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ABSTRACT

The seasonal abundance of various life stages of Diaprepes abbreviatus (L.), was monitored in separate years in adjacent irrigated and non-irrigated citrus plantings, as well as thickets of Brazilian-pepper located near Poinciana, FL in Osceola County. Adult emergence, estimated by weekly catches in cone-shaped ground traps, occurred throughout the year with a peak in mid-June in both citrus and Brazilian-pepper plantings. Onset of adult emergence coincided with an increase in soil moisture and temperature. Trap counts were highest when soil water potential increased to 3-5 centibars at a depth of 15-30 cm and soil temperature averaged 22-24°C. In the non-irrigated citrus planting, the adult emergence peak was of shorter duration, but of greater magnitude, compared to the irrigated planting. Although the alternate host, Brazilian-pepper, produced fewer weevils than did citrus, the seasonal emergence pattern was virtually the same.

Adult abundance within the citrus plantings was also monitored weekly using modified Tedders traps. The number of adults captured approximated the number caught in ground traps. Adult number caught weekly changed seasonally, particularly in the fall when adult populations were the highest. Ground traps caught a larger number of adults in the spring. The number of egg masses collected weekly in the tree canopy and the number of neonates caught weekly beneath the tree canopy were both correlated with the number of adults captured weekly in modified Tedders traps. These data suggest that adults caught in modified Tedders traps provide a reliable indicator for estimating the seasonal abundance of all life stages within a citrus planting. Larvae of different instars, pupae, and tender adults were recovered from the soil rhizosphere after periodic tree removal. No diseased or parasitized life stages were observed in the study. Most life stages were present in the soil at each sample date, but the proportion of larvae in various instars changed seasonally. The implications of this study for understanding the population dynamics of D. abbreviatus are discussed in relation to current and future IPM strategies.

Key Words: Diaprepes abbreviatus, population dynamics, citrus, Brazilian-pepper

RESUMEN

Se realizó un monitoreo sobre la abundancia estacional de varias etapas de vida de Diaprepes abbreviatus (L.) en años separados en siembras de cítricos irrigadas y no irrigadas en campos adjacentes, así como en matorrales de la pimienta de Brasil (Brazilian peppertree, Schinus terebinthifolius) cerca de Poinciana, Florida en el condado de Osceola. La emergencia de los adultos, estimada por el número de adultos atrapados en las trampas de forma de un cono puestas sobre el suelo, aconteció a través del año con el punto más de la población ocurriendo en el medio de junio en siembras de cítricos y en Schinus terebinthifolius. El inicio de la emergencia de los adultos coincidió con un aumento en la humedad del suelo y de la temperatura. El número de Diaprepes atrapados por trampa fué el más elevado cuando el potencial del agua de suelo aumentó a 3-5 centibarras a una profundidad de 15-30 cm y a un promedio de la temperatura del suelo de 22-24°C. En siembras de cítricos no irrigados, el punto más alto de la emergencia de los adultos duró menos tiempo, pero fué de mayor magnitud, comparada con las siembras no irrigadas. Aunque el hospedero alternativo, Schinus terebinthifolius produjo menos picudos de lo que fueron producidos en los cítricos, el patrón de emergencia estacional fué virtualmente el mismo.

También, se realizó un monitoreo semanalmente sobre la abundancia de adultos entre la misma sierra de cítricos usando trampas modificadas del tipo “Tedders”. El número de adultos capturados fué aproximadamente el número recolectado en las trampas del suelo. El número de adultos recolectados semanalmente cambió según la estación, particularmente en el otoño cuando la población de adultos fué la más alta. Las trampas de suelo capturaron un mayor número de adultos en la primavera. El número de masas de huevos recolectados semanalmente en la copa del árbol y el número de neonatas (larvas recién nacidas) capturados semanalmente debajo la copa del árbol fueron ambos correlacionados con el número de
adultos capturados semanalmente en las trampas modificadas de tipo “Tedders”. Estos datos sugieren que los adultos capturados en las trampas modificadas de tipo “Tedders” proveen un indicador confiable para estimar la abundancia estacional de todas las etapas de vida dentro de una siembra de cítricos.

Larvas de diferentes estados, pupas y adultos tenebrosos fueron recobradas de la rizófora después de la eliminación de árboles hecha periódicamente. No fueron observadas las etapas de vida con enfermedades o parasitadas en el estudio. La mayoría de las etapas estuvieron presentes en el suelo en cada fecha de muestreo, pero la proporción de larvas en las varias etapas cambió según la estación. Se discuten las implicaciones de este estudio para entender la dinámica de la población de *D. abbreviatus* en relación con las estrategias actuales y futuras de Manejo Integrado Plagas (MIP).

*D. abbreviatus* (L.) a root weevil native to the Caribbean region (O’Brien & Wibmer 1982) is a major localized pest of commercial citrus, ornamental plants, and some agronomic crops in Florida since its introduction in 1964 (Woodruff 1964, McCoy 1999). It has recently spread to citrus and ornamentals in Texas (French & Skaria 2000) and has been intercepted frequently by regulatory officials in California (Kris Godfrey, personal communication). Currently, it ranks as a high risk pest of California agriculture. The adult, egg, and neonate stages appear on above-ground parts of the host plant and all larval stages, pupae and teneral adults occur below ground (Wolcott 1936). Although this weevil can be univoltine on citrus, the life cycle varies in duration with many over-lapping generations and many different life stages may be present simultaneously. Upon hatching, neonates fall from tree and enter the soil. The small neonates feed on fibrous roots, whereas later instars feed on larger structural roots, causing deep grooves as they consume the outer bark and cambium layer. From year to year root injury appears to accumulate, and feeding sites can serve as infection courts for root rot diseases such as *Phytophthora* spp. (Graham et al. 1996, 2002), thereby exacerbating economic losses.

Current integrated pest management (IPM) strategies for weevil suppression include horticultural practices such as irrigation and fertilization, *Phytophthora* control in the soil, and a mix of control tactics aimed at reducing larval and adult populations (McCoy & Duncan 2000). Monitoring of adults using visual counts or modified Tedders traps has been deployed to better time foliar and soil applications of adulticides and larvicides in citrus groves (Stansly et al. 1997, Duncan et al. 2001). Although trapping has been useful in determining seasonal patterns of adult emergence from the soil, a lack of knowledge of the developmental biology and ecology of *D. abbreviatus* has limited our understanding of trap accuracy and population dynamics of various life stages in the field.

The purpose of this 2-yr study was to assess the relative abundance of various life stages of *D. abbreviatus* in adjacent distinct citrus plantings and in Brazilian-pepper thickets surrounding the citrus using different monitoring methods. Brazilian-pepper is a host to both adults and larvae of *Diaprepes* (Simpson et al. 1996) where they feed on leaves and roots, respectively. Relative abundance of different life stages was then related to abiotic factors such as air temperature, soil temperature, rainfall, and soil moisture. No irrigation was deployed in the first year. This information was deemed fundamental to the development and application of various IPM strategies.

**Materials and Methods**

**Experimental Site**

Independent studies were conducted near Poinciana, FL in Osceola County in two distinct plantings of Hamlin orange grafted to Swingle citrumelo rootstock set at 6.1 × 8.5 m. These adjacent plantings, designated north (40 acres) and south (50 acres), were planted on two-row beds, in a poorly drained alfisol soil type classified as Floridana fine sand (68.8% sand, 11.8% silt, 19.4% clay). The surface layer was 35.6 cm loam and the subsurface layers 76.2 cm gray fine sand followed by clay. The soil had a low to moderate organic matter content with a pH of 4.8. Both plantings had been infested with *D. abbreviatus* for at least 10 yr according to local reports. In the north planting, an estimate of weevil larvae in the soil was made in early February 2000, shortly before our study was begun. Three trees were removed and soil sieved to recover larvae. These trees produced 83–86 late instar larvae/0.4 m² of soil. Although tree decline was severe in the north planting at this time, high larval populations in combination with poor horticultural care (no irrigation) resulted in greater tree decline by years end. The rapidity of tree decline and death in the north planting in 2000, necessitated moving our study to the south planting in 2001. In so doing, our original experimental plan to replicate by year was lost.

In the north planting, 150 productive trees with decline symptoms were purposely selected for studies in 2000. These trees were non-irrigated and received no chemical treatments dur-
ing the study period. The grove was mowed regularly to reduce weed growth and experimental trees received a herbicide application (glyphosate at standard rate) as needed to prevent weed growth at the tree canopy margin and beneath the tree. Some trees were pruned lightly to improve accessibility beneath the tree. One fertilizer application was applied to the healthy trees in mid-April using a standard citrus mixture (6-6-6). Brazilian-pepper trees, *Schinus terebinthifolius* Raddi, were growing wild throughout the north planting along with other woody weed hosts of *D. abbreviatus*.

By contrast, the south planting used for study in 2001, was highly productive and had been under regular grove care that included irrigation on a need basis, fertilization twice per year, and regular weed and pest control. Fertilization and weed control only were continued in 2001 following the same schedule.

### Monitoring Adult Weevils

In each planting, seasonal adult emergence from the soil and adult abundance in the grove were monitored using cone-shaped screened ground traps (0.9 m base dia.) and modified pyramidal Tedders traps (Tedders & Wood 1994, McCoy et al. 2000), respectively. A trap of each type was placed as pairs opposite each other beneath a tree, midway between the tree trunk and the canopy dripline. Within each planting, 100 randomly selected trees received a pair of traps. Traps were monitored weekly from 17 March through 18 December 2000 and from 6 March through 11 December 2001. In 2001, an additional 100 cone-shaped ground traps covered with mylar plastic to simulate no irrigation, were monitored weekly within the grove from 6 March through 29 August.

The whole citrus planting was bordered on three sides by pasture that harbored various alternate host plants for *D. abbreviatus* such as tal- low tree, *Sapium sebiferum* L., coffee weed, *Sesbania exaltata* (Raf.) Rydb. ex A. W. Hill, and the fore-mentioned Brazilian-pepper. In two thickets of Brazilian-pepper located in the adja-
cent pasture within 90 m of the south citrus planting, 100 ground traps were distributed randomly beneath trees and monitored from 6 March through 11 December 2001 only.

### Monitoring Oviposition in the Tree Canopy

In 2001 only, egg mass abundance within the accessible part of the tree canopy was monitored weekly by systematic 15 min visual inspections of each of 20 trees from 6 March through December 2001. Inspection entailed searching for detection of attached or folded leaves glued by female weevils during oviposition.

### Monitoring Neonate Fall to the Soil Surface

The seasonal abundance of neonates falling from the trees was assessed using modified pitfall traps (funnel traps). Traps consisted of a 20.3 cm diameter plastic funnel attached to 3.1 × 50.0 cm length of PVC pipe support elevated about 30 cm above the ground. A screw top conical tube (50 ml), containing 5 ml of ethylene glycol, was attached to the funnel tip using duct tape to serve as a collection unit.

Eight traps were placed under each tree in the cardinal directions, four traps at 30 cm from the trunk and 4 traps at 30 cm from the dripline. Larvae were collected from 25 trees in 2000 and 20 trees in 2001; and collection tubes were changed weekly. In the laboratory, neonates were identified and counted. *Diaprepes abbreviatus* neonates were identified by the frontal suture joining the epicranial suture to form an inverted “Y” on the head capsule (Beavers & Woodruff 1971). Trapping was performed weekly from 1 May through 26 December 2000 and from April through 31 December 2001.

### Monitoring Weevil Life Stages in the Soil

The seasonal changes in relative abundance of the various life stages of *D. abbreviatus* in the soil was assessed by periodically removing four or more trees and recovering all detectable life stages from the soil beneath the extracted trees. Trees were topped using a chainsaw and the remaining roots and surrounding soil were removed using a back hoe. Most of the soil adhering to the roots was removed by shaking and/or probing with a shovel. Soil from the roots and beneath the tree was then placed into buckets for subsequent sieving. Approximately 0.59 m³ of soil was collected per tree to a depth of 30 cm according to the procedures of Duncan et al. (1996). All life stages of *D. abbreviatus* except larvae younger than fifth instar were visually detectable and recovered from the soil using a motor-driven shaker and 0.64-cm mesh sieve. The numbers of larvae, pupae and adults recovered from each tree were recorded. Larval instar was determined by head capsule width measurement (Quintela et al. 1998). All healthy larvae exhibiting normal behavior were recorded as ‘live’. Live larvae exhibiting abnormal behavior and dead larvae were placed in a disposable Petri dish (50 × 9 mm) on moistened filter paper. Cadavers were examined microscopically every other day for 7 d to detect characteristic signs indicative of bacterial, fungal, or nematode infection (Lacey & Kaya 1999).

### Weather Monitoring

Soil temperature and moisture were continuously monitored at 15 and 30 cm depths using
paired thermistors and calcium block transducers set to record hourly readings. Rainfall (amount and duration) was measured using a tipping-bucket rain gauge linked to a day recorder. Free water applied via irrigation was also manually recorded. Ambient air temperature and relative humidity were recorded using a weekly chart hygrothermograph. Weekly data were integrated and stored in a T21X micrologger (Campbell Scientific Inc., Logan, Utah).

Statistical Analysis

SAS System for Windows, release 6.11 was used for analysis (SAS Institute Inc. 1990). Proportions of adults captured in the two trap types were compared with contingency table analysis and $\chi^2$ tests using PROC FREQ, SAS System for Windows 6.11 (SAS Institute Inc. 1990). Correlation analysis used PROC CORR.

RESULTS

Adult Weevil Emergence and Abundance

Cone-shaped ground traps, designed specifically to catch emerging adults, captured 385 weevils from mid-July through the end of October in the non-irrigated north planting in 2000. The same type traps caught 428 weevils from mid-May through the end of November in the irrigated south planting in 2001. Peak adult emergence occurred in mid-June in the non-irrigated planting and at approximately the same time the following year in the irrigated planting (Figs. 1A and B). Adult emergence in the non-irrigated planting was closely-related to soil moisture (Fig. 1A). When soil water potential at a depth of 15-30 cm increased to 3-5 centibars emergence began shortly thereafter and continued throughout the summer. In late September, soil temperature began to decline and adult emergence dropped off, even though soil moisture remained at or below 10 centibars (Fig. 1A). Low levels of adult emergence did occur in the early spring when soil temperatures were 2-4EC lower than during the mid-summer period. Adult emergence in the irrigated planting in 2001 was lower in magnitude, but more continuous throughout the year (Fig. 1B). Soil water potential of <4 centibars combined with soil temperatures ranging from 22-24EC in the summer appeared to favor a longer emergence period in the irrigated grove (Fig. 1B) compared to the non-irrigated grove (Fig. 1A).

Tedders traps, designed to catch both emerging adults and those attracted from the surrounding area, captured 3113 weevils from mid-March to mid-December in the non-irrigated planting in 2000. The same type of trap caught 7337 in the irrigated planting in 2001. Although a greater number of adults were caught in Tedders traps, both traps detected a strong late spring emergence in both years (Figs. 1 and 2). However, only Tedders traps detected a late season peak in adult abundance in September and October that exceeded the spring peak in magnitude (Figs. 2A and C).

Although there was a significant correlation between the number of adults caught using the 2 trap types when all year 2000 data was pooled ($r = 0.6682, df = 39, P = 0.0001$), this relationship disappeared during the active emergence period (22 June through 12 October) when >90% of adult captures were recorded ($r = 0.2682, df = 15, P = 0.2979$). In 2001 in the irrigated planting, there was no significant correlation between catches from the two trap types even when all data were pooled ($r = 0.2305, df = 38, P = 0.1524$). By comparison, total weevils captured in cone traps were 428, 1297, and 100, respectively, in irrigated, non-irrigated citrus and Brazilian-pepper, respectively, in the south planting in 2001. The seasonality of adult emergence under the three conditions was quite similar when percent cumulative emergence was compared from mid-March to late August (Fig. 3A). However, trap counts for certain sample dates were significantly different for percent cumulative emergence ($P < 0.05$) between irrigated citrus and non-irrigated citrus based on contingency table analysis and the $\chi^2$ test. There was no difference between non-irrigated citrus and Brazilian-pepper (Fig. 3A). Exceeding cumulative adult emergence increased from 29 August to mid-November in both irrigated citrus and Brazilian-pepper plantings with no significant difference between sites (Fig. 3B). Sampling was terminated in August in the non-irrigated citrus planting to prevent further water stress to the trees.

Egg Mass Abundance

A total of 811 egg masses of *D. abbreviatus* was detected on leaves in 2001 in the irrigated planting from 13 June through 11 December with a peak in mid-Sept (Fig. 2D). The seasonal pattern for the number of egg masses recovered during weekly monitoring was similar to the number of adults caught in modified Tedders traps (Figs. 2C and D). The correlation between egg mass number and adult number as indicated by modified Tedders trap counts was highly significant ($r = 0.7885, df = 38, P = 0.0001$).

Neonate Abundance

In 2000 in the non-irrigated planting, 2408 neonates were recovered from modified pitfall traps compared to 4841 in the irrigated planting in 2001. Neonate drop was detected from 12 June through 18 December, 2000 in the non-irrigated planting and 28 May through 31 December 2001 in the irrigated planting (Figs. 2B and E). Neo
Fig. 1. Weekly records of soil moisture, soil temperatures, and numbers of adult weevils captured in cone traps: (A) non-irrigated citrus planting, 2000, and (B) the irrigated citrus planting, 2001.
nate drop was significantly correlated with numbers of adults caught in Tedders traps in both years (year 2000, \( r = 0.3971, df = 32, P = 0.0201 \); year 2001, \( r = 0.8467, df = 32, P = 0.0001 \)) but neither egg mass nor neonate abundance were correlated with numbers of adults caught in cone traps (neonates vs. cone traps, year 2000, \( r = 0.0143, df = 32, P = 0.9360 \); year 2001, \( r = -0.0884, df = 32, P = 0.6190 \); and egg masses vs. cone traps, \( r = 0.0462, df = 38, P = 0.7769 \)).

Abundance of Subterranean Life Stages

In the non-irrigated citrus planting in 2000, 1253 life stages of D. abbreviatus were recovered from 0.59 m$^3$ of sieved soil collected from the tree rhizosphere of each of 32 trees. In the irrigated planting in 2001, 785 life stages were recovered from 28 trees using the same procedures. Many fourth instar larvae and younger stages probably escaped detection. No diseased or parasitized larvae were found among live and dead larvae held in the laboratory for symptom expression. All detectable larval instars were present in the soil rhizosphere throughout most of the year, but the proportion of the population represented by different life stages often changed seasonally (Figs. 4 and 5). Pupae and teneral adult recovery was more erratic.

In the non-irrigated citrus planting in 2000, the only instar that failed to show significant change in proportional representation during the year was the eighth instar (\( n = 319 \)) whereas, in the irrigated planting in 2001, the only instar failing to
show this change was the eleventh instar (n = 17; contingency table analysis, P > 0.05; Fig. 5). The scarcity of tenth and eleventh instars during the emergence period suggest an earlier onset to pupation. Pupae and adults showed strong seasonal peaks in abundance that correlated to peak adult emergence as monitored with ground traps. Moreover, in 2001 in the irrigated planting, ninth instars were extremely rare in October, whereas fourth to eighth instars were unusually abundant at that time.

**DISCUSSION**

Comparison of data from both irrigated and non-irrigated studies suggest that the onset and magnitude of adult emergence of *D. abbreviatus* can be delayed by soil moisture deficit. This response to moisture can be related to more frequent rainfall (Figs. 1A and B) or free water via irrigation supplemented with rainfall (Fig. 3). A positive effect of soil moisture on weevil emergence has been suggested in the literature from the Caribbean (McCoy 1999) and Florida (Beavers & Selhime 1975). In the laboratory, Lapointe & Shapiro (1999) found that soil moisture ranging from 20-80% affected larval survival and pupation. Our results represent the first field study where soil moisture was linked to seasonal adult emergence. The effect of soil moisture on adult weevil emergence is well-documented for the pecan weevil, *Curculio caryae* (Horn) (Harris & Ring 1980). Generally, pecan weevils emerge in large numbers after rainfall. Furthermore, Harris & Ring (1980) found that drought hardened clay soil impeded weevil emergence but irrigation allowed normal emergence. In addition, they found that weevil emergence can be postponed by withholding moisture, but cannot be accelerated by the addition of moisture before the normal emergence window.

In many citrus groves located in central and east coastal areas of Florida, one annual peak of adult abundance has been detected using Tedders traps (Stansly et al. 1997, Duncan et al. 2001), single peak population density generally occurred typically in the May/June period. In the non-irrigated and irrigated citrus plantings in 2000 and 2001 respectively, adult abundance estimated using Tedders traps demonstrated as two distinct seasonal peaks, one in late spring and another in late summer, the latter being the greatest. These data suggest that the fall peak cannot be explained by adult emergence. Since adult weevils are relatively long-lived in the field, at least some of the late-summer peak in adult abundance might be due to an accumulation of older weevils. However, this trend has not occurred in other locations where adult abundance was monitored with Tedders traps (Stansly et al. 1997, Duncan et al. 2001). A more likely explanation for the fall peak is adult migration from alternate host plants. As shown in Fig. 3, adults can emerge con-
continuously from alternate hosts such as Brazilian-pepper throughout the summer.

As previously mentioned, the Tedders trap is widely used in scientific research to trap adult weevils (Duncan et al. 2001). When placed beneath the tree midway between the canopy margin and the tree trunk, Tedders traps are effective for measuring changes in adult citrus root weevil abundance within a season, and the onset of adult emergence from the soil (Stansly et al. 1997, Duncan et al. 2001, Nigg et al. 2001). These data show conclusively a close association of adult trap counts, egg mass counts, and neonate counts substantiating the conclusions of the above authors. Tedders traps, therefore, provide useful estimates, not only of adult number, but also reproductive activity in the field.

Entomopathogenic fungi and nematodes can reduce weevil populations in the soil under natural conditions; however, their spatial distribution and persistence in the soil is variable (McCoy & Duncan 2000). In independent studies conducted in both the north and south citrus plantings to determine the field efficacy of commercial preparations of entomopathogenic nematodes against Diaprepes larvae in the soil, no control was obtained. In addition, only a few larvae recovered from the soil were parasitized by native nematodes and no microbe infections were diagnosed in the laboratory (McCoy et al. 2002). These findings agree with the present data that indicate no soil-inhabiting natural enemies were found in wild larvae collected from the rhizosphere of extracted trees. The alfisol soil type found in these citrus plantings has fine particle size and may be antagonistic to these natural enemies which, in turn, might partially explain why overall weevil densities were exceptionally high and tree injury so severe in both plantings.

Our data on the number of neonates dropping from trees into funnel traps compared to the number of adult weevils being captured under trees in cone-shaped emergence traps suggests that mortality during this part of the life cycle might often exceed 98%. In 2000, in the non-irrigated grove, the number of dropping neonates averaged 376.25 per m$^2$; whereas the number of emerging adults averaged 6.05 per m$^2$, for a survival rate of 1.61%. In 2001, in the irrigated grove, the number of dropping neonates averaged 945.51 per m$^2$; whereas the number of emerging adults averaged 6.73 per m$^2$, for an average survival rate of 0.71%.

Our data on the various developmental stages of D. abbreviatus have important practical implications for timing the application of various control agents used as foliar or soil treatments. Since prolonged dry weather can delay adult emergence and, conversely, extended periods of high soil moisture can extend emergence, seasonal adult emergence can be highly variable from year to year. It would appear that monitoring with ground traps or Tedders traps is worthwhile. Since trapping is not grower friendly, a weather related model might be of benefit in timing foliar treatments to control emerging adults as they enter the tree canopy. If adult emergence is prolonged by favorable soil conditions, the benefits of foliar sprays to control adults will be lost because of their generally short residual activity and the possible negative side effects of multiple applications. Future control strategies might be more effective if they could target the teneral adult stage in the soil.

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**Fig. 5.** Statistical comparison of the percentage of the population of each weevil life stage recovered in time from the soil rhizosphere in non-irrigated (2000) and irrigated (2001) citrus plantings. For each life stage, bars with common letters are not significantly different at the $P = 0.05$ level based on contingency table analysis and $\chi^2$ test.
Peak neonate drop to the soil occurred from mid-July through October in both citrus plantings (Fig. 2), suggesting that soil barrier treatments might be more effective when applied at this time of the year (McCoy & Duncan 2000). As is true for foliar sprays for adult control, the residual effect of a soil barrier treatment with chemicals is too short to assure prolonged efficacy, suggesting the need for multiple applications during the period of highest neonate drop.

A broad range of larval instars occur in the tree rhizosphere throughout the year. The fact that all larval stages are present year round with overlapping generations presents enormous problems when attempting chemical control since susceptibility and appropriate dosage vary with larval age. Although entomopathogenic nematodes also exhibit differential larval susceptibility to Diaprepes, they will infect virtually all instars (Shapiro et al. 1999) at levels unachievable by chemicals.

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