Larval Growth of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) and Resulting Root Injury to Three Citrus Varieties in Two Soil Types

STEVEN ROGERS, C. W. MCCOY, AND J. H. GRAHAM

Citrus Research and Education Center, University of Florida, IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850

**ABSTRACT** Larval growth and intraspecific competition of *Diaprepes abbreviatus* (L.) larvae and consequent root injury in container-grown citrus in the greenhouse were evaluated. Roots of Carrizo citrange, *Citrus sinensis* L. Osbeck × Poncirus trifoliata (L.) Raf.; Cleopatra mandarin, *C. reticulata* Blanco, and Swingle citrumelo, *C. paradisi* Macf. × *P. trifoliata* (L.) Raf. rootstock seedlings grown in Candler fine sand and potting soil were colonized with different populations of *D. abbreviatus* larvae. Larvae were exposed to the seedlings for 79 d. Larval growth and development increased steadily for 70 d on all rootstock-soil combinations, at which time most larvae were instars 6–8. Most feeding injury occurred to roots when larvae were between instars 3 and 6. Larval weight reached a plateau at 70 d, but often declined between 70 and 79 d. When larvae were small, injury to seedlings developed slowly, primarily on fibrous roots, then feeding increased rapidly, often resulting in total consumption of both fibrous root and bark tissue. Although not statistically significant, root injury developed slightly slower on Swingle citrumelo compared with Carrizo and Cleopatra rootstocks, but damage was comparable by 79 d. Little or no difference in consumptive benefit to the larvae was found between the rootstocks. Based on larval weight days, little feeding injury occurred during the first 21 d, but increased rapidly between 21 and 60 d. Soil type affected the rate of larval growth and development, with potting soil contributing to greater growth rates. Detritus in potting soil provided little or no nutritional resource, suggesting that the effect of potting soil on larval development was primarily physical. In addition, fewer inoculated larvae per seedling exhibited greater weight gains than higher infestation densities, suggesting that intraspecific competition for nutritional resources influenced larval development.

**KEY WORDS** *Diaprepes abbreviatus*, citrus, development, injury, damage

*Diaprepes abbreviatus* (L.), a polyphagous broad nose weevil, has been reported to attack >270 plant species throughout the Caribbean region (Woodruff 1964, Beavers et al. 1979). During the past 35 yr, *D. abbreviatus* has become established on many horticultural crops in Florida, including sugarcane, woody ornamentals, and >10% of the citrus grown commercially (Simpson et al. 1996). Neonates of *D. abbreviatus* usually invade the soil after hatching from eggs and falling from leaves inhabited by adults (Wolcott 1933). Injury inflicted by larvae on roots can lead to plant death or decline.

*Diaprepes abbreviatus* larvae feed on all rootstocks commonly budded to *Citrus* spp. (Shapiro and Gottwald 1995, Grosser and McCoy 1996, McCoy et al. 1996). Larger larvae even devour bark from the crown area of some root systems, causing girdling and eventual death of the tree (Whitwell 1991). Decline symptoms associated with *D. abbreviatus*-induced root injuries, such as leaf yellowing or premature leaf drop, twig dieback, off-blooming, fruit drop, and alternate bearing, resemble tree declines caused by other tree diseases and root disorders, such as those caused by citrus blight, tristeza, or *Phytophthora* spp. (Quintela et al. 1998). Feeding injuries caused by *D. abbreviatus* larvae also have been associated with the infectious diseases caused by *Phytophthora* spp. (Rogers et al. 1996, Graham et al. 1997).

Rational approaches to root weevil management in the field are predicated on an understanding of larval feeding behavior and the interactions within and between *Diaprepes* larvae and other organisms in the rhizosphere of different rootstock hosts under different environmental conditions. Although concurrent experiments are being conducted to determine the pathogenic relationship between *D. abbreviatus* root injuries and soil pathogens such as *Phytophthora* spp. (Rogers et al. 1996, Graham et al. 1997), this study was initiated to examine the specific citrus root-herbivore dynamics of *D. abbreviatus* larvae. Further, research describing differential resistance of citrus rootstocks has yielded variable results (Shapiro and Gottwald 1995, Shapiro et al. 1997). Thus, the objectives of this study were to examine temporal development of root injury and examine the effect of different larval densities on the relationship between root consumption and larval growth on three commercially important citrus rootstocks.
Materials and Methods

About 600 6-mo-old citrus rootstock seedlings propagated in the greenhouse were used in this experiment. Rootstocks were selected based on their resistance to Phytophthora spp., to generate baseline data for comparison with root diseases (Graham 1990). Carrizo citrange, Citrus sinensis L. Osbeck × Poncirus trifoliata (L.) Raf (Phytophthora-susceptible); Cleopatra mandarin, C. reticulata Blanco (Phytophthora-susceptible); and Swingle citrumelo, C. paradisi Macf. × P. trifoliata (L.) Raf. (Phytophthora-resistant) were obtained from a local citrus nursery as bare root seedlings (Roland Dilley and Son, Avon Park, FL). Each seedling was transplanted into a single 125-cm³ Conetainer (Stuewe and Sons, Corvallis, OR) containing Candler fine sand (Typic quartzsands, 96.5% sand, 2% silt, 1.5% clay, pH 6.8, organic matter content of 1% and extractable P of 3.5 (µg/g soil) or a commercial potting soil (Metro mix 500, Scotts, Marysville, OH), 25% peat, 40% processed ash, 25% vermiculite, 10% proprietary, pH limed to 7.0). Plants used in the experiments were selected by visual inspection for within-variety uniformity of shoot and canopy size. Seedlings were maintained in the greenhouse at 25°C and were fertilized weekly with a water soluble 20-20-20 (N-P-K) fertilizer (Peter’s) and watered regularly.

Neonate larvae of D. abbreviatus (~48 h old) were obtained from eggs laid by field-collected adult females confined to screen cages in a greenhouse at 27 ± 2°C. Either two or five neonate larvae were placed in Eppendorf tubes that were used to scatter larvae onto the soil surface of each Conetainer. An equal number of noninoculated seedlings also were included in the treatment regime. All inoculations were made on the same day. Treatments were blocked according to larval infestation, soil type, and rootstock to form 18 treatment combinations, including the control. Treatment groups consisted of nine cohorts replicated four times. At each interval of 7–14 d after infestation, a cohort from each treatment was taken from the greenhouse and evaluated by carefully removing each plant from the soil and placing it on a shallow examination tray. A spatula was used to remove the soil from around the roots and recover the larvae. Larvae were counted and roots were visually rated for feeding injury using the Horsfall and Baratt rating system (Horsfall and Barratt 1945). Ratings for the Horsfall and Baratt system ranged from 0 to 11, inclusive. Each incremental rating change corresponded to an exponential change in percentage injury to the root system (0 = 0% injury, 1 = 0–3%, 2 = 3–6%, 3 = 6–12%, 4 = 12–25%, 5 = 25–50%, 6 = 50–75%, 7 = 75–87%, 8 = 87–94%, 9 = 94–97%, 10 = 97–100% and 11 = 100% injury). These values were used to mathematically transform visual assessments of larval feeding injury to mean percentage injury. For each cohort, mean larval weight/treatment was obtained by weighing total lar-
val biomass and dividing by the number of larvae recovered from the soil. Dry root biomass was obtained by clipping roots from the shoots at 0.5 cm above the soil line and air drying for 10 d before weighing on an Mettler AE-160 analytical balance (Mettler Instrument, Heightstown, NJ). Determinations of percentage root injury and root loss in grams were made by subtracting measured root injury and loss from the untreated controls in each respective group. Measures of both root loss (using the Horsfall and Baratt system) and root loss (measured in grams) were included because, although each approach quantifies root damage, each measures two different aspects of the damage. The visual Horsfall and Baratt system measures overall damage to the root system volumetrically, whereas root loss in grams measures grams biomass lost because of the feeding larvae. The two measures are correlated but may or may not be coincident.

Fenna (1942) showed that detritus in plant growth media could provide a nutritional resource for the larvae of certain root weevil species. To test this possibility with D. abbreviatus, neonates were placed in Candler sand and potting soil with and without Carrioz seedlings and maintained in the previously described manner. After 21 d, seedlings were removed from the soil and the soil was sifted and examined for larvae.

Statistical Analyses. Statistical analyses were based on a randomized factorial analysis of variance (ANOVA) design based on soil type, cultivar, and infestation treatment. Quintela et al. (1998) identified the relationship between head capsule size, instar, and weight. Using larval weights measured in these experiments, instars were estimated from the relationship between instar and larval weight as described in Quintela et al. 1998 (Fig. 1). Results for larval weight, root injury, root loss, and larvae recovery were analyzed by ANOVA (α = 0.05) and means were compared using the Fisher protected least significant difference (LSD) and the Tukey-Kramer test (SAS Institute, 1998). Larval weight days were expressed as the cumulative weight of larvae in grams feeding on the roots over time,

\[ LD = LD_{t-i} + (LT_i \times N/2) \]  

where LD is larval weight days; \( LD_{t-i} \) is cumulative weevil weight days of larvae at the previous sample date; \( LT_i \) is total weight of larvae at current sample date and \( N \) is number of days between previous and current sample dates. Larval growth between sample dates was assumed to be linear.

Results

In this study, young larvae were observed to feed on fibrous roots, whereas later instars fed on larger lateral roots, causing deep lateral grooves while masticating the outer bark and cambium layers. Different infestation levels resulted in different numbers of larvae
being recovered between 21 and 60 d (Fig. 2), with the greatest number of larvae being recovered between 21 and 42 d. The percentage recovery was greater from the five-larvae infestation. After 54 d, however, the number of recovered larvae declined to \(\leq 1\) larva per seedling and in most cases was not different between infestations of two and five larvae. Before 21 d, the number of larvae recovered from the soils increased because of ease of recovery with increasing size. Because of their very small size, neonate larvae were virtually impossible to detect in the soil and their initial injury to roots was exceptionally difficult to quantify. However, feeder roots did exhibit signs of minute, although visually detectable, injury. This low-level feeding on fibrous roots corresponds to the initial lag in the injury progress curve. Root injury became much easier to detect as the larvae increased in size after 21 d, and many root tissues were completely consumed by 79 d (Figs. 3 and 4). Thus, \(\approx 3\) wk of larval feeding occurred before either root loss or visually assessed percentage root injury increased to easily detectable levels. Feeder root injury subsequently increased to where it gradually became measurable, and injury eventually spread to the tap root. Scarcer root resources limited food by this time, contributing to the reduced rate of injury development by 79 d.

Fresh weight gain of developing larvae increased steadily to \(\approx 70\) d into the experiment. However, larval weight often remained constant or decreased between 70 and 79 d (Fig. 5). Based on cumulative larval weight days, larval weight gain and feeding injury showed little or no increase within the first 21 d while larvae were observed to be feeding on fibrous roots. However, larval weight days increased after larvae began feeding on the tap and structural root systems (Fig. 6). By 79 d, although not statistically significant, the trend was that the greatest larval weight days accumulation occurred for larvae reared on Carrizo rootstock in potting soil. Although statistical difference in larval weight days across infestation density was observed, cumulative larval weight days for larvae reared on Cleopatra rootstock was significantly lower than for the other two rootstocks \((F = 13.695; df = 2, 54; P < 0.0001)\). The greatest larval weight gain \(>79\) d was observed on Carrizo rootstock planted in potting soil (Figs. 5 and 6).

Although larval weights increased \(\approx 1\) mg/d in the early instars, daily weight gains for some larvae increased by more than a factor of 10 after the larvae reached instars 2–3 (21 d into the infestation). Averaged across cultivar and infestation density, there was a significant effect of soil type on larval weight gain.
Fig. 4. Loss of root weight in grams over time from larval feeding by *D. abbreviatus* on three citrus rootstocks in two soil types. Values presented were calculated by subtracting the gram root weight for each sample date from the uninfested control. ■ 0 larvae per root; ● 2 larvae per root; ▲ 5 larvae per root. Vertical bars represent one standard error of the mean.

(F = 10.268; df = 1, 40; P < 0.0027 at t = 42; F = 5.469; df = 1, 40; P = 0.0246 at t = 79). Both intermediate and final larval weights in Candler sand (∼51 mg at t = 42 and ∼88 mg at t = 79) were significantly lower than in Metro potting mix (∼72 mg at t = 42 and ∼41 mg at t = 79).

Generally, mean larval weight gain for rootstock and soil was coincident with an increase in root injury. The trend in larval weight gain was more nearly linear than increases in root injury (Figs. 3 and 5). Although variable, the percentage root injury assessed using the Horsfall and Baratt rating system (Horsfall and Barratt 1945) increased rapidly after 21 d (Fig. 3). Feeder root consumption by neonates was exceptionally difficult to detect until ∼14–21 d after infestation, and the lag in detectable root injury up to ∼14 d after infestation was attributed to their low feeding rates on the fibrous roots. Averaged across soil type and cultivar, there was a significant effect of infestation density on bark injury development. Although individuals in the two infestation treatments obtained greater final weights (t = 79), the two larvae infestations resulted in significantly less bark injury compared with seedlings infested with five larvae (F = 55.871; df = 2, 556; P < 0.0001), but there was no statistical difference in root loss between the Candler and potting soil types (F = 0.826; df = 1, 556; P < 0.0001).

Discussion

Mean larval weight gains, root injury, and larval survivorship were different depending on the conditions under which they developed. Of the three environmental backgrounds studied—rootstock, soil type, and infestation density—larval developmental differences were clearly evident in the latter two. Consistent differences in larval development across
the three rootstocks in this study were not seen. These results contrast with Shapiro and Gottwald (1995), but not with Shapiro et al. (1997). Results from Shapiro and Gottwald (1995) may be predicated on the fact that the experiment was terminated prematurely (i.e., had their larvae been allowed to feed longer, then the differential damage observed might have become less pronounced). Soil type and infestation density affected the level of root injury, but higher infestation levels resulted in competition between larvae for root resources.

As measured by head capsule size, Quintela et al. (1998) observed that *D. abbreviatus* undergo 11 instar stages, and the weights of larvae on unrestricted artificial diets often exceed 500 mg by ~75 d. In comparison, they found that most larval weight gain on plant roots, up to ~300 mg, occurred from about instar 6–7, or 30–95 d after neonates were introduced to roots. Weight declines of 20–50 mg occurred between 70 and 80 d in several cases. Larvae underwent a period of lethargy from instar 8 to pupation, where larval weights tended to remain constant or even decrease in some circumstances. Within the 79 d of our experiment, instar 8 and a weight of ~250 mg were the maxima estimated to have occurred (Figs. 1 and 5). Our data also showed that the greatest weight gain occurred between instars 6 and 8, ~35–70 d after neonate infestation. We also observed that larval weight remained constant or decreased between 70 and 79 d. This later lethargic period may have involved an initiation of pupation, but was probably strongly influenced also by the loss of root resources on which larvae could continue feeding. These studies also demonstrated that increases in root injury occurred most rapidly during instars 6–8, suggesting that these instars have greater resource requirements and thereby cause the greatest damage to citrus roots. Our data also suggest that *D. abbreviatus* may be an indeterminate molter.

Generally, increases in grams of root loss corresponded with increases in percentage root injury. The most rapid rates of loss occurred between 21 and 70 d, after the larvae exceeded about instar 3. A decrease in the rate of root loss (i.e., root regeneration) was evident on at least one occasion in all treatment combinations (Fig. 4). Excluding experimental variation, two factors occurring singly or in combination could be responsible for this phenomenon: a stress-response regrowth of roots that exceeded the rate of larval consumption; or a physiological change in larval development resulting in a reduced feeding rate. Quintela et al. (1998), however, did not demonstrate reductions in larval development during time periods comparable with when the root regeneration was observed. Reductions in larval development in this experiment during these time periods likewise were not measured. Therefore, the most plausible explanation seems to be a regrowth of roots that exceeded the rate of root consumption by the larvae. It is not known what

---

**Fig. 5.** Mean larval weight gain of *D. abbreviatus* for three rootstocks in two soil types infested with two and five larvae per unit. ■ 2 larvae per root system; ●, 5 larvae per root system. Vertical bars represent one standard error of the mean.
factors might have induced this regrowth to occur, but root regrowth does have several very important practical implications. Graham (1995) demonstrated that tolerance to Phytophthora root rot was expressed as a greater capacity to regenerate roots under certain environmental conditions, so root regeneration under conditions of stress are not implausible with D. abbreviatus. If Phytophthora root rot is managed, frequent, light irrigation and fertigation regimes also could help induce root regeneration and tree recovery from larval injury, especially if such materials are applied during the driest periods of the year.

Potting soil appeared to provide a medium more conducive to larval feeding and development than Candler sand. Gustin and Schumacher (1989) demonstrated that increased bulk density of soils beyond 1.1 mg/m³ in sandy soils physically limited the movement of first-instar western corn rootworm (Coleoptera: Chrysomelidae) because of closer compacting of the soil particles. The potting soil was more loosely packed than the Candler sand, making it significantly easier for larvae to move about through the root zone. Because there was no clear evidence that potting soil detritus provided a nutritional benefit to the developing larvae in our experimental series, it is hypothesized that the benefit conferred by the potting medium was primarily physical. Based on the observed packing density, the diffusion rates of moisture and oxygen through the potting soil was likely greater, and these factors in concert may have been more conducive for the vigorous metabolic activity required by the feeding and growing larvae.

Fenna (1942) showed that detritus in plant growth media could provide a nutritional resource for larvae of certain root weevil species. After 21 d in our experiment, all larvae grown in Candler fine sand or potting mix alone perished, whereas most or all larvae grown in the presence of the Carrizo roots survived, irrespective of the medium in which they were grown. This extensive mortality after 21 d showed that the nutritional benefit to D. abbreviatus larvae from the potting soil detritus was marginal at best and could not sustain larval development in the absence of a seedling root food source.

Averaged across soil and rootstock, larvae infested at two per seedling accumulated biomass more rapidly than those in the five-larval infestations. Because root systems were standardized to size, the differences in larval development must have been caused by crowding or other competitive effects. These effects were observed even when larval survival was high and root resources were not limiting (Figs. 2 and 4).

Larval recovery decreased over time (Fig. 2). This decrease was more pronounced in treatments inoculated with five larvae, also suggesting that some type of resource competition was involved. Although inoculation densities were constant (2 and 5), different groups within an infestation cohort encountered dif-

Fig. 6. Development of larval days for D. abbreviatus infested on three citrus rootstocks in two soil types. □ 2 larvae per root; ● 5 larvae per root. Vertical bars represent one standard error of the mean.
different levels of natural mortality and therefore different intensities of competition. That is, competition became a function not only of the size and numbers of larvae inhabiting the rhizosphere (crowding density), but also of the proportion of root tissue available to individual larva because of such crowding. For example, five larvae of 50 mg each and 100 cm of linear root tissue available would have experienced greater competition than two larvae of 50 mg and 100 cm of linear root tissue. Intraspecific competition for essential resources thus appeared to have led to variations in density-dependent increases in death rates where spatial and nutritional resources were constant.

Current field data indicate that the natural survival rate of neonate D. abbreviatus larvae is extremely low, perhaps approaching 10^{-3} (C.W.M., unpublished data). Such low neonate survival ratios suggest that the probability of subterranean larva-to-larva contact in commercial fields is very low, especially on trees with large root systems, so competitive interactions between larvae for food and space such as those observed in this experiment would likely not occur in nature. An exception might be if biochemical or environmental factors served to create preferred feeding niches for the larvae, such as near the crown areas of the tree root systems.

Acknowledgments

We gratefully acknowledge the technical assistance of Carmi Louzada, without whose efforts this research would not have been possible. This research was supported in part by a grant from the Florida Citrus Production Research Advisory Council. This is Florida Agricultural Experiment Station Journal Series No. N-01643.

References Cited


Received for publication 12 November 1998; accepted 2 December 1999.