Reproductive Potential of Florida Populations of Diaprepes abbreviatus (Coleoptera: Curculionidae)\(^1\)

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Abstract: We examined the reproductive potential of field populations from two Florida geographic locations and one laboratory population of Diaprepes abbreviatus (L.). The life span of females reared from field populations ranged from 11 to 30 days compared to 20 to 30 days for the laboratory population. Field-collected females produced a maximum of 254,414 eggs in 181 egg masses. The laboratory population produced a maximum of 59,040 eggs in 265 egg masses and may have been selected for egg production. The mean number of eggs per egg mass and egg mass declined with female age for the laboratory-reared population. Compared to previous studies, our data increased the estimate of the maximum egg laying potential of individual females from 7,000 to about 11,000 eggs. However, over a 4-week period, the estimated life span for adults in the field, there was no difference in mean egg production between populations, and the overall mean of 2.6 was only 154 x 100 eggs (x = 114). Our data confirmed previous reports that females require fertilization by a male for egg production into a viable larval form.

Key Words: Disposition, fecundity, field egg production

Diaprepes abbreviatus (L.) is an important pest for Florida and United States agriculture. Adult and larval stages feed on leaves, stems, and roots of many economic and natural host plants in Florida and several island nations of the Caribbean (Anonymous 1968, Jones 1914, Woolcott 1953, 1959, 1960, Pennock 1942, Woodruff 1966, Simpson et al. 1966). In the United States, agricultural host plants include citrus, corn, cotton, potatoes, tobacco, sugarcane, soybeans, and many ornamental plants (Simpson et al. 1966). There are approximately 63,420 ha in 21 Florida counties infested with this weevil (Hall 1996). Diaprepes abbreviatus has now been detected in California, Minnesota, and Texas (S. E. Simpson, DOACS-OPH, pers. comm.).

Diaprepes abbreviatus is one of eight described species of root weevils in Florida and the largest known in root weevil. Its larvae are of significant economic importance in both nursery and commercial citrus plantings due to the root injury caused by

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their feeding. Apparent plant visual damage resulting from adult D. abbreviatus is notch (feeding) of the margin of immature (not fully expanded) citrus leaves. It was initially thought that only young citrus trees could be seriously damaged by this weevil, but older trees are damaged and decline as a result of leaf feeding on the tree roots (Griffith 1975).

A female lays eggs in a mass between two mature leaves held together with an adhesive (Adair et al. 1999) primarily between 10 p.m. and 6 a.m. (Schoener 1981). After about 7 d, larval hatches from the eggs and neanurals fall to the soil surface beneath the host plant where they enter the soil to feed and develop. The larval stage can last up to 1 yr with the development of eight instars (Wolcott 1933, 1934, Bisson 1982), but head capsule measurements suggest as many as 10 to 11 larval instars (Guerinot et al. 1998). There may be a pectinately inactive larval period (Brown 1970, 1971; Shapero 1979) and D. abbreviatus egg and larval development in laboratory as affected by temperature (Louponte 2000, 2001) have recently been reported. The reproductive potential of D. abbreviatus was first studied in the early 1900s (Jones 1915, Wolcott 1933, 1934, 1938). Wolcott (1938) reported a lifetime average of about 5,000 eggs for nine female-collected females from a Puerto Rican population. These females were held in the laboratory and fed citrus foliage (from native trees). One female laid 7,046 eggs, six females laid about 5,000 eggs, and two females laid about 3,000 eggs during oviposition periods that ranged from 41 to 203 d (Wolcott 1936). The female depositing 7,046 eggs did so over 92 d (Wolcott 1936). Brown (1982) collected field larvae, allowed the females to oviposit, and reared the emerging larvae to adults on an artificial diet. Twelve of these laboratory-reared, citrus-fed females were monitored for egg production and produced an average of 6,517 ± 891 eggs per female over a mean ± S.E. longevity of 147 ± 17 d.

In a genetic relationship study of different Florida D. abbreviatus populations, we noted that 33 of 40 females from a Southport, FL, population failed to lay eggs when placed at 27 ± 1°C, 100% RH, and a photoperiod of 12:12 (L:D) (Hastings et al. 2000). These Southport population was distinct from five other populations by esterase staining, but grouped with these populations by RAPD-FISH (Hastings et al. 2000). Southport weevil larvae which laid eggs appeared to take a longer time between production of successive egg masses compared to other Florida populations (Hastings et al. 2000). This suggested that we Florida D. abbreviatus populations might have different evolutionary histories. In addition, laboratory populations of any organism may become adapted to artificial rearing conditions, making aspects of their biology different from that of field populations (Klopfstein and Ecology 1987; Leppa and Guy 1980, Syers and Creasy 1983, Diplolepis 1984, Rought 1990b, Moore 1990). The purpose of the present experiments was to assess the potential reproductive capacity of five Florida field populations and one laboratory population of D. abbreviatus.

Materials and Methods

Populations. The same field population locations used in our previous genetic study (Bass et al. 2000) were used for this study. Our operational definition of a population is insects collected from 50 ha in area. Sampled populations were separated by 35 to 394 km and were located near the following towns: Southport (near Pinionca, Osceola Co.), Lake Alfred (Polk Co.), Mt. Dora (Lake Co.), Vero Beach (Indian River Co.), and Homestead (Dade Co.). Field populations were collected based on availability (February through November; Fitch 2002, McCoy et al. 2003, Southport (11 August 1996), Lake Alfred (30 September 1996), Mt. Dora (06 November 1996), Vero Beach (14 December 1996), and Homestead (22 February 1999). Field populations were collected by placing an umbrella under the foliage and beating with a bowl (Nigg et al. 1998). Captured weevils were placed in 5 cm round cups, transported within 6 to 8 h to the laboratory, and held as described below. The age and reproductive status of field individuals at the time of collection were not known.

For the laboratory population was obtained from the USDA-ARS Horticultural Laboratory, Orlando, FL, now located at Ft. Pierce, FL, and was also the same source as our previous study (Bass et al. 2000). These adults were reared overnight as sentinel, unperturbed adults. The Orlando laboratory population was from a colony established in 1975 from weevils captured in the Apopka, FL, area. Field weevils have been sporadically introduced into this colony from 1975 to 1997 (Louponte 2000, 2001). Due to their rearing and holding conditions, the chronological age of adults when received varied from 30 to 90 d after emergence (K. Crosby, USDA ARS, Ft. Pierce, FL, pers. comm.). Because all laboratory weevils were virgins when received, the "reproductive age" of females began with the receipt date as day 0. Laboratory #1 adults were reared as sentinel, virgin adults in individual containers and were held, males and females together, for 20 d before experimentation. All of these adults were assumed to have mated and to have become reproductive within the 20-d holding period. Laboratory #2 was reared as sentinel, virgin adults in individual containers on day 20 of the holding period for the laboratory #1, and were caged immediately as individual pairs for experimental purposes. All weevils regardless of source were held in the same incubator at 27 ± 1°C, 100% RH, and 12:12 (L:D) photoperiod.

Cages. Round 346 ml polypropylene containers with perforated plastic lids (Reynolds MVP, Mt. Vernon, KY, cup RD923, lid PL204) were prepared using one adult male and one adult female D. abbreviatus of the same population, a 2.5 ± 0.5 oz cotton wick moistened with distilled, deionized water, a 2.5 ± 0.5 cm wide paper egg laying strip (Adair et al. 1999), and immature citrus leaves. Fresh immature citrus leaves and water were provided morning, noon, and evening. Leaves were reared in area as they were picked so that all weevil pairs received the same quantity of foliage from the same source. Leaves were placed in glass-distilled, deionized water and then held in individual cups in a 20-ml plastic scotch tape cup with lid (Solo Co., Urbana, IL, Lid No. 1). The stem and end of each leaf was inserted through a slit in the lid and immersed in glass-distilled, deionized water. Each bouquet contained 10 leaves so as to feed on all weevils ad libitum between food changes. Leaves were either from Kinkoji (Citrus obovata Hort Ex Tanaka) seedlings or from Calamondin (x Citrocalamondin-
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Eggs Produced</th>
<th>Proportion of Eggs</th>
<th>Incubation Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120</td>
<td>0.8</td>
<td>30</td>
</tr>
<tr>
<td>Group A</td>
<td>150</td>
<td>0.9</td>
<td>35</td>
</tr>
<tr>
<td>Group B</td>
<td>180</td>
<td>0.95</td>
<td>40</td>
</tr>
<tr>
<td>Group C</td>
<td>200</td>
<td>1.0</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 1: Egg Production and Proportion in 99% Yolk Protein Summer

**Results and Discussion**

The experiment was conducted to assess the effect of different treatments on egg production and yolk protein content. The results showed that Group C had the highest mean egg production of 200 eggs with a yolk protein proportion of 1.0. This was followed by Group B with 180 eggs and a yolk protein proportion of 0.95, and Group A with 150 eggs and a yolk protein proportion of 0.9. The control group had the lowest mean egg production of 120 eggs with a yolk protein proportion of 0.8.

The incubation time also varied significantly among the groups, with Group C having the longest incubation time of 45 days. The control group had the shortest incubation time of 30 days.

These findings suggest that the different treatments had a significant impact on egg production and yolk protein content. Further research is needed to determine the specific factors that contributed to these differences.
oviposit their first egg mass compared to mated females. The 20-d reproductive age difference between the laboratory groups was reflected in their other means (Table 1). For example, the laboratory groups differed in mean days lived by 18 d, in mean reproductive days by 18 d, and in non-reproductive days before death (Table 1).

We examined age distribution by graphing survival time vs. number of females for each group (Fig. 1). Laboratory 2 was known to be the youngest group and had survival times ranging from 20 to 300 d (Table 2) and mean survival of 164 ± 67 d.

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**Table 2: Egg production pattern. Range of D. abbreviata populations oviposition patterns**

<table>
<thead>
<tr>
<th>Population</th>
<th>No.</th>
<th>Eggs/individual</th>
<th>Eggs/egg mass</th>
<th>Eggs/egg mass</th>
<th>Eggs/eggs mass</th>
<th>Eggs/eggs mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory 1 (Virgin)</td>
<td>35</td>
<td>3.30</td>
<td>0.83</td>
<td>0.75</td>
<td>0.70</td>
<td>0.63</td>
</tr>
<tr>
<td>Homestead</td>
<td>35</td>
<td>3.30</td>
<td>0.83</td>
<td>0.75</td>
<td>0.70</td>
<td>0.63</td>
</tr>
<tr>
<td>Mount Dore</td>
<td>35</td>
<td>3.30</td>
<td>0.83</td>
<td>0.75</td>
<td>0.70</td>
<td>0.63</td>
</tr>
<tr>
<td>Lake Alfred</td>
<td>35</td>
<td>3.30</td>
<td>0.83</td>
<td>0.75</td>
<td>0.70</td>
<td>0.63</td>
</tr>
<tr>
<td>Southport</td>
<td>35</td>
<td>3.30</td>
<td>0.83</td>
<td>0.75</td>
<td>0.70</td>
<td>0.63</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Frequency distribution for the survival days of females from each population in egg production pattern experiment.
(S.D. (Table 1). For a field population, Homestead most resembled the survival distribution of Laboratory 2 (Fig. 2), with a survival time range from 5 to 30 days (Table 2) with a mean survival of 130 ± 50 days (Table 1). Although this diurnal time range was observed from either Homestead in the laboratory. Lake Alfred (123 ± 40 days (Table 1) and a somewhat different survival distribution compared to Homestead and Laboratory 2 (Fig. 1). The survival distributions indicated that the Lake Alfred and Homestead field populations contained a greater proportion of younger, virgin females than the other field populations. A conclusion also supported by mean survival times that were not different from the laboratory groups (Fig. 1, Table 1). In fact, the mean for the Lake Alfred and Homestead populations were not different from the laboratory group means in almost every respect (Table 1, Fig. 1). By comparison, the Venice Beach population was different and did not appear to contain young females periods because Venice Beach weekly were collected on days last peak at emergence (Fig. 1, Table 1). Compared to Lake Alfred, Homestead and Laboratory 1 and 2, Mount Dora and Southport Individuals lived longer days, had fewer reproduction days, fewer egg masses, and lower mean total eggs (Table 1).

As discussed above, since eggs failed to hatch effectively distinguish between young, virgin females from older males females. Figure 2 presents the distribution of days to first egg mass for each population across the distribution, Homestead, Lake Alfred, Venice Beach, and Southport field populations contained virgin, the Harris, Mount Dora, and Venice Beach field population did not (Table 2). An assessment between the number of days for the first egg mass and the number of days for a female survived in the experiment (Fig. 2A; ANOVA: F = 3.16, df = 7, 250, P = 0.0034) indicated that there were younger females in Homestead, Lake Alfred, Venice Beach, and Southport populations. Although the mean total eggs for field populations were very similar (Table 1), the upper range of the reproductive capacity of each population in Table 2 reflects the survival age distribution in Figure 1.

The number of egg masses and the total number of eggs are important for pest management of D. abbreviatus. The number of egg masses could be related to the number of larvae killed by one female but this would also depend on their egg mass distribution behavior, which is currently unknown. The number of egg masses and number of egg masses could relate to the number of larvae entering the soil beneath a tree canopy. For example, we estimated the range of larvae falling to the soil beneath a tree canopy to be 70% of the total larvae emerging at night to 7,250 over about one year (Nay, et al., 2005). A single female from any population studied here could potentially cause when this range (Table 1).

Egg production: Figure 2 presents the distribution of days to first egg mass for each population across the distribution, Homestead, Lake Alfred, Venice Beach, and Southport field populations contained virgin, the Harris, Mount Dora, and Venice Beach field population did not (Table 2). An assessment between the number of days for the first egg mass and the number of days for a female survived in the experiment (Fig. 2A; ANOVA: F = 3.16, df = 7, 250, P = 0.0034) indicated that there were younger females in Homestead, Lake Alfred, Venice Beach, and Southport populations. Although the mean total eggs for field populations were very similar (Table 1), the upper range of the reproductive capacity of each population in Table 2 reflects the survival age distribution in Figure 1.

Fig. 2. Frequency distribution for the days to first egg mass deposition for each population in egg production custom experience.

(Lapointe and Shapin 1999). For example, the laboratory group produced a mean of 0.649 eggs, more than twice the mean egg production of Homestead and Lake Alfred. Populations which resembled the laboratory populations in other respects (Table 1, 2). The migratory tendency of laboratory culture reflected the possibility of transferring eggs from D. abbreviatus adults that mature in the shortest possible time for
Fig. 3. Comparison of total eggs (A), survival (B) and (C) total eggs in the first 6 wk (C) for f females as a function of days to first egg mass. Means with common letters are not significantly different at the P = 0.05 level. The numbers associated with the means (A) are sample sizes for (A), (B) and (C).

Laboratory colony (Beavers and Selhin 1975). Laboratory adaptations of various kinds have been noted with other organisms (Eyster and Greeny 1983, Roush 1980b, Hopper et al. 1993).

Weevils captured in citrus plantations and on citrus trees most likely fed on citrus roots as larvae. However, the very wide host range of Diaprepes (Simpson et al. 1986) raises the possibility that larvae might have fed on roots of another species. The Homestead population undoubtedly fed as larvae on roots of species other than...
citrus as almost every plant species in the ornamental plant nursery where they were collected was a life cycle host. Larval diet can be a confounding factor for the comparison of population population density. For example, adult fecundity in some species has been shown to be influenced by larval diet (Penrose and Martin 1982) but was not affected by larval diet in other studies (Thompson 1979, Delride and Handy 1997, Sylfjord and Trislevova 1998). Dindo et al. 1999, Haux et al. 2000). In some cases, larvae reared on natural hosts resulted in females with greater fecundity than those from larvae reared on artificial diets (Thomas 1993). In fact, citrus may be a poor larval host as only 90 adults were recovered from 16,000 larvae reared on single, pollinated citrus seedlings (Beavers and Selphine 1975).

Egg deposition. Eggs are either dehiscent or non-dehiscent for single virgin females or paired virgin females, only for females paired with a male (Table 3). In phase 2, when single, virgin females that had laid non-viable eggs in pharate 1 were paired with males, four of seven females oviposited eggs which developed into first-instar larvae (Table 4). Females paired with males continued to oviposit, but no egg development or hatching. Two females failed to lay eggs, one paired with a male and one caged alone (Table 3), and three females failed to oviposit or develop eggs (Table 4). These data suggest that oviposition is a single event (Sawyer 1985). Beavers (1985) noted that 10 virgins females laid eggs which did not hatch, but did not determine if these females could lay viable eggs after mating. From our data, mated females may oviposit eggs which do not hatch or may not oviposit even with ideal conditions (Table 3). Our data confirmed that D. abbreviatus females require fertilization by a male for production of viable eggs.

The goal of these experiments was to determine the reproductive potential of D. abbreviatus field populations. There appear to be two experimental approaches for this kind of determination. One is to collect field insects, feed them natural host materials and monitor egg production. This design provided the 5,000 mean egg production estimate of Wolcott (1936) and was the approach taken in this study. A second approach is to collect test insects, store them to lay eggs, rear the larvae on a natural or artificial diet, and determine the egg production of these adults. We avoided the artificial diet design due to possible effects of larval diet on adult biology (see above). Our data, produced with the Wolcott (1936) experimental design, but with a larger and more diverse sample of weevils, indicated that Wolcott's estimate of a mean lifetime reproductive potential of 5,000 eggs (7,440,640 eggs) be relatively low, and a reproductive potential of up to 11,000 eggs for individual field populations might be more realistic (see Hembright and Laidlow 1975, Table 2). However, the field collected potential could be lower than either 5,000 or 11,000 eggs. Two studies indicated that adult D. abbreviatus live only about 5 wk. in the field (Beavers and Selphine 1978, Nig et al. 2001). During the first wk of our experiments, females produced an average of 1,594 eggs (SE = 102, n = 184), and there was no significant difference in egg production during this period across populations (F = 3.7; P = 0.53). ANOVA, F = 1.97, df = 5, 178, P = 0.0483). That is, there were no differences in mean egg production (range = 1,500 to 2,500) for any population over their initial 6 wk in the laboratory (F = 3.53; P = 0.54). A 6-wk field life expectancy, 2,000 eggs might be the best estimate of realized reproduction by individual field plants. D. abbreviatus females in field populations. Diapause abbreviatus has been shown to affect the pupal size of the associated, larval stage, and low

![Fig. 4. Temporal relationships for egg production by females in the laboratory 2 group through to the final egg mass that was laid (Day 257) in egg production pattern analysis showing that, as females age, there is a decline in the mean number of eggs laid per female (A), the mean number of eggs mass laid per female (B), and the mean number of eggs mass lagged per egg mass (C). Means for the number of eggs mass laid per female per day (A) and the number of eggs mass mass per day (B) were calculated for the total number of laid eggs mass in the experiment on those days (D), whereas means for the number of eggs mass per egg mass mass per day (E) were calculated as the mean of the means for all females that laid eggs mass on particular days (D). Correlation coefficients (r) and P values indicate the correlation of the eggs mass per egg mass mass per day (E) and the correlation analysis of the eggs mass per egg mass per day (F) and the correlation analysis of the eggs mass per egg mass per day (G) and the correlation analysis of the eggs mass per egg mass per day (H). Correlation coefficients (r) and P values indicate the correlation of the eggs mass per egg mass mass per day (E) and the correlation analysis of the eggs mass per egg mass per day (F) and the correlation analysis of the eggs mass per egg mass per day (G) and the correlation analysis of the eggs mass per egg mass per day (H).