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SOIL LIMING AND FLOODING EFFECTS ON *DIAPREPES* ROOT WEEVIL LARVAL SURVIVAL AND CITRUS SEEDLING GROWTH

HONG LI[1], JAMES P. SYVERTSEN, CLAY W. MCCOY,
ROBIN J. STUART AND ARNOLD W. SCHUMANN
University of Florida, IFAS
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850

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Abstract. **Soil acidity and tree stress from flooding can occur simultaneously with *Diaprepes abbreviatus* (L.) root weevil infestations in citrus groves. We conducted a greenhouse study in 2003 to determine the effect of flooding duration on the outcome of soil liming, and the interaction of soil liming and flooding treatments on *Diaprepes* larval survival and seedling growth. We used a Floridana sandy soil (pH 4.8) from a citrus grove damaged by *Diaprepes* root weevil in Osceola County. The treatments consisted of three soil pH levels (non-limed, and target pH of 6 and 7), two citrus cultivars (Swingle citrumelo (*Citrus paradisi* Macfad. Raf. × *Poncirus trifoliata* (L.)) and Carrizo citrange (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata*), flooding durations of 0 or 40 days, and *Diaprepes* larval infestations at 0 or 5 larvae for 55 days. Dolomite ($CaCO_3 \cdot MgCO_3$) was used for liming. Treatments were arranged using 15 replicates in a completely randomized design. Soil pH increased by 0.5-0.9 units for the target pH 6, and 0.7-1.0 units for the target pH 7. The linear effect of liming treatment significantly increased soil pH ($P < 0.01$). Larval survival rate was up to 100% for low soil pH (< 5.1) but decreased to 60% at the highest measured soil pH of 5.7. Total larval weight decreased significantly from 60 mg to 18 mg per seedling when soil pH increased from 5.1 to 5.7. Shoot dry weight was higher at the target pH 7 than at other pH treatments ($P < 0.05$). Our data suggested that increasing soil pH by 1 unit could decrease *Diaprepes* larval survival while promoting citrus growth.**

Soil acidity and soil waterlogging are problems in citrus production in Florida (Boman and Obreza, 2002). Soil in Florida is generally acidic because of high rainfall and the use of acid forming fertilizers such as ammonium nitrate NH_4NO_3. Although citrus soils are often sandy, excess rainfall can result in soil flooding on low-lying flatwoods with poor drainage (Li et al., 2004a). Soil acidity (Kidder, 2003) and waterlogging (Jackson, 1990; Syvertsen et al., 1983) can have negative impacts on tree growth. Within 20-30 d of flooding, leaves of two citrus variety seedlings, Swingle and Smooth Flat Seville, were water stressed and shoots ceased growth (Li et al., 2003a). In a poorly drained citrus grove, leaf stomatal conductance was significantly lower in a flooded area than in a non-flooded area (Li et al., 2004a). Also, severely declined trees were associated with high soil Fe concentration (Li et al., 2004b), and it is known that soil Fe concentrations increase significantly when soil pH drops from 6 to 5 (Adams, 1984).

Soil acidity and tree stress from flooding can occur simultaneously with *Diaprepes* root weevil infestation in Florida citrus groves. For example, an orange grove at Southport near Poinciana in Osceola County, central Florida, has been damaged by *Diaprepes* root weevil over the last 10 years (Nigg et al., 2001; McCoy et al., 2003; Stuart et al., 2003). In this grove, the soil is strongly acid (pH 4.8) and flooding occurred in Dec. 2002 and in Jan. 2003 (Li et al., 2004a). We found that a low density of *Diaprepes* adults was significantly correlated with areas high in soil Ca and Mg concentrations (Li et al., 2003b). Thus, high concentrations of Ca and Mg in soil may not favor *Diaprepes* larval survival. Because soil liming with dolomite ($CaCO_3 \cdot MgCO_3$) has been practiced every year and a large amount of Ca and Mg have been brought to the soil in the grove, it has been suggested that soil liming practices might have influenced *Diaprepes* larval survival.

Growers can improve the quality of acid soils by liming to adjust pH to the levels beneficial to citrus. Previous flooding duration (Li et al., 2003a) and floodwater pH (Shapiro et al., 1997) also could be environmental factors influencing *Diaprepes* larval survival. It is not clear whether low soil pH was caused by flooding, and soil waterlogging might have influenced *Diaprepes* larval survival in citrus groves. Separating plant stresses from soil acidity, waterlogging and root weevil larval feeding requires controlled environmental studies in the greenhouse.

We hypothesized that flooding events may influence the effectiveness of soil liming, and that correcting soil pH may have an impact on *Diaprepes* root weevil larval survival. Our objective was to determine the effects of flooding on soil liming outcome and seedling growth, and to quantify the effect of increased soil pH on *Diaprepes* root weevil larval survival and larval growth. If increasing soil pH improves soil quality for citrus and decreases *Diaprepes* larval survival, then liming could become part of an environmentally friendly management strategy for *Diaprepes* root weevil in Florida citrus.

Materials and Methods

Soil liming procedure. We conducted a greenhouse study of liming × flooding × larval feeding from 12 Sep. 2003 to 29 Jan. 2004 at the Citrus Research and Education Center, University of Florida. The treatments consisted of three soil pH levels (grove field soil, pH 4.8, and target pH 6 and 7), two citrus rootstock varieties, two levels of flooding duration (0 or 40 d), and two levels of *Diaprepes* larval infestation (0 or 5 larvae). The two rootstock varieties were Swingle citrumelo (*Citrus paradisi* Macfad. Raf. × *Poncirus trifoliata* (L.)) and Carrizo citrange (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata*).

We used a flatwoods Floridana sandy soil, classified as Siliceous, Hyperthermic, Arenic Argiaquolls Alfisols in the study. The soil was collected from a depth of 0-0.3 m in a citrus grove near Poinciana, Osceola County, Florida (28°22'N, 81°58'W). The grove has been damaged by *D.* root weevil over the last ten years. The soil had a mean pH of 4.8, cation exchangeable capacity of 15 Cmol kg^{-1}, and a base saturation of 57% of soil weight. Other characteristics of this soil can be found in Li et al. (2004a).

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[1]Corresponding author; e-mail: hongli@crec.ifas.ufl.edu.

The liming requirements (LR) for the target soil pH was established using the equation as follows: LR (kg ha^{-1}) = CEC (BS_2-BS_1) × L, where CEC is soil cation exchangeable capacity, BS_1 is soil base saturation before liming, BS_2 is the expected base saturation after liming, and L is soil depth (Barber, 1984). We assumed an expected BS of 85% for the target pH 6, and 90% for the target pH 7. Soil depth L was 0.3 m based on the soil sampling depth (Li et al., 2004a). Limestone (dolomite $CaCO_3 \cdot MgCO_3$) was used as the lime material. The dolomite consisted of 54% $CaCO_3$ (39% Ca), and 46% $MgCO_3$ (28% Mg). We also did a series of liming tests for calibrating the LR calculated from the equation above using the techniques described in Barber (1984). The liming tests were to verify the LR for a target soil pH of 5, 5.5, 6, 6.5 and 7 determined using the above equation. The soil-lime/water (1:1) mixture was incubated for two weeks, and pH was measured every two days. A curve of measured soil pH was plotted against the target soil pH to obtain the adjusted LR. The dolomite rates to meet the adjusted LR were 12 Mg ha^{-1} for the target pH 6, and 20 Mg ha^{-1} for the target pH 7 for a soil depth of 0.3 m. Lime and soil were dried at 70 °C in the oven over night before use.

Flooding and larval feeding procedures and data measurements. The 3-month old seedlings of Swingle citrumelo and Carrizo citrange were obtained from a commercial nursery (Reed Bros Nursery, Dundee, Fla.). Seedlings were selected for uniformity of root density and canopy size for each variety. Each seedling was transplanted into a single 130-cm^3 pot. Seedlings were irrigated and fertilized using the materials and methods described in Li et al. (2003a). Seedlings were allowed to recover from being transplanted and to acclimate for 40 d before flooding.

The flooding treatment seedlings were submerged to 2 cm above the tops of the pots in a 0.8 × 0.5 × 0.5 m container. The shoots remained in the atmosphere in the greenhouse. The flooded and non-flooded treatments had 15 replicate seedlings per pH treatment and per variety, and were arranged in a completely randomized design. After the flooding procedure was completed, seedlings were drained for a week then five 1-d old active neonate larvae were inoculated onto the surface of each pot receiving the treatment. We used flooding and larval infestation procedures as described in Li et al. (2003a). The larval feeding period lasted 55 d.

Soil redox potential (E_h) was measured about every 4 days using an oxidation-reduction probe (ORP, Model 290A, Orion Res., Inc., Boston, Mass.). Leaf stomatal conductance (g_s) was measured using a Delta-T porometer (Delta-T Devices, Cambridge, UK). For each seedling, the first fully expanded leaf situated about 3 cm below the shoot tip, was selected as a representative leaf for measurement. For all treatments the g_s was measured on fixed leaves throughout the experiment.

Larval survival rate, larval weight, and root injury rating by flooding and larval feeding were evaluated at the end of the experiment using methods described in Li et al. (2003a). Root rating was visually determined relative to a non-flooded and non-larvae infested control as an undamaged reference (Fig. 1A). Whole seedling root rating was classified by percentage damage as 0% (control), 0-25%, 25-50%, 50-75%, and >75% damaged roots (Fig. 1B). Leaf area at the end of the experiment was determined using a LI-COR leaf area meter model LI-3000 (Lambda Instruments, Lincoln, Nebr.). Whole seedlings were oven dried at 70 °C until a constant weight was achieved and then dry weights of stems, leaves and roots were determined.

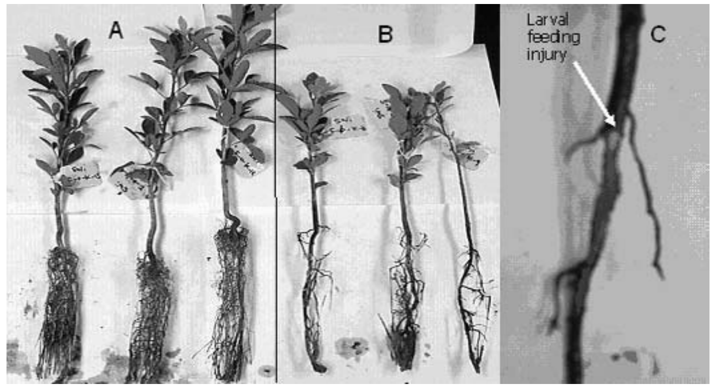

Fig. 1. Non-flooded and non-larval infested (reference) roots (A), seedlings damaged by flooding for 40 d and larval feeding for 55 d (B), and a root injured by *Diaprepes* larval feeding (C).

Fresh weight of the soil was determined for each seedling after the removal of surviving larvae. Soils were oven dried at 90 °C for determining dry weight. Gravimetric soil water content was calculated as the difference between fresh and dry weight of the soil. Soil pH of each seedling was determined with soil:water 1:1 using an Orion pH meter (Model 105, Orion Res., Inc., Boston, Mass.).

Analysis of variance of plant, root, soil and larval data was conducted using PROC GLM, descriptive statistics of all variables were determined using PROC UNIVARIATE, and treatment means were compared using the LSD test (SAS Institute, 1990).

Results

Soil redox potential and leaf stomatal conductance. Soil redox (oxidation-reduction) potential (E_h) remained high at about +400 mV for non-flooded seedlings in the atmosphere. However, under floodwater the E_h value decreased from +300 mV to -80 mV within 4 d of flooding and then decreased to -200 mV by the end of flooding. When seedlings were removed from floodwater, the E_h increased to +300 mV within 24 h in the atmosphere. The E_h was similar for both Swingle (-228 mV to + 400 mV) and Carrizo (-216 mV to + 412 mV). The difference in soil E_h was only significant between flooded and non-flooded seedlings ($P < 0.01$).

Leaf stomatal conductance (g_s), a measure of the leaf resistance to loss of water vapor through the stomata, varied daily between 320-600 mmol $m^{-2}s^{-1}$ for Swingle and Carrizo before flooding. However, leaf stomatal conductance decreased to below 100 mmol $m^{-2}s^{-1}$ within 10 d of flooding, and the g_s value was only about 35 mmol $m^{-2}s^{-1}$ for flooded seedlings by the end of flooding (data not shown). By the end of flooding, the g_s for the non-flooded treatments was still about 4 times higher than the value for the flooded treatments. Leaf stomatal conductance was slightly higher in Swingle than Carrizo. There was no significant difference in g_s between the pH treatments.

Soil pH vs. larval survival and growth. Final measured pH values were 4.8 ± 0.13 (n = 30) for the non-limed soil, 5.4 ± 0.21 (n = 30) for the target pH 6, and 5.7 ± 0.26 (n = 30) for the target pH 7. As compared to initial soil pH, the pH value increased from 0.5 to 0.9 units for the target pH 6, and from 0.7 to 1.1 units for the target pH 7. This polynomial equation shows that only the linear effect of the liming treatment was

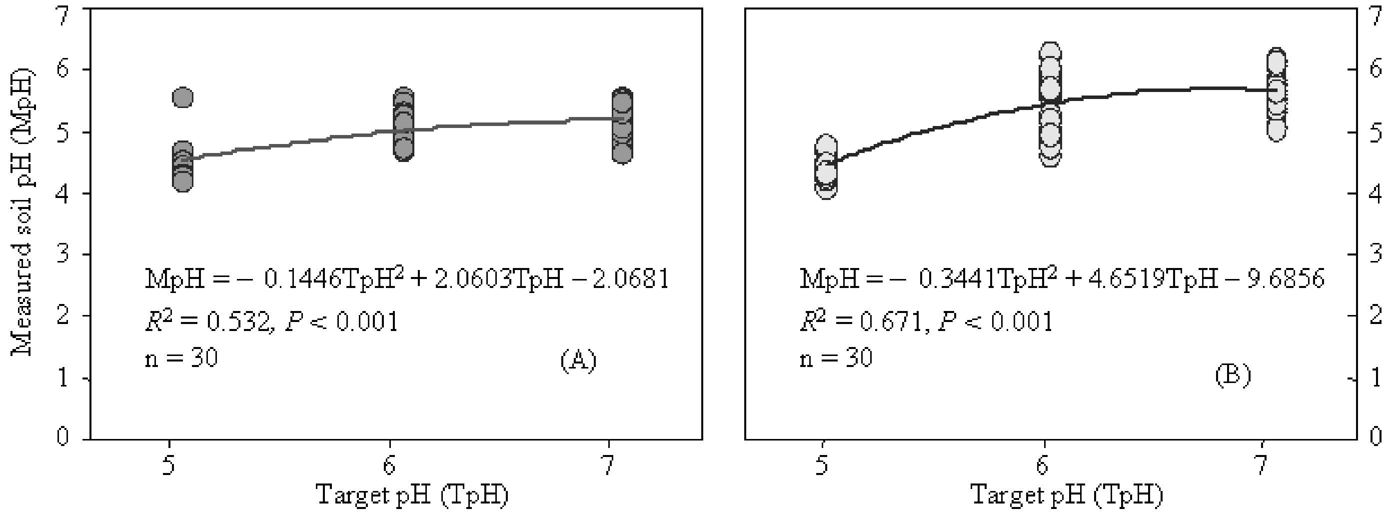

Fig. 2. Final measured soil pH vs. target pH. Non-flooded 145 d (A), and flooded 40 d and non-flooded 105 d (B) in Swingle.

significant on soil pH. ($P < 0.001$, Fig. 2A) and a plateau appeared at a target pH between 6 and 7 under both non-flooded (Fig. 2A) and flooded conditions (Fig. 2B). Flooded soil pH was on average 0.3 units higher (Fig. 2B) than non-flooded soil pH (Fig. 2A). Flood water pH increased gradually because oxygen was depleted with time after submergence, which resulted in higher pH in flooded soil than in non-flooded soil.

There were significant interactions between rootstock variety and flooding treatments on measured soil pH (Fig. 3). Soil from the flooded Carrizo seedlings (CAR-F) had the highest response but the non-flooded Swingle seedlings (SWI-NF) had no response to the liming treatments (Fig. 3). Within each target pH, the measured soil pH for each variety had little variation with a small standard error (0.03-0.09 pH units), which was not shown in Fig. 3.

The survival rate of the 56-d old larvae ranged from 0 to 100% per seedling for low soil pH in the range of 4.5 to 5.1, and 100% survival only occurred in low soil pH smaller than 5.1 (Fig. 4A). Larval survival was reduced from 80% to 60% per seedling with higher soil pH in the range of 5.2-5.8 in the non-flooded Swingle (n = 30, Fig. 4A) and from 40 to 20% per seedling in the non-flooded Carrizo when soil pH increased from 5.1 to 5.7 (n = 30, graph non-shown). The polynomial equation describing larval survival rate showed a significant reduction by the quadratic effect of soil pH (Fig. 4A). Although there was no measured data to show whether larval survival and weight could be further decreased with higher soil pH, the equations suggested that soil pH greater than 5.8 might further decrease larval survival.

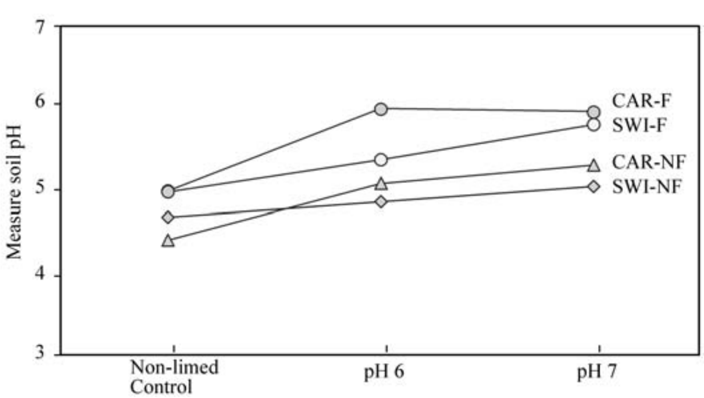

Fig. 3. Interaction between variety and flooding treatments. CAR-F, Carrizo-flooded, CAR-NF, Carrizo-non-flooded, SWI-F, Swingle-flooded, and SWI-NF, Swingle-non-flooded treatments. Each point represents the mean of 15 measurements.

Larval weight is a measure of larval growth. Total larval weights ranged from 0 to 60 mg per seedling (Fig. 4B) and larval weights were generally proportional to larval survival. Total larval weight was as high as 60 mg per seedling for soil pH < 5, and the weight was reduced to a minimum of 12 mg per seedling when the soil pH increased from 5.1 to 5.7 (non-flooded Swingle). Larval weight was also significantly reduced by the quadratic effect of the liming treatment (Swingle, non-floooded, Fig. 4B). Because larval weight was proportional to larval survival, the equation (Fig. 4B) suggested that a soil pH greater than 5.8 might have the effect of further decreasing larval growth.

In addition, larval weight also increased with soil water content and larval weight was twice as high (81 mg per seedling) at a soil water content of 31% (previously flooded Swingle, n = 10) compared to a lower soil water content (17-22%).

Shoot growth and root injury. Shoot length growth was 2.9 ± 1.5 cm for Swingle (n = 90) and 2.1 ± 1.9 cm for Carrizo (n = 90) for a period of four months with 40 d of flooding and 55 d of larval infestation. Shoot length grew significantly faster in Swingle than Carrizo ($P < 0.05$). There was little growth (only 0.1 cm) for the flooded treatments from the period of flooding until larval infestation.

Increased soil pH had a significant effect on seeding growth for Swingle under both flooding and non-flooding conditions. Shoot growth increased significantly from 2.8 cm to 3.6-4.3 cm when measured soil pH increased 0.5-1 unit ($P < 0.01$). Shoot dry weight increased from 2 g to 4 g with increasing soil pH from 4.8 to 5.8 ($P < 0.05$). Shoot dry weight was significantly higher for target pH 6 and target pH 7 than the control under non-flooded conditions (Fig. 5A). Under flooded conditions, target pH 7 had a significantly higher shoot dry weight than the other treatments (Fig. 5B). Root injury by larval feeding was not pronounced (Fig. 1C).

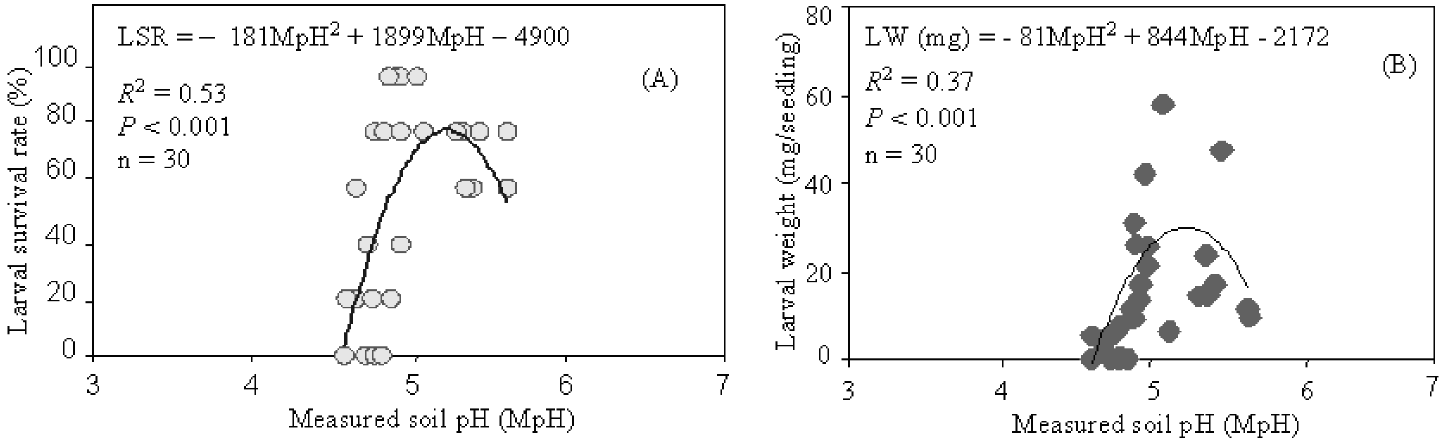

Fig. 4. *Diaprepes* larval survival rate vs. measured soil pH (A), and larval weight vs. measured soil pH (B) in Swingle, non-flooded seedlings. LSR, larval survival rate; and LW, larval weight.

Discussion

Both negative soil oxidation-reduction potential and very low leaf stomatal conductance were signs of plant water stress from root injury. The mechanisms through which limestone (dolomite $CaCO_3 \cdot MgCO_3$) reacts with acid soils are complex. The more efficient liming effect for target pH 6 than target pH 7 (Fig. 2) may be because the duration of the experiment was not long enough for all the applied dolomite to react with soil hydrogen. There was 66% more dolomite applied to the target pH 7 than the target pH 6. Also, high levels of organic matter (8%, n = 50 soil samples, Li et al., 2003b) may have complicated the liming outcome as higher liming requirements are needed for soil with high organic matter content (Adams, 1984).

The floodwater used in the experiment had a pH close to 7, which may have contributed to a higher pH in flooded treatments than non-flooded treatments (Fig. 3). The flooded seedlings were under floodwater (pH 7) for 40 d while the non-flooded seedlings received a fertilizer solution of pH 5.5 every other day during the same period. Usually, natural floodwater is from rainfall, which can have an acid pH with excessive hydrogen to acidify soil. In any case, floodwater pH should be similar to rainfall pH to examine whether natural flooding events have any influence on soil pH.

Although the interactions between rootstock variety and liming treatments (Fig. 3) suggested that Carrizo roots may absorb more hydrogen than Swingle, there was no significant difference in shoot length growth or larval survival between seedling varieties. However, *Diaprepes* larval survival and larval weights tended to decrease in soil pH higher than 5.2 (Fig. 4), and it is possible that larval survival and weights could be even lower in slightly acid soil (pH 6.1-6.5) than in a moderately acid soil (pH 5-5.5). Thus, raising soil pH may be a treatment to decrease the impact of the *Diaprepes* root weevil in Florida citrus.

Soil pH controls many of the chemical and biological properties of soil (Adams, 1984). Our shoot dry weight results (Fig. 5) suggested that the target pH 7 was likely a good treatment for seedling growth under the flooded and non-flooded conditions. In light of this study, it would be useful to conduct further studies to examine whether liming soil to a pH 6-6.5 (optimum soil pH for citrus growth) could reduce *Diaprepes* larval survival while increasing citrus seedling growth. If raising soil pH could also be a treatment for control of *Diaprepes* root weevil and citrus tree decline, it could be a horticulturally and environmentally friendly component in a management program of the *Diaprepes* root weevil in Florida citrus.

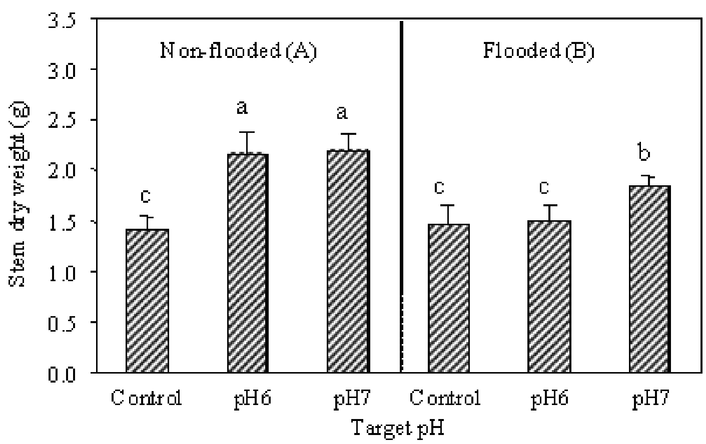

Fig. 5. Comparison of stem dry weight by target pH for non-flooded (n = 45 seedlings, A) and flooded (n = 45 seedlings, B) seedlings of Swingle citrumelo. Each bar represents the mean and standard error of 15 measurements. Common letters above bars indicate no significant difference at $P < 0.05$.

Conclusions

Flooding influenced soil liming outcome. Water saturation stress from flooding reduced soil oxidation-reduction potential and citrus leaf stomatal conductance and injured seedling roots. Soil pH in the range of 5.2-5.7 decreased *Diaprepes* larval survival and larval growth compared to lower soil pH (4.5-5.1). The target pH 7 was likely a good treatment for seedling shoot growth under flooded and non-flooded conditions. It would be useful to conduct further experiments to determine if liming soil to a higher pH between 6-6.5 would help lower *Diaprepes* larval survival and increase seedling growth.

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