SOIL REDOX POTENTIAL AND LEAF STOMATAL CONDUCTANCE OF TWO CITRUS ROOTSTOCKS SUBJECTED TO FLOODING AND ROOT WEEVIL FEEDING

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Abstract. In 2002, a greenhouse study was conducted to determine the effects of different flooding durations on soil redox potential, and to compare the interaction of flooding and larval root weevil (Diaprepes abbreviatus L.) feeding on citrus seedling leaf stomatal conductance and root damage. The experimental design was completely randomized with two rootstocks (Swingle citrumelo (SWI) and Smooth Flat Seville (SFS)), four flooding durations (0, 10, 20, and 30 days), and larval feeding for 42 days. Plants were flooded, drained for a week, and then five neonate larvae per seedling were introduced onto the soils. Flooding significantly reduced soil redox potential (P < 0.001); and flooding, rootstock variety, and their interaction significantly affected leaf stomatal conductance (P < 0.001). After flooding, soil redox potential dropped from +2000 mV to below -100 mV within 1-3 days, and plant leaf stomatal conductance declined from 260 mmol m⁻²s⁻¹ to 60 mmol m⁻²s⁻¹ after 30 days for SWI. Swingle appeared to be more tolerant of water stress by flooding than SFS. Survival of Diaprepes larvae was higher in flooded treatments than non-flooded treatments (P < 0.05). Flood damaged seedlings were more susceptible to root injury by larval feeding than non-flooded seedlings. Treatments flooded for 10 days had higher stomatal conductance and lower root injury from larval feeding than those flooded for 30 days. We conclude that negative soil redox potential and a decrease of leaf stomatal conductance might be useful as early indicators of plant water stress from flooding and root damage from weevil larval feeding.

Flood events may have long-term negative impacts on plant-soil systems (Kozlowski, 1984). In Florida, excess rainfall and poor soil drainage can result in periodic soil flooding. Soil waterlogging reduces soil oxidation-reduction potential (Patrick et al., 1996; Svertsen et al., 1983) and increases plant water stress (Jackson, 1990; Kozlowski and Pallardy, 1979; Oren et al., 2001; Svertsen et al., 1983). The root weevil, Diaprepes abbreviatus (L.), is a serious pest and a particularly challenging obstacle to the continued profitability of the citrus industry in Florida (McCoy et al., 2003). In a periodically flooded citrus grove, the distribution of Diaprepes adults has been linked to soil characteristics associated with flooding (Li et al., 2003), but the responses of flooded versus non-flooded plants to larval feeding by this weevil are unknown.

Neonate larvae of Diaprepes (D.) drop from egg masses located in the citrus canopy, enter the soil, and feed on tree roots (McCoy et al., 2003; Rogers et al., 2000; Stuart et al., 2003). However, because of their small size, neonate larvae are virtually impossible to detect in the soil (McCoy et al., 2003; Stuart et al., 2003), and their initial injury to roots can be exceptionally difficult to quantify (Rogers et al., 2000). The ability of citrus seedlings to tolerate feeding by D. larvae differs among varieties (Nigg et al., 2001). Rogers et al. (2000) estimated that injury to different rootstocks growing in well-drained soil ranged from 50 to 80% by 40 d after infestation by 2-5 D. neonate larvae, with many root tissues being completely consumed after 79 d. However these seedlings had not been exposed to flooding.

The Diaprepes root weevil has been dispersed primarily by nursery stock into more than 12,000 ha of citrus in Florida since it was first detected in 1964 (Bas et al., 2000). Many growers are unaware of larval root injury because obvious shoot and root symptoms are not apparent. Since plant flooding and larval feeding occur underground, it is necessary to monitor plant and soil stress under anaerobic conditions caused by flooding to understand the role of water-logging in potentially predisposing citrus roots to feeding injury by weevil larvae. This understanding could provide growers with a tool to quantify plant stress along a flooding gradient in the field, and suggest management options to reduce damage and improve citrus production.

The objectives of the study were to (i) examine temporal changes in soil redox (oxidation-reduction) potential and plant leaf stomatal conductance under flooding and D. larval feeding conditions, and (ii) to compare the effects of citrus variety, flooding duration, and D. larval feeding on plant water stress and root injury in the greenhouse. Our hypothesis (H₁) was that flood damaged roots are more susceptible to D. larval feeding pressure than undamaged roots.

Materials and Methods

Plant flooding and Diaprepes larval feeding procedure. The study was conducted in a greenhouse at the Citrus Research and Education Center, University of Florida, Lake Alfred, Fla., during Oct.-Dec. 2002. The treatments were two citrus rootstock varieties, four flooding durations at 0 (NF), 10 (F10), 20 (F20) and 30 (F30) d, and two infestation levels of D. larvae at 0 (ND) and 5 larvae (D) per seedling. Seedlings of Swingle citrumelo (SWI, Poncirus trifoliata (L.) Raf. × Citrus paradisi Macfad.) and Smooth Flat Seville (SFS, C. aurantium L.), two commercial citrus rootstock varieties in Florida, were selected for study.

Six-month-old SWI and SFS seedlings were obtained from a commercial nursery (Reed Bros Nursery, Dundee, Fla.) in mid-August 2002. The peat moss based soil-less potting media was gently washed from the roots, and bare-root seedlings were transplanted into sterilized Candler fine sand. Each seedling was transplanted into a single 130-cm³ pot. Candler sand is a typic quartzszamment containing 96.5% sand, 2% silt, 1.5% clay, pH 6.8, organic matter content of 1%, and extractable P of 5.5 mg·kg⁻¹. Plants used in the study were selected for uniformity of root density and canopy size. Seedlings were maintained in the greenhouse at about 25/20 °C day/night temperature, and irrigated and fertilized every other day. Fertilizer was a mixture

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of nutrient solution with a pH of 4.5 and an electrical conductivity of 250 mS·m⁻¹. Per liter of nutrient solution there were 237 mg N, 31 mg P, 90 mg K, 398 mg Ca, 18 mg Mg, 42 mg S, and additional recommended micronutrients of B, Cu, Zn, Mn and Mo. About 30 mL of fertilizer or water was applied to a seedling each time, which was sufficient to cause leaching.

On 1 Oct. 2002, the 30-d (F30) flooding treatments were submerged in water in a 1.5 × 0.5 × 0.8 m flooding container to 2 cm above the tops of pots; the shoots remained in the atmosphere. The water surface was covered with polyvinyl pieces to reduce water evaporation and minimize gas exchange. The remaining plants received diluted nutrient solution fertilizer (50%) alternating with water every other day. After 10 d, the 20-d (F20) pots were submerged and, 10 d later, the 10-d (F10) flooding treatments were submerged. There were eight replicates (plants) per variety per flooding duration. A total of 48 seedlings were submerged. A completely randomized design was used to arrange the treatments as defined by variety and flooding duration in the greenhouse. Submergence of all replicates for a particular flooding duration was simultaneous. The temperature of the flooding water was 25 ± 2 °C during the entire flooding period. The control (non-flooded) plants continued to receive 50% nutrient solution fertilizer and water on alternate days. On 30 Oct., all flooded plants were taken out of the water, and allowed to drain for a week.

Neonate larvae of Diaprepes were obtained from eggs laid by field-collected adults confined to screen cages at a temperature of 25 ± 2 °C. On 6 Nov., just before the infestation, active larvae were selected using the light drop procedure (Quintela and McCoy, 1997). Five active neonate larvae were placed in a tube and then scattered onto the soil surface of each pot receiving this treatment. Larvae moved into the soils immediately after inoculation. There was a no-larvae control (ND) that consisted of eight replicates for each variety. Irrigation and fertilization were applied to all seedlings at the same rate as during the flooding period. Maximum air temperature in the greenhouse was 28-35 °C throughout the flooding and larval infestation periods.

Forty-two days after the inoculation, root injury percentage and relative root density were evaluated. Each plant was removed from the pot and placed on a shallow examination tray. A spatula was used to gently remove the soil from around the roots. Larvae were removed from the soil and counted for each plant. Larval instar was not determined. A larval-feeding root-injury rating of 0 to 3 was visually determined (0 = 0% injury, 1 = 0-3%, 2 = 3-6%, and 3 = 6-12%, Rogers et al., 2000). A density root rating of 1 to 4 was also visually determined (1 = dense, 2 = moderate, 3 = declined, and 4 = severely declined roots). Because the larval infested seedlings were either non-flooded or flooded for different durations before infestation, the treatments constituted the following combinations: non-flooded and infested (NF-D); 10d-flooded and infested (F10-D); 20d-flooded and infested (F20-D); and 30d-flooded and infested (F30-D). There was also a non-flooded and non-infested (NF-ND) control.

Soil and plant measurements and data analysis. Flooding water temperature was measured using an Omega HH64 Thermometer (Omega Engineering Inc., Stamford, Conn.). Soil redox (i.e., oxidation-reduction) potential was measured using the method of Patrick et al. (1996) with a combination electrode of Orion mV meter (Model 290A, Orion Research Inc., Boston, Mass.). The electrode was calibrated using a standard solution of 240 mV. The redox potential was measured in each pot between 1000 and 1100 h. Measurements were taken every day during the flooding period and twice per week during the larval infestation period.

Leaf stomatal conductance (gₜ) was measured using a Delta-T porometer (Delta-T Devices, Cambridge, UK). For each seedling, the first fully expanded leaf situated about 3 cm below the shoot tip, was selected as a representative leaf for measurement. Measurements were taken every day during the flooding period and twice per week during the larval infestation period between 0900 and 1000 h. Total shoot length of each seedling was measured from the top of the pot to the shoot tip using a ruler every 2 weeks throughout the experimental period.

Analysis of variance of plant, soil, and root measurements were conducted using PROC GLM, and descriptive statistics and correlation analysis of all variables were determined using PROC UNIVARIATE and PROC CORR (SAS Institute, 1990).

Results

Temporal patterns of soil redox potential by flooding and larval infestation. A positive soil redox potential (+) means that soils are more capable of oxidation than the standard hydrogen electrode (Eₒ = 0). The soil redox potential patterns decreased into the negative range as quickly as 24 h under the flooded anaerobic conditions (Fig. 1). There were three decreases of soil redox potential following the beginning of the 30-d (F30), 20-d (F20), and 10-d (F10) flooding treatments (Fig. 1). The redox potential dropped from 200 mV to -100 mV within 3 d for the F30 flooding treatment. However, anoxic soil conditions (complete lack of oxygen) were attained within 2 d of flooding for the F20, and within 1 d for the F10 (Fig. 1). In the first 10 d of flooding, the soil redox potential varied between -5 ± 136 mV (n = 72). Late in the 20- and 30-d flooding treatments, the soil redox potentials were reduced to -123 ± 36 mV (n = 66). The variations in soil redox potential were similar for the two varieties (Fig. 1).

When pots were removed from the floodwater, there was an increase of soil redox potential to a positive value (200 mV) within one day in the greenhouse atmosphere (Fig. 1). The soil redox potential became constant (220-238 mV) during the soil draining and larval infestation period. In the non-flooded treatment, the soil redox potential remained high.

Fig. 1. Temporal patterns of soil redox (oxidation-reduction) potential throughout the experiment. SWI, rootstock Swingle; SFS, rootstock Smooth Flat Seville; NF, non-flooded; F30, 30 day-flooded; F20, 20 day-flooded; F10, 10 day-flooded seedlings.
was significantly greater in F10-254 (P < 0.001). The effect of larval infestation on leaf stomatal conductance in SWI during the experiment, and there was a significant difference in soil redox potential was most pronounced between F10 and F30 (P < 0.001).

Leaf stomatal conductance by variety, flooding duration, and larval feeding. Plant leaf stomatal conductance (g) is a measure of the physiological state of the plant in response to environmental variations and stresses (Kozlowski and Pallardy, 1979). The measured stomatal conductance varied with rootstock variety and flooding duration (Table 1). Leaf stomatal conductance tended to decrease in all flooded seedlings (Fig. 2a). In the non-flooded control treatments, stomatal conductance decreased slightly with time (Fig. 2a) as leaf resistance (r) to water loss increased with age (g = 1/r). During the first 10 d of flooding, the g values were similar between the control and the F30 treatments (Fig. 2a). However, in the F30 SWI treatment, the g values decreased to 141 ± 46 mmol m⁻²s⁻¹ (n = 64) at 20 d then to 71 ± 30 mmol m⁻²s⁻¹ (n = 64) within 30 d of flooding. This represented a drop of 50% in stomatal conductance for every 10 d of flooding. There was a similar trend of decreasing leaf conductance in the F30 treatments in SFS (graph not shown). In general, mean leaf stomatal conductance decreased with flooding duration in the order of NF < F10 < F20 < F30 (Fig. 2a).

After the plants recovered from flooding, leaf stomatal conductance increased and then decreased again following larval infestation (Fig. 2b). The non-flooded no-larvae control treatment (NF-ND) had the highest stomatal conductance (177 ± 50 mmol m⁻²s⁻¹, n = 54), followed by the non-flooded with larval treatment (NF-D, 153 ± 62 mmol m⁻²s⁻¹, n = 62). For the flooded SFS seedlings with larvae treatments, the mean g, decreased in the order of F10-D < F20-D < F30-D (Fig. 2b). There was a similar trend for leaf stomatal conductance in SWI during the larval feeding period (data not shown).

Effects of variety, flooding duration, and the interaction between variety and flooding duration were significant for leaf stomatal conductance (Table 1). Leaf stomatal conductance averaged 162 ± 56 mmol m⁻²s⁻¹ for SWI and 77 ± 28 mmol m⁻²s⁻¹ in SFS during the experiment, and there was a significant difference in g between the two varieties (Table 1). Only linear effects of flooding duration treatment within SWI and SFS were significant (P < 0.001). Leaf stomatal conductance was significantly higher in F10 than F30 for SWI (P < 0.001) and SFS (P < 0.001). The effect of larval infestation on leaf stomatal conductance was significant (P < 0.001), and stomatal conductance was significantly greater in F10-D than F30-D (P < 0.001).

Table 1. Analysis of variance for plant leaf stomatal conductance (g) and soil redox potential during flooding period.

<table>
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<td>8.0**</td>
<td>43.1**</td>
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ns, not significant; *P < 0.05; **P < 0.01.

Effects of Diaprepes larval feeding on previously flooded seedlings. In SWI, larval survival was significantly greater in the flooded soil (4.4 ± 0.5 for the F30) than that non flooded soil (3.0 ± 1.1 for the NF; Fig. 3a). The lowest larval survival was found in the non-flooded soils in SWI (Fig. 3a) and in SFS (data not shown). Larval survival was similar in SFS (4.1 ± 0.7) and SWI (3.9 ± 1.1).

Root injury to the non-flooded and previously flooded plants ranged from 0 to 12%. Root injury was slightly greater in SWI than in SFS. Root injury by larval feeding on the non-flooded roots ranged from 0 to 3% in SFS (Fig. 3b) and in SWI (graph not shown). Root injury by larvae for the 30-d flooded roots ranged from 3 to 12% in SFS (Fig. 3b) and in SWI (graph not shown). Larval feeding injury was similar at 3-6% (i.e., rating 1-2) for the 10- and 20-day flooded roots in SFS (Fig. 3b) and in SWI (graph not shown).

The root density of the two rootstocks was reduced by flooding and larval feeding. The non-flooded and non-infested roots were rated as dense (i.e., rating 1), and the root density declined to rating 3 for the seedlings previously flooded for 30 d. Roots previously flooded for a shorter duration (10 d) had a higher root density than those flooded for longer periods (20-30 d). This was similar to the patterns of larval feeding injury (Fig. 3b). The effects of larval infestation were very significant on larval survival (P < 0.001), root injury (P < 0.001), and root density (P < 0.001). The interaction between variety and larval infestation was significant for larval survival (P < 0.005). The comparison also showed that larval survival, larval root injury, and root density were significantly greater for the F10 vs. F20 (P < 0.001), and F 10 vs. F30 (P < 0.001).

Discussion

Plant roots can easily be suffocated if gas exchange is impeded, and the most common impediment to gas diffusion is water saturating the root environment (Kozlowski, 1984). In the present study, when soil was waterlogged (flooded to the soil surface), submerged roots were not in contact with oxygen. Initially, flood water contained oxygen, but this was depleted within hours and the soil redox potential became negative within one day (Fig. 1). Oxidation-reduction occurs simultaneously, and soil redox potential is directly related to soil aeration (Patrick et al., 1996; Ulloa et al., 2000). In the present study, as the waterlogged soils became devoid of O₂, anoxic soil conditions (Eh drops below zero mV) were attained within 1-3 d of flooding (Fig. 1). Svvertsen et al. (1983) reported that the soil redox potential dropped below zero mV within a week of flooding, and that the redox potential reached a minimum Eₜ of -250 mV after 3 weeks of flooded conditions. The different results in these studies were probably because soil redox potential varies with soil type, pH, and temperature, and, consequently, the Eh became stabilized within different time periods (Patrick et al., 1996).

The increase of plant stress (or decrease of leaf stomatal conductance) from flooding (Fig. 2) could initially be caused by the negative soil redox potential under the anaerobic conditions (Fig. 1). Soil redox potential could influence water and nutrient availability along with soil physical, chemical and biological processes (Patrick et al., 1996). Since gas exchange between the soil and atmosphere became negligible, plant stress occurred from the reduction of oxygen, and then the complete lack of oxygen during short-term flooding. In the present study, leaf stomatal conductance was significantly greater in
F10 than F30 (Fig. 2), indicating that seedlings damaged by longer floods experienced more water stress. This was likely a consequence of altered respiration in the absence of oxygen.

It is interesting to note that the lower leaf stomatal conductance in the seedlings previously flooded compared to those not flooded (Fig. 2b) may have been related to the fact that root injury by larval feeding was more severe in the previously flooded treatments than in the non-flooded treatments (Fig. 3b). The flood damaged seedlings were more susceptible to water stress (Fig. 2b), and non-flooded roots were more resistant to larval feeding than were flooded roots (Fig. 3b). Furthermore, seedlings previously flooded for a shorter duration (10-20 d) suffered less from water stress (Fig. 2b) and larval feeding injury (Fig. 3b) than those previously flooded for a longer duration. Flooded seedlings favored larval survival (Fig. 3a) as larval root feeding was more pronounced (i.e., higher feeding %) in the previously flooded seedlings (Fig. 3b).

Some Florida soils are very poorly drained and subject to flooding for a period of more than 4 months (SCS, 1979). It would be interesting to examine potential associations of flooding, root feeding by *Diaprepes* larvae and infections by *Phytophthora* (Graham et al. 2003) or other pathogens (Nigg et al., 2001). Graham et al. (2003) reported that Swingle citrumelo typically is not susceptible to *Phytophthora*, but it readily succumbs to the more aggressive *Phytophthora* species when grown in wet soils with root feeding by *Diaprepes* larvae.

**Conclusions**

Flooding significantly reduced soil redox (oxidation-reduction) potential and increased plant water stress. Temporal patterns of leaf stomatal conductance were a function of rootstock variety, flooding duration, and *Diaprepes* larval feeding. Previously flood damaged seedlings were more susceptible to larval feeding pressure. It would be interesting to monitor the
development of leaf stomatal conductance during a flooding duration longer than 30 d under these experimental conditions. A negative soil redox potential and a decrease of leaf stomatal conductance could be used as early indicators of plant water stress from flooding and root damage by the feeding of weevil larvae.

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