The Ability of Coptera haywardi (Ogloblin) (Hymenoptera: Diapriidae) to Locate and Attack the Pupae of the Mediterranean Fruit Fly, Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), under Seminatural Conditions

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In Latin America, the diapriid Coptera haywardi (Ogloblin) attacks the pupae of tephritid fruit flies. Anastrepha spp. are among its natural hosts, but in the laboratory it also develops in the exotic Mediterranean fruit fly, Ceratitis capitata (Wiedemann). Field cage tests demonstrated that C. haywardi could locate and parasitize Mediterranean fruit fly pupae under seminatural conditions as found in a Guatemalan coffee plantation. A mean of 18.3% of the pupae buried artificially at depths of ~5 mm were parasitized by C. haywardi, while those buried at 15 mm suffered 3.2% parasitism. In a laboratory experiment, larvae that buried themselves to pupate were not significantly more likely to be parasitized than artificially buried pupae, although they may have left a physical or chemical trail that betrayed their presence. Thus, the artificial burial of pupae is unlikely to grossly underestimate C. haywardi efficacy in the field. Another field cage test found that mortality levels due to unsuccessful parasitoid attacks were similar to those resulting from successful parasitism. Thus, the actual effect of a mass-release might be considerably greater than that suggested from parasitism data alone. The results are considered sufficiently positive to encourage further testing of C. haywardi as a biological control agent of the Mediterranean fruit fly.

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INTRODUCTION

Augmentative releases of braconid larval–pupal parasitoids have suppressed populations of various tephritid fruit fly pests (e.g., Wong et al., 1991; Sivinski et al., 1996; Montoya et al., 2000). A pupal parasitoid may be a useful addition to such releases, because insects that escape one form of natural enemy might be vulnerable to another (Sivinski, 1996). Mixed mass-releases of sterile male Mediterranean fruit flies, Ceratitis capitata (Wiedemann), braconid larval parasitoids, and the pteromalid pupal parasitoid Pachycrepoides vindemiae (Rondani) have been popular with Costa Rican agriculturalists (Camacho, 1992, 1998). The role that the exotic P. vindemiae plays in control remains to be demonstrated (see Guillen et al., 2001), and it is possible that such a generalist species with an extremely broad host range may negatively impact nontarget flies such as tachinid parasitoids and pollinators.

Coptera haywardi (Ogloblin) is a diapriid pupal parasitoid and one that has attractive qualities as a biological control agent in augmentative release programs (Sivinski et al., 1998). First among these attributes is its relative specificity. While most pupal parasitoids of Diptera are ectoparasitic generalists (e.g., P. vindemiae), the endoparasitic C. haywardi develops only in Tephritidae (Sivinski et al., 1998). To date, the outstanding difficulty facing its use in augmentation programs is its inability to develop in irradiated hosts (Menezes et al., 1998). Host irradiation is a means of eliminating unparasitized flies in many, although not all, fruit fly parasitoid mass-rearing systems (e.g., Sivinski et al., 1996).

C. haywardi has been collected from southern Mexico through much of South America (Ovruski et al., 2000). Across its range, it attacks the pupae of native Anastrepha spp. resulting from the larvae that develop in a number of native and introduced fruits (e.g., Lopez et al., 1999). In the laboratory it develops in the Mediterranean fruit fly (Sivinski et al., 1998), a species...
introduced into the New World during historical times (e.g., Gilstrap and Hart, 1987 and citations). To pursue the possibility of augmentative releases of C. haywardi to suppress Mediterranean fruit fly populations in Central America and elsewhere, it is first necessary to demonstrate its capacity to locate and parasitize host pupae under natural conditions, particularly under the conditions prevailing in coffee plantations, the principal source of Mediterranean fruit flies in Latin America.

Here we report on a test of the capacity of field-caged C. haywardi to attack Mediterranean fruit fly pupae artificially buried at two different depths. The field cages were located in a coffee plantation in the volcanic highlands of Guatemala, a region where augmentative braconid parasitoid releases have been employed in the recent past to suppress Mediterranean fruit fly populations (e.g., Sivinski et al., 2000a), and where future releases of C. haywardi might be reasonably contemplated. Because Mediterranean fruit fly pupae in the field cage were to be buried by hand, and because it is possible that larval burrowing into the soil to pupate may leave a chemical or physical trail exploited by foraging parasitoids, an experiment was performed in the laboratory (MOSCAMED Laboratorio La Aurora, Ciudad de Guatemala) to determine whether parasitism by C. haywardi of artificially buried pupae was similar to that inflicted on self-buried pupae.

**MATERIALS AND METHODS**

**Study Site**

Field cage tests on the ability of C. haywardi to parasitize Mediterranean fruit fly under seminatural conditions were carried out in Finca El Capetillo, ~12 km SW of Antigua, Departamento Sacatepéquez, Guatemala, at an altitude of 1388 m. C. haywardi is most abundant at relatively high altitudes in nature, and along one altitudinal transect in Mexico it was found at 600–1000 m (Sivinski et al., 2000b). A hygrotherograph, placed near the experimental site for the duration of the tests, recorded temperatures ranging from 9 to 26°C and relative humidities from 38 to 98%. The vegetation in the vicinity consists for the most part of coffee bushes grown under various species of shade trees.

**Study Insects**

C. haywardi were from a colony initiated with individuals collected in the vicinity of Xalapa, Veracruz, Mexico and maintained for ~2 years (~20 generations) at the MOSCAMED Aurora facility in Guatemala City. Mediterranean fruit flies were obtained at the MOSCAMED mass-rearing facility at El Pino, Guatemala, where they had been colonized for over 100 generations.

Laboratory Comparison of Parasitism in Artificially Buried and Self-Buried Pupae

To determine whether burrowing larvae leave physical or chemical trails that might be exploited by foraging C. haywardi, moistened soil was placed in 400-ml plastic cups to a depth of 10 mm. In 10 such cups, 25 >2-day-old Mediterranean fruit fly pupae were placed at the 5 mm level and then buried under 5 mm of soil. On the previous day, in a second set of 10 cups, 25 late instar larvae were placed on the surface of the soil and allowed to bury themselves and pupate. This schedule ensured that the artificially buried and self-buried pupae were of the same age. On the following day, 10 female C. haywardi adults were placed in the cups. Females were 5–10 days of age, had been caged with males, and had been previously given access to Mediterranean fruit fly pupae. Cups and insects were kept at ambient temperature (~25°C) and relative humidity (~65%). After 5 days, female parasitoids were removed, and the pupae from the top 5 mm of soil in the self-burial cups were separated from those occupying the bottom 5 mm. This insured that parasitism of the artificially buried pupae could be compared to that of self-buried pupae that had dwelt at least as deeply in the soil and were similarly vulnerable to burrowing C. haywardi.

Emergence of adult insects from the various cohorts (artificially buried at 5 mm, self-buried at <5 mm, and self-buried at >5 mm) was monitored for an additional 30 days. Adult insects were counted, and the mean percentage parasitism (adult parasitoids/adult flies + adult parasitoids), mean Mediterranean fruit fly survival (adult flies/total pupae, both surviving and nonsurviving), and percentage parasitoid production (adult parasitoids/total pupae, both surviving and nonsurviving) of the artificially buried and the >5 mm self-buried cohorts were compared by Student t tests following arcsine transformation of the square roots of the data (SAS Institute, 1988). The values for percentage parasitism and percentage parasitoid production differ because substantial numbers of puparia failed to produce an adult insect of either species. Because this mortality might have been due in part to unsuccessful parasitoid attacks (examinations of puparia known to have been stung did not reveal recognizable oviposition scars, so attacked but unparasitized pupae were difficult to identify), means of both percentage parasitism and percentage parasitoid production were analyzed.

**Field Cage Comparison of Parasitism of Pupae Buried at Different Depths**

The comparison of parasitism of pupae buried at different depths was carried out in 2.1-m-high × 2.5-m-wide field cages constructed from nylon mesh (12 threads/cm) and placed over a single, ~2-m-high, fruiting coffee bush. Soil from near the cages was placed ~5
TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage parasitism</th>
<th>Percentage fly survival</th>
<th>Percentage production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificially buried pupae at 5 mm</td>
<td>74.9 (6.2)</td>
<td>17.6 (4.3)</td>
<td>45.0 (3.6)</td>
</tr>
<tr>
<td>Self-buried pupae at 5-10 mm</td>
<td>81.8 (4.5)</td>
<td>11.3 (2.8)</td>
<td>56.8 (5.7)</td>
</tr>
<tr>
<td>t, df, P</td>
<td>0.90, 18, &gt;0.37</td>
<td>-1.12, 18, &gt;0.24</td>
<td>1.7, 18, &gt;0.09</td>
</tr>
</tbody>
</table>

Prior to the introduction of *C. haywardi*, food was provided in the form of a honey and water solution that had been poured into 40-cm-long plastic tubes sealed on both ends with cotton wicks. One tube was placed on the soil surface in each cage, and two others were suspended from branches of each of the coffee bushes. Subsequently, field-caged parasitoids were observed feeding on the solution. On the following day, the soil was remoistened and 100 male and 100 female *C. haywardi* adults were introduced into each cage. After 48 h the soil inside the cages was again remoistened. Five days after introducing the parasitoids the pupae were sifted from the soil. The cohorts of pupae from each of the cages were individually maintained at the Aurora Laboratory in a plastic 400-ml cup containing moistened saw dust. They were kept at ambient temperature (25°C) and relative humidity for 30 days. During this time adult insects emerging from the cups were collected and counted. Comparison of the mean parasitism rates at the two depths was accomplished by Student *t*-test following arcsine transformation of the square roots of the data (SAS Institute, 1988).

Field Cage Estimate of Mortality from Unsuccessful Parasitoid Attacks

To determine whether pupal mortality not due to the emergence of a parasitoid was due to unsuccessful parasitism attempts, a field cage experiment similar to the one above was conducted in same area. In this instance, 100 pairs of *C. haywardi* adults were placed in four field cages containing 248 pupae buried at 5 mm, while no parasitoids were added to another four cages with identical numbers of pupae. Subsequent treatment of the pupae was the same as described above. A comparison of the numbers of flies that died following incomplete development in the two treatments was performed through a contingency \( \chi^2 \) test (Zar, 1974).

RESULTS

Laboratory Comparison of Parasitism of Artificially Buried and Self-Buried Pupae

Percentage parasitism (adult parasitoids/total pupae) of Mediterranean fruit fly by *C. haywardi* was not significantly higher in cohorts of pupae that had buried themselves to depths of 5 mm or more than in those that had been artificially buried under 5 mm of soil (Table 1). Neither were there any significant differences in Mediterranean fruit fly survival (adult flies/total pupae, both surviving and nonsurviving) or parasitoid production (adult parasitoids/total pupae, both surviving and nonsurviving) (Table 1). These results are conservative. The self-buried pupae could be deeper/less vulnerable, but not shallower/more vulnerable, than the artificially buried and do not disprove the hypothesis that *C. haywardi* uses physical and/or chemical cues left by burrowing larvae to locate hosts. They do indicate that these clues are not necessary for successful foraging.
Field Cage Comparison of Parasitism of Pupae Buried at Different Depths

C. haywardi was able to locate and parasitize artificially buried pupae of Mediterranean fruit fly under the environmental conditions of a Guatemalan coffee plantation (Fig. 1). Mean parasitism of pupae buried at 5 mm (18.3%; SE = 3.8%) was higher than that of pupae buried at 15 mm (3.2%; SE = 1.1%) (t = 3.18; df = 7; P < 0.02). Significantly more pupae buried at 5 mm (mean = 76.6; SE = 13.2) had failed to eclose by the end of the experiment than had pupae buried at 15 mm (mean = 27.5; SE = 9.5) (t = −2.9; df = 7; P < 0.03).

Field Cage Estimate of Mortality from Unsuccessful Parasitoid Attacks

Abiotic factors, such as higher temperatures and lower humidities, could contribute to the greater non-parasitoid-emergence mortality at shallower depths. However, biotic factors, including more frequent unsuccessful parasitism attacks by C. haywardi, might also have played a role. In a subsequent experiment, pupae buried at 5 mm in field cages were either left by themselves or exposed to C. haywardi. Under both conditions, some adults did not emerge on schedule. Dissections revealed either fully developed pupae that had failed to emerge from the puparia or pupae that had failed to complete development. The number of undeveloped pupae relative to the number of developed pupae was significantly higher in flies that had been exposed to parasitism (Fig. 2; χ² = 5.7, P < 0.025). The difference in percentage arrested development between exposed and unexposed pupae (7.9%; percentage in flies exposed to parasitoids = 11.9% vs percentage in control flies = 4.0%) was similar to the mean level of successful parasitism during the experiment (6.0%; SE = 2.7). This suggests that mortality associated with unsuccessful parasitism may have been as high as or higher than that resulting from successful parasitoid attacks.

DISCUSSION

How well did C. haywardi perform in the field cage test and is this species a candidate for augmentative release? The answers depend in part on how closely the field cage environment resembled conditions typical of the field itself. Laboratory experiments found no evidence that C. haywardi required any chemical or physical trails left by burrowing larvae to discover its hosts. Therefore, artificial burial of test pupae should not have a priori led to unrealistic parasitism rates. Because the pupae were exposed throughout their period of greatest vulnerability, (2–7 days of age; Sivinski et al., 1998), it is unlikely that longer tenure in the field cages would have substantially increased parasitism. Thus, the field cage results were unlikely to grossly underestimate the mortality that might have been inflicted on a natural population under similar conditions.

On the other hand, two physical factors might contribute to the relatively high field cage parasitism and could potentially lead to overestimation of the effects of augmentation. First, soil type and compaction may influence the ability of a burrowing parasitoid to locate its hosts (see Hodgson et al., 1998). The soil in the field cages was disturbed to bury the pupae, and this might have made the flies more accessible than would typically be the case. We might argue that the soil is also likely to be disturbed by the burrowing activity of the

FIG. 1. The means and standard errors of mortality (uneclosed pupae) suffered by Mediterranean fruit fly pupae buried at different depths in field cages.

FIG. 2. The numbers of uneclosed pupae that either had completed development but failed to emerge or had failed to complete development following either exposure to Coptera haywardi in field cages or no exposure to the pupal parasitoid in field cages.
larvae, but at this time we have no data to compare the two soil states. A more compelling rebuttal is the finding of Guillen et al. (2001) that artificial compaction of soil had no effect on parasitism by C. haywardi in the laboratory. Second, while the burials were within the range of naturally occurring pupation depths, other larvae burrow more deeply prior to pupation. Given the drastic decrease in parasitism and associated mortality at the deeper of the two field cage burial depths, it is possible that numbers of naturally occurring pupae, located even further under the ground, are still less likely to be attacked (see Hodgson et al., 1998; Guillen et al., 2001).

In summary, there is no evidence that parasitism in the field cages was underestimated because of the removal of important foraging cues or that disturbed soils within the field cages led to overestimating parasitism. However, the depths of our artificially buried pupae probably do not reflect the entire depth range of naturally occurring pupae, and, to the extent that pupae occur at more than 15 mm under the surface, mortalities might be lower in natural habitats.

In addition to the mortality inflicted by C. haywardi, during both successful and unsuccessful attacks, the cost of producing and dispersing the parasitoids influences the practicality of its use in augmentative biological control. Even a small increase in Mediterranean fruit fly parasitism might be economically justified if the costs to produce it are very low. For example, it is possible to integrate the rearing of C. haywardi into the mass-rearing of braconid larval parasitoids (Menezes et al., 1998). After exposure to the commonly mass-reared braconid Diaclasmimorphe longicaudata, tephritid larvae have been allowed to pupate and then presented to C. haywardi. The combined numbers of the two species in the next generation approximately doubled parasitoid production. Because the pupae that were parasitized by C. haywardi would have otherwise produced only flies or been discarded, there were no costs to providing hosts. The costs of the second exposure to parasitism, in terms of labor, cages, and adult parasitoid maintenance, remain to be determined.

In conclusion, it can be difficult to decide whether to pursue further investigations into the use of a particular biological control agent. Part of the difficulty is the interpretation of parasitism rates. That is, can data gathered under one set of circumstances, particularly semiarificial circumstances at what may be high parasitoid densities, be extrapolated to predict performance in a variety of other habitats and what level of mortality is needed to significantly suppress the growth of pest populations under these various conditions? While these may be laborious questions to address in the case of parasitoids of subtropical dipteran pupae, we wish to emphasize again that augmentation of an efficient parasitoid species could be a valuable addition to fruit fly biological control and well worth the labor (Sivinski, 1996).

Given the potential value of augmentative releases of pupal parasitoids, it is our impression, from field collections (Lopez et al., 1999), laboratory tests (Guillen et al., 2001), and the above field cage tests, that C. haywardi is an unusually attractive candidate for larger-scale testing of mixed larval and pupal parasitoid releases (see the comparison with P. vindemiae in Guillen et al., 2001). This is particularly true if the apparently substantial host mortality due to unsuccessful attacks is added to parasitism. We propose that research into augmentative biological control employing C. haywardi concentrate on determining effective release rates and increasing rearing efficiency (i.e., further decreases in production costs and quality control).

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