Augmented releases of adult Diachasmimorpha longicaudata (Ashmead) to control the Caribbean fruit fly Anastrepha suspensa (Loew) were carried out in two urban Florida locations: Key Biscayne and Clewiston. In the former, 60,000 adult parasitoids/km²/week were released near host trees. Fruit fly populations during winter and much of spring appeared to be suppressed to 5–10% of levels found during a previous survey of the Key Biscayne site and a neighboring control area. Parasitism levels were difficult to quantify, but were generally higher in release areas. Sampling difficulties also create problems in demonstrating causation. The Clewiston releases were less consistent in number and size, but previous to their temporary suppression, Caribbean fruit flies were apparently also substantially suppressed. A second species of braconid, Doryctobracon areolatus (Szepligeti), common in the Clewiston area control sites, was apparently nearly eliminated from the release site. Sex ratios of wasps recovered from sampled fruit were male-biased in both release areas though the factor(s) responsible are unknown. Augmented releases of D. longicaudata may be particularly useful in suppressing Caribbean fruit fly populations in areas where more traditional methods such as insecticide-bait sprays and sterile male releases are impractical.

KEY WORDS: biological control; augmented release; Anastrepha suspensa; Diachasmimorpha longicaudata; Doryctobracon areolatus; Tephritidae; Braconidae.

INTRODUCTION

Populations of several tephritid fruit fly pests have been reduced by the introduction of natural enemies (reviewed by Gilstrap and Hart, 1987). For example, numbers of oriental fruit fly Bactrocera (= Dacus) dorsalis (Hendel) in Hawaii during the 1940s and 1950s reached only 6–8% of their epidemic peak following the establishment of several braconid parasitoids (Newell and Haramoto, 1968). In Florida, Caribbean fruit fly, Anastrepha suspensa (Loew), populations were reduced ca. 40% by the introduction of the opine braconid Diachasmimorpha longicaudata (Ashmead) (Baranowski, 1987). Originally recovered from Bactrocera species, D. longicaudata is native to Malaysia, India, New Britain, Borneo, Saipan, and the Phillipine Islands (Bess, 1961; Clausen et al., 1965). It is a late-instar larval–pupal parasitoid that locates maggots within fruit by their feeding sounds (Lawrence, 1981). In the southern peninsula of Florida, D. longicaudata accounts for ca. 95% of Caribbean fruit fly parasitism (Sivinski, 1991). To the north, near Lake Okeechobee, it is frequently surpassed in numbers by another introduced opine Doryctobracon areolatus (Szepligeti) (Sivinski et al., unpublished data; see Baranowski et al., 1993).

While introduced parasitoids have caused considerable mortality, A. suspensa remains a threat to the export of citrus to California, Arizona, and Japan and makes the commercial growing of guava (Psidium guajava L.) and peaches (Prunus persica Batsch) prohibitively expensive. The reasons for insufficient levels of control are probably those that inhibit the efficacy of fruit fly parasitoids around the world (Debouzie, 1989; Wharton, 1989). These include relatively low fecundity of the parasitoid, and their poor tracking of fly populations over space and time due to dispersal difficulties and, in some cases, lack of diapause (see Baranowski et al., 1993).
A means of mitigating these difficulties is to augment parasitoid populations when and where needed. The infestation of olives by overwintering Bactrocera oleae (Gmel.) in Corfu, Greece is limited by spring time releases of Pyssalina (= Opious) concors (Szepligeti) (Kapatos et al., 1977). In Hawaii, augmented releases of Diachasmimorpha tryoni (Cameron) significantly suppressed populations of Mediterranean fruit fly (Ceratitis capitata (Wiedemann)) (Wong et al., 1991, 1992).

We report here on the augmented release of Diachasmimorpha longicaudata directed against the Caribbean fruit fly. Unlike the previous Hawaiian releases, adult parasitoids rather than parasitized fly puparia were placed in the field. We used, for the first time on a large scale, the irradiation of fly larvae prior to exposure to parasitoids to prevent a mixture of fertile flies and parasitoids destined for release (Sivinski and Smittle, 1990; R. E. Burns, unpublished data).

**METHODS**

Parasitoids were released in Key Biscayne, Florida, a low-lying and partially forested island approximately 1 km off the coast of metropolitan Miami. The center of the island is a 5.3-km² residential area with a high density of fruit fly hosts. These included 20 guava, 334 Surinam cherry (Eugenia uniflora L.), 25 loquat (Eriobotrya japonica (Thumb.) Lindl.), and several hundred tropical almond trees (Terminalia catappa L.). Releases were also made at Clewiston, Florida, a town on the southwest shore of Lake Okeechobee. It is an “ecological island” surrounded by sugar cane plantations with only an occasional host, such as wild guavas, within 2 km. The 13.7-km² release area contained 125 Surinam cherry, 59 loquat, and 51 guava plants.

Because releases were made at or near fruiting host plants, the condition of the plants was monitored biweekly and this information was used to calculate the proportion of the wasps to be put in any particular location within the release area. Thus, specific release sites varied in their number and location from week to week. The simultaneous control for Key Biscayne consisted of an identical set of “trap tree” species in suburban areas of South Miami, Florida, on the mainland side of Biscayne Bay. Immokalee and LaBelle, Florida, towns within 60 km of Clewiston, served as controls for the Clewiston release site. Fly populations in Key Biscayne and Clewiston were monitored in the manner described below for 1 year prior to any treatment.

Glass McPhail traps, the standard means of sampling Anastrepha spp., were filled with a solution of 300–500 ml of water and 20 g of a torula yeast and borax bait and hung in eight of each of the previously mentioned fruit hosts. These were visited weekly when flies were sexed and counted and traps were cleaned and refilled. At the same time, insects present in the fruit of the trap trees were sampled in the following manner. Ripe fruit from trees or freshly fallen fruit without insect emergence holes was taken. To avoid influencing local insect density by oversampling the number of fruits at a site, no more than 10% of fruits in all stages of development or 50% of ripe fruits were taken. If fewer than 10 fruits were present, none were taken. To increase sample sizes, up to 10 other plants of a species were sampled on any given week. In Clewiston and its control areas this sometimes included Calamondin (X Citrofortunella mitis J. Ingram and H. E. Moore) and grapefruit (Citrus paradisi Mack). Fruits from a particular site and date were weighed to the nearest gram and then placed individually on moist vermiculite in plastic cups at 25–27°C and 60–80% humidity. After 3 days, larvae that had left the fruit were separated and the fruit was returned to the vermiculite. Seven days after sampling, the fruits were discarded and additional pupae in the vermiculite were recorded. Pupae were held in vermiculite for a period of 4 weeks. The adult insects were then counted and identified.

The two periods of pupal removal each served a special purpose. Because only mature larvae are susceptible to attack by the braconid parasitoids present in Florida, samples of fruit containing younger larvae underestimate potential parasitism. Ideally, only those maggots leaving the fruit at sampling time or collected as pupae in the field are assured of having been completely exposed to larval parasitoids. This sampling technique proved impractical on a large scale and the 3-day larval emergence sample was a compromise. Larvae leaving fruit within the 3-day period were probably in their third instar at the time of collection (under standard rearing conditions, R. E. Burns, unpublished data). Thus, they were old enough to have been exposed to attack, although they did not suffer the entire period of vulnerability experienced by larvae left in the field (see also Lathrop and Newton, 1933 and Kapatos et al., 1977). The 7-day-long period, corresponding to the Caribbean fruit fly larval developmental period, gave an estimate of the total number of larvae infesting a particular fruit sample.

The numbers of parasitoids released were based on early versions of Knippling's (1992) model for Caribbean fruit fly control. We initially attempted releases of at least four parasitoids for every fly and preferred a 10:1 ratio. In practice, it is difficult to quantitatively estimate or predict the number of fruit present and, with previous knowledge of infestation levels, determined the larval fly population and predicted the adult population. We began releasing an estimated 60,000 adult parasitoids of both sexes/km²/
week in Key Biscayne and maintained this level throughout the study period (January 1-August 14, 1992; when Hurricane Andrew eliminated the host trees, holding facilities, and fruit fly population). At the same time, releases in Clewiston began in the range of an estimated 20,000/km²/week but numbers fluctuated with parasitoid availability and releases were temporarily suspended in late March because of rearing difficulties. For the purposes of this report, Clewiston will only be considered from January through April. These release rates in the two sites bracket Knipling's (1992) estimate of the ca. 30,000 parasitoids/km²/week required to suppress and eventually eradicate a low population of Caribbean fruit flies. The actual ratios of parasitoids to flies, even in Key Biscayne with its steady release rate, almost certainly varied with changes in fly populations over time.

Parasitoids were reared by the Florida Division of Plant Industry in a manner derived from that of Wong and Ramadan (1992) (Burns, 1991). Prior to presentation to parasitoids, fly larvae were exposed to 4 kr of gamma radiation. This was done to prevent the eclosion of adult flies in lots of parasitoids (Sivinski and Smittle, 1990). In order that adult parasitoids could be released, pupae were shipped overnight to the IFAS Tropical Research and Education Center, Homestead, Florida and the Division of Plant Industry facility in LaBelle, Florida. Here, pupae were held during a 5- to 7-day emergence period (with the first emergences occurring on Day 1). Adults were fed throughout on a diet of honey and water. Cages consisted of screen bags (32 x 32 cm) that were loaded directly in vans and taken to release sites (Sivinski et al., 1994).

Quality control of parasitoids consisted of percent emergence and testing the ability to parasitize fly larvae under both laboratory and seminatural conditions in field cages. In tests of ability to parasitize there were no significant differences between shipped parasitoids, parasitoids reared at the Homestead release site, and wild parasitoids obtained from the field outside release areas (Sivinski et al., in preparation). Tests were performed at the Homestead rearing center and the Division of Plant Industry, Gainesville.

During the 12 months previous to the adult parasitoid release in Clewiston and for a 6-month period, 6 months prior to adult releases in Key Biscayne, parasitized pupae were placed in fruiting host trees. This was done at a rate similar to that for adult parasitoid releases and in a manner adopted from that of Wong et al. (1991). A 1-gallon (3.8 liter) plastic bucket and lid were painted silver to reflect light and moderate interior temperatures. Twenty 1.5-cm holes were drilled around its circumference, 2 cm below the rim. Inside were placed one to six plastic cups containing up to 100 ml of parasitized pupae and paper toweling. The paper prevented shifting and spillage of pupae and provided a resting place for eclosing adults. Pupae were put in the field the day before the first estimated adult emergences. Buckets were hung in shaded areas and the connecting wire smeared with a sticky material, "Tack-Trap" (Animal Repellents, Inc., Griffin, GA), to prevent ants and other predators from entering the bucket. New buckets and pupae were set out weekly but each bucket was left for 2 weeks to ensure maximum parasitoid emergence. Thus, a host tree typically bore two buckets containing different age pupae. Buckets kept in cages at holding sites in Homestead and LaBelle, but otherwise set up identically to those taken to the field, showed emergence rates similar to those found in pupae kept in the laboratory following shipping (Sivinski et al., in preparation). Monitoring the effects of placing parasitized pupae in the field on adult fly numbers, parasitism, and larval density was done in a manner identical to that already described.

RESULTS

After 5 weeks of adult parasitoid releases, fruit fly populations in Key Biscayne were lower than those of controls (often 5% or less) and remained consistently below those of the South Miami control area (Figs. 1 and 2). A similar pattern occurred when comparisons were made of fruit fly numbers in Key Biscayne in 1990 when no controls were applied to numbers in 1992 during parasitoid releases (Figs. 3 and 4). An unusual freeze occurred in Key Biscayne during December 1989 and the low fly numbers during the first months of 1990 may reflect a loss of early season fruit.

The situation in Clewiston was more complex. Parasitoid releases in Clewiston were erratic in size due to parasitoid supply problems and were temporarily suspended at one point in March. However, up to that point, fly numbers in the release area were considerably below those in control areas (which historically harbored fewer flies than the release site; Holler and Harris, 1993, Fig. 5).

As noted earlier, accurate levels of parasitism for fruit flies are difficult to calculate, but in general, parasitoids were recovered more often in release areas than in control areas (Table 1). An exception occurs for Surinam cherry. This may be due to a possible density-dependent foraging success on flies infesting Surinam cherries by D. longicaudata. Higher fruit infestation levels were correlated with higher parasitism in the control area (Fig. 6, but parasitism levels tended to be higher in release areas compared to similar fruit infestation levels in the control area. Intercepts of the two slopes were significantly different (F = 6.5; P = 0.008).

The sex ratios of D. longicaudata reared from fruit collected in both release areas was male-biased compared to those in control locations (Key Biscayne = 65% male (0.04) vs Miami = 48% (0.04); t = 3.02, df = 51,
Infestation of fruit by fly larvae as reflected in maggots per gram of host fruit was consistently low during the early part of the year in Clewiston but showed more fluctuation in Key Biscayne (Figs. 7 and 8). However, fruit species showed different infestation levels, with the most dramatic difference occurring in loquat (Fig. 9).

Placing parasitized fruit fly pupae in emergence buckets hung on host trees proved less effective in Florida than in a previous study in Hawaii (see Wong et al., 1991, 1992). While it is difficult to predict what might have occurred to fly populations in Clewiston...
had the area not been exposed to parasitized pupae, there is no suggestion of their efficacy. For example, peak fly numbers were higher during the 1991 treatment year than they were in the previous year when no parasitoids were released (x 108/trap/week vs x 28/trap/week). During the early part of the year when adult parasitoid releases in Clewiston appeared to have a substantial effect, flies were more abundant when the parasitoid-release treatment consisted of parasitized pupae than adult parasitoids (Fig. 10). However, the fly population was somewhat depressed compared to the Immokalee-LaBelle control areas at the beginning of 1992; i.e., the end of pupal releases and the start of adult parasitoid releases in Clewiston (see Fig. 5).

**FIG. 3.** The average number of Caribbean fruit flies captured in Key Biscayne during 1990 when no treatment was applied and during 1992 when adult parasites were released.

**FIG. 4.** The ratio of Caribbean fruit flies captured per week in Key Biscayne during 1990 when no treatment was applied and during 1992 when adult parasitized were released.
DISCUSSION

Our results provide evidence that the integrated pest management of fruit flies can benefit from augmented parasitoid releases; though causation between releases and pest suppression is difficult to prove due to inherent problems in sampling parasitism. For instance, Florida faces difficulties in exporting citrus to Arizona, Texas, California, and particularly, Japan because of possible infestation by Caribbean fruit flies. In the past, shipments were fumigated with ethylene dibromide (EDB) to guarantee they contained no fly larvae. EDB has been found to be carcinogenic, and its use has been suspended by the EPA. In response, the state has initiated a fly-free zone protocol whereby a trapping system assures that no fly is present in citrus groves during harvest. Thus, growers can certify their harvest as free of Caribbean fruit fly. The largest populations of A. suspensa occur in the “dooryard” fruit examined in this study and are typically found in urban and suburban areas. Fly-free zones are in the greatest danger when adjacent to these areas. If urban fly populations can be suppressed to the point that they do not pose a threat to agricultural areas, then fly-free zones might be more easily protected or expanded.

There are special problems associated with such a suppression program. The traditional method of fruit fly control, bait sprays containing malathion, is unpopular in inhabited areas, particularly if repeated indefi-

![FIG. 5.](image1)
The average numbers of Caribbean fruit flies captured in McPhail traps per week in Clewiston during adult parasite releases and in control areas.

![FIG. 6.](image2)
The proportion of Caribbean fruit flies parasitized by Diachasmimorpha longicaudata over the density of flies in Surinam cherry fruit. Black circles represent the south Miami control and white circles the Key Biscayne adult parasite release.

### TABLE 1

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<tr>
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<th>Key Biscayne release</th>
<th>South Miami control</th>
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<tbody>
<tr>
<td>Guava</td>
<td>0.53 (0.15) 198</td>
<td>0.38 (0.16) 160</td>
</tr>
<tr>
<td>Loquat</td>
<td>1.0 (0) 372</td>
<td>0.69 (0.06) 418</td>
</tr>
<tr>
<td>Tropical almond</td>
<td>0.58 (0.04) 827</td>
<td>0.11 (0.11) 133</td>
</tr>
<tr>
<td>Surinam cherry</td>
<td>0.38 (0.05) 2971</td>
<td>0.48 (0.05) 1138</td>
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nitely. An alternative, the release of sterile flies for population suppression, has raised objections from citrus producers. They are concerned that released flies will be captured in traps used to certify their groves as fly-free. Difficulties or delays in identification of irradiated flies could jeopardize their shipments. An alternative that raises none of the above objections is the augmented release of parasitoids.

In addition to situations where augmented parasitoid releases answer a special need, this study suggests that parasitoids could fill a role in fruit fly eradication programs. Knipling (1992) modeled releases of parasitoids alone and combined releases of sterile flies and parasitoids on pest populations and found the two controls together were more effective than either one separately. Female flies that escape mating with sterile males produce offspring vulnerable to high densities of parasitoids. A more general model by Barclay (1987) reached a similar conclusion. In a series of sequential experiments in Hawaii, Wong et al. (1992) found 1.3× reductions in Mediterranean fruit fly numbers following releases of D. tryoni and a 4.7× reduction after releases of sterile males. A 12.3-fold reduction occurred after combined releases. There may be a similar or even greater potential for control in the integrated use of D. longicaudata and sterile Anastrepha suspensa.

One point of departure between Knipling’s parasitoid-
The intended purpose of Caribbean fruit fly suppression is to form a buffer between high pest populations and a crop rather than directly lower infestation in the crop itself. The male-biased sex ratios of parasitoids reared from fruit in release areas suggests that improvements can be made in either release rates or eclosion/adult handling techniques. Such sex ratios among parasitoids are not uncommon in the laboratory (e.g., Rotary and Gerling, 1973) and are generally thought to be due to either the failure of some females to mate, or high densities that result in competitive interference, leading females to produce male eggs. In addition, males are also more likely to survive the superparasitism that could occur under crowded conditions (Wylie, 1966; Viktorov and Kochetova, 1972). Which, if any, of these factors or combination of factors is responsible for the male-biased sex ratio in the release areas is unknown. Density alone is not likely to be the critical factor. There is a tendency for female-biased sex ratios in the unnaturally high densities that occur during mass-rearing of D. longicaudata (e.g., Wong and Ramadan, 1992; R. Burns, unpublished data). A laboratory study of the related Psyttalia concolor failed to find a density effect on progeny sex ratio (Avilla and Albajes, 1983). In the control areas there was no relationship between an estimate of the relative abundance of parasitoids to fly larvae (percent parasitism) and the sex ratio of weekly samples of parasitoids reared from the field (South Miami, \( r^2 = 0.02, P = 0.47 \); Immokalee, \( r^2 = 0.0002, P = 0.93 \); LaBelle, \( r^2 = 0.009, P = 0.56 \)).

A second effect on the parasitoid fauna was an apparent drastic decline of Doryctobracon areolatus in the Clewiston release areas. Over the adult release year this insect constituted 63 and 85% of the parasitoid fauna in the neighboring Immokalee and LaBelle controls. During the same period only 1.8% of the parasitoids in Clewiston were D. areolata. The effect of simplification of the parasitoid fauna in Florida ecosystems is unknown, although there is a precedent for such replacement by successively more competitive braconids introduced into Hawaii for tephritid control (see Gilstrap and Hart, 1987 and references).

It is not clear why the placement of parasitized pupae had such little effect compared to adult parasitoid releases. A previous success using pupae in Hawaii employed a different, but closely related, parasitoid, Diachasmimorpha tryoni (Wong et al., 1991). We have little more than anecdotes to offer for the Florida situation. Despite efforts to exclude predators, ants, frogs, wasps, and cockroaches were not uncommonly found in or near release buckets. Parasitoids fed, sheltered, and allowed to mate in the laboratory would also be less likely to suffer mortality during the first days of life than those that need to disperse to find food sources and sexual partners after eclosing in the field.
A serious problem facing biological control studies of fruit flies is the difficulty in estimating parasitism levels. As noted earlier, removal of fruit from the field during sampling decreases the period in which larvae are susceptible to attack. The result is an underestimate of parasitism. This is further complicated by the common phenomenon of different age larvae occupying a piece of fruit so that some flies emerging from fruit were never vulnerable to parasitoids. Our compromise of considering larvae that had left fruit within 3 days of sampling yielded data that were generally consistent with expectations from an augmented release. Wong et al. (1991) dissected fruit and removed mature larvae for rearing. They obtained perhaps more favorable results, finding 47% parasitism by Diachasmimorpha tryoni in a release area compared to 14.2% in a control. A more ideal solution to the difficulty of measuring parasitism is to expose infested fruit in the field and collect pupae that have suffered all the dangers that a tephritid would encounter during its maturation. In a small-scale test of field-exposed fruit using guava, parasitism increased from ~14% (fruit removed from the field and held on vermiculite) to ~80% (fruit left in the field on vermiculite) (Sivinski and Baranowski; unpublished data). Unfortunately, an attempt to use this method on a wide scale and under harsh environmental conditions proved difficult. The need for a practical technique remains a priority.

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