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ScienceDirect
Applied Soil Ecology 35 (2007) 553–565
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Controlling factors of environmental flooding, soil pH and *Diaprepes abbreviatus* (L.) root weevil feeding in citrus: Larval survival and larval growth

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Received 28 February 2006; received in revised form 22 September 2006; accepted 22 September 2006

Abstract

The underlying influences of soil flooding, pH level and soil-inhabiting *Diaprepes abbreviatus* (L.) root weevil larval feeding in citrus were examined in two separate greenhouse studies, rootstock × flooding × *Diaprepes*-larvae (RFD) and liming × rootstock × flooding × *Diaprepes*-larvae (LRFD). Our objectives were to determine the combined effects of soil flooding and pH level on survival and growth of *Diaprepes* root weevil larvae to gain insights of insect-environmental relations for the weevil control. We used a Floridana sandy loam (pH 4.8) from a citrus grove infested by *Diaprepes* root weevil in center Florida. The RFD experiment consisted of two citrus rootstocks (Swingle and Smooth Flat Seville), three flooding durations (0, 20, and 40 days) and two larval infestation rates (0 and 5 larvae) for 40-day feeding. The LRFD experiment consisted of two citrus rootstocks (Swingle and Carrizo), three pH levels (non-limed control, and target pH 6 and 7), two flooding durations (0 and 40 days), and two larval rates (0 and 5 larvae) for 56-day feeding. Dolomite (54% $CaCO_3$ and 46% $MgCO_3$) was used for soil liming in the LRFD. Treatments were arranged with 15 replicates in a completely randomized design. In the RFD, flooded soil pH was 0.3 units higher than non-flooded soil and larval survival was the lowest in the longest flooded treatment ($P < 0.05$). In the LRFD, soil pH increased 0.5–0.9 units for the target pH 6, and 0.7–1.1 units for the target pH 7. The effects of rootstock, liming and flooding treatments and their interactions were significant on soil pH and larval survival ($P < 0.05$). Larval survival decreased from 80% to 60% with increasing soil pH from 4.8 to 5.7. Total larval weight per seedling decreased significantly from 0.060 g to 0.012 g when the soil pH increased from 5.1 to 5.7. Flooding reduced larval survival and growth, and increasing acidic soil pH by 1 unit would be an option for controlling soil acidity and for promoting integrated management of *Diaprepes* root weevil in citrus.

Keywords: Citrus rootstock; Liming; *Diaprepes* root weevil; Flooding; Larval survival; Soil pH

1. Introduction

Soil acidity and environmental flooding from poor drainage would occur with infestation of the root weevil *Diaprepes abbreviatus* (L.) in citrus production areas including Florida (Li et al., 2004a, 2004b, 2006a). Developing biological and cultural tools to reduce the survivability of soil-inhabiting *Diaprepes* larvae have been in the center of the citrus integrated pest

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doi:10.1016/j.apsoil.2006.09.013

management program in Florida. Some citrus soils in Florida are strongly acidic (Obreza and Collins, 2002; Li et al., 2004a). Factors causing high soil acidity in the area include high rainfall, aluminium from soil reacting with water to give free hydrogen, low elevation causing waterlogging or flooding, and common use of acidic forming fertilizers such as NH_4NO_3. Soil acidity can have negative effects on many plant root systems (Adams, 1984; Bohn et al., 2001). The potential interactions between *Diaprepes* weevil infestation with flooding and soil acidity in citrus have initiated studies on management practices to control this pest (Li et al., 2004a, 2006a).

Diaprepes root weevil larvae pupae in soil and subsequently feed on citrus tree roots, which can break the resistance of structural roots to infection by *Phytophthora* spp. and lead to tree decline or death (Rogers et al., 2000; Graham et al., 2003; McCoy et al., 2003; Stuart et al., 2004). Due to their small size, neonate larvae are virtually impossible to detect in the soil and their initial injury to roots can be difficult to quantify (Jones and Schroeder, 1983; Rogers et al., 2000). Entomopathogenic nematodes have been tested for more efficient ways to control *Diaprepes* larvae (Duncan et al., 2003; Stuart et al., 2004). It was reported that soil-inhabiting *Diaprepes* larval survival and growth were related to citrus rootstock, soil type and soil moisture in the greenhouse studies (Rogers et al., 2000; Li et al., 2004b, 2006a). In the field, the emerging *Diaprepes* adult weevil from the soil was positively correlated with soil water content, clay, silt, and soil organic matter content, and negatively correlated with sand content, pH, P, Zn, and Cu (Li et al., 2005). It is not known, however, whether the distribution patterns of *Diaprepes* root weevils were associated with flooding, or whether differences in responses of flooded and non-flooded trees to larval feeding would influence differently larval growth and survival.

The soil environment influenced the abundance of most herbivorous insects (Orians and Fritz, 1996; Lower et al., 2003), and had profound impacts on subsequent survival of larvae (Riis and Esbjerg, 1998; Hatch and Blaustein, 2000; Watanabe et al., 2002; Suemoto et al., 2005). The causes related to larval survival were complex. Some experimental data showed that early larval survival was more sensitive to acid stress (low pH) and high Al and NO_3 and Cl salt solutions had the toxicological effects on the number of surviving larvae (Schrader et al., 1998; Hatch and Blaustein, 2000). In a poor drained citrus grove in Florida where soil was strongly acidic and frequently waterlogged, trees were severely damaged by *Diaprepes* root weevils and the density of active *Diaprepes* adults was significantly high in areas low in Ca and Mg concentrations (Li et al., 2004a). In other citrus grove, soil pH was near neutral and density of active *Diaprepes* adults was also correlated to soil pH and Mg (Li et al., 2006b). Would high soil pH and Ca and Mg concentrations not favor *Diaprepes* larval survival? Whether flooding and waterlogging have contributed to such low soil pH, and periodic flooding has affected *Diaprepes* larval survival? The lack of understanding the underlying factors that govern larval survival would be an important gap in the management strategy of this pest.

Growers need to improve the quality of these acid soils by adjusting pH to the levels suitable to citrus tree growth. May liming have impact on *Diaprepes* root weevil larval survival and adult emergence? Would the flooding complicate the liming treatments? We therefore hypothesized that high soil pH from adding lime would not favor *Diaprepes* larval survival, and flooding events may influence soil liming effectiveness and *Diaprepes* larval survival. In this study we investigated (i) the effect of flooding on soil characteristics and liming outcome, and (ii) the underlying effects and interactions of flooding duration and soil pH level on the survival and growth of *Diaprepes* root weevil larvae in citrus seedlings. We expected that larvae grow better in non-flooded soil conditions. An increased understanding of the underlying factors that govern larval survival would contribute to establish a strategy of using cultural practices for integrated management of this root weevil.

2. Materials and methods

2.1. Floridana sandy loam in Diaprepes root weevil infested citrus grove

We conducted two greenhouse studies of *Diaprepes* root weevil larval feeding on flooded and non-flooded citrus rootstock seedlings at the Citrus Research and Education Center, University of Florida during May 2003–January 2004. We used a Floridana sandy loam, a flatwoods citrus soil in both greenhouse studies. The Floridane sandy loam was classified as Siliceous, Hyperthermic, Arenic Argiaquolls Mollisol (USDA-NRCS, 2003). The soil was sampled from a 20-yr old ‘Hamlin’ orange (*Citrus sinensis* (L.) Osb.) grove (28°07′4″N, 81°21′10″W) in Osceola County, Central Florida. The citrus trees were infested by *Diaprepes* root weevil since the last ten years, (McCoy et al., 2003). In, addition, the grove was in a poorly drained depression and flooding occurred depending on rain patterns (Li et al., 2004a, 2005).

In this Floricana sandy loam, it was reported that *Diaprepes* root weevil neonates dropping from the tree canopy to the soil varied between 370 and 940 neonates per m^2 of soil surface, and the soil under the trees contained an average of 50 visual larvae per m^3 of fresh soil (McCoy et al., 2003). Adult emergence from the soil averaged 12.9 weevils per m^2 of soil surface per year (Li et al., 2005), and 8–96 active adult weevils per $34 \times 25\ m^2$ per week under the tree canopy (Li et al., 2006b). The use of this Floridana sandy loam in the greenhouse studies was to further provide information about the influence of flooding and pH level in larval survival and larval growth.

The Floridana sandy loam was sampled in the depth of 0–0.3 m at 203 cone traps (for capturing *Diaprepes* adult weevils emerging from the soil) in the grove (Li et al., 2005). The 203 soil samples were air-dried. Soil characterizations showed that the Floridana sandy loam was strongly acid (pH 4.8 ± 0.4, $n = 203$). The soil contained 540 g kg^{-1} sand, 158 g kg^{-1} silt, 302 g kg^{-1} clay, 92 g kg^{-1} organic matter, 58% base saturation, 35 mS m^{-1} electrical conductivity, 16 Cmol kg^{-1} cation-exchangeable capacity, and 20, 103, 285, 1418 and 28 mg kg^{-1} of Mehlich-1 exchangeable P, K, Mg, Ca and Fe, respectively. These data were the mean of 203 samples.

2.2. Rootstock × flooding × *Diaprepes*-larvae experiment

The rootstock × flooding × *Diaprepes*-larvae (RFD) experiment was conducted in the greenhouse during May–August 2003. The treatments consisted of two citrus rootstock varieties, three levels of flooding duration (0, 20 and 40 days), and two levels of *Diaprepes* neonate larval feeding (0 and 5 larvae). The two rootstock varieties were Swingle citrumelo (*Citrus paradisi* Macfad. × *Poncirus trifoliata* (L.) Raf.) and Smooth Flat Seville (*Citrus aurantium* L.) (Table 1). Seedlings of the two rootstocks were obtained from a commercial nursery (Reed Bros Nursery, Dundee, FL). The 3-month-old seedlings were selected for uniformity of root density and canopy size for each variety.

Fifteen seedlings of each rootstock variety were used for each flooding treatment (Table 1). The peat moss based soil less potting media was gently washed from the roots, and bare-root seedlings were each transplanted into a single 130-cm^3 plastic pot with the air-dried Floridana sandy loam. The initial soil–pot gap (distance from the soil surface to the top of the pot) was 1 cm. The treatments were arranged by variety and flooding duration in a completely randomized design. The transplanted seedlings were maintained for 40 days before starting the flooding procedure. Seedlings were irrigated using tap water and fertilized using a commercial nutrient solution (pH 5) on alternative days by the methods described in Li et al. (2004b, 2006a).

There were three separate procedures, which were flooding, draining, and larval feeding, for each experiment (Table 1). The seedlings for the flooding treatments were submerged in a 0.8 × 0.5 × 0.5 m plastic tub for each flooding treatment. Seedlings were flooded simultaneously by submerging the entire tray of

Table 1
Treatments and procedures for the Rootstock × Flooding × *Diaprepes*-larvae (RFD) experiment, and for the Liming × Rootstock × Flooding × *Diaprepes*-larvae (LRFD) experiment

Treatments and replicates	Greenhouse experiments			
	Rootstock × Flooding × *Diaprepes*-larvae (RFD)		Liming × Rootstock × Flooding × *Diaprepes*-larvae (LRFD)	
Rootstock variety	SWI[†]	SFS[†]	SWT[†]	CAR[†]
Liming target pH	–	–	Control, 6, 7	Control, 6, 7
Flooding duration (days)	0, 20, 40	0, 20, 40	0, 40	0, 40
Replicates (reps)	15	15	15	15
Total seedlings	3 × 15 = 45	3 × 15 = 45	3 × 2 × 15 = 90	3 × 2 × 15 = 90

Diaprepes larval feeding (40 days in RFD and 56 days in LRFD experiments) using previous flooding treatment seedlings

	NF (SWI-SFS)	F (SWI-SFS)	NF (SWI-CAR)	F (SWI-CAR)
Non-Diaprepes (ND) reps[†]	5	10	5	10
Diaprepes (D, 5 larvae) reps[†]	5	10	5	10
Total seedlings	2 × 3 × 5 = 30	2 × 3 × 10 = 60	2 × 3 × 2 × 5 = 60	2 × 3 × 2 × 10 = 120

[†] SWI, rootstock Swingle; SFS, rootstock Smooth Flat Seville; CAR, rootstock Carrizo; F, flooded; NF, non-flooded; D: *Diaprepes* larval infestation.

pots. The water level was maintained 2 cm above the top of the pots and the shoots remained in the well ventilated atmosphere in the greenhouse. When flooding duration was completed, seedlings were removed from the tub and allowed to drain for a week. *Diaprepes* neonate larvae were then introduced to seedlings for the test of larval survival and larval growth. The soils were not flooded during the larval feeding period. The greenhouse was maintained at an air temperature of 26 ± 6 °C and a relative humidity of 35 ± 5% during the RFD experiment.

2.3. Liming×Rootstock×Flooding×Diaprepes-larvae experiment

The liming × rootstock × flooding × *Diaprepes*-larvae (LRFD) experiment was conducted in the greenhouse during Aug. 2003–Jan. 2004. Since the emergence of *Diaprepes* adult weevils was positively correlated to the soil pH in the grove (Li et al., 2005), the LRFD experiment was intended to determine if higher soil pH level could influence *Diaprepes* larval survival. The treatments consisted of two citrus rootstock varieties, three soil pH levels (initial soil pH 4.8 (control), target pH 6, and target pH 7), two levels of flooding duration (0 and 40 days), and two levels of *Diaprepes* neonate larvae (0 and 5 larvae) for 56 days of feeding. The two rootstock varieties were Swingle citrumelo (*Citrus paradisi* Macfad. × *Poncirus trifoliata* (L.) Raf.) and Carrizo citrange (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.) (Table 1).

The liming requirements (LR) for target soil pH was established using the equation as follows: LR (kg ha^{-1}) = CEC (BS_2–BS_1) × L, where CEC was cation-exchangeable capacity, BS_1 was base saturation before liming, BS_2 was the expected base saturation after liming, and L was soil depth (Adams, 1984; Bohn et al., 2001). 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. A commercial dolomite, containing 54% $CaCO_3$ (39% Ca) and 46% $MgCO_3$ (28% Mg), was used as the sources of lime. We also did a series of liming test for calibrating the LR calculated from the equation above. The liming test was to target soil pH of 5, 5.5, 6, 6.5 and 7 with liming requirements determined for 50, 100, 150, and 200 g of soil, and the soil-lime:water (1:1) mix was incubated for four weeks in the laboratory. During the incubation, the mix soil pH was measured every two days. A curve of measured soil pH was plotted against the target soil pH then the LR determined using the above equation was further adjusted. The dolomite rates were 12 Mg ha^{-1} for the target pH 6 and 20 Mg ha^{-1} for the target pH 7. Lime and soil were dried at 70 °C in the oven over night then were weight and hand mixed.

The treatments in the LRFD experiment were also arranged by rootstock variety and flooding duration using 15 replicates in a completely randomized design (Table 1). Seedlings of Swingle and Carrizo, obtained from the Reed Bros Nursery (Dundee, FL), were also 3-month old and selected for uniformity of root density and canopy size for each variety. Seedling preparation, transplanting, maintenance, and the flooding procedure were done using the methods described in the RFD experiment. The greenhouse was maintained at an air temperature of 22 ± 8 °C and at a relative humidity of 30 ± 8% during the LRFD experiment.

2.4. Larval feeding test, soil measurements and data analysis

Diaprepes larval feeding test was begun after draining the flooded seedlings for a week in each experiment. *Diaprepes* neonates were obtained from eggs laid by field-collected adults confined to screen cages at a temperature of 25 ± 2 °C in the laboratory. We used 1-day-old neonates in both experiments. The initial neonate weights were determined using three sets of 100 neonates of 1-day old. For high vigor, the neonates were selected within an hour before the larval infestation using the light drop procedure (Quintela and McCoy, 1997). Five active neonate larvae were carefully placed in a tube then scattered onto the soil surface of the seedling pot. The inoculated neonates moved into the moist soil quickly and exhibited positive geotaxis. With a 1-cm gap space between the soil surface and the top of the pot, no further steps were taken to prevent the escape of neonates from pots prior to soil penetration, as shown in previous studies (Jones and Schroeder, 1983; Rogers et al., 2000).

Per flooding treatment, 10 random seedlings were infested using the selected *Diaprepes* neonate larvae (D), and 5 seedlings received no larvae (ND), as shown in Table 1. Larvae were allowed for feeding on citrus seedling roots for 40 days in the RFD experiment, and 56 days in the LRFD experiment to test larval survival and growth in a longer period of feeding. During the larval feeding periods, all seedlings including the controls received the same rates of fertilization and irrigation in both experiments.

At the end of the experiment, larval survival rate and larval weight were evaluated using the methods described in Rogers et al. (2000). Each plant was removed from the pot and placed on a shallow examination tray. A spatula was used to gently remove

Table 2
Rootstock × Flooding × *Diaprepes*-larvae (RFD) experiment. Comparison of soil–pot cap, pH, larval survival and larval weight between the treatments for rootstock Swingle (SWI) and Smooth Flat Seville (SFS). Data are mean ± standard deviation

Flooding and Diaprepes larval feeding Treatment	Rootstock Swingle (SWI)			
	Soil–pot gap (cm)[†]	Soil pH[†]	Larval survival (%)[†]	Larval weight (g)[†]
NF-D[††]	1.8 ± 0.4 c	4.8 ± 0.2 c	72 ± 17 a	0.17 ± 0.28 a
F20-D[††]	3.0 ± 0.7 b	4.9 ± 0.1 b	64 ± 21 ab	0.13 ± 0.05 b
F40-D[††]	3.3 ± 0.8 a	5.1 ± 0.6 a	62 ± 22 ab	0.08 ± 0.04 c
Rootstock Smooth Flat Seville (SFS)				
NF-D[††]	1.9 ± 0.4 c	4.8 ± 0.2 c	78 ± 18 a	0.11 ± 0.08 b
F20-D[††]	3.1 ± 0.6 b	4.9 ± 0.2 b	54 ± 34 b	0.08 ± 0.06 c
F40-D[††]	3.4 ± 0.8 a	5.2 ± 0.5 a	16 ± 25 c	0.02 ± 0.03 d
SWI (all treatments)[††]	2.7 ± 1.4 b	4.9 ± 0.6 b	67 ± 19 a	0.13 ± 0.21 b
SFS (all treatments)[††]	2.8 ± 1.5 b	5.0 ± 0.5 a	49 ± 36 b	0.07 ± 0.09 c

[†] Mean in the same letter at the same column is not significantly different at $P < 0.05$. Soil–pot gap LSD = 0.3 cm, soil pH 0.08; larval survival LSD = 16%, and larval weight = 0.03 g.

[††] NF, non-flooded control; F20, 20-day flooded; F40, 40-day flooded. D: *Diaprepes* larval infestation.

the soil from around the roots to count larvae. Weights of surviving larvae per seedling were determined using a Mettler AMI00 balance (Mettler Instrument Crop., Hightstown, NJ). Soil of each seedling was collected after removal of all survival larvae then the soil was air-dried. Soil pH of each seedling was determined in soil:water (1:1) using the Orian pH meter.

During the flooding period, floodwater pH was measured using an Orion pH meter (Orion Research Inc., Boston, MA). Soil–pot gap (distance between the soil surface and the top of the pot) was measured using a ruler before and after of the flooding procedure. We did other measurements (soil redox potential, leaf stomatal conductance, shoot length, leaf area, dry matters of leaves, stems and roots, and root damage rating for both experiments), which were not shown here.

Homogeneity of the data was confirmed using the Bartlett test. Normality and residual distribution of the data were verified using PROC UNIVARIATE (SAS Institute, 1990). The effects of treatments on soil pH, larval survival and larval growth were determined by analysis of variance using the General Linear Models procedure. Comparison of treatment means was done using the Least Significant Difference (LSD) test, and correlation analysis was done using PROC CORR (SAS Institute, 1990).

3. Results

3.1. Soil–pot gap and soil pH variation

The soil–pot gap increased on average 0.8 cm in the non-flooded pots, 2.3 cm after 20 days of flooding, and 2.6 cm after 40 days of flooding and a week of draining, as compared to the initial soil–pot gap value (1.0 cm) in the RFD experiment (Table 2). As a loam, the Floridana sandy loam would be compacted after the flooding-draining procedure. In this RFD experiment where the soil was not limed, flooded soil pH increased by 0.1 units after 20 days of flooding and by 0.3 units in Swingle and Smooth Flat Seville after 40 days of flooding. The difference in soil–pot gap and soil pH were significantly between the flooding treatments for the two rootstocks (Table 2).

In the LRFD experiment, the soil–pot gap was similar to that shown in Table 2 for the treatments after 40 days of flooding and 7 days of draining. The measured pH values were equally 0.3 units higher in the flooded control than in the non-flooded control in Swingle (5.09 versus 4.75) and also in Carrizo (4.77 versus 4.46) at the end of the experiment (Table 3). Among the target pH treatments, only one (flooded target pH 6 in Carrizo) reached the target pH level (Table 3). The measured soil pH was significantly higher (LSD = 0.065, $t = 1.98$, $\alpha = 0.05$) in Carrizo (pH 5.34) than in the Swingle (pH 5.22). Combining the measurements in two rootstocks, the soil pH values were 4.77 ± 0.13 for the control, 5.44 ± 0.21 for the target pH 6, and 5.65 ± 0.26 for the target pH 7, and the difference in pH was significant among the liming treatments (LSD = 0.0798, $t = 1.98$, $\alpha = 0.05$). 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.

Differences in soil pH between liming treatments, rootstocks and flooding treatments were significant

Table 3
Liming × Rootstock × Flooding × Diaprepes-larvae (LRFD) experiment. Descriptive statistics of final measured soil pH related to the target liming treatments in two citrus rootstocks Swingle and Carrizo. $n = 15$ per rootstock per liming treatment

Treatment	Measured soil pH					
	Non-flooded			40-day flooded		
	Control	Target pH 6	Target pH 7	Control	Target pH 6	Target pH 7
Rootstock Carrizo						
Mean	4.46	5.18	5.42	4.77	6.14	6.16
CV	1.95	4.81	4.39	2.29	3.33	3.57
Kurtosis	−1.12	−0.80	−0.91	−1.04	0.05	−1.27
Skewness	−0.36	0.16	0.21	0.07	0.70	0.45
Minimum	4.30	4.81	5.08	4.61	5.86	5.84
Maximum	4.57	5.62	5.87	4.97	6.56	6.41
Rootstock Swingle						
Mean	4.75	4.95	5.14	5.09	5.49	5.95
CV	5.45	3.16	5.59	2.51	3.72	6.51
Kurtosis	10.7	5.01	−1.20	−1.20	0.16	−1.32
Skewness	3.03	1.96	−0.12	−0.31	0.28	0.50
Minimum	4.50	4.78	4.68	4.86	5.13	5.47
Maximum	5.63	5.41	5.62	5.24	5.93	6.54

(Table 4). The overall average pH was 0.61 units higher (LSD = 0.0651) in the flooded treatment (5.59, $n = 90$) than in the non-flooded (4.98, $n = 90$). There was an interaction between rootstock variety and flooding treatment on soil pH, appearing between the control and target pH 6 (Fig. 1). Soil in the flooded Carrizo showed the highest response but the non-flooded Swingle had little response to the liming. At each target pH, the measured soil pH in each rootstock had little variation with a small standard deviation (pH 0.11–0.39), which gave a very small coefficient of variation (Table 3). Except the non-flooded treatment in the Swingle, all limed soil pH data had a kurtosis and a skewness close to one (Table 3).

Soil water content was higher in the limed treatments (Table 4). As plotted against the target pH liming treatments (TpH), the measured soil pH (MpH) was linearly and quadartically related to the TpH, shown by the polynomial equations as follows:

$$
\begin{array}{llll}
\text{Carrizo:} & \text{Non}-\text{flooded} & \text{MpH} = -0.2396\text{TpH}^2 + 3.3561\text{TpH} - 6.3295 & R^2 = 0.8092^{**}, \quad n = 45 \\
 & \text{Flooded} & \text{MpH} = -0.7092\text{TpH}^2 + 9.1776\text{TpH} - 23.392 & R^2 = 0.9332^{**}, \quad n = 45 \\
\text{Swingle:} & \text{Non}-\text{flooded} & \text{MpH} = 0.0022\text{TpH}^2 + 0.01681\text{TpH} + 3.8568 & R^2 = 0.3186^{*}, \quad n = 45 \\
 & \text{Flooded} & \text{MpH} = 0.0246\text{TpH}^2 + 0.1332\text{TpH} + 3.8098 & R^2 = 0.6542^{**}, \quad n = 45 \\
\text{Carrizo+Swingle:} & \text{Non}-\text{flooded} & \text{MpH} = -0.1187\text{TpH}^2 + 1.7621\text{TpH} - 1.2363 & R^2 = 0.5232^{**}, \quad n = 90 \\
 & \text{Flooded} & \text{MpH} = -0.3423\text{TpH}^2 + 4.6554\text{TpH} - 9.7909 & R^2 = 0.7087^{**}, \quad n = 90
\end{array}
$$

The linear effects of liming treatments could increase soil pH more strongly in Carrizo ($P < 0.001$) than in Swingle ($P < 0.05$), and it was indicated also by the greater coefficients in the polynomial equations in Carrizo. The quadratical effects of TpH could decrease the soil pH in Carrizo but not in Swingle based on the pH equations. A plateau appeared after the target pH-6 level under non-flooded conditions in both rootstocks (graph not shown). The pH polynomial models had greater coefficients of determination in Carrizo than in Swingle.

3.2. Larval survival and larval growth

In the RFD experiment, larval survival was significantly higher in Swingle (66 ± 19%) than in Smooth Flat Seville (49 ± 36%) (LSD = 12%, $t = 1.98$, $\alpha = 0.05$). In Swingle, larval survival was similarly high among the flooding treatments (Table 2). In Smooth Flat

Table 4
Liming × Rootstock × Flooding × *Diaprepes*-larvae (LRFD) experiment. Comparison of *Diaprepes* larval survival and soil water content of different liming treatments. The larval infestation period was 56 days in non-flooded or in flooded rootstock Swingle (SWI) and Carrizo (CAR). Data were mean and standard deviation of 15 measurements

Treatment	*Diaprepes larval* survival and soil water content			
	Non-flooded		40-day flooded	
	Larval survival[†] (%)	Soil water content[†] (g g^{-1})	Larval survival[†] (%)	Soil water content[†] (g g^{-1})
Rootstock Swingle (SWI)				
Control[††]	60 ± 32 c	0.20 ± 0.03 b	51 ± 34 c	0.18 ± 0.04 b
Target pH 6	64 ± 44 c	0.22 ± 0.05 a	40 ± 32 d	0.20 ± 0.10 a
Target pH 7	72 ± 17 b	0.23 ± 0.06 a	66 ± 28 b	0.21 ± 0.06 a
Rootstock Carrizo (CAR)				
Control[††]	84 ± 23 a	0.21 ± 0.05 ab	52 ± 33 c	0.18 ± 0.06 b
Target pH 6	82 ± 15 a	0.23 ± 0.04 a	51 ± 25 c	0.17 ± 0.04 b
Target pH 7	74 ± 27 b	0.21 ± 0.03 ab	82 ± 11 d	0.19 ± 0.04 ab
SWI (mean)[††]	69 ± 31 b	0.22 ± 0.11 a	52 ± 32 c	0.20 ± 0.13 a
CAR (mean)[††]	80 ± 21 a	0.21 ± 0.09 ab	62 ± 23 b	0.19 ± 0.10 ab
SWI + CAR (mean)	74 ± 26	0.22 ± 0.12	57 ± 27	0.19 ± 0.14

[†] Mean in the same letter at the same column is not significantly different at $P < 0.05$. Larval survival LSD = 12%, and soil water content = 0.03 g g^{-1}.

[††] Control, non-limed and non-flooded control; SWI, rootstock Swingle; CAR, rootstock Carrizo.

Seville, larval survival was significantly higher in the non-flooded control (78 ± 18%) than in the flooded treatments, and the larval survival rate significantly decreased with the flooding duration (Table 2). Larval survival was the lowest in the longest previously flooded 40 days (16 ± 25%), which was only less than 1/4 of the survival rates in the control and in the 20-day flooding treatment in Smooth Flat Seville. Increasing pH by 0.3 units and soil–pot gap by 1.8 cm in the 40-day flooding treatment corresponded to the lowest larval survival rate of 16% in Smooth Flat Seville (Table 2).

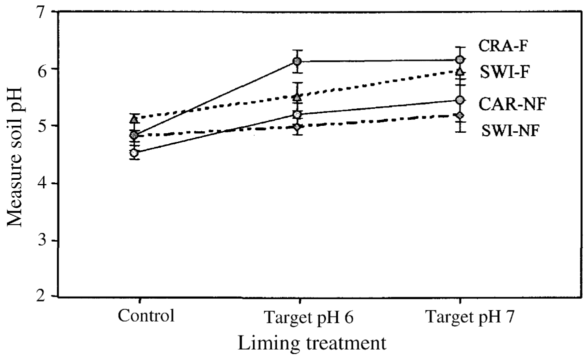

Fig. 1. Interactions between rootstocks (Carrizo and Swingle) and flooding treatments (non-flooded and flooded) on soil pH in the Liming × Rootstock × Flooding × *Diaprepes*-larvae (RLFD) experiment. Each point represents the mean and standard deviation of 15 measurements. CAR-F, flooded Carrizo; CAR-NF, non-flooded Carrizo; SWI-F, flooded Swingle; SWI-NF, non-flooded Swingle.

Total weight of larvae averaged between 0.11–0.17 g per seedling in the non-flooded control in both rootstocks (Table 2), a growth of 240–370 times after 40 days of feeding on seedling roots. The initial larval weight was on average 0.00045 g (5 of 1-day old neonates) only. Larval weight decreased with the duration of previous flooding, and the lowest larval weight was also in the longest flooded treatment (40 days) in Smooth Flat Seville (Table 2). With lower survival rate, the total weight of the larvae in the SFS was only about half of the weight values in Swingle (Table 2). Per seedling total larval weight was more correlated to survival rate in Smooth Flat Seville ($r = 0.68$, 0.72, and 0.93) than in Swingle ($r = 0.25$, 0.42, and 0.21 for the control, 20 and 40-day flooding treatments, respectively).

In the LRFD experiment, larval survival in the non-limed and non-flooded control for Swingle and Carrizo averaged 72%, which was comparable to the rates in the non-flooded control in the RFD experiment (72–78%, Table 2). The target pH 6 level had the lowest (mean 40%) but highly variable (standard deviation 32%) survival rate among all treatments. The larval survival was significantly lower and more variable in Swingle than in the Carrizo (Table 4). Together larvae survival was 17% lower in the flooded (57%) than in the non-flooded (74%) by combining two rootstocks. Larval weight seemed to increase with soil water content as larval weight was significantly higher (0.081 g per seeding) with a higher

Table 5
Effects of liming, citrus rootstock, flooding duration, and *Diaprepes* larval infestation and their interactions on soil pH, larval survival, and larval weight in Liming × Rootstock × Flooding duration × *Diaprepes*-larvae (LRFD) experiment

Sources	d.f.	Measured soil pH[†]	Larval survival[†]	Larval weight[†]
Liming (L)	2	241.06^{**}	8.61^{**}	3.70^{*}
Rootstock (R)	1	10.28^{**}	4.95^{*}	0.96 ns
Flooding (F)	1	294.16^{**}	8.22^{**}	36.79^{**}
Diaprepes larvae (D)	1	1.33 ns		
L × R	2	35.63^{**}	0.16 ns	0.38 ns
L × F	2	17.74^{**}	4.72^{*}	1.94 ns
R × F	1	0.52 ns	2.54 ns	0.47 ns
L × R × F	2	6.64^{**}	5.84^{**}	0.98 ns
L × R × F × D	2	2.42^{*}		
sModel R^2[†]	23	0.87^{**}	0.42^{**}	0.41^{**}
cv[††]		4.13	48.6	75.2
Mean[††]		5.29	57.2	0.0332
RMSE[†††]		0.22	29.0	0.0250

[†] *F* values. Not all the interactions were listed for the model. *Diaprepes* larvae did not include in the model for the variables larval survival and larval weight because there were no data on larval survival and weight for the control (zero larvae). ns, non significant, and * and **significant at $P < 0.05$ and $P < 0.01$.

[††] CV, coefficient of variation; Mean, larval survival in percent and larval weight in g.

[†††] RMSE: root mean square error.

soil water content (0.31 g kg^{-1}) than with a lower soil water content (0.17–0.22 g kg^{-1}) in the flooded Swingle.

The effects of liming, rootstock and flooding treatments were significant on soil pH and larval survival, and most of the interactions of liming, rootstock and flooding treatments with *Diaprepes* larval infestation were significant on soil pH in the LRFD experiment (Table 5). *Diaprepes* larvae was not included in the model of analysis of variance for the variables of larval survival and weigh because there were no data for the control (zero larvae input). The interactions between liming and flooding treatments and their interaction with rootstock were significant on larval survival but not on larval weight (Table 5).

3.3. Larval survival frequency and regression pattern related to soil pH

The analysis of frequency showed that in the LRFD experiment, up to 60% of the seedlings ($n = 10$) had a survival rate of 100% in the non-limed and non-flooded control, compared to 40% of the seedlings having a survival rate of 80–100% in the non-flooded target pH 6 and 7 in Carrizo (Fig. 2A). In the flooded treatments, no seedling had 100% survival rate in the non-limed control and the target pH 6 but most of the seedlings had a survival rate of 80% in the target pH 7 (Fig. 2B). In Swingle, the analysis frequency for all liming treatments showed that 50% of the seedlings ($n = 30$) had a high survival rate of 80–100% in the non-flooded treatments (Fig. 3A), and 50% of the seedlings ($n = 30$) had a rate of 40–100% in the flooded treatments (Fig. 3B).

The regression of larval survival rate against measured soil pH in the LRFD experiment showed that the larval survival was random (0–100%) for soil pH from 4.5 to 5.1, and 100% survival rate occurred only in soil pH lower than 5.1 in the non-flooded (Fig. 4A). The larval survival tended to decrease with increase of pH in Swingle ($n = 30$, Fig. 4A) and in Carrizo ($n = 30$, Fig. 4B). Per seedling larval survival rate reduced from 80% to 60% with higher soil pH between 5.2 and 5.8 in Swingle (Fig. 4A) and from 100 to 40% in Carrizo when soil pH increased from 5.1 to 5.7 (Fig. 4B). Larval survival was reduced by soil pH greater than 5.3 based on the polynomial equations. However, it was not clear whether larval survival could further decrease with soil pH greater than 5.7 because no soil pH greater than this value was measured (non-flooded seedling).

The regression patterns of larval weight appeared to decline significantly against the measured soil pH in Swingle (Fig. 4C) and the decrease was rather than slight in Carrizo (Fig. 4D). Larval weight was also significantly reduced at soil pH >5.3 (Fig. 4C and D). Per seedling total larval weight decreased from 0.060 g at soil pH <5 to 0.012 g when the soil pH increased from 5.1 to 5.7 in the non-flooded Swingle. Per seedling larval weights were only significantly higher in the flooded treatments for the two rootstocks (Fig. 5). Total larval weights were lower in the non-flooded treatments (0.023 ± 0.014 g), which were only 50% of the weights

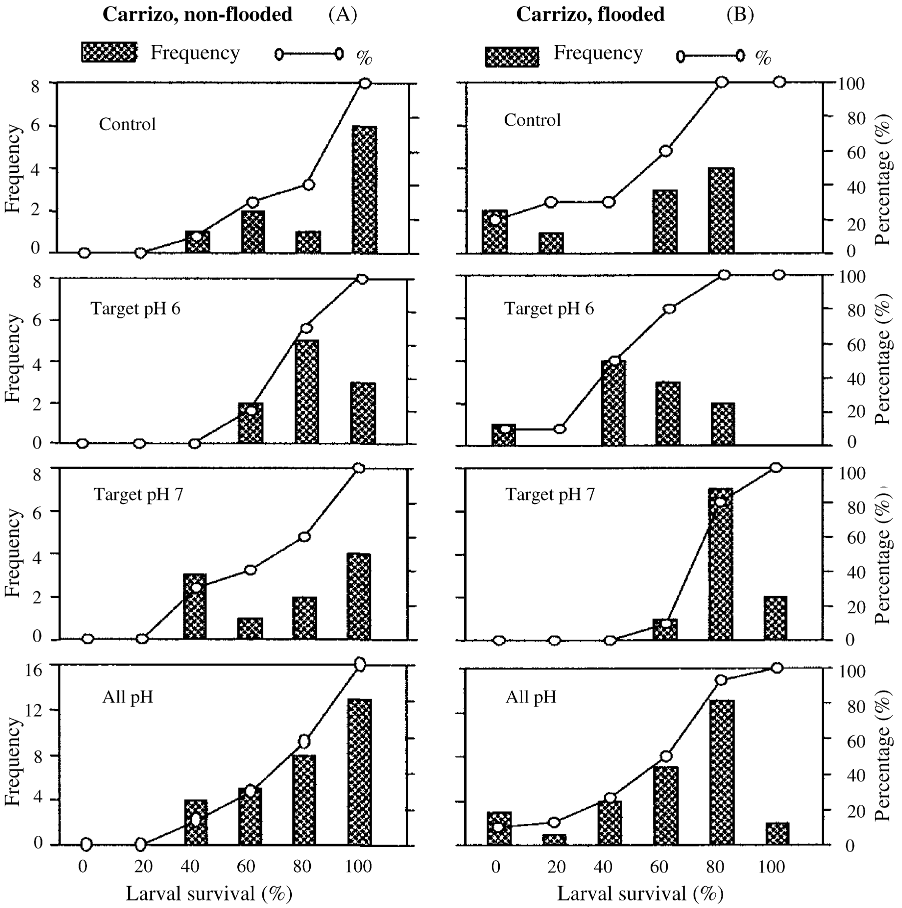

Fig. 2. Frequency analysis of larval survival for liming treatments in non-flooded (A) and flooded (B) rootstock Carrizo in the Liming × Rootstock × Flooding × *Diaprepes*-larvae (RLFD) experiment.

in the flooded (0.064 ± 0.078 g) in Swingle. Changes in total larval weight were also higher in flooded treatments (0.060 ± 0.051 g) compared to the weights in non-flooded (0.024 ± 0.013 g) in Carrizo. Larval weights were the highest and most variable in the non-limed flooded treatment in Swingle (Fig. 5A).

Only the effects of liming and flooding treatments were significant on larval weight (Table 5), and there was no effect of rootstock because the larval weights were similar between the two rootstocks (Fig. 5). The similar patterns of larval weight to larval survival (Fig. 4) could be explained by their correlation relationships, shown in Table 6. The correlation coefficients were all positively significant ($0.44 < r < 0.90$, Table 6), which suggested that total larval weight (or larval growth) was proportional with larval survival rate.

Table 6

Pearson correlation coefficients for larval survival rate and larval weight in the Liming × Rootstock × Flooding × *Diaprepes*-larvae (LRFD) experiment.

Treatments	Rootstock Carrizo		Rootstock Swingle	
	Non-flooded†	40-day flooded†	Non-flooded†	40-day flooded†
Pearson correlation coefficients (r)				
Control†	0.78**	0.90**	0.90**	0.61**
Target pH6†	0.88**	0.74**	0.57*	0.44*
Target pH7†	0.60**	0.65**	0.61**	0.90**

†*, significant at probability $P < 0.05$, and **, significant at $P < 0.01$.

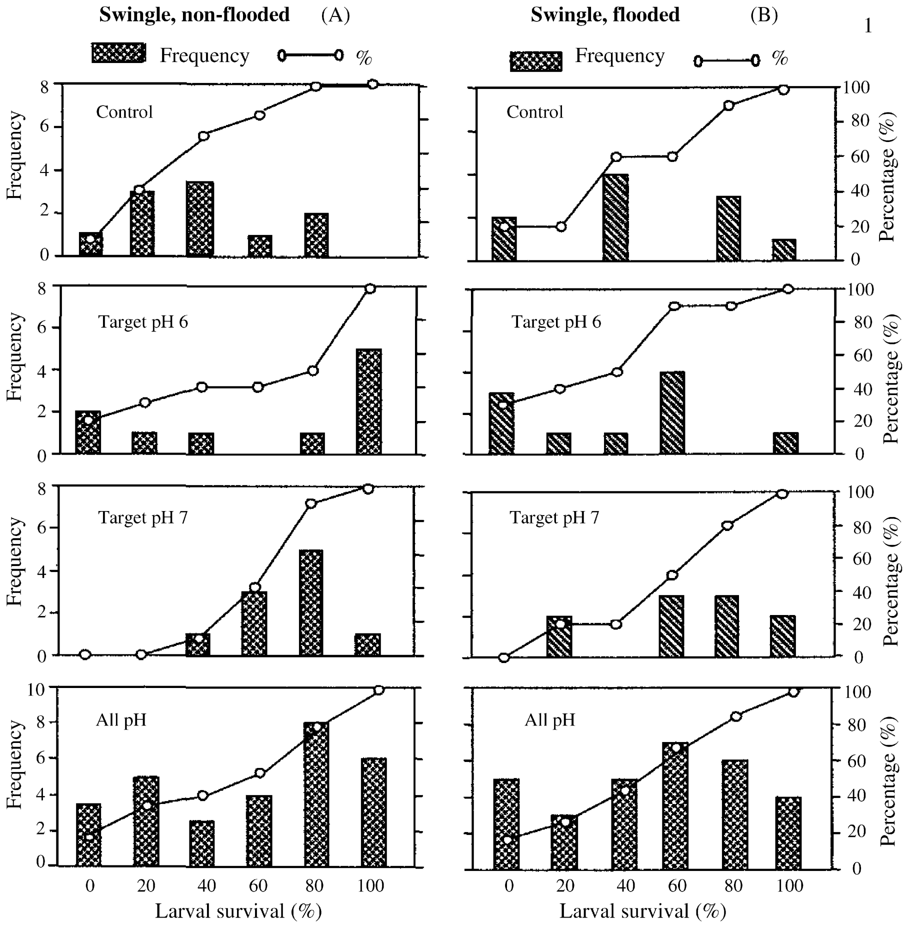

Fig. 3. Frequency analysis of larval survival for liming treatments in non-flooded (A) and flooded (B) rootstock Swingle in the Liming × Rootstock × Flooding × *Diaprepes*-larvae (RLFD) experiment.

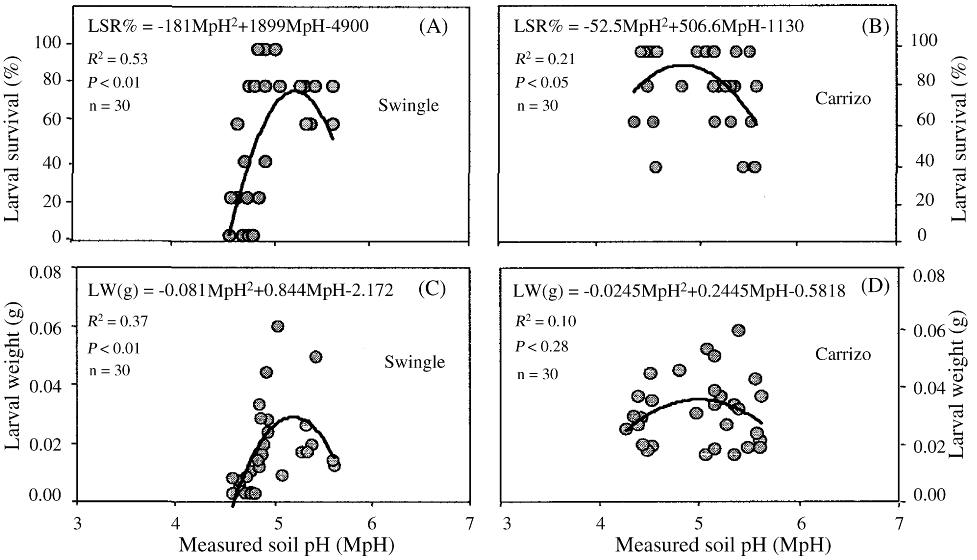

Fig. 4. Regression of *Diaprepes* larval survival and larval weight against measured soil pH for non-flooded seedlings of rootstock Swingle (A and C) and rootstock Carrizo (B and D) in the Liming × Rootstock × Flooding × *Diaprepes*-larvae (RLFD) experiment.

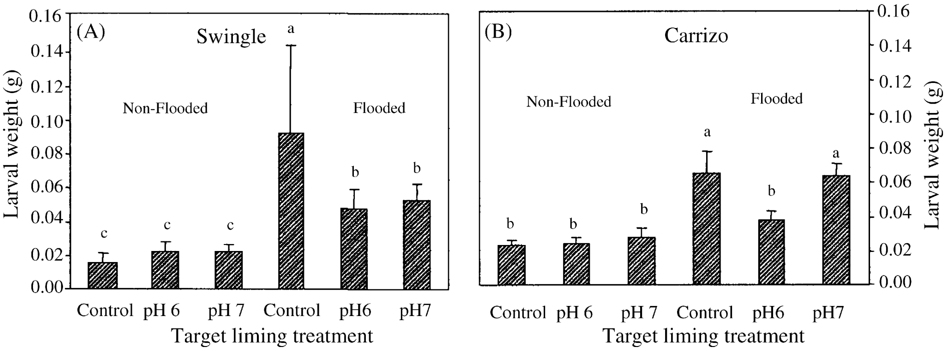

Fig. 5. *Diaprepes* larval weights of different liming treatments after 56 days of infestation in non-flooded or flooded seedlings of rootstock Swingle (A) and Carrizo (B). Each bar represents the mean and standard error of 15 measurements.

4. Discussion

4.1. Larval survival and growth versus soil pH levels

Data from both non-liming (RFD) and liming (LRFD) studies showed that *Diaprepes* root weevil larval survival and growth could reduce with changes of pH to higher levels in the acidic soil. With non-liming (RFD experiment), where soil pH was higher (5.2 ± 0.5) with a gain of 0.3 units after the 40 days of flooding, the larval survival could be low (26%, 2-rootstock mean) and total larval weight per seedling could also be low (0.042 g, 2-rootstock mean) after 40 days of feeding on the 40-day flooded seedlings (Table 2). With liming (LRFD experiment), after 56 days of feeding on the 40-day flooded seedlings *Diaprepes* larval survival was two times higher (59%, 2-rootstock mean, Table 4) but total larval weight per seedling was only equal (0.048 g, 2-rootstock mean) to the numbers in the RFD experiment. Also, in the non-limed and non-flooded control in the LRFD experiment, total larval weights per seedling was only 0.024 g (2-rootstock mean) after 56 days of feeding, which was only 17% of the larval weights for the non-flooded and no liming control (0.14 g, 2-rootstock mean) in the RFD experiment. The 1-day old larvae used in the two experiments were two different populations. It was not clear if the liming had prohibited the larval growth (weight gain) or there was difference in vitality between the two populations, which would influence the larval survival (Jones and Schroeder, 1983; Schrader et al., 1998; Hatch and Blaustein, 2000; Suemoto et al., 2005).

Significantly lower larval survival and weight gain with higher soil pH, larger soil–pot gap (Table 2) and lower soil water content (Table 4) in the 40-day flooded treatments suggested that larval survival and growth might not only have been influenced by soil pH but also other environmental characteristics, such as soil compaction and soil water content. Although soil compaction was not measured in the two studies, the flooded soils could have been more compacted because after the flooding–draining procedure the soil–pot gap became 1.2–1.5 cm greater in the flooded treatments than in the non-flooded control (Table 2). Flooded soil contained less water (Table 4), which was also an indicator of soil compaction after flooding.

Compacted soil would pose problems of reduced porosity and poor aeration that would be less suitable to larval survival and growth. In other studies, surviving numbers and activity levels of larvae were significantly reduced in the treatment with a low pH of 5, high nitrate exposure levels of 20 mg L^{-1} (Hatch and Blaustein, 2000). It was also reported that *Diaprepes* larval survival was high (76–85%) after 40 days of feeding on 20-day flooded seedlings of two citrus rootstocks in a Candler fine sandy soil with 965 g kg^{-1} of sand (Li et al., 2006a). Larval survival could be influenced by soil type, soil moisture and other soil characteristics (Riis and Esbjerg, 1998; Rogers et al., 2000). As suggested in Li et al. (2006a), flooded soil would be less aerated that might lead to lower larval survival than in the non-flooded soil.

The practical implication of our results would be control of *Diaprepes* weevil larvae from raising acidic soil pH. The decrease of larval survival and larval weights within a soil pH of 5.3–5.7 (Fig. 4) suggested that increasing pH by 0.5–1 unit might be useful for control of this root weevil in the strongly acidic soil. The more pronounced effects of soil pH on larval survival and growth in Swingle than in Carrizo (Fig. 4) was the results of the interactions between the rootstock, liming and flooding treatments (Table 5). Although the R^2 values were not high ($R^2 < 0.33$, Fig. 4), these model coefficients of determination were significant except for the larval weight in Carrizo (Fig. 4D). It is to mention that although larval survival and larval weight appeared dispersal as plotted against the measured soil pH, their trends were generally decline with soil pH (Fig. 4).

Further, could larval survival and weights be even lower at a slightly acid soil (pH 6.0–6.5) than in a moderately acid soil (pH 5.3–5.7)? In the citrus groves, weekly density of *Diaprepes* weevil adult population was 2.7 times higher (0.0393 adult weevils m^2) in the strong acidic (pH 4.8) Floridana sandy loam than in the near neutral (pH 6.6) Ona sand (0.0144 adult weevils m^2) in Florida (Li et al., 2006b). Also, density of *Diaprepes* adults captured in Tedders traps decreased with increasing Ca and Mg concentrations in citrus grove, where dolomite was applied (Li et al., 2004a). There is certainly a need for further information about whether larval survival could be even lower in soil pH of 6.0–6.5, a level more suitable to citrus tree growth.

4.2. Soil pH, larval survival and larval weight versus soil flooding

Larvae survived better under non-flooded conditions (2-rootstock mean, 75%, Table 2; 67%, Table 4) than under flooded conditions (2-rootstock mean, 49%, Table 2; 52%, Table 4). The significantly lower survival and weight of larvae in flooded treatments in the both experiments (Table 2, Table 4, and Fig. 5) suggested that flooding might be beneficial by reducing larval survival. Because of the depletion of oxygen from the floodwater with time of submergence, pH of the floodwater became higher in the longer duration of flooding (Table 2). The floodwater used in the studies had a pH close to 7, which might also be a possible reason for higher pH in flooded treatments than in non-flooded treatments. Usually, natural floodwater from rainfall is acidic with excessive hydrogen to acidify soil. Li et al. (2006a) suggested that floodwater pH should be similar to rainfall pH to examine whether natural flooding events have any influence on soil pH.

The significant interaction of rootstocks and flooding treatments on soil pH (Table 5) was because the responses to liming treatments of the rootstock Carrizo were greater than Swingle (Fig. 1). There might be a difference in capacity of absorption of hydrogen between the two rootstocks because the higher soil pH in Carrizo (Fig. 1) suggested that this rootstock might absorb more hydrogen than Swingle. Also, the significant interaction between liming, rootstock and flooding treatments on larval survival (Table 5) would explain why larval survival was significantly higher in Carrizo (80 ± 21% in non-flooded and 62 ± 23% in flooded, Table 4) than in Swingle (69 ± 31% in non-flooded and 52 ± 32% in flooded, Table 4). It would be useful further conducting a one factor experiment to quantify separately liming effect on larval survival.

The correlation coefficients between larval survival rate and weight gain (Table 6) showed that this correlation had no trend of depending on rootstock, liming level or flooding duration. Because larvae subsequently feed on roots, seedling root mass and root quality might explain these differences in larval weight gains. Also, higher soil water content in the limed soil (target pH 6–7, Table 4) agreed with the idea that liming could raise soil water holding because of adding large amounts of Ca to the soil (Adams, 1984; Li et al., 2001). A possible explanation for only 1/4 of the liming treatments reaching the target pH levels (Table 3) might be because of heterogeneity distribution of lime in the soil, which could be because of leaking lime from the top to the bottom of container through seedling watering and fertilizing. The more efficient liming effect for the target pH 6 than the target pH 7 (Fig. 1) suggested that all applied limestone dolomite might have not completely reacted with hydrogen ions yet because there was 66% more dolomite applied to the target pH 7 than the target pH 6 treatments. Also, the high levels of soil organic matter content (8%) might have affected the liming outcome. High organic matter content would mean high exchangeable acidity and high buffering capacity in the soil. Soil with a high buffering capacity would require larger amount of lime to increase the pH than soils with a low buffering capacity (Adams, 1984; Bohn et al., 2001). It would be useful to further quantify buffering capacity and exchangeable acidity of this soil for determining a more efficient lime rate.

Soil pH is known as a chemical indicator of soil quality. Soil pH controls many of the biological, chemical and physical properties of soil (Adams, 1984; Bohn et al., 2001). However, the mechanism allowing an insect to survive and the mechanisms through which limestone reacts with acid soils are complex. Adjusting soil pH levels to citrus needs through liming is a common cultural practice. Our data have suggested a possibility of continuing decrease of larval survival to occur in a higher soil pH than 5.7 until a level more suitable to citrus tree growth. Understanding the critical plant and soil factors that determine survival and growth of larvae has been considered for developing successful pest management strategies (Lower et al., 2003). Our results can implicate in raising acidic soil pH for control of pest larvae. If raising soil pH could be a treatment for *Diaprepes* root larval control, then improving soil liming would be a cultural option for integrated management of this root weevil.

5. Conclusions

Survival and growth of *Diaprepes* root weevil larvae could be affected by soil flooding duration and pH level. Flooding might be beneficial by reducing larval survival because the potential of neonate larval growth was lower in the flooded soils than in the non-flooded soils. Increasing in soil–pot gap and decreasing in soil water content suggested soil compaction in flooded soil. Flooding also affected the soil liming outcome. The effects of liming, rootstock and flooding and their interactions were significant on soil pH and larval survival. The rootstock Carrizo had a stronger response to liming treatments than Swingle but no difference in larval weight was found between rootstocks. *Diaprepes* larval survival and larval growth varied with soil pH, and larval survival and weight showed a trend to decrease with soil pH range greater than 5.7, a level more suitable to citrus tree growth than the initial soil pH 4.8. The implications of our results in practices would be raising soil pH from strongly acidic to slightly acidic range to reduce *Diaprepes* larval survival and using a cultural tool of improving soil pH for integrated pest management in acidic soil. Future examinations include whether a target soil pH within 6–6.5 (optimum soil pH for citrus growth) would reduce *Diaprepes* larval survival and growth. More data are needed for establishing the relationships between larval survival and larval growth elated to soil flooding and pH levels, and for quantifying a liming rate to correct soil pH and to control *Diaprepes* root weevil larval population.

Acknowledgement

We thank Florida Citrus Production Research Advisory Council for the financial support for this study.

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