in Apopka in 1964 (Woodruff 1964). It is believed to have entered Florida from Puerto Rico in a shipment of ornamental plants (Woodruff 1985). Puerto Rico probably is the center of origin of this species (Lapointe 2000a, Obrien and Kovarik 2000), but it also is found in Trinidad, Tobago, Venezuela, and Mexico, though its presence in these other locations is disputed. In the United States, it is found in Florida, Texas (Knapp et al. 2001, Skaria and French 2001), and southern California (Klunk 2005). In Florida, Diaprepes root weevil occurs in 23 counties in the south and central parts of the state (Anonymous 1996, Peña 1997, Weissling et al. 2004).

Diaprepes root weevil is an abundant and a serious pest of citrus and sugarcane in its home range of Puerto Rico (Woodruff 1964). Infestations of this insect nearly put many central Florida ornamental plant nurseries out of business during the 1970s (Schroeder 1994). In 1996 it was reported to infest approximately 24,281 ha (60,000 acres) of citrus, and control costs and losses exceeded \$2,965 per ha (\$1,200 per acre) (Stanley 1996). The weevil has cost the Florida citrus industry an estimated \$72 million annually (Anonymous 1996, Stanley 1996). Diaprepes root weevil continues to be a long-term threat to several agronomic and horticultural crops because of inadequate management strategies.

Diaprepes root weevil 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. 2000b, Mannion et al. 2003, Godfrey et al. 2006, C.G. Martin personal observation and unpublished data). Some plants support only one stage of the insect; for

example *Ardesia crenata* Sims, which supports the larval stage. However, many economically important plants support all stages of the weevil from egg to adult, such as sweet potato, *Ipomoea batatas* (L.) Lam., and buttonwood, *Conocarpus erectus* L. (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 *Diaprepes* root weevils were common on field-grown ornamental plants. Plants with the highest percentage of egg masses were live oak (*Quercus virginiana* Mill.), silver buttonwood, (*C. erectus* L. variety *sericeus* Fors.Ex DC), and black olive (*Bucida buceras* L.). The highest adult population densities were found on black olive, dahoon holly (*Ilex cassine* L.), and *Bauhinia* sp. (Mannion et al. 2003).

Insect herbivory often affects leaf gas exchange including net CO<sub>2</sub> assimilation, stomatal conductance, transpiration, and internal CO<sub>2</sub> concentration (the partial pressure of CO<sub>2</sub> in the substomatal space) of host plants (Andersen and Mizell 1987, Schaffer and Mason 1990, Schaffer et al. 1997). Measurements of leaf gas exchange can provide quantitative measures of insect damage to plants prior to the appearance of any visual symptoms. The effects of insect herbivory on leaf gas exchange can vary according to feeding guild (Welter 1989). Root (1973) and Welter (1989) classified arthropods according to their feeding-damage type or guild, such as mesophyll feeders, phloem feeders, stem borers, root feeders, or direct leaf consumers. *Diaprepes* and other root weevils such as *Pachneus litus* (Germ.) and *Artipus floridanus* Horn (Coleoptera: Curculionidae) have two feeding guilds. Larvae are in the root-feeder guild, whereas adults are in the direct-leaf-consumer guild (Syvertsen and McCoy 1985).

In a review of arthropod herbivory effects on leaf gas exchange, most studies claimed that herbivory harms this process, although insignificant or beneficial effects of herbivory on leaf gas exchange have been noted (Welter 1989). Syvertsen and McCoy (1985) found that leaf feeding by *A. floridanus* adults decreased photosynthesis and transpiration of citrus trees. According to

Welter (1989), leaf feeding by adults, which are in the direct-leaf-consumer guild, often tend to increase photosynthesis (when measured on a leaf area basis). However, this effect is atypical compared to the other guilds, such as stem borers and root-feeders, which tend to decrease photosynthesis.

As young *Diaprepes* root weevil larvae work their way from small roots to larger lateral and main roots, they often form deep grooves when feeding on the larger roots, and they consume the outer bark and cambial layers (McCoy et al. 2002). Roots may be girdled causing root death, or the crown may be girdled causing tree death (McCoy et al. 2002). Larval girdling often kills small citrus trees by destroying their ability to take up nutrients (Wolcott 1936, 1948; Quintela et al. 1998). However once the larvae are controlled, damage especially to smaller roots, which grow more rapidly than larger roots, may be outgrown in a few weeks to a few months. Gouges in cambial layers and girdling, however, may cause long-term deformity from which plants may never recover.

Although the host range of *Diaprepes* root weevil includes citrus, ornamental plants, and grasses (Simpson et al. 1996), the majority of previous research with this insect has focused on citrus and there have been relatively few reports of the effects of larval feeding on plant species other than citrus. In a previous study of ornamental plants, root feeding by *Diaprepes* root weevil larvae did not significantly affect photosynthesis, transpiration, stomatal conductance, or root, stem, and leaf dry weights of live oak; however, it did significantly reduce root, stem, and leaf dry weights of pygmy date palm (*Phoenix roebelenii* O'Brien) (Diaz et al. 2006). Diaz et al. (2006) also observed that root feeding by *Diaprepes* root weevil reduced photosynthesis, transpiration, stomatal conductance, root dry weight and often stem and leaf weights of green buttonwood trees.

The purpose of the present study was to further examine the effects of *Diaprepes* root weevil feeding on leaf gas exchange and growth of woody ornamental plants. The hypothesis tested was that larval herbivory reduces leaf gas exchange and biomass of all the plant species tested.

### **Materials and Methods**

The experiment was conducted in the spring and summer of 2006 in Homestead, FL, using plants in 11-liter plastic containers placed on ground cloth in an outdoor site exposed to full sun. There were two treatments (non-infested plants and plants infested with *Diaprepes* root weevil larvae), six replications per treatment, and four plant species for a total of 48 plants.

**Plant material.** Mahogany (*Swietenia mahagoni* Jacq.), Surinam cherry (*Eugenia uniflora* L.), pond apple (*Annona glabra* L.), and green buttonwood trees were purchased in February 2006. Pond apple trees were purchased in 4-liter containers from a commercial nursery (Fort Pierce, FL) and repotted into 11-liter containers 5 d after purchase. Surinam cherry and mahogany were purchased in 11-liter containers from a commercial nursery (Miami, FL). Buttonwood was also purchased in 11-liter containers from a commercial nursery (Homestead, FL). At the time treatments were initiated buttonwood and mahogany plants were each 2 years old, Surinam cherry trees were approximately 1.5 years old and pond apple were approximately 0.5 years. All plants were grown in a nursery mix consisting of 40% Florida peat, 30% pine bark, 20% cypress sawdust, and 10% sand. On the same day that plants were purchased, about one-third to one-half the foliage of the buttonwood and Surinam cherry plants was pruned to improve and standardize the size of the plants. On the first infestation date (10 March), plant heights (mean  $\pm$  SD) were  $43 \pm 9$ ,  $101 \pm 8$ ,  $35 \pm 5$ , and  $51 \pm 14$  cm for buttonwood, mahogany, Surinam cherry and pond apple, respectively. The experiment was conducted for 146 d (March – July). Plants were irrigated for 30 min twice per day and fertilized 2 d before beginning the

experiment (28 February 2006) with liquid fertilizer (Peters 20-20-20 with micronutrients, United Industries, St Louis, MO) according to the manufacturers recommended rate. Plants were fertilized again on day 69 with the same fertilizer and rate as stated above. Pests other than *Diaprepes* root weevil were controlled by manual removal of the insects.

**Larval infestation.** Each plant in the infested treatment was infested with 20 *Diaprepes* root weevil larvae on each of two occasions: day 9 (10 March 2006) and day 84 (24 May 2006). The reason for the second infestation was the lack of significant differences in leaf gas exchange (net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, and internal CO<sub>2</sub> concentration) between treatments, and thus the assumption that the larval population may have not been sufficient to detect differences between treatments. To infest the plants, one larva was placed individually into each of 20 holes, 5-10 cm deep made in the potting medium in each container. Holes were 4-8 cm from the stem, and 2.5 cm apart. Based on mean head capsule widths (Quintela et al. 1998), larvae were second through fifth instars (fourth instar average) for the 10 March infestation and fifth through ninth instars (seventh instar average) for the 24 May infestation. Control plants were not infested with larvae.

**Data collection.** Data collected included the number of larvae, pupae, and adults recovered per plant, larval head capsule widths, leaf gas exchange (net CO<sub>2</sub> assimilation, transpiration, stomatal conductance and internal CO<sub>2</sub> concentration), plant height and caliper (trunk diameter), and fresh and dry weights of roots, stems, and leaves and the number of trunks per container at the end of the study. Trunk caliper 10 cm above soil line and plant height to the top of the highest leaf above the soil line were measured for all plants prior to *Diaprepes* root weevil infestation. Trunk diameters were measured with a micrometer (0-25 mm Electronic Digital Micrometer, Marathon Co., Richmond Hill, Ontario, Canada). Soil temperature was recorded at 1 h intervals from day 43 (12 April 2006) to day 146 (25 July) with sensors

(StowAway® Tidbit® temploggers, Onset Co., Pocasset, MA) placed in the soil of four plants that were not included in the experiment but were maintained under the same experimental conditions. Sensors were placed at a soil depth of 6 cm two-thirds the distance from the center to the outer edge of the container.

Leaf gas exchange was measured on two fully expanded, recently mature leaves per plant with a CIRAS-2 portable gas analyzer (PP Systems, Amesbury, MA). Recently mature refers to a leaf that has fully expanded and hardened off within the preceding few weeks of measurement. These leaves usually were between the fourth and tenth node below the stem tip. Gas exchange values of the two leaves were averaged and the mean value per plant (replication) was used for statistical analyses. All leaf gas exchange measurements were made between 08:30 and 15:45h. However, on each measurement date, all measurements of each plant species (including infested and non-infested treatments) were made within 65 min of each other. During gas exchange measurements, the photosynthetic photon flux was maintained at  $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  with a halogen lamp attached to the leaf cuvette, and the reference  $\text{CO}_2$  concentration in the cuvette was kept constant at  $375 \mu\text{mol mol}^{-1} \text{CO}_2$ . Leaf gas exchange was measured 8 d (buttonwood and mahogany) and 1d (Surinam cherry and pond apple) before insect infestation and at 2-wk intervals after infestation.

Four weeks after the last leaf gas exchange measurement, trunk diameter and plant height were again recorded, and increases in these variables from before the first infestation were calculated. One week later, plants were harvested. Stems were cut off 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. Recovered larvae were preserved in 75% ethyl alcohol. Fresh root, stem, and leaf weights were determined (Mettler PE 3600 Delta Range®, Mettler Co., Highstown, NJ) immediately after the plants were harvested. Roots,

stems, and leaves were then dried in an oven (Memmert, Wisconsin Oven Co., East Troy, WI) at 75°C for 2 d to a constant weight.

**Experimental design and statistical analyses.** The experiment was arranged as a completely randomized design with two treatments (infested and non-infested) and six single-plant replications per treatment. There were four plant species tested for a total of 48. For all plant physiology and growth variables, infested and non-infested treatments were compared within each species with a standard T-test. Interactions between species and treatment were not tested because we were only interested in main effects. However, larval survival and size were compared among plant species using analysis of variance (ANOVA) and Waller-Duncan K-ratio test. Statistical analysis was conducted with SAS statistical software Version 9.1 (PROC T-TEST and GLM, SAS Institute, Cary, NC, 2003).

## Results

During the experimental period, mean daily soil temperatures varied between 21 and 32°C with monthly averages between 24 and 28°C (Figure 2-1).

For green buttonwood trees, initially there were no significant differences in net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, or internal CO<sub>2</sub> concentration between infested and non-infested treatments except at wk 4 when infested plants had significantly higher stomatal conductance ( $t = 2.4$ ,  $df = 6$ ,  $P = 0.048$ ) and internal CO<sub>2</sub> concentrations ( $t = 2.4$ ,  $df = 10$ ,  $P = 0.04$ ) than non-infested plants (Figure 2-2c, d). At 14 wk, plants in the non-infested treatment had significantly higher transpiration ( $t = -3.3$ ,  $df = 10$ ,  $P = 0.009$ ), stomatal conductance ( $t = -3.4$ ,  $df = 10$ ,  $P = 0.0065$ ), and internal CO<sub>2</sub> concentration ( $t = -2.8$ ,  $df = 9$ ,  $P = 0.022$ ) than plants in the infested treatment (Figure 2-2b, c, d). By wk 18, infested plants had significantly higher net CO<sub>2</sub> assimilation ( $t = 3.0$ ,  $df = 9$ ,  $P = 0.015$ ) than non-infested plants (Figure 2-2a). Hence, after the second infestation (wk 14-18) with larger larvae than the first

infestation, treatment differences in net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, and internal CO<sub>2</sub> concentration occurred more often than before the second infestation (wk 2-12). Non-infested buttonwood plants had significantly greater root, stem, and leaf fresh and dry weights than infested plants (Table 2-1).

For mahogany, transpiration ( $t = -2.7$ ,  $df = 10$ ,  $P = 0.023$ ) and stomatal conductance ( $t = -2.5$ ,  $df = 9$ ,  $P = 0.037$ ) were significantly higher for non-infested than infested plants at 16 wk (Figure 2-3b, c) and no other leaf gas exchange variables were affected by treatment (Figure 2-3a, d). Fresh and dry weights of roots, stems, and leaves were higher for non-infested plants than infested plants, but these differences were only significant for leaves (Table 2-1).

For Surinam cherry, net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance were generally higher for non-infested than infested plants; however, some of these differences were significant only after the second infestation (Figure 2-4). Surinam cherry gas exchange values that were significantly higher for non-infested than infested plants included net CO<sub>2</sub> assimilation at wk 14 ( $t = -5.0$ ,  $df = 10$ ,  $P = 0.0005$ ) and at wk 18 ( $t = -9.4$ ,  $df = 10$ ,  $P = <0.0001$ ), transpiration at wk 14 ( $t = -4.1$ ,  $df = 9$ ,  $P = 0.0026$ ), and stomatal conductance at wk 14 ( $t = -5.0$ ,  $df = 10$ ,  $P = 0.0006$ ). Internal CO<sub>2</sub> concentration, however, was higher for infested than non-infested plants with significant differences at wk 14 ( $t = 2.6$ ,  $df = 10$ ,  $P = 0.028$ ) and at wk 18 ( $t = 2.9$ ,  $df = 8$ ,  $P = 0.02$ ) (Figure 2-4d). Fresh and dry weights of roots, stems, and leaves were consistently higher for non-infested than infested Surinam cherry plants, but differences were only significant for roots and leaves (Table 2-1).

For pond apple, there were no significant differences between infested and non-infested plants in net CO<sub>2</sub> assimilation (range 1.8 to 14.9  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration (range 1.1 to 7.5  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), stomatal conductance (range 28 to 506  $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), or internal CO<sub>2</sub> concentration (range 188 to 265  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ). Non-infested pond apple trees tended to

have greater leaf, stem and root fresh and dry weights than infested plants, though differences were not statistically significant (Table 2-1). Pond apple did support populations of *Diaprepes* root weevil larvae (Figure 2-5) and exhibited damage from root feeding.

Mahogany and pond apple each had one trunk, whereas buttonwood and Surinam cherry were multi-trunked. There was no significant effect of treatment on increase in plant height or stem diameter for any of the plant species tested. The range of plant heights for buttonwood, mahogany, Surinam cherry, and pond apple was 13.7-38.5 cm, 15.6-55 cm, 23.2-37.3 cm, and 0.06-11.6 cm, respectively, and range of stem diameters was 1.5-5.1 mm, 1.2-4.8 mm, 2.9-4.9 mm, and -0.64 to 3.24 mm, respectively. However, the increase in plant height and stem diameter for each species tended to be lower in infested than in non-infested plants.

The total number of larvae, pupae, and adults recovered did not differ significantly among plant species ( $F = 1.6$ ;  $df = 3, 23$ ;  $P = 0.22$ ) and averaged 3.7-7.2 per plant depending on the species (Figure 2-5). No life stages were recovered from non-infested plants. There was a significant difference in head capsule widths of larvae recovered from different plant species ( $F = 3.1$ ;  $df = 3, 23$ ;  $P = 0.049$ ) with the largest larvae collected from Surinam cherry (mean  $2.59 \pm 0.19$  mm; 9<sup>th</sup> instar) and the smallest collected from mahogany ( $2.29 \pm 0.06$  mm; 8<sup>th</sup> instar) (Figure 2-6).

## Discussion

Differences in net CO<sub>2</sub> assimilation between *Diaprepes*-infested and non-infested treatments suggest that the order of susceptibility to larval feeding damage of the host plants tested is Surinam cherry followed by buttonwood, then mahogany with pond apple the least affected. However, differences in biomass (fresh and dry weights) between infested and control treatments indicated a slightly different order of susceptibility to larval feeding damage with reductions in biomass greatest for buttonwood followed by Surinam cherry then mahogany,

again with pond apple the least affected by larval feeding. Diaz et al. (2006) found that buttonwood was more susceptible to larval feeding than live oak or pygmy date palm. Hence, mahogany, pond apple, and live oak all seem to be less susceptible than buttonwood to *Diaprepes* root weevil larval feeding.

Out of the 40 insects added per pot, the 3.7-7.2 insects recovered is about 9-16% survivorship. The time period was 137 d for the first 20 insects combined with 62 d for the second 20 insects. Low survivorship similar to levels we found is not uncommon. For example, Diaz et al. (2006) recovered 6-10 *Diaprepes* root weevil larvae per container 90 d after infesting with 40 fifth-to-sixth-instar larvae and thus had 15-25% survivorship. Cannibalism is common among *Diaprepes* root weevil larvae and may help account for these low survival rates. In addition, predators such as ants, spiders, earwigs, hemipterans, and lady beetles are known to attack *Diaprepes* root weevil larvae (Whitcomb et al. 1982, Richman et al. 1983a, b, Tryon 1986, Jaffe et al. 1990b, McCoy et al. 2000, Stuart et al. 2002). Predators such as fire ants (*Solenopsis* sp.) (Hymenoptera: Formicidae) and spiders (many unknown spp.) (Arachnida: Araneae) have commonly been seen at the study site. Brown anole lizards (*Anolis sagrei* Duméril & Bibron) (Reptilia: Polychrotidae), also abundant at the site, prey on soft-bodied, slow-moving insect larvae similar in size to *Diaprepes* root weevil larvae and hence may also eat *Diaprepes* root weevil larvae. In addition, younger larvae seem more inclined than older larvae to emigrate from the pots. Hence, emigration of larvae may have been more problematic after the first infestation than after the second infestation because younger larvae were used. Cannibalism, predators, pathogens, and emigration may have all contributed to the low larval survivorship in the present study. We attempted to provide enough larval feeding pressure to allow significant treatment effects without causing excessive cannibalism and therefore infested with 20 larvae per container two times.

All recovered adults were found buried in soil presumably in a resting period. Lapointe (2000b) tested the effects of constant 22, 26, and 30°C temperatures on *Diaprepes* root weevil larval survival and developmental rates on an artificial diet and found the highest survival rates occurred at 22 and 26°C with lowest survival at 30°C. The average monthly soil temperatures for the present study were 24-28°C, or within 2°C of 26°C. Rates of larval development and survival for the present study were thus fairly close to their maximum rates. Data for larval recovery were difficult to interpret for plant species vulnerability to larval feeding injury because differences between treatments were not significant for the sum of larvae, pupae, or adults and minimally significant for larval size (head capsule widths). However, they do suggest that buttonwood, Surinam cherry, and mahogany were more vulnerable to larval feeding injury than pond apple. The greater numbers of insects recovered and/or larger head capsule widths for buttonwood, Surinam cherry and mahogany than pond apple indicates that pond apple is not as strong a host for *Diaprepes* root weevil larvae as the other three species. Overall, buttonwood and Surinam cherry were the most susceptible species to larval root feeding by *Diaprepes* root weevil, followed by mahogany and then pond apple.

According to Welter (1989), the effects of herbivory by insects in the root-feeding guild has been less thoroughly documented than the effects of herbivory by above-ground guilds, such as phloem feeders and gall formers. In addition, the effects of herbivory by nymphs and larvae on leaf gas exchange (Schaffer et al. 1997, Diaz et al. 2006) has been less frequently studied than the effects of herbivory by adult insects (Syvertsen and McCoy 1985, Boucher and Pfeiffer 1989). Results obtained for green buttonwood in the present study were generally comparable to those of Diaz et al. (2006) who observed higher net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance (on a leaf area basis) for non-infested plants than plants infested with *Diaprepes* root weevil larvae. However, during 2 to 4 wk following each of two infestations in the present

study, all four gas exchange values for buttonwood increased in infested compared to non-infested plants, and ended the period mostly higher for infested than non-infested plants. Overall, all four gas exchange values for buttonwood were thus significantly higher for infested than non-infested plants the same number of times that values for non-infested plants were significantly higher than those of infested plants. For buttonwoods, a compensatory increase in leaf gas exchange after infestation may have resulted in increased net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, and internal CO<sub>2</sub> concentration in infested relative to non-infested plants. Diaz et al. (2006) also found evidence of compensatory response where buttonwood leaf gas exchange values increased in a long-term response to larval feeding injury. Similar to Diaz et al. (2006), we did not observe a compensatory increase in the other plant species tested.

Nigg et al. (2001a) tested the responses of seven varieties of citrus rootstock seedlings to *Diaprepes* root weevil larval feeding. They compared fresh and dry weights, trunk diameters, and larval recovery of infested and non-infested plants of each variety to determine relative tolerance of each variety to larval feeding on roots. At 90 d, Cleopatra mandarin, Sun Chu Sha mandarin, and sweet lime were tolerant of *Diaprepes* root weevil larval feeding. However, at 168 d, none of the seven citrus rootstock varieties were tolerant or resistant to larval feeding. Hence, instead of using host plant resistance, Nigg et al. (2001a) recommended controlling adults to reduce larval feeding to tolerable levels.

Several studies by Li et al. (2003a, 2006, and 2007b) also used *Diaprepes* root weevil larvae to test effects of larval feeding (and flooding) on plant gas exchange, biomass, and/or larval growth on citrus varieties in a laboratory or greenhouse environment. They used much smaller numbers of larvae than Nigg et al. (2001a), Diaz et al. (2006), or the present study and much smaller sizes of larvae than Diaz et al. (2006) or the present study. However, they also

used much smaller pots (about 5 cm cube) and plants (6 mo old seedlings). Although much fewer and/or smaller larvae were used, they were sufficient to yield the appropriate results given the smaller plant and pot sizes. Similar results have been reported for another root-feeding larval pest, the western corn rootworm, *Diabrotica vergifera vergifera* Leconte (Coleoptera: Chrysomelidae) (Riedell and Reese 1999, Urias-Lopez et al. 2000). Riedell and Reese (1999) found that in the tassel stage of corn, larval feeding by rootworms lowered stomatal conductance significantly relative to non-infested plants, although net CO<sub>2</sub> assimilation was unchanged. While larvae were actively feeding, net CO<sub>2</sub> assimilation was lower in root-damaged than in undamaged corn plants. Net CO<sub>2</sub> assimilation was also lower for severely infested than moderately infested or non-infested plants. In addition, the development of adventitious roots as a compensatory growth response occurred more in moderately infested than in non-infested or severely infested corn plants. Hence, the severity of root damage and the level of compensatory growth played important roles in mediating shoot growth and the level of net CO<sub>2</sub> assimilation (Riedell and Reese 1999).

Schaffer et al. (1997) also reported similar results for the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), which are also herbivorous larvae, but in the mesophyll feeding guild (Welter 1989). In plants infested with citrus leafminers, the duration of leafmining and the number of larvae per leaf were each correlated with greater leaf area damage and reductions in net CO<sub>2</sub> assimilation (Schaffer et al. 1997). Ingham and Detling (1986) artificially defoliated 55% of the foliage of sideoats gramma grass, *Bouteloua curtipendula* (Michx.) Torr., and infested roots of a different treatment with a root feeding nematode, *Tylenchorhynchus claytoni* Steiner. Root biomass, shoot biomass, and tiller number were each reduced by artificial defoliation or by root-feeding nematodes. Net CO<sub>2</sub> assimilation and

transpiration, however, significantly increased in the remaining foliage after artificial defoliation, but were unaffected by nematode root feeding.

As noted, Welter (1989) stated that whole-leaf consumption by adults, which are in the leaf consumer feeding guild, often tends to increase net CO<sub>2</sub> assimilation when measured on a leaf-area basis, although this effect is atypical compared to the other guilds, such as root-feeders, which tend to decrease net CO<sub>2</sub> assimilation. With regard to whole-leaf consumption, increased net CO<sub>2</sub> assimilation may be explained by the availability of the same quantity of nutrients such as nitrogen delivered to a smaller leaf area after defoliation by the insects. This may render more nitrogen and other nutrients available per remaining leaf area to synthesize chlorophyll and supply other reactions that increase net CO<sub>2</sub> assimilation.

The results of this study confirmed the findings of Simpson et al. (1996) that buttonwood, Surinam cherry, and mahogany are host plants of *Diaprepes* root weevil. Perhaps most noteworthy, pond apple was able to support *Diaprepes* root weevil larvae. To our knowledge this is the first report of this species as a host. Furthermore, this is the first member of the entire plant family, Annonaceae, to be reported as a host to *Diaprepes* root weevil larvae. Pond apple has been suggested as a potential flood-tolerant rootstock for commercial *Annona* species (Nuñez-Elisea et al. 1999). Thus, its ability to host *Diaprepes* root weevil larvae may be a consideration when selecting rootstocks for commercial *Annona* species. Furthermore, other commercial fruit crop species in the Annonaceae such as cherimoya (*Annona cherimoya* Mill.), ilama (*Annona diversifolia* Saff.), sugar apple (*A. squamosa* L.), and atemoya (*A. squamosa* X *A. cherimoya*) (Popeno 1920, Morton 1987) may be also vulnerable to feeding by *Diaprepes* root weevil larvae and their susceptibility should be tested in areas where *Diaprepes* root weevil infestation poses a threat.

The results of this study can help prioritize decisions for pest management of buttonwood, Surinam cherry, mahogany, and pond apple plants. For example, pond apple may never require treatment to control *Diaprepes* root weevil larvae, while Surinam cherry and buttonwood may benefit from such a treatment. Future studies should investigate susceptibility to damage from *Diaprepes* root weevil larval feeding for other economically valuable plant species often visibly infested with adult weevils, such as *Bulnesia arborea* (Jacq.) Engl. (Zygophyllaceae) and black olive (*B. buceras*).

Table 2-1. Effects of Diaprepes root weevil larvae on root, stem, and leaf fresh and dry weights of buttonwood, mahogany, Surinam cherry, and pond apple.

Species	Treatment	Fresh Weight (g) (mean $\pm$ SD)			Dry Weight (g) (mean $\pm$ SD)		
		Root	Stem	Leaf	Root	Stem	Leaf
Button-wood	Non-infested	78.3 $\pm$ 11.4	89.8 $\pm$ 9.6	129.0 $\pm$ 29.2	68.8 $\pm$ 11.7	62.1 $\pm$ 7.5	83.7 $\pm$ 12.9
	Infested	57.3 $\pm$ 14.0	48.6 $\pm$ 20.3	76.5 $\pm$ 21.5	48.7 $\pm$ 12.7	35.4 $\pm$ 13.7	55.4 $\pm$ 12.9
	<i>P</i> <sup>a</sup>	0.018	0.0027	0.0061	0.018	0.0032	0.0035
Mahoga-ny	Non-infested	97.7 $\pm$ 47.4	192.1 $\pm$ 81.3	61.2 $\pm$ 6.1	73.5 $\pm$ 34.3	108.6 $\pm$ 49.9	47.9 $\pm$ 5.4
	Infested	53.1 $\pm$ 26.9	119.5 $\pm$ 65.4	37.1 $\pm$ 9.1	44.6 $\pm$ 20.4	68.4 $\pm$ 36.4	32.0 $\pm$ 6.5
	<i>P</i>	0.08	0.12	0.0005	0.11	0.14	0.0011
Surinam cherry	Non-infested	34.5 $\pm$ 10.9	89.0 $\pm$ 31.9	135.4 $\pm$ 42.1	30.5 $\pm$ 9.2	66.8 $\pm$ 21.4	75.6 $\pm$ 17.0
	Infested	15.4 $\pm$ 10.0	65.1 $\pm$ 26.3	64.0 $\pm$ 43.3	12.5 $\pm$ 7.4	47.3 $\pm$ 19.3	44.0 $\pm$ 21.5
	<i>P</i>	0.01	0.019	0.016	0.0043	0.13	0.019
Pond apple	Non-infested	16.8 $\pm$ 16.4	17.7 $\pm$ 17.7	3.6 $\pm$ 2.5	13.4 $\pm$ 12.5	14.3 $\pm$ 13.2	16.8 $\pm$ 16.4
	Infested	11.5 $\pm$ 5.0	13.5 $\pm$ 7.0	2.9 $\pm$ 1.3	9.7 $\pm$ 4.1	11.5 $\pm$ 5.7	11.5 $\pm$ 5.0
	<i>P</i>	0.48	0.61	0.56	0.51	0.65	0.55

<sup>a</sup> Significance level determined with a standard T-test; n=6.

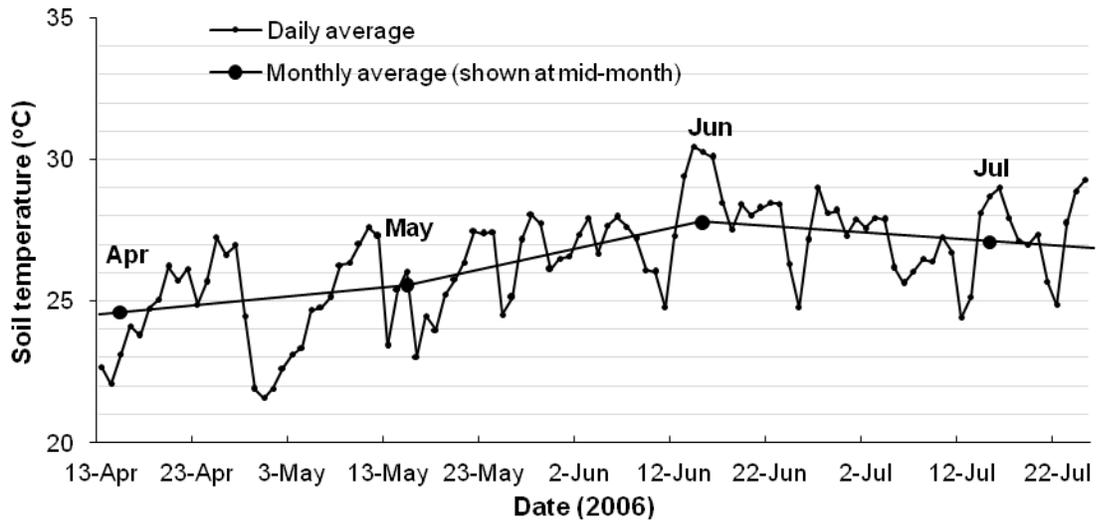


Figure 2-1. Average temperature 6 cm below the soil surface during the experiment.

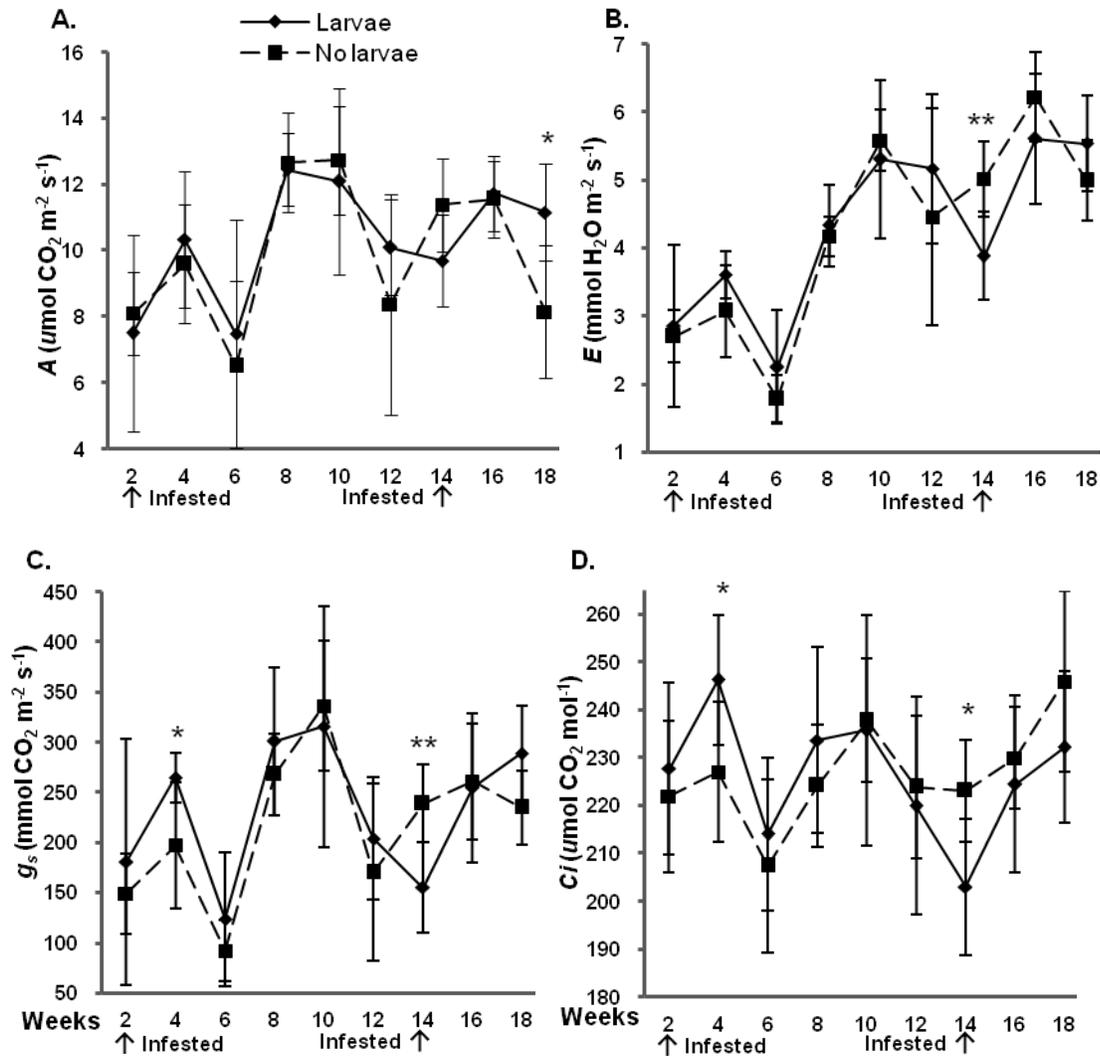


Figure 2-2. Effect of *Diaprepes* root weevil larval feeding on net CO<sub>2</sub> assimilation ( $A$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and substomatal partial pressure of CO<sub>2</sub> ( $C_i$ ) of green buttonwood plants. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$  according to a standard T-test.

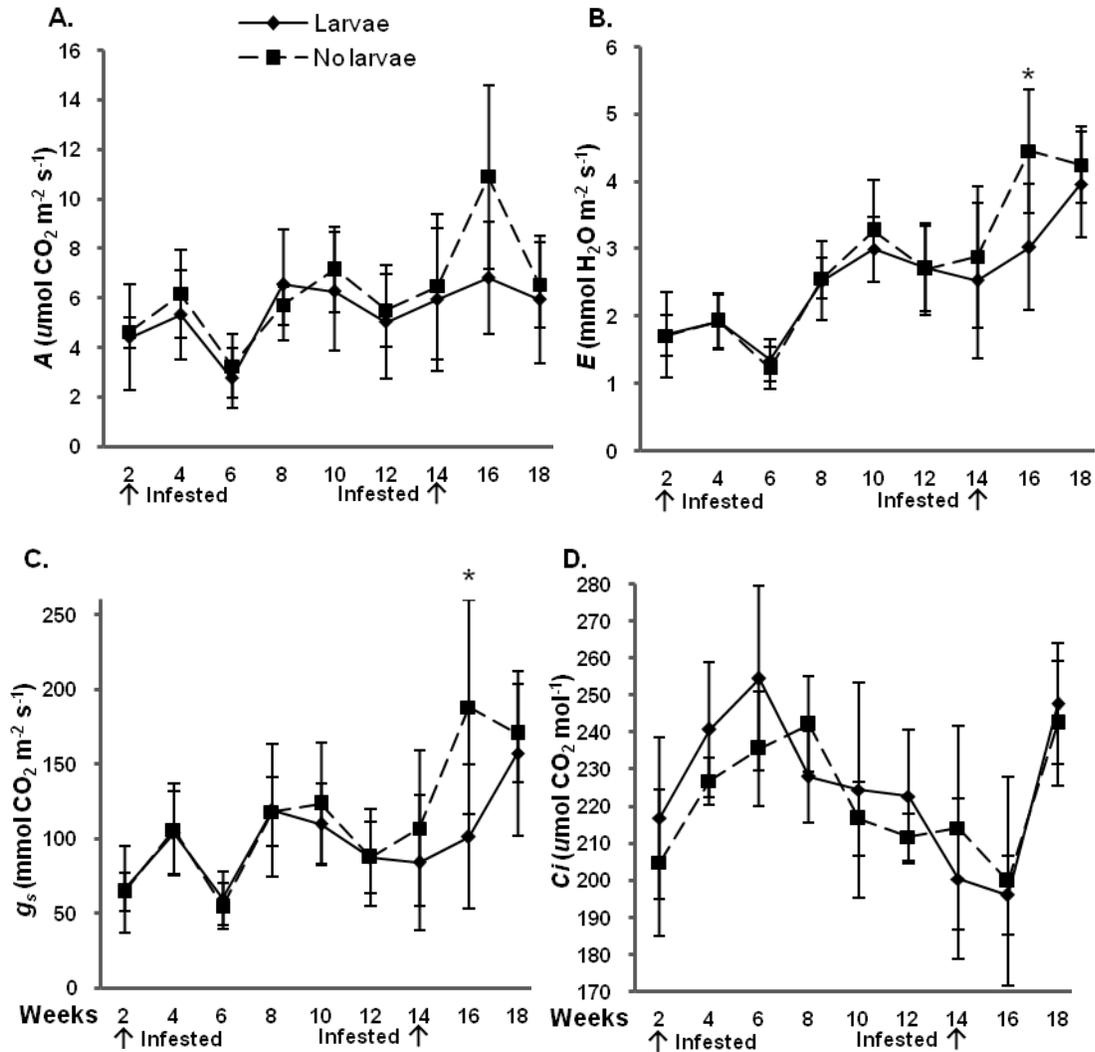


Figure 2-3. Effect of *Diaprepes* root weevil larval feeding on net CO<sub>2</sub> assimilation ( $A$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and substomatal partial pressure of CO<sub>2</sub> ( $C_i$ ) of mahogany trees. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$  according to a standard T-test.

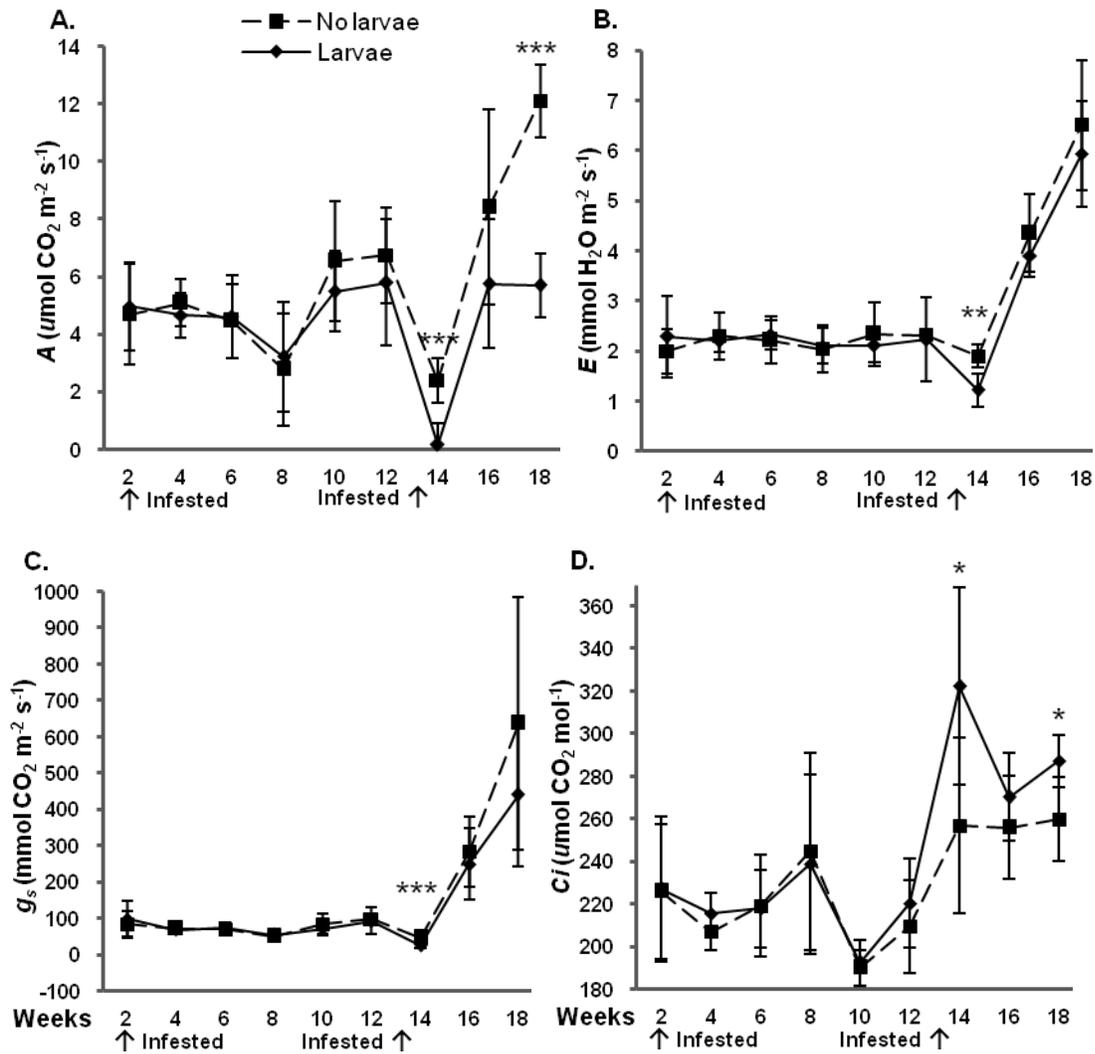


Figure 2-4. Effect of *Diaprepes* root weevil larval feeding on net CO<sub>2</sub> assimilation ( $A$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and substomatal partial pressure of CO<sub>2</sub> ( $C_i$ ) of Surinam cherry plants. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$  according to a standard T-test.

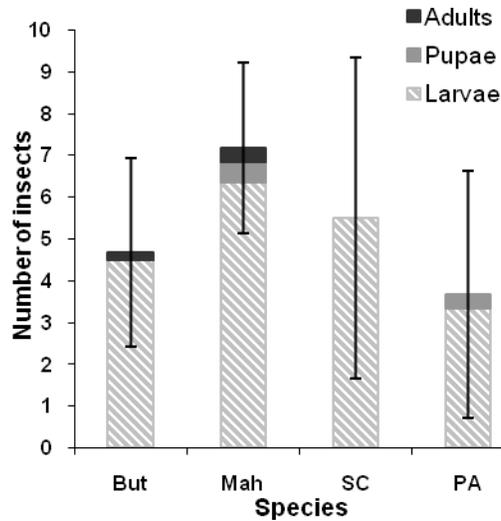


Figure 2-5. Average sum of larvae, pupae, and adults of *Diaprepes* root weevil per plant species. But = buttonwood, Mah = mahogany, SC = Surinam cherry, and PA = pond apple. Bars represent means  $\pm$  SD. Differences in total number of insects recovered were not significant among plant species.

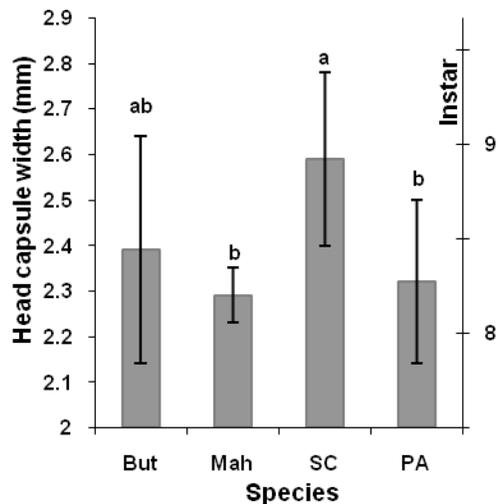


Figure 2-6. Mean head capsule widths and resulting instars of *Diaprepes* root weevil larvae recovered from infested plants. But = buttonwood, Mah = mahogany, SC = Surinam cherry, and PA = pond apple. Bars represent means  $\pm$  SD. Different letters indicate significant differences among species according to ANOVA and Waller-Duncan multiple range tests ( $P \leq 0.05$ ).

CHAPTER 3  
EFFECTS OF FLOODING AND SOIL TYPE ON THE PHYSIOLOGY AND GROWTH OF  
FOUR WOODY ORNAMENTAL PLANT SPECIES IN SOUTH FLORIDA

**Introduction**

A significant portion of agriculture in south Florida occurs in low-lying areas with the water table only a few meters below the soil surface (Schaffer 1998). Changes in water delivery practices to Everglades National Park as a result of the Everglades Restoration Project have resulted in elevating the water table in these areas (Schaffer 1998). This has increased the severity, duration, and extent of flooding in areas with tropical fruit orchards and ornamental plant nurseries. For agriculture to remain viable in these areas, it is important to understand how flooding affects crop physiology, growth, and yield to help identify flood-adapted crops and production systems (Schaffer 1998).

Flooding or waterlogging of the root zone typically depletes soil oxygen content (Kozlowski 1997). Oxygen content in soil can be indirectly determined by measuring the redox potential of the soil; redox potentials of +300 mV or more indicate aerobic conditions, whereas redox potentials of less than +200 mV suggest anaerobic conditions (Ponnamperuma 1972, 1984). The effects of flooding on physiology and growth of a woody, perennial plant species can vary among soil types and is partly based on the rates of soil oxygen depletion and other factors such as soil pH (Schaffer et al. 1992, Kozlowski 1997). In plant nurseries in south Florida, woody, perennial ornamental plants are grown either in potting medium in containers or in the field. Field plants are generally grown in marl soils, which are classified as Biscayne soil (loamy, carbonatic, hyperthermic, shallow Typic Fluvaquents) (Nobel et al. 1996, Li 2001). These marl soils are derived from limestone in areas that alternate several months of flooding during the wet season with several months of non-flooded conditions during the dry season. The

resulting “calcite mud” soil is high in calcium, has pH of 7.4-8.4, and has poor drainage (Li 2001).

Responses of woody, perennial plants to flooding include senescence, shoot dieback, premature leaf abscission, decreased cambial growth, and the suppression of leaf formation and expansion (Kozlowski 1997). Flooding also inhibits root formation and branching, and the growth of roots and mycorrhizae. Root mortality, loss of mycorrhizae, and suppressed root metabolism help to reduce the absorption of macronutrients. In addition, flooding reduces net CO<sub>2</sub> assimilation and the transport of carbohydrates within plants, and decreases flower bud initiation, anthesis, and fruit size, set, quality, and growth (Kozlowski 1997). Flooding often causes a change in the allocation of photosynthates within plants. For example, Yamamoto and Kozlowski (1987) found that height and diameter growth of flooded *Acer platanoides* L. seedlings were suppressed, whereas bark growth was increased, which suggests that flooding affects carbohydrate partitioning. Early measurable plant responses to flooding include reductions in net CO<sub>2</sub> assimilation and stomatal conductance. Therefore, measuring these leaf gas exchange variables can help quantify damage from flooding prior to the appearance of any visual symptoms.

Green buttonwood (*Conocarpus erectus* L., Combretaceae), mahogany (*Swietenia mahagoni* Jacq., Meliaceae), pond apple (*Annona glabra* L., Annonaceae), and Surinam cherry (*Eugenia uniflora* L., Myrtaceae) are widely grown in southern Florida. Green buttonwood and mahogany trees are native to southern Florida and are grown as lawn and street trees (Watkins and Sheehan 1975). Surinam cherry is also a widely planted shrub in southern Florida and is planted on a small scale as a fruit crop. Pond apple is a tree native to south Florida, parts of the Caribbean, and Central and South America. Although typically not grown as a commercial fruit crop (Popeno 1920, Morton 1987), this species is very tolerant of flooded soil conditions

(Schaffer 1998, Núñez-Elisea et al. 1999, Ojeda et al. 2004) and greatly increases the flood-tolerance of commercial *Annona* species when used as a rootstock (Núñez-Elisea et al. 1999).

The purpose of this study was to test effects of flooding on growth and physiology of green buttonwood, mahogany, pond apple, and Surinam cherry trees in a nursery potting medium and in the marl soil typically found in field nurseries in southern Florida.

### **Materials and Methods**

Two tests were conducted at the Tropical Research and Education Center, University of Florida, Homestead, from 2006 to 2007. The first test was conducted outside during the spring and summer of 2006 with buttonwood and mahogany. The second test was also conducted outside but in the late fall, winter, and spring 2006-2007 with pond apple and Surinam cherry.

**Plant material.** All plants were obtained from local commercial nurseries. Before initiating treatments, buttonwood, mahogany, and Surinam cherry trees were approximately 2 years old, and pond apple trees were approximately 1 year old. At the beginning of the experiment, plant heights (mean  $\pm$  SD) were  $44 \pm 11$ ,  $96 \pm 14$ ,  $77 \pm 20$ , and  $56 \pm 11$  cm for buttonwood, mahogany, pond apple, and Surinam cherry, respectively. Half the plants of each species (12 out of 24 plants) were repotted into 11-liter plastic containers filled with a marl soil native to south Florida 77 d before initiating the first test. The marl soil was collected from a fallow agricultural field in Homestead, FL and was sieved to remove large objects. For each plant species, the remaining half (12 of 24) plants remained in the same 11-L containers in which they were purchased, or if purchased in smaller containers they were repotted into 11-L containers filled with the same nursery potting medium. The potting media for each species were standard commercial mixes and thus not necessarily the same for each species. For green buttonwood, the medium was composed of 60% Florida peat and 40% hardwood chips, by volume. The potting medium for mahogany contained 25% Florida peat, 65% pine bark, and

10% coarse sand, by volume. For pond apple and Surinam cherry the medium contained 40% Florida peat, 20% pine bark, 20% cypress sawdust, and 20% sand, by volume. All trees in both tests were fertilized with liquid fertilizer (Peters 24-8-16 with micronutrients, United Industries, St Louis, MO) at the manufacturer's recommended rate for plants in general. Buttonwood and mahogany trees were each fertilized 9 May 2006 (29 d before beginning the test), while pond apple and Surinam cherry were each fertilized 22 November 2006 (19 d before beginning their test). No pesticides were used; pests were controlled by hand removal.

**Flooding treatments.** For each species, six of the twelve plants in each soil type were flooded by placing them into 19-liter plastic buckets filled with tap water to 10-cm above the soil surface. The other six plants in each soil type were left unflooded as controls. Flood period durations were 23 d for buttonwood and mahogany and 41 d for pond apple and Surinam cherry. Flood durations were determined by the appearance of physiological indicators of plant stress on at least one of the plant species tested, such as reduced photosynthesis and wilting. All trees were irrigated by overhead sprinkler during time intervals when plants were not flooded. Buttonwood and mahogany were irrigated by overhead sprinklers for 30 min twice a day, whereas pond apple and Surinam cherry were irrigated for 30 min once a day until day 103, when irrigation was changed to 30 min twice a day. During flood periods, however, non-flooded trees were manually irrigated with 0.5 liter of water per plant every 2 d, and flooded trees were not irrigated except by maintaining flood water levels as described above.

**Soil temperature and redox potential.** Soil temperature was measured at 1-h intervals throughout the experiment with sensors located 6 cm below the soil surface (StowAway<sup>®</sup> Tidbit<sup>®</sup> temploggers, Onset Co., Pocasset, MA). Soil temperature was determined using four sensors for buttonwood and mahogany, and three sensors for pond apple and Surinam cherry. Soil redox potential was measured with a platinum combination electrode attached to a portable volt meter

(Accumet #AP62, Fisher Scientific, Pittsburgh, PA). Measurements were made by inserting the electrode into a polyvinyl chloride (PVC) pipe (20 cm long x 22 mm wide) that protruded 4 cm above the soil surface and was placed in the soil 2 cm from the edge of the pot. Soil redox potential was recorded at a mean depth of 6 cm below soil surface and measured in 3 pots (replications) per flooded treatment (soil type), or six plants per plant species. Soil redox potential was measured daily for the first 6 or 7 d of flooding, then at intervals of 3 to 8 d, until plants were unflooded.

**Leaf gas exchange and chlorophyll index.** Net CO<sub>2</sub> assimilation and stomatal conductance were measured on two fully expanded, recently matured leaves or leaflets (between the fourth and tenth node below the stem) per plant with a CIRAS-2 portable gas analyzer (PP Systems, Amesbury, MA). For net CO<sub>2</sub> assimilation and stomatal conductance measurements, the photosynthetic photon flux was maintained at 1000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  with a halogen lamp attached to the leaf cuvette, and the reference CO<sub>2</sub> concentration into the cuvette was kept constant at 375  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub>. Mahogany has pinnately compound leaves with 6-8 similarly-sized leaflets per leaf, whereas all the other plant species tested have simple leaves (Watkins and Sheehan 1975, Wunderlin 1998). For leaf gas exchange measurements, mahogany leaflets were randomly selected from all positions on the leaf. For each plant species, leaf gas exchange of plants in each treatment was initially measured a few days prior to flooding and periodically until a few days after plants were unflooded. The first measurement of leaf gas exchange for buttonwood, mahogany, pond apple, and Surinam cherry was 6 d, 4 d, 6 d, and 4 d, respectively, before flooding began. Leaf gas exchange measurements for buttonwood, mahogany, pond apple, and Surinam cherry were spaced at intervals of 2-9 d, 2-9 d, 3-11 d, and 4-11 d, respectively, on a separate day for each plant species. Leaf gas exchange was measured 12 times for buttonwood and mahogany and 19 times for pond apple and Surinam cherry.

**Plant growth.** For plants in each treatment, stem diameter was measured 10 cm above soil surface, and for plants with multiple stems at this height, diameter of the largest stem was recorded. Plant height was measured from the soil surface to the plant apex. Stem diameter and plant height were measured prior to flooding and again immediately before harvest. For buttonwood, harvest began 84 d after flooding was initiated and 61 d after unflooding. For mahogany, harvest began 88 d after flooding was initiated and 65 d after unflooding. For both pond apple and Surinam cherry, harvest began 128 d after flooding was initiated and 87 d after unflooding. For all plants, roots, stems, and leaves were oven-dried at 75°C to a constant weight, and dry weights were determined for each plant organ. For buttonwood, pond apple, and Surinam cherry, leaf dry weights included leaf blades and petioles, but for mahogany, leaf dry weights included leaflets, petiolules, rachises, and petioles. Additionally, the number of inflorescences per plant was determined for buttonwood and the numbers and weights of flowers and fruits (including pedicels) per plant were determined for pond apple and Surinam cherry.

**Experimental design and statistical analysis.** Each plant species was analyzed separately. For each plant species, the experimental design was a 2 (soil type) x 2 (flooding treatment) factorial with six single-plant replications per treatment for a total of 24 plants of each species arranged in a completely randomized design. Data were first analyzed by a two-way analysis of variance (ANOVA) to assess flooding and soil type interactions. In each soil type, flooded versus non-flooded treatments were compared using standard T-test, and within each flooding treatment, effects of soil type were assessed by a standard T-test (PROC T-TEST and GLM, SAS Institute, Cary, NC, 2003).

## **Results**

Range of mean daily soil temperatures was 24.4-30.5°C for buttonwood and mahogany and 10.3-25.0°C for pond apple and Surinam cherry. Soil redox potential for green buttonwood

plants flooded in marl soil ranged from +310 mV on the first day of flooding to -234 mV on day 14 (Figure 3-1a), and in potting medium, soil redox potential varied from +176 mV on the first day of flooding to -234 mV on day 4 (Figure 3-1a). Soil redox potential for flooded mahogany in marl soil varied from +296 mV on the first day of flooding to -106 mV on day 21 and in potting medium from +221 mV on the first day of flooding to -239 mV on day 4 (Figure 3-1b). For pond apple, soil redox potentials in flooded marl soil ranged from +153 mV on the first day of flooding to -98mV on day 18 (Figure 3-1c) and in potting medium ranged from +152 mV on the first day of flooding to -163 mV on day 7. Soil redox potentials for Surinam cherry in marl soil ranged from +219 mV on the first day of flooding to -162 mV on day 18 and in potting medium ranged from +152 mV on the first day of flooding to -270 mV on day 7 (Figure 3-1d).

**Leaf gas exchange and chlorophyll index.** For green buttonwood, there was a significant interaction between flooding and soil type ( $P \leq 0.05$ ) for net CO<sub>2</sub> assimilation and stomatal conductance on one or more measurement dates. Net CO<sub>2</sub> assimilation was significantly lower for plants in flooded than in non-flooded marl soil (Figure 3-2a) and in flooded marl soil compared to flooded potting medium (Figure 3-2c) on several measurement dates. For non-flooded buttonwoods, differences in net CO<sub>2</sub> assimilation between potting medium and marl soil were not significant in 11 of 12 weeks, but in week 10, net CO<sub>2</sub> assimilation was significantly higher for plants in marl soil than in potting medium (Figure 3-2d). Green buttonwood in potting medium performed equally well under flooded and non-flooded conditions: there were no significant differences between flooded and non-flooded plants in net CO<sub>2</sub> assimilation (Figure 3-2b) or stomatal conductance (Figure 3-3b). For green buttonwood in marl soil, stomatal conductance was significantly lower for flooded than for non-flooded plants on weeks 3-7 (Figure 3-3a). Also, stomatal conductance of flooded green buttonwood was significantly lower in marl soil than potting medium in weeks 6 and 7, but significantly higher in week 8 (Figure 3-

3c). For non-flooded green buttonwood plants, stomatal conductance was significantly lower for potting medium than marl soil treatment combinations in weeks 3, 4, 8, and 10 (Figure 3-3d).

For mahogany there was a significant interaction between flooding and soil type ( $P \leq 0.05$ ) for net CO<sub>2</sub> assimilation and stomatal conductance on one or more measurement dates.

Mahogany net CO<sub>2</sub> assimilation was consistently lower for flooded plants in marl soil than for either non-flooded plants in marl soil (Figure 3-4a) or flooded plants in potting medium (Figure 3-4c). These differences in net CO<sub>2</sub> assimilation for mahogany were significant between flooded and non-flooded plants in marl soil in weeks 2-8 and 10 and between plants flooded in marl soil and those flooded in potting medium weeks 3-7 (Figure 3-4 a, c). For mahogany in potting medium, net CO<sub>2</sub> assimilation was significantly lower for flooded than non-flooded plants only on week 12, and differences were not significant for the other 11 of 12 wk (Figure 3-4b). For non-flooded mahogany, there were no significant differences in net CO<sub>2</sub> assimilation between plants in potting medium and those in marl soil (Figure 3-4d). Stomatal conductance of mahogany plants in marl soil was significantly lower for flooded than for non-flooded plants in weeks 2-10, but in potting medium, there was no significant difference between flooded and non-flooded plants (Figure 3-5a and b). Stomatal conductance of flooded mahogany was significantly lower in marl soil than in potting medium in weeks 3-5 and 7, but for non-flooded plants, there were no significant differences in stomatal conductance between marl soil and potting medium (Figure 3-5c and d).

For pond apple, there was a significant interaction between flooding and soil type ( $P \leq 0.05$ ) for net CO<sub>2</sub> assimilation and stomatal conductance on one or more measurement dates. For pond apple in marl soil, net CO<sub>2</sub> assimilation was significantly higher in flooded than in non-flooded plants on weeks 10, 12, and 14 (Figure 3-6a). Net CO<sub>2</sub> assimilation of pond apple plants in potting medium was significantly higher for flooded than non-flooded plants on weeks 7, 10,

12, and 14-16 (Figure 3-6b). Net CO<sub>2</sub> assimilation of pond apple was significantly higher for plants flooded in marl soil than plants flooded in potting medium on weeks 2 and 3 (Figure 3-6c). Also, net CO<sub>2</sub> assimilation for non-flooded pond apple plants was significantly higher for plants in marl soil than plants in potting medium on weeks 2-5, 7-8, 10, and 15-16 (Figure 3-6d). There were no significant differences in stomatal conductance between flooded and non-flooded pond apple plants in marl soil or between flooded plants in marl soil and flooded plants in potting medium (Figure 3-7a and c). Stomatal conductance of pond apple in potting medium was significantly higher for flooded than non-flooded plants on weeks 7, 12, and 14 (Figure 3-7b). Stomatal conductance of non-flooded pond apple was significantly higher for marl soil than potting medium in weeks 2 and 5-9 (Figure 3-7d).

For Surinam cherry, there was a significant interaction between flooding and soil type ( $P \leq 0.05$ ) for net CO<sub>2</sub> assimilation and stomatal conductance on one or more measurement dates. Net CO<sub>2</sub> assimilation of Surinam cherry was significantly lower for flooded than non-flooded plants in marl soil on weeks 4-12 (Figure 3-8a). For Surinam cherry plants in potting medium, net CO<sub>2</sub> assimilation was significantly lower for flooded than non-flooded plants in weeks 4-11 (Figure 3-8b). Net CO<sub>2</sub> assimilation of flooded Surinam cherry was also significantly lower for plants in marl soil than in potting medium on weeks 4-6 and 8-13 (Figure 3-8c). Net CO<sub>2</sub> assimilation for non-flooded Surinam cherry was significantly lower for plants in marl soil than in potting medium, but only in weeks 11 and 18, whereas differences were insignificant in the remaining 17 of 19 wk (Figure 3-8d). Stomatal conductance of Surinam cherry in marl soil was significantly lower for flooded than non-flooded plants in weeks 5-12 (Figure 3-9a). In potting medium, stomatal conductance of Surinam cherry was significantly lower for flooded than non-flooded plants on weeks 8-9 (Figure 3-9b). Stomatal conductance for flooded Surinam cherry plants was significantly lower in marl soil than potting medium in weeks 6, 8-13, and 17 (Figure

3-9c). For non-flooded Surinam cherry plants, there was no significant difference in stomatal conductance between plants in potting medium and those in marl soil in 18 of 19 measurements; however, on week 11, stomatal conductance for plants in marl soil was significantly lower than those in potting medium (Figure 3-9d).

**Plant growth.** For green buttonwood, there was a significant ( $P \leq 0.05$ ) interaction between flooding and soil type for leaf dry weight and plant height. However, there were no significant interactions between flooding and soil type for root, stem, and total plant dry weight, stem diameter, or the number of inflorescences per plant. Therefore, dry weights and plant heights were not pooled, whereas stem diameters, and the number of inflorescences were pooled for statistical analyses. For green buttonwood in marl soil, leaf dry weight was significantly lower for flooded than non-flooded plants, but there were no significant differences between flooded and non-flooded plants in root, stem, or total dry weights (Figure 3-10a). In potting medium, there were no significant differences in dry weights due to flooding (Figure 3-10b). Green buttonwood root, stem, leaf, and total dry weights were significantly lower for flooded plants in marl soil than for flooded plants in potting medium (Figure 3-10c). Root stem, leaf, and total dry weights of non-flooded green buttonwood plants were significantly lower in marl soil than in potting medium (Figure 3-10d). Stem diameter of green buttonwood was significantly lower for non-flooded than flooded plants with soil types pooled (Figure 3-11a). Buttonwood stem diameter was also significantly lower for plants in marl soil than those in potting medium with flooded and non-flooded plants pooled (Figure 3-11b). Hypertrophic (swollen) lenticels and small numbers of adventitious roots, fewer than 10 per plant and up to 15 cm long, were often observed on flooded green buttonwood plants in marl soil and/or potting medium. For green buttonwood in marl soil, plant height was lower for flooded than for non-flooded plants, but not significantly (Figure 3-11c). Also, plant height of flooded green buttonwood was

significantly lower in marl soil than in potting medium (Figure 3-11e). There were no significant differences in plant height between flooded and non-flooded green buttonwood in potting medium (Figure 3-11d), nor between marl soil and potting medium for non-flooded plants (Figure 3-11f). There were no significant effects of flooding or soil type on the number of inflorescences per buttonwood plant (range 0-170).

For mahogany, there was a significant interaction ( $P \leq 0.05$ ) between flooding and soil type for leaf dry weight and stem diameter. However, there was no interaction for root, stem, and total plant dry weight and plant height. Therefore, mahogany dry weights and stem diameter were not pooled, whereas plant height was pooled for analyses. For mahogany in marl soil, leaf dry weight was significantly lower for flooded than for non-flooded plants (Figure 3-12a). Also, leaf dry weight of flooded plants was significantly lower in marl soil than in potting medium (Figure 3-12c). There were no significant differences in root, stem, or total dry weights between flooded and non-flooded mahogany plants in marl soil or between marl soil and potting medium for flooded plants (Figure 3-12a and c). There were no significant differences in dry weights between flooded and non-flooded mahogany plants in potting medium (Figure 3-12b), nor between marl soil and potting medium for non-flooded plants (Figure 3-12d). Stem diameter of mahogany in marl soil was significantly lower for flooded than for non-flooded plants (Figure 3-13a), and for non-flooded plants in potting medium than non-flooded plants in marl soil (Figure 3-13d). There were no significant differences in stem diameter between flooded and non-flooded mahogany plants in potting medium (Figure 3-13b), nor between marl soil and potting medium for flooded plants (Figure 3-13c). There were no significant differences in plant height between flooded and non-flooded mahogany plants (Figure 3-13e), but plant height was significantly lower in marl soil than in potting medium (Figure 3-13f).

For pond apple, there was no significant flooding and soil type interaction ( $P \leq 0.05$ ) for root, stem, leaf, or total plant dry weight, inflorescence number or weight, plant height, or stem diameter. Therefore, all these variables were pooled for analyses. Pond apple root, stem, leaf, and total dry weights were significantly greater in marl soil than in potting medium (Figure 3-14b), but flooding did not significantly affect these variables (Figure 3-14a). Pond apple stem diameter was significantly greater in flooded than in non-flooded plants (Figure 3-15a), but there were no significant differences between marl soil and potting medium (Figure 3-15b). In addition, flooded pond apple plants exhibited swollen stem lenticels. There was no significant effect of flooding or soil type on height of pond apple plants (range -5 to 5.8 cm), on the number of flowers and fruit per plant (range 0-1), or on weight of flowers and fruit per plant (range 0-0.55 g).

For Surinam cherry, there was a significant interaction ( $P \leq 0.05$ ) between flooding and soil type for leaf dry weight, stem diameter, plant height, and flower and fruit weight. However, there were no significant interactions for root, stem, and total plant dry weights and the number of flowers or fruit per plant. Therefore, data for these variables were not pooled for analyses. Stem, leaf, and total dry weights of Surinam cherry in marl soil were significantly lower for flooded than non-flooded plants, but there was no significant difference in root dry weight (Figure 3-16a). In potting medium, there were no significant differences between flooded and non-flooded Surinam cherry plants in root, stem, leaf, or total dry weights (Figure 3-16b). Flooded Surinam cherry in marl soil had significantly lower root, stem, leaf, and total plant dry weights than plants in potting medium (Figure 3-16c). In addition, Surinam cherry root, stem, and total dry weights were significantly lower for non-flooded plants in marl soil than for non-flooded plants in potting medium, but there was no significant difference in leaf dry weight (Figure 3-16d). Surinam cherry stem diameter in marl soil was significantly lower for flooded

for non-flooded plants (Figure 3-17a). Stem diameter of flooded Surinam cherry was significantly lower for plants in marl soil than for plants in potting medium (Figure 3-17c). There were no significant differences in stem diameter between flooded and non-flooded Surinam cherry plants in potting medium (Figure 3-17b), nor between marl soil and potting medium for non-flooded plants (Figure 3-17d). Height of Surinam cherry plants in marl soil was significantly lower for flooded than for non-flooded plants (Figure 3-18a). Also, height of Surinam cherry was significantly lower for plants flooded in marl soil than for those flooded in potting medium (Figure 3-18c). There were no significant differences in height between flooded and non-flooded Surinam cherry plants in potting medium (Figure 3-18b), nor between marl soil and potting medium for non-flooded plants (Figure 3-18d). There were no significant differences between any pair of treatment combinations in number of Surinam cherry flowers and fruit per plant. Also, for Surinam cherry, there were no significant differences in flower and fruit weight per plant between flooded and non-flooded plants in marl soil (Figure 3-19a), nor between marl soil and potting medium for non-flooded plants (Figure 3-19d). However, flower and fruit weight of Surinam cherry in potting medium was significantly higher for flooded than for non-flooded plants (Figure 3-19b). In addition, flower and fruit weight for flooded Surinam cherry plants was significantly lower in marl soil than in potting medium (Figure 3-19c).

### **Discussion**

The most apparent trend in gas exchange data for buttonwood, mahogany, and Surinam cherry was for net CO<sub>2</sub> assimilation and stomatal conductance to be lower in flooded marl soil than in non-flooded marl soil or flooded potting medium. For buttonwood, mahogany, and Surinam cherry plants, flooding reduced net CO<sub>2</sub> assimilation and stomatal conductance much more in marl soil than in potting medium. Similarly, growth of green buttonwood, mahogany, and Surinam cherry plants was often significantly less for plants in marl soil than plants in

potting medium, especially when flooded. Flooded marl soil apparently provided a much less favorable environment for net CO<sub>2</sub> assimilation and stomatal conductance than did either non-flooded marl soil or flooded potting medium for these plant species, and this was manifested in reduced gas exchange and growth. A possible reason is reduced oxygen availability in flooded marl soil than in non-flooded marl soil or flooded potting medium.

Green buttonwood was one of two species most compromised by planting in marl soil compared to potting medium, especially when marl soil was flooded (the most compromised species was Surinam cherry). This is surprising given the native habitat of green buttonwood, which includes tidal swamps of central and south Florida (Wunderlin 1998), and in south Florida, tidal swamps frequently have marl soil. As suggested from its native range, buttonwood is fairly tolerant of flooding, though it also thrives in non-flooded, moderately moist soil, which is common for landscape plants. The native habitat of buttonwood frequently has flooding combined with marl soil, but is the environment in which buttonwood evolved and should be best adapted.

Flooding green buttonwood initially reduced net CO<sub>2</sub> assimilation and stomatal conductance much more in marl soil than in potting medium. However, this trend was reversed for stomatal conductance beginning 3 wk after unflooding plants, rendering it higher for plants in flooded marl soil than flooded potting medium. Hence, there was evidence of flood-induced compensatory increase in stomatal conductance in green buttonwood. Compensatory increase in leaf gas exchange of stressed plants has been noted with buttonwood in response to larval feeding by Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) (Diaz et al. 2006, Chapter 2). Buttonwood was the only plant species that increased leaf gas exchange as a compensatory reaction to a stress because of flooding or insects compared to several other plant species tested in this and other studies (Diaz et al. 2006, Chapter 2).

Diaz (2005) tested effects of flooding and larval infestation on net CO<sub>2</sub> assimilation, stomatal conductance, and fresh and dry root weights of green buttonwood and live oak in potting medium. Diaz (2005) found that flooding significantly reduced net CO<sub>2</sub> assimilation and stomatal conductance beginning 1 wk after plants were flooded, although flooding did not significantly affect root, stem, or leaf fresh or dry weights. For green buttonwood in the present study, net CO<sub>2</sub> assimilation and stomatal conductance were not significantly different between flooded and non-flooded plants in potting medium. Minimal differences were also found in the present study between flooded and non-flooded green buttonwood in potting medium in dry weights, plant heights, and number of inflorescences per plant. Results of green buttonwood gas exchange and growth in potting medium in the present study generally agree with growth data from Diaz (2005) suggesting the plant grows equally well in flooded and non-flooded potting medium in the time periods of the studies. However, these results also suggest that green buttonwood in potting medium varied somewhat in response to flooding: Diaz (2005) found that flooding reduced green buttonwood gas exchange, and in the present study and flooding increased plant trunk diameters. Although green buttonwood in potting medium varied in its response to flooding, it generally performed as well in flooded as in non-flooded potting medium in gas exchange and growth.

In the present study, pond apple plants developed swollen stems and hypertrophic lenticels in response to flooding. Hypertrophic lenticels have been shown to benefit flooded plants in two ways: 1) they exchange gases in flood water through breaks in the closing layers (Hook et al. 1970, Hook 1984); and 2) they release potentially toxic compounds from plants, such as acetaldehyde, ethylene, and ethanol (Chirkova and Gutman 1972). Often, swollen stems of flooded plants indicate increased aerenchyma, which can facilitate movement of O<sub>2</sub> from shoots to submerged roots. Because swollen stem lenticels and aerenchyma tissue presumably

increase the stem girth, a greater stem diameter increase in flooded than in non-flooded soils may indicate adaptation to flooding.

Núñez-Elisea et al. (1999) tested effects of flooding on net CO<sub>2</sub> assimilation and growth of pond apple plants, two other *Annona* sp. seedlings, and four scion/rootstock combinations in Krome gravelly soil and potting medium. Marl soil used in the present study and Krome gravelly soil used by Núñez-Elisea et al. (1999) are similar in pH (7.4-8.4) and both have a very calcium-rich composition, but marl soil is more poorly drained than Krome gravelly soil (Li 2001). Núñez-Elisea et al. (1999) found that *A. squamosa* L. and *A. reticulata* L. did not tolerate flooding as rootstocks or seedlings, but when grafted onto pond apple, *A. reticulata* and two other scions tolerated flooding. In the present study, pond apple was very tolerant to soil flooding. Núñez-Elisea et al. (1999) found that flooded trees, especially pond apple, developed hypertrophic lenticels and thicker stems that were caused by enlarged xylem cells, but with reduced xylem density. This concurs with observations in the present study that flooded pond apple developed hypertrophic lenticels and significantly thicker stems under flooded conditions.

Ojeda et al. (2004) investigated effects of root-zone temperature and flooding on the physiology and growth of pond apple and soursop. Both plant species had roots in temperature-controlled chambers with canopies exposed to ambient conditions in a sunny greenhouse with 6 wk flooding for flooded treatments (Ojeda et al. 2004). Both studies were similar in their use of a potting medium and their duration of flood period. Pond apple was more flood-tolerant than soursop, and only trees with morphological adaptations such as enlarged trunk bases, hypertrophic stem lenticels, and adventitious roots survived continuous flooding (Ojeda et al. 2004). Overall, the present study, Núñez-Elisea et al. (1999), and Ojeda et al. (2004) all agree that pond apple exhibits good flood tolerance by developing morphological adaptations in response to flooding, such as thicker trunks and hypertrophic lenticels.

Green buttonwood and pond apple developed significantly larger stem diameters when flooded than when not flooded, and hypertrophic stem lenticels were conspicuous under flooded conditions for both species. For green buttonwood, more adventitious roots were observed on flooded than non-flooded plants. These data suggest that green buttonwood and pond apple were flood-adapted by increasing stem diameters and numbers of swollen stem lenticels and/or adventitious roots in response to flooding. Pond apple is well adapted to flooding and marl soil (Schaffer 1998, Núñez-Elisea et al. 1999, Ojeda et al. 2004) and is potentially graft-compatible with commercial *Annona* spp. grown for fruit if compatible interstocks are used (Núñez-Elisea et al. 1999). Therefore, pond apple has potential as a rootstock for commercial *Annona* species in poorly drained soil, particularly in calcareous soils of south Florida (Schaffer et al., 2006).

Mahogany gas exchange and growth were similar to those of buttonwood and Surinam cherry with lower values in flooded marl soil than in either non-flooded marl soil or flooded potting medium. Mahogany net CO<sub>2</sub> assimilation, stomatal conductance, dry weight, and plant height tended to respond better in potting medium than in marl soil, especially when flooded. However, mahogany stem diameter increase was significantly greater in non-flooded marl soil than in either flooded marl soil or non-flooded potting medium, and trees did not develop adventitious roots. Hence, mahogany did not show adaptation to flooding, which may reflect its native habit of coastal hammocks (Wunderlin 1998), which are typically not flooded and may lack marl soil (Li 2001).

The trend for Surinam cherry gas exchange to be significantly greater in non-flooded than flooded potting medium contrasts with buttonwood and mahogany which had minimal differences between these treatment combinations. Hence, Surinam cherry seems to be the least flood-tolerant plant species tested in this study. Similar to mahogany, Surinam cherry did not show adaptation to flooding as shown by the absence of adventitious roots when flooded and

significantly larger stem diameters in non-flooded marl than in flooded marl soil. This may reflect the habitat in which Surinam cherry is typically found when not cultivated, “disturbed hammocks,” which are typically non-flooded and hence do not have marl soil (Wunderlin 1998, Li 2001).

Based on leaf gas exchange, growth, and signs of morphological adaptation to flooding of the species tested, the order of flood tolerance to marl soil was as follows: pond apple showed the highest, followed by buttonwood, then mahogany, and then Surinam cherry; and in the potting media used in this study, the order was pond apple and buttonwood shared the highest flood tolerance followed by mahogany, and then Surinam cherry.

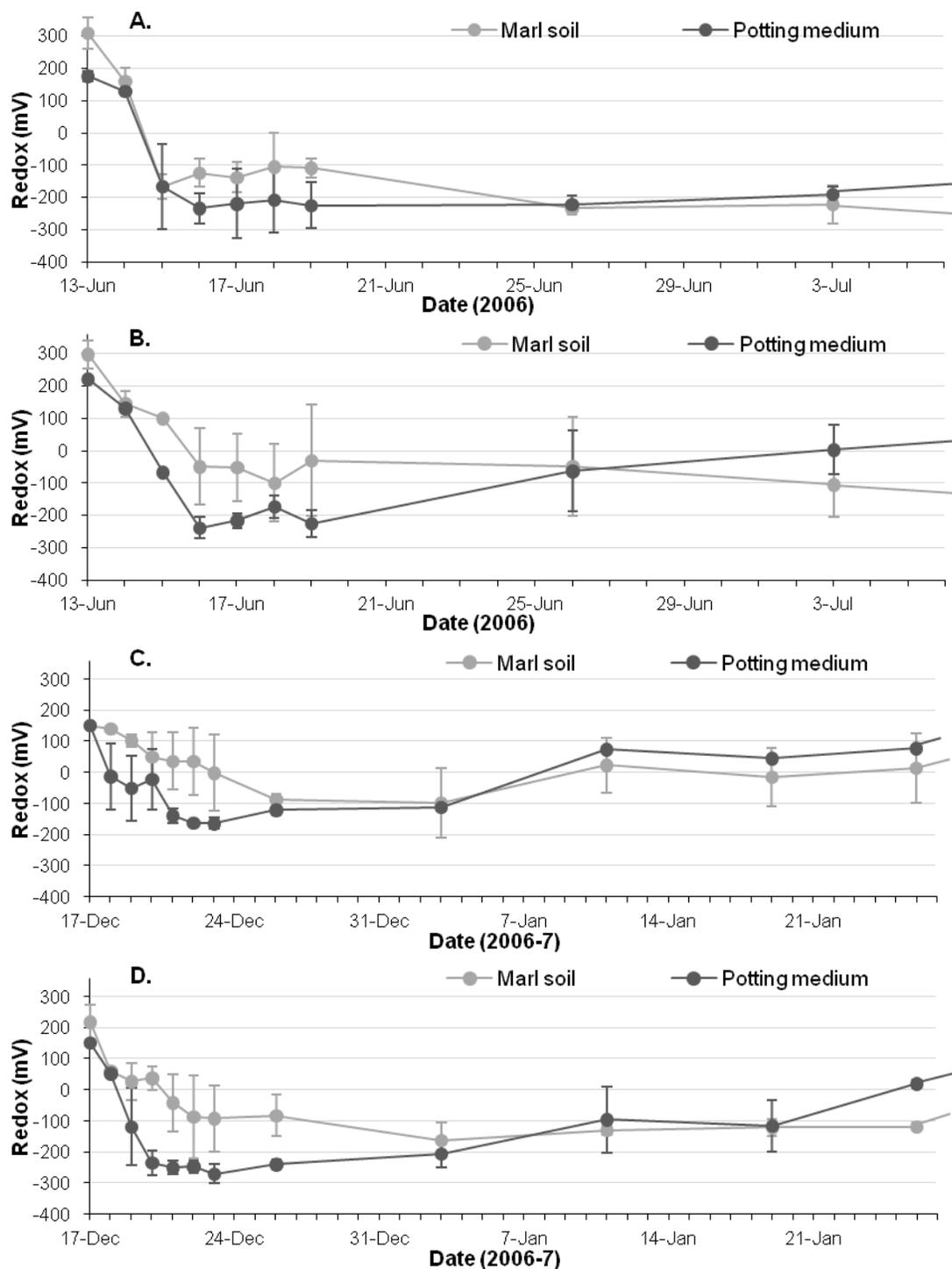


Figure 3-1. Soil redox potential. A) green buttonwood B) mahogany C) pond apple and D) Surinam cherry. Each point represents the mean  $\pm$  SD of three measurements per measurement date for each flooded treatment combination per plant species.

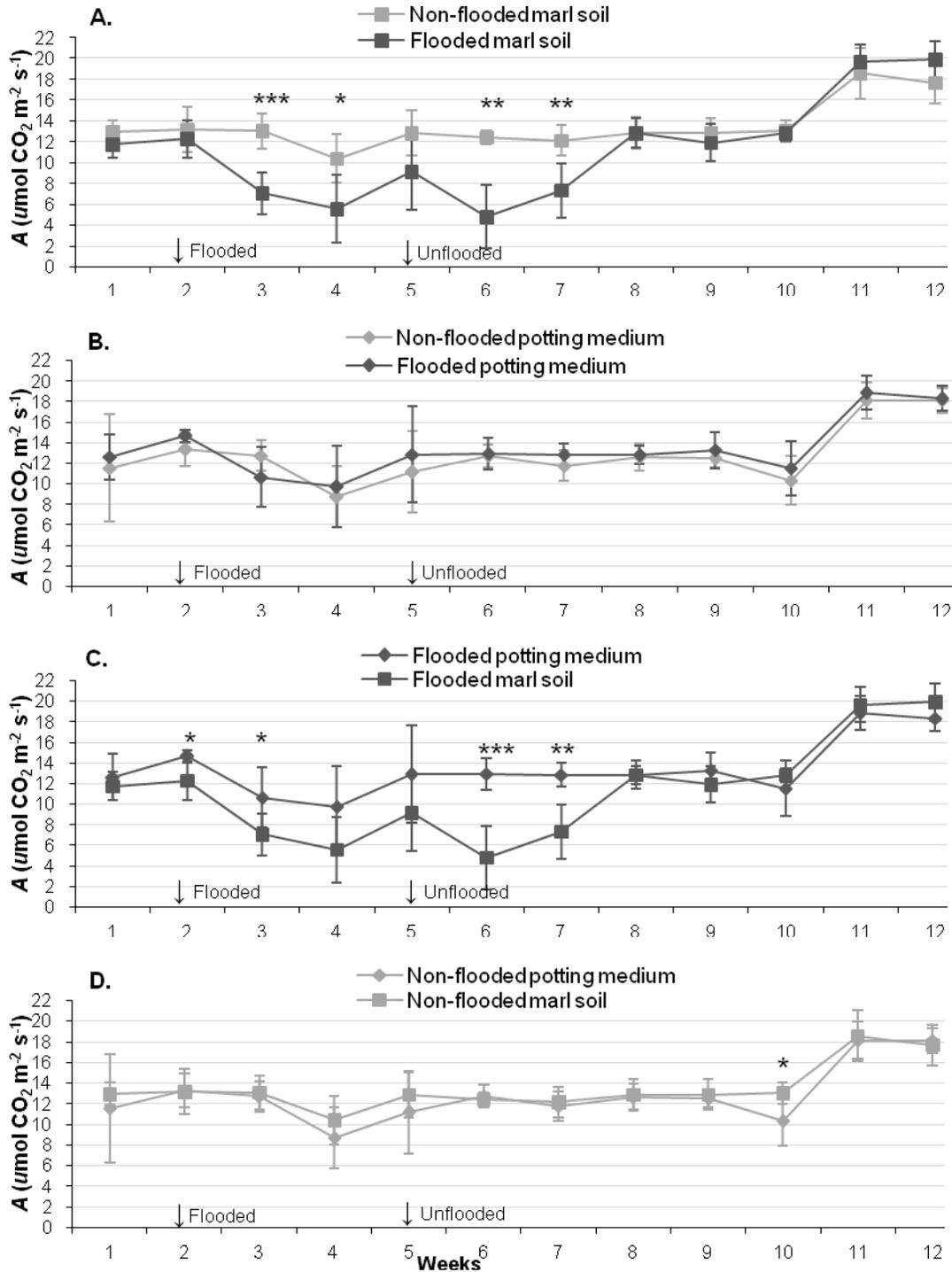


Figure 3-2. Effects of flooding and soil type on net CO<sub>2</sub> assimilation (*A*) of green buttonwood plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

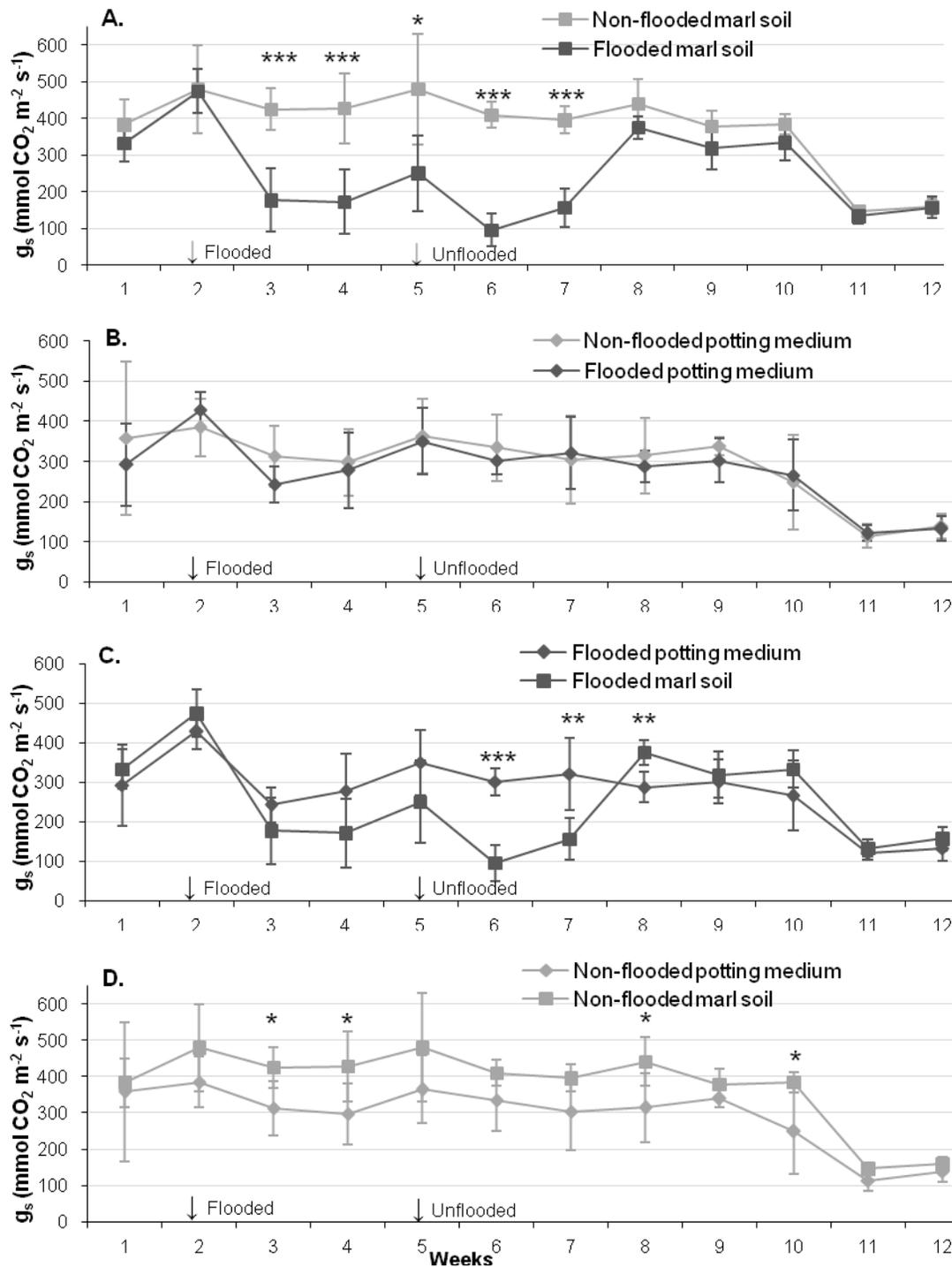


Figure 3-3. Effects of flooding and soil type on stomatal conductance ( $g_s$ ) of green buttonwood plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

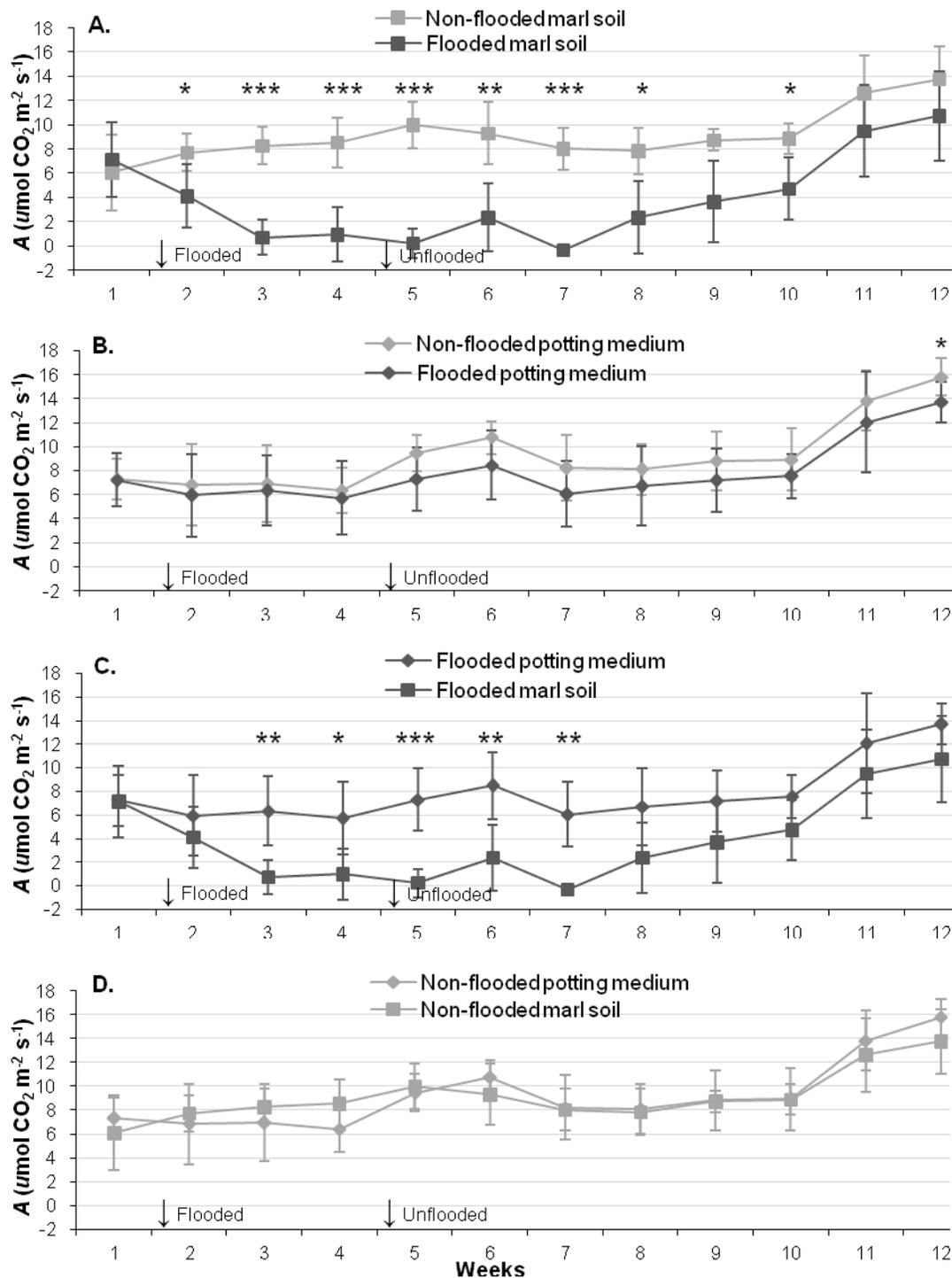


Figure 3-4. Effects of flooding and soil type on net CO<sub>2</sub> assimilation (*A*) of mahogany plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

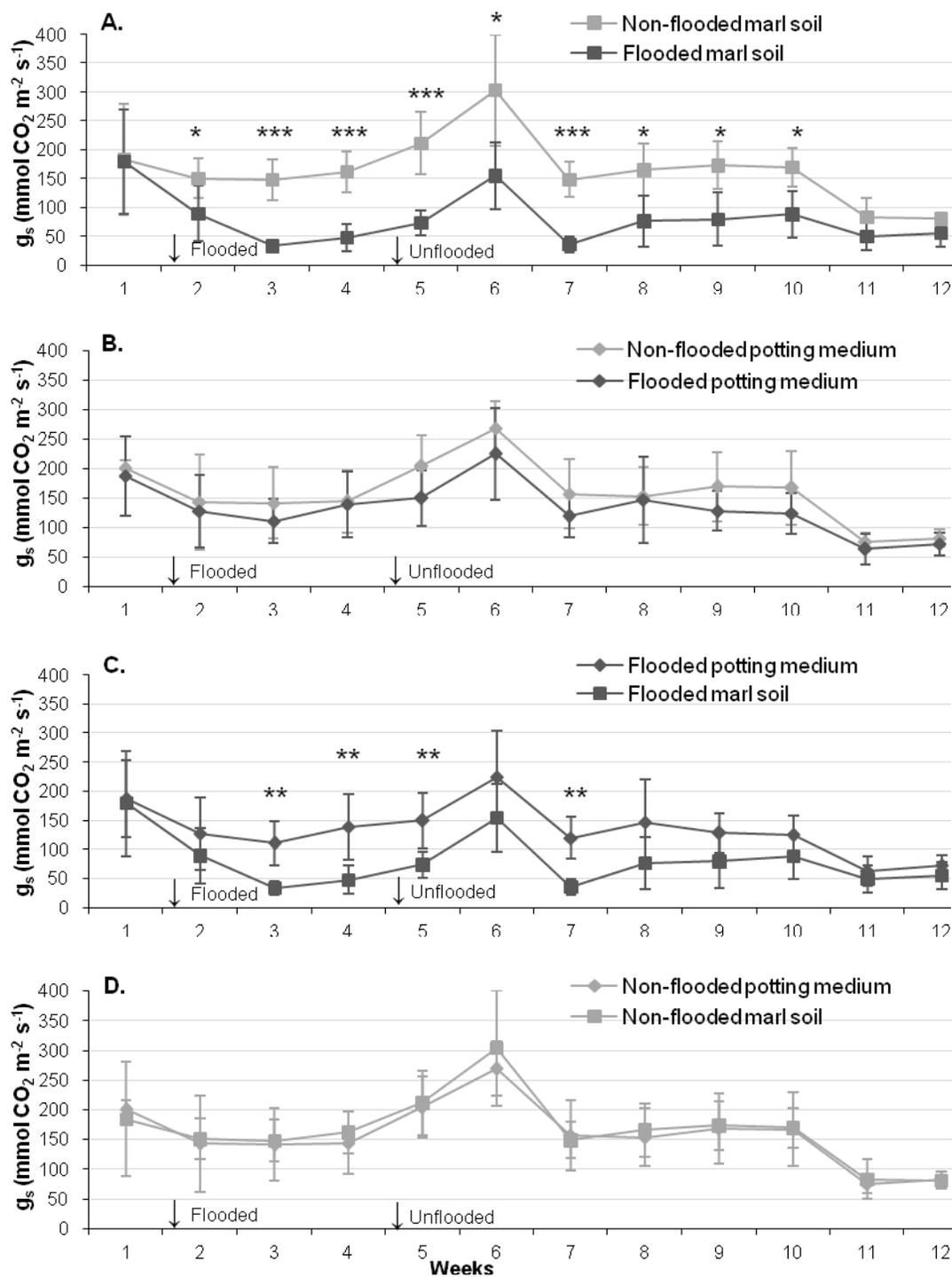


Figure 3-5. Effects of flooding and soil type on stomatal conductance ( $g_s$ ) of mahogany plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

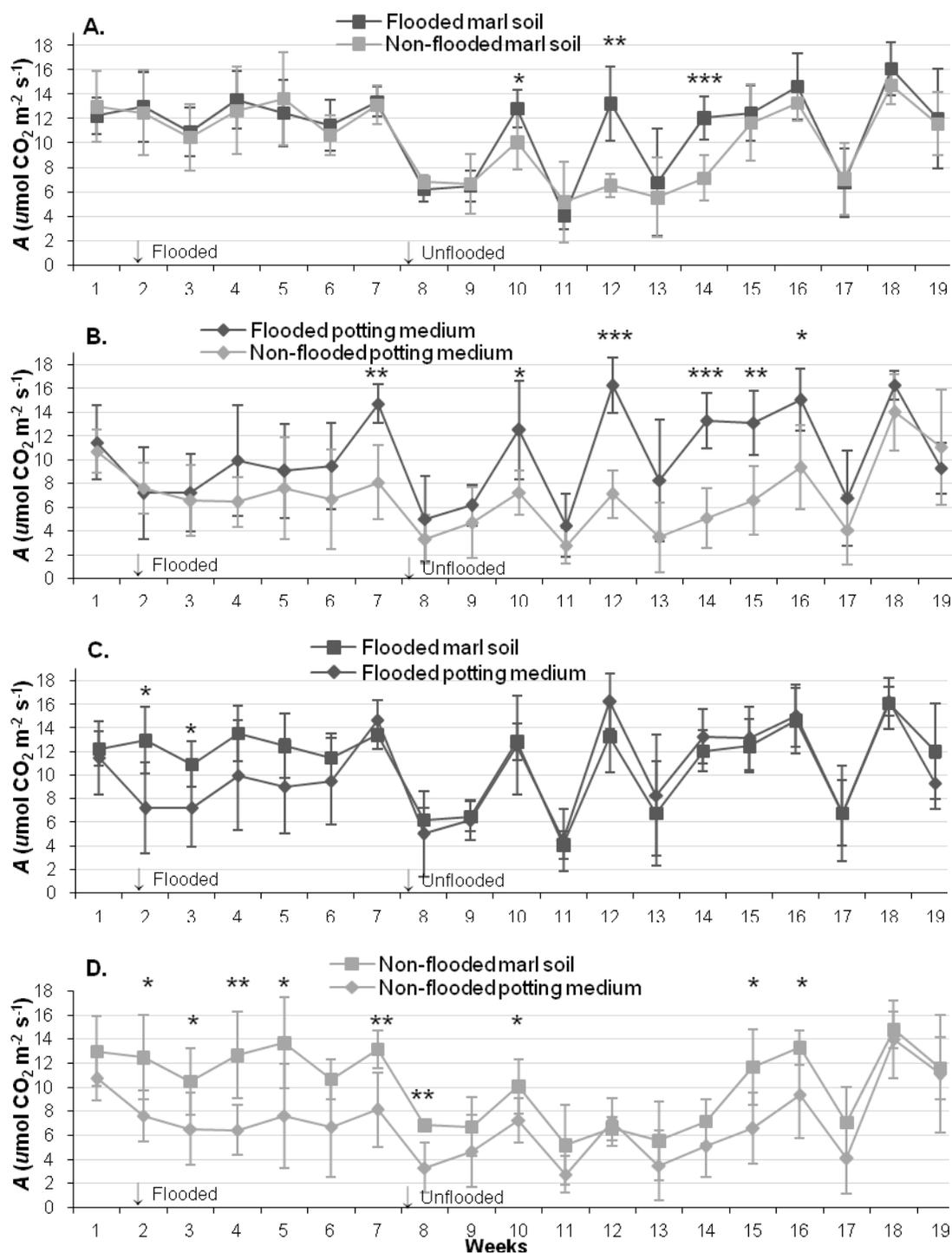


Figure 3-6. Effects of flooding and soil type on net CO<sub>2</sub> assimilation (*A*) of pond apple plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

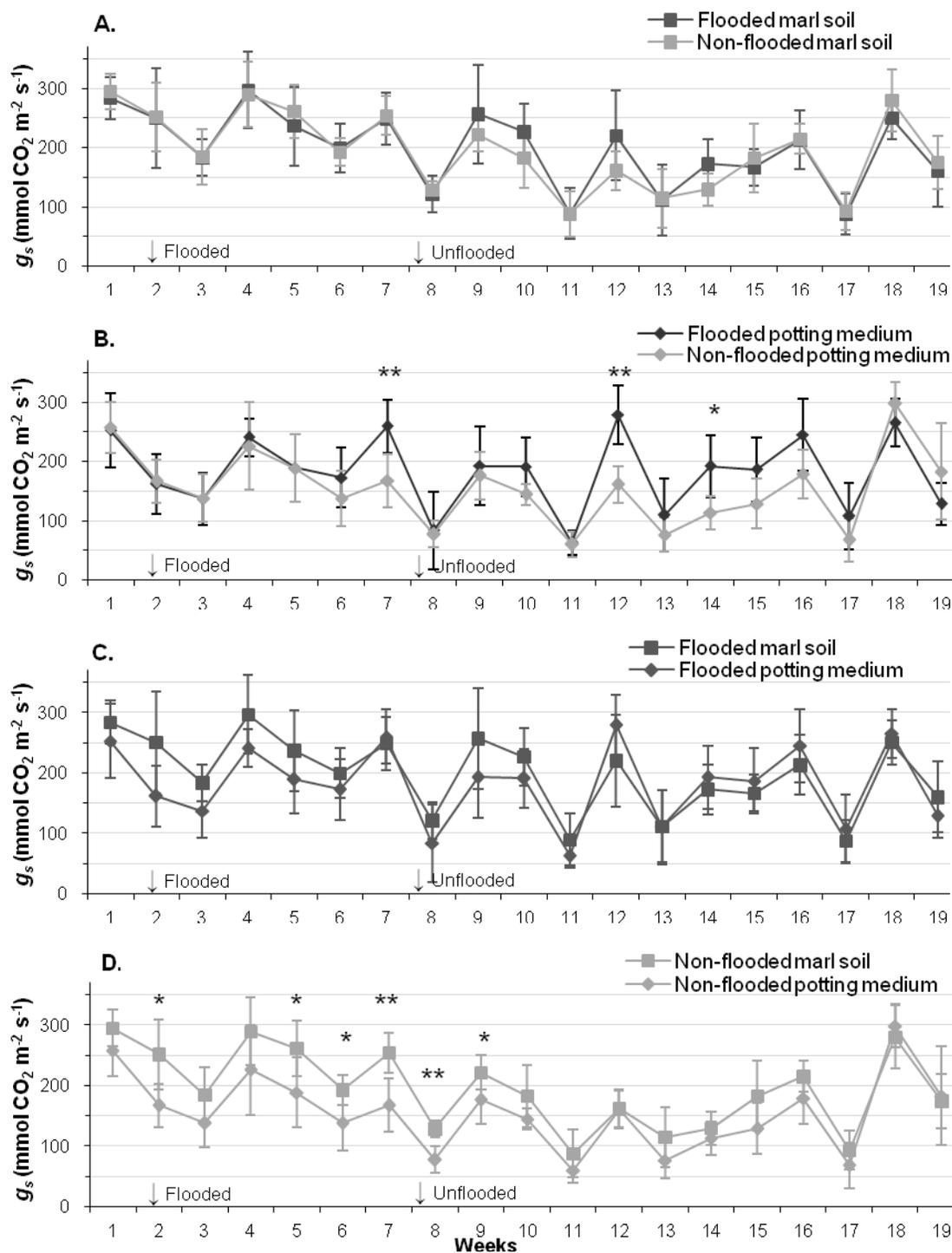


Figure 3-7. Effects of flooding and soil type on stomatal conductance ( $g_s$ ) of pond apple plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

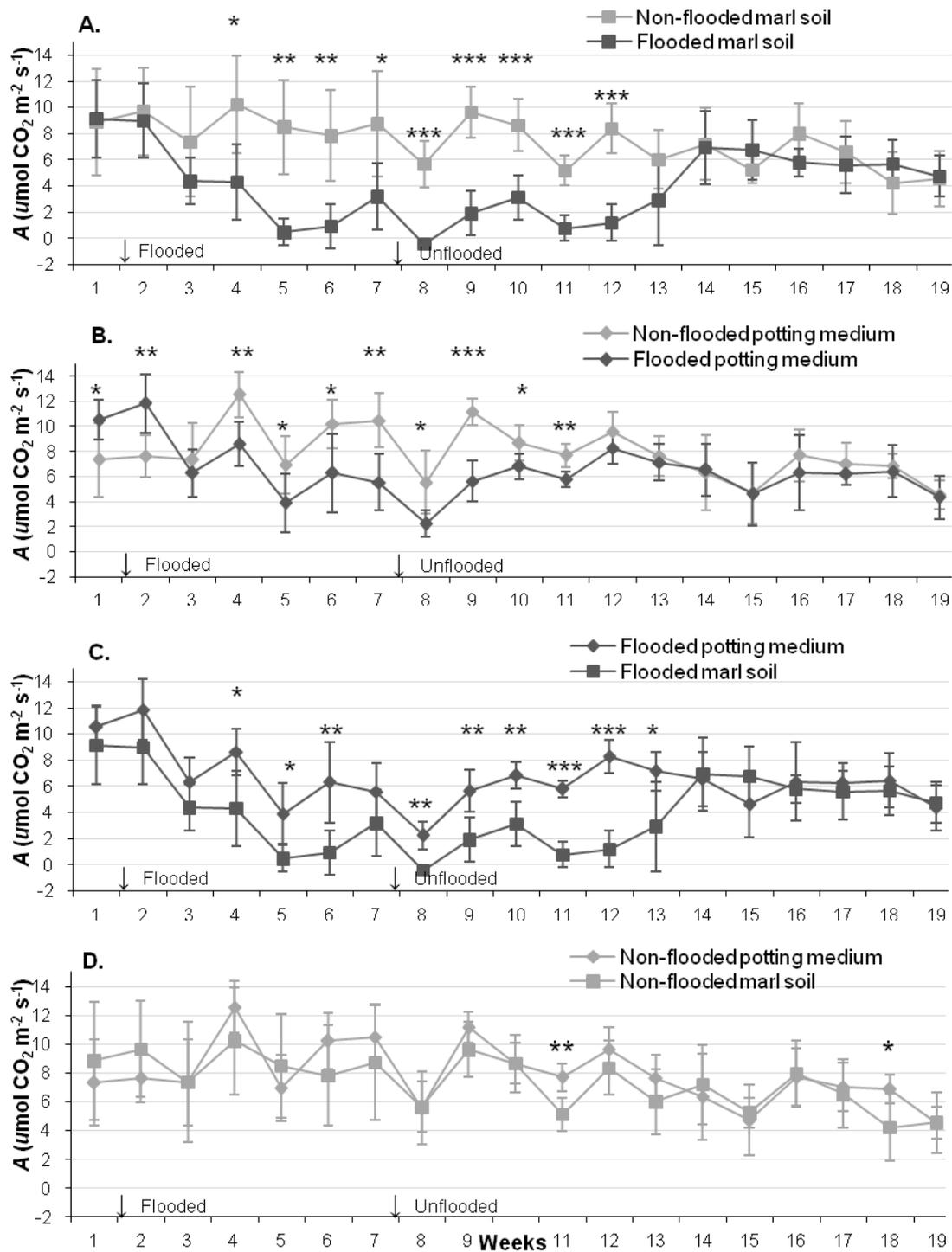


Figure 3-8. Effects of flooding and soil type on net CO<sub>2</sub> assimilation (*A*) of Surinam cherry plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

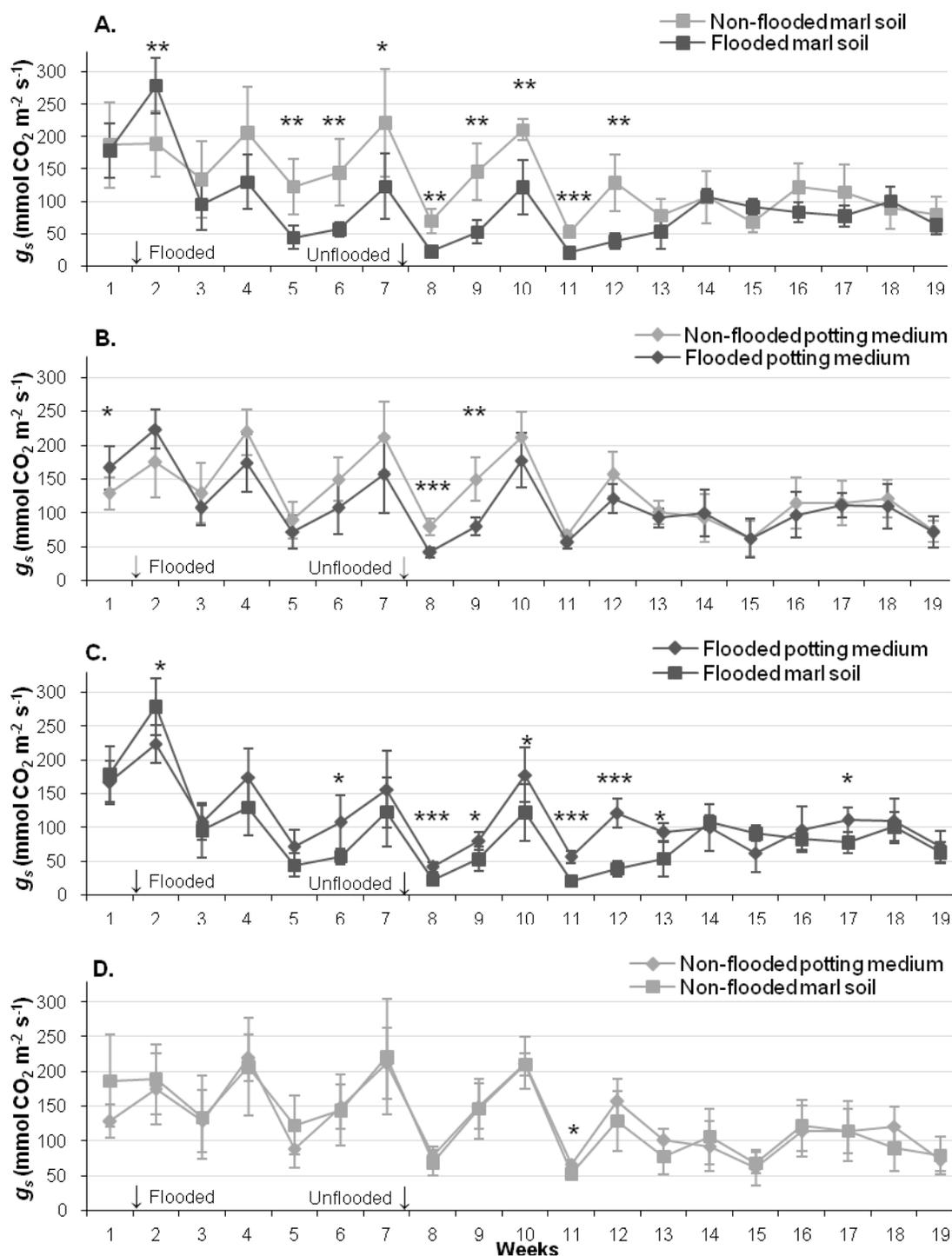


Figure 3-9. Effects of flooding and soil type on stomatal conductance ( $g_s$ ) of Surinam cherry plants. Symbols represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

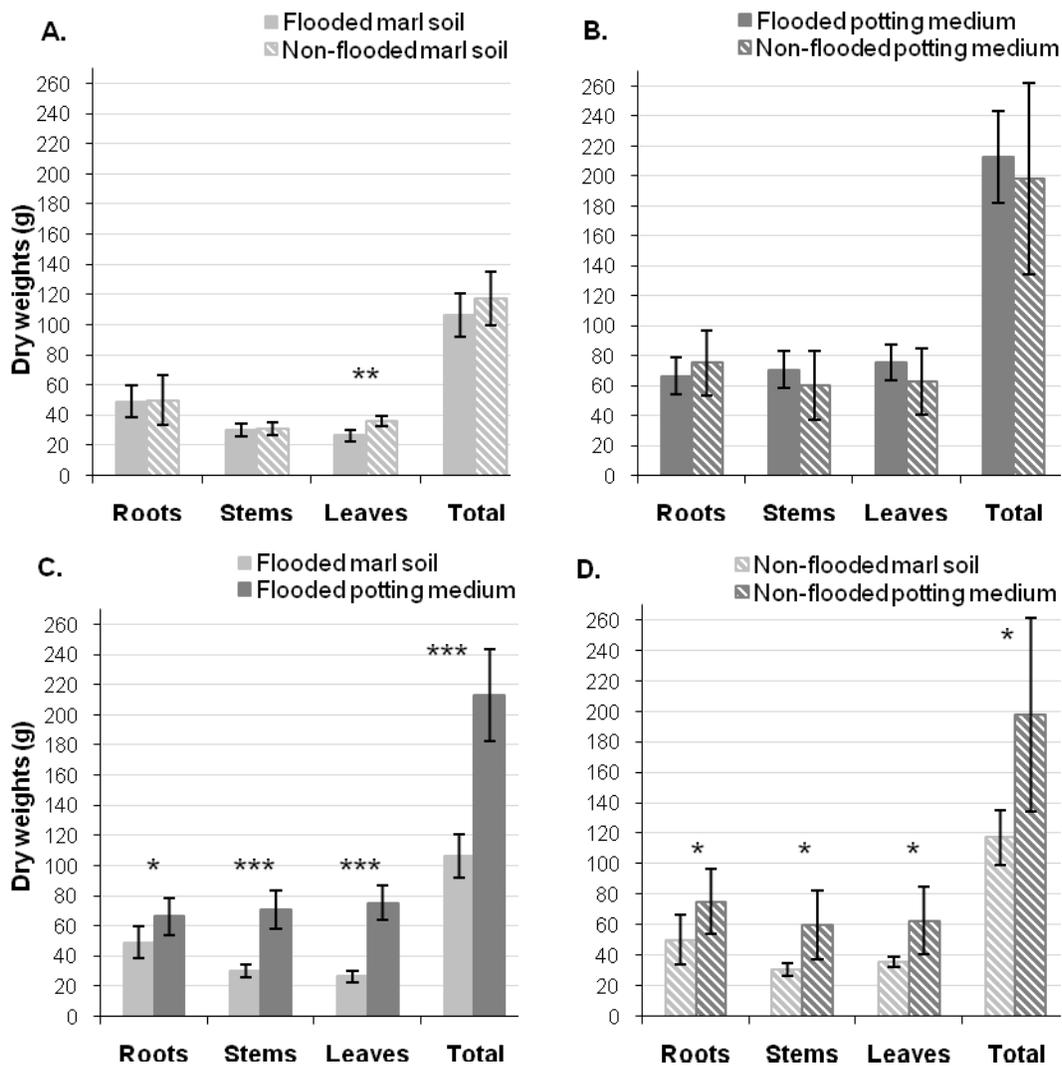


Figure 3-10. Effects of flooding and soil type on dry weights of green buttonwood plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

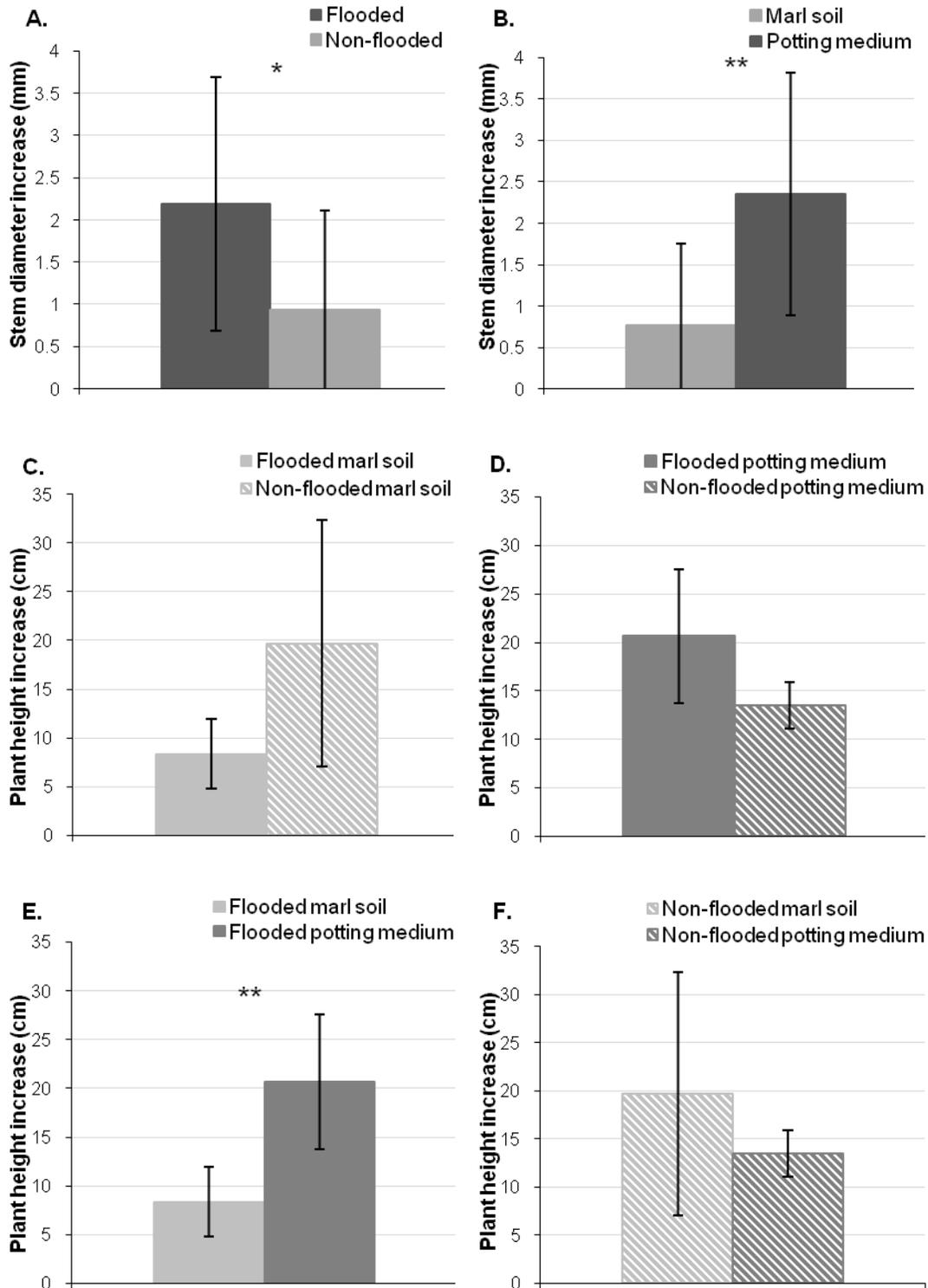


Figure 3-11. Effects of flooding and soil type on (A, B) increase in stem diameter and (C, D, E, and F) increase in plant height for green buttonwood plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

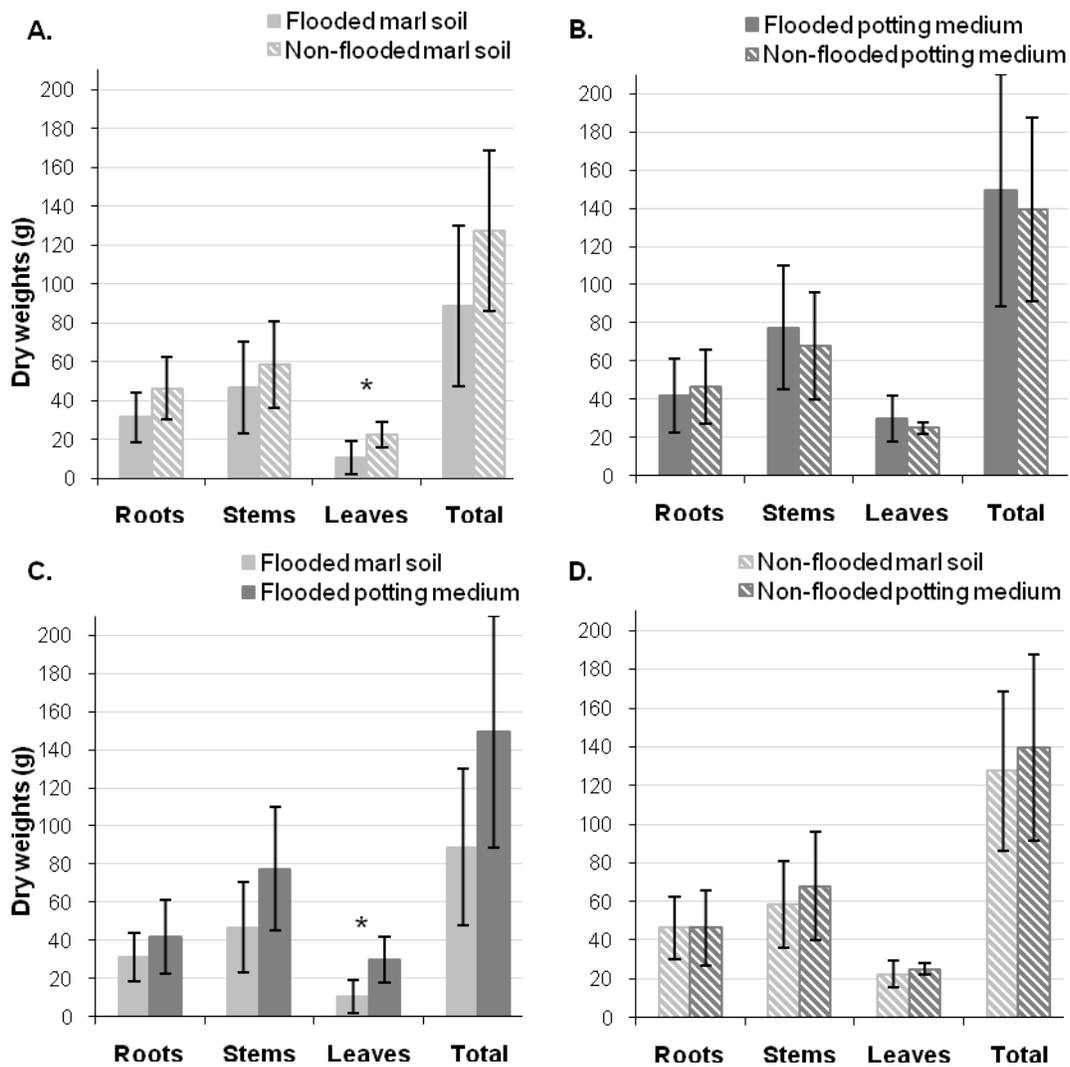


Figure 3-12. Effects of flooding and soil type on dry weights of mahogany plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

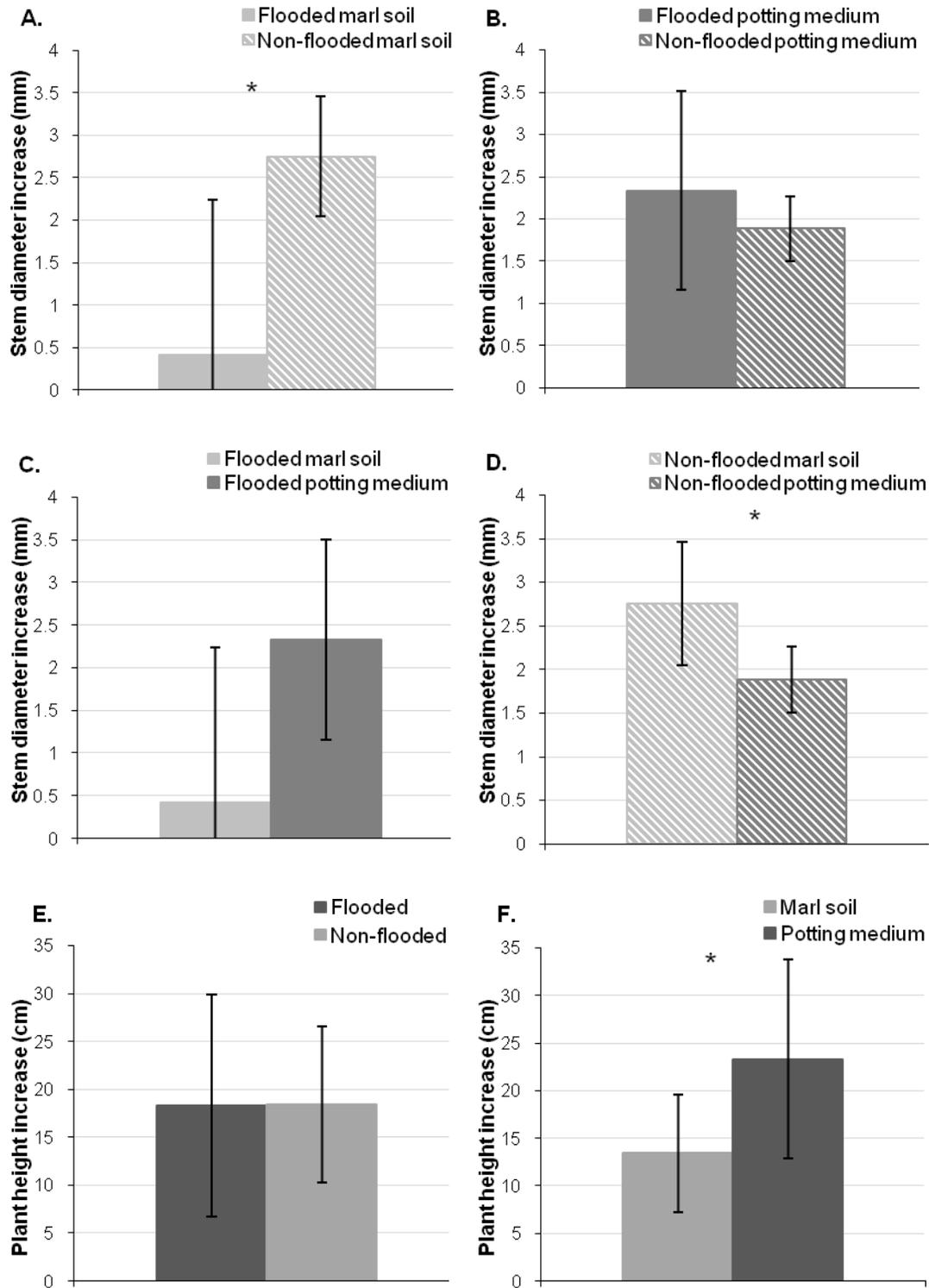


Figure 3-13. Effects of flooding and soil type on (A-D) increase in stem diameter and (E-F) increase in plant height for mahogany plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

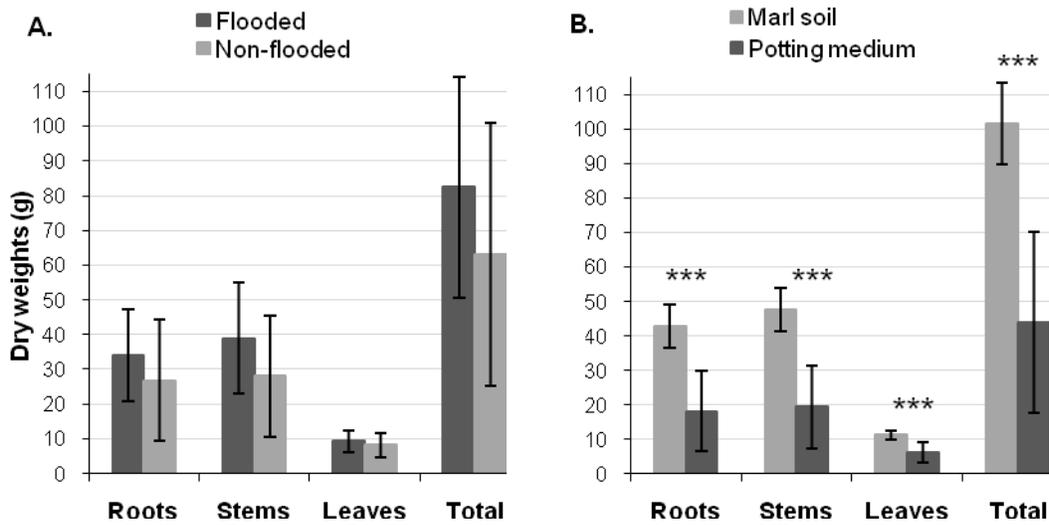


Figure 3-14. Effects of flooding and soil type on dry weights of pond apple plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

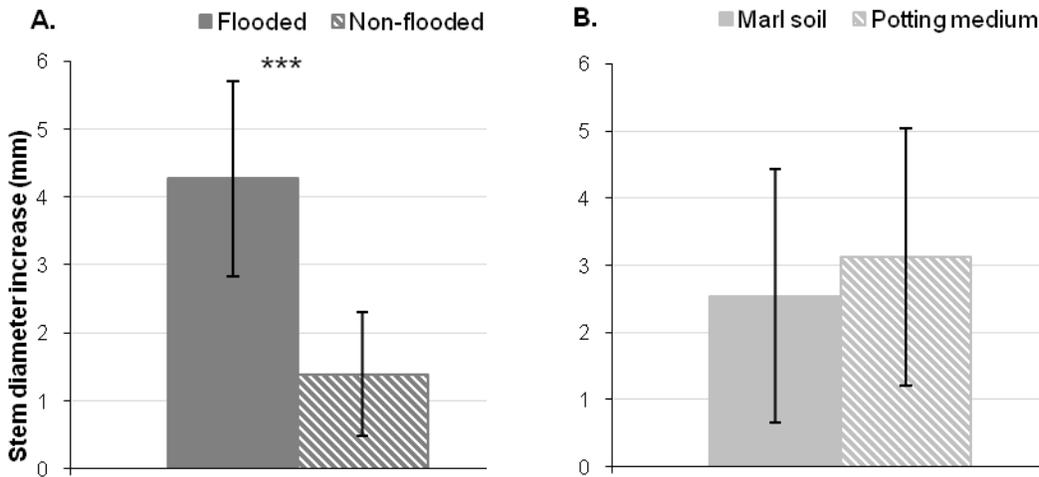


Figure 3-15. Effects of flooding and soil type on increase in stem diameter for pond apple plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

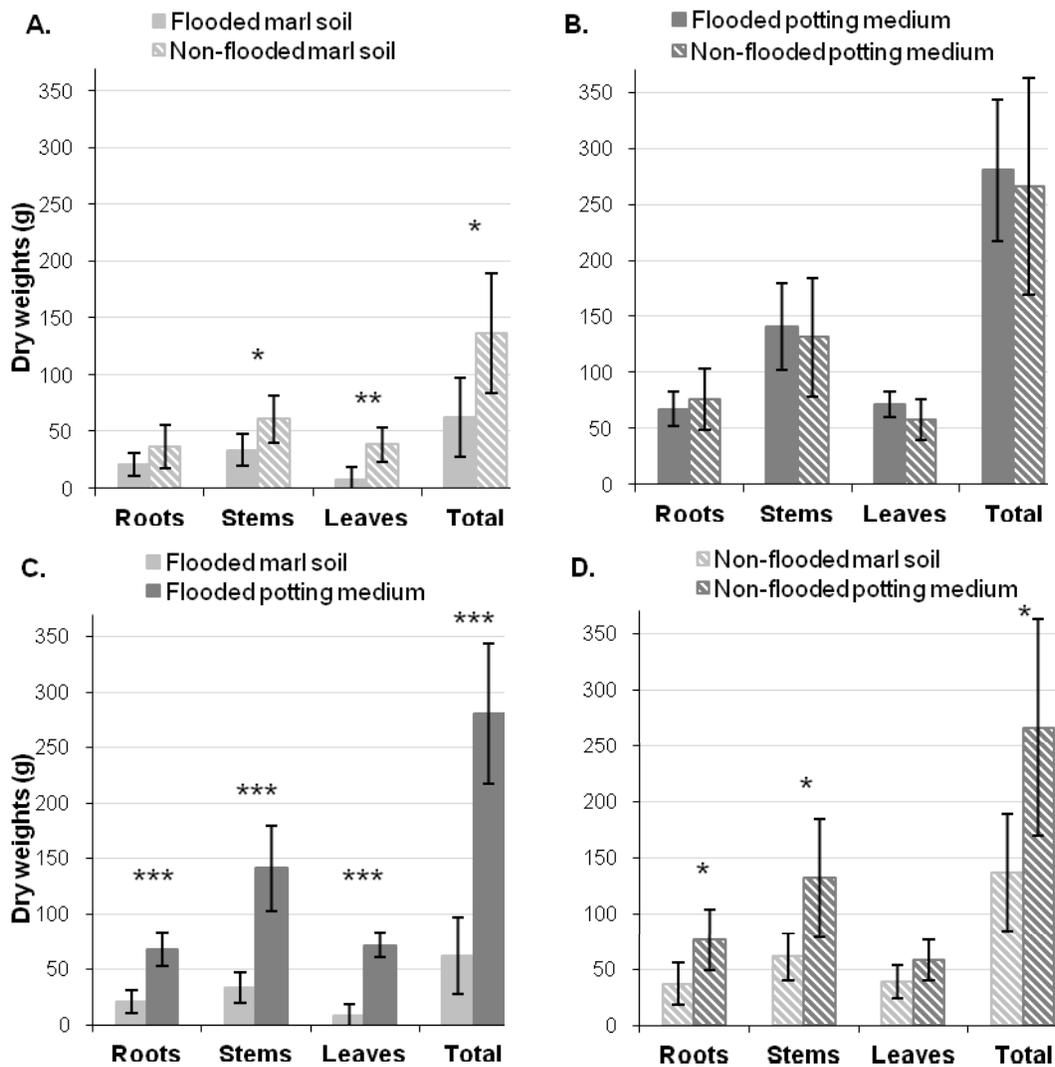


Figure 3-16. Effects of flooding and soil type on dry weights of Surinam cherry plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

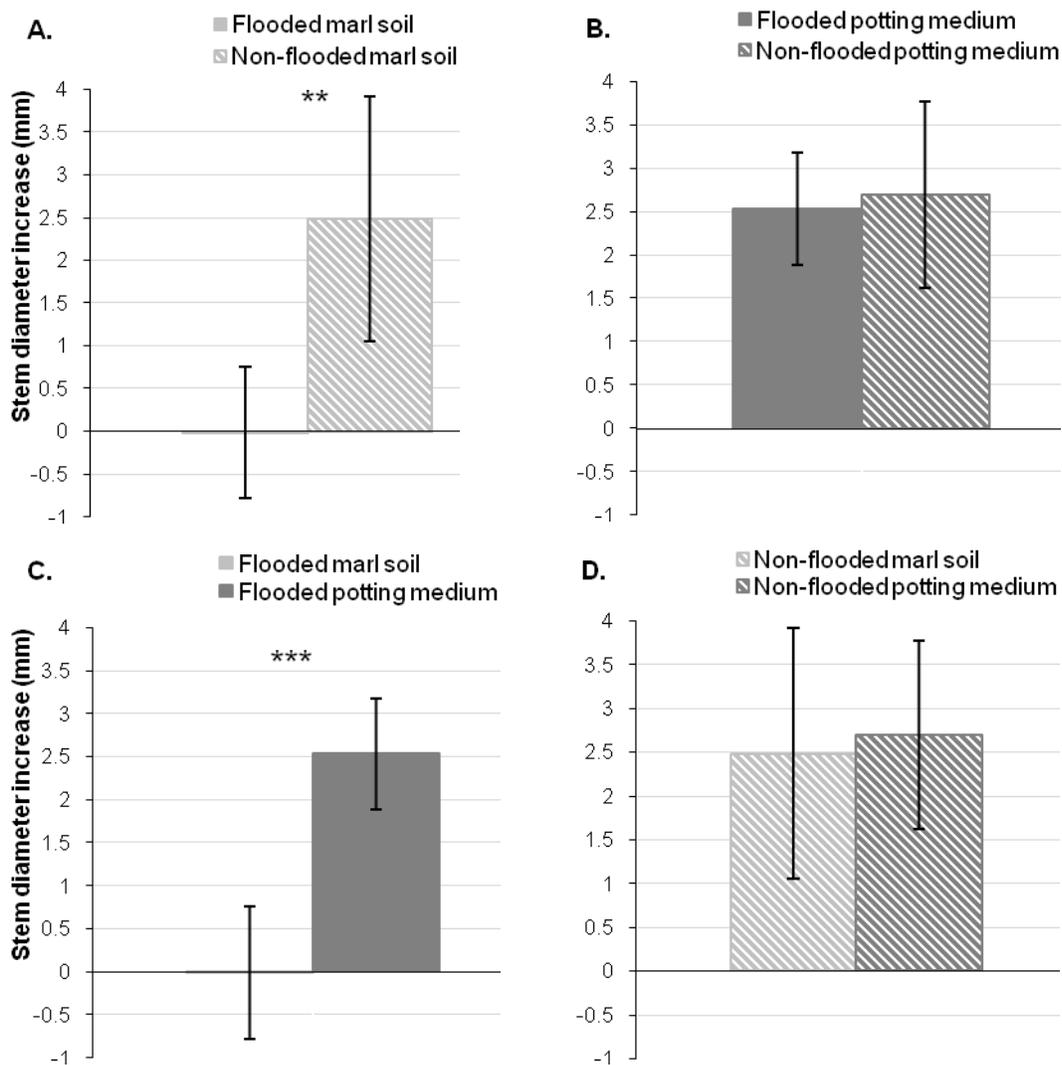


Figure 3-17. Effects of flooding and soil type on increase in stem diameter for Surinam cherry plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

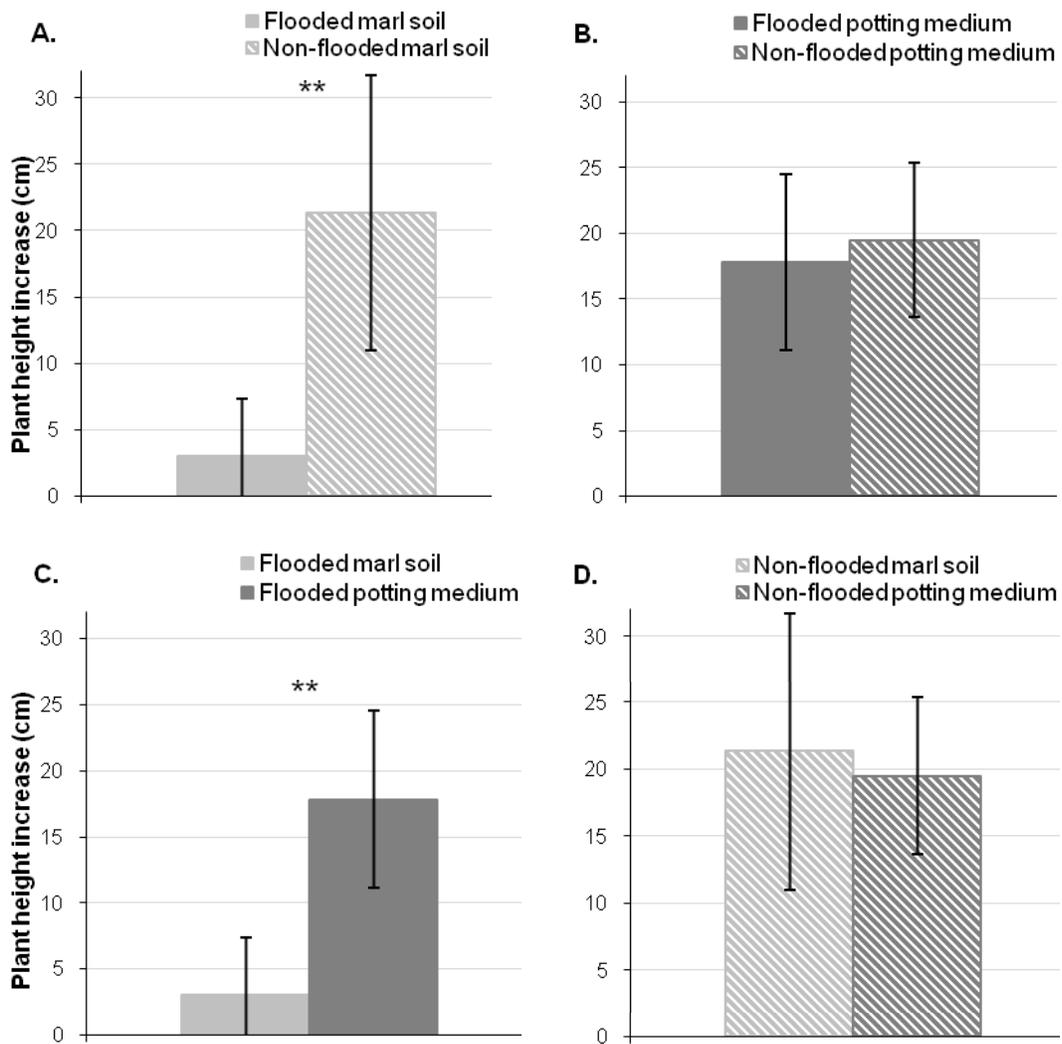


Figure 3-18. Effects of flooding and soil type on increase in plant height for Surinam cherry plants. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

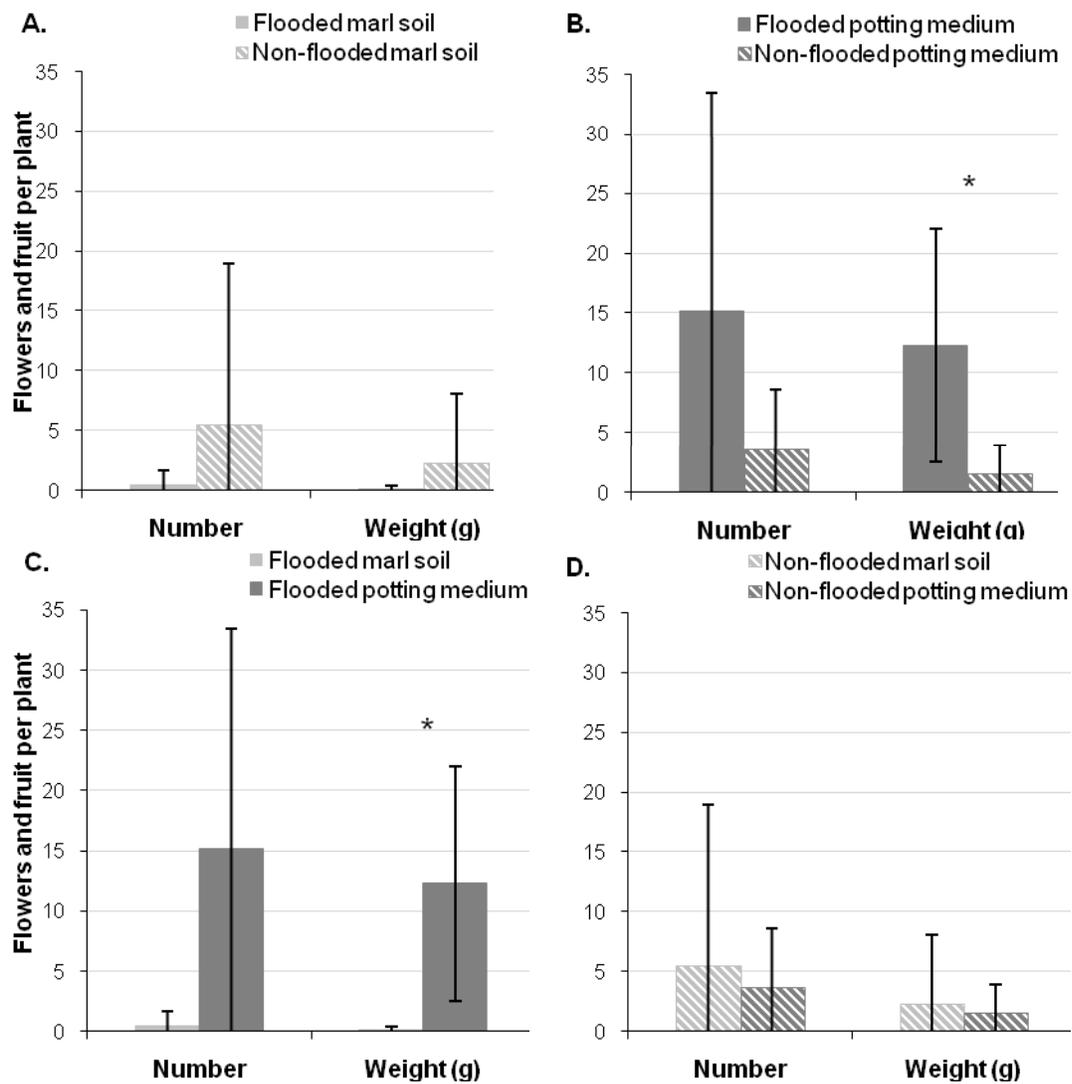


Figure 3-19. Effects of flooding and soil type on the number and weight of inflorescences per Surinam cherry plant. Bars represent means. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , and NS non-significant according to a standard T-test.

CHAPTER 4  
SURVIVAL OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)  
LARVAE ON GREEN BUTTONWOOD TREES IN FLOODED MARL SOIL OR POTTING  
MEDIUM.

**Introduction**

Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), is a serious pest of citrus and sugarcane in its home range of Puerto Rico (Woodruff 1964). Since its introduction to Florida, it has been a continuous problem in citrus (Stanley 1996). Diaprepes root weevil also feeds on a wide host range of ornamental plants and grasses (Simpson et al. 1996) which also commonly provide an avenue of spreading this pest to new locations with the movement of infested plants. Management of this pest on ornamentals is necessary to reduce the risk of spreading this pest to new areas. Currently, it has been reported in Texas (Knapp et al. 2001, Skaria and French 2001) and southern California (Klunk 2005).

Flooding is sometimes used in southern Florida sugarcane fields to control various pests (Cherry 1984; Hall and Cherry 1993). It has been suggested that flooding may also be useful for controlling Diaprepes root weevil larvae in sugarcane fields (Hall and Cherry 1993, Shapiro et al. 1997). Lapointe and Shapiro (1999) examined the effects of soil moisture on Diaprepes root weevil development and survival and found that the poorest larval survival would be expected under flooded conditions. Waterlogged soils are 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).

On the other hand, flooding of the root zone may exacerbate the effects of root feeding by Diaprepes root weevil larvae. Li et al. (2003a) not only found survival of Diaprepes root weevil larvae was significantly higher in previously flooded soil than in non-flooded soil, but flood-damaged seedlings were more susceptible to larval feeding injury than non-flooded seedlings. Similarly, Li et al. (2006) investigated the effects of flooding and soil type on larval survival and

showed that larval survival was higher in sandy soil than in loam soil. Plants flooded for at least 20 d were more stressed and more prone to *Diaprepes* root weevil larval feeding injury after removal of plants from flooding than non-flooded control plants (Li et al. 2006).

The objective of this study was to determine the survival of *Diaprepes* root weevil larvae in flooded marl soils and in a flooded nursery potting medium with green buttonwood (*Conocarpus erectus* L., Combretaceae) serving as a food source. Green buttonwood is widely grown as an ornamental tree or shrub in southern Florida and is native to the tidal swamps of central and south Florida (Watkins and Sheehan 1975, Wunderlin 1998). Green buttonwood has also been reported as a host of both adult and larval *Diaprepes* root weevil (Mannion et al. 2003).

### **Materials and Methods**

The experiment was conducted in the winter and spring of 2007 in Homestead, FL, using *Diaprepes*-infested green buttonwood trees in 4-liter containers filled with either marl soil or potting medium in an outdoor, open site. Plants were obtained from a commercial nursery in December 2006 and replanted (12 January 2007). At the time treatments were initiated, buttonwood plants were approximately 6-12 mo old.

Each plant was repotted into a 4-liter plastic container with half the plants (16 out of 32) in a nursery potting medium (40% Florida peat, 30% pine bark, 20% cypress sawdust, and 10% sand) and the other half (16 plants) in marl soil. The marl soil was obtained from a fallow agricultural field (Homestead, FL). Plants were fertilized (13 February 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 *Diaprepes* root weevil were removed manually. A total of 24 plants were used in this study with two soil treatments (potting medium and marl soil) and two flood treatments (flooded and non-flooded) arranged in a 2 x 2 factorial design with six single-plant replications per treatment. An

additional eight “monitoring” plants (four flooded plants in each soil type) were used for periodic destructive harvest to assess larval survival on one plant in each soil type at each assessment time. The monitoring plants helped determine when to remove plants from flooding and when to harvest and evaluate plants. The monitoring plants were not included in the statistical analysis.

**Larval infestation.** Six weeks after repotting (23 February 2007), each container was infested with 15 *Diaprepes* root weevil larvae raised on an artificial diet and supplied by the Florida Division of Plant Industry, Gainesville. Larvae used to infest plants were fourth through sixth instars (mean fifth instar) based on head capsule width (Quintela et al. 1998). 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.** On day 17 of the experiment (11 March 2007), one-half the number of plants in each soil type (marl soil or potting medium) were flooded by placing the container with each plant into a larger 19-liter plastic container filled with tap water with the level maintained 10 cm above soil surface (24 cm total depth). There were six flooded plants and six non-flooded (control plants) for each soil type. 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 of the monitoring plants. 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). Non-flooded plants were irrigated by overhead sprinkler 30 min once a day until day 29 (23 March), when irrigation times were changed to 30 min twice a day. Flooded plants were irrigated only when not flooded.

**Data collection.** Data collected included soil temperature, numbers of live and dead larvae recovered per plant, and larval head capsule widths. Soil temperature was recorded at 1 h

intervals throughout the experiment with sensors (StowAway® Tidbit® temploggers, Onset Co., Pocasset, MA). The sensors were placed in the soil of three 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 plants were unflooded and harvested, roots were removed from the soil, and the soil 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 using a microscope micrometer with 50 micrometer units per mm and 80 x magnification. All data for percentage of larvae found alive were based on live/total ratios, and not live/found or found/total, because of the presumption that the larvae not found were dead and decomposed.

**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 one treatment combination (flooded potting medium), data for head capsule widths were analyzed with a one-way ANOVA with three 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 (PROC T-TEST and GLM, SAS Institute, Cary, NC, 2003).

## **Results and Discussion**

For plants used for monitoring purposes only, mean percentages of live larvae found in the flooded marl and the flooded potting medium, respectively were 47 and 20 on flood day 4; 87 and 7 on flood day 7; 60 and 7 on flood day 10; and 27 and 0 on flood day 24. The latter sample on day 24 was the first day for which survivorship was less than 30% in both soil types.

Therefore, all flooded test plants were harvested 2 wk after that date, 38 d after flooding treatments commenced, and 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 (Figure 4-1). The mean percentage survival out of 15 original larvae per container was significantly lower in flooded potting medium than in flooded marl soil ( $t = 4.58$ ,  $df = 5$ ,  $P = 0.006$ ) (Figure 4-1c). In non-flooded soil, there were no significant effects of soil type on percentages of live larvae recovered (Figure 4-1d). Flooding significantly reduced the mean percentage of larvae surviving compared to non-flooded conditions in marl soil ( $t = -5.45$ ,  $df = 9$ ,  $P = 0.0004$ ) (Figure 4-1a) and in potting medium ( $t = -6.36$ ,  $df = 5$ ,  $P = 0.0014$ ) (Figure 4-1b).

There were significant differences in head capsule widths between treatments ( $F = 37.3$ ;  $df = 2, 17$ ;  $P < 0.0001$ ). 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 (Figure 4-2). 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 (2000b) examined the effects of constant temperatures on *Diaprepes* root weevil 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 2000b). Mean daily soil temperatures during the treatment period of the present study ranged from 16 to 25°C with monthly averages 17.9 to 21.7°C (Figure 4-3). 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, Chapter 2). In addition, flooding buttonwoods in potting medium significantly reduced photosynthesis, transpiration, and stomatal conductance beginning 1 wk after flooding (Diaz 2005). In another study with buttonwood, flooding did not cause significant differences in photosynthesis, stomatal conductance, or dry weights of plants grown in potting medium (Chapter 3). However, when grown in marl soil, flooding significantly reduced photosynthesis, stomatal conductance, and leaf dry weight compared to non-flooded plants. However, the native tidal-swamp habitat of buttonwood is frequently flooded and on marl soil, but is the environment in which buttonwood evolved and should be best adapted.

Comparing *Diaprepes* root weevil larval survival in flooded marl soil with flooded potting medium was difficult because of the high proportion of larvae not recovered. However, this was expected because larvae quickly decompose when they die. Survival of *Diaprepes* root weevil 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 *Diaprepes* root weevil larvae 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 *Diaprepes* root weevil larvae in flood-tolerant to

moderately flood-sensitive plant species. This regime is especially suggested for plants tolerant or moderately tolerant to flooding. However, it may not be practical to flood plants in containers. When not flooded, soil type such as marl soil or potting medium did not affect *Diaprepes* root weevil larval survival or growth (ie. head capsule widths or instars) 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 to 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 of *Tomarus subtropicus* (Blatchley) (Coleoptera: Scarabaeidae) (Cherry 1984) and wireworm larvae *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae) (Hall and Cherry 1993). Shapiro et al. (1997) exposed *Diaprepes* root weevil 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 wk) on larval mortality. Mean mortality exceeded 90% by 3 wk at 24 and 27°C and by 5 wk at 21°C, but was only 46% after 5 wk at 18°C (Shapiro et al. 1997). In addition, soil pH increased significantly with time and mortality (Shapiro et al. 1997). Li et al. (2004 and 2007b) found that pH increased with increasing flood period, which is related to the reduced oxygen content of flooded soil, and not necessarily the *Diaprepes* root weevil larvae used. In other studies, wireworm larvae (*M. communis*) had 80% mortality after 6 wk of submergence at 27°C (Hall and Cherry 1993), whereas scarab grubs (*T. subtropicus*) had 100% mortality after ~1 wk (5-10 d) of submergence

(Cherry 1984). 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). Flooding may be useful for controlling *Diaprepes* root weevil larvae in sugarcane fields, although only in the summer and fall when floodwater temperatures are close to their maximum (27°C) (Hall and Cherry 1993, Shapiro et al. 1997).

Lapointe and Shapiro (1999) tried to determine levels of soil moisture that optimized production of *Diaprepes* root weevil adults in the laboratory. Optimal survival to pupation occurred at 30-70% soil moisture (Lapointe and Shapiro 1999). About 60-65% of larvae survived to pupation under these ideal moisture conditions (Lapointe and Shapiro 1999). The poorest survival of larvae occurred in low (20%) and in high (80%) soil moisture levels (Lapointe and 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 Shapiro's et al. (1997) study, larvae of *Diaprepes* root weevil were exposed directly to flooding. However, Li et al. (2003a, 2006, 2007b) and Diaz (2005) unflooded plants before infestation with larvae. Although, Diaz's (2005) study and the present study were both conducted in Homestead, FL, Diaz (2005) infested soil with 40 fifth to sixth instar larvae per container in two infestations of 20 larvae each that were 1 mo apart. Also Diaz unflooded plants 1 d before they were initially infested with larvae so both stresses were not simultaneous. There were no significant differences in larval recovery 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.

Li et al. (2003a, 2006, 2007b) also examined the effects of *Diaprepes* root weevil larval infestation and flooding on plant growth, larval survival, and other characteristics in a greenhouse environment. Flooding also occurred before larval infestation so both stresses were not simultaneous (Li et al. 2003a, 2006, 2007b). They used seedlings of Swingle citrumelo (*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.) and one other citrus rootstock. Pot sizes were small (5 cm<sup>3</sup>) as were plants (seedlings) and sizes and numbers of larvae (neonates, 0 or 5 per pot). They also had similar flood periods (0-40 d), larval feeding periods (40-56 d), and pots in trays were flooded to 3 cm above soil line. For Li et al. (2003a), survival of *Diaprepes* root weevil 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 larval survival averaged 25% higher in sandy soil than in loam soil in plants previously flooded for 20 d.

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. (2006) found that plants flooded for at least 20 d were more stressed and more prone to *Diaprepes* root weevil larval feeding injury after removal of plants from flooding than non-flooded control plants. Their results suggest that avoidance of flooding and early control of *Diaprepes* root weevil larvae may help protect young plants. Li et al. (2007b) studied the effects of flooding and soil pH on the growth and survival of *Diaprepes* root weevil larvae. When not limed, flooding increased the average soil pH up to 0.3 units for the longest flooded (40-d) treatment (Li et al. 2007b). In another study by Li et al.

(2007b), citrus seedlings flooded 40 d had the lowest larval survival rates and larval weights compared to seedlings flooded for shorter flood durations, which may reflect higher soil pH at longer flood durations. Here, larval survival and growth were significantly decreased by pre-applied flooding (Li et al. 2007b). 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 significantly 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. 2007b). Other factors such as soil type, compaction, bulk density, and water content may also influence larval survival and growth (Riis and Esbjerg 1998, Rogers et al. 2000, Li et al. 2007b). 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 *Diaprepes* root weevil (Li et al. 2007b). Flooding was also recommended as a possible control method in citrus (Li et al. 2007b). Flooding may hence reduce larval survival while plants are flooded. However, depending on soil pH, water-stressed plants may be more susceptible to *Diaprepes* root weevil larval feeding when un-flooded 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. (2007b), 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 *Diaprepes* root weevil survival. Soil pH in the range of marl soil was not investigated in the foregoing studies. Perhaps marl soil offers a pH range more favorable to larval survival and growth than our potting medium. This may help account for higher larval survival rates in flooded marl soil than in flooded potting medium found in the present study. 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 *Diaprepes* root weevil larvae in the pH range of marl soil (7.4-8.4) compared to 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. (2007b).

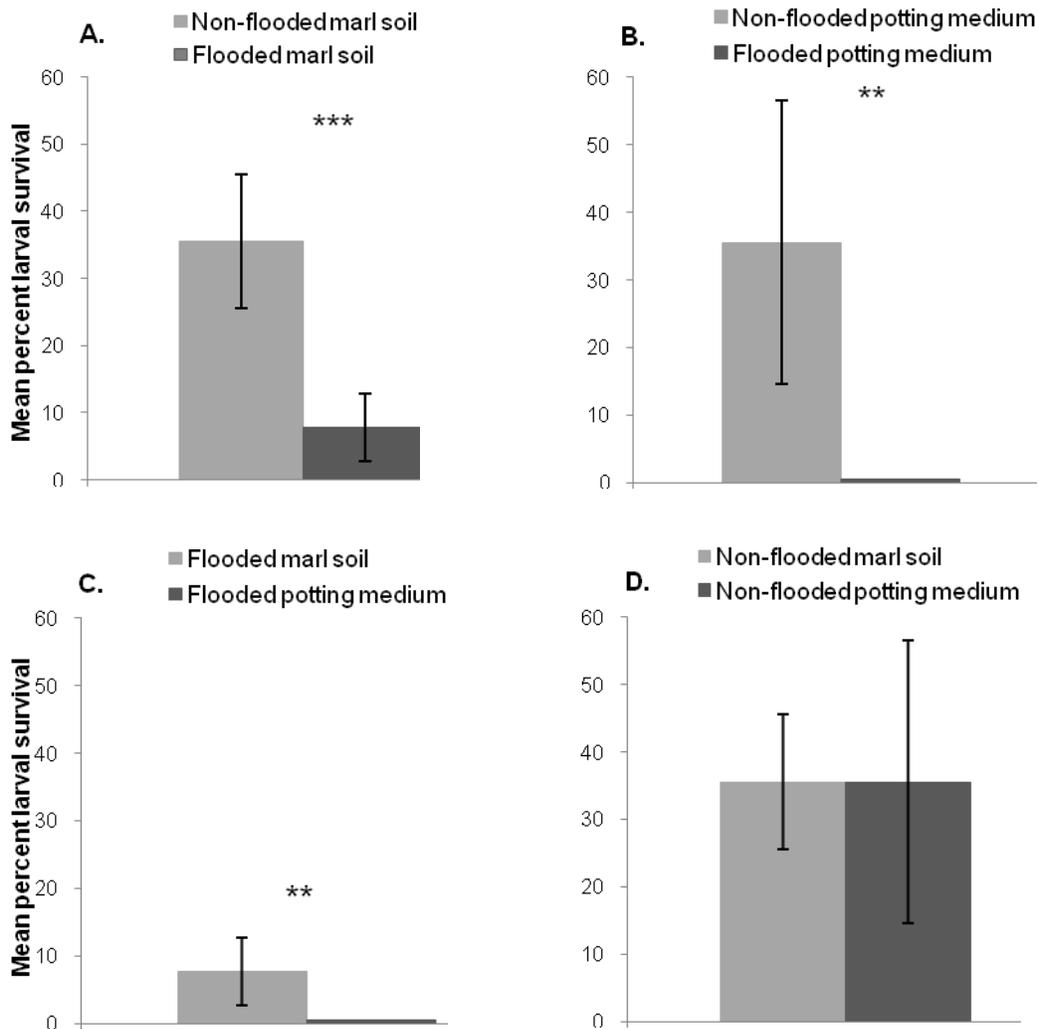


Figure 4-1. Effects of flooding and soil type on percentages of 15 *Diaprepes* root weevil larvae added to each container that were found live at harvest based on ratios of live/total. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$  according to a standard T-test.

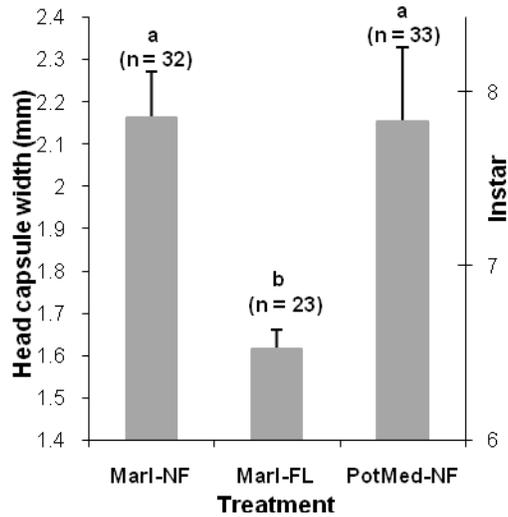


Figure 4-2. Mean head capsule widths and instars ( $\pm$  SD) of *Diaprepes* root weevil larvae found at harvest. Values for live and dead larvae were pooled. N is the total number of larvae found in each treatment. X-axis symbols are Marl-NF (non-flooded marl soil), Marl-FL (flooded marl soil), and PotMed-NF (non-flooded potting medium).

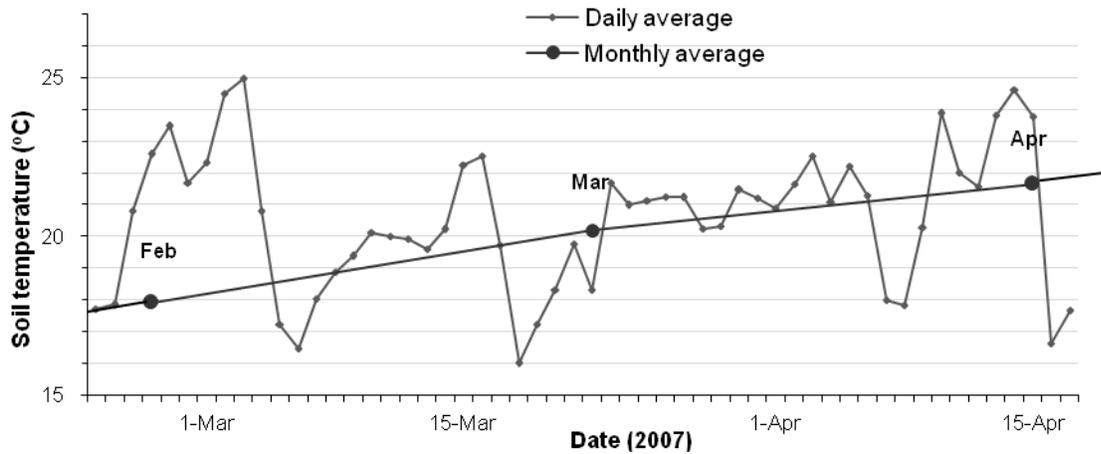


Figure 4-3. Soil temperature during the experiment. Each point is the average of three temperature sensors each buried 6 cm below the soil surface in potted plants not used in the experiment but held under the same environmental conditions and with the same media as in the experiment.

CHAPTER 5  
EFFECTS OF HERBIVORY BY *DIAPREPES ABBREVIATUS* (L.) (COLEOPTERA:  
CURCULIONIDAE) LARVAE AND FLOODING ON LEAF GAS EXCHANGE AND  
GROWTH OF GREEN BUTTONWOOD AND SWINGLE CITRUMELO PLANTS

**Introduction**

*Diaprepes abbreviatus* L. (Coleoptera: Curculionidae: Entiminae), commonly called Diaprepes root weevil, is an important and economic pest of citrus and other horticultural crops (Simpson et al. 1996). In the United States, it is found in Florida, Texas (Knapp et al. 2001, Skaria and French 2001), and southern California (Klunk 2005). In Florida, Diaprepes root weevil is found in 23 counties in the south and central parts of the state (Anonymous 1996, Peña 1997, Weissling et al. 2004).

Diaprepes root weevil 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. 2000b, Mannion et al. 2003, Godfrey et al. 2006). Not all host plants support all stages of Diaprepes root weevil, however, many economically important plants support all stages of the weevil from egg to adult, such as sweet potato, *Ipomoea batatas* (L.) Lam., and (green) buttonwood (*Conocarpus erectus* L.) (Simpson et al. 1996). In a survey of ornamental plant nurseries in southern Florida, egg masses, feeding damage, and adult weevils were common (Mannion et al. 2003). Diaprepes root weevil larvae feed on the roots of their host plants starting with small roots when they are young and move to larger, lateral and main roots as they mature (McCoy et al. 2002). Roots may be girdled causing severe root damage or death which impacts the ability of the plant to take up nutrients (McCoy et al. 2002). This type of damage often kills small citrus trees (Wolcott 1936, 1948; Quintela et al. 1998).

Tropical agriculture in southern Florida, particularly between Biscayne and Everglades National Parks, tends to occur in low-lying areas with high water tables, which are prone to

periodic flooding (Schaffer 1998). Many of the woody ornamental plant nurseries which include green buttonwood are grown in pots in a standard potting medium or in the field in these flood-prone areas. Green buttonwood is native to the tidal swamps of central and south Florida (Watkins and Sheehan 1975, Wunderlin 1998) so it tolerates flooding well, though it also thrives in non-flooded, moderately moist soils, which are common for landscape plants.

Considerable research has examined combinations of *Diaprepes* root weevil infestation, rootstock variety, soil flooding, soil type, and pH effects on leaf gas exchange and growth of citrus rootstocks and/or *Diaprepes* root weevil larval survival (Li et al. 2003a, 2004, 2006, 2007b). However, there is no published research on interactions between *Diaprepes* root weevil larval feeding and soil flooding that simultaneously occurs on woody ornamental plants including buttonwood. At least half the Rutaceous fruit trees in commerce in Florida are grown on Swingle citrumelo or Swingle (*Poncirus trifoliata* (L.) Raf. x *Citrus paradisi* Macf.) (Rutaceae) rootstock (Auscitrus 2004, F. Davies pers. comm. 2008). Swingle has moderate to low flood tolerance, though it may be more flood-tolerant than another popular rootstock, Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb., Rutaceae) (Auscitrus 2004, Li et al. 2004).

The objectives of this study were to determine interactions and effects of short-term (continuous) and cyclically flooded soil on damage from *Diaprepes* root weevil larvae to green buttonwood and Swingle citrumelo plants. An additional objective was to compare effects on green buttonwood with those of Swingle citrumelo, a species known to be sensitive to flooding and larval infestation. The hypothesis was that leaf gas exchange, growth, and *Diaprepes* root weevil larval survival rates are lowered more by short-term than by cyclical flooding and that flooding exacerbates damage caused by *Diaprepes* root weevil.

## Materials and Methods

The experiment was conducted in Fall 2008 in Homestead, FL with plants in 11-liter plastic containers placed on ground cloth in an outdoor site exposed to full sun. The two plant species used in these studies were green buttonwood and Swingle citrumelo.

**Plant material.** Green buttonwood and Swingle citrumelo trees (obtained from a commercial nursery) were approximately 2 years old and 1 year old, respectively, when treatments were initiated. Initial plant heights for green buttonwood 6 d before infestation in short-term flooded and cyclically flooded treatments, respectively, were  $126 \pm 13$  cm and  $122 \pm 11$  cm (mean  $\pm$  SD). For Swingle citrumelo, initial plant heights for short-term flooded and cyclically flooded treatments, respectively, were  $141 \pm 17$  cm and  $132 \pm 14$  cm. The potting medium for green buttonwood and Swingle citrumelo was Fafard mix 2 (70% Canadian peatmoss (porous, fluffy), 20% perlite, and 10% vermiculite).

**Flooding treatments.** For each plant species, plants were exposed to short-term or cyclical flooding by submerging 11-L containers with the plants into 19-L buckets filled with tap water with water levels maintained at 10 cm above the soil surface. Cyclical flooding involved alternating periods of flooding with dry periods. For plants in short-term or cyclical flooding treatments, one-half of the total number of plants were left unflooded as controls. Thus, for each test with short-term or cyclical flooding, there were two test plants per replication that were flooded (one infested and one non-infested) and two test plants per replication that were non-flooded (one infested and the other non-infested). Short-term flooded plants were flooded 2 d consecutively in a single flood cycle followed by 5 d without flooding. Cyclically flooded plants were flooded for 2 d followed by a 5-d “drying period” resulting in a 7-d cycle that was repeated 3 times. Hence tests with short-term and cyclically flooded treatments were performed concurrently during the first 13 d after infestation (31 Oct – 13 Nov), but beyond this point, they

were different. Total days flooded for short-term and cyclic flooding was 2 d and 6 d, respectively. All plants (flooded and non-flooded) were irrigated by overhead sprinkler for 30 min twice per day.

**Larval infestation.** For each plant species, one-half the short-term flooded plants and one-half the cyclically flooded plants were infested with *Diaprepes* root weevil larvae on day 4 (31 October 2008). Each of 40 plant containers was infested with 20 larvae raised on an artificial diet supplied by Florida Division of Plant Industry, Gainesville. Larvae used to infest plants were about 28 d old and averaged  $1.15 \pm 0.14$  mm in head capsule width, 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 in the soil, 3-10 cm deep, 4-8 cm from the stem, and 3 cm apart. 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 two air sensors and two soil sensors (StowAway<sup>®</sup> Tidbit<sup>®</sup> temploggers, Onset Co., Pocasset, MA placed in the soil (soil temperature) and canopies (air temperature) of plants not included in the experiment but in similar potting media, the same container type, and located next to the test plants. The two air sensors were each placed in canopies 66-71 cm above the soil surface. The two 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.

Soil redox potential was measured with a metallic combination electrode (Accumet Model 13-620-115, Fisher Scientific, Pittsburgh, PA) attached to a portable volt meter (Accumet model AP62, Fisher Scientific, Pittsburgh, PA). Soil redox potential was measured daily during each flood period for two flooded, infested plants and two flooded, non-infested plants. The four measurements were averaged to calculate mean redox potential for short-term flooded or cyclically flooded treatments for each plant species. Measurements of redox potential were

made by inserting the electrode into a polyvinyl chloride (PVC) pipe inserted into the soil 2 cm from the edge of the pot. Soil pH was measured for all flooded plants two times per flood period with a platinum combination electrode attached to the same portable volt meter used for redox measurements. For each flood cycle, the first pH measurement was on the same day plants were flooded and the second was 2 d later on the day they were unflooded. The exception was Swingle citrumelo plants during the first flood cycle, where pH was measured 1 d after plants were flooded, and again 1 d later when plants were unflooded.

**Plant data collection.** Net CO<sub>2</sub> assimilation, transpiration and stomatal conductance were measured on two fully expanded, recently mature leaves or leaflets per plant with a CIRAS-2 portable gas analyzer (PP Systems, Amesbury, MA). Values of the two 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. All leaf gas exchange measurements were made between 8:30 and 16:45 h. During gas exchange measurements, the photosynthetic photon flux was maintained at 1000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  with a halogen lamp attached to the leaf cuvette and the reference CO<sub>2</sub> concentration in the cuvette was kept constant at 375  $\mu\text{mol mol}^{-1}$ . Swingle citrumelo has compound leaves with three leaflets per leaf, and the terminal leaflet is larger than lateral leaflets (Hutchison 1974, Wunderlin 1998), however, buttonwood has simple leaves. All leaf gas exchange measurements for Swingle citrumelo were taken on the large terminal leaflets.

Plant height was measured to the top edge of the highest leaf above the soil surface prior to infestation and initiation of flooding. Stem diameter was measured 10 cm above soil surface prior to infestation and initiation of flooding, and for plants with multiple stems at this height, diameter of the largest stem was recorded. Between initial measurements of plant height or stem diameter and harvest, 18-19 d lapsed for short-term flooded plants, which was presumed

insufficient to show a measurable difference in plant height or stem diameter. However, for cyclically flooded plants, 33-34 d lapsed between initial and final measurement of stem diameter and plant height, which was believed sufficient to show a difference in these variables. Hence, the second and final measurement of stem diameter and plant height was not made for short-term flooded plants but was performed for cyclically flooded plants 4 d before harvest began, or 28 November 2008.

All plants in the short-term flooded test were harvested from days 17 to 18 (13-14 November 2008), or 13-14 d after infesting, 7-8 d after initially flooding plants, and 5-6 d after unflooding plants. Plants in the cyclically flooded test were harvested from days 36 to 37 (2-3 December 2008), or 32-33 d after infesting, 26-27 d after initially flooding plants, and 10-11 d after the final unflooding of plants. At harvest, stems were cut off 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 were then 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 at 75°C for 5 d to a constant weight and dry weights were determined. For buttonwoods, leaf dry weight included leaf blades and petioles, and for Swingle citrumelo, leaf dry weight included leaflets, petiolules, and petioles. Root damage was evaluated for infested Swingle citrumelo plants using a visual rating system in which 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 larval herbivory generally did not significantly affecting leaf gas exchange or growth in this species, unlike with Swingle citrumelo.

**Experimental design and statistical analysis.** For each plant species, there were four treatments in each short-term and cyclically flooded test: 1) flooded infested, 2) flooded non-infested, 3) non-flooded infested, and 4) non-flooded non-infested in a 2 (flooding treatment) x 2 (infestation treatment) factorial design. For each plant species, there were five single-plant replications per treatment. A two-way analyses of variance (ANOVA) was used to determine flooding and infestation interactions. Standard T-tests were used to compare flooded versus non-flooded and infested versus 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 (PROC T-TEST and GLM, SAS Institute, Cary, NC, 2003).

## **Results**

**Air and soil temperature, soil redox potential, and floodwater pH.** During the test period, mean daily temperatures ranged from 13.0°C to 24.3°C for the air and 16.8°C to 27.7°C in the soil with monthly averages from 15.8°C to 22.8°C for air and 19.8°C to 25.1°C in the soil (Figure 5-1a). Soil redox potential for short-term flooded green buttonwood plants ranged from +195 mV to +140 mV, and for short-term flooded Swingle citrumelo plants, from +376 mV to +182 mV (Figure 5-1b). For cyclically flooded plants, soil redox potential during the first flood period ranged from +193 mV to +162 mV for green buttonwood and from +378 mV to +165 mV for Swingle citrumelo plants (Figure 5-1b). During the second flood period, soil redox potential ranged from +597 mV to +166 mV for green buttonwood and from +498 mV to +174 mV for Swingle citrumelo (Figure 5-1b). During the third (final) flood period, soil redox potential ranged from +508 mV to +153 mV for green buttonwood and from +523 mV to +193 mV for Swingle citrumelo (Figure 5-1b). For combined short-term and cyclically flooded green buttonwood and Swingle citrumelo plants, pH of floodwater was 7.21-7.78.

**Leaf gas exchange.** For Swingle citrumelo or green buttonwood, there were no significant flooding and larval infestation interactions except for net CO<sub>2</sub> assimilation for short-term flooded green buttonwood on the first measurement date. To test responses to flooding, treatments for larval infestation were pooled and to test responses to larval infestation, flooding treatments were pooled. For cyclically flooded green buttonwood, net CO<sub>2</sub> assimilation ( $t = -2.21$ ,  $df = 13$ ,  $P = 0.0451$ ) and stomatal conductance ( $t = -2.70$ ,  $df = 17$ ,  $P = 0.0154$ ) were significantly higher for non-flooded than flooded plants on the fifth (final) measurement date (Figure 5-2). However, there were no significant differences between infested and non-infested, cyclically flooded green buttonwood plants in net CO<sub>2</sub> assimilation (range 1.7-11.9  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) or stomatal conductance (range 24-150  $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). There were also no significant differences between infested and non-infested or flooded and non-flooded, short-term flooded green buttonwoods in net CO<sub>2</sub> assimilation (range 3.5-12.5  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) or stomatal conductance (range 39-184  $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Transpiration was significantly higher for non-infested than infested, short-term flooded green buttonwood plants on the second measurement date, after infestation but before flooding ( $t = -2.50$ ,  $df = 15$ ,  $P = 0.0245$ ) (Figure 5-3a). Transpiration for cyclically flooded green buttonwood was significantly higher for non-flooded than flooded plants on the third measurement date, after infestation and the first flood cycle, but before the second flood cycle ( $t = -2.24$ ,  $df = 18$ ,  $P = 0.0384$ ) (Figure 5-3b). There were no significant differences in transpiration between short-term flooded and non-flooded green buttonwood plants (range 1.0-4.4  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) nor between infested and non-infested, cyclically flooded plants (range 0.75-4.38  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ).

There were no significant differences in net CO<sub>2</sub> assimilation between flooded and non-flooded or infested and non-infested Swingle citrumelo plants in either short-term treatments (range 2.6-11.1  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) or cyclical treatments (range 1.7-10.5  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). For

cyclically flooded Swingle citrumelo plants, transpiration ( $t = -2.64$ ,  $df = 17$ ,  $P = 0.0170$ ) and stomatal conductance ( $t = -3.10$ ,  $df = 17$ ,  $P = 0.0064$ ) were significantly higher for non-infested than infested plants on the fifth (final) measurement date (Figure 5-4a and b). However, there were no other significant differences between flooded and non-flooded or infested and non-infested Swingle citrumelo plants in transpiration or stomatal conductance for short-term flooding (ranges in transpiration 1.6-3.9 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> and stomatal conductance 43-143 mmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) or cyclical flooding (ranges in transpiration 1.3-3.5 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> and stomatal conductance 32-130 mmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>).

**Plant growth.** There were no significant flooding and infestation interactions for stem diameter and plant height of either plant species. For tissue dry weights, the only significant flooding and larval infestation interaction was for root dry weights of cyclically flooded Swingle citrumelo ( $F = 4.87$ ;  $df = 1$ ;  $P = 0.0422$ ). Therefore, dry weights of cyclically flooded Swingle citrumelo were not pooled for analysis, whereas all other dry weights, stem diameter, and plant height data were pooled for each plant species.

There were no significant effects of cyclical flooding or larval infestation on increase in stem diameter or plant height for either buttonwood (stem diameter range -0.64 to 2.05 mm, plant height range -2.9 to 7.3 cm) or Swingle citrumelo (stem diameter range -1.19 to 0.88 mm, plant height range -0.83 to 2.82 cm). There were also no significant effects of flooding or larval infestation on root, stem, leaf, or total dry weight for short-term buttonwood (ranges for roots 46-100 g, stems 91-178 g, leaves 49-106 g, and total 200-369 g), short-term Swingle (ranges for roots 26-46 g, stems 69-111 g, leaves 10-20 g, and total 109-172 g), cyclical 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 weight for cyclical Swingle (ranges for stems 65-112 g, leaves 8.7-20.5 g, and total 102-173 g). However, root dry weight for cyclical Swingle citrumelo was significantly higher for

flooded, infested than for non-flooded, infested plants ( $t = 3.16$ ,  $df = 5$ ,  $P = 0.0234$ ) (Figure 5-5a). Root dry weight of Swingle citrumelo was also significantly higher for non-flooded, non-infested than for non-flooded, infested plants in the cyclical flooding test ( $t = -3.65$ ,  $df = 8$ ,  $P = 0.0066$ ) (Figure 5-5b).

For green buttonwood, there was no significant effect of short-term or cyclical flooding on percent larval survival or head capsule width of recovered larvae (Figure 5-6a and b). For Swingle citrumelo, there was also no significant effect of short-term flooding on percent larval survival or head capsule width of recovered larvae (Figure 5-6a and b). However, cyclically flooded Swingle citrumelo plants had significantly reduced percent survival ( $t = -2.44$ ,  $df = 6$ ,  $P = 0.0488$ ) and head capsule width ( $t = -4.32$ ,  $df = 5$ ,  $P = 0.0064$ ) of *Diaprepes* root weevil larvae than non-flooded plants (Figure 5-6a and b). Root damage rating was significantly higher for non-flooded than cyclically flooded Swingle citrumelo plants ( $t = -3.09$ ,  $df = 5$ ,  $P = 0.0269$ ) (Figure 5-7), but there was no significant difference between non-flooded and short-term flooded plants.

## Discussion

In the present study, the average monthly soil temperatures were 0.9 to 6.2°C below the ideal developmental temperature of 26°C for *Diaprepes* root weevil and up to 2.2°C below the ideal survival temperatures of 22 to 26°C for this weevil (Lapointe 2000b). Lapointe (2000b) found the highest larval 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. In that study, increasing temperatures above 26°C slowed development and decreased survival rates (Lapointe 2000b). 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.

A measure of oxygen abundance in soil is the redox potential. 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<sub>2</sub> depletion in the soil and other factors such as soil pH (Schaffer et al. 1992). Well-drained, well oxygenated soils have redox potentials of +300 mV or more, whereas flooded soils have redox potentials of +200 mV or less (Ponnamperuma 1972, 1984). Redox potentials measured for short-term flooded and cyclically flooded soils in this study indicated that while there was a decline in soil O<sub>2</sub> content during flooding periods, soils did not become very depleted of oxygen. This may have been caused by the short duration of flooding periods, which included a single 2-d flood period for short-term flooded soil and three 2-d flood periods each separated by 5 d without flooding for cyclically flooded soil. All mean soil redox potentials for flooded plants were aerobic (above +300 mV) on the first day of every flood cycle except for short-term flooded and cyclically flooded green buttonwoods on the first flood cycle. On the second day of each flood cycle, all means for short-term and cyclically flooded green buttonwood and Swingle citrumelo plants were cumulatively between +193 mV and +281 mV, and they were between +140 and +193 on the third (final) day. All mean soil redox potentials hence ranged from +140 to +597 mV, and were anaerobic to aerobic (Ponnamperuma 1972, 1984).

Duration of flooding and larval infestation periods in the present study were relatively short compared to previous studies, where green buttonwood was exposed to 14-35 d of flooding followed by 90 d infestation (Diaz 2005), and Swingle citrumelo was exposed to 20-40 d of flooding followed by 40-56 d of larval infestation (Li et al. 2004, 2007b). The shorter duration of flooding and larval infestation in the present study may help account for fewer significant differences in leaf gas exchange, growth, and larval recovery compared to similar tests with flooding and/or *Diaprepes* root weevil infestation of the same plant species. However, for short-

term flooded green buttonwood plants in the present study, fourth to sixth instar larvae caused sufficient feeding pressure to significantly reduce transpiration in infested compared to non-infested plants on one measurement 5 d after infestation. This was the only significant difference in leaf gas exchange, growth, or larval recovery noted for short-term flooded green buttonwood or Swingle citrumelo trees.

For cyclically flooded green buttonwood, transpiration was significantly higher for non-flooded than flooded plants on the third measurement, and net CO<sub>2</sub> assimilation and stomatal conductance were significantly higher for non-flooded than flooded plants on the fifth (final) measurement. Transpiration and stomatal conductance for cyclically flooded Swingle citrumelo plants were each significantly higher for non-infested than infested plants on the fifth (final) measurement. Most of these significant differences in leaf gas exchange for cyclically flooded green buttonwood and Swingle citrumelo plants occurred on the final measurement, indicating effects of flooding and insects were cumulative.

There were no significant differences between flooded and non-flooded green buttonwood plants in increase of stem diameter and no adventitious roots were observed, but net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance all were significantly lower for flooded than non-flooded plants on at least one measurement. In another study (Chapter 3), when green buttonwoods in potting medium were flooded for a longer duration (23 d), stem diameter increase was significantly greater in flooded than non-flooded plants. Also, adventitious roots and hypertrophic lenticels were seen on flooded plants, but there were no significant differences in net CO<sub>2</sub> assimilation or stomatal conductance. Increased stem diameter, hypertrophic stem lenticels, and adventitious roots in flooded green buttonwood combined with almost no significant differences in net CO<sub>2</sub> assimilation or stomatal conductance were also observed in another study (Chapter 6). Effects of flooding may be different when longer flood periods are

applied than those of the present study: for example, over 3 wk of short-term flooding, which is long enough for green buttonwoods to develop thicker stems, adventitious roots, and hypertrophic lenticels (Chapters 3 and 6). In addition, Swingle citrumelo has moderate to low flood tolerance (Auscitrus 2004), whereas green buttonwood is relatively flood tolerant. The short flood durations in the present study may have not allowed sufficient time for green buttonwood to flood-adapt, hence it was less flood tolerant than Swingle citrumelo; however, flooding reduced infestation more effectively on Swingle than on green buttonwood.

Green buttonwood gas exchange and growth was strongly reduced by *Diaprepes* root weevil larval feeding in other studies with multiple plant species (Diaz 2005, Diaz et al. 2006, Chapter 2). However, Swingle citrumelo was even more susceptible than green buttonwood to larval herbivory in the present study. For cyclically flooded Swingle citrumelo plants, three 2-d flood periods significantly reduced larval growth and survival. However, these 2-d flood periods did not significantly affect larval growth or survival in cyclically flooded green buttonwood or in short-term flooded green buttonwood or Swingle Citrumelo plants. Therefore, three 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 *Diaprepes* root weevil larvae without affecting leaf gas exchange or growth of trees on Swingle citrumelo rootstock. However, these short-term flood periods would probably not benefit green buttonwoods.

In the present study, root dry weight of cyclically flooded Swingle citrumelo plants was significantly greater for flooded infested than for non-flooded infested plants and significantly greater for non-flooded non-infested than non-flooded infested plants. This suggests that larval herbivory decreased root dry weight more in non-flooded than in flooded plants. This is supported by significantly higher root damage ratings for non-flooded than cyclically flooded Swingle citrumelo root balls. Greater root dry weight for flooded infested than non-flooded

infested Swingle citrumelo plants suggests that flooding may reduce effects of *Diaprepes* root weevil larval herbivory. Both head capsule width and percent survival of recovered larvae were significantly greater for non-flooded than cyclically flooded Swingle citrumelo plants. Reduced larval feeding and growth on cyclically flooded Swingle citrumelo plants may have allowed for increased root dry weight in flooded over non-flooded plants, which, unlike short-term flooded plants, seemed to have sufficient time for these differences to become apparent.

In the present study, all data for percentage of larvae found alive were based on live/total ratios, and not live/found or found/total, because of the presumption that the larvae not found were dead and decomposed. Previous results suggest that flooding for at least 3 d for plants grown in potting medium, would help control *Diaprepes* root weevil larvae in flood-tolerant to moderately flood-sensitive plant species (Chapter 4).

Shapiro et al. (1997) found that mean mortality of flooded unfed *Diaprepes* root weevil larvae exceeded 90% by 3 wk at 24 and 27°C, and by 5 wk at 21°C, but was only 46% after 5 wk at 18°C (Shapiro et al. 1997). Similarly, larvae of the wireworm *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae) had 80% mortality after 6 wk of submergence at 27°C (Hall and Cherry 1993), whereas scarab grubs *Tomarus subtropicus* (Blatchley) (Coleoptera: Scarabaeidae) had 100% mortality after ~1 wk (5-10 d) of submergence (Cherry 1984). Flooding is sometimes used in southern Florida sugarcane fields to control the foregoing larvae, *T. subtropicus* (Cherry 1984) and *M. communis* (Hall and Cherry 1993). Flooding may be useful for controlling *Diaprepes* root weevil larvae in sugarcane fields in the summer and fall when floodwater temperatures are close to their maximum (27°C) (Hall and Cherry 1993, Shapiro et al. 1997). Hence, flooding potentially may help control *Diaprepes* root weevil larvae in flood-tolerant ornamental plants including green buttonwood.

Lapointe and Shapiro (1999) determined that optimal survival to pupation of *Diaprepes* root weevil occurred at 30-70% soil moisture. About 60-65% of larvae survived to pupation under these ideal moisture conditions (Lapointe and Shapiro 1999). The poorest survival of larvae occurred in low (20%) and in high (80%) soil moisture levels (Lapointe and Shapiro 1999). Thus, lowest 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%.

Larvae of *Diaprepes* root weevil were exposed directly to flooding in the present study. However, Li et al. (2003a, 2006, 2007b) and Diaz (2005) unflooded plants before infestation with larvae. Diaz (2005) tested effects of flooding and *Diaprepes* root weevil larval infestation on leaf gas exchange and weight of green buttonwood plants, but unflooded plants 1 d before larval infestation so both stresses were not simultaneous. Overall, leaf gas exchange and plant dry weights observed by Diaz (2005) seemed to decrease more due to flooding than larval infestation in green buttonwood. There were no significant differences in the mean number of larvae recovered between pre-flooded and non-flooded green buttonwood plants, which may reflect similar soil water content between the treatments during infestation (Diaz 2005). Hence, whether or not flooding was applied before infestation with *Diaprepes* root weevil larvae may be less important than the soil water content during larval infestation, although soil pH and food plant quality may also affect results.

In a previous study (Chapter 4), survival of *Diaprepes* root weevil larvae was significantly higher in flooded marl soil than in flooded potting medium. This may have been caused by more favorable pH for larval survival in flooded marl soil (pH = 7.4-8.4; Li 2001) than in flooded potting medium (pH = 6.0; Chapter 4). In the present study, the relatively high soil pH that increased during the flood period and the well-aerated potting medium may have allowed similar

larval survival rates and growth in flooded and non-flooded potting media for green buttonwood. A possible reason why pH effects were different in green buttonwood than Swingle citrumelo may be differences in edaphic habitats in which they are adapted.

Li et al. (2003a, 2006, 2007b) examined the effects of *Diaprepes* root weevil larval infestation and flooding on plant growth, larval survival, and other characteristics of Swingle citrumelo and one other citrus rootstock in a greenhouse. Flooding occurred before larval infestation so both stresses were not simultaneous. Li et al. (2003a) found survival of *Diaprepes* root weevil larvae was significantly higher in previously flooded 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) investigated the effects of flooding and soil type on larval survival and found that for plants previously flooded 20 d, larval survival averaged 25% higher in sandy soil than in loam soil. Soil pH 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. (2006) found that Single citrumelo plants flooded for at least 20 d were more stressed and more prone to *Diaprepes* root weevil larval feeding injury after removal of plants from flooding than non-flooded control plants. Their results suggest that avoidance of flooding and early control of *Diaprepes* root weevil larvae may help protect young plants. Li et al. (2007b) also studied the effects of flooding and soil pH on the growth and survival of *Diaprepes* root weevil larvae. Citrus seedlings flooded 40 d had the lowest larval survival rates and larval weights compared to seedlings flooded for shorter flood durations, which may reflect higher soil pH during longer flood durations. Here, larval survival and growth were significantly decreased by pre-applied flooding (Li et al. 2007b). 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 significantly 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. 2007b). 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. 2007b). 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 *Diaprepes* root weevil (Li et al. 2007b). Flooding was also recommended as a possible control method in citrus (Li et al. 2007b). Flooding may hence reduce larval survival while plants are flooded. However, depending on soil pH, flood-stressed plants may be more susceptible to *Diaprepes* root weevil larval feeding when un-flooded than non-stressed plants that were either never flooded or flood-tolerant and previously flooded. Hence, pre-applied flooding may either increase or decrease larval survival based on soil moisture, pH, and plant health while soil is infested.

In the present study, flooding slightly increased the soil pH. A pH increase was also noted by Li et al. (2004, 2007b) for previously flooded compared to previously non-flooded soil in a flatwoods Floridana sandy soil (Li et al. 2004) and in Floridana sandy loam (Li et al. 2007b). Here, pH was increased 0.3 units above the mean pH (5.0) for non-flooded soil after 40 d flooding, resulting in less favorable conditions for growth and survival of *Diaprepes* root weevil larvae. As suggested above by Li et al. (2007b), increasing the soil pH from 4.8 to 5.7 decreased larval survival and/or weight. Thus, higher pH of floodwater in the present study (7.21-7.78) and in previous studies that used marl soil (Chapter 4) (7.4-8.4) would appear less favorable for *Diaprepes* root weevil larval survival than pH 4.8 to 5.7 in Li et al. (2007b). In Chapter 4, higher *Diaprepes* root weevil larval survival rates were observed in flooded marl soil (pH 7.4-8.4) than in flooded potting medium (pH 6.0). Therefore, the higher pH of marl soil (pH = 7.4-8.4)

compared to potting medium (pH = 6.0) may have been more favorable to larval survival when plants were flooded. In summary, cyclical flooding for three 2-d cycles seems more likely to control larvae in Swingle citrumelo than in green buttonwood.

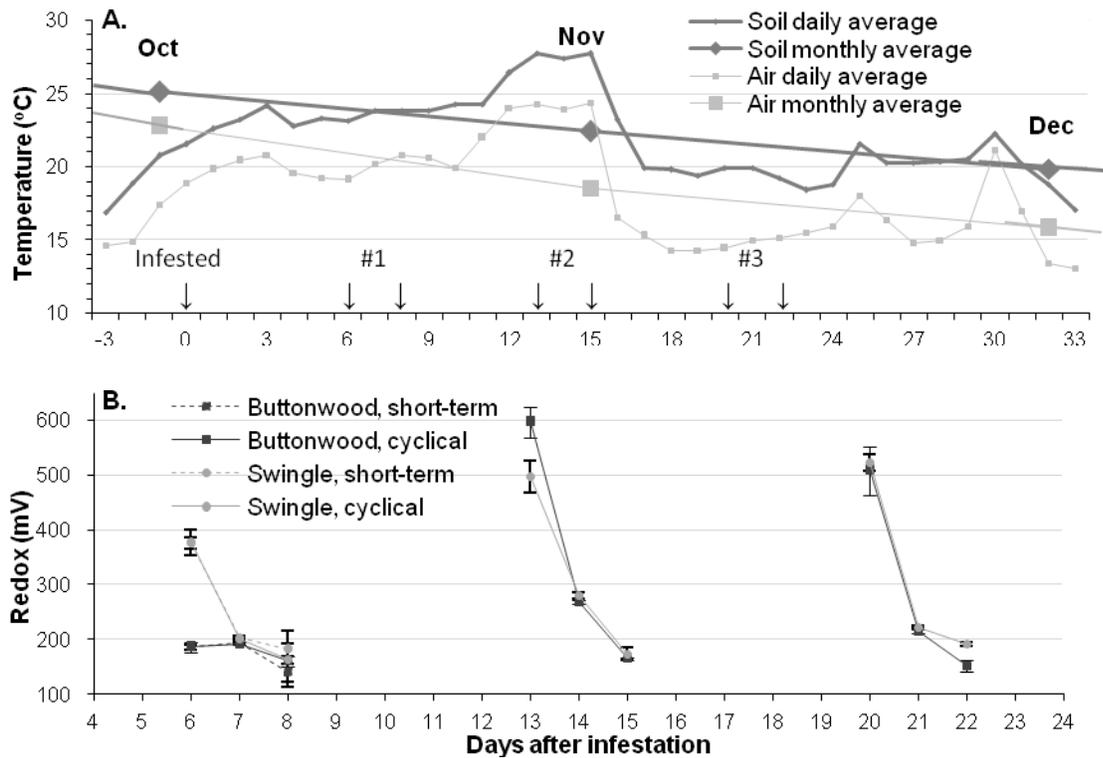


Figure 5-1. A) temperature and B) soil redox potential during the experiment. For temperature, each point is the average of two sensors. For redox potential, each point represents the mean  $\pm$  SD of four measurements. For temperature, successive flood cycles are denoted by pairs of arrows with the number of the flood cycle above the arrows. Each flood cycle in A) corresponds to an individual graph in B).

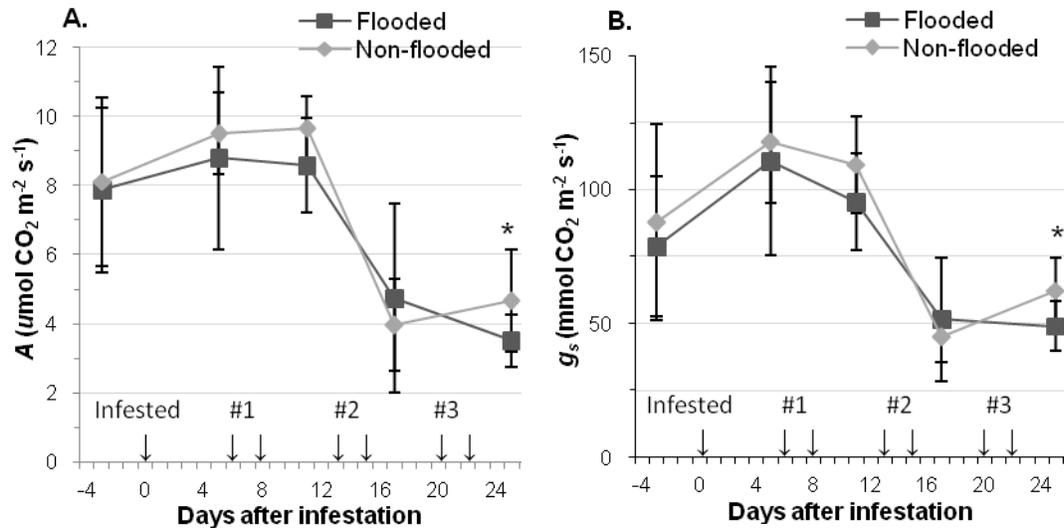


Figure 5-2. Effects of flooding on A) net CO<sub>2</sub> assimilation and B) stomatal conductance for cyclically flooded green buttonwood trees. Symbols represent means  $\pm$  SD. Successive flood cycles are denoted by pairs of arrows with number of the flood cycle shown above the arrows. Asterisks indicate significant differences between treatments at  $P \leq 0.05$  according to a standard T-test.

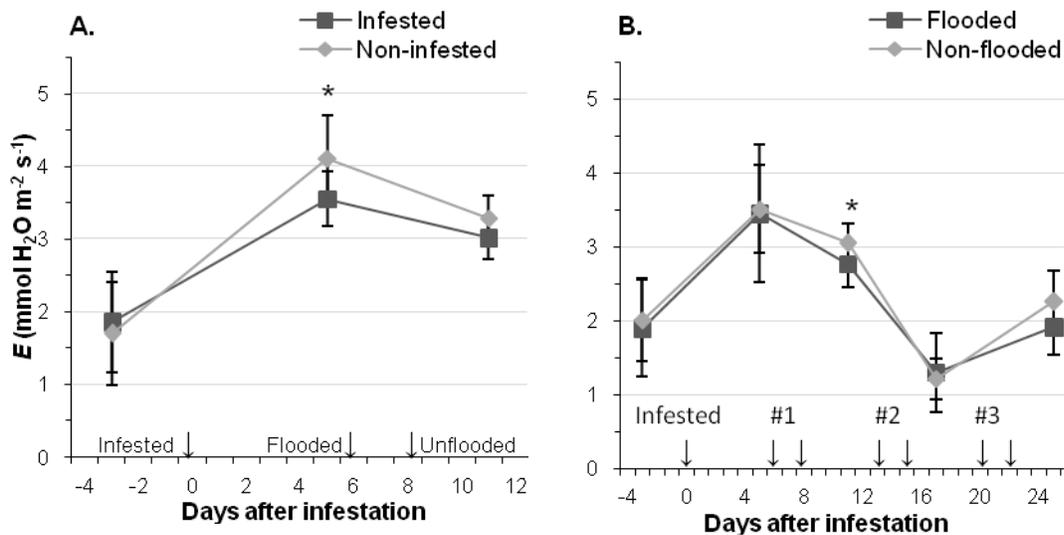


Figure 5-3. Effects of larval infestation or flooding on transpiration for A) short-term flooded and B) cyclically flooded green buttonwood plants. Symbols represent means  $\pm$  SD. For cyclically flooded plants, successive flood cycles are denoted by pairs of arrows with the number of the flood cycle shown above the arrows. Asterisks indicate significant differences between treatments at  $P \leq 0.05$  according to a standard T-test.

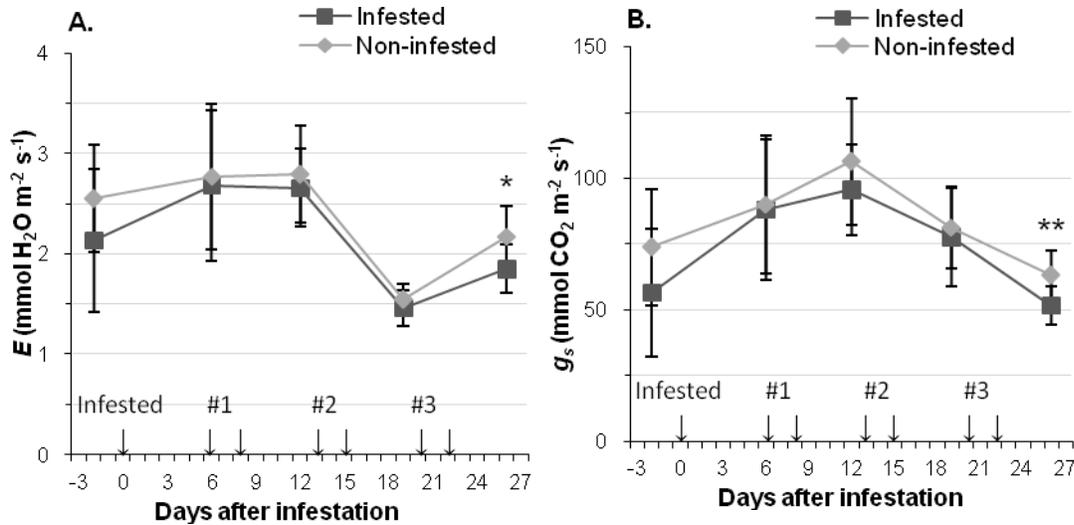


Figure 5-4. Effects of larval infestation on A) transpiration and B) stomatal conductance of cyclically flooded Swingle citrumelo trees. Symbols represent means  $\pm$  SD. Successive flood cycles are denoted by pairs of arrows with the number of the flood cycle above the arrows. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$  and \*\*  $P < 0.01$  according to a standard T-test.

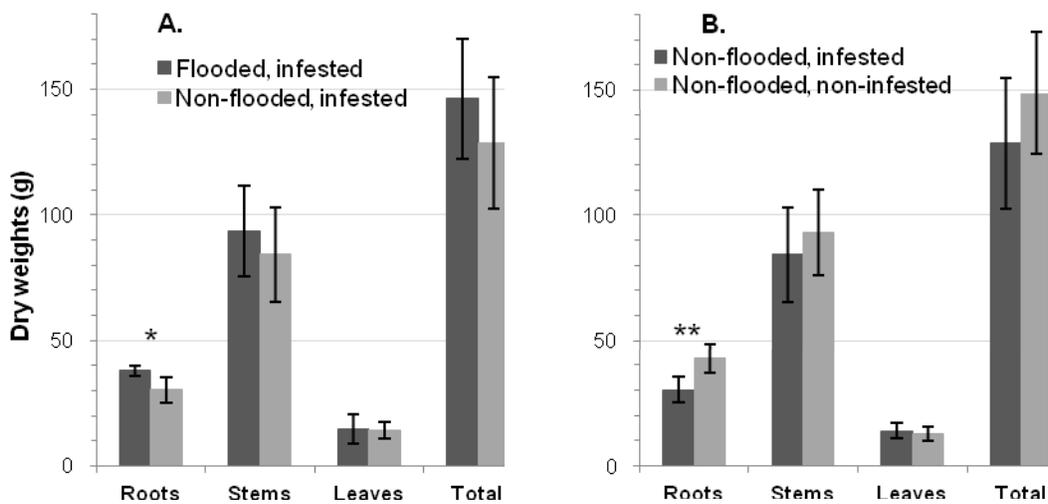


Figure 5-5. Effects of flooding and *Diaprepes* root weevil larval infestation on dry weights of cyclically flooded Swingle citrumelo plants. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$  and \*\*  $P < 0.01$  according to a standard T-test.

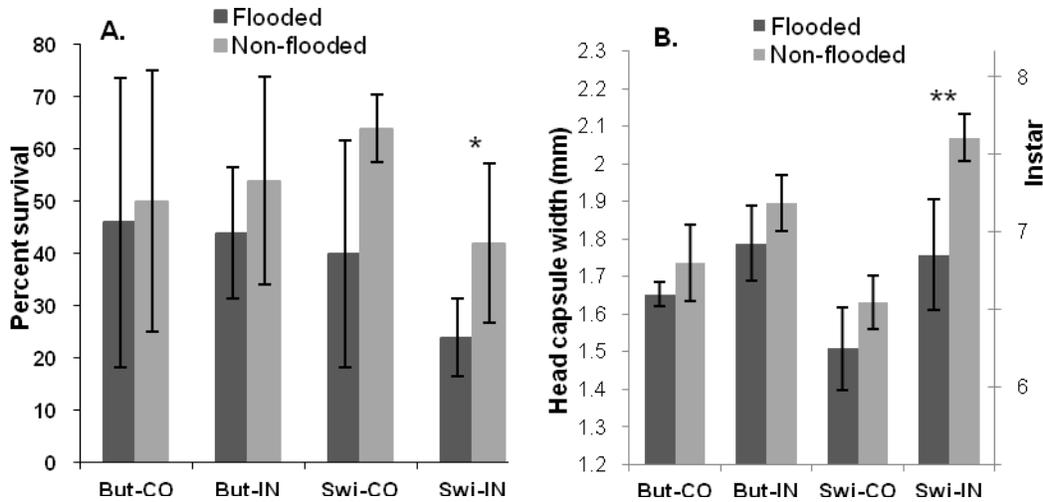


Figure 5-6. Effects of flooding on A) percent survival and B) head capsule width of *Diaprepes* root weevil larvae recovered at harvest. X-axis symbols are short-term (continuously) flooded green buttonwood (But-CO), cyclically flooded (intermittent) green buttonwood (But-IN), short-term (continuously) flooded Swingle citrumelo (Swi-CO), and cyclically flooded (intermittent) Swingle citrumelo (Swi-IN) plants. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$  and \*\*  $P < 0.01$  according to a standard T-test.

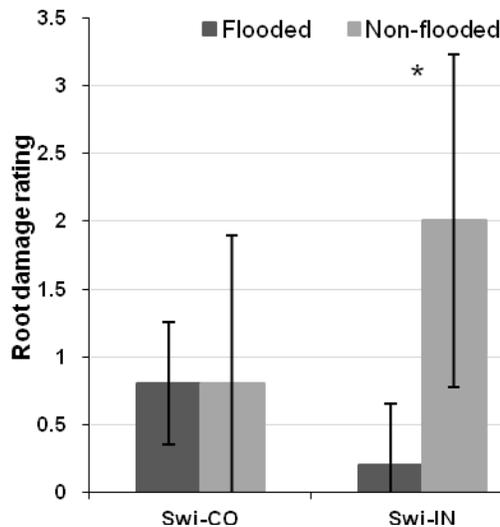


Figure 5-7. Visual damage ratings for *Diaprepes* root weevil larvae feeding on Swingle citrumelo roots. Damage rating symbols are 0 = no visible damage, 1 = minimal visible damage, 2 = moderate visible damage, and 3 = maximum visible damage. X-axis symbols include short-term (continuously) flooded Swingle plants (Swi-CO), and cyclically flooded (intermittent) Swingle (Swi-IN). Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at  $P \leq 0.05$  and  $P < 0.01$  according to a standard T-test.

CHAPTER 6  
LEAF DAMAGE AND PHYSIOLOGICAL RESPONSES OF SELECT WOODY  
ORNAMENTAL PLANTS TO ADULT FEEDING BY *DIAPREPES ABBREVIATUS*  
(COLEOPTERA: CURCULIONIDAE) AND SOIL FLOODING

**Introduction**

*Diaprepes abbreviatus* L. (Coleoptera: Curculionidae: Entiminae) commonly called Diaprepes root weevil, was first found in Florida in a citrus nursery in Apopka in 1964 (Woodruff 1964). Currently in the United States, it is found in Florida, Texas (Knapp et al. 2001, Skaria and French 2001), and southern California (Klunk 2005). In Florida, Diaprepes root weevil is found in 23 counties in the south and central parts of the state (Anonymous 1996, Peña 1997).

The host range of Diaprepes root weevil is very large including at least 317 varieties, 280 species, 180 genera, and 68 families of plants (Simpson et al. 1996, 2000, Knapp et al. 2000b, Mannion et al. 2003, Godfrey et al. 2006). The large host range creates many management challenges for this pest. Although not all host plants support all life stages, many economically important plants support all stages of the weevil from egg to adult (Simpson et al. 1996). In addition to the management challenges, the wide host range also provides a common avenue of moving this pest to new areas. Many common ornamental plant hosts grown in south Florida, such as green buttonwood, are known hosts of Diaprepes root weevil, and if not controlled, may play a role in spreading this pest through the movement of infested plants. Management of Diaprepes root weevil in ornamental plant nurseries is important and necessary to provide plant protection and to reduce the risk of spread.

The most obvious feeding damage by adult weevils includes notching along leaf margins of especially young, tender leaves (McCoy et al. 2002, Wolcott 1936, 1948). This can result in moderate to severe defoliation of host plants such as young, replanted citrus trees (Quintela et al.

1998, McCoy et al. 2002, Mannion et al. 2003). Unlike larval root feeding, however, prolonged adult feeding does not seem to economically reduce yields of mature citrus trees (McCoy et al. 2002). However, for ornamental plant species, excessive foliar damage makes them less attractive at the time of sale and can reduce sales (C. Mannion pers. comm.).

Insect herbivory often affects leaf gas exchange of host plants (Andersen and Mizell 1987, Schaffer and Mason 1990, Schaffer et al. 1997). Syvertsen and McCoy (1985) studied photosynthesis, transpiration, and water use efficiencies of citrus infested with adult *A. floridanus*, another common weevil in Florida. They found that when weevil population densities were more than one weevil per leaf, herbivory reduced water use efficiency up to 20%. They also found that when adult *A. floridanus* increased consumption of citrus leaf area, photosynthesis and water use efficiency decreased. Because water use efficiency decreased more rapidly than photosynthesis, drought stress from injured leaves may have resulted in reduced photosynthesis.

Responses of woody plants to flooding include senescence, shoot dieback, premature leaf abscission, decreased cambial growth, and suppression of formation and expansion of leaves and internodes (Schaffer et al. 1992, Kozlowski 1997). In addition, flooding reduces photosynthesis, carbohydrate transport, initiation of flower buds, anthesis, and fruit size, set, quality, and growth (Schaffer et al. 1992, Kozlowski 1997). Flooding often causes a change in the allocation of photosynthates within plants. For example, flooding suppressed height and diameter growth of flooded seedlings of *Acer platanoides* L. (Aceraceae), whereas bark growth increased, which suggests that flooding affects carbohydrate partitioning (Yamamoto and Kozlowski 1987). Thus, flooding may reduce photosynthates, which help to produce leaves and root masses that provide food for insect feeding. Flooding may therefore indirectly reduce the initial food available to insects as well as subsequent products of photosynthesis used to repair feeding damage.

Flooding may therefore reduce herbivory from insects because the plants may be more toxic and less nourishing, hence, more repulsive and less attractive than non-flooded plants. However, flood-induced production of compounds such as ethanol can also attract insects (Schroeder and Weslien 1994).

The main objectives of our study include investigating effects of flooding and adult *Diaprepes* root weevil feeding on leaf gas exchange and to determine if flooding affects host preference by adult weevils or predisposes trees to increased damage or feeding by adult weevils. The host plants included in these studies are green buttonwood *Conocarpus erectus* L. (Combretaceae), mahogany (*Swietenia mahagoni* Jacq., Meliaceae), pond apple (*Annona glabra* L., Annonaceae), and Surinam cherry (*Eugenia uniflora* L., Myrtaceae) which are widely grown in south Florida as fruit crops or ornamental plants (Watkins and Sheehan 1975, Wunderlin 1998, Nuñez-Elisea et al. 1999). Both green buttonwood and pond apple are native to south Florida and can tolerate flooding (Watkins and Sheehan 1975, Wunderlin 1998, Nuñez-Elisea et al. 1999).

### **Materials and Methods**

Three separate tests with four plant species were conducted at the Tropical Research and Education Center, University of Florida, Homestead, from 2007 to 2008. The first test began in the summer 2007 using green buttonwood and Surinam cherry; and ended in fall 2007 for Surinam cherry and early winter 2008 for green buttonwood. The second test was conducted in the spring and summer of 2008 using mahogany, and the third test was conducted during the summer and fall 2008 with pond apple. The objective of these tests was to look at effects on each plant species but not to make comparisons among plant species.

**Plant material.** Green buttonwood trees were purchased in 4-liter containers from a commercial nursery in February 2007 then transplanted into 11-liter containers between 19

February and 11 April 2007, about 3-5 mo before initiating the study. Surinam cherry plants were purchased in 11-liter containers from a commercial nursery in February 2006, with multiple plants per container, which is a typical practice in Florida nurseries. Trunks were divided (each with a separate root system) and transplanted into groups of four trunks per 11-liter container about 4 mo prior to the test. Mahogany trees were obtained by digging seedlings from the field in Homestead, FL, and repotting them into 11-liter containers before beginning the experiment. Pond apple trees were purchased from a commercial nursery in 11-liter containers and transplanted to 15-L plastic containers immediately after purchasing them. At the time treatments were initiated, approximate trees ages were 1-year-old for green buttonwood and 3-years-old for Surinam cherry, pond apple, and mahogany. At the beginning of the experiment, plant heights (mean  $\pm$  SD) were  $91 \pm 14$ ,  $71 \pm 4$ ,  $127 \pm 11$ , and  $130 \pm 17$  cm for green buttonwood, Surinam cherry, mahogany, and pond apple, respectively. Green buttonwood, Surinam cherry, and mahogany plants were grown in plastic containers in a standard potting medium (40% Florida peat, 20% pine bark, 20% cypress sawdust, and 20% sand), and pond apple was grown in a similar medium (40% Florida peat, 50% pine bark, and 10% sand).

Green buttonwood, Surinam cherry, and mahogany were fertilized 8 May 2007, 79 d before initiating treatments, with a timed release fertilizer (Osmacote Plus<sup>®</sup> 15-9-12, Scotts, Marysville, OH) according to the manufacturer's recommended rate. On the same date, a foliar iron spray (Sequestrene 138 Fe<sup>®</sup> with 6% chelated iron, Becker Underwood, Ames, IA) was applied at the manufacturer's recommended rate. Surinam cherry was again fertilized 9 July 2007, 16 d before initiating treatments with a liquid fertilizer (Miracle-gro<sup>®</sup> 15-30-15 with micronutrients, Stern's Miracle-Gro Products, Port Washington, NY) at the recommended rate.

Mahogany trees developed heavy infestations of the woolly whitefly, *Aleurothrixus floccosus* Maskell (Hemiptera: Aleyrodidae) in fall 2007, about 6 mo before the test began. To

control this pest, plants were sprayed 29 November 2007 with an insect growth regulator (Distance<sup>®</sup>, Valent Co., Walnut Creek, CA) at the manufacturer's recommended rate. This was mixed with Prescription Treatment<sup>®</sup> Ultra Fine Oil (Whitmire Micro-Gen Research Laboratories, St. Louis, MO) at the manufacturer's recommended rate. This combination controlled the insect, and it was a minor pest during the experiment. Pond apple trees were also infested with larvae of the citrus fruit piercer moth, *Gonodonta nutrix* (Cramer) (Lepidoptera: Noctuidae), which caused most of the foliar damage observed for non-infested plants and similar damage initially for infested plants. These larvae were manually removed as needed.

## **Treatments**

**Infestation treatments.** For all four plant species, aluminum screen cages (60 cm x 60 cm x 1.2 m) were used to contain adult *Diaprepes* root weevil. Groups of four screen cages were attached to form cubes, 1.2-m on each side, and placed on 1.2 m x 1.2 m pallets. Two plants (one flooded and one non-flooded) were placed in each cage. The standard aluminum screen in these cages had an aperture size of 1.5 mm x 1.5 mm and blocked approximately 30% of incoming sunlight (Burger et al. 2007). All pallets of screen-enclosed plants were located in an outdoor site exposed to full sun and on landscape fabric to control weeds.

Twenty *Diaprepes* root weevil adults (10 males and 10 females) were released into each of six screen cages, one cage per replication. All adults were collected from 3 to 22 d before infestation from canopies of trees growing in fields at commercial plant nurseries in Homestead, FL. Adults for each test were maintained in 30-cm x 30-cm x 30-cm plexiglass cages until their use for infestation. They were provided a diet of green buttonwood leaves and water in 2 vials, and cages were cleaned three times per week. Surinam cherry and mahogany plants were infested with adults after flooded plants exhibited early signs of physiological stress as indicated by slight reduction in net photosynthesis. Flooding treatments are described below. Plants were

infested on day 11 of flooding for Surinam cherry and day 31 of flooding for mahogany. Green buttonwoods did not exhibit reduction in net CO<sub>2</sub> assimilation after 25 d of flooding, hence they were found to be relatively flood tolerant and were infested then. Pond apple trees are known to be very flood tolerant (Schaffer 1998, Núñez-Elisea et al. 1999, Ojeda et al. 2004, Chapter 3), hence, we did not wait for symptoms of flooding stress to appear, and trees were infested after 2 d of flooding. Durations of infestation were 81 d, 148 d, 50 d, and 23 d for Surinam cherry, green buttonwood, mahogany, and pond apple, respectively. For green buttonwood and Surinam cherry plants, all the cages were infested, whereas for mahogany and pond apple, one-half the total number of cages were infested. After conducting the test for buttonwood and Surinam cherry, it was realized that treatments without the weevils were necessary for comparison, hence, non-infested treatments were added to the tests with mahogany and pond apple.

**Flooding treatments.** One flooded and one non-flooded plant of the same species were placed together in a screen-enclosed cage. The container of each flooded plant was submerged into a 19-liter plastic bucket filled with tap water to 10 cm above soil surface. Plants were flooded July 30 2007 for green buttonwood and Surinam cherry, 21 May 2008 for mahogany, and 18 Sep 2008 for pond apple. Flood durations were 73 d for Surinam cherry, 180 d for green buttonwood, 80 d for mahogany, and 24 d for pond apple. Flood durations were determined by the appearance of physiological indicators of plant stress, such as reduced photosynthesis and wilting. All plants were irrigated for 30 min twice per day by overhead sprinkler.

### **Data Collection**

Data collected included numbers of egg clusters, feeding damage to foliage, soil temperature and redox potential, photosynthesis, transpiration, stomatal conductance of water vapor, plant height and stem diameter (at 10-cm above the soil surface), and leaf, stem, and root fresh and dry weights. For Surinam cherry, the leaf chlorophyll index (leaf greenness) was also

measured just before harvest. For green buttonwood, the number of adventitious roots originating above soil level were recorded twice (30 d after treatment initiation and at harvest, day 197). Mahogany has pinnately compound leaves with 6-8 leaflets per leaf, whereas all the other plant species tested have simple leaves (Watkins and Sheehan 1975, Wunderlin 1998). For leaf gas exchange measurements, an attempt was made to randomly select mahogany leaflets from all positions on the leaf.

**Soil temperature and redox potential.** Soil temperature was recorded at 1 h intervals throughout the experiments with sensors (StowAway<sup>®</sup> Tidbit<sup>®</sup> temploggers, Onset Co., Pocasset, MA) located at a soil depth of 6 cm two-thirds the distance from the center to the outer edge of the pot. Redox potential (*Eh*) of the soil solution was measured with a platinum combination electrode attached to a portable volt meter (Accumet #AP62, Fisher Scientific, Pittsburgh, PA). Measurements were made by inserting the electrode into a polyvinyl chloride (PVC) pipe (20 cm long x 22 mm wide) placed in the soil 2 cm from the edge of the pot. Soil redox potential was recorded at a mean depth of 6 cm below soil surface. Soil redox potential was measured for four flooded plants per plant species. Redox potential was measured daily during the first 6-7 d of flooding and thereafter at intervals of 3-12 d (mostly 6-8 d) until plants were unflooded.

**Leaf gas exchange and chlorophyll index.** Leaf gas exchange measurements included net CO<sub>2</sub> assimilation (photosynthesis), transpiration, and stomatal conductance. Leaf gas exchange was measured on two recently mature, fully expanded, hardened-off leaves or leaflets per plant with a CIRAS-2 portable gas analyzer (PP Systems, Amesbury, MA). For each plant, gas exchange values of two leaves or leaflets were averaged to provide a single plant value used as a replication. All leaf gas exchange measurements were made between 10:45 and 17:15 for Surinam cherry, 10:30 and 17:30 for green buttonwood, 14:00 and 19:30 for mahogany, and 14:15 and 18:15 for pond apple. During gas exchange measurements, the photosynthetic photon

flux in the leaf cuvette was maintained at  $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  with a halogen lamp attached to the cuvette, and the reference  $\text{CO}_2$  concentration in the cuvette was kept constant at  $375 \mu\text{mol mol}^{-1} \text{ CO}_2$ . Leaf gas exchange was measured prior to flooding plants and then periodically during the flooding period. A final leaf gas exchange measurement was made after plants had been unflooded and uninfested. The first leaf gas exchange measurements were made 2 d, 5 d, 5 d, and 2 d before flooding green buttonwood, Surinam cherry, mahogany, and pond apple, respectively. Surinam cherry was the only plant species tested in which there was a visible difference in leaf color between flooded and non-flooded plants. Hence for Surinam cherry plants, leaf chlorophyll index, a measurement of leaf “greenness,” was determined with a SPAD meter (model 502, Minolta Inc., Japan), on three leaves per plant at harvest (day 107, 08 Nov 2007).

**Egg clusters and leaf damage.** Egg clusters were counted and removed from each plant within infested cages and the percentage of leaf damage from feeding was visually estimated for all plants. Relative to release dates of adult *Diaprepes* root weevil, initial assessments of number of egg clusters and leaf damage during infestation were made 5 d, 7 d, 5 d, and 7 d after releasing adults onto green buttonwood, Surinam cherry, mahogany, and pond apple plants, respectively. Insects remained in cages until no egg clusters were found on at least half the infested plants, and mean numbers of egg clusters per plant did not differ significantly between flooded and non-flooded treatments for two consecutive weeks. Adults were removed 81 d, 148 d, 50 d, and 23 d after their introduction for Surinam cherry, green buttonwood, mahogany, and pond apple, respectively.

Upon removal from cages, all male and female adults were counted from each cage. Final counts of egg clusters and damage were made 1-5 d after all adults were removed from cages. Egg clusters were removed by detaching two leaves or leaflets enclosing them, which resulted in

different numbers of leaves or leaflets removed per treatment (plant) for each replication per measurement date. Neither green buttonwood nor Surinam cherry had significant differences in numbers of egg clusters between flooded and non-flooded treatments on any measurement date, and the few leaves removed between treatments were not believed sufficient to significantly affect results, such as leaf fresh weight. For mahogany, however, we compensated for unequal leaf removal among treatments by removing the same number of leaves from each plant as the plant with the highest number of egg clusters in a given replication. For pond apple, egg clusters were removed by wiping them off with a cloth instead of by removing leaves they were attached to because pond apples had much larger leaves than leaves or leaflets of the other plant species. However, pond apple leaves were sensitive to handling and often fell off during evaluation, hence, the same number of leaves were removed from all treatment combinations as in the plant with the most abundant leaf drop to equalize reduction of leaves.

**Plant growth.** Stem diameter and plant height were measured for all plants at the beginning and end of each experiment. Stem diameter was measured 10 cm above the soil surface, and for plants with multiple stems at this height, diameter of the largest stem was recorded. Plant height was measured from the soil surface to the top of the highest leaf or branch. Plants were left flooded until at least one plant exhibited physical stress and would likely die if not removed from flooding. Surinam cherry and mahogany were unflooded 73 d and 80d, respectively, after flooding began. Buttonwood and pond apple, however, did not exhibit physical signs of flooding stress and were unflooded 180 d and 24 d, respectively, after flooding.

After adults were removed from screen cages, plants were removed from the cages and placed in the open on weed control cloth for measurements of leaf damage, number of egg clusters, and leaf gas exchange. At the end of the treatment period, fresh weights of roots, stems, leaves, and adventitious roots (green buttonwood), were determined. In addition, for green

buttonwood and Surinam cherry, numbers of flowers and fruit (including pedicels) were determined per plant. Roots, stems, and leaves were then oven dried at 75°C to a constant weight and dry weights were determined. For buttonwood, pond apple, and Surinam cherry, leaf fresh and dry weights included leaf blades and petioles, but for mahogany, leaf fresh and dry weights included leaflets, petiolules, rachises, and petioles.

**Experimental design and statistical analyses.** For green buttonwood and Surinam cherry, plants were either flooded or non-flooded and all plants were infested with *Diaprepes* root weevil adults. For tests with mahogany and pond apple, however, there were two flooding treatments (flooded or non-flooded), and each flooding treatment had two adult infestation treatments (infested or non-infested) in a 2 x 2 factorial design. Data were analyzed separately for each plant species, and there were six single-plant replications per treatment or treatment combination. For each test with mahogany and pond apple, a two-way analysis of variance (ANOVA) was used to determine if there were significant interactions between flooding and insect infestation treatments. Flooding and insect infestation treatment main effects were compared using standard T-tests. All statistical analyses were done with SAS statistical software Version 9.1 (PROC T-TEST and GLM, SAS Institute, Cary, NC, 2003).

## Results

During the test period for green buttonwood and Surinam cherry, mean daily soil temperatures were 3.5°C to 26.1°C with monthly averages of 14.8°C to 24.6°C (Figure 6-1a).

**Green buttonwood.** Soil redox potential for flooded buttonwood ranged from +123 mV to -288 mV (Figure 6-2a). There were generally no significant differences between flooded and non-flooded green buttonwoods in net CO<sub>2</sub> assimilation (range 0.51-18.8  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration (range 0.61-9.10  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), or stomatal conductance (range -117 to 1262  $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Root dry weight was significantly lower in flooded than non-flooded

buttonwood plants ( $t = -2.79$ ,  $df = 7$ ,  $P = 0.0276$ ) (Figure 6-3). There were no significant differences between flooded and non-flooded buttonwood plants in fresh weight of roots (range 154-358 g), stems (range 304-536 g), leaves (range 178-408 g), or total weight (range 673-1247 g), or in stem, leaf, or total dry weight (Figure 6-3). Stem diameter of buttonwood was significantly lower in non-flooded than flooded plants ( $t = 3.54$ ,  $df = 6$ ,  $P = 0.0134$ ), but there was no significant difference in plant height between treatments (Figure 6-4). There was no significant effect of flooding on the number of inflorescences per buttonwood plant (range 0-109). Significantly more adventitious roots were found on flooded than on non-flooded buttonwoods at 24 d after flooding ( $t = 4.19$ ,  $df = 5$ ,  $P = 0.0085$ ) and at harvest ( $t = 2.92$ ,  $df = 5$ ,  $P = 0.0331$ ) (Figure 6-5a). Also, a significantly higher percentage of total fresh weights ( $t = 5.45$ ,  $df = 5$ ,  $P = 0.0028$ ) and dry weights ( $t = 5.9$ ,  $df = 5$ ,  $P = 0.002$ ) composed of adventitious roots were found on flooded than on non-flooded green buttonwoods at harvest (Figure 6-5b). Mean percentage of total weight composed of adventitious roots was 2.1% for fresh weight and 2.9% for dry weight. Buttonwood plant containers averaged 1.5-1.8 separately rooted plantlets, differences between treatments were not significant, and all of them survived to harvest (Figure 6-6).

Maximum mean foliar damage (foliage missing because of adult feeding) was 32% (non-flooded) and 33% (flooded), and these occurred in the four measurements immediately preceding harvest (Figure 6-7a). There was no significant difference in percent damage from adult *Diaprepes* root weevil feeding between flooded and non-flooded buttonwoods on any date measured. Mean maximum number of egg clusters per plant per evaluation date for buttonwood was 22 (non-flooded) and 21 (flooded), and these occurred during evaluation dates 6-11 (Figure 6-7b). There were no significant differences in number of egg clusters between flooded and non-flooded treatments of buttonwood. At the end of the test, mean ratio of female to total recovered

adults from buttonwood was 0.62, and the difference between numbers of males and females was not significant (Figure 6-8). Although not statistically compared, buttonwood appeared to have the most egg clusters per plant of four plant species in the present study, and adult *Diaprepes* root weevil seemed to live the longest on buttonwood compared to the other plant species.

**Surinam cherry.** Soil redox potential for flooded Surinam cherry ranged from +126 mV to -261 mV (Figure 6-2b). Surinam cherry net CO<sub>2</sub> assimilation was significantly lower for flooded than non-flooded plants on 9 of 14 measurement dates (Figure 6-9a). Transpiration and stomatal conductance were each significantly lower for flooded than non-flooded plants on 7 of 14 measurement dates (Figure 6-9b and c). Leaf chlorophyll index was significantly lower for flooded than for non-flooded Surinam cherry plants ( $t = -2.58$ ,  $df = 9$ ,  $P = 0.0296$ ) (Figure 6-10). Surinam cherry leaf fresh weight ( $t = -4.19$ ,  $df = 7$ ,  $P = 0.0035$ ), total fresh weight ( $t = -2.38$ ,  $df = 9$ ,  $P = 0.0398$ ), and leaf dry weight ( $t = -4.39$ ,  $df = 8$ ,  $P = 0.0021$ ) were each significantly lower for flooded than for non-flooded plants (Figure 6-11). However, there were no significant differences between root or stem fresh weights or between root, stem, or total dry weights (Figure 6-11). Stem diameter ( $t = -2.76$ ,  $df = 10$ ,  $P = 0.0205$ ) and plant height ( $t = -4.55$ ,  $df = 10$ ,  $P = 0.0011$ ) were also significantly lower for flooded than non-flooded Surinam cherry plants (Figure 6-4). There was no significant effect of flooding on the number of flowers and fruit per Surinam cherry plant (range 0-6). Surinam cherry in non-flooded containers averaged 100% survival of separately rooted plantlets per container, whereas in flooded containers, only 38% survived, and differences between treatments were significant ( $t = -7.42$ ,  $df = 5$ ,  $P = 0.0007$ ) (Figure 6-6b).

Maximum mean percent damage to Surinam cherry foliage from adult *Diaprepes* root weevil was 33% for non-flooded plants and 20% for flooded plants during the final three measurement dates before harvest (Figure 6-12a). Feeding damage was significantly higher for

non-flooded than flooded Surinam cherry plants in weeks 5 and 7-14 (Figure 6-12a). Average maximum number of egg clusters per Surinam cherry plant was 8.0 for non-flooded and 4.7 for flooded plants on measurement dates 2-6, but there were no significant differences between treatments (Figure 6-12b). The mean ratio of female to total recovered adults from Surinam cherry at the end of the test was 0.71 with significant differences between numbers of males and females ( $t = 2.98$ ,  $df = 8$ ,  $P = 0.0169$ ) (Figure 6-8). Among the four plant species tested, Surinam cherry was the only one with a significant difference between numbers of males and females. In addition to having the highest mean percentage of adults that were female among four plant species tested (71%), Surinam cherry yielded the highest mean total number of adults recovered (8.5 adults), but these differences among plant species were not statistically compared.

**Mahogany.** Mean daily soil temperatures ranged from 24.5°C to 28.9°C with monthly averages of 26.8°C to 27.4°C (Figure 6-1b). Soil redox potential for flooded mahogany plants ranged from +67 mV to -297 mV (Figure 6-2c). There were no significant interactions ( $P \leq 0.05$ ) between flooding and infestation for net CO<sub>2</sub> assimilation, transpiration, or stomatal conductance on any measurement date. There were also no significant interactions between flooding and infestation treatments for stem diameter, plant height, root, stem, leaf, or total fresh weights or dry weights. However, there were significant flooding and infestation interactions for the percentage of leaf damage from adult feeding and the number of egg clusters per plant on one or more measurement dates. Therefore, percent damage from adult feeding and number of egg clusters per plant were not pooled, whereas all the other data were pooled for analysis.

Net CO<sub>2</sub> assimilation was significantly higher for non-flooded than flooded mahogany trees during the final four measurements (Figure 6-13a). Transpiration ( $t = -2.59$ ,  $df = 21$ ,  $P = 0.0168$ ) and stomatal conductance ( $t = -2.53$ ,  $df = 21$ ,  $P = 0.0196$ ) were each significantly higher for non-flooded than flooded mahogany during the ninth of 11 measurements (Figure 6-13b and

c). Net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance tended to be higher for infested than for non-infested mahogany during weeks 8-11 with all significant differences occurring in week 9 of 11 measurements in the test (Figure 6-14). This was 65 d after flooding and 35 d after infesting with adult *Diaprepes* root weevil (Figure 6-14). At the end of the treatment period, increases in stem diameter ( $t = -6.72$ ,  $df = 22$ ,  $P = <0.0001$ ) and plant height ( $t = -2.54$ ,  $df = 14$ ,  $P = 0.0233$ ) were each significantly higher for non-flooded than flooded mahogany plants (Figure 6-15). There were no significant differences between infested and non-infested mahogany plants in increase in stem diameter (range -0.73 to 4.49 mm) or plant height (range 6.5-44.1 cm). Leaf fresh weight ( $t = -2.99$ ,  $df = 18$ ,  $P = 0.0077$ ), total fresh weight ( $t = -2.12$ ,  $df = 21$ ,  $P = 0.0457$ ), leaf dry weight ( $t = -3.09$ ,  $df = 18$ ,  $P = 0.0062$ ), and root dry weight ( $t = -2.85$ ,  $df = 18$ ,  $P = 0.0105$ ) were each significantly higher for non-flooded than flooded mahogany plants (Figure 6-16 a and c). There were no significant differences between non-flooded and flooded mahogany plants in root or stem fresh weights or in stem or total dry weights (Figure 6-16 a and c). Mahogany leaf fresh weights ( $t = -2.44$ ,  $df = 19$ ,  $P = 0.0248$ ) and leaf dry weights ( $t = -2.31$ ,  $df = 19$ ,  $P = 0.0320$ ) were each significantly higher for non-infested than infested plants (Figure 6-16 b and d), but there were no significant differences in root, stem, or total fresh or dry weights.

Mean maximum percentage of feeding damage from adult *Diaprepes* root weevil on mahogany foliage was 42% (non-flooded, infested) and 25% (flooded, infested) in weeks 6 and 9, respectively (Figure 6-17). There was significantly more leaf damage on non-flooded infested than on flooded infested mahogany plants in weeks 1-8 (Figure 6-17a). However, there were no significant differences in leaf damage between non-flooded non-infested and flooded non-infested plants (Figure 6-17b). Also, significantly more damage occurred on flooded, infested than on flooded, non-infested mahogany plants in weeks 3-9 (Figure 6-17c), and on non-flooded,

infested than on non-flooded, non-infested plants in all 9 wk (Figure 6-17d). Mean maximum number of egg clusters per mahogany plant was 14.5 egg clusters per measurement (non-flooded, infested) and 11.2 (flooded, infested), and both maxima occurred on the second measurement date (Figure 6-18). Number of egg clusters per mahogany plant was significantly higher for non-flooded infested than for flooded infested plants in week 1 ( $t = -2.32$ ,  $df = 8$ ,  $P = 0.0486$ ), with no significant differences between treatments for each of the remaining 8 wk (Figure 6-18a). There were no significant differences in number of egg clusters between non-flooded non-infested and flooded non-infested mahogany plants (Figure 6-18b). Also, number of egg clusters per mahogany plant was significantly higher for flooded infested than flooded non-infested plants in weeks 2-4 (Figure 6-18c), and for non-flooded infested than for non-flooded non-infested plants in weeks 1-6 (Figure 6-18d). Mean ratio of female to total recovered adults from mahogany at the end of the test was 0.57 with no significant difference between the number of males and females (Figure 6-8).

**Pond apple.** Mean daily soil temperatures ranged from 23.4°C to 27.7°C with monthly averages of 24.9°C to 26.4°C (Figure 6-1c). Soil redox potential for flooded pond apple varied from +189 mV to -260 mV (Figure 6-2d). There was a significant interaction between flooding and infestation treatment ( $P \leq 0.05$ ) for net CO<sub>2</sub> assimilation on one or more measurement dates. However, there were no significant interactions ( $P \leq 0.05$ ) between flooding and infestation treatments for transpiration, stomatal conductance, percent leaf damage, egg clusters per plant, stem diameter, plant height, root, stem, leaf, or total fresh or dry weights. Therefore, all pond apple data were pooled for statistical analysis except for net CO<sub>2</sub> assimilation data.

Non-flooded, infested pond apple plants had a significantly higher net CO<sub>2</sub> assimilation rate than flooded, infested plants, but only on week 5 ( $t = -2.90$ ,  $df = 10$ ,  $P = 0.0160$ ) (Figure 6-19). There were no other significant differences between flooded and non-flooded or infested

and non-infested pond apple plants in net CO<sub>2</sub> assimilation (range 4-22  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Also, there were no significant differences between flooded and non-flooded or infested and non-infested pond apple plants in transpiration (range 1.5-5.5  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) or stomatal conductance (range 25-196  $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Fresh root weight ( $t = -2.50$ ,  $df = 16$ ,  $P = 0.0237$ ) and dry root weight ( $t = -4.10$ ,  $df = 22$ ,  $P = 0.0005$ ) were each significantly greater for non-flooded than flooded pond apple plants (Figure 6-20). However, there were no significant differences between flooded and non-flooded pond apple plants in stem, leaf, or total fresh or dry weight (Figure 6-20). Also, there were no significant differences between infested and non-infested pond apple plants in root, stem, leaf, or total fresh weight (ranges 127-232 g, 306-487 g, 60-176 g, and 543-848 g, respectively) or root, stem, leaf, or total dry weight (ranges 41-69 g, 104-162 g, 21-47 g, and 176-273 g, respectively). There were no significant differences in increase in stem diameter or plant height between flooded and non-flooded pond apple plants (Figure 6-15) or between infested and non-infested plants (stem diameter range 0.22-3.96 mm, plant height range -19 to 18 cm). No adventitious roots were observed on flooded pond apple, possibly because of the short duration of the experiment.

Based on diagnosis of a specimen that was collected from the same nursery on the same date that pond apple trees were purchased, pond apples in the present study were infested with the fungal stem and leaf rot, *Phomopsis* (sp. or spp.) (Coelomycota) (A. Palmeteer, Plant Disease Clinic, University of Florida Tropical Research and Education Center). This resulted in about 10-30% pond apple foliage loss by the end of the experiment, whereas average maximum leaf damage from adult *Diaprepes* root weevil was about 4.1 %. There were no significant differences between flooded and non-flooded or infested and non-infested pond apple plants in mean feeding damage from adult *Diaprepes* root weevil (range based on 1 standard deviation was 0.8-6.8%). Mean maximum number of egg clusters per infested pond apple plant was 0.9 on

the second measurement date, when it was significantly higher than for non-infested plants ( $t = 3.53$ ,  $df = 11$ ,  $P = 0.0047$ ) (Figure 6-21). There were no other significant differences in number of egg clusters between flooded and non-flooded or between infested and non-infested pond apple plants. Mean ratio of female to total recovered adults from pond apple at the end of the test was 0.40 with no significant differences between numbers of males and females (Figure 6-8). Pond apple was the only plant species tested with more males than females collected at the end of the treatment period, and it had the lowest survival rate of adult insects, although there were no statistical comparisons among plant species.

### **Discussion**

A measure of oxygen abundance in the soil is redox potential. The effects of flooding on the physiology and growth of a woody, perennial plant species can vary among soil types and are partly based on the rates of soil O<sub>2</sub> depletion and other factors like soil pH (Schaffer et al. 1992). Well-drained, well oxygenated soils have redox potentials of +300 mV or more, whereas flooded soils have redox potentials of +200 mV or less (Ponnamperuma 1972, 1984). Cumulatively for all plant species in the present study, mean soil redox potential of flooded plants ranged from +189 mV to -297 mV, indicating that flooded soils were hypoxic.

To counteract detrimental effects of flooding, such as reduced photosynthesis, many plants have evolved anatomical or morphological adaptations including hypertrophic (swollen) stem lenticels, development of aerenchyma tissue, and adventitious (above-ground) roots (Kozlowski 1997). Hypertrophic lenticels benefit flooded plants by increasing O<sub>2</sub> transport to roots (Hook et al. 1970, Hook 1984) and serving as excretory sites for potentially toxic compounds, such as acetaldehyde, ethylene, and ethanol produced during anaerobic root metabolism (Chirkova and Gutman 1972). Aerenchyma tissue occurs in root epidermal layers and shoot cortexes of flooded highbush blueberries (*Vaccinium corymbosum* L., Ericaceae) (Abbott and Gough 1987, Crane and

Davies 1989), and it facilitates movement of O<sub>2</sub> from shoots to submerged roots. However, according to Nuñez-Elisea et al. (1999), flooded pond apple plants did not develop aerenchyma tissue (air pathways or intercellular spaces), but they grew adventitious roots and hypertrophic lenticels on submerged roots.

For buttonwoods in the present study, significantly more adventitious roots were found on flooded than on non-flooded plants. However, root dry weight (including adventitious roots) was significantly lower for flooded than non-flooded buttonwood plants. This may have resulted from resource partitioning under flooded conditions (Yamamoto and Kozlowski 1987, Kozlowski 1997). Although root dry weight of buttonwood was significantly lower in flooded than non-flooded plants, stem diameter was significantly lower in non-flooded than flooded plants. Buttonwood developed enlarged stem diameters and adventitious roots when flooded. These adaptations can increase oxygen absorption and transport to flooded roots, and they may indirectly avoid injury from anaerobic compounds and help with nutrient transport (Kozlowski 1997). Thus, enlarged stem diameters and adventitious roots in buttonwood may have contributed to minimal differences observed between flooded and non-flooded plants for most gas exchange and growth variables measured. With exception of root dry weight and stem diameter, there were generally no significant differences in net CO<sub>2</sub> assimilation transpiration, stomatal conductance, plant height, root fresh weights, or in stem, leaf, or total fresh and dry weights between flooded and non-flooded green buttonwood. Flooded green buttonwood plants were very similar in appearance to non-flooded plants suggesting equal performance and foliage quality. Diaz (2005) found that flooding green buttonwoods in potting medium significantly reduced photosynthesis, transpiration, and stomatal conductance beginning 1 wk after flooding, although flooding did not significantly affect root, stem, or leaf fresh or dry weights. In another study with green buttonwood, flooding did not cause significant differences in photosynthesis,

stomatal conductance, or dry weights of plants grown in potting medium (Chapter 3). The present study found similar results, although flood periods were longer (180 d for the present study versus 23 d for Chapter 3) and all plants were infested with *Diaprepe* adults in the present study, unlike in previous chapters.

Although we did not compare leaf gas exchange or plant growth between plant species statistically, differences in net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance between flooded and non-flooded plants suggest the order of susceptibility to flooding is Surinam cherry, followed by mahogany, with green buttonwood and pond apple the least affected. Differences in plant growth between flooded and non-flooded treatments in infested or pooled cages suggest the order of susceptibility to flooding is Surinam cherry and mahogany most affected and buttonwood and pond apple least affected. Hence, overall differences in gas exchange and growth between flooded and non-flooded plants in infested or pooled cages suggest the order of susceptibility to flooding is Surinam cherry, then mahogany, with green buttonwood and pond apple least affected.

Although pond apple was among the plant species least affected by flooding based on leaf gas exchange and plant growth data, it was still more susceptible than in a previous study (Chapter 3), which had 41 d of flooding instead of 24 d in the present study. For example, net CO<sub>2</sub> assimilation of pond apple in the previous study was significantly higher in flooded potting medium than non-flooded potting medium on 6 of 19 measurements. However, in the present study, the only significant difference was significantly lower net CO<sub>2</sub> assimilation for flooded infested than non-flooded infested pond apple plants on one measurement. A similar difference was found with pond apple growth, such as stem diameter, which significantly increased with flooding in the previous, but not in the present study. The longer flood duration in the previous

study (41 d) seemed to favor more adaptation and less susceptibility to flooding by pond apple than the shorter duration (24 d) in the present study.

Diaz et al. (2005) employed similar methods to the present study in evaluating adult *Diaprepes* root weevil feeding damage on green buttonwood, but obtained variable results. In one test, feeding by adult *Diaprepes* root weevil significantly increased photosynthesis, transpiration, and stomatal conductance after 2 mo (Diaz et al. 2005). In the second test, feeding by adult *Diaprepes* root weevil significantly decreased root dry weight, but there were otherwise no significant differences in root, stem, or leaf fresh or dry weights in either test.

Mahogany was unique among plant species tested because *Diaprepes* root weevil adults fed on stems as well as leaves. They left gouges in the outer stem and bark and chewed up petioles resulting in many more leaves and leaflets killed above the wounds than by feeding alone. Mahogany had the highest maximum mean adult feeding damage and second highest maximum mean number of egg clusters per plant, although adults often avoided older leaves in favor of younger leaves. On buttonwood and Surinam cherry foliage, however, adults generally ate both young and old leaves, avoided petioles and bark, and tended to not kill uneaten leaves. Diaz et al. (2005) found that *Diaprepes* root weevil adults fed more on green buttonwood than on live oak leaves, which may have been less susceptible to adult feeding than buttonwood because the leaves were tougher and more difficult for insects to chew. All adults died within 1 mo on live oaks in the study by Diaz et al. (2005), whereas 96% of adults died after 23 d infestation on pond apple in the present study, probably because of starvation in both studies. In the field, however, adult weevils were observed successfully feeding on live oak leaves during flushing, when leaves were less rigid and thus consumable (Diaz et al. 2005). However, on pond apple in the present study, leaves were regenerated continuously and soft leaves were always available, which sometimes had a few small notches from *Diaprepes* root weevil leaf feeding. In addition,

adults tended to congregate on screen tops and sides, also on plant stems, but they avoided pond apple leaves, unlike with the other plant species. Live oaks are a host plant for adult *Diaprepes* root weevil (Simpson et al. 1996, Mannion et al. 2003, Diaz et al. 2005), but pond apple has not been reported as a host for adults.

Although an objective of the present study was not to compare plant species, differences in average maximum feeding damage suggest mahogany was most susceptible to damage by adult *Diaprepes* root weevil, followed by buttonwood and Surinam cherry, with pond apple the least affected. However, adults laid the most egg clusters per plant and appeared to live the longest on buttonwood compared to the other plant species tested, whereas pond apple had the fewest egg clusters and shortest adult lifespan. Hence, differences in the maximum number of egg clusters per treatment per plant species indicate a slightly different order of susceptibility to adult *Diaprepes* root weevil: number of egg clusters per plant was greatest for green buttonwood, then mahogany, then Surinam cherry, and was lowest for pond apple. Overall differences in these maximum means among treatments and plant species suggest the order of susceptibility to combined feeding damage and number of egg clusters is mahogany and green buttonwood, then Surinam cherry, with pond apple the least affected.

Because buttonwood plants are highly attractive for adult oviposition (Simpson et al. 1996, Mannion et al. 2003), the resulting larval infestation may be sufficiently high to significantly reduce photosynthesis and growth (Diaz et al. 2006, Chapter 2). However, buttonwood is a landscape plant for which aesthetics are quite important. Therefore, adult insects may be more problematic than larvae on buttonwoods because foliar feeding damage makes them less attractive and can reduce sales.

Responses of woody plants to flooding include suppressed formation and expansion of leaves, reduced photosynthesis and carbohydrate transport, and changes in the allocation of

photosynthates within plants (Kozlowski 1997). Thus, flooding may reduce photosynthates, which help to produce leaves and root masses that provide food for larvae and adults of *Diaprepes* root weevils. Flooding may therefore indirectly reduce the initial food available to larvae and adults as well as subsequent products of photosynthesis used to repair feeding damage. Surinam cherry generally performed much better in non-flooded than flooded conditions on most gas exchange and growth parameters measured. It was also one of two plants species in the present study in which adults fed significantly more on non-flooded than on flooded plants in the same cage. According to Bernays and Chapman (1994), most phytophagous insect species are confined to certain plant parts, which typically determine physical and chemical attributes to which insects respond. Because adult *Diaprepes* root weevil are foliage feeders, better quality of foliage as food may have caused *Diaprepes* root weevil adults to prefer non-flooded over flooded Surinam cherry and mahogany foliage in the present study. There is no indication in the literature that *Diaprepes* root weevil is attracted to ethanol or other products of flooding, anaerobic metabolism, or associated microbes.

Short plant height and compact foliage of Surinam cherry may have aided within-cage movement of *Diaprepes* root weevil adults to plants they preferred for feeding, although they oviposited in statistically equal numbers on non-flooded and flooded plants. *Diaprepes* root weevil adults significantly preferred non-flooded over flooded Surinam cherry plants for feeding. Hence, significant differences between non-flooded and flooded plants in a given cage for oviposition may have occurred if plants were spaced farther apart and in larger cages. Possible reasons why non-flooded Surinam cherry plants were preferred over flooded plants include differences in chemicals emitted by the two flood treatments, such as attractants emitted by non-flooded plants or repellents emitted by flooded plants, but this was not measured. However, Wee et al. (2008) showed that healthy, non-flooded plants emitted chemicals that attracted another

weevil with similar feeding guilds and phenology to *Diaprepes* root weevil, the Fuller's rose weevil, *Pantomorus cervinus* (Boheman) (Coleoptera: Curculionidae). Wee et al. (2008) tested behavioral and electrophysiological responses of Fuller's rose weevil to host plant volatiles from leaves of sweet lemon (*Citrus limonum* Risso., Rutaceae) and white clover (*Trifolium repens* L., Fabaceae). Wee et al. (2008) identified eight monoterpene volatiles and a mixture of them in lemon leaf oil and two green leaf volatiles from white clover leaves, and all these chemicals were detectable by Fuller's rose weevil (Wee et al. 2008). However, none of these compounds seemed to be byproducts of flooding, anaerobic metabolism, or associated microbes (Fulton and Erickson 1964, Wang et al. 1967, Hook et al. 1971, Rowe and Catlin 1971, Culbert and Ford 1972, Ponnampereuma 1984, Kozlowski 1997).

Non-flooded mahogany and Surinam cherry plants both tended to have more fresh, whole (mostly uncut) leaf material than flooded plants as shown by their significantly higher leaf fresh and dry weights. Hence, *Diaprepes* root weevil adults may have preferred non-flooded over flooded mahogany and Surinam cherry leaves because they produced volatiles in the concentration and mixture needed to attract adults. In addition, Schroeder and Beavers (1985) found that adult male *Diaprepes* root weevil produced an aggregation pheromone that attracts both males and female adults. Hence, greater leaf damage found on non-flooded than flooded Surinam cherry and mahogany plants may have resulted from initial attraction from both plant volatiles and insect aggregation pheromones causing adults to aggregate and feed more on non-flooded than on flooded plants.

Ingham and Detling (1986) artificially defoliated 55% of the foliage of sideoats gramma grass, *Bouteloua curtipendula* (Michx.) Torr. Root biomass, shoot biomass, and tiller number were each reduced by artificial defoliation, though net CO<sub>2</sub> assimilation and transpiration significantly increased in the remaining foliage after artificial defoliation. With mahogany in the

present study, net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance were each significantly higher in infested than non-infested plants on the 9<sup>th</sup> (of 11) measurement. However, mahogany fresh and dry leaf weights were each significantly higher for non-infested than infested plants, but there were no significant differences in root, stem, or total fresh or dry weights, stem diameter, plant height. As noted, Welter (1989) stated that whole-leaf consumption by adults, which are in the leaf consumer feeding guild, often tends to increase net CO<sub>2</sub> assimilation when measured on a leaf-area basis, although this effect is atypical compared to the other guilds, such as root-feeders, which tend to decrease net CO<sub>2</sub> assimilation. With regard to whole-leaf consumption, increased net CO<sub>2</sub> assimilation may be explained by the availability of the same quantity of nutrients such as nitrogen delivered to a smaller leaf area after defoliation by the insects. This may render more nitrogen and other nutrients available per remaining leaf area to synthesize chlorophyll and supply other reactions that increase net CO<sub>2</sub> assimilation.

Mahogany, like Surinam cherry, was not flood-adapted because it had significantly higher net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, and growth variables such as stem diameter in non-flooded than in flooded treatments. Hence, the root environment may have been less aerobic than with buttonwood or pond apple, which were more flood adapted. Flooded mahogany roots may therefore have created more toxins, such as ethanol and formaldehyde (Kozlowski 1997), which may repel *Diaprepes* root weevil adults; this is suggested by the feeding preference exhibited by weevils for non-flooded over flooded mahogany. Mahogany was one of two plant species in which *Diaprepes* root weevil adults showed a significant preference of non-flooded over flooded plants based on foliar damage. Surprisingly, it was the only plant species in which adults also had an ovipositional preference of non-flooded over flooded plants based on number of egg clusters, although significant only for one measurement.

Possible reasons for this preference include a less favorable chemical environment in flooded than in non-flooded plants for attracting adults to feed and oviposit on leaves.

As noted, adult *Diaprepes* root weevil fed on mahogany differently than on the other plant species by eating the outer stem and bark in addition to petioles and leaflet blades. However, adults tended to avoid older leaves in favor of younger leaves. Diaz et al. (2005) showed that adult *Diaprepes* root weevil preferred younger over older leaves of live oak suggesting they may have preferred the softer texture of younger leaves because they were easier to consume than the harder, mature leaves. Differences in leaf chemistry (phagostimulents, etc.) may have also rendered new leaves better tasting than old leaves. By similar mechanisms, mahogany bark on young stems may be more attractive to adult *Diaprepes* root weevil for feeding than bark on other plant species tested.

The results of this study confirmed the findings of Simpson et al. (1996) that green buttonwood, Surinam cherry, and mahogany are host plants of adult *Diaprepes* root weevil. However, pond apple was not found to be a host for adult *Diaprepes* root weevil in the present study. Adults in the present study tended to congregate on screen tops instead of on pond apple foliage and were rarely seen feeding on leaves. In addition, very few characteristic semicircular leaf notches were found along pond apple leaf margins, and these notches usually appeared much smaller than on the other plant species. *Diaprepes* root weevil adults also laid the fewest egg clusters and lived the shortest lifespan on pond apple than observed on green buttonwood, Surinam cherry, and mahogany. Hence, the test with pond apple was the shortest of the four tests in the present study. However in another study (Chapter 2), pond apple was found to be a larval host for *Diaprepes* root weevil, although without significantly affecting leaf gas exchange or plant growth. Although pond apple can support *Diaprepes* root weevil larval development, it

does not seem to attract the adults, which are needed to oviposit and thereby infest pond apple with larvae.

Nigg et al. (2001a) recommended controlling adult *Diaprepes* root weevil to reduce larval feeding to tolerable levels, and pond apple may already have leaf chemistry rendering this step (and accompanying costs) unnecessary. Pond apple is well adapted to flooding and marl soil (Schaffer 1998, Núñez-Elisea et al. 1999, Ojeda et al. 2004) and has potential as a rootstock for commercial *Annona* spp., which are not as flood-tolerant as pond apple (Núñez-Elisea et al. 1999). The present study provides additional evidence that pond apple may be a good choice for commercial rootstock of other *Annona* spp. because adult *Diaprepes* root weevil rarely oviposited on pond apple, hence, it would seem unlikely to become infested. However, commercial fruit tree species grafted as scions on pond apple rootstock should be tested for adult feeding and oviposition behavior because once used as a rootstock, the scion species would determine if adults would oviposit and thus supply roots with larvae. However, even if this happens, once a scion species is grafted onto pond apple, economic losses may not occur because the larvae may not significantly affect plant gas exchange or growth (Chapter 2).

Flooding has been studied as a means of controlling *Diaprepes* root weevil larvae, such as in sugar cane fields, where flooding may provide effective control, although only in the summer and fall when floodwater temperatures are close to their maximum (27°C) (Hall and Cherry 1993, Shapiro et al. 1997). The results of this study can help prioritize decisions for pest management of green buttonwood, Surinam cherry, mahogany, and pond apple plants. For example, pond apple may never require treatment to control *Diaprepes* root weevil larvae, while Surinam cherry and green buttonwood may benefit from such a treatment. Future studies should investigate susceptibility to damage from larvae and adult *Diaprepes* root weevil feeding for other widely planted landscape plant species often visibly infested with weevils, such as *Bulnesia*

*arborea* (Jacq.) Engl. (Zygophyllaceae) and black olive (*B. buceras*). Mannion et al. (2003) found adults, feeding damage, and egg masses on *B. buceras*. Because of the tendency of *Diaprepes* root weevil adults to disperse slowly, and to feed and oviposit on the same plants they are found on, the presence of large populations of adult weevils in these tree species suggests high production of eggs and larvae.

For future *Diaprepes* root weevil adult choice tests, particularly in relation to host plant flooding, it would be beneficial to identify *Diaprepes* root weevil attractants or repellents for flooded plants because flooding sometimes significantly affected adult feeding and/or oviposition preferences. This would help determine whether the attractants or repellents are ethanol or something else or could potentially be used to help manage the pest.

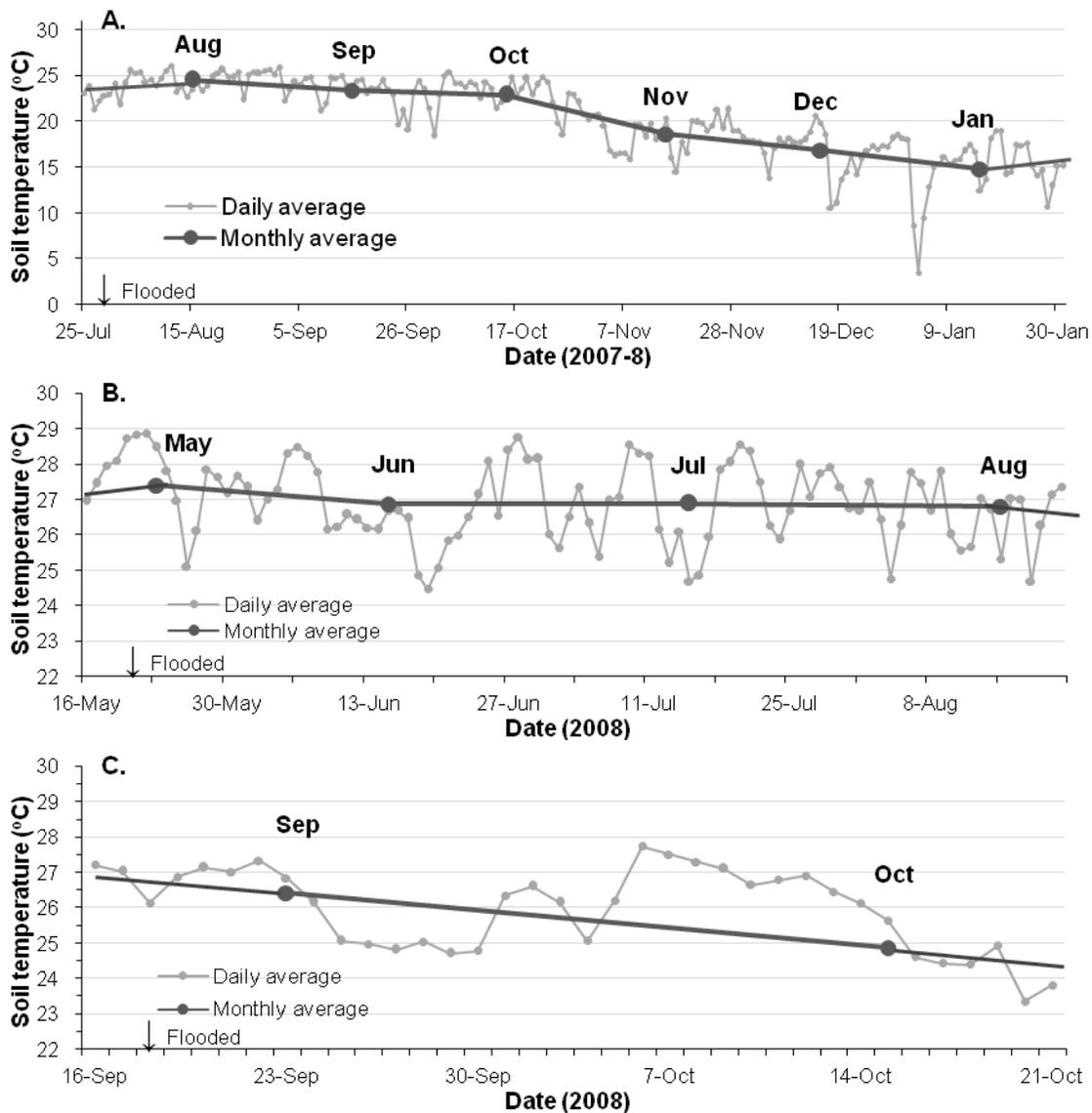


Figure 6-1. Soil temperatures during the experiment. A) green buttonwood and Surinam cherry, B) mahogany, and C) pond apple. Each point is the average of one sensor for green buttonwood, Surinam cherry, and mahogany (A-B), and three sensors for pond apple (C).

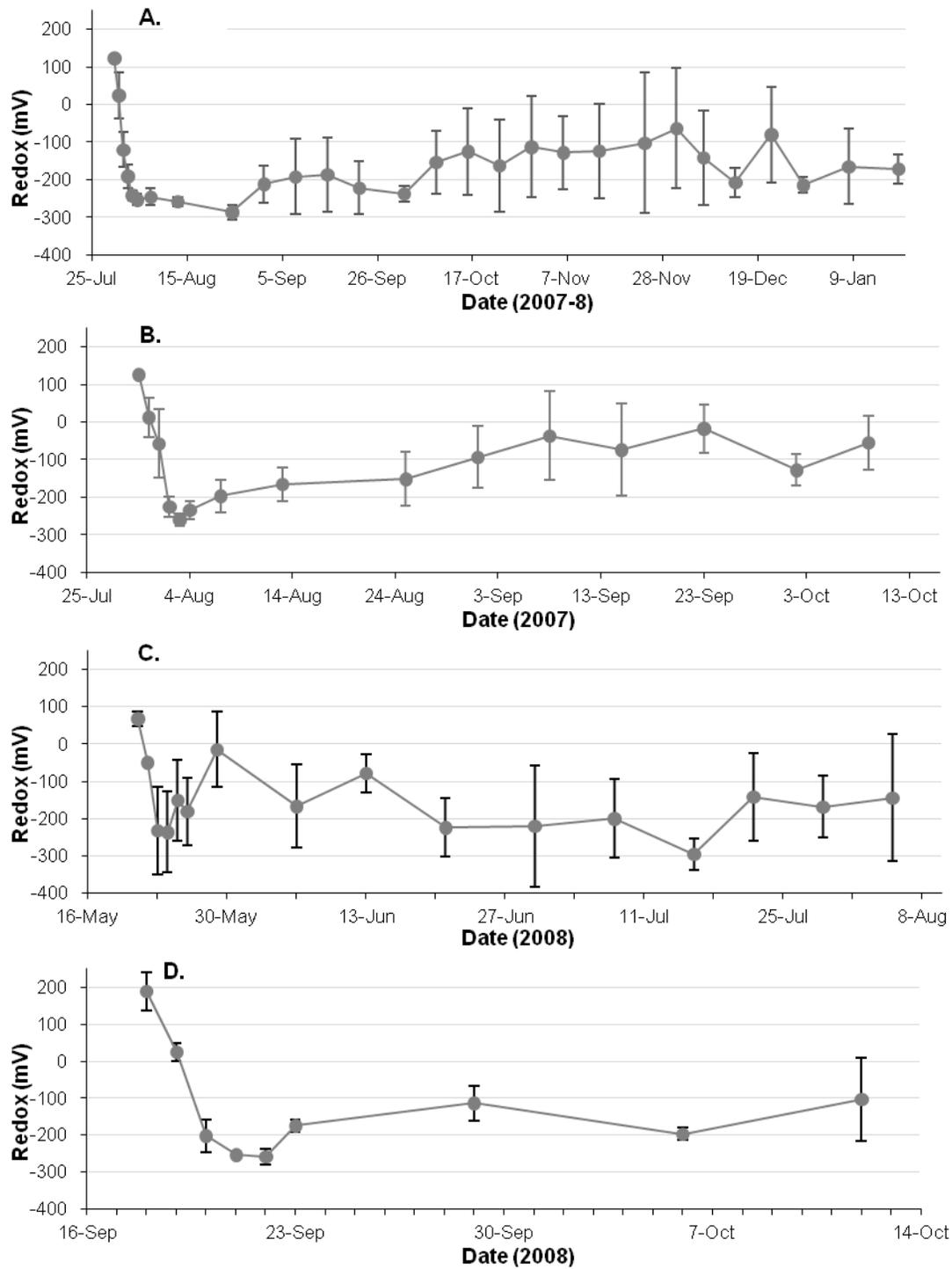


Figure 6-2. Soil redox potential. A) green buttonwood, B) Surinam cherry, C) mahogany, and D) pond apple. Each point represents the mean  $\pm$  SD of four sensors.

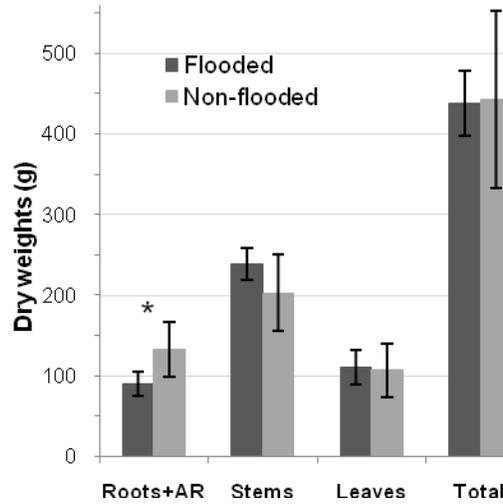


Figure 6-3. Effect of flooding on dry weights of infested green buttonwood plants. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

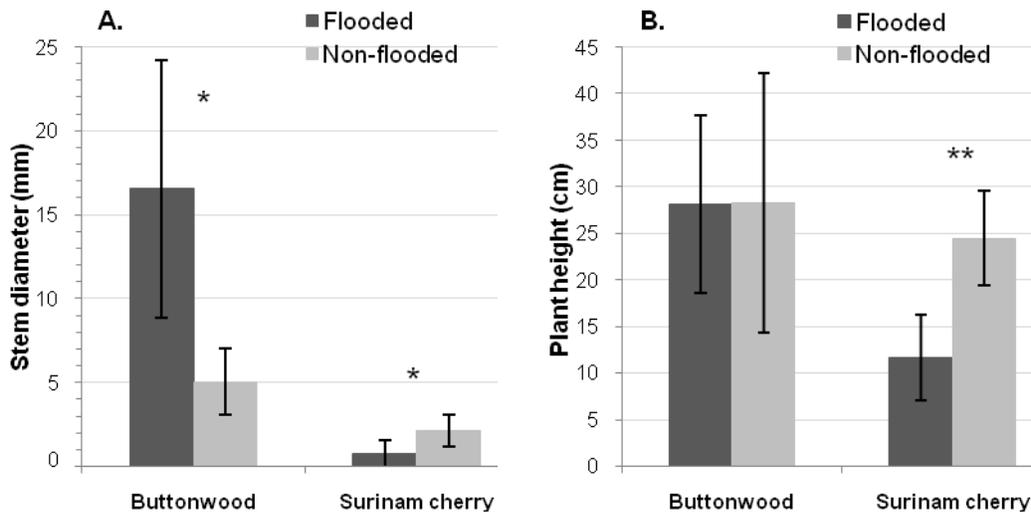


Figure 6-4. Effects of flooding on increases in A) stem diameter and B) plant height for infested green buttonwood and Surinam cherry plants. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments within plant species at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

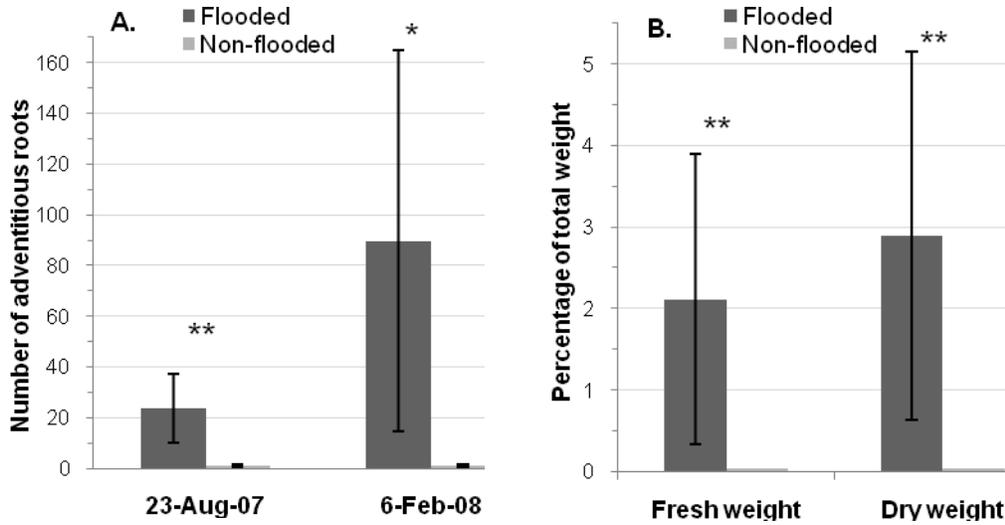


Figure 6-5. Effects of flooding on infested green buttonwood trees. A) number of adventitious roots and B) their percentage of total weight. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

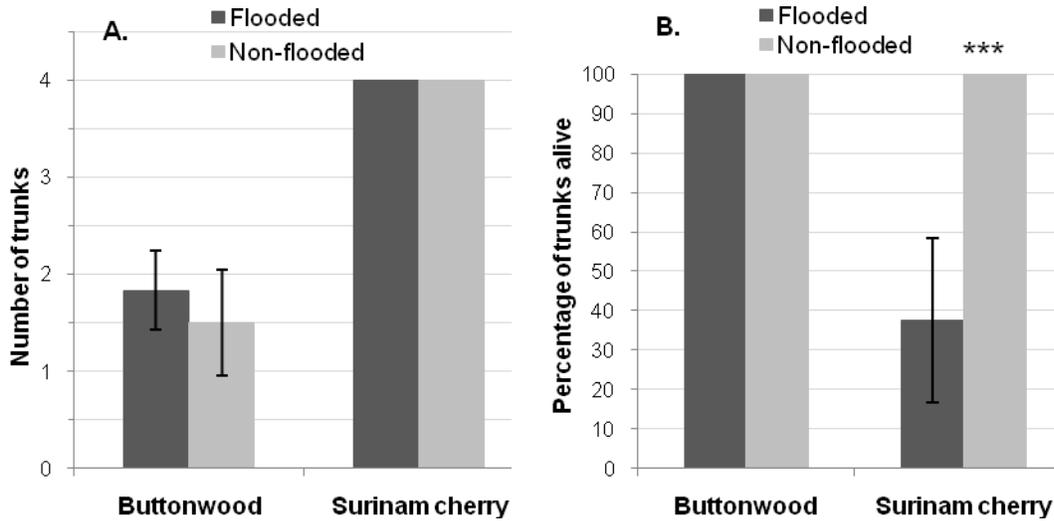


Figure 6-6. A) number of trunks and B) effects of flooding on percentage of trunks found alive at harvest for infested green buttonwood and Surinam cherry plants. Mahogany and pond apple each had one trunk. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

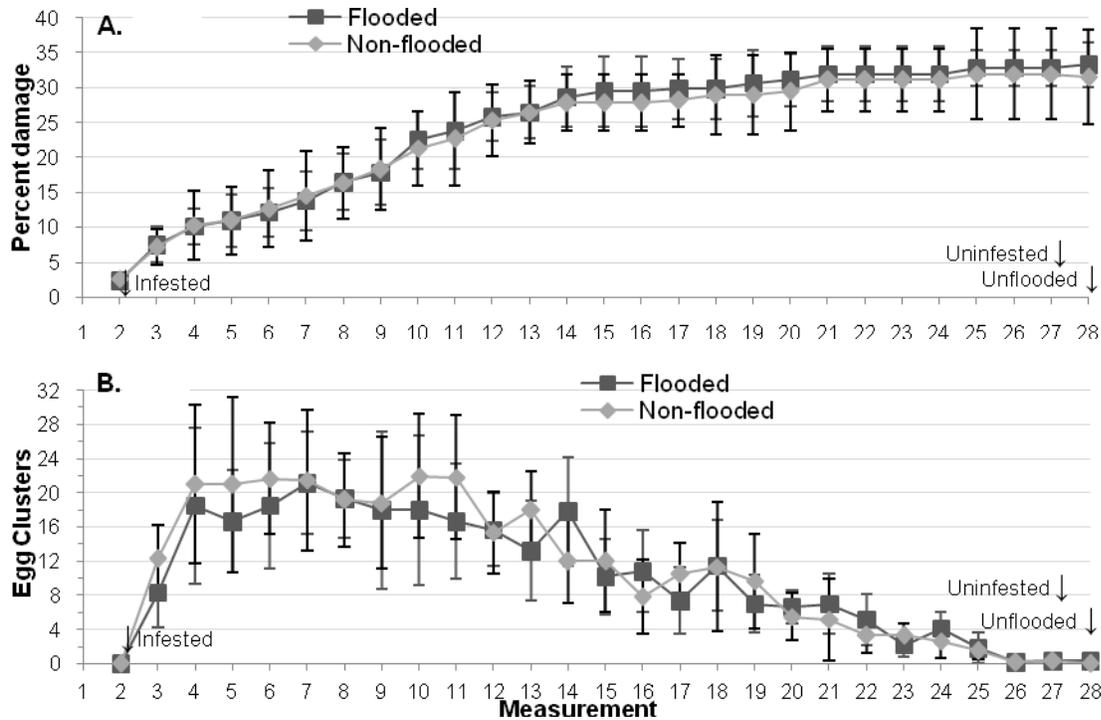


Figure 6-7. Effects of flooding on infested green buttonwood plants. A) Diaprepes root weevil adult feeding damage and B) number of egg clusters per plant. Symbols represent means  $\pm$  SD.

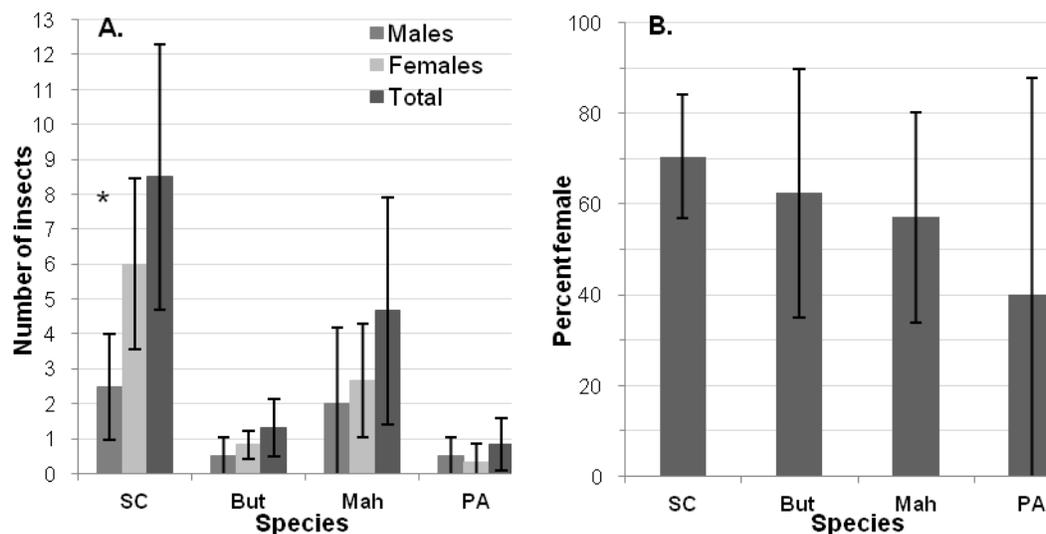


Figure 6-8. Effects of flooding and infestation on A) number and sex of adult Diaprepes root weevil recovered at the end of the test and B) percentage of adults at the end of the test that were female (a measure of sex ratio). Bars represent means  $\pm$  SD. Asterisks indicate significant differences between numbers of male and female adults recovered within plant species at  $* P \leq 0.05$  according to a standard T-test. But = green buttonwood, Mah = mahogany, SC = Surinam cherry, and PA = pond apple.

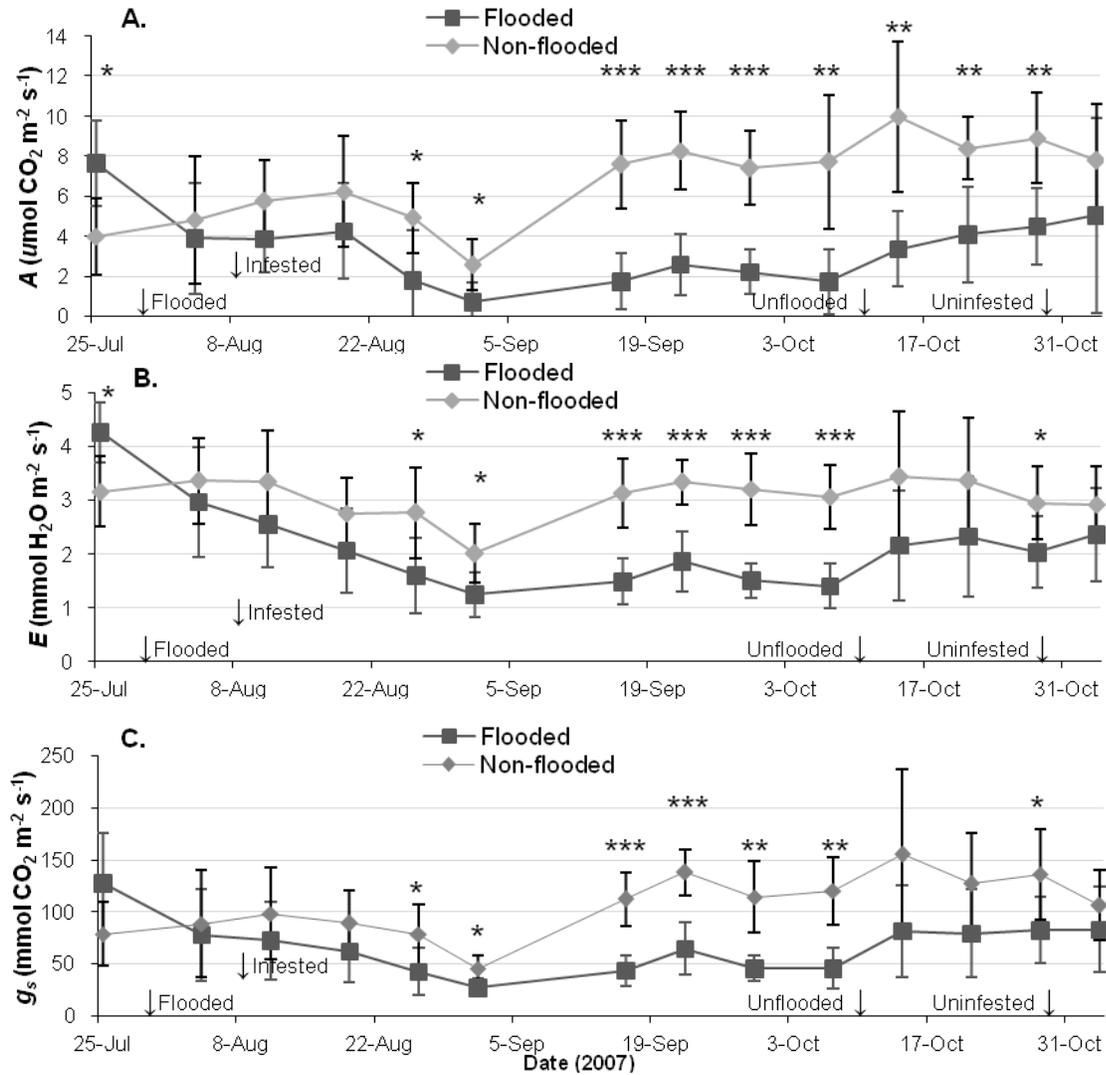


Figure 6-9. Effects of flooding on infested Surinam cherry plants. A) net CO<sub>2</sub> assimilation, B) transpiration, and C) stomatal conductance. Symbols represent means ± SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

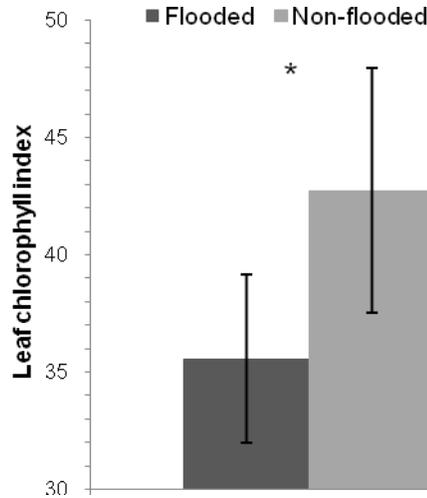


Figure 6-10. Leaf chlorophyll index for infested Surinam cherry plants measured 8-Nov-07 (pre-harvest). Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$  according to a standard T-test.

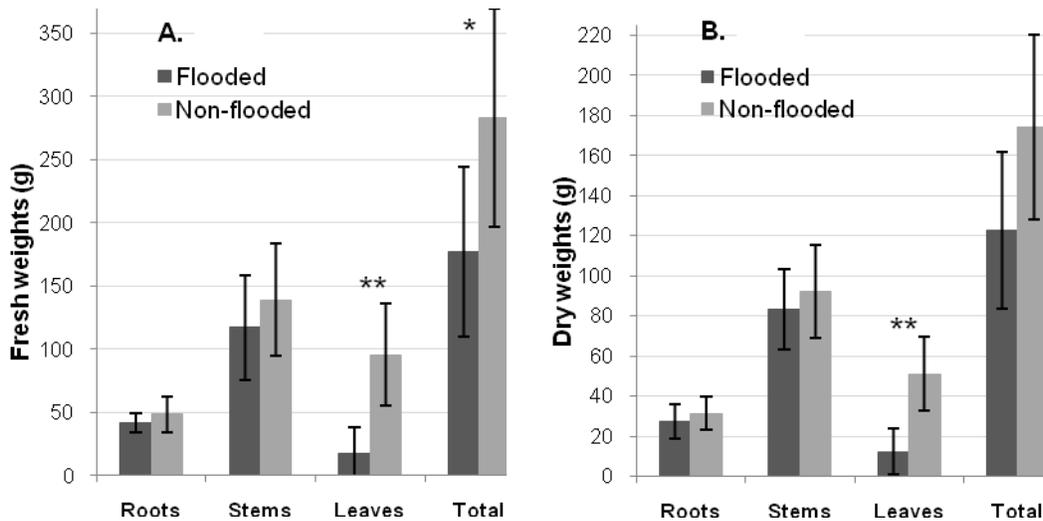


Figure 6-11. Effects of flooding on infested Surinam cherry plants. A) fresh weights and B) dry weights. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

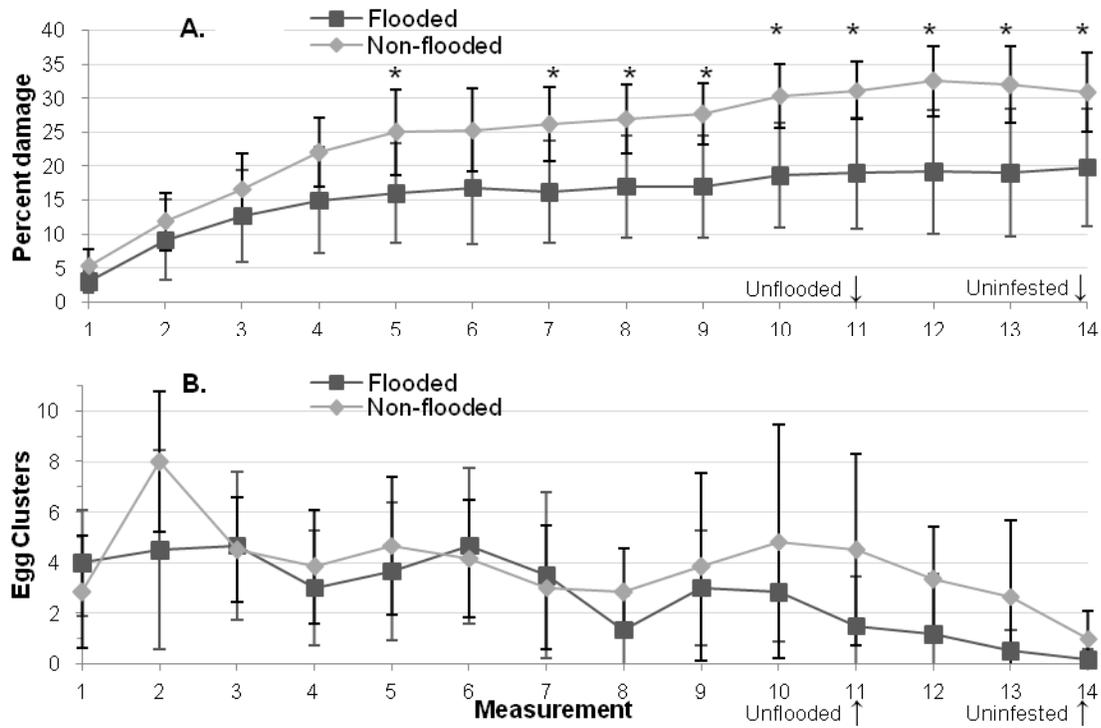


Figure 6-12. Effects of flooding on *Diaprepes*-infested Surinam cherry plants. A) adult feeding damage and B) number of egg clusters per plant. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

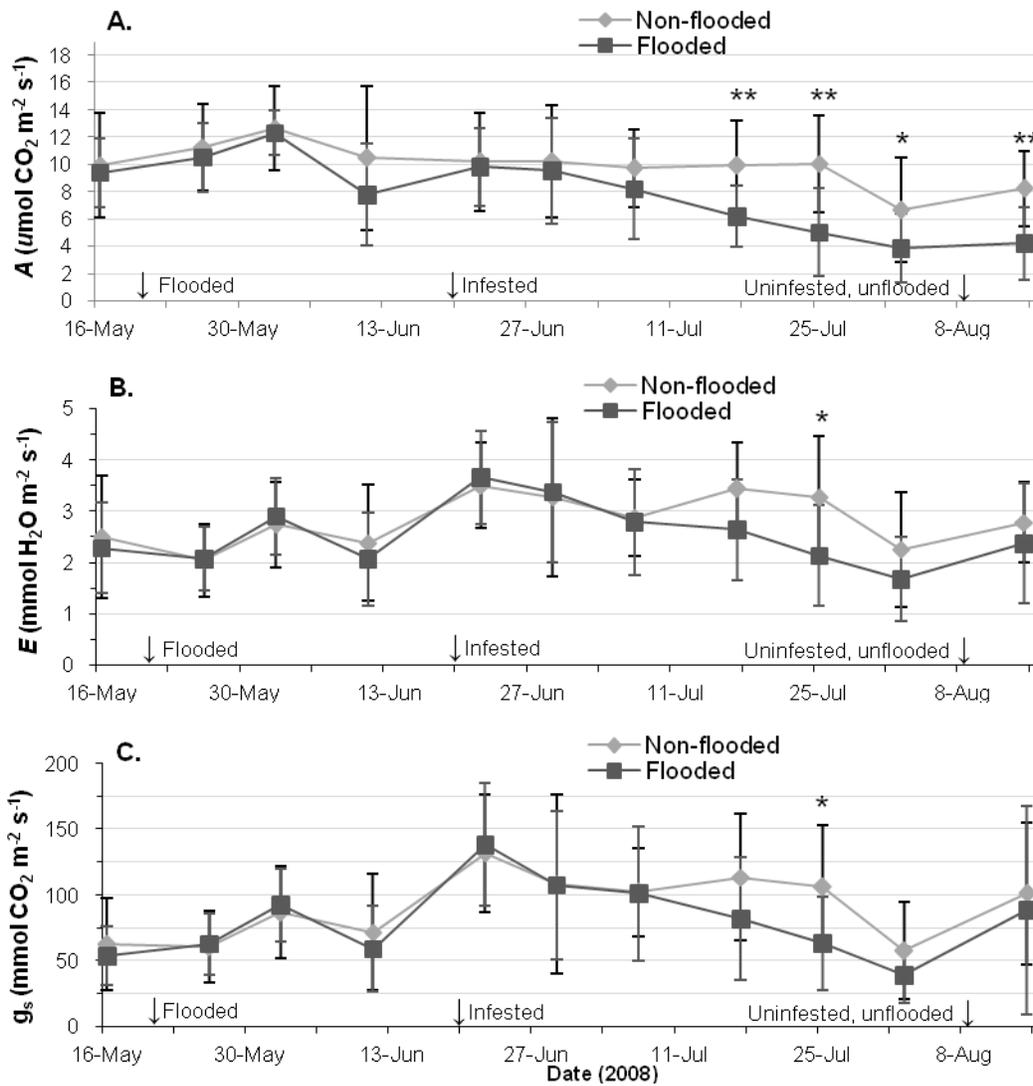


Figure 6-13. Effects of flooding on mahogany trees. A) net CO<sub>2</sub> assimilation, B) transpiration, and C) stomatal conductance. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

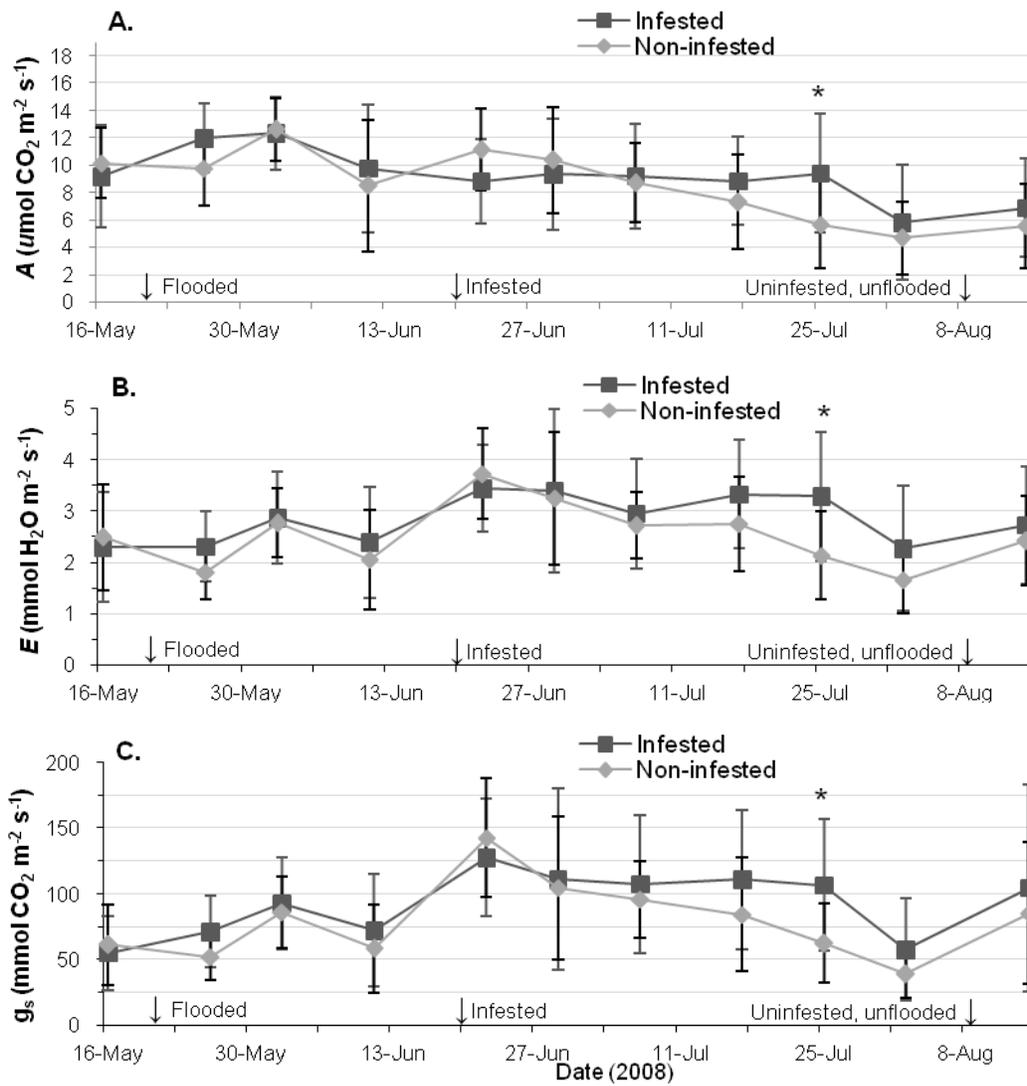


Figure 6-14. Effects of infestation on mahogany trees. A) net CO<sub>2</sub> assimilation, B) transpiration, and C) stomatal conductance. Symbols represent means ± SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

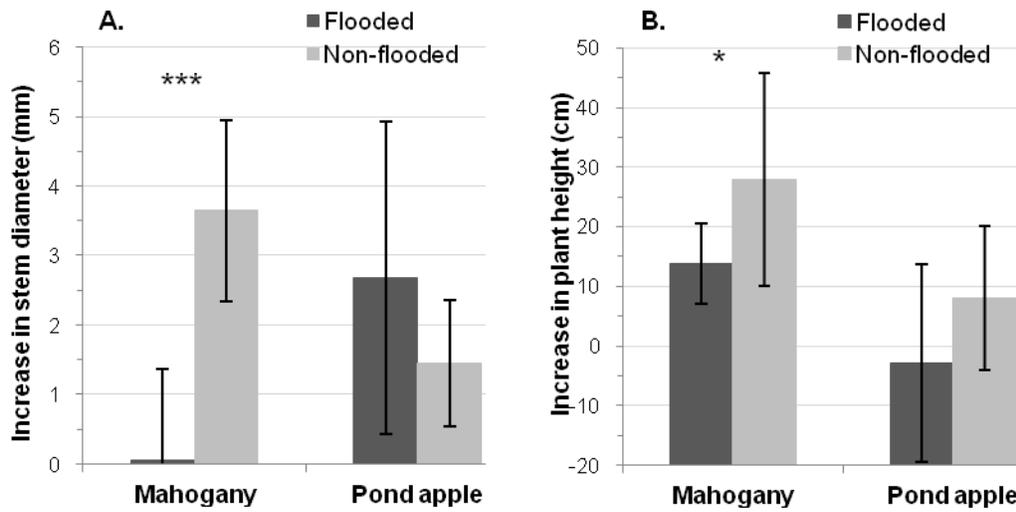


Figure 6-15. Effects of flooding on mahogany and pond apple plants. A) increase in stem diameter and B) increase in plant height. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

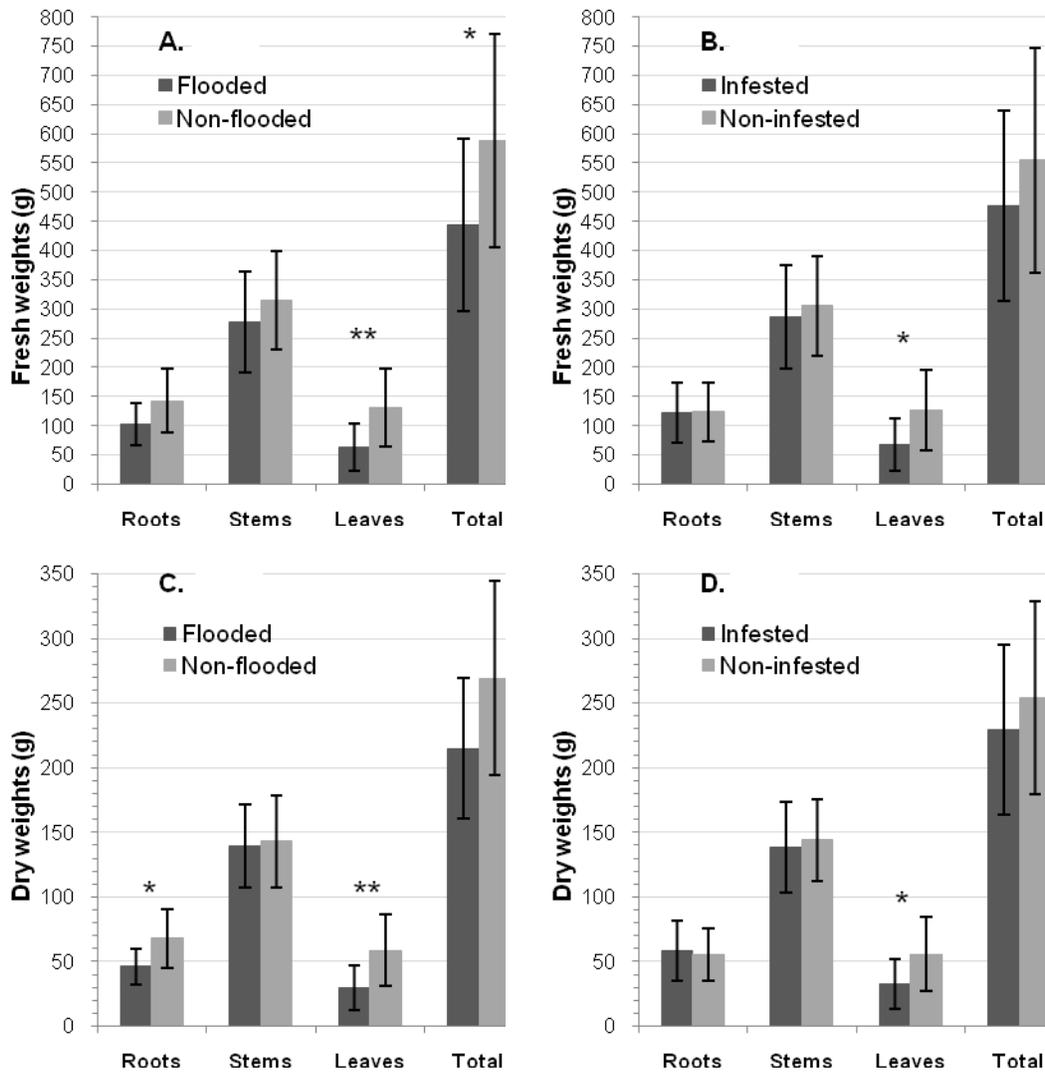


Figure 6-16. Effects of flooding and infestation on mahogany trees. A-B) fresh weights and C-D) dry weights. Bars represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

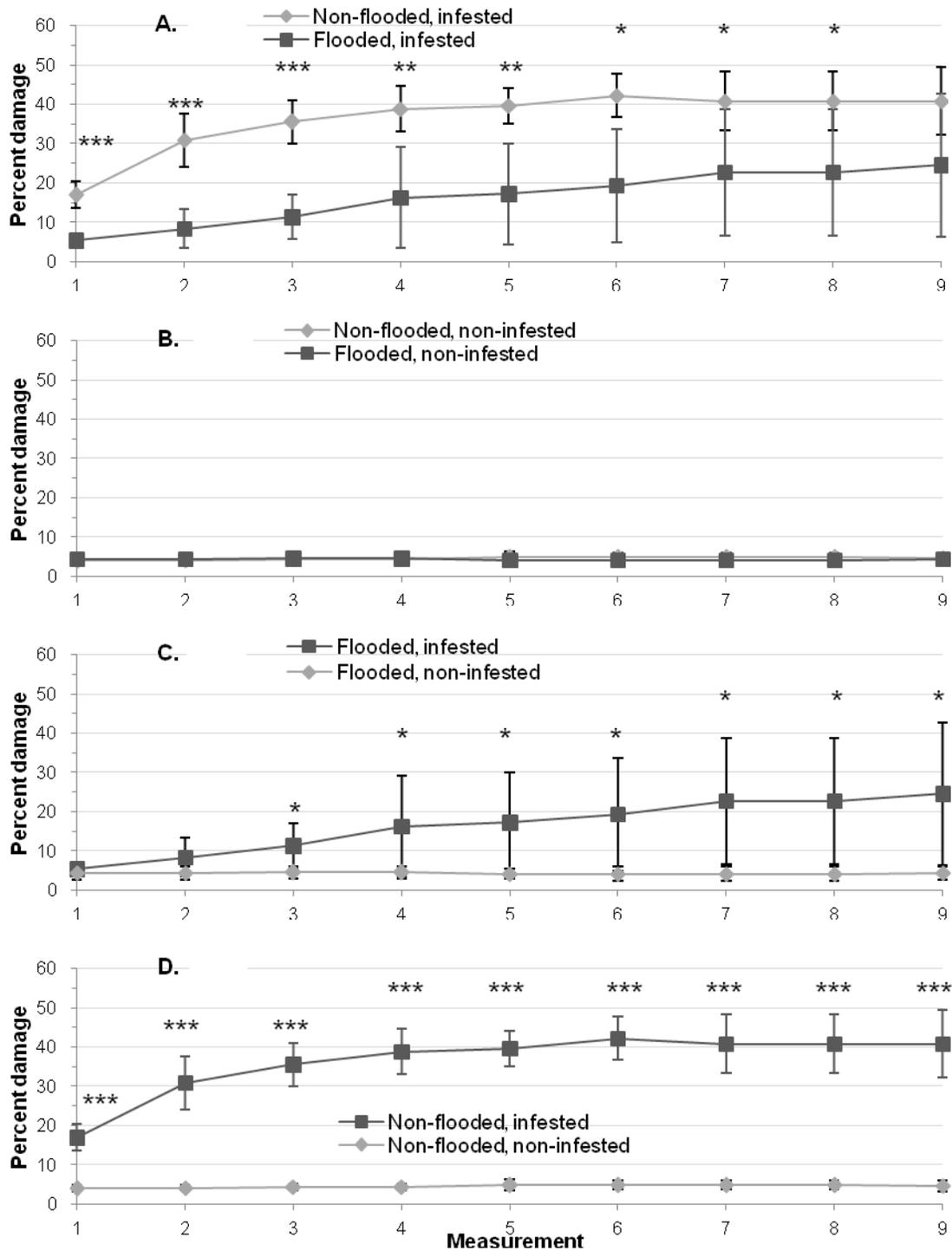


Figure 6-17. Effects of flooding and infestation on adult *Diaprepes* root weevil feeding damage on mahogany trees. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

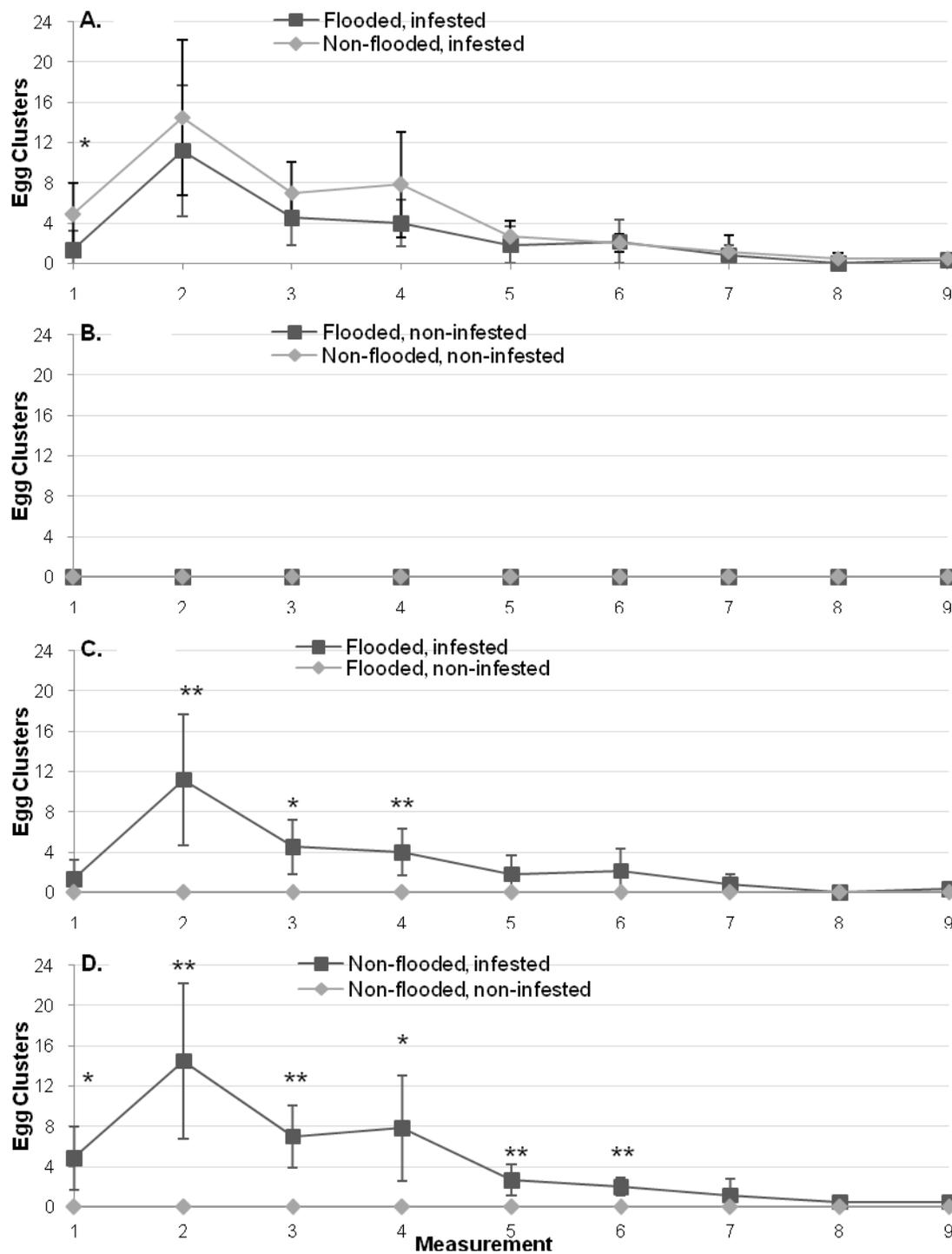


Figure 6-18. Effects of flooding and infestation on number of *Diaprepes* root weevil egg clusters per mahogany tree. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

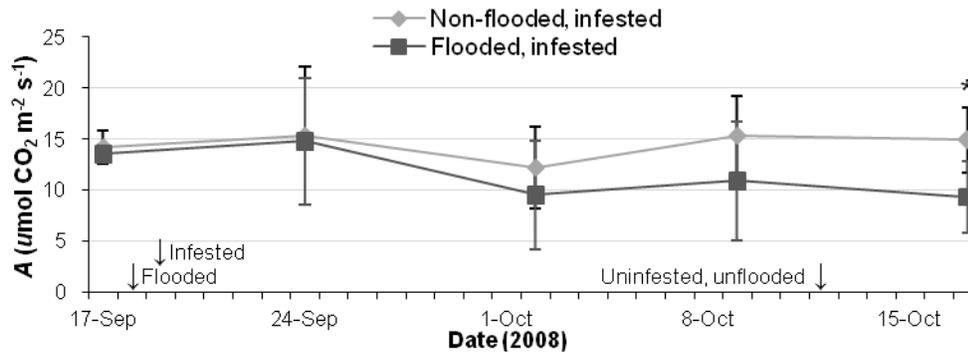


Figure 6-19. Effects of flooding on net CO<sub>2</sub> assimilation of infested pond apple trees. Symbols represent means ± SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

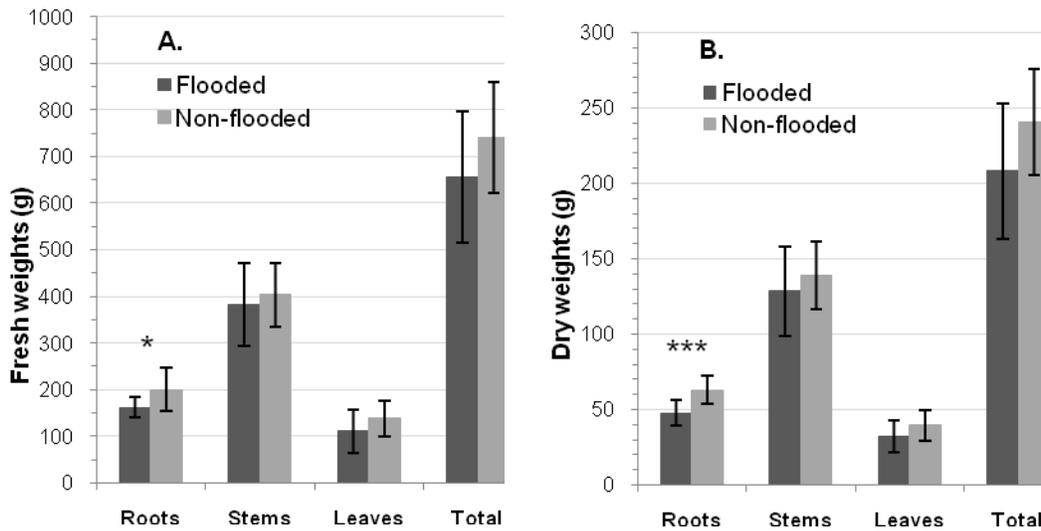


Figure 6-20. Effects of flooding on pond apple plants. A) fresh weights and B) dry weights. Bars represent means ± SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

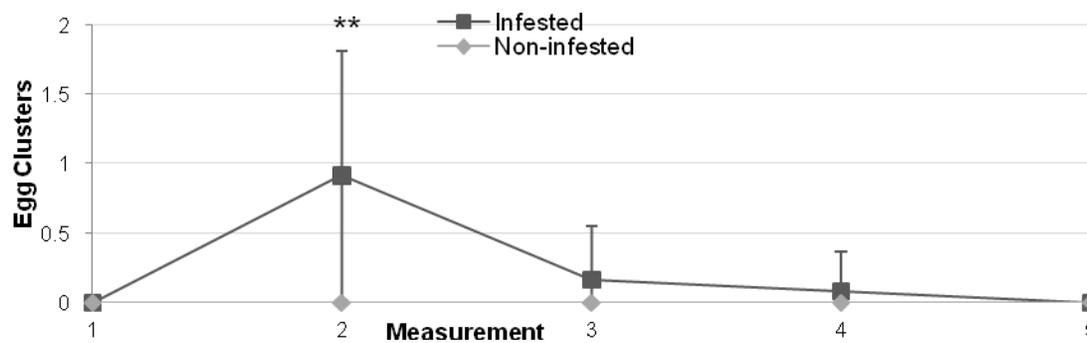


Figure 6-21. Effect of adult *Diaprepes* root weevil infestation on number of egg clusters per pond apple plant. Symbols represent means  $\pm$  SD. Asterisks indicate significant differences between treatments at \*  $P \leq 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  according to a standard T-test.

## CHAPTER 7 CONCLUSIONS

The goal of this project was to determine effects of *Diaprepes* root weevil herbivory, flooding, and the interaction of these two stresses on leaf gas exchange and growth of select woody ornamental plant species. Leaf gas exchange included net CO<sub>2</sub> assimilation, transpiration, stomatal conductance of water vapor, and internal CO<sub>2</sub> concentration. Diaz (2005) and Diaz et al. (2005, 2006) investigated effects of larval and adult *Diaprepes* root weevil herbivory with and without flooding on leaf gas exchange and growth of woody ornamental plants. However, they left several unanswered questions about the growth and physiological responses of ornamental plants to short-term flooding, cyclical flooding, and soil type. These questions included: 1) what are the physiological and growth responses of ornamental plants to only flooding; 2) what is the response of *Diaprepes* root weevil larvae in marl soil typical of south Florida field nurseries compared to nursery potting soils; 3) would intermittent (cyclical) flooding affect *Diaprepes* root weevil herbivory differently than short-term (continuous) flooding; 4) what are the effects of long-term feeding and multiple generations of *Diaprepes* root weevil (at least 2 mo for adults and 8 mo for all stadia combined) ; 5) can a better comparison be made for effects of flooding versus *Diaprepes* root weevil herbivory on ornamental plants; and 6) does flooding and the resulting anaerobic respiration attract adult *Diaprepes* root weevil.

To help clarify relationships between *Diaprepes* root weevil herbivory, host plant physiology, and flooding, and to answer some of these questions, I proposed the following hypotheses: 1) larval herbivory reduces leaf gas exchange and growth of green buttonwood (*Conocarpus erectus* L.), mahogany (*Swietenia mahagoni* Jacq.), Surinam cherry (*Eugenia uniflora* L.), and pond apple (*Annona glabra* L.), which are frequently grown as ornamental or fruit plants in southern Florida; 2) flooding reduces plant growth and leaf gas exchange more in

marl soil than in a standard nursery potting medium; 3) flooding reduces larval survival more in marl soil than in a standard potting medium; 4) survival rates of *Diaprepes* root weevil larvae and plant biomass are reduced more by short-term than by cyclical flooding; and 5) flooding reduces leaf gas exchange, growth, leaf feeding damage, and attraction to and oviposition on host plants by adult *Diaprepes* root weevil.

Based on differences in leaf gas exchange and plant growth between infested and non-infested plants of the four species tested, green buttonwood and Surinam cherry shared the greatest vulnerability to *Diaprepes* root weevil larval feeding, followed by mahogany, with pond apple the least affected. For green buttonwood, larval feeding reduced net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, and internal CO<sub>2</sub> concentration by 10-54% just 3 h after infestation with large, seventh instar larvae. However, 4 wk later, net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance were 11-37% higher for infested than non-infested plants on leaf area bases, which may exemplify physiological compensation to insect herbivory within leaves. For Surinam cherry plants, larval feeding reduced net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance by 7-32%. For mahogany and pond apple, there were few or no significant differences in leaf gas exchange between infested and non-infested plants.

For all plant species, mean root and shoot fresh and dry weights were higher for non-infested than infested plants with significant differences most frequent for green buttonwood (37-85% higher), followed by Surinam cherry (37-143% higher), mahogany (49-84% higher), and pond apple (24-46% higher), which had no significant differences. There were significant differences among plant species in *Diaprepes* root weevil mean head capsule widths, thus larval instars, of larvae recovered from soil with the largest larvae from Surinam cherry ( $2.59 \pm 0.19$  mm) and the smallest from mahogany ( $2.29 \pm 0.06$  mm).

Based on combined leaf gas exchange and biomass data, Surinam cherry performed best in potting medium compared to marl soil, followed by buttonwood, mahogany, and pond apple. Based on leaf gas exchange, growth, and signs of morphological adaptation to flooding, order of flood tolerance in marl soil was pond apple with the highest, followed by buttonwood, then mahogany, then Surinam cherry; in potting medium the order was pond apple and buttonwood with the greatest flood tolerance, followed by mahogany, then Surinam cherry. The most apparent trend was for gas exchange and growth of green buttonwood, mahogany, and Surinam cherry to be significantly lower for plants growing in flooded marl soil than in either non-flooded marl soil or flooded potting medium. In contrast to green buttonwood, mahogany, or Surinam cherry, pond apple net CO<sub>2</sub> assimilation, stomatal conductance, and plant growth (more consistently than the other plant species) tended to be higher for flooded than non-flooded plants and for plants in marl soil than in potting medium. Green buttonwood and pond apple each developed significantly larger stem diameters in flooded than in non-flooded conditions and swollen stem lenticels under flooded conditions. Adventitious roots were also observed on flooded green buttonwood trunks, but were absent from non-flooded plants. Hence, green buttonwood and pond apple were the two most flood-adapted plant species possibly because they are native to areas that are often flooded.

In marl soil, significantly more *Diaprepes* root weevil larvae survived after 38 d in non-flooded than in flooded conditions. Similarly, more larvae survived in non-flooded than in flooded potting medium, from which no larvae were recovered. Larval survival rates were significantly higher in flooded marl soil than 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 were therefore at least one instar smaller than larvae recovered from non-flooded marl soil or non-

flooded potting medium. In summary, flooding marl soil or potting medium reduced survival and often growth of *Diaprepes* root weevil larvae.

Flooding with three 2-d cycles appeared more likely to control *Diaprepes* root weevil larval infestation in Swingle citrumelo than in green buttonwood plants. Most significant differences noted for each plant species were in response to cyclical flooding and after all flood cycles had ended. The effects of flooding may be cumulative for green buttonwood and effects of insects may be cumulative for Swingle citrumelo because cyclical flooding caused most of the significant differences noted for green buttonwood and all for Swingle citrumelo. Contrary to similar studies, green buttonwood was the most flood-sensitive plant species, possibly because flood periods were too short for plants to develop morphological or anatomical adaptations such as increased stem aerenchyma, hypertrophic stem lenticels, or adventitious roots. Swingle citrumelo was the most sensitive plant species to *Diaprepes* root weevil larval feeding. Flooding significantly reduced percent survival and head capsule widths of recovered larvae, but only for cyclically flooded Swingle citrumelo plants, which also had significantly less root damage on flooded than on non-flooded plants.

For green buttonwood, there were generally no significant differences between flooded and non-flooded plants in net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, plant height, number of inflorescences per plant, root, stem, leaf, or total fresh weights, or stem, leaf, or total dry weights. However, root dry weight (including adventitious roots) was significantly lower for flooded than non-flooded green buttonwood plants. In contrast, the number of adventitious roots and their percentage of fresh and dry weights were significantly higher in flooded than non-flooded plants. In addition, stem diameter was significantly higher in flooded than in non-flooded green buttonwood plants. These results suggest that green buttonwood adapted to flooding, possibly through increased development of adventitious roots and aerenchyma tissue,

which is suggested by enlarged stem diameter in flooded plants. These adaptations rendered most of the leaf gas exchange, plant growth, and hence possibly food quality and preference by adult *Diaprepes* root weevil the same between flooded and non-flooded green buttonwood plants.

For Surinam cherry, however, net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, leaf chlorophyll index, leaf and total fresh weights, leaf dry weights, plant height, trunk survival, and stem diameter were higher for non-flooded than flooded plants. Adult feeding damage, but not the number of egg clusters, was significantly higher for non-flooded than flooded Surinam cherry plants. This may have resulted from non-flooded Surinam cherry plants being healthier and more appealing to the insects.

Similar to Surinam cherry, mahogany results were significantly higher for non-flooded than flooded plants on at least one measurement each for net CO<sub>2</sub> assimilation, transpiration, stomatal conductance, plant height, stem diameter, leaf and total fresh weight, and leaf and root dry weight. Net CO<sub>2</sub> assimilation, transpiration, and stomatal conductance were significantly greater for infested than non-infested mahogany plants, but only on week 9 of 11. Mahogany fresh and dry leaf weights were significantly greater for non-infested than infested plants. Adult *Diaprepes* root weevil preferred non-flooded over flooded mahogany plants based on feeding damage, which was similar to Surinam cherry. In addition, significantly more egg clusters per plant were laid on non-flooded than flooded mahogany plants on 1 of 9 measurements. Thus, mahogany was the only plant species in which *Diaprepes* root weevil adults showed an ovipositional preference within infested cages on at least one measurement. Mahogany was also unique because adults fed on stems and petioles, causing more leaf mortality than just from leaf feeding.

Although pond apple is a flood-tolerant species, it apparently was not flooded long enough to develop enlarged stems or adventitious roots as adaptations to flooding. Hence, net CO<sub>2</sub>

assimilation was significantly higher in non-flooded infested than flooded infested pond apple plants on 1 of 5 measurements, and fresh and dry root weights were significantly higher for non-flooded than flooded plants. Pond apple had the least feeding damage (4.1%) and lowest number of egg clusters (0.9) per plant. Hence, adult *Diaprepes* root weevil would seem unlikely to infest and oviposit on pond apple plants. Although pond apple can support *Diaprepes* root weevil larvae through pupation, it is unlikely to become infested with larvae.

Based on combined gas exchange and growth data, Surinam cherry showed the highest susceptibility to flooding, followed by mahogany, with buttonwood and pond apple the least affected. Based on combined maximum feeding damage and number of egg clusters per plant, buttonwood and mahogany shared the highest susceptibility to adult *Diaprepes* root weevil, followed by Surinam cherry, with pond apple the least affected. Ovipositional preference of *Diaprepes* root weevil adults for non-flooded over flooded mahogany plants was observed based on egg cluster distribution. Also, a significantly higher percentage of insect damage in non-flooded than flooded plants on most measurement dates was unique to mahogany and Surinam cherry, the two plant species most affected by flooding.

Significant interaction occurred between flooding and larval infestation during the treatment period, but only for root dry weight of cyclically flooded Swingle citrumelo. For infested plants, root dry weight was significantly higher for flooded than non-flooded plants, and with non-flooded plants, root dry weight was significantly higher for non-infested than infested plants. These results suggest that flooding for three 2-d cycles decreased loss of root mass from larval feeding, hence, flooding may help control *Diaprepes* root weevil larval infestation in Swingle citrumelo.

For adult infestation of pond apple plants, significant flooding and infestation interaction occurred only for net CO<sub>2</sub> assimilation on one measurement date, and flooding significantly

decreased net CO<sub>2</sub> assimilation on this measurement date for infested plants. Also, pond apple plants had minimal adult feeding damage from *Diaprepes* root weevil (4.1% mean maximum), and they lacked adaptations to flooding, for example: they had statistically equal stem diameters between flooded and non-flooded plants. Hence, this interaction for pond apple was believed to have resulted mainly from lack of adaptation to flooding, possibly because of short flood duration (24 d). This flood period would probably not help to control *Diaprepes* root weevil in pond apples because of low adult feeding damage with no significant differences between flooded and non-flooded or infested and non-infested plants. This flood duration is also not suggested because of occasional reduction in leaf gas exchange and plant growth in flooded compared to non-flooded pond apples. For adult infestation of mahogany plants, significant flooding and infestation interaction occurred for percentage of leaf damage from adult feeding and number of egg clusters per plant on two or more measurement dates. Flooding mahogany plants decreased leaf feeding damage and number of egg clusters per plant. However, flooding is not recommended for *Diaprepes* root weevil control in mahogany (either) because it reduces leaf gas exchange and plant growth, thus plant health.

Overall, plant damage, physiology, and growth of the plants tested were affected by host plant, flooding, and soil type; larval survival and preference were also affected by host plant, flooding, and soil type; and adult host preference was affected by host plant and flooding.

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## BIOGRAPHICAL SKETCH

Cliff G. Martin was born in Miami Florida in 1956. In his early years, he lived in Panama, Canal Zone, which is where his interest in entomology first blossomed because of the many huge *Morpho* and beautiful heliconian butterflies, leaf-cutter and giant Ponerine ants, giant wasps and spiders, and other entomological wonders in the jungle. Years later in Tallahassee, FL, his interest in horticulture ignited when he took up bonsai as a hobby. He graduated high school in 1975 and earned his B.S. degree from Florida A&M University in 1979 in ornamental horticulture. His ambition was to work in the nursery industry. For 11 years, he worked in the nursery and landscape industry in various horticultural labor positions. In the Fall 1981, however, he obtained an assistantship and entered an M.S. degree program in horticulture at Iowa State University, but chose to leave after one semester. He moved to San Luis Obispo County, CA, where he served as a ranch hand, at a cemetery, and on a fire engine crew for several years, and then to Phoenix, AZ in 1987, where as an employee and a contractor, he improved and diversified his landscape skills and experience.

In 1991, he got his first entomology job to inspect cotton boll weevil traps with the State of Arizona for 10 months. In 1992, he performed botanical surveys in Saguaro National Monument--one of his favorite jobs. These experiences have shown him the value of independence and creativity as important assets in living a safe, practical, and interesting lifestyle on a limited budget.

In 1993, he started a non-thesis M.S. graduate program. He finished his degree in entomology in 1996 and got a job with the USDA Plant Variety Protection Office in Beltsville, MD (1996-1998). He then obtained an assistantship at the University of Florida in the Department of Entomology in weed biological control. To satisfy a longing for the outdoors and botany, he took as many botany electives as possible and identified and prepared hundreds of

herbarium specimens to learn plants. Upon graduating, he worked on a 3-mo botanical survey at Ozark National Scenic Riverways in Missouri (2004), and like with Saguaro National Monument (1992), loved the botany. He then moved to Fort Myers, FL, in September 2004 to do clean-up labor after Hurricane Charlie. He subsequently applied to the University of Florida to do a Ph.D. and was offered an assistantship to work on *Diaprepes* root weevil and its effects on plants.