Parasitism and Predation on Eggs of *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) in Cowpea: Impact of Endosulfan Sprays

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**ABSTRACT**

The impact of endosulfan sprays in cowpea on parasitism and predation of *Leptoglossus phyllopus* (L.) eggs was determined by placing egg masses in the field after insecticide treatment at early, mid- and late pod-fill in 1997 and 1998. The parasitoids, *Gryon carinatifrons* (Ashmead), *G. pennsylvaniaicum* (Ashmead), and *Ooencyrtus ?leptoglossi* Yoshimoto were recovered from the egg masses. *G. carinatifrons* was the most abundant species, composing 83.9% of the parasitoids recorded. Predators included fire ants, *Solenopsis invicta* Buren, that tended to remove the eggs entirely and the snowy tree cricket, *Oecanthus fultoni* Walker, which chewed eggs and left chorion fragments on the substrate. Parasitism of *L. phyllopus* eggs was significantly reduced by endosulfan treatments, but predation was not affected. Parasitism generally was low early in the season and increased as the season progressed. Predators caused more egg mortality than did parasitoids and *S. invicta* was the major predator. These results support the hypothesis that parasitism and predation are important mortality factors of *L. phyllopus* in cowpea.

**KEY WORDS**  *Leptoglossus phyllopus*, *Gryon carinatifrons*, *G. pennsylvaniaicum*, *Ooencyrtus ?leptoglossi*, *Solenopsis invicta*, *Oecanthus fultoni*, cowpea

Indigenous insect parasitoids and predators are important sources of mortality for herbivorous insect pests (Smith & Mittler 1967, Batra 1982, Jones 1982). Often, however, the effectiveness of these entomophagous species is limited in the field by insecticides (DeBach 1974). Most insecticides are toxic to non-target beneficial arthropods as well as to the target pests (Barlett 1963, Stern 1963, Croft & Brown 1975, Wilkinson et al. 1975, Orr 1988, Smart et al. 1989, Terry et al. 1993). Van den Bosch (1966) reported that applications of insecticides destroy more beneficial arthropods than any other agricultural practice and most beneficials are more susceptible to insecticides than are the target pests (Croft 1990). Smilanick et al. (1996) reported that methamidophos residue significantly affected pentatomid egg parasitoids, *Trissolcus basalis* (Wollaston) and *T. utahensis* (Ash-
mead) in tomato (Lycopersicon esculentum Mill.). Also, chlorfenapyr and methyl parathion applied to soybean (Glycine max [L] Merrill) was highly toxic to the heteropteran predators, Geocoris punctipes (Say), Nabis roseipennis Reuter and Podisus maculiventris (Say) (Boyd & Boethel 1998). Similarly, Barlett (1963) reported that endosulfan was highly toxic to parasitic Hymenoptera and coccinellids. Thus, pest resurgence and outbreaks of secondary pests are often major consequences of destruction of natural enemies by insecticides (Metcalf 1994). Despite these negative effects, insecticides are still the most commonly-used control measure against insect pests of cowpea (Vigna unguiculata [L.] Walpers) (Chalfant 1976, Stacey 1979).

The leaffooted bug, Leptoglossus phyllopus (L.), is a pest of cowpea in the southeastern United States (Bissell 1929, Fery & Schalk 1981). Feeding by both nymphs and adults greatly reduces seed quality and yield (Schalk & Fery 1982). Control of this bug and other sucking bug pests is necessary to prevent substantial reductions in the marketable yield of cowpea in the southern United States (Stacey 1979). One of the most frequently used insecticides is endosulfan, which is used mainly for control of the cowpea curculio (Chalcodermus aeneus Boh.). However, the impact of endosulfan on natural enemies of sucking bugs in cowpea has not been investigated.

The objectives of this study were to determine parasitism and predation on eggs of L. phyllopus in cowpea and to evaluate the impact of endosulfan sprays on parasitism and predation on L. phyllopus eggs.

Materials and Methods

Field tests were conducted in 1997 and 1998 at Clemson University’s Coastal Research and Education Center, Charleston, South Carolina. The two treatments were endosulfan-treated and untreated control. Endosulfan was applied using a backpack sprayer at 4.21 kg/cm² at the recommended rate of 0.56 kg a.i./ha. Three applications were made in a season, at early pod-fill (46 days after planting [DAP]), mid-pod-fill (53 DAP), and at late pod-fill (59 DAP). The plot size used in 1997 was 4 by 9 m with 1 m between plots and 4 m between blocks. A second test was conducted on larger (20 by 35 m) plots in 1998, in addition to carrying out tests on the smaller plots as in 1997. Plots and blocks in the larger plots were separated by 4 m alleys. The larger plots were included in 1998 to try to reduce the possible effects of arthropod movements from sprayed into adjacent control plots. Plots were laid out in randomized complete block design with four replications. All plots were planted on 26 May in 1997 and 1998.

Adults and nymphs of L. phyllopus were collected from the field on several host plants such as thistle (Cirsium spp.), Pyrrhopappus carolinianus (Walter) DC., and tomato. Bugs were reared in the laboratory in Plexiglass cages (51 by 53 by 36 cm) at 28.7 ± 0.5°C and 55.8 ± 2.0% RH with 14:10 [L:D]h photoperiod. Fresh green beans (Vigna spp.) and sunflower seeds (Helianthus spp.), supplemented with corn (Zea mays L.) and okra (Abelmoschus esculenta [L.]), were given as food. Water was provided by a moistened wick in a plastic cup of water. Wooden applicator sticks (15 cm long by 0.2 cm diameter), inserted in lumps of modeling clay, were placed in the cages as oviposition sites (Mitchell & Mitchell 1986). Applicator sticks bearing egg masses were removed from the cages daily and kept in a freezer at −20°C until ready to be placed in the field. Powell & Shepard (1982)
found that freezing eggs of *Nezara viridula* (L.) did not affect their acceptability as hosts for the parasitoid, *T. basalis*.

The number and location of eggs on the stick were noted. Often, there was more than one egg mass per stick but these were treated as one mass. Mean number of eggs per stick ranged from 10 to 101 eggs in 1997 and 13 to 135 in 1998. Five egg masses were placed in each plot. Sticks bearing eggs were attached to stems or petioles with paper clips and marked with surveyor’s tape for ease of locating eggs later. Eggs were placed in the field during early pod-fill, mid-pod-fill and late pod-fill 24 h after chemical applications were made, and collected five days later. Eggs remaining on sticks were counted and all missing eggs and those that were partially removed were presumed attacked by predators. Recovered eggs were held in test tubes covered with nylon mesh and maintained in the rearing room at 28.7 ± 0.5°C and 55.8 ± 2.0% RH with 14:10 [L:D] h photoperiod until parasitoids emerged. Parasitism was determined by counting parasitoids that emerged or their exit holes and by dissecting whole eggs to detect parasitoids that had not emerged. Eggs were classified as: 1) eaten, 2) chewed, with part of the chorion remaining, 3) parasitized, with the presence of an exit hole or parasitoid, 4) intact, hemicylindrical in shape with no exit hole or parasitoid found within and, 5) collapsed, triangular in shape with collapsed sides and no exit hole or parasitoid (Mitchell et al. 1999). Percent parasitism per egg mass was calculated as parasitized eggs divided by parasitized plus intact eggs × 100. Chewed, eaten and collapsed eggs were excluded from calculations of parasitism. Scelionid parasitoids were identified using keys provided by Masner (1983) and were confirmed by Dr. Walker A. Jones of the USDA, Weslaco, Texas. Dr. J. B. Woolley of Texas A&M University, College Station, identified the encyrtid parasitoids. Percent predation per egg mass was calculated as number of eggs that were chewed plus number of completely eaten eggs, divided by total number of eggs in the mass placed in the field × 100.

Estimates of predator density were determined by visual observations made during the time eggs were in the field at 0730 h, 1230 h, and at 1730 h, and any predator near the egg masses or feeding on them was recorded. Observation data were augmented by pitfall traps in 1998. Traps were made from plastic cups (11.5 × 10 × 4 cm), which were half-filled with ethylene glycol (Super Tech Antifreeze/Coolant, Alsip, Illinois). Cups were buried into the soil so that the lip of the cup was at ground level. Two traps were placed in each plot, two times during the season. Trap contents were collected after 5 d and arthropods were identified.

**Statistical analyses.** The data were averaged over number of egg masses to obtain means for analysis. Percentages were transformed to arcsine square root values to ensure homogeneity of variances before analyses (Gomez and Gomez 1984). Data for parasitism and predation were analyzed using the multivariate analysis of variance (MANOVA)/ANOVA for repeated measures, with pod-fill stages as the repeated measures (SAS Institute 1996). Means for main factors were separated using Fisher protected least significant difference (LSD) test (*P* < 0.05).

**Results**

The scelionid parasitoids, *Gryon carinatifrons* (Ashmead) and *G. pennsylvanicum* (Ashmead) were reared from the field-exposed egg masses in 1997. In
addition, an encyrtid, *Ooencyrtus ?leptoglossi* Yoshimoto was recovered from the eggs in 1998. *Gryon carinatifrons* was the most abundant parasitoid species, making up 83.9% of all parasitoids that emerged. *Gryon pennsylvanicum* and *O. ?leptoglossi* comprised 13.9% and 2.2% respectively. Only one parasitoid of all species emerged from a single egg. Voucher specimens of parasitoids were deposited in the Entomology Department Arthropod Museum at Clemson University.

The red imported fire ant, *Solenopsis invicta* Buren, was the most abundant predator based on pitfall trap collections (Table 1) and visual observations in the field. The ants were regularly seen removing eggs from the substrate. The snowy tree cricket, *Oecanthus fultoni* Walker chewed *L. phyllopus* eggs leaving chorion fragments on the substrate. The results from pitfall traps and visual observations also revealed that *Hippodamia convergens* Guérin-Méneville (Coccinellidae), *Colomegilla maculata* (De Geer) (Coccinellidae), *Labidura riparia* (Pallas) (Labi-duridae), *Gryllus* spp., *Geocoris* spp., *Nabis* spp., *P. maculiventris*, and *Pardosa milvina* (Hentz) (Lycosid spiders) were common in the field (Table 1), but they were not observed feeding on *L. phyllopus* eggs. Predators (probably *S. invicta*) that removed the entire eggs were responsible for 85.5% of total predation while chewing predators accounted for 15.5%.

In 1997, there was no significant interaction between the main factors of spray treatment and pod-fill stage (MANOVA, Wilks Lamda value = 0.5964; $F = 1.6217$, df = 4, 22; $P = 0.2043$). The main effects or season-long effects of treatment on percent parasitism and predation for the 2-yr study are summarized in Table 2. Although no significant effects of endosulfan treatment were detected when parasitism and predation were considered simultaneously (MANOVA, Wilks Lambda value = 0.1329; $F = 6.5215$, df = 2, 2; $P = 0.1330$), separate ANOVAs revealed that the insecticide significantly reduced percent parasitism ($F = 14.10$, df = 1, 12; $P = 0.0330$), but not percent predation ($F = 0.03$, df = 1, 12; $P = 0.8713$). Partial correlation matrices generated with the PRINTE option in the MANOVA for treatment showed that parasitism and predation were not significantly correlated with each other for either year. There was a significant effect of pod-fill stage (MANOVA, Wilks Lambda value = 0.1468; $F = 8.8543$, df = 4, 22; $P = 0.0002$). Parasitism was significantly lower at early pod-fill than at mid- or late pod-fill ($F = 31.19$, df = 2, 12; $P = 0.0001$). However, predation was not different at any of the pod-fill stages ($F = 0.74$, df = 2, 12; $P = 0.4964$) (Fig. 1).

In 1998, in the $4 \times 9$ m plots, there was no significant overall treatment effect on parasitism and predation were detected (MANOVA, Wilks Lambda value = 0.5166; $F = 2.1523$, df = 4, 22; $P = 0.1082$). Neither insecticide treatment (MANOVA, Wilks Lambda value = 0.2888; $F = 2.4617$, df = 2, 2; $P = 0.2889$) nor pod-fill stage (MANOVA, Wilks Lambda value = 0.7796; $F = 0.7293$, df = 4, 22; $P = 0.5816$; Fig. 2) significantly influenced rates of parasitism and predation. The apparent lack of significant effects of endosulfan treatment in these plots was probably due to the large variation in percent parasitism, which ranged from 0-100% in control plots (Table 2).

In the larger plots ($20 \times 35$ m) in 1998, no significant overall treatment effect on parasitism and predation were detected (MANOVA, Wilks Lambda value = 0.0716; $F = 12.9720$, df = 2, 2; $P = 0.0716$). However, examination of the ANOVAs again showed a significant reduction in percent parasitism ($F = 16.08$, df = 1, 12; $P = 0.0278$) but not percent predation ($F = 1.10$, df = 1, 12; $P =$
with endosulfan treatment (Table 2). Pod-fill stage (MANOVA, Wilks Lambda value = 0.3149, \( F = 4.3004, \text{df} = 4, 22; P = 0.0101 \)) and treatment by pod-fill stage interaction (MANOVA, Wilks Lambda value = 0.2677, \( F = 5.1308, \text{df} = 4, 22; P = 0.0457 \)) both showed significant effects on rates of parasitism and predation. Parasitism was significantly lower at early pod-fill than at mid- or late pod-fill (\( F = 5.26, \text{df} = 2, 12; P = 0.0229 \)). Percent predation was higher at late pod-fill than at mid pod-fill (\( F = 4.14, \text{df} = 2, 12; P = 0.0430 \)) (Fig. 3).

Neither percent chewed eggs nor those removed differed significantly between endosulfan-treated and untreated plots in 1997 (chewed: \( F = 0.20, \text{df} = 1, 12; P = 0.6849 \); removed: \( F = 0.01, \text{df} = 1, 12; P = 0.9240 \)), 1998 small plots (chewed: \( F = 0.91, \text{df} = 1, 12; P = 0.9567 \); removed: \( F = 2.47, \text{df} = 1, 12; P = 0.2140 \)) or 1998 large plots (chewed: \( F = 7.36, \text{df} = 1, 12; P = 0.0730 \); removed: \( F = 0.49, \text{df} = 1, 12; P = 0.5355 \)) (Table 3).

Table 2. Season-long percent parasitism and predation (mean ± SEM) on eggs of *L. phyllopus* in cowpea plots receiving endosulfan sprays and in control plots.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Percent parasitism (Mean ± SEM)</th>
<th>Percent predation (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1997 (4 by 9 m plots)</td>
<td></td>
</tr>
<tr>
<td>Sprayed</td>
<td>12</td>
<td>7.1 ± 2.2a</td>
<td>18.5 ± 4.3a</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>14.5 ± 3.2b</td>
<td>22.0 ± 6.0a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1998 (4 by 9 m plots)</td>
<td></td>
</tr>
<tr>
<td>Sprayed</td>
<td>12</td>
<td>2.6 ± 1.0a</td>
<td>32.1 ± 5.0a</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>21.8 ± 8.7a</td>
<td>48.3 ± 7.9a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1998 (20 by 35 m plots)</td>
<td></td>
</tr>
<tr>
<td>Sprayed</td>
<td>12</td>
<td>4.2 ± 1.9a</td>
<td>24.6 ± 4.8a</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>14.4 ± 4.2b</td>
<td>34.3 ± 4.8a</td>
</tr>
</tbody>
</table>

Within column means ± SE followed by the same letter are not significantly different (\( P > 0.05 \)).
Discussion

These studies revealed that three endosulfan treatments generally impacted negatively on parasitoids but not predators of *L. phyllopus* eggs (Table 2). In two out of the three tests, parasitism was lower in treated plots compared with controls. These results agree with those of Justo (1994), who reported that parasitism of *N. viridula* eggs by *T. basalis* in tomato was significantly reduced by esfenvalerate treatments compared with controls. Likewise, he found that predation was not significantly different between these treatments. Croft & Brown (1975) reported that parasitoids are more susceptible to insecticides than predators.

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**Fig. 1.** Mean (± SEM) percent parasitism and predation on eggs of *L. phyllopus* at different stages of pod-filling in cowpea in 1997. Parasitism and predation were measured by placing five egg masses at each pod-fill stage in treated and untreated plots for 5 d. Means are for eight plots. (A) Percent parasitism, (B) Percent predation. Bars with different letters are significantly different (*P* < 0.05; LSD).
haps this is because parasitoids, unlike generalist predators, search extensively for their prey, which increases their contact with insecticide residues. Barlett (1963) tested the toxicity of several insecticides and reported that endosulfan was highly toxic to hymenopteran parasitoids.

Parasitism was generally low at early pod-fill and increased as the season progressed. Similar phenologies were reported for Gryon flavipes (Ashmead) on eggs of the rice ear bug, Leptocorisa oratorius (Fabricius) (Rothschild 1970), G. carinatifrons and G. pennsylvanicum on L. phyllopus eggs in cowpea (Mitchell et al. 1999), and for egg parasitoids of the harlequin bug, Murgantia histrionica Hahn (Ludwig & Kok 1998). Taylor (1975) and Matteson (1981) also reported that

Fig. 2. Mean (± SEM) percent parasitism and predation on eggs of L. phyllopus at different stages of pod-filling in cowpea in 1998. Parasitism and predation were measured by placing five egg masses at each pod-fill stage in treated and untreated plots for 5 d. Means are for eight plots. (A) Percent parasitism, (B) Percent predation. Bars with different letters are significantly different ($P < 0.05$; LSD).
parasitism on eggs of *Clavigralla tomentosicollis* Stål by *Gryon gnidus* Nixon was low when bug eggs first appeared in the field but peaked late in the season. They observed that this mortality did not prevent the bug population from reaching damaging levels. A previous study showed that early pod-fill is the most susceptible stage of cowpea to pod-sucking bug damage (Abudulai & Shepard 2001). Thus, the low rates of parasitism at early pod-fill in the present study may not have reduced *L. phyllopus* populations enough to prevent economic damage in cowpea.

More eggs were removed than were chewed, which indicated that predators such as *S. invicta* that removed eggs contributed more to *L. phyllopus* egg mor-

Fig. 3. Mean (± SEM) percent parasitism and predation on eggs of *L. phyllopus* at different stages of pod-filling in cowpea in 1998. Parasitism and predation were measured by placing five egg masses at each pod-fill stage in treated and untreated plots for 5 d. Means are for eight plots. (A) Percent parasitism, (B) Percent predation. Bars with different letters are significantly different (*P* < 0.05; LSD).
tality than did chewing predators such as *O. fultoni*. Other workers also have reported that *S. invicta* are major predators of insect eggs in the field (Regsdale et al. 1981, Stam et al. 1987, Justo 1994, Zenger & Gibb 2001). Although we did not observe many of the predator species that were collected in pitfall traps feeding on egg masses, it is possible they contributed to total egg predation in the field. Using an enzyme linked immunosorbent assay (ELISA), Regsdale et al. (1981) reported that *G. punctipes*, *C. maculata* and *P. maculiventris* were important predators of *N. viridula* eggs in soybean.

Overall percent predation on *L. phyllopus* eggs was higher than was percent parasitism (Table 2). However, predators probably removed both parasitized and unparasitized eggs, which complicates calculation of parasitism.

In conclusion, endosulfan treatment affected *L. phyllopus* egg mortality by parasitoids, but not by predators. However, both predators and parasitoids are important sources of *L. phyllopus* egg mortality. Because parasitoid populations build up late in the season after most bug damage to cowpea has occurred, their potential as a source of mortality may not be fully realized. Therefore, augmentative or inundative releases of parasitoids early in the season, coupled with cultural techniques that enhance the build-up of natural enemies in alternate hosts and judicious use of less toxic insecticides may be part of an integrated pest management program for *L. phyllopus* in cowpea.

### Acknowledgments

We thank Walker A. Jones of USDA Welasco, Texas, for verifying our scelionid parasitoid identifications and J. B. Woolley, Texas A&M University, College Station, for determining our encyrtid specimens. We are also grateful to W. C. Bridges for statistical advice and Mark Schaffer for field assistance. Funding was provided by USAID Grant DAN-1310-G-

**Table 3. Season-long percent predation (mean ± SEM) on eggs of *L. phyllopus* by predators that chewed eggs and left part of chorion on substrate and by predators that removed eggs, in cowpea plots receiving endosulfan sprays and in control plots.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Predation (%) (Mean ± SEM)</th>
<th>Predation (%) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(eggs chewed)</td>
<td>(eggs removed)</td>
</tr>
<tr>
<td>1997 (4 by 9 m plots)</td>
<td>12</td>
<td>2.1 ± 1.0a</td>
<td>16.3 ± 4.5a</td>
</tr>
<tr>
<td>Sprayed</td>
<td>12</td>
<td>4.1 ± 1.7a</td>
<td>18.0 ± 6.0a</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>3.0 ± 1.3a</td>
<td>29.4 ± 5.0a</td>
</tr>
<tr>
<td>1998 (4 by 9 m plots)</td>
<td>12</td>
<td>2.8 ± 1.2a</td>
<td>45.5 ± 8.1a</td>
</tr>
<tr>
<td>Sprayed</td>
<td>12</td>
<td>5.9 ± 2.1a</td>
<td>18.6 ± 3.6a</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>10.0 ± 2.2a</td>
<td>24.2 ± 4.4a</td>
</tr>
<tr>
<td>1998 (20 by 35 m plots)</td>
<td>12</td>
<td>5.9 ± 2.1a</td>
<td>18.6 ± 3.6a</td>
</tr>
</tbody>
</table>

Within column means ± SE followed by the same letter are not significantly different (*P > 0.05*).
SS-6008-00 through the Bean/Cowpea CRSP Program at Michigan State University. Technical Contribution Number 4518 of the South Carolina Agriculture and Forestry Research System.

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