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Citrus Tree Abiotic and Biotic Stress and Implication of Simulation and Modeling Tools in Tree Management

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ABSTRACT

Plant abiotic and biotic stress is related to unfavorable and environmental constraints. As a warm climate tree fruit crop, citrus (*Citrus sinensis* (L.) Osb.) is adapted to a wide variety of soil types and growth conditions. However, when waterlogging, soil acidity and root weevil infestation occur simultaneously, citrus roots can be injured from anaerobic disturbance, oxygen deprivation and root injury, which can lead to tree decline. Multi-year spatial overlay patterns of plants, insects and soils may yield management insights for reducing plant biotic and abiotic stresses. This paper attempts to summarize abiotic and biotic stress of citrus trees associated with soil anaerobe, soil waterlogging, environmental acidity and *Diaprepes abbreviatus* root weevil infestation, and to give an overview of the development of new biological tools such as greenhouse simulation and model prediction tools for integrated fruit production of citrus. Greenhouse simulation studies and a series of multi-year studies at citrus orchard scale have been conducted across center and southern counties in Florida. The results showed that citrus tree decline was correlated with anaerobe and high soil Fe concentrations ($P < 0.05$), and citrus tree biotic and abiotic stress is directly reflected by low leaf stomatal conductance, flooding root damage, weevil larval root feeding injury, and anaerobic-related soil redox potential. Citrus rootstock roots were injured up to three weeks of submergence and flooded-roots were more susceptible to *Diaprepes* root weevil feeding than non-flooded roots. Time series analysis reveals that root adult weevil population outbreaks were associated with warm air temperatures across a period of three years ($r = 0.49$, $P < 0.0067$), suggesting that warming conditions would contribute to more tree biotic stress. Greenhouse simulation tools and time series forecast models have the implication in reducing biotic and abiotic stress of citrus trees.

Keywords: citrus root injury, citrus rootstocks, citrus root weevil, insect-environmental relations, larval survival, leaf stomatal conductance, soil oxidation-reduction potential, time series analysis

CONTENTS

INTRODUCTION

Plant stress is the state of a plant under the conditions of a force applied and damage is the result of too high a tress that can no longer be compensated for (Lichenthaler 1996; Gutschick 1999; Bray *et al*. 2000; Mittler 2006; Laughlin and Abella 2007). Plant stress is defined as an external factor that exerts a disadvantageous influence on a plant, and the concept of stress is intimately associated with that of stress tolerance that is the plant's fitness to cope an unfavorable environment and an attack (Taiz and Zeiger 2002; Mittler 2006; Li *et al*. 2008). Abiotic and biotic stress conditions may result in extensive loss in citrus production (Olsen *et al*. 2000; Fernandez-Ballester *et al*. 2003; Li *et al*. 2006; Syvertsen and Hanlin 2008). Most stresses faced by plants are either abiotic (e.g. anaerobe, flooding, acidity, salinity, heat, drought, or nutrient deficits) or biotic (e.g. insect, disease or pathogen), which are related to environmental conditions (White 1984; Mattson and Haack 1987; Dorn *et al*. 1999; Li *et al*. 2003a, 2003b; Ramirez-Rodriguez 2005; Garrett *et al*. 2006; Li *et al*. 2006, 2008). Plants show some degree of stress when exposed to unfavorable environments and these stresses can collectively contribute to affect plant growth and crop productivity (Blum 1996; Taiz and Zeiger 2002; Jones *et al*. 2003; Li *et al*. 2003b, 2005, 2006, 2007a). It is estimated that up to 82% of potential crop yields are lost due to abiotic stress annually (Bray *et al*. 2000).

In North America, citrus production totals 14.9 billion kilograms, representing 18% of world total production. Florida produces 82% of total citrus production in North America. Citrus, a warm climate fruit tree in the Rutaceae family, performs best in subtropical climates, where there is a slight change of season but little or no chance of freezing weather.

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Invited Review

Citrus is adapted to a wide range of soil types and is more tolerant of high or low pH. Citrus trees are grown on almost pure sand in central Florida, to organic muck near the Everglades, to loamy, heavy, high-pH soils in the San Joaquin Valley of California (Rieger 2005).

Citrus fruits obtain their highest internal quality (juice content, sugar and acid levels) in Florida subtropical humid climates, while irrigated citrus fruits achieve best external quality in California Mediterranean climate (Rieger 2005). However, citrus species are susceptible to a number of disease and insect infestation (Olsen *et al.* 2000) and citrus trees generally do not tolerate soil flooding for more than a few days without injury (Rieger 2005). Temperature and soil water are the main environmental factors controlling citrus tree health and quality (Falivene *et al.* 2006; Syvertsen and Hanlon 2008). Florida citrus soils range from well-drained Entisols on relatively high, rolling landscapes to poorly-drained Alfisoils and Spodosols on low-lying flatwoods (Obreza and Collins 2002). Most flatwoods soils contain high levels of active hydrogen because of high rainfall, aluminum from soil reacting with water to give free hydrogen (Li *et al.* 2007b).

Biology of citrus root weevil *Diaprepes abbreviatus* (L.)

The root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), originally from the Caribbean, has become a major pest of citrus and other agricultural crops in Florida in recent years (Nigg *et al.* 2001; Futch 2003; Graham *et al.* 2003; McCoy *et al.* 2003; Nigg *et al.* 2003; Stuart *et al.* 2004). *Diaprepes* root weevil adults are citrus leaf feeders of all tree varieties and females deposit eggs in masses glued between leaves in the citrus canopy. Hatching neonates fall to the soil surface, and move into the soil where they feed on roots and subsequently pupate (Nigg *et al.* 2001; McCoy *et al.* 2003). *Diaprepes* larval growth is consistently fast at ambient temperature of 22-26°C (Lapointe 2000). Growth of *Diaprepes* larvae could be an increase of 36-375 times of their weights as 1-day-old neonates within 30 days of infestation, and 240-370 times within 40 days of infestation on citrus roots in room temperature in the greenhouse (Li *et al.* 2003a, 2006, 2007b). The time for single generation from *Diaprepes* oviposition to adult emergence is estimated to be about 22 weeks (or 150 days) at 26°C under the laboratory conditions (Lapointe 2000).

Diaprepes root weevil adults might be attractive to local trees where their emerging sites are in citrus orchards as the weevils move relatively little (Nigg *et al.* 2003). In a citrus orchard in central Florida, a total of 619 adult weevils were captured and then 580 adults were marked and released. Over a period of 10 weeks of release, 146 weevils (or 25% of the released weevils) were re-captured (Nigg *et al.* 2003). It was reported that 40% of recaptured marked adult weevils moved within 0-24 m, 41% of the re-capture adults moved within 25-72 m from the release points. The movement of all recovered adult weevils was within 72 m from the release point during this release period (Nigg *et al.* 2003). This movement information has been useful for characterization of relationships between citrus trees, soils and *Diaprepes* root weevil. If the root weevil adults tend to remain relatively close to where they emerge from the soil, then the root weevil population might be related to soil and water variables that may influence the performance of trees on which the weevils feed (Li *et al.* 2003b, 2005).

Synthesis of citrus tree abiotic and biotic stress

Plant abiotic stress is signaled by low leaf water potential and stomatal conductance (Jones *et al.* 2003; Li *et al.* 2004a). Abiotic stress of citrus trees associated with flooding, waterlogging, high soil acidity and unbalanced nutrient has received much attention in Florida (Syvertsen *et al.* 1983; Obreza and Collin 2002; Li *et al.* 2004a). Citrus rootstock physiological growth process and stomatal regulation are substantially influenced by flooding and high acidity (Li *et al.* 2006). Among the most important factors that could influence citrus tree health status, air/soil temperatures, soil water, pH, and Fe, Mg and Ca concentrations were associated with *Diaprepes* weevil development patterns (Li *et al.* 2004a, 2007a).

Since *Diaprepes* larvae, pupae and teneral adults are soil-habiting, soil physical and chemical characteristics could influence larval development and adult weevil density. This finding has been consistent in several greenhouse simulation studies (Rogers *et al.* 2000; Li *et al.* 2003b, 2004b, 2006, 2007b), and a series of field studies in different citrus orchards across central and southern counties in Florida (Nigg *et al.* 2001, 2003; McCoy *et al.* 2003; Li *et al.* 2003a, 2004a, 2007a, 2007c, 2007d). Citrus trees were vulnerable to attack by the *Phytophthora-Diaprepes* weevil complex in fine-textured, poorly drained soils (Graham *et al.* 2003). A variety of approaches of chemical and biological controls have been tested but as no effective and safe control methods have been found, this species is still spreading. Because of the small size, neonate larvae are virtually impossible to detect in the soil and the initial injury to roots can be difficult to quantify (Jones and Schroeder 1983; Rogers *et al.* 2000).

Citrus tree decline symptoms are not apparent until the larvae are well-established on the roots and extensive damage has occurred (Graham *et al.* 2003). Individually, biotic stress of adult weevil leaf feeding and larval root feeding pressures of *Diaprepes* root weevil have been the subject of intensive research in Florida (Nigg *et al.* 2001; Graham *et al.* 2003; Li *et al.* 2003a; Nigg *et al.* 2003; Li *et al.* 2006, 2007b). It is reported that *Diaprepes* root weevil larvae can consume 20-80% of the citrus seedling roots within six weeks of infestation (Rogers *et al.* 2000). Long period feeding of *Diaprepes* larvae can break the resistance of citrus roots to infection by *Phytophthora* spp., and both larval feeding and disease can lead to tree decline to an unproductive state or death by extensive larval root injury (Graham *et al.* 2003).

Temperature would be likely to have a significant effect on occurrence, timing, development, dispersal and movement of insect pests (White 1984; Mattson and Haack 1987; Viens and Bosch 2000; Li *et al.* 2007c, 2008). Warm temperature can cause insect outbreak, change insect behavior and influence efficacy of insecticides on pest control (Vicens and Bosch 2000; Amarasekare and Edelson 2004; Peacock *et al.* 2006). High air and soil temperatures can substantially cause high density of *Diaprepes* root weevil adult population in citrus orchards in Florida (Li *et al.* 2007c).

Reducing citrus tree abiotic and biotic stress

Management of root weevil pest is often obtained by repeated foliar application of chemical treatments when pest densities exceed an economical threshold that requires treatments (Graham *et al.* 2003; Byers and Castle 2005). Typically, in the absence of early detection methods and effective management tools, citrus growers control the *Diaprepes* weevil population by four applications of insecticides each year, and usually uniform rate is applied over the orchard (Li *et al.* 2007d). However, the excess use of costly pesticides can harm the environment (Byers and Castle 2005).

Understanding plant, soil and insect relations has implication in reducing plant stress (Li *et al.* 2008). In Florida, simulation studies in the greenhouse have shown citrus seedling root injury under both flooding anaerobic effects and *Diaprepes* root weevil larval feeding pressure (Li *et al.* 2003a, 2006, 2007b). Field scale studies *Diaprepes* root weevil life cycle, neonate dropping from tree canopy to soil, adult weevil emergence from soil, active adult weevil population and biological control have been the research focus for control of this pest (Lapointe 2000; Rogers *et al.* 2000; Nigg *et al.* 2001; Graham *et al.* 2003; McCoy *et al.* 2003; Nigg *et al.* 2003; Stuart *et al.* 2004). Orchard scale studies of citrus tree, soil and *Diaprepes* root weevil were primary

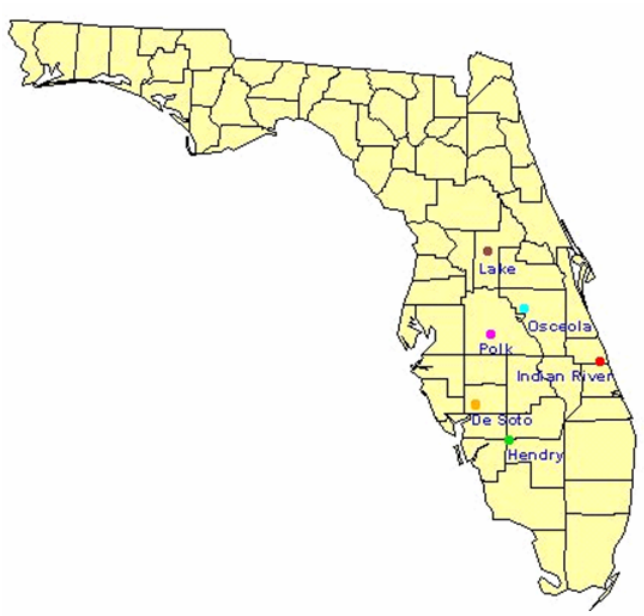

Fig. 1 Citrus orchards that relationships of citrus trees, soils and *Diaprepes* root weevil were assessed during 2001-2004. *Diaprepes* root weevil adult population has been surveyed across six counties in center and southern Florida: Lake County, Osceola County, Polk County, Indian River County, DeSoto County and Hendry County. Some of the data were reported in Futch (2003) and Li *et al*. (2003a, 2004a, 2005, 2007a, 2007c, 2007d).

done in the six counties across center and southern Florida: Lake County, Osceola County, Polk County, Indian River County, DeSoto County and Hendry County (**Fig. 1**).

Citrus tree abiotic and biotic stress problems have been assessed in different orchards across center, southern and south-western counties of Florida (Nigg *et al*. 2001, 2003; Graham *et al*. 2003; Li *et al*. 2003a, 2004a, 2006, 2007a). The measurements include the susceptibility of citrus rootstocks to *Phytophthora* disease associated with *Diaprepes* larval root feeding injury (Graham *et al*. 2003), tree health rating, tree canopy volume and orange fruit yield related to soil physical and chemical conditions and *Diaprepes* adult population development (Li *et al*. 2004a, 2005). The effects of flooding and larval feeding on citrus rootstock shoot growth and root injury, multi-year spatial and temporal overlay patterns of citrus tree decline, *Diaprepes* adult weevil emergence from soils, active adult population, soil flooding pattern, water table, soil water content, pH, and soil Fe, Ca, Mg, K, P, Cu and Zn concentrations are analyzed, quantified and mapped (Li *et al*. 2003a, 2004a, 2007a, 2007b, 2007c, 2007d).

This paper attempts to summarize the susceptibility of citrus trees to abiotic and biotic factors (flooding, waterlogging, soil acidity, unbalanced nutrient in soil) and citrus tree management information from county-scale field studies and greenhouse simulation studies conducted in Florida. This paper also attempts to discuss about using leaf stomatal conductance, soil redox potential, soil chemical and physical characteristics and root weevil survival data in development of management tools for reducing citrus tree decline from abiotic and biotic stress. Time series models and exponential growth models would be useful for reducing the cost of field monitoring and for less frequent spray for integrated citrus tree management.

CITRUS TREE DECLINE UNDER ANAEROBE, HIGH SOIL IRON AND ROOT WEEVIL FEEDING

The simultaneous occurrence of soil habiting root weevil, soil acidity, waterlogging and water-related soil chemical components has been a challenge for sustainability management of citrus orchards. Citrus tree decline in relation to soil

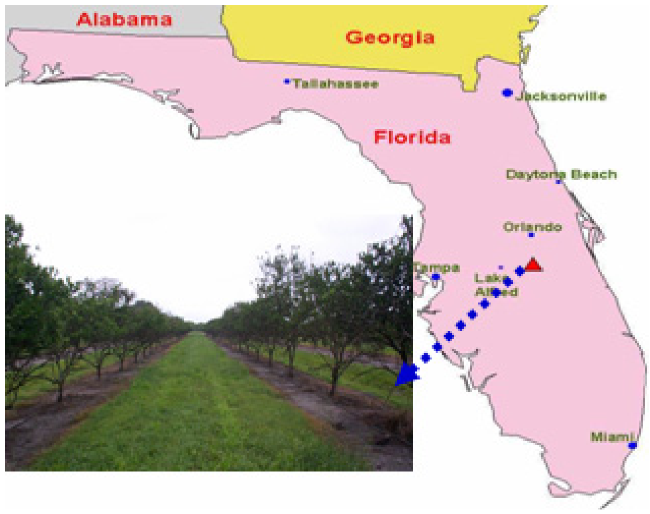

Fig. 2 Citrus orchard layout in Osceola County. The trees were 20-year old ‘Hamlin’ orange trees on Single rootstock.

conditions and *Diaprepes* root weevil infestation was assessed in a 9.5-ha citrus orchard in Osceola County, Central Florida (28° 22′ N, 81° 58′ W) in 2002 (**Fig. 2**). The site was located about 500 m in the south of Lake Tohopekaliga, the largest lake (76 km²) in Osceola County. The citrus trees were ‘Hamlin’ orange trees on Swingle citrumelo rootstock (*Citrus paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.), 20-yr old, planted on raised 2-row beds with drainage furrows between the beds, 17 m apart (**Fig. 3**). In the study area, there were a total of 1409 mature trees and 758 young trees replanted in 2000 for research purposes (**Fig. 3**). All trees received regular liming, irrigation and fertilization based on regional recommendation but no chemical treatments for pest control (Li *et al*. 2004a).

The mature trees had been infested by *Diaprepes* root weevil during the last 10 years in the orchard. Two years (2000 and 2001) of data showed that dropping numbers of *Diaprepes* root weevil larvae from the tree canopy averaged 370 and 940 neonates per m^2 of soil surface under tree canopy (McCoy *et al*. 2003; Nigg *et al*. 2003). Larval numbers in the soil under tree canopy were on average of 50 larvae per m^3 of fresh soil, measured by tree removal (n = 60 trees) and sieve sampling around the central root area (McCoy *et al*. 2003).

The soils, formed in the flatwoods sediments, were classified as a loamy Mollisol (USDA-NRSC 2003). Flooding occurred, depending on rain pattern, because of high rainfall (1380 mm per year) and low elevation (depression at the lake), causing soil redox potential (E_h) far below zero (-260 mV) in flooded areas (**Fig. 4**). Water table depth, a component for distinguishing the soil unsaturated and saturated zones, varied between 0.6-1.4 m across the orchard during dry period (**Fig. 5A**). Soil electrical conductivity (SEC) showed a negative correlation with water table depth as the SEC was high in the deep water table areas (**Fig. 5B**).

The soils (0-0.3 m in the depth) were sampled near (0.3 m) tree trunk to evaluate the relationship between tree decline and soil water and nutrient conditions (Li *et al*. 2004a). A total of 50 soil samples were taken in a 34 x 25 m grid across the orchard. The soils were air-dried and Mehlich I extracted cations and anions (P, K, Mg, Ca, B, Zn, Mn, Fe and Cu) were analyzed using an inductively coupled argon plasma emission spectrophotometer (Horwitz 2000). As the result of waterlogging, the soil in the orchard was highly acidic (pH 4.9 ± 0.4) and high in Fe concentrations (36 ± 14 mg kg^{-1}). Acid soils contain high levels of active hydrogen and/or aluminum in relation to Ca and Mg, and plants have a limited tolerance to low pH (Kidd and Proctor 2001). Soil liming adds a considerable amount of Ca and Mg to the soil but too much Ca and Mg in the soil can interfere with the availability of other nutrients (Sopher and Baird 1982; Bohn *et al*. 2001). Iron (Fe), a micronutrient for plant growth, de-

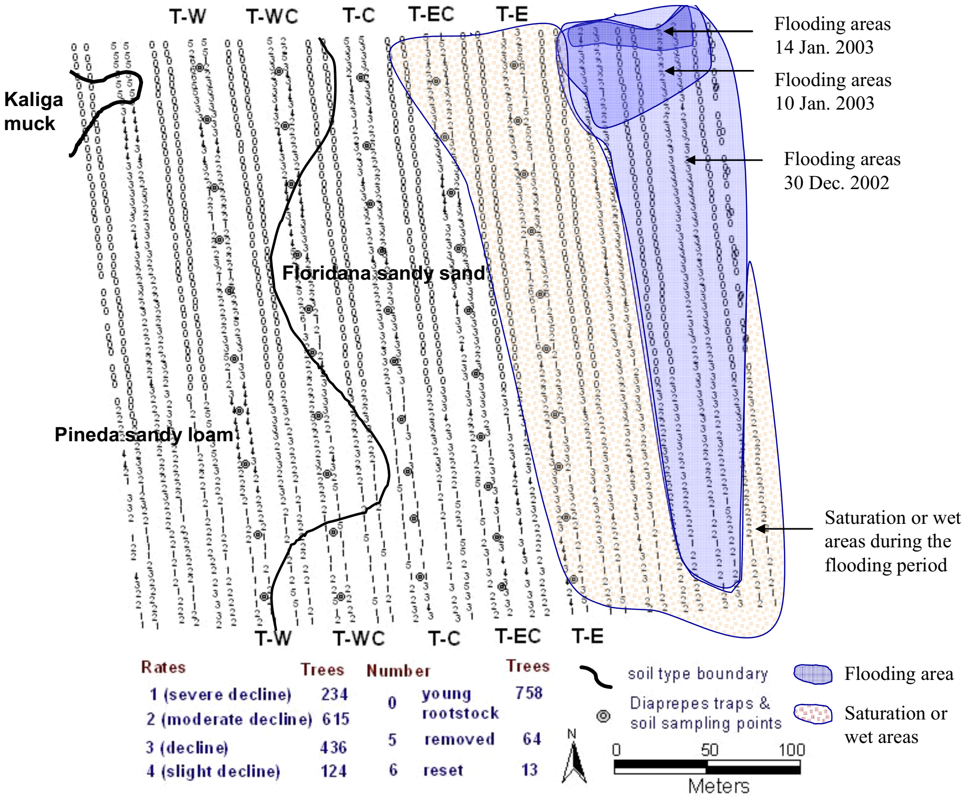

Fig. 3 Osceola citrus orchard study. Map of the citrus tree ('Hamlin' orange trees on Swingle rootstock) rating at the Osceola study site in a 9.5-ha citrus orchard with soil type boundary, tree, tree bed and Tedders trap locations, tree rating, and flooding areas. T, transect (tree bed); T-W, west transect; T-WC, west-center transect; T-C, center transect; T-EC, east-center transect; T-E, east transect. Re-printed from **Li H, Futch SH, Stuart RJ, Syvertsen JP, McCoy CW** (2007a) Association of soil iron with citrus tree decline and variability of soil pH, water, magnesium and *Diaprepes* Root weevil: two-site study. *Environmental and Experimental Botany* **59**, 321-333, ©2007, with kind permission from Elsevier Publisher.

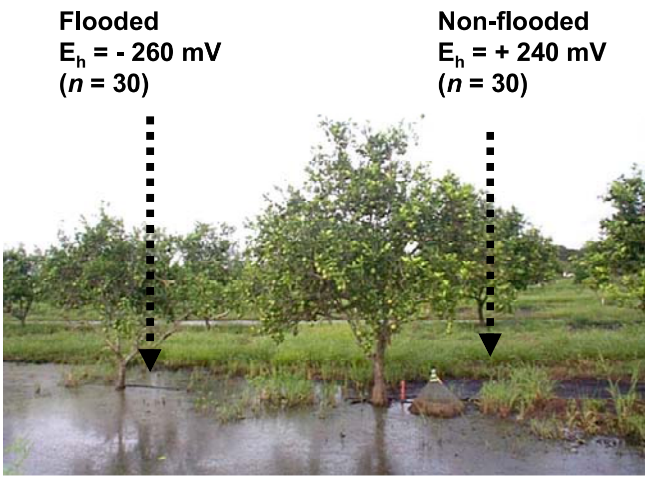

Fig. 4 Osceola citrus orchard study. Flooding occurred after a period of rain. Flooded anoxic soil had a negative soil redox potential E_h that caused tree decline.

creases significantly when soil pH increases from 5 to 6 (Sopher and Baird 1982; Bohn *et al*. 2001). High concentrations of Fe^{2+} in the soil solution could form a plaque to affect absorption of nutrients by roots (Liu *et al*. 2007a).

Citrus tree decline was assessed by visual quantification of tree decline symptoms using the characteristics defined by Blazquez (1991). The tree ranking was assessed using a numerical 1-4 ranking system as follows: 1 = severe decline, 2 = moderate decline, 3 = decline, and 4 = slight decline. The tree characteristics in rating was: 1 = canopy easily seen through, flush on major limbs only or on less than half of the tree, leaves small); 2 = moderate decline (canopy easily seen through, flush on secondary and higher limbs scattered around the entire canopy, leaves small); 3 = decline (well-defined canopy, more than half of which cannot be seen through, flush on secondary and higher limbs, leaves large); and 4 = slight decline (well-shaped and well-defined canopy that cannot be seen through, flush on secondary and higher limbs, leaves large and green) (Li *et al*. 2004a). More severe decline and decline trees were situated in the flooded area than non-flooded areas (**Fig. 3**).

The regression plot of tree health rating (THR) against soil Fe concentration showed that tree decline was linearly correlated to soil Fe at the Osceola site (**Fig. 6**). More than 50% of the severely decline (rating 1) and moderately decline (rating 2) trees were situated with a high soil Fe concentration of 40-80 mg kg^{-1}. Severely decline and moderately decline trees were 60.2% of the total infested trees. Only two trees of rating 3 (decline trees, or 30.9% of total infested trees) were with a soil Fe concentration > 40 mg kg^{-1}. All the healthier trees (rating 4, slight decline) were in areas low in Fe concentrations between 13 and 39 mg kg^{-1} (Li *et al*. 2007a).

Severe citrus tree decline was found in shallow water table areas and flooded areas, where the stomatal conductance (g_s) of mature, top young leaves of the citrus rootstock seedlings decreased from 152 mmol $m^{-2}s^{-1}$ to 94 mmol $m^{-2}s^{-1}$ under the flooded conditions (**Fig. 7**). However, leaf water potential P_a did not differ among trees in the flooded areas (0.66 ± 0.18 MPa) vs. the non-flooded areas (0.66 ± 0.10 MPa) (Li *et al*. 2004a).

Stepwise multiple linear models for tree health rating (THR) related to SWC (g kg^{-1}), soil pH, and soil Fe (mg kg^{-1}) showed the trends as follows:

$$THR = 4.6598 - 0.00304SWC - 0.03503pH - 0.0326Fe \quad R^2 = 0.26,\ P < 0.0087 \qquad [1]$$

The model Eq. [1] was also significant (F = 4.37, df = 3, 46), and the estimate parameters were significant for the intercept (P < 0.0343), and Fe (P < 0.0017) (Li *et al*. 2007a).

Too much Fe in soil could affect plant growth as shown

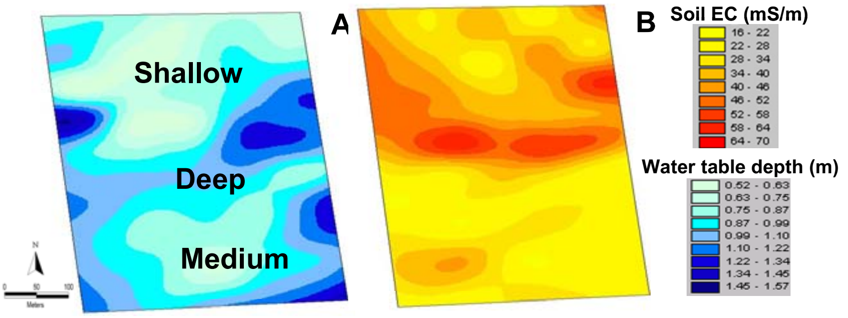

Fig. 5 Osceola citrus orchard study. Spatial patterns of water table (**A**) and soil electrical conductivity (**B**) across the Oseceola citrus orchard.

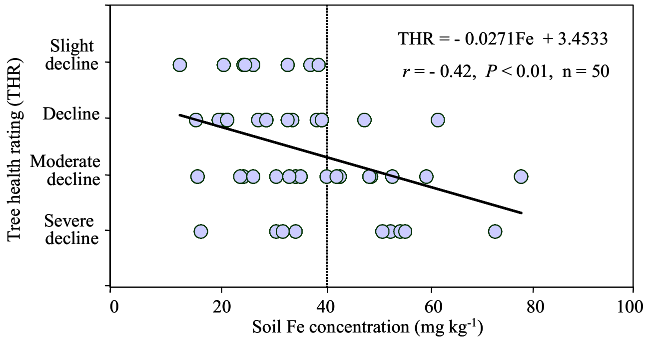

Fig. 6 Osceola citrus orchard study. Regression relationship of soil Fe concentration vs. tree decline rating (1 = severe decline, 2 = moderate decline, 3 = decline, and 4 = slight decline) on the Mollisol at the Osceola site. Re-printed from **Li H, Futch SH, Stuart RJ, Syvertsen JP, McCoy CW** (2007a) Association of soil iron with citrus tree decline and variability of soil pH, water, magnesium and *Diaprepes* Root weevil: two-site study. *Environmental and Experimental Botany* **59**, 321-333, ©2007, with kind permission from Elsevier Ltd.

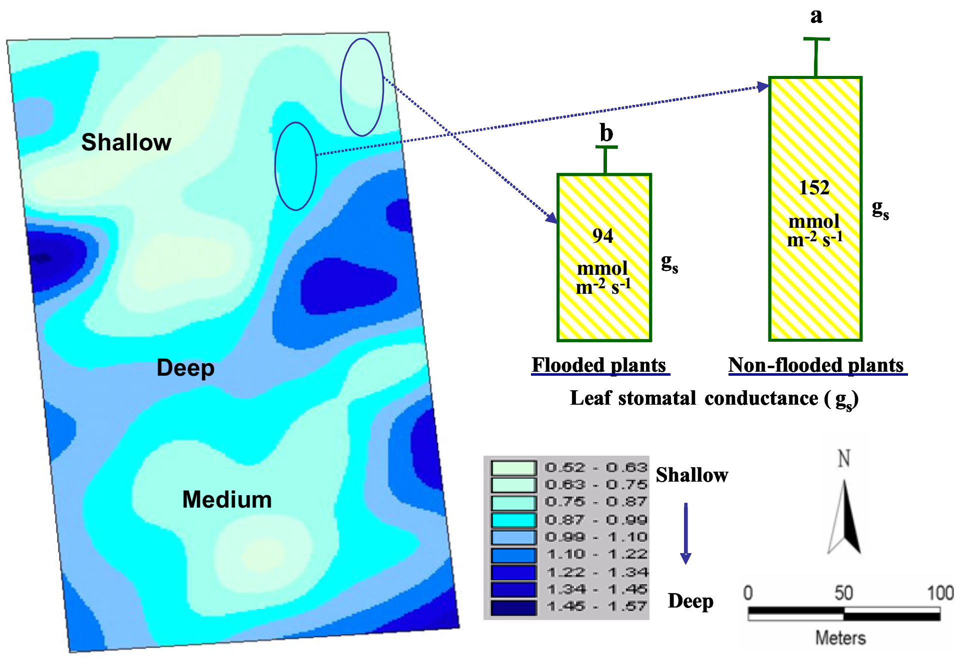

Fig. 7 Osceola citrus orchard study. Comparison of plant leaf stomatal conductance (g_s) in flooding and non-flooding areas. Re-printed from **Li H, Syvertsen JP, Stuart RJ, McCoy CW, Schumann A, Castle WS** (2004a) Soil and *Diaprepes* root weevil spatial variability in a poorly drained citrus grove. *Soil Science* **169**, 650-662 ©2007, with kind permission from Lippincott, Williams & Wilkins, Wolters Kluwer Publisher.

by the severely declined trees in areas high in Fe concentration. The high level of extractable soil Fe could be due to the low soil pH (4.9), high SWC (260 g kg^{-1}) and flooding (Li *et al.* 2007a). Soil Fe concentration was negatively correlated with water table depth (r = - 0.38, P < 0.01), showing that high soil Fe concentration was associated with high soil water content. Soil Fe is more soluble at a low pH level, and a large amount of Fe can become available under anaerobic conditions (Bohn *et al.* 2001). The increase of extractable soil Fe concentrations with increasing SWC and decreasing soil pH are consistent with the Fe chemistry mechanism described in Sopher and Baird (1982) and Bohn *et al.* (2001). These might explain why severely declined trees were associated with high soil Fe concentrations > 40 mg kg^{-1} (**Fig. 6**). Thus, increasing soil pH and improving soil drainage could also result in decreasing soil Fe level to reduce tree decline (Li *et al.* 2007a).

CITRUS TREE ABIOTIC STRESS FROM POOR SOIL REDOX POTENTIAL AND LEAF STOMATAL CONDUCTANCE

Plants demand oxygen, water and nutrients for growth. It is established that flooding can result in oxygen deprivation in the root system, and lead to disturb plant-soil system equilibrium, damage in roots and arrest plant growth (Pezeshki and Delaune 1998; Oren *et al.* 2001; Li *et al.* 2003b, 2004a, 2006). Soil oxidation-reduction (redox) potential (E_h), can be reduced by soil waterlogging, which translated into a greater demand for oxygen within the soil and increased plant water stress (Pezeshki and Delaune 1998; Li *et al.* 2003b, 2006). Plant physiological responses to flooding stress are reflected by to reduced leaf stomatal conductance (g_s), leaf gas exchange, leaf water potential (Oren *et al.* 2001; Li *et al.* 2003b; Mielke *et al.* 2003; Blanke and Cooke 2004: Li *et al.* 2004a). Flooding induced stomatal closure and leaf conductance and leaf turgor potential were signi-ficantly reduced in flooded 2-yr old sour orange plants (Ruiz-Sanchez *et al.* 1996). In a poorly drained citrus grove, flooded trees were more water stressed than non-flooded trees, as indicated by leaf g_s, which was significantly lower in an area flooded for three weeks than in a non-flooded area (Li *et al.* 2003a).

In citrus, plant environmental stress from flooding can occur simultaneously with root weevil infestations (Li *et al.* 2003b, 2004a, 2004b, 2006). It is reported that the *Diaprepes abbreviatus* (L.) root weevil can be dispersed nursery rootstock into citrus groves and injury inflicted by *Diaprepes* larvae on roots has resulted in plant decline and death (Jones and Schroeder 1983; Quintela and McCoy 1997;

Table 1 Greenhouse study. Experimental treatments and procedures with two citrus rootstocks Swingle (SWI) and Smooth Flat Seville (SFS).

1. Flooding procedures (one seedling in each 130 cm^3 pot)	
Treatments	
Rootstock variety	SWI, SFS
Flooding (days) non-larvae	0 (non-flooding & non-larval feeding), 8 reps per variety
+ 5 larvae after flooding	0, 10, 20 and 30-day flooding and 40-day larval feeding, 8 reps per variety
2. Draining for one week for flooded seedlings after flooding termination.	
3. Larval feeding for 40 days using flooded and non-flooded seedlings after draining procedures.	

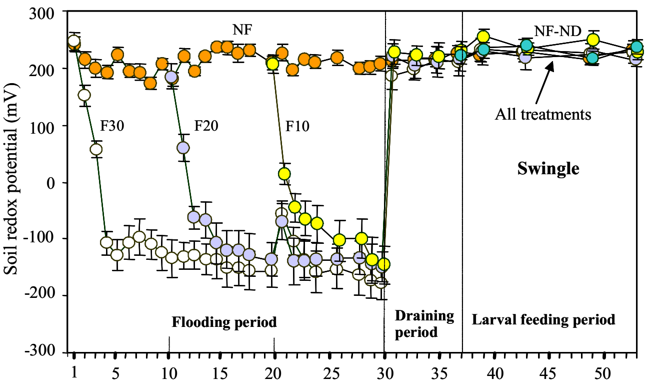

Fig. 8 Greenhouse study. Temporal patterns of soil redox potential for Swingle throughout Experiment I. NF, non-flooded; F30, 30-day flooded; F20, 20-day flooded; F10, 10-day flooded treatments. Each point represents the mean and standard error of 8 measurements. Re-printed from **Li H, Syvertsen JP, Stuart RJ, McCoy CW, Schumann A** (2006) Water stress and root injury from simulated flooding and *Diaprepes* root weevil feeding in citrus. *Soil Science* **171**, 138-151 ©2006, with kind permission from Lippincott, Williams & Wilkins, Wolters Kluwer Publisher.

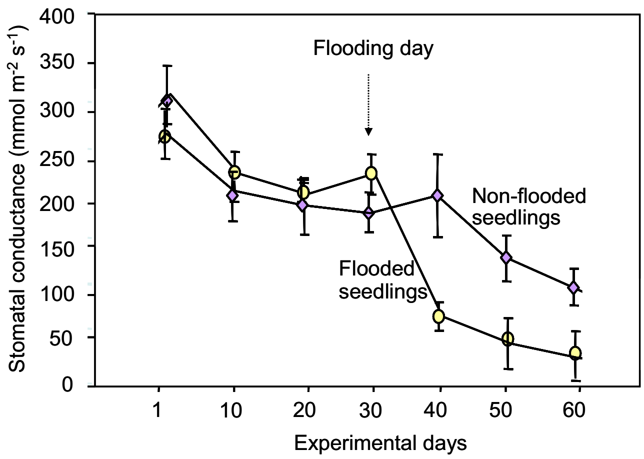

Fig. 9 Greenhouse study. Mean and standard error of leaf stomatal conductance (g_s) two citrus rootstock varieties (Swingle and Smooth Flat Seville) by flooded and non-flooded seedlings across the period of 60 days. Each point represents the mean of n = 48 for flooded seedlings and n = 16 for non-flooded seedlings.

Rogers *et al*. 2000). The soil environment influenced plant growth and the abundance of most herbivorous insects (Orians and Fritz 1996). Flooding increased soil pH, decreased nutrient availability and leaf dry matter yield (Li *et al*. 2003b; Yoo and James 2003) but leaf beetle larval pupal weight was not influenced by nutrient or flooding conditions to the plant (Lower *et al*. 2003).

Flooding events and soil waterlogging can be critical stress factors for citrus trees. In Florida, citrus is often cultivated on low-lying flatwoods soils with poor drainage (Obreza and Collins 2002). Citrus root injuries from larval feeding were associated with larval density, rootstock variety, soil type and moisture and root injury to different rootstocks growing in well-drained soil ranged from 50 to 80% by 40 days after infestation by 2-5 *Diaprepes* neonate larvae, and many root tissues were completely consumed after 79 days (Rogers *et al*. 2000). However, does plant growth or root injury be related to larval survival? Are the responses of flooded trees to larval feeding different than non-flooded trees? Are flood-damaged seedlings more vulnerable or susceptible to larval feeding injury than non-flooded seedlings? Does the combination of flooding and larval feeding complicate treatments for weevil control?

A greenhouse simulation study was conducted in the greenhouse house to investigate changes of citrus soil redox potential, leaf stomatal conductance, plant growth and larval survival under flooding and *Diaprepes* larval feeding treatments, how the combined effects of soil flooding and *Diaprepes* larval feeding on citrus tree root damage. It is expected that plants and larvae to grow better in non-flooded conditions than in flooded conditions, because flooding prohibits gas exchange in the plant-soil system and flooded soil is compacted. The studies attempted to quantify how long seedling plants can tolerate flooding stress.

The greenhouse study was conducted at the Citrus Research and Education Center, University of Florida. Three-month old seedlings of two commercial citrus rootstock varieties, Swingle citrumelo (*Citrus paradisi* Macfad x *Poncirus trifoliata* (L.) Raf.) and Smooth Flat Seville (*Citrus aurantium* L.) were used in the study (**Table 1**). The treatment consisted of four levels of flooding duration (0, 10, 20 or 30 days), and two levels of *Diaprepes* larvae (0 or 5 larvae per seedling), feeding for 40 days (**Table 1**). The design was 2 varieties × 4 flooding durations × 8 replicates, arranged in a completely randomized design. There were a total of 80 seedlings. Seedlings were selected for uniformity of root density and canopy size for each variety before transplanting into a 130-cm^3 plastic pot for submergence. Soil was a Candler fine sand, pH 6, containing 965 g kg^{-1} (or 97%) of sand, similar to the average sand content of citrus soils in Florida (Li *et al*. 2003b, 2006).

The flooding treatments were applied by submerging seedlings to 2 cm above the tops of seedling pots and the shoots remained in the atmosphere. The 30-day treatments were submerged for 10 days then the 20-day treatments were flooded. After flooding for 10 more days, the 10-day treatments were submerged (Li *et al*. 2006). As a result, the flooding treatments were completed at the same time. All flooded seedlings were removed from the water then put for drainage (**Table 1**).

All flooding treatments (0, 10, 20 or 30 days) were infested by 5 *Diaprepes* neonate larvae per seedling a week of drainage. There was a control for non-flooding and non-larval feeding (NF-ND), which consisted of 2 varieties × 8 replicates. One-day old *Diaprepes* larvae were inoculated to the soil for all feeding treatments a week after completing the flooding procedure (**Table 1**). The seedlings were not flooded during the larval feeding period. Except the flooding period, all treatments received the same rates of fertilizers and waters. Temperatures in the greenhouse varied between 28 ± 4°C throughout the experiment (Li *et al*. 2006).

The results of the study showed that the soil redox potential (E_h), measured using the Orion oxidation-reduction probe, decreased abruptly following submergence of each treatment and the E_h became negative within 1-3 days of flooding (**Fig. 8**). The soil E_h varied within -100 and -180 mV and it showed a complete lack of oxygen that was attained as quickly as 1 day after submergence (Li *et al*. 2006). Leaf stomatal conductance (g_s), measured using the Delta-T A4 porometer, decreased significantly within in-

Table 2 Greenhouse study. Analysis of variance for citrus seedling leaf stomatal conductance (g_s) and soil redox potential (E_h) during flooding period of two rootstocks Swingle and Smooth Flat Seville.

Sources	*df*	Soil E_h†	Leaf g_s†
Model	42	236**	7.25**
Rep	7	0.55 ns	2.69*
Variety (V)	1	0.47 ns	89.5**
Flood (F)	3	3286**	7.30**
Rep x V	7	0.44 ns	1.9 ns
V x F	3	8.0**	43.1**
Rep x F	21	0.56 ns	1.5 ns
R^2		0.99	0.94

†: ns, not significant; *, $P < 0.05$; **, $P < 0.01$.

Re-printed from **Li H, Syvertsen JP, McCoy CW, Schumann A** (2003b) Soil redox potential and leaf stomatoal conductance of two citrus rootstocks subjected to flooding and *Diaprepes* root weevil feeding. *Proceedings of the Florida State Horticultural Society* **116**, 252-256, ©2003, with kind permissions from *Florida State Horticultural Society.*

creasing flooding duration (**Fig. 9**). The g_s values started to decrease after 2 days of flooding and then decreased consistently with duration of flooding (Li *et al.* 2006). The g_s value was high (350 mmol m^{-2} s^{-1}) before flooding but it was as low as 40 mmol m^{-2} s^{-1} by the end of 30 days of flooding (**Fig. 9**). The analysis of variance, determined using PROC GLM procedure (SAS, 1990), showed that flooding treatment had the significant effects on soil E_h (F = 3286, df = 3, $P < 0.0001$) and citrus leaf g_s (F = 7.3, df = 3, $P < 0.01$). The interaction between variety and flooding was also significant on soil E_h (F = 8.0, df = 3, $P < 0.01$) and citrus leaf g_s (F = 43, df = 3, $P < 0.001$).The ontrast showed also that soil E_h and citrus leaf g_s were significantly different between Flooding treatments (**Table 2**).

Shoot growth was significantly higher in the control (non-flooded) than in flooded treatments for both rootstocks (**Fig. 10A, 10B**), which was a result of stronger root development in the non-flooded seedlings (**Fig. 10C, 10D**). Shoot growth of citrus tree seedlings arrested during the flooding period. Root injury, estimated by classifying the whole seedling root system by percentage damage as 0% (control), 0-25%, 25-50%, 50-75%, and >75% damage, was mainly attributed to flooding anaerobic (Li *et al.* 2006). Flooded soil pH was found to increase with flooding duration. The pH value of 40-day floodwater increased by 0.7 units by the end of a 40-day flooding period compared to 0.3 units for the 20-day flooding treatments. However, changes in flooding water had no significant effects on seedling shoot growth (Li *et al.* 2006).

The effect of flooding was significant on shoot growth and root rating for the two rootstock varieties (Li *et al.* 2006). With initial shoot lengths of 39.4 ± 3.9 cm in SWI and 27.6 ± 6.4 cm in SFS, shoot lengths grew faster in SWI (2.7 ± 1.5, 1.5 ± 1.2, and 0.3 ± 0.6 cm) than in SFS (0.3 ± 0.5, 0.4 ± 0.7, and 0.3 ± 0.4 cm) for the F10, F20 and F30 treatments, respectively, during the flooding period. However, during the larval infestation period, shoot growth was greater in SFS (3.1 ± 5.1, 1.4 ± 1.8, and 0.1 ± 0.1 cm) than in SWI (0.2 ± 0.1, 0.1 ± 0.1, and 0.3 ± 0.3 cm) for the previously flooded F10, F20 and F30 treatments, respectively. There were significant differences in shoot growth for larval feeding treatments compared to non-larval feeding treatments (**Table 3**).

Citrus rootstocks could vary in their responses to flooded soil conditions and citrus leaf hydraulic conductivity and stomatal conductance were reduced significantly under flooded anaerobic conditions (Syvertsen *et al.* 1983; Li *et al.* 2003b). It was reported that the ability of citrus seedlings to tolerate larval feeding differs among rootstocks, and the correlation between root loss and larval weight gain was significantly positive (Li *et al.* 2004b). The assessment of the degree of plant physical stress from soil flooding events by measuring plant leaf stomatal conductance (g_s) has yielded the insights of relationship of soil waterlogging and citrus tree decline in the humid environment. Citrus rootstocks showed a maximum capacity of three weeks to resist to flooding damage but ten days of flooding showed the significant effect on lowing leaf stomatal conductance and slowing shoot growth of citrus rootstocks (Li *et al.* 2006).

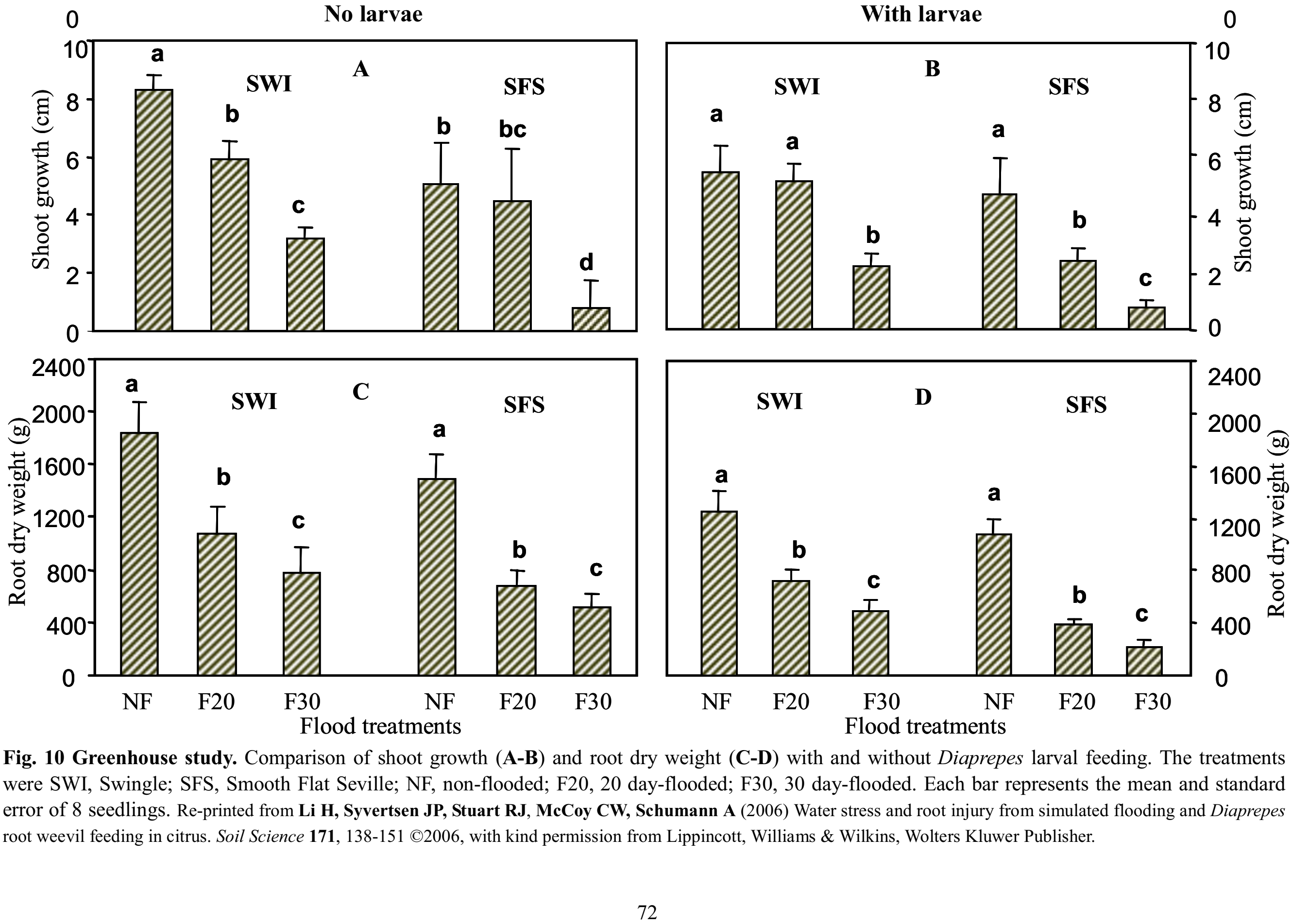

Fig. 10 Greenhouse study. Comparison of shoot growth (**A-B**) and root dry weight (**C-D**) with and without *Diaprepes* larval feeding. The treatments were SWI, Swingle; SFS, Smooth Flat Seville; NF, non-flooded; F20, 20 day-flooded; F30, 30 day-flooded. Each bar represents the mean and standard error of 8 seedlings. Re-printed from **Li H, Syvertsen JP, Stuart RJ, McCoy CW, Schumann A** (2006) Water stress and root injury from simulated flooding and *Diaprepes* root weevil feeding in citrus. *Soil Science* **171**, 138-151 ©2006, with kind permission from Lippincott, Williams & Wilkins, Wolters Kluwer Publisher.

Table 3 Greenhouse study. Contrast for soil redox potential (E_h), leaf stomatal conductance (g_s), shoot length, larval survival, and root rating for flooding and non-flooding treatments of two citrus rootstocks (Swingle and Smooth Flat Seville). Seedlings n = 8 per flooding and non-flooding treatment per rootstock.

Contrasts	*df*	E_h†	g_s†	Shoot length†	Larval survival†	Root rating†
Flooding period						
NF vs. F††	1	1018**	8.22**	15.6**		
F10 vs. F20††	1	7.96**	4.6* ns	4.7*		
F10 vs. F30††	1	6395**	17.1**	7.6**		
Larval feeding period						
ND vs. D†††	1	45.7**	3.7 ns	8.6**		6.8**
NF-ND vs. NF-D†††	1	2.5 ns	0.1 ns	3.2 ns		6.0*
F10-D vs. F20-D†††	1	1.4 ns	5.7*	1.2 ns	26.4**	27.1**
F10-D vs. F30-D†††	1	3.5 ns	14.0**	2.1 ns	461.7**	41.3**

† *F* values. ns, non significant, and * and **significant at $P < 0.05$ and $P < 0.01$.
†† F10, 10-day flooded; F20, 20-day flooded; F30, 30-day flooded; F40, 40-day flooded treatments.
††† N*D*, no-*Diaprepes* larvae; NF, non-flooded treatments.
Re-printed from **Li H, Syvertsen JP, Stuart RJ, McCoy CW, Schumann A** (2006) Water stress and root injury from simulated flooding and *Diaprepes* root weevil feeding in citrus. *Soil Science* **171**, 138-151 ©2006, with kind permission from Lippincott, Williams & Wilkins, Wolters Kluwer Publisher.

CITRUS TREE TOLERANCE TO LARVAL FEEDING AND ROOT WEEVIL LARVAL SURVIVAL

Because root waterlogging and larval feeding occur underground, the identification of specific underground infestation sites for root weevils might reduce the area to treat and could contribute to reduced treatment costs for chemical and biological control (Li *et al.* 2004a). As *Diaprepes* neonate larvae invade the soil after hatching from eggs laid by adults in the citrus canopy and larvae feed on tree roots and subsequently pupate in the soil, injury inflicted by *Diaprepes* larvae can result in tree decline and death (Rogers *et al.* 2000). However, the initial injury to roots can be difficult to quantify. It is reported that the ability of citrus seedlings to tolerate larval feeding differs among rootstocks, and the correlation between root loss and larval weight gain was significantly positive (Rogers *et al.* 2000; Li *et al.* 2004b). Root injury of different rootstock seedlings growing in well-drained sandy soil varied between 50 to 80% within 40 days of inoculation of 2-5 *Diaprepes* neonate larvae, and many root tissues were completely consumed after 79 days (Rogers *et al.* 2000).

The relative vulnerability of citrus rootstock seedlings to *Diaprepes* root weevil larval feeding injury, determined in the above greenhouse simulation study (methods detailed in previous section), showed that root injury by larval feeding increased with the duration of previous flooding. The injury ranged between 0-3% of roots for the non-flooded rootstock seedlings, 0-6% of roots for the 10-day flooded and 20-day flooded seedlings, and 3-12% of roots for the 30-day flooded seedlings in both Swingle and Smooth Flat Seville varieties (Li *et al.* 2006).

These measurements showed that citrus seedling root injury was attributed to both flooding and larval infestation. As compared to the control (non-flooded) seedling roots, whole seedling root damage by flooding and larval feeding increased with flooding duration. About 25-50% of root damage was found in rootstock seedlings previously flooded for 20 days, and root damage increased significantly to 50-75% for rootstock seedlings that were previously flooded for 30 days (Li *et al.* 2006).

Larval survival was significantly different between the previously flooded treatments ($P < 0.05$, Li *et al.* 2006). With an initial infestation of 5 neonate larvae per seedling, the lowest larval survival was found in the non-flooded treatment (60 ± 22%), and the survival rate was significantly greater in the longest flooded treatments, 30-day flooded (88 ± 10%) for both varieties (Li *et al.* 2006). In addition, difference in larval survival was found between the two citrus rootstock varieties (Swingle, 78 ± 22%; and Smooth Flat Seville, 82 ± 14%).

Total weight of survival larvae varied between 25 and 182 mg per seedling. The initial larval weight was on average 0.5 mg (5 1-day old neonates). Larval weight increased by 50-364 times after 40 days of feeding on seedling roots, indicating a strong growth (or feeding) potential of neonate larvae. Larval growth was the highest in the non-flooded treatments. It might be because less flooding compaction would mean better aeration in the sandy soil that could be more favorable to larval survival (Li *et al.* 2006). Flooded and waterlogged soils were typically more compacted with higher bulk density than non-flooded soil (Saqib *et al.* 2004).

In other separate greenhouse study with a loamy soil, larval survival was significantly lower, 66 ± 19% for Swingle and 50 ± 22% for Smooth Flat Seville seedlings flooded 20-40 days (Li *et al.* 2007b). It was reported that an increase of 0.5 unit pH in the flooded soil could reduce significantly larval survival (Li *et al.* 2007c). 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). A significant increase in soil pH was found in flooded soil (Yoo and James 2003).

It is suggested that 20-day flooding duration is the threshold for citrus rootstock seedling plant water stress and root injury from flooding damage (Li *et al.* 2004a). Citrus rootstock seedling roots could be injured by waterlogging and larval feeding. A negative soil redox potential and a decrease in leaf stomatal conductance could be an early indicator of plant water stress and root damage from flooding, and soil type and soil texture could also be factors affecting larval survival in the field. There is a need for more information about the associations of waterlogging, redox potential, soil pH, soil texture, and larval survival for citrus rootstock protection.

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, 2006). 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 Blaustain 2000). *Diaprepes* root weevil larval survival and growth could reduce from 80% to 60% when soil pH increased from 4.8 to 5.6 in acidic soil (Li *et al.* 2007c).

IMPLICATION OF TIME SERIES MODEL IN ROOT WEEVIL CONTROL FOR REDUCING CITRUS BIOTIC STRESS

Economic costs from citrus tree infestation of *Diaprepes* root weevil and the associated root disease in citrus could be as much as $600 per hectare (Graham *et al.* 2003). The related management for tree protection has been sprayed with four applications of insecticides each year with one application in each season. However, field monitoring of insect populations for treatment determination could be critical because of the time and labor cost. Mathematic models were useful to define problems, understand the systems, and

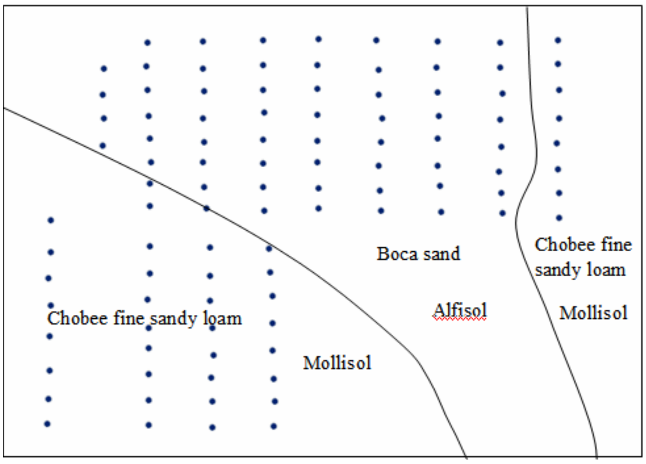

Fig. 11 Hendry citrus orchard study. Soil units (Boca sand, Alfisol; Chobee fine sandy loam, Mollisol), soil type boundaries and trap locations for monitoring *Diaprepes* root weevil adults after emerging from the soil (n = 100) under Swingle rootstock tree canopy. Re-printed from **Li H, Futch SH, Syvertsen JP, McCoy CW** (2007d) Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* (L.) root weevil in citrus. *Soil Biology and Biochemistry* **39**, 2436-2447 ©2007, with kind permission from Elsevier Ltd.

make predictions for purposes of insect and soil management, which have shown an ability to predict insect development patterns to reduce costs from monitoring field data (Worner 1991; Tobin *et al.* 2001; Byers and Castle 2005; Crowder and Onstad 2005; Li *et al.* 2007a, 2007d). These models included time-step simulation models, non-linear degree-day models, and best-fit polynomial and exponential regression models.

If *Diaprepes* root weevil development patterns were associated with time and air/soil temperatures (Li *et al.* 2007c), mathematical equations derived from their correlations in subsequent years would be useful for the weevil control through predicting its development pattern with time (Li *et al.* 2007d). The development of *Diaprepes* root weevil population was monitored in a flatwoods citrus orchard in Hendry County, south Florida (26° 44′ 37″ N, 81° 31′ 33″ W). The citrus trees were 10-year old 'Hamlin' orange trees on 'Swingle' citrumelo rootstocks, planted in high density (3 × 8 m), 2-bed rows. The trees had been infested by *Diaprepes* root weevils during the 6 years prior to the beginning of the study, and the orange fruit yield in the orchard varied between 36-50 Mg ha^{-1} (Li *et al.* 2007d).

The *Diaprepes* root weevil population was monitored in a 30 × 12 m grid using 100 pyramidal Tedders traps across a period of three years (2001-2003). During the study period, the citrus trees received regular grove care including irrigation, fertilization, and pest control using the regional recommendation (Li *et al.* 2007d). In the *Diaprepes* root weevil monitoring areas, there were two soil types, Boca sand (Alfisols) and Chobee fine sandy loam (Mollisols), formed in thick beds of sandy and loamy marine sediments (USDA-NRCS 2003). Boca sand zoned between Chobee fine sandy loam (**Fig. 11**). The Boca soil is a moderately permeable soil and the Chobee soil is a slowly permeable soil. Due to low elevation in the depression, the Boca sand and Chobee loam were poorly drained. The drainage system in the orchard consists of drainage furrows in every 4 rows of trees for evacuating surface water (Li *et al.* 2007d).

The data showed that a total of 962, 945 and 549 adult weevils were trapped in 2001, 2002 and 2003, respectively (Li *et al.* 2007d). The weekly adult density varied between 5.5-9.6 weevils per trap (**Table 4**). The weevil population was more abundant in the east and south than other areas across the orchard each year (**Fig. 12**). The higher weevil density was found in the Mollisol soil, and the interpolated areas of high weevil density were limited to the Mollisol (Chobee loam) and some transition areas across the Alfisol (Boca sand) during the three years (**Fig. 12**).

The weevil population peaked in the spring when the temperatures were warm up to 25°C each year. For example, the adult population peaked in last week of April (112 weevils) and the second week of May in 2001 (103 weevils). The active weevil density was on average 0.023 ± 0.018 weevils m^{-2} across the three years and the highest density was 0.091 weevils m^{-2}, monitored in 2001. Difference in weevil density between the three years was significant (ANOVA, $F = 8.90$, $df = 2, 297$, $P < 0.0002$), and the contrasts showed a significant difference in weevil density for 2001 vs. 2003 ($F = 13.90$, $P < 0.0002$), and 2002 vs. 2003 ($F = 12.78$, $P < 0.0004$) (Li *et al.* 2007d).

From the start of the year to the population peak in the spring, the captured adult *Diaprepes* weevils showed an exponential pattern each year. Up until the population peak (within the first 19-21 weeks of the year), the growth of the weevil population (*Dia*) with time (t, week) could be described by the exponential equations as shown in Li *et al.* (2007d) as follows:

$$2001: Dia = 7.747e^{0.1333t} \; (R^2 = 0.72, P < 0.01) \quad [2]$$
$$2002: Dia = 8.2465e^{0.1461t} \; (R^2 = 0.70, P < 0.01) \quad [3]$$
$$2003: Dia = 3.259e^{0.1557t} \; (R^2 = 0.54, P < 0.05) \quad [4]$$

The outbreak of *Diaprepes* adult weevils from the start of the year (mid February) to the peak in the spring (April-May) exhibited an exponential trend of insect growth (**Fig. 6**). The threshold weekly temperatures for the root weevil outbreak were 20-27°C as mean air temperature and 22-29°C as mean soil temperature (Li *et al.* 2007d). The exponential growth of the *Diaprepes* weevil population showed the best fit by the 3-yr mean population ($R^2 = 0.81$, $P < 0.01$, Li *et al.* 2007c). The growth of a population was exponential in theory when populations were in the initial growth

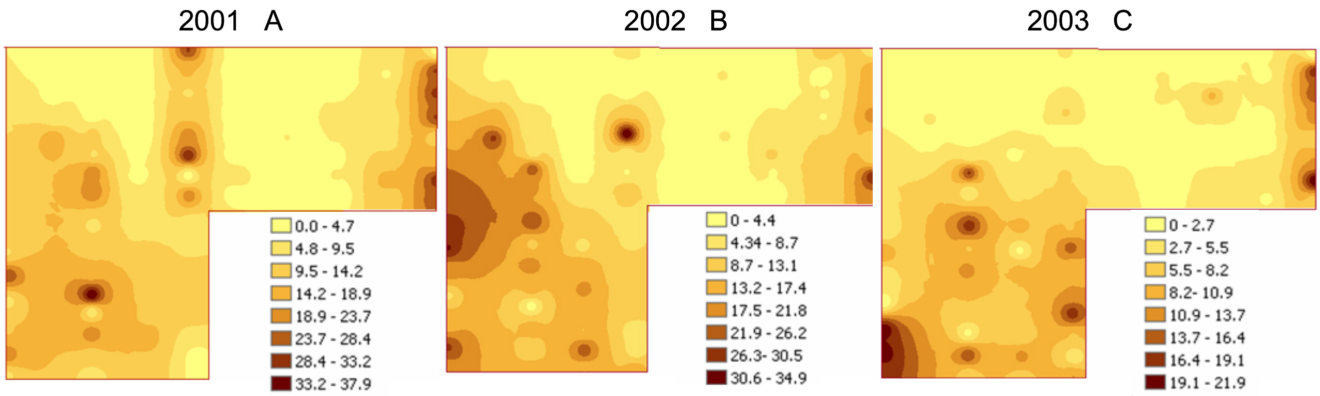

Fig. 12 Hendry citrus orchard study. Interpolated spatial patterns of *Diaprepes* adult root weevil density (adults per 30 × 12 m^2) in 2001 (**A**), in 2002 (**B**) and in 2003 (**C**). Re-printed from **Li H, Futch SH, Syvertsen JP, McCoy CW** (2007d) Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* (L.) root weevil in citrus. *Soil Biology and Biochemistry* **39**, 2436-2447 ©2007, with kind permission from Elsevier Ltd.

Table 4 Hendry citrus orchard study. Descriptive statistics of weekly *Diaprepes* root weevil population monitored in 2001, 2002 and 2003, gravimetrical soil water content (SWC), time domain reflectory volumetric water content (TDR), clay, sand, silt, soil organic matter content (SOM), and Mehlich-I extractable nutrient variables determined in 2003 (n = 100).

Variable	Mean	SD	Min.	Max.	Kurtosis	Skewness	CV (%)
Dia01†	9.6	9.0	0	38.0	0.7	1.2	93.2
Dia02†	9.5	8.5	0	35.0	0.2	1.0	90.3
Dia03†	5.5	5.6	0	22.0	1.0	1.3	101.3
SWC††	0.052	0.038	0.003	0.167	0.294	1.039	73.5
TDR††	0.071	0.065	0.020	0.270	2.252	1.735	91.4
Sand††	906	44	754	986	0.5	-0.7	4.9
Clay††	34	28	1.0	102	-0.2	0.5	83.4
Silt††	60	39	8.0	246	5.3	2.0	65.3
SOM††	10.1	5.7	2.0	24.7	-0.8	0.4	56.4
CEC††	7.8	6.1	1.8	26.5	2.5	1.7	78.2
pH††	6.3	0.7	4.8	7.5	-0.9	-0.3	11.8
P††	27	16	5.5	70	-0.3	0.6	57.9
K††	38	30	5.0	179.5	3.4	1.3	79.0
Mg††	78	54	10	237	-0.3	0.8	69.7
Ca††	1056	1106	174	4799	4.5	2.3	104.7
Mn††	4.2	1.3	1.7	8.0	-0.3	0.2	31.5
Fe††	31	6.8	14.1	45.8	-0.2	0.2	22.1

† Weekly *Diaprepes* adult populations in 2001, in 2002, and in 2003, respectively.

†† SWC, gravimetric soil water content (kg kg^{-1}), TDR, volumetric soil water content (m^3 m^{-3}), sand, clay, silt in g kg^{-1}, organic matter content (g kg^{-1}), CEC in (Cmol kg^{-1}), and P, K, Mg, Ca, Mn and Fe in mg kg^{-1}.

Re-printed from **Li H, Futch SH, Syvertsen JP, McCoy CW** (2007d) Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* (L.) root weevil in citrus. *Soil Biology and Biochemistry* **39**, 2436-2447 ©2007, with kind permission from Elsevier Ltd.

Table 5 Hendry citrus orchard study. Correlation between weekly mean *Diaprepes* root weevil density in 2001, 2002, 2003, and 3-yr weekly mean *Diaprepes* root weevil density related to gravimetrical soil water content (SWC), time-domain-reflectory volumetric soil water content (TDR), soil texture (sand, clay, and silt), organic matter content (SOM), pH, cation exchange capacity (CEC), and macro and minor soil nutrients (P, K, Mg, Ca, Mn and Fe).

Variables	Dia01†	Dia02†	Dia03†	3-yr Dia†
	Pearson correlation coefficient (r)††			
Dia02†	0.63**	1		
Dia03†	0.47**	0.56**	1	
3-yr Dia†	0.88**	0.86**	0.77**	1
SWC	0.37**	0.52**	0.42**	0.51**
TDR	0.36**	0.47**	0.39**	0.48**
Sand	-0.34**	-0.34**	-0.33**	-0.40**
Clay	0.38**	0.52**	0.55**	0.56**
Silt	0.11 ns	0.01 ns	-0.03 ns	0.05 ns
SOM	0.44**	0.55**	0.55**	0.60**
pH	-0.23*	-0.04 ns	-0.11 ns	-0.17 ns
CEC	0.38**	0.57**	0.54**	0.57**
P	0.23*	0.35**	0.29**	0.34**
K	0.42**	0.53**	0.55**	0.58**
Mg	0.49**	0.65**	0.53**	0.65**
Ca	0.32**	0.52**	0.48**	0.50**
Mn	0.28**	0.26**	0.14 ns	0.28**
Fe	0.14 ns	0.32**	0.19 ns	0.24*

† *Diaprepes* root weevil population in 2001, 2002, 2003, and 3-yr total population (3-yr Dia). n = 100.

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

Re-printed from **Li H, Futch SH, Syvertsen JP, McCoy CW** (2007d) Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* (L.) root weevil in citrus. *Soil Biology and Biochemistry* **39**, 2436-2447 ©2007, with kind permission from Elsevier Ltd.

phases at the start of the season when competition and damage-induced plant stress should have less influence (Byers and Castle 2005). It is suggested that increases in temperature have a number of implications for meteorology-dependent insect pests in space and time (Li *et al*. 2007c).

The study found that the weevil density was 0.40, 0.42 and 0.027 weevils per m^2 in the slowly permeable Mollisol against 0.017, 0.014 and 0.0064 weevils per m^2 in the moderately permeable Alfisol in 2001, 2002 and 2003, respectively. The honestly significant difference (HSD) was 0.0067 weevils per m^2 in the slowly permeable Mollisols and 0.0028 weevils per m^2 in the moderately permeable Alfisols (Li *et al*. 2007d). The soil analysis found also that soil physical and chemical characteristics were variable (**Table 5**) and soil water content, organic matter, and Mg, K and P concentrations were high within the Mollisol (**Fig. 13**). The correlation analysis found that *Disprepes* root weevil adult population development was positively correlated ($P < 0.05$) to soil water content, clay content, organic content, and P, K, Mg, Ca and Mn concentrations (**Table 5**).

Since most life stages including larvae, pupae and teneral adults occur in soil (McCoy *et al*. 2003; Nigg *et al*. 2003), and characteristics of the soil and the citrus trees on which root weevil feed can directly or indirectly influence adult weevil development (Li *et al*. 2004a, 2005, 2007a, 2007d). Soil nutrients influence citrus tree canopy performance (Alva *et al.* 2003) and soil organism cycles (Klironomos *et al*. 1999). The weevils might be more attracted to bigger, healthier, fuller trees for feeding and egg laying for more abundant adult occurrence (Li *et al*. 2004a, 2007a, 2007d).

Using the moving average forecast model (SAS Institute 1993), the future *Diaprepes* population pattern against time (t) is estimated as the average of the last N monitoring of the underlying time series, which can be the 3-yr means of the weekly *Diaprepes* field monitoring data. The simple un-weighted moving average model is described by the equation as shown in Li *et al*. (2007d) as follows:

$$Dia_{t+1} = \frac{\overline{Dia_t} + \overline{Dia_{t-1}} + \overline{Dia_{t-2}} + ... + \overline{Dia_{t-j}}}{N} \quad [5]$$

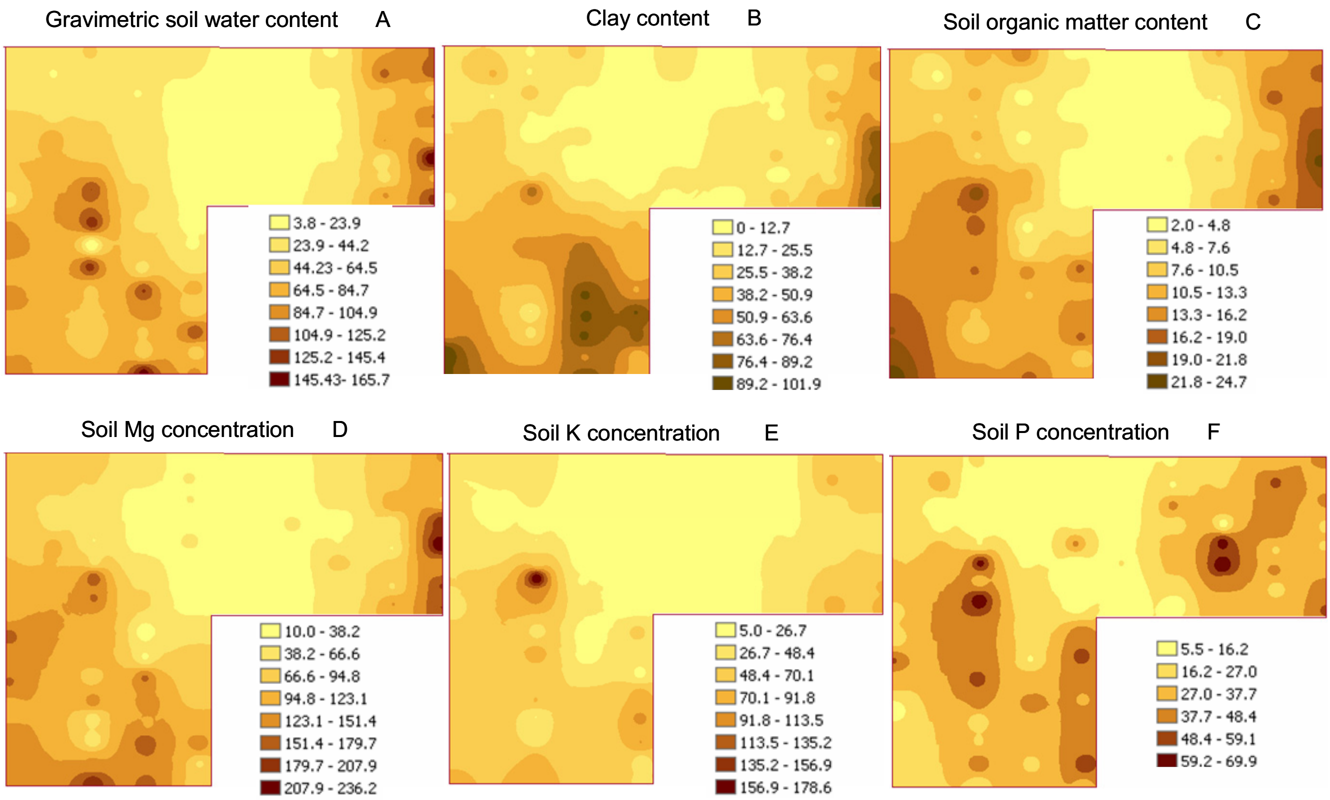

Fig. 13 Hendry citrus orchard study. Interpolated spatial patterns of gravimetric soil water content (kg kg^{-1}, **A**), clay content (g kg^{-1}, **B**), soil organic matter content (g kg^{-1}, **C**), and Melich-I extractable soil Mg (mg kg^{-1}, **D**), K (mg kg^{-1}, **E**) and P concentration (mg kg^{-1}, **F**). Re-printed from **Li H, Futch SH, Syvertsen JP, McCoy CW** (2007d) Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* (L.) root weevil in citrus. *Soil Biology and Biochemistry* **39**, 2436-2447 ©2007, with kind permission from Elsevier Ltd.

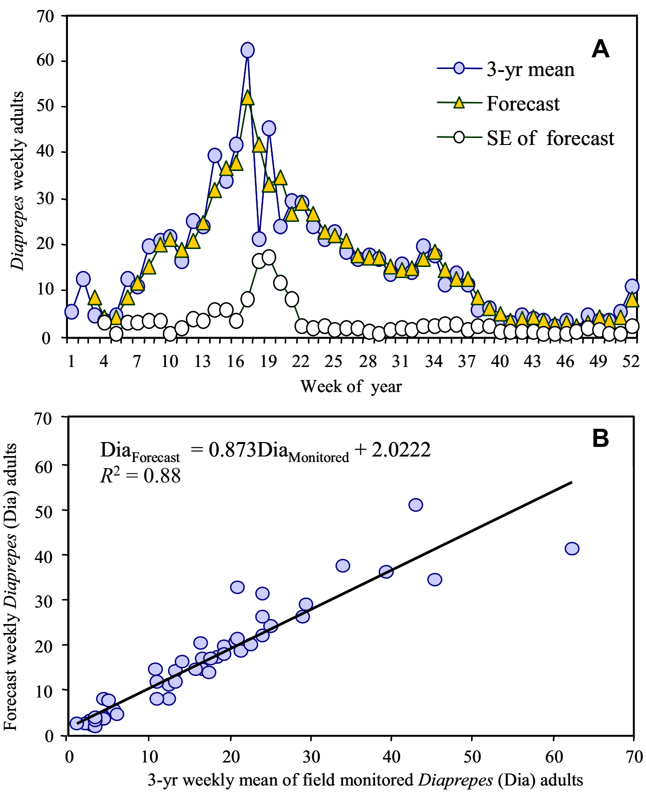

Fig. 14 Hendry citrus orchard study. Moving average model forecast of development patterns of *Diaprepes* adult weevil population and the 3-yr mean *Diaprepes* adult population monitored in the citrus orchard (**A**), and regression relationship of the forecast data and the field monitoring data (**B**). Re-printed from **Li H, Futch SH, Syvertsen JP, McCoy CW** (2007d) Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* (L.) root weevil in citrus. *Soil Biology and Biochemistry* **39**, 2436-2447 ©2007, with kind permission from Elsevier Publisher.

where Dia_{t+1} is the forecast for the *Diaprepes* root weevil for a future period (week), $\overline{Dia}$ is the 3-yr mean of weekly monitoring data of *Diaprepes* root weevil population, t is the number of weeks, j is the total of consecutive number of weeks of monitoring, and N is the moving average interval of the period (week). The moving average interval $N = 2$ was used n the moving average forecast estimations.

The time series analysis was done using PROC EXPAND procedure (SAS Institute 1993), which has generated a new time series of weekly *Diaprepes* weevil population by computation of the moving average of the original series (multiple year mean of weekly *Diaprepes* weevil monitoring data). The standard errors of the un-weighted moving average forecast data can also estimated from the PROC EXPAND procedure (Li *et al*. 2007d).

The generated new series by the moving average model using Eq. [5] demonstrates that the future temporal patterns of the *Diaprepes* weevil population would be highly variable with time, comparable to the field observation patterns shown by the 3-yr mean weekly *Diaprepes* data (**Fig. 14**). However, the forecasted peak, with a lag time step of one week compared to the field monitored peak, exhibited a smoother change with time steps than the 3-yr field dataset (**Fig. 14A**). The moving average forecast varies between 16.0 ± 11.9 (range 2.1-52) weevils, which is close to the mean and standard deviation (15.8 ± 12.7 weevils) of the 3-yr field dataset. The standard errors (SE) of the moving average forecast data followed the yearly peak in early spring (**Fig. 14A**). The moving average model removed seasonal and irregular variation to show the smoothed trend patterns (**Fig. 14**), which quantified the variability (growth, peak, and decrease) of weevil development for making management decisions. When plotted against the 3-yr mean field data, the regression shows that the forecast data are strongly related to the multi-year field monitoring data ($R^2 = 0.88$, **Fig. 14B**) (Li *et al*. 2007d).

Models probably would be adequate for a specific situation in predicting populations, their damage and control costs (Byers and Castle 2005). For practical management, the moving average forecast model would be useful for predicting future development of the weevil population across a period of a year. The forecast trend (**Fig. 14**) suggests that equal timing of insecticide applications would not be efficient based on the weevil temporal pattern, and insecticides should be applied in the spring, in the summer, and early in the fall. Also, higher rates of insecticides should be applied in the spring, and lower rates should be applied in the summer and in the fall. Such rates need to be further determined in future study (Li *et al*. 2007d).

Models are often incorporated in a decision-making framework (Worner 1991; Tobin *et al*. 2001; Li *et al*. 2007a, 2007d). The established time series model [Eq. 5], the multivariate linear stepwise model [Eq. 1] and the exponential models [Eq. 2-4] can be implicated into practices for reducing citrus tree biotic stress. One strategy can be using temporal development patterns of *Diaprepes* adult population to determine insecticide application timing (Li *et al*. 2007a, 2007c, 2007d). The other can be using soil characteristics for controlling the root weevil for tree protection (2007d). Time series moving average forecast and soil characteristics-based simple and multivariate models, have the implications for variable rate and less frequent sprays to contribute to pesticide risk reduction, reducing citrus tree biotic stress (Li *et al*. 2007d, 2008).

As abiotic and biotic stresses of citrus trees and its root weevil survival are associated with time and environmental soil variables, the mathematical equations derived from tree, soil and weevil variable correlations in subsequent years would be useful for integration with soil unit management zones, environmental mapping, and autocorrelation analysis for reducing citrus tree abiotic and biotic stress through improving management of the orchard and the root weevil.

CONCLUSIONS

Citrus tree abiotic and biotic stress is a combination of unfavorable constraints from soil anoxia from flooding, waterlogging, unbalanced nutrient levels and root weevil feeding injury. The combined effects of these stress factors and *Diaprepes* root weevil development with time have given an insight how strongly the trees, soils and insects can be directly and indirectly related with each other. Flooding may be beneficial by reducing larval survival but it can be critical for citrus plant survival, based on the notion that flooding could significantly reduce soil redox potential, plant leaf stomatal conductance, and shoot growth. A negative soil redox potential and a decrease of leaf stomatal conductance could be used as early indicators of plant water stress from waterlogging. The information obtained from the greenhouse simulation study may be useful for predicting the combined damage of flooding and larval feeding on mature trees, although flooding events can be more frequent, flooding duration cannot be controlled, and mature tree roots might be more resistant to waterlogging damage and larval injury at orchard scale.

Citrus susceptibility to flooding stress and root weevil larval feeding determined through the simulation of greenhouse studies has helped understanding within what time range seedling plants can tolerate flooding stress. Flooded roots would be more susceptible to injury by *Diaprepes* root weevil larvae than non-flooded roots because flooded roots became weaker. The combination of flooding and larval feeding might complicate treatments for weevil control. A 20-day flooding period was a critical duration for plant water stress tolerance because leaf stomatal conductance could be significantly reduced and roots could be damaged during a flooding period of 20 days or longer.

Warm temperature conditions can contribute to the citrus root weevil outbreaks. Synthesis of tree root weevil and soil variable relations in space and time via time series model would be useful for reducing the cost of field monitoring and for achieving improved integrated pest management. Using this approach, time series model predictions will help for less frequency of spraying from excess use of costly pesticides that can harm the environment, and for reducing field insect monitoring that is time consuming. Regulation of soil water regime, nutrient levels and leaf water potential by stomata and development of simulation and modeling management of tools have the implication for reducing citrus tree decline and for achieving improved integrated pest management in citrus.

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