Abundance of *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) Neonates Falling to the Soil Under Tree Canopies in Florida Citrus Groves

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ABSTRACT The purpose of these experiments was to estimate the number and distribution of *Diaprepes abbreviatus* (L.) neonate larvae dropping from the canopy of infested citrus trees. The number of neonates was monitored in the field using passive funnel traps in two simultaneous experiments and a separate experiment for an additional year. In one experiment, traps were placed from trunk to dripline in the cardinal directions under each of five trees (132 traps total). In a second experiment, eight traps were placed under each tree in the cardinal directions, one trap 30 cm from the trunk and one trap 30 cm from the dripline/direction for 25 trees (200 traps total). Larvae were collected weekly for 50 wk in conical tubes containing ethylene glycol as a preservative. Traps closer to the tree trunk captured more larvae than traps nearer the dripline. The area under the tree canopy was positively correlated with the total estimated number of larvae captured per tree. The estimated number of total larvae/tree over the course of our experiments ranged from 955 to 7,290. The highest number of neonate larvae observed in 1 wk was $67 \pm 6/m^2$. There was an inverse relationship between the number of traps beneath a tree and the number of trees that needed to be sampled to estimate mean population density with a given precision. However, there was a direct relationship between number of traps/tree and the total number of traps needed for a given precision. This passive technique could be used to quantify the destructive larval stage and to assess *D. abbreviatus* management strategies.

KEY WORDS larval monitoring, funnel trap, root weevil

THE DIAPREPES ROOT WEEVIL, *Diaprepes abbreviatus* (L.) is an insidious pest of citrus, sugarcane, and economic crops of the tropics and subtropics (Simpson et al. 1996). Originally described from the Caribbean, this weevil was first discovered in Florida in 1964, and has now spread to 22 Florida counties and at least 94 plant nurseries (citrus and ornamental). This area includes ≈72,727 ha (160,000 acres) of which 22,727 ha (50,000 acres) are citrus (Hall 1995, Simpson et al. 1996).

Male and female adult weevils feed on the young leaves of citrus. Eggs are glued in a mass between two leaves (generally mature leaves) (Fennah 1942). After hatching, the neonate larvae drop to the ground, feed on the roots, and emerge as adults 6 mo to 2 yr later (Fennah 1942, Griffith 1975). Root damage can be severe and, therefore, the larva is the economically important stage of *D. abbreviatus* (Fennah 1942, Woodruff 1968, Beavers et al. 1979, Hall 1995, Anonymous 1997). Larvae of *D. abbreviatus* have been found feeding on >40 plant species in 20 plant families (Simpson et al. 1996).

To prevent movement of *D. abbreviatus* larvae from infested nurseries, a quarantine on untreated plants was imposed on *D. abbreviatus*-infested nurseries in 1968 and remains in effect today. Heptachlor and dieldrin were the first insecticides used for nursery soil incorporation to control *D. abbreviatus.* Currently, Bifenthrin (Talstar) is required to be incorporated at 25 ppm into the potting soil of *D. abbreviatus*-infested nurseries to control larvae. Wide area treatment of commercial citrus with heptachlor, dieldrin and chlordane was a quarantine treatment from 1968 to 1979, but this program failed to contain this weevil. There are no integrated pest management strategies to control this weevil (McCoy and Simpson 1994).

One of the proposed integrated pest management strategies to manage this weevil is the use of resistant citrus rootstocks that would permit continued citrus production in *D. abbreviatus*-infested areas (Beavers and Hutchison 1985). Experiments with different citrus rootstock seedlings have suggested that some citrus rootstocks are differentially sensitive to *D. abbreviatus* larval feeding (Beavers and Hutchison 1985, Shapiro and Gottwald 1995, Nigg et al. 1999a, 2001a,

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Table 1. *Diaprepes abbreviatus* **larval inoculation rates in rootstock resistance studies**

Reference	Surface area	Larval inoculation	Experimental time	Total larvae/m^2
Schroeder et al. 1979	176.7 cm^2	10 to 50/d	14 d	565–2825
Beavers and Hutchison 1985	176.7 cm^2	100—one time	56 d	5659
McCoy et al. 1995	56.7 cm^2	20—one time	7 d	3526
McCoy et al. 1995	324.1 cm^2	10—2 times	105 d	618
McCoy et al. 1995	1551.5 cm^2	20—5 times	70 d	645
Shapiro et al. 1997	346.2 cm^2	10—one time	35 + 45 d	289
Shapiro and Gottwald 1995	346.2 cm^2	10—one time	44 d	289
Grosser and McCoy 1996	284.9 cm^2	15—one time	45 d	526
Rogers et al. 1996	5.98 cm^2	3—one time	35 d	5017
Rogers et al. 1996	5.98 cm^2	5—one time	35 d	8361
Lapointe et al. 1999	201.0 cm^2	10—one time	35 d	498
Lapointe et al. 1999	12.6 cm^2	1—one time	30 d	794
Lapointe et al. 1999	12.6 cm^2	2—one time	30 d	1587
Lapointe et al. 1999	12.6 cm^2	4—one time	30 d	3175
Nigg et al. 1999b	176.7 cm^2	10—3 times	90 d	1698
Nigg et al. 2001b	176.7 cm^2	10—24 times	720 d	13,560
Nigg et al. 2001c	176.7 cm^2	20—6 times	168 d	6791
This study 2002	79,000 cm^2	at least weekly in season	350 d	402 ± 31

2001b). However, there are no field data to guide the numbers of larvae to use in greenhouse experiments; numbers have varied widely (Table 1). Despite the importance of larval damage, estimates of numbers of neonate larvae dropping from the canopy in the field are not available. The purposes of these experiments were to estimate the abundance and distribution of *D. abbreviatus* neonate larvae dropping from the canopy of infested Florida citrus trees, and to establish a correlation between neonate and adult density.

Materials and Methods

Field Site. Three field trials were conducted near Poinciana, FL (Osceola County) in a declining mature planting of 'Hamlin' oranges (*Citrus sinensis* (L.) Osbeck) grafted to *Swingle citrumelo* rootstock (X *Citroncirus* 'Swingle'). The grove was planted on two-row beds with a spacing of 6.1 × 8.5 m and was equipped with under-tree micro emitter irrigation. The outer edge of the tree canopy was ≈0.75 m above the ground on all experimental trees. The spodosol soil type for the grove was classified as Delray loamy fine sand; the surface layer ≈35.6-cm loam and the subsurface layer ≈76.2-cm gray fine sand. The spodosol soil was poorly drained with a low to moderate organic content and a pH of 4.8. The trials were within 40 m of one another. Leaf feeding by adult *D. abbreviatus* was evident on trees throughout the grove. No pest control was applied to this site.

Experimental Design. Although adult abundance can be monitored by beating into an umbrella (Nigg et al. 1999a, 1999b, 2001a), we passively monitored adults to avoid any disturbance which could affect oviposition. We visually inspected each tree in the experiment from 3 to 5 min to determine the total adult weevils/tree on each sampling day. For larval monitoring we used a modified pitfall trap (Fig. 1) (Southwood 1978). Plastic funnels (20.3 cm diameter) were placed under the canopy in the cardinal direc-

Fig. 1. (A) Traps installed under a citrus tree. (B) Trap detail.

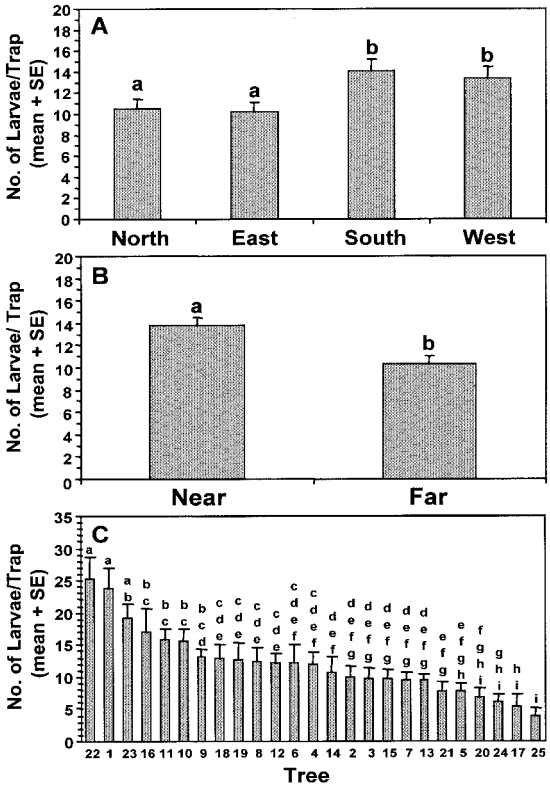

Fig. 2. Results of the 25-tree experiment indicating that more larvae were captured in the south and west quadrants under the tree canopy than in the north and east (A), that more larvae were captured closer to the trunk than closer to drip line (B), and that there were significant differences in the number of larvae captured per tree (C). Analysis involved a 3-factor ANOVA with mean separations using LSD. Bars with common letters were not significantly different at the $P = 0.05$ level.

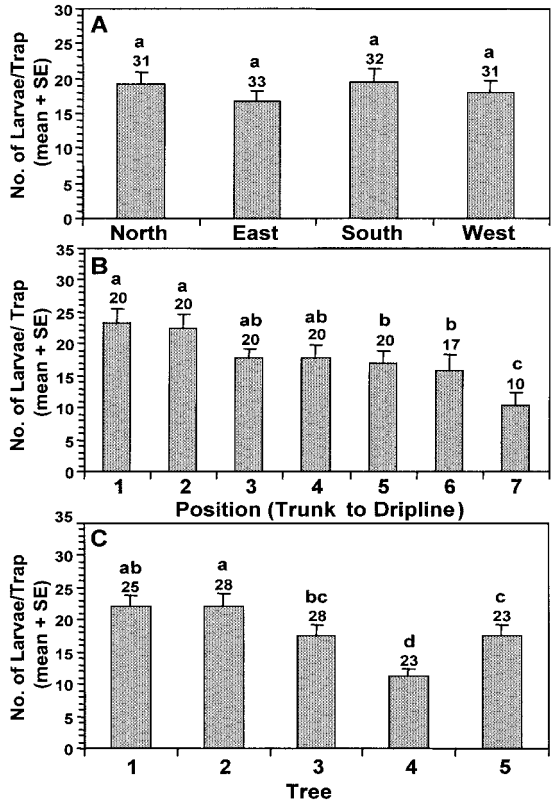

Fig. 3. Results of the 5-tree experiment indicating no significant differences in the number of larvae captured in different quadrants around trees, (A [$P = 0.8534$]), that more larvae were captured near to the trunk than farther away (B [$P = 0.0001$]), and that there were significant differences in the number of larvae captured per tree (C [$P = 0.0001$]). Analysis involved a 3-factor ANOVA with mean separations using LSD. Bars with common letters were not significantly different at the $P = 0.05$ level. Numbers above the bars are number of samples.

tions (north, east, south, and west). A 50-ml screwed capped conical tube containing 5 ml of antifreeze as a preservative (ethylene glycol/diethylene glycol, Super Tech, Alsip Pkg. Inc., Alsip, IL) was attached with duct-tape to the bottom of each funnel. Each tube with funnel attached was inserted into a 3.1 × 50-cm PVC pipe that was inserted vertically into the soil so that the outside edges of the funnel were 30 cm above the ground (Fig. 1). Each tube and PVC pipe was numbered. Tubes were removed, capped, and new tubes were installed once each week.

One experiment, termed the 5-tree experiment, consisted of five randomly chosen trees located two beds apart from one another. Funnel traps were placed from the trunk to the edge of the canopy dripline to form a continuous row in each of the cardinal directions. There was a total of 132 funnels for the 5-tree experiment (23–30/tree dependent on trunk to dripline distance). These trees were monitored from 1 May 2000 to 16 April 2001 (50 wk).

A second experiment consisted of 25 trees; termed the 25-tree experiment. Eight funnels were placed beneath each tree as described above. For each cardinal direction, one funnel was placed 30 cm from the trunk, and the second funnel was placed 30 cm inside the dripline. The tubes for this experiment were removed and replaced, and adults were monitored with the same methodology as the 5-tree experiment. The 25-tree experiment was monitored from 12 June 2000 to 16 April 2001 (44 wk).

A third, 20-tree experiment ($n = 20$), termed the 20-tree experiment, was conducted from 1 May 2001 to 30 April 2002. This experiment used eight funnel traps/tree (as per the 25-tree experiment), 20 trees, and was conducted in a separate area of the grove from the 5-tree and 25-tree experiments. This experiment was conducted to provide additional adult abundance data and to confirm our observations of the previous year. Adult and larval numbers were monitored as described above.

Weather Monitoring. The amount and duration of rainfall was monitored with a tipping bucket rain gauge and a day recorder (Weathertronics model

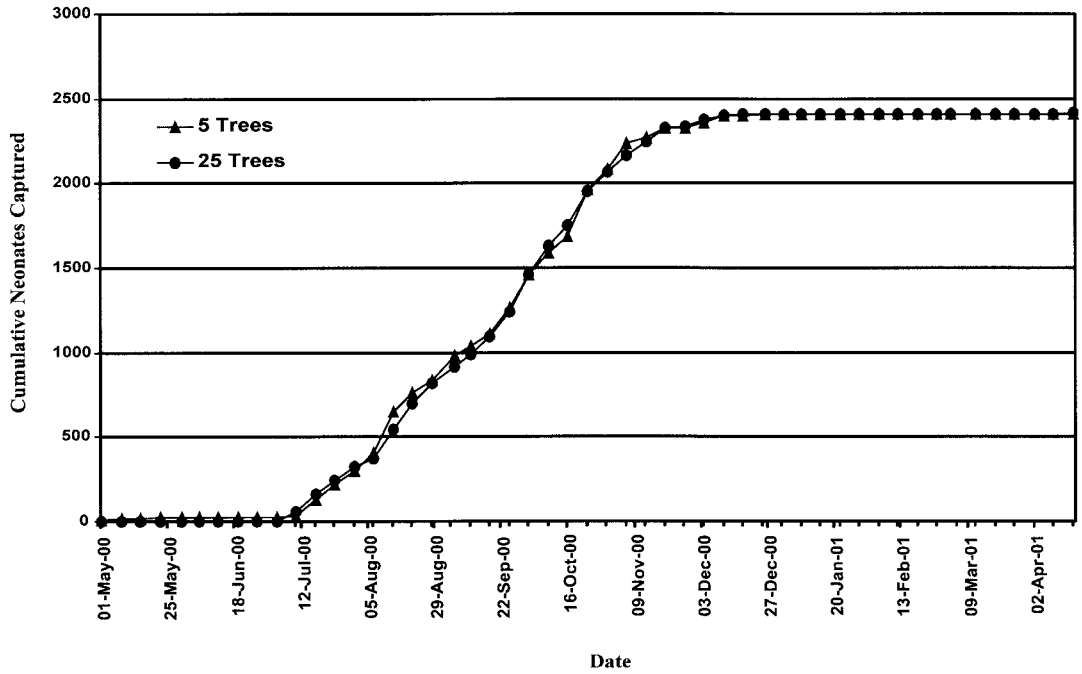

Fig. 4. Cumulative larval capture from five trees with funnels installed from trunk to dripline, 132 funnels total. ▲ Sampling began 1 May. Total capture = 2407. No larvae captured after 26 December 2000. Cumulative actual larval capture from 25 citrus trees with eight traps installed per tree, 200 funnels total. ● Sampling began 12 June. Total capture = 2,416.

6110, Weathertronics Inc., Raleigh, NC). Ambient air temperature and relative humidity were recorded using a weekly chart hygrothermograph (Bendix model 594, Bendix, Inc., Baltimore, MD).

Data and Statistical Analyses. Each tube was emptied in aliquots, and each aliquot was inspected for *D. abbreviatus* larvae under a binocular microscope. Each larva was identified as a *D. abbreviatus* larva by head suture pattern and to larval instar by head capsule measurement (Quintela et al. 1998).

Data from the 5-tree study were analyzed using 3-factor analysis of variance (ANOVA; PROC GLM, SAS Institute 2000) in which there were four levels for direction (north, east, south, and west), seven levels for distance (numbered from trunk to dripline), and five levels for trees. Trees were used as a blocking factor ($n = 5$). Data were square root transformed before analysis. Data from five traps were omitted from the analysis because they represented traps farthest from the trunks of the largest trees (i.e., trap positions 8 and 9) and were not well replicated among trees for direction and position categories.

Data from the 25-tree and 20-tree studies were analyzed using 3-factor ANOVA (PROC ANOVA, SAS Institute 2000) in which there were four levels for direction (north, east, south, and west), two levels for distance (near and far), and 25 or 20 levels for trees. Trees were used as a blocking factor ($n = 20$ or 25). Data were square root transformed before analysis. Means separation was with Fishers least significant difference (LSD) test (SAS Institute 2000).

The tree canopy was approximated as being a half prolate spheroid above a cylinder, and the volume of the tree canopy was calculated using the formula: canopy volume = $\pi R^2(2X/3 + Y)$, where X = HT – HD and Y = HD – HS with R = half the tree diameter at the widest point, HT = the overall tree height, HS = the skirt height, and HD = the height from the ground to the widest point of the tree. The total number of neonates/tree was calculated as the total sample catch from all traps under a tree divided by the area sampled (i.e., the total funnel area) multiplied by the total area under the canopy. The total area under the canopy was calculated as a circle (πR^2), where R was half the average of four measurements of the diameter of the canopy dripline made at various compass points. The correlations between canopy measurements, area under the canopy and larval numbers were performed with SAS PROC CORR (SAS Institute 2000).

Analyses of Minimum Sample Sizes for Population Estimates. Data from the 25-tree experiment were fitted to Taylor's Power Law (TPL; $s^2 = a(\bar{x})^b$) to estimate the relationship between the variance (s^2) and mean (x) across different intervals of time (Taylor 1961, Duncan et al. 2001). The means and variances for numbers of larvae/funnel/tree/wk and numbers of larvae/funnel/tree/mo were determined for cases in which eight, four, two, or one funnels were used per tree. Data from funnels at the south and west quadrants were used for the case of four funnels; only the near funnels in the south and west quadrants were used for the two-funnel case; and data from the near

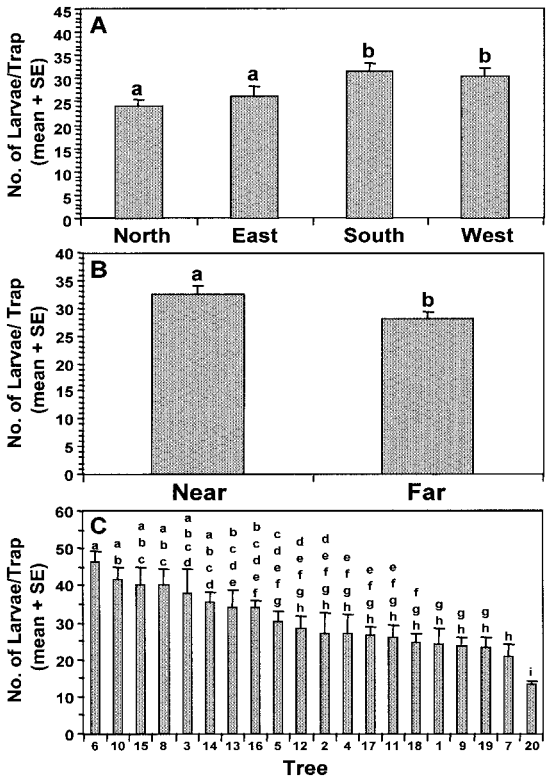

Fig. 5. Results of the 20-tree experiment indicating that more larvae were captured in the south and west quadrants around a tree than in the north and east (A [$P = 0.0016$]), that more larvae were captured near to the trunk than farther away (B [$P = 0.0034$]), and that there were significant differences in the number of larvae captured per tree (C [$P = 0.0001$]). Analysis involved a 3-factor ANOVA with mean separations using LSD. Bars with common letters were not significantly different at the $P = 0.05$ level.

west funnel was used for the one-funnel case. Log_n variance was regressed on $\log_n$ mean to derive parameter estimates of Taylor's Power Law. The number of trees and number of funnels/tree needed to estimate the mean population density with a confidence interval half-length equal to 50% of the mean ($P = 0.05$) was estimated from the formula

$$n = (z^2_{\alpha/2} a \bar{x}^{b-2}) / (0.5CI/\bar{x})^2$$

where n = sample size, z = the standard normal variate (1.96, $P = 0.05$), a and b are TPL parameters, and CI = confidence interval (Duncan et al. 2001). These data were used to design the third experiment. Graphing was with Sigma Plot (SPSS 2000).

Results and Discussion

In the 25-tree study, there was a total of 6,800 individual funnel trap samples. In these trap samples there were 2,408 neonates trapped. The number of neonates caught per trap ranged from 0 to 37 (mean = 12.04, SE = 0.533). Only four traps captured no neonates in the 5- and 25-tree experiments.

The ANOVA for the 25-tree study produced a highly significant result ($F = 7.19$; df = 31, 168; $P = 0.0001$). The results for all three main effects were highly significant (direction, $F = 7.73$; df = 3, 168; $P = 0.0001$; distance, $F = 23.32$; df = 1, 168; $P = 0.0001$; tree, $F = 7.23$; df = 24, 168; $P = 0.0001$). The interaction between direction and distance was not significant ($F = 0.92$; df = 3, 168; $P = 0.4313$). Mean separation using Fisher LSD indicated that trap counts were significantly higher in the south and west quadrants than in the north and east quadrants (Fig. 2A), and significantly higher near the trunk than farther away (Fig. 2B). The number of neonates captured per tree was highly variable and differed significantly among some trees (Fig. 2C).

For the 5-tree study, there was a total of 5,380 individual funnel trap samples. A total of 2,407 neonates were trapped. The number of neonates captured per trap ranged from 3 to 45 (mean = 18.23, SE = 0.819).

The ANOVA for the 5-tree study produced a highly significant result ($F = 2.65$; df = 31, 95; $P = 0.0002$). In contrast to the 25-tree study, the result for the main effect of direction was not significant ($F = 0.26$; df = 3, 95; $P = 0.8534$), but the results for the main effects of distance and tree were highly significant (distance, $F = 5.46$; df = 6, 95; $P = 0.0001$; tree, $F = 9.41$; df = 4, 95; $P = 0.0001$). The interaction between direction and distance was not significant ($F = 0.78$; df = 18, 95; $P = 0.7226$). Means for trap counts in the various directions are shown in Fig. 3A. Mean separation using LSD indicated that trap counts were higher near the trunk than farther away from the trunk (Fig. 3B), possibly because *D. abbreviatus* females oviposit on older leaves and older leaves are deeper in the tree canopy. As for the 25-tree study, the number of neonates captured per tree was highly variable and differed significantly among some trees (Fig. 3C).

There was no significant correlation between canopy volume and either the mean number of neonates/trap for each tree ($r = 0.1740$, df = 28, $P = 0.3577$) or for the total estimated number of neonates captured per tree ($r = 0.3344$, df = 28, $P = 0.0709$) when data from the 5- and 25-tree studies were pooled. There was also no significant correlation between the area under the canopy and the mean number of neonates/trap for each tree ($r = 0.3142$, df = 28, $P = 0.0909$), but there was a significant correlation between the area under the canopy and the total estimated number of neonates captured per tree ($r = 0.5624$, df = 28, $P = 0.0012$).

The number of neonates/square meter ranged from 124 to 783 for an estimated total range of 955–7,290 neonates/tree over the 30 trees in the 5- and 25-tree experiments. The larval capture pattern of the 5- and 25-tree experiments was identical (Fig. 4). These 30 trees had an average canopy volume of 15 ± 1 m^3, an average area under the dripline of 7.9 ± 0.3 m^2, an average estimated total catch of $3{,}240 \pm 302$ larvae and an average total larval catch of 402 ± 31/m^2. Using the highest neonate catch/tree in any week, yielded an average of 67 ± 6 (SE) larvae/square meter in that

Table 2. Estimated parameters for Taylor's Power Law derived from numbers of *D. abbreviatus* larvae caught in funnel traps during periods of 1 wk and 1 mo

Traps tree^{-1}	Interval	Taylor's Power Law parameter				r^2	*P*
		a	SE(A)*	b	SE (*b*)		
1	Week	1.95228	0.077	1.33	0.063	0.94	0.0001
2	Week	1.19961	0.064	1.33	0.052	0.96	0.0001
4	Week	0.69143	0.068	1.30	0.045	0.97	0.0001
8	Week	0.47855	0.085	1.32	0.048	0.96	0.0001
1	Month	0.60835	0.182	1.23	0.162	0.92	0.0010
8	Month	0.17552	0.197	1.28	0.077	0.98	0.0001

* SE(A) = standard error of intercept ($\log_n a$); SE (*b*) = standard error of the slope.

week, which extrapolates to 268 larvae/m^2 in a 4-wk month.

In the 20-tree study, a total of 4,841 neonates were trapped. The number of neonates caught per trap ranged from 9 to 63 (mean = 30.26, SE = 0.997). The ANOVA produced a highly significant result ($F = 5.53$; df = 26, 133; $P = 0.0001$). The results for all three main effects were highly significant (direction, $F = 5.36$; df = 3, 133; $P = 0.0016$; distance, $F = 8.90$; df = 1, 133; $P = 0.0034$; tree, $F = 6.21$; df = 19, 133; $P = 0.0001$). The interaction between direction and distance was not significant ($F = 0.29$; df = 3, 133; $P = 0.8344$). Mean separation using LSD indicated that trap counts were significantly higher in the south and west quadrants than in the north and east quadrants (Fig. 5A), and significantly higher near the trunk than farther away (Fig. 5B). The number of neonates captured per tree was highly variable and differed significantly among some trees (Fig. 5C). These data mirror the 25-tree experiment data.

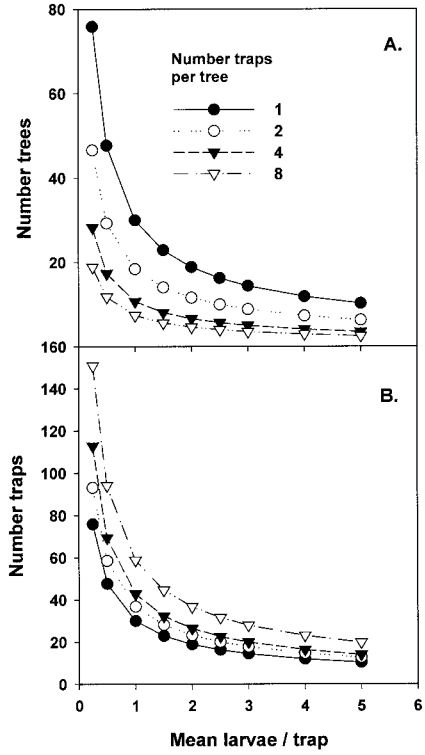

Fig. 6. Minimum sample sizes (A = number trees and B = number traps) needed using various numbers of traps/tree to estimate numbers of larvae/trap/tree/wk with a confidence interval half-length equal to 50% of the mean ($P = 0.05$) as estimated from the formula $n = (z^2\ a\bar{c}^{b-2})/(0.5\ CI/\bar{x})^2$ where n = minimum sample size, z = the standard normal variate (1.96, $P = 0.05$), *a* and *b* are TPL parameters (Table 1), and CI = confidence interval.

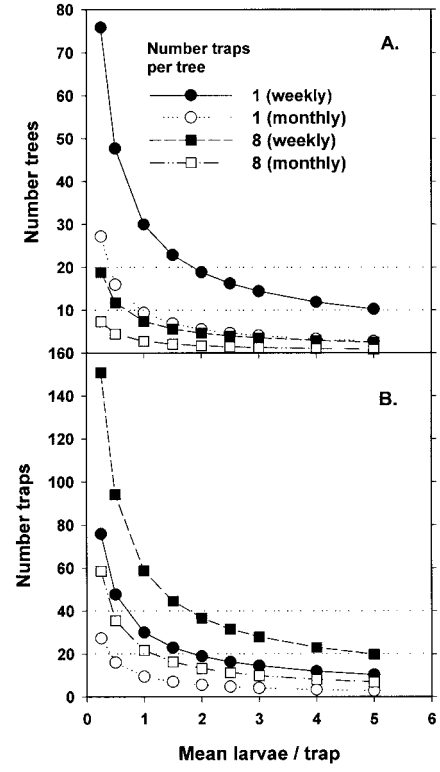

Fig. 7. Comparison of minimum sample sizes (A = number trees and B = number traps) needed using various numbers of traps/tree to estimate numbers of larvae/trap/tree/wk or per month with a confidence interval half-length equal to 50% of the mean ($P = 0.05$) as estimated from the formula $n = (z^2\ a\bar{x}^{b-2})/(0.5\ CI/\bar{x})^2$ where n = minimum sample size, z = the standard normal variate (1.96, $P = 0.05$), *a* and *b* are TPL parameters (Table 2), and CI = confidence interval.

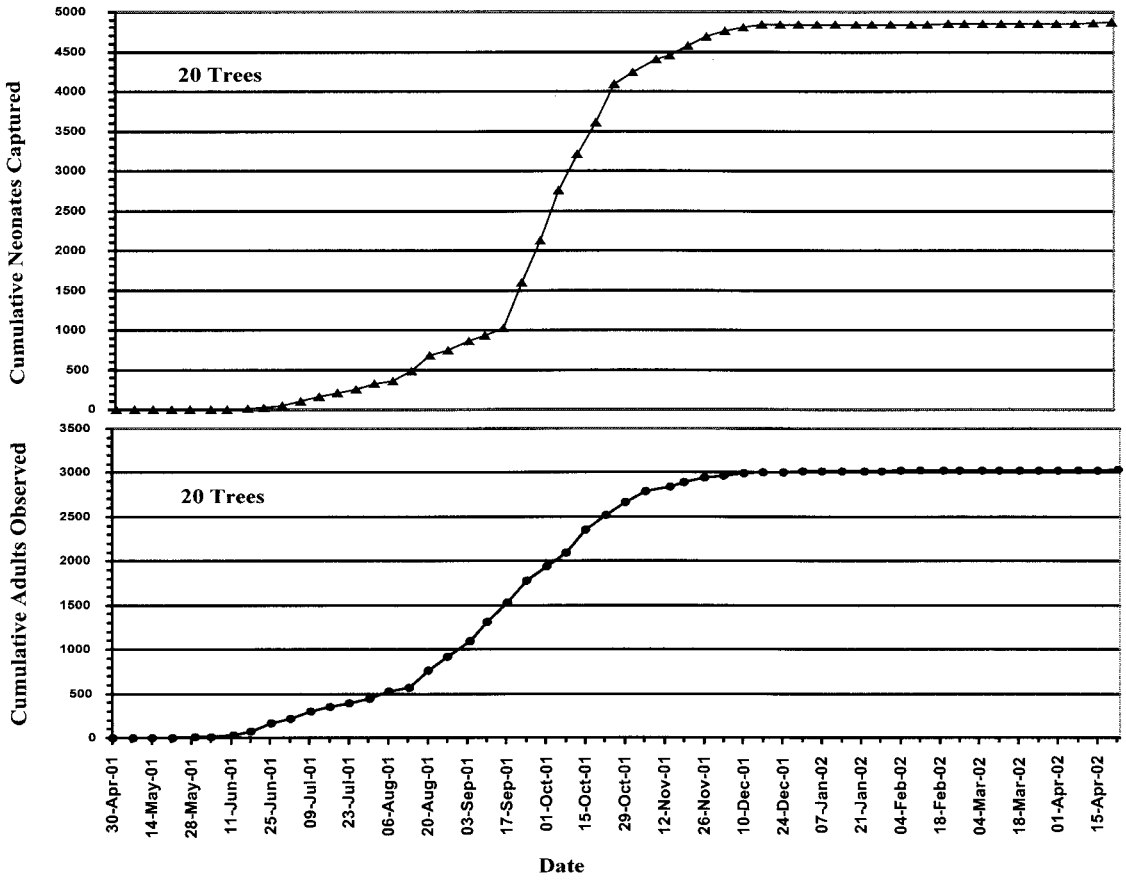

Fig. 8. Cumulative adults observed versus cumulative neonates captured in the 20-tree experiment.

Estimating Sample Size. Estimates of the parameter *b* from Taylor's Power Law were very similar (range, 1.30–1.33) for cases in which number of funnels/tree ranged from one to eight, whereas the parameter *a* varied inversely with funnel numbers (Table 2). Estimates of the parameter *b* are congruent with that estimated for adult *D. abbreviatus* (1.33) collected using modified Tedders traps (Duncan et al. 2001).

Numbers of trees required for population measurement at a specified precision were inversely related to the numbers of funnels/tree for all weevil densities (Fig. 6A). At the lowest mean population density considered (0.25 weevils/funnel/tree/wk), 76 trees with one funnel/tree were estimated to be adequate to achieve a confidence interval half-length: mean ratio ≈0.5, whereas 19 trees were required for the case of eight funnels/tree. However, the total number funnels needed for each case was directly related to the number of funnels used per tree (Fig. 6B). At the lowest population density, 76 funnels were adequate if one funnel was used per tree, compared with 152 funnels for the case of eight funnels/tree.

Fewer trees and funnels were required to measure means on a monthly basis, rather than weekly (Fig. 7A–B, Table 1). At low population density (0.25 weevils/funnel/tree), 28 trees and funnels were needed for the case of a single funnel/tree, and eight trees and 64 funnels were needed when eight funnels/tree were used.

In greenhouse studies, we have used 10 or 20 neonate larvae/inoculation in 15-cm diameter pots to assess *D. abbreviatus* pesticide treatments and rootstock resistance. Based on a 176.7-cm^2 area/pot, greenhouse larval pressure was 566 or 1,133 larvae/m^2, which is approximately two to four times the maximum larval catch/wk that we measured in the field in the current study (Nigg et al. 1999b, 2001a, 2001b). A different perspective of this difference is illustrated by the average total of 402 larvae/m^2 for our field study versus 1,698–13,560 larvae/m^2 total in our greenhouse assessments (Nigg et al. 1999b, 2001a, 2001b). Other experiments designed to examine citrus rootstock resistance to *D. abbreviatus* have used ≈0.5–33 times the actual field larval pressure (Table 1). The larval inoculations in Table 2 do not match field conditions for larvae/m^2 or for the seasonal frequency of larval inoculation. That is, in previous resistance studies larval numbers were too high and the length of the studies, in general, was too short (Table 1). Based on this study, future plant resistance studies with *D. abbreviatus* need fewer larvae/inoculation, an inoculation gradient from low to high larval numbers, more frequent in-

oculations, a period with no inoculation, and longer studies to more closely mirror field conditions.

Only neonate larvae and adult weevils were captured in these studies. The presence of adults mirrored the presence of larvae. That is, once adults were observed in the trees, larvae were captured until adults disappeared (Fig. 8). Based on adult observations and larval captures, the ratio of adults and resulting larvae remained relatively constant throughout the year (Fig. 8). Our data suggest that for control of larvae through foliar adult or soil larval pesticides, applications should be made when either adults are observed or larvae are captured.

The seasonal pattern of adult presence in our study differed from previous studies. We observed adults from July through December (Fig. 4). McCoy and Duncan (2000) and Adair (2000) observed a peak of adult weevil capture in adult traps in April and May. Duncan et al. (2001) observed a peak adult weevil capture in March and April. A variety of factors probably contributed to these differences. Our method of observation was passive observation of adults. Previous adult monitoring has been with traps. Our experiments were conducted in dry years for Florida and we note here that adult emergence began with the summer rain.

This is the first study to quantify the number of neonate larvae of *D. abbreviatus* dropping from the citrus canopy in the field. When adults were present larvae were also present. This suggests that control measures for adults and larvae should be taken when adults or larvae are present.

Our larval monitoring technique appears to be suitable for estimating the relative and perhaps the absolute number of neonate larvae entering the soil under an infested citrus tree. The ability to quantify the destructive larval stage of *D. abbreviatus* means this passive technique could be used to assess management technologies for *D. abbreviatus.*

For the future, more data are needed to confirm the usefulness of our larval monitoring technique to assess adult and larval management methods.

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