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AUGMENTATION OF PARASITOIDS FOR BIOLOGICAL CONTROL OF CITRUS BLACKFLY IN SOUTHERN TEXAS

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ABSTRACT

Two parasitoid species, *Amitus hesperidum* Silvestri and *Encarsia opulenta* (Silvestri), were released in an augmentative program to control citrus blackfly, *Aleurocanthus woglumi* Ashby, in the citrus growing areas of southern Texas. Releases were made with laboratory-reared and field insectary parasitoids. Six citrus groves were closely monitored, and evaluations made during and after releases suggested that both parasitoid species became reestablished and exerted control over pest populations. Dissection of citrus blackfly immatures led us to suggest that *E. opulenta* increased in larger numbers than *A. hesperidum*, and that a stable host-natural enemy relationship became established.

Key Words: *Aleurocanthus woglumi*, *Amitus hesperidum*, *Encarsia opulenta*, augmentative biological control

RESUMEN

Dos especies de parasitoides, *Amitus hesperidum* Silvestri y *Encarsia opulenta* (Silvestri) fueron liberadas en un programa de aumentó para el control de la mosca prieta de los cítricos. *Aleurocanthus woglumi* Ashby, en áreas donde siembran los citricos en el sur de Texas. Las liberaciones fueron hechas usando parasitoides criados en el laboratorio y los del insectario del campo. Un monitoreo preciso de seis huertos de cítricos fue hecho, y las evaluaciones hechas durante y después de la liberación que sugerieron que ambas especies de parasitoides se re-establecieron y ejercieron un control sobre la población de la plaga. La disección de inmaduras de la mosca prieta de los cítricos tambien sugerió que el *E. opulenta* se aumento en números mas altos que el *A. hesperidum*, y que una relación estable entre el hospedero y el enemigo natural fue establecida.

The citrus blackfly, *Aleurocanthus woglumi* Ashby, first invaded the Lower Rio Grande Valley of Texas in 1955 on residential citrus (Smith et al. 1964), and again near Brownsville in 1971 in both residential citrus and commercial groves (Hart et al. 1973). An augmentative biological control program to establish parasitoids was initiated in 1974 with release of three species, *Amitus hesperidum* Silvestri (Hymenoptera: Platygasteridae), *Encarsia* (= *Prospaltella*) *opulenta* (Silvestri), and *E. clypealis* (Silvestri) (Hymenoptera: Aphelinidae). These releases were made from laboratory-reared and field-collected cultures in Mexico (Hart 1978). Evaluations undertaken from 1977-1982 indicated a widespread distribution of *E. opulenta*, but few *A. hesperidum*, and no *E. clypealis*, suggesting local competitive displacement by *E. opulenta* in groves with effective parasitoid regulation (Summy et al. 1983).

Citrus blackfly population densities remained stable under excellent biological control until the mid 1980s (Summy et al. 1983). Following a severe freeze in December 1983, citrus blackfly densities surged while concomitant parasitoid densities apparently remained low. Citrus blackfly populations reached damaging levels during the 1988 and 1989 seasons, especially in central valley groves (French et al. 1990). A biological control program of parasitoid augmentation was initiated in June 1989, and results suggested reestablishment of *E. opulenta* and *A. hesperidum* (Meagher et al. 1991). However, another severe freeze in December 1989 halted evaluation efforts.

Although most commercial groves had no citrus blackfly populations after the freeze (unpublished data), populations were discovered in citrus nurseries and on residential citrus trees in early 1990 (French & Meagher 1992). Newly planted and commercial groves located near residential areas soon were infested with dense, largely unparasitized citrus blackfly populations (unpublished data). An augmentation program was initiated in 1992 to increase biological control efficacy in commercial citrus groves. This was accomplished by releasing parasitoids into residential citrus so that "field insectaries" could be developed. Commercial groves were then sampled with yellow sticky traps (Harlan et al. 1979; Summy et al. 1986) and leaf observation, so that groves containing large citrus blackfly densities could be identified as release sites. The final step was to transfer laboratory- and field insectary-produced parasitoids into infested groves. This report describes the seasonal progression of the host and its natural enemies in commercial citrus groves in southern Texas.

MATERIALS AND METHODS

Groves Sampled

Grapefruit and orange groves selected for this study were located throughout the Lower Rio Grande Valley in both Cameron and Hidalgo counties. They included groves near Bayview (10 ha., 'Rio Red' grapefruit, sampled 21 July 1992-21 March 1995; 7.2 ha., 'Marrs' and 'Valencia' orange, sampled 23 November 1993-21 March 1995), Donna (16.2 ha., 'Ruby Red' and 'Star Ruby' grapefruit, 27 July 1992-22 February 1994), Mercedes (16.2 ha., 'Ruby Red' grapefruit and 'Valencia' orange, 7 December 1993-21 March 1995), Edinburg (8.3 ha., 'Ruby Red' grapefruit, 22 July 1992-12 April 1994), Mission-grapefruit (12.1 ha., 'Rio Red' grapefruit, 15 July 1992-22 March 1994), and Mission-orange (3.4 ha., 'Marrs' orange, 15 July 1992-22 March 1994).

Parasitoid Releases

Augmentation of both *A. hesperidum* and *E. opulenta* was accomplished with laboratory-produced and field-collected specimens from Florida (Florida Department of Agriculture, Division of Plant Industry, Gainesville) and field insectary-produced parasitoids from Texas. Both the cup and paper bag methods of French et al. (1990) and Meagher et al. (1991) were used to disperse parasitoids. From January 1992-February 1993, over 92,000 *A. hesperidum* and 18,000 *E. opulenta* were released throughout the citrus growing region, and selected releases of both species of parasitoids were made later during 1993.

Citrus Blackfly Sampling

Citrus blackfly infestation and parasitization levels were sampled by two techniques. First, the percentage of leaves infested with citrus blackfly was determined by examining four branches of eight trees. The total number of leaves and number with live citrus blackfly immature stages were recorded. Selection of each branch was based on directional orientation (quadrants: southeast, southwest, northwest, northeast). Five of the trees were 'station' trees that were sampled each time; an additional three trees were selected randomly each sample date. This sampling technique was not conducted in the Bayview-orange grove. Analysis of variance (PROC GLM, LSD mean separation test, SAS Institute 1995) was used to examine variation among trees or among quadrants. Parasitization was calculated by dissecting and examining a subsample of at least 100 fourth stage nymphs ('pupae').

Beginning in late 1993, an additional sampling technique was developed to provide a closer examination of citrus blackfly parasitization. These samples were taken in the grapefruit and orange sections of the Bayview grove and in the Mercedes grove. From each tree, one hundred leaves that contained citrus blackfly pupae were collected. From this collection, ten leaves containing pupae were selected and returned to the laboratory for dissection. Each pupa was categorized as live, dead, or emerged citrus blackfly; or live, dead, or emerged parasitoid. Dead citrus blackfly and parasitoids were represented by desiccated remains. Emerged individuals were represented by either the characteristic pupal exuvia split by citrus blackfly or circular exit holes created by parasitoids. Parasitization by species was not identified, although exit hole numbers per pupa at times provided species information. Generally, two exit holes indicated the presence of *A. hesperidum*, although rarely an individual larva of both species was found live in one host pupa. Parasitoid adults searching on leaves were noted during sampling.

RESULTS

Citrus blackfly-infested leaves exhibited significant inter- and intra-tree variation when each grove was analyzed individually ($P < 0.0001$; $P < 0.003$, respectively). The northwest quadrant of the tree always contained more infested leaves than the southeast quadrant (Table 1). These results are in agreement with previous studies in Florida and Texas which suggested high inter-tree variation (Dowell & Cherry 1981), although Gilstrap et al. (1980) found more citrus blackfly in the northwestern quadrant of the tree. Although our sampling described clear quadrant differences in population level, we agree with other researchers that collection of leaves for population sampling or parasitoid efficacy should be from all tree areas, especially when pest densities are low (Cherry & Fitzpatrick 1979; Gilstrap et al. 1980).

The Donna, Edinburg and two Mission grove locations selected for monitoring in 1992 had initial citrus blackfly populations below 10% infested leaves (Fig. 1). Leaf samples from these groves showed maximum citrus blackfly infestations ranging from 44.5 to 82.1%, but leaf samples taken at the end of sampling showed little to no citrus blackfly infestation (Table 2). Parasitization from both species ranged from 62.1-100% in these groves, but by the end of sampling in 1994 was much reduced due to the scarcity of hosts.

Population dynamics of host and parasitization in the Bayview grove showed low numbers of

TABLE 1. PERCENT CITRUS BLACKFLY-INFESTED LEAVES BY TREE QUADRANT FROM CITRUS GROVES IN THE LOWER RIO GRANDE VALLEY, TEXAS, 1992-1995.

			Tree quadrant			
Grove	*df*	F	Northwest	Northeast	Southwest	Southeast
Bayview	3, 1271	7.2	18.2 ± 1.5 a	15.3 ± 1.4 b	16.0 ± 1.4 ab	12.2 ± 1.3 c
Donna	3, 775	30.1	19.9 ± 1.7 a	17.9 ± 1.7 a	13.3 ± 1.3 b	8.4 ± 1.1 c
Mercedes	3, 491	5.6	81.1 ± 2.2 a	80.7 ± 2.2 a	77.9 ± 2.6 a	73.0 ± 2.8 b
Edinburg	3, 759	21.0	23.1 ± 1.9 a	20.2 ± 1.8 b	19.9 ± 1.7 b	12.8 ± 1.4 c
Mission-grapefruit	3, 775	4.9	32.7 ± 2.5 a	31.8 ± 2.5 a	31.1 ± 2.5 a	28.8 ± 2.4 ab
Mission-orange	3, 806	3.1	19.2 ± 1.7 a	17.9 ± 1.6 ab	15.7 ± 1.6 b	15.8 ± 1.6 b

Means (± SE) within the same row followed by the same letter are not significantly different, LSD ($P > 0.05$).

citrus blackfly initially when sampled in summer 1992 (Fig. 2). Citrus blackfly populations rose to over 60% leaves infested by late fall 1993, until increasing parasitization appeared to reduce host populations. A closer examination of grapefruit trees showed proportionally high levels of para-

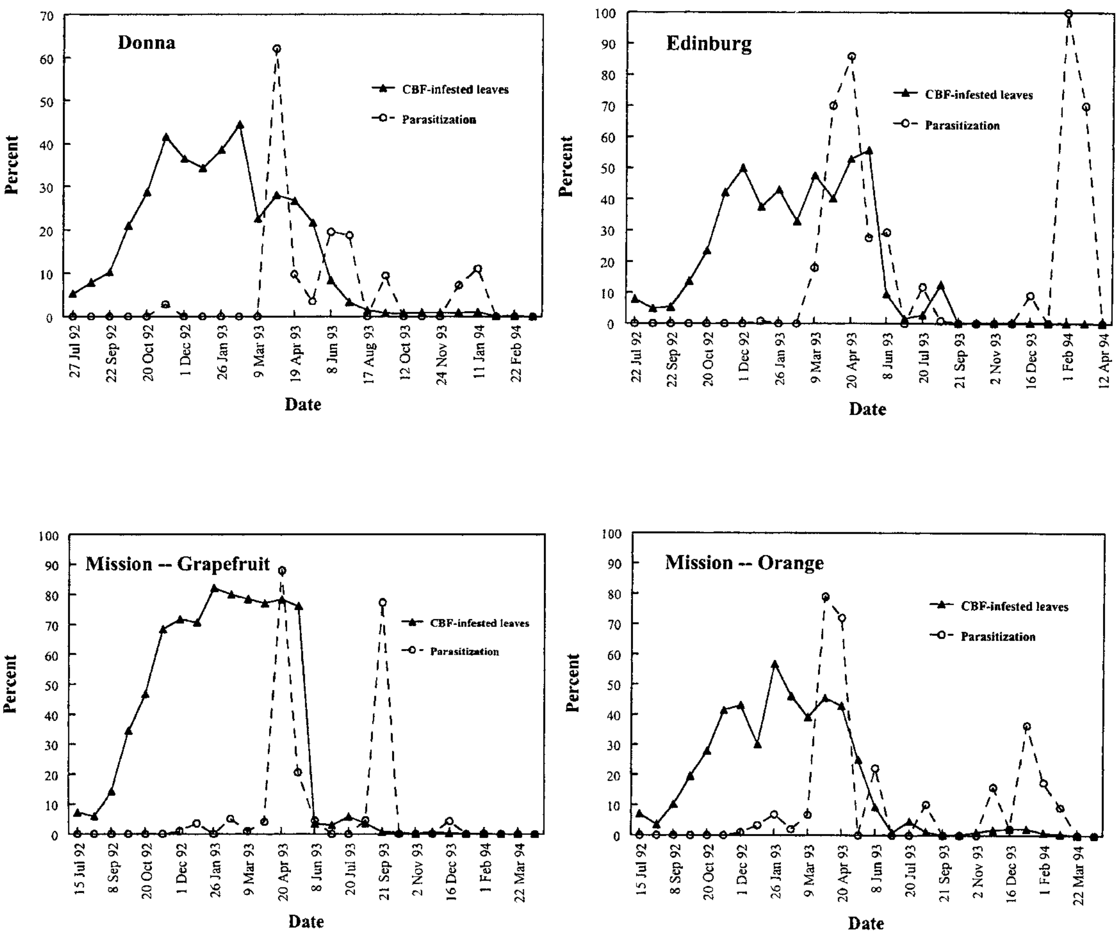


Fig. 1. Population densities of citrus blackfly (percent leaves infested) and parasitization (percent fourth stage parasitized) due to *Amitus hesperidum* and *Encarsia opulenta*, in three Lower Rio Grande Valley, Texas citrus groves, 1992-1994.

TABLE 2. HIGHEST AND FINAL PERCENT CITRUS BLACKFLY-INFESTED LEAVES AND PERCENT PARASITIZATION BY *E. OPULENTA* AND *A. HESPERIDUM* FROM GRAPEFRUIT AND ORANGE GROVES IN THE LOWER RIO GRANDE VALLEY, TEXAS, 1992-1995.

Grove	Highest infested leaves (%)	Highest parasitization (%)	Final infested leaves (%)	Final parasitization (%)
Bayview	65.5 ± 5.9	79.3	2.8 ± 0.5	48.7
Donna	44.5 ± 5.4	62.1	0.03 ± 0.03	0
Mercedes	100.0 ± 0	92.9	68.1 ± 3.1	59.2
Edinburg	55.7 ± 4.3	100.0	0	0
Mission-grapefruit	82.1 ± 2.5	88.0	0	0
Mission-orange	56.8 ± 5.2	79.0	0	0

sitization occurred during late winter 1993 and spring 1994, with over 50 immature parasitoids per leaf found in the 15 February, 29 March, and 19 April samples, and 65 per leaf in the 20 June sample (Fig. 3 a, b). Adults from many of the parasitized pupae during this period had already emerged. Adult *A. hesperidum* was the predominate species observed on leaves during early sampling, and although *E. opulenta* adults were present, their numbers did not increase until fall 1994. The highest citrus blackfly population density also occurred during spring and summer 1994, with a peak of 109.7 ± 30 live immatures found in the 10 May sample (Fig. 3a). The grower, without our recommendation, applied an unknown insecticide in February and May, resulting in mortality of both citrus blackfly and parasitoids (20.7 ± 6.8 dead parasitoid immatures per leaf, 20 June sample) (Fig. 3b). Populations of citrus blackfly, *A. hesperidum*, and *E. opulenta* all declined after the 20 June sample. Samples taken in early 1995 showed low populations levels of the host, but active populations of both parasitoids species.

Overall population density of citrus blackfly was lower in the Bayview oranges, peaking at 26.7 ± 8.4 live immatures per leaf in late 1994 (Fig. 3c). As in the grapefruit, high mortality occurred during June through August as a result of an insecticide application. However, both citrus blackfly and parasitoid activity increased in the late fall. Over 10 live or emerged parasitoids per leaf were found by the end of the study, including both *A. hesperidum*, and *E. opulenta* (Fig. 3d).

Sampling in the Mercedes grove showed initial citrus blackfly populations already close to 40% infested leaves (Fig. 2). This grove was the only one that had high levels of citrus blackfly at the conclusion of sampling, although parasitization was also high (Fig. 2). Intensive sampling in late 1993 through early 1994 showed medium levels of live and dead citrus blackfly, with low levels of parasitization (Fig. 4a). By spring, citrus blackfly populations increased to >100 live pupae per leaf in May. Populations remained high through summer and early fall, peaking with an average of 120.3 live and 3.8 emerged citrus blackfly pupae per leaf in October 1994. However, parasitoid activity was increasing by August, as exemplified by observation of searching adult *E. opulenta* on leaves. Parasitization increased through fall and into 1995, with >200 live or emerged parasitoid immatures found in the 1 November sample (Fig. 4b). Citrus blackfly populations decreased after the 22 November sample, and from the 19 December sample to the conclusion of the study, over 70 live or emerged immature parasitoids (predominately *E. opulenta*) per leaf were documented.

DISCUSSION

Release of these and other exotic parasitoid species against citrus blackfly in Mexico during the early 1950s formed the resource for future releases in the Western Hemisphere. By the end of 1953, over 300 million adults (242 million of *A. hesperidum* alone) were dispersed in Mexico (Flanders 1969). The discovery of the pest in Ft. Lauderdale, FL residential citrus during January, 1976 led to the release of *A. hesperidum*, *E. opulenta*, and *E. clypealis* from laboratory cultures in General Teran, Mexico (Hart et al. 1978). Since then, over 250,000 parasitoids from laboratory colonies, field collections, and movement of infested and parasitized citrus leaves were released in Florida from October 1979 through May 1980 (Nguyen et al. 1983). Laboratory cultures and field collections from Florida formed the basis for the material that was augmented into Texas citrus.

Our results suggest the successful establishment of *A. hesperidum* and *E. opulenta* in the Texas citrus agroecosystem following severe freezes, and the reduction of citrus blackfly populations. Insecticide applications, especially within the grapefruit trees at Bayview, limited our ability to follow natural enemy interactions. However, postbloom and summer selective pest management chemical sprays have been shown to have only short term influence on citrus blackfly natural enemy populations (Fitzpatrick et al. 1978, 1979).

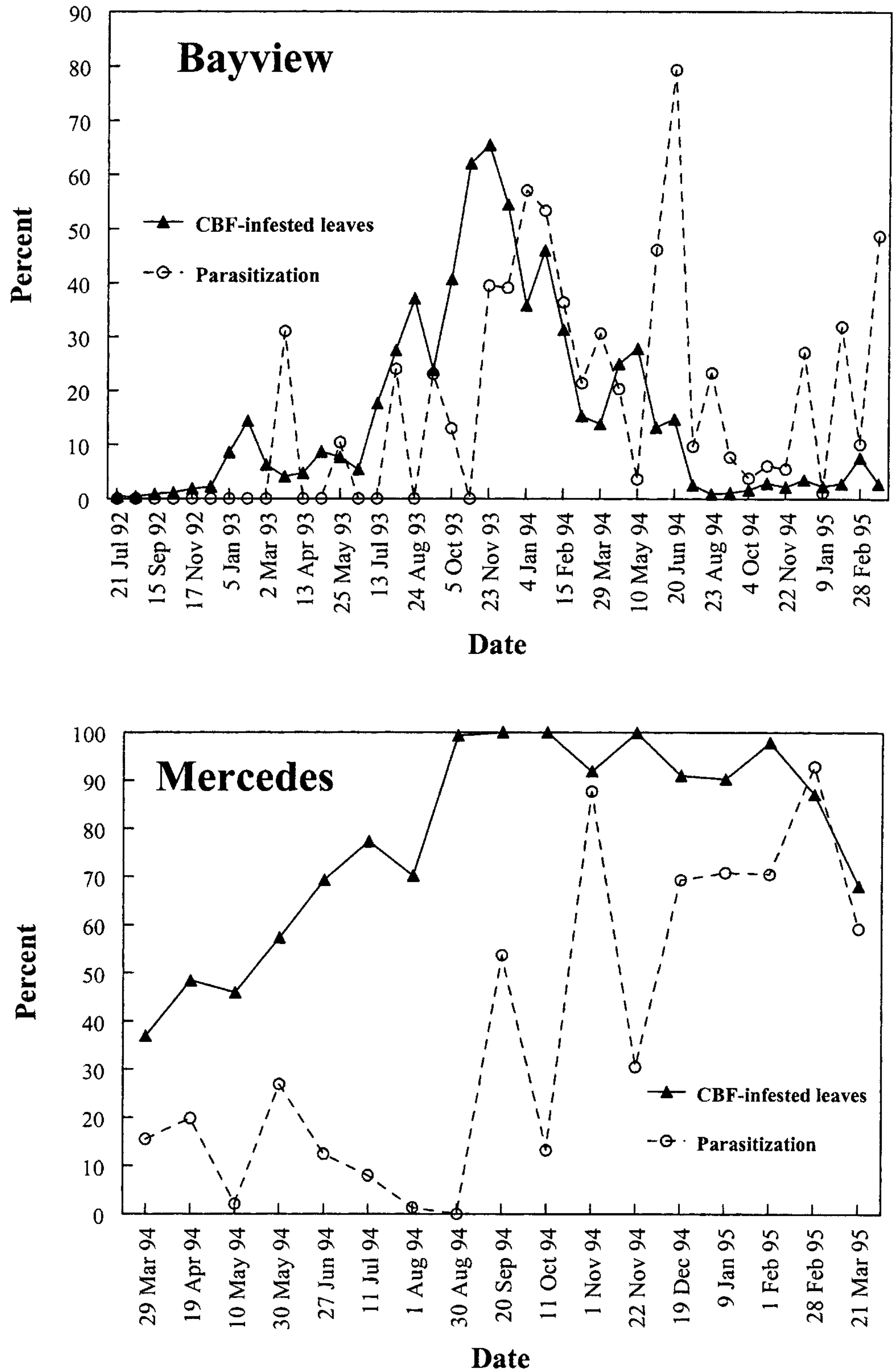


Fig. 2. Population densities of citrus blackfly (percent leaves infested) and parasitization (percent fourth stage parasitized) due to *Amitus hesperidum* and *Encarsia opulenta*, in two Lower Rio Grande Valley, Texas citrus groves, 1992-1994.

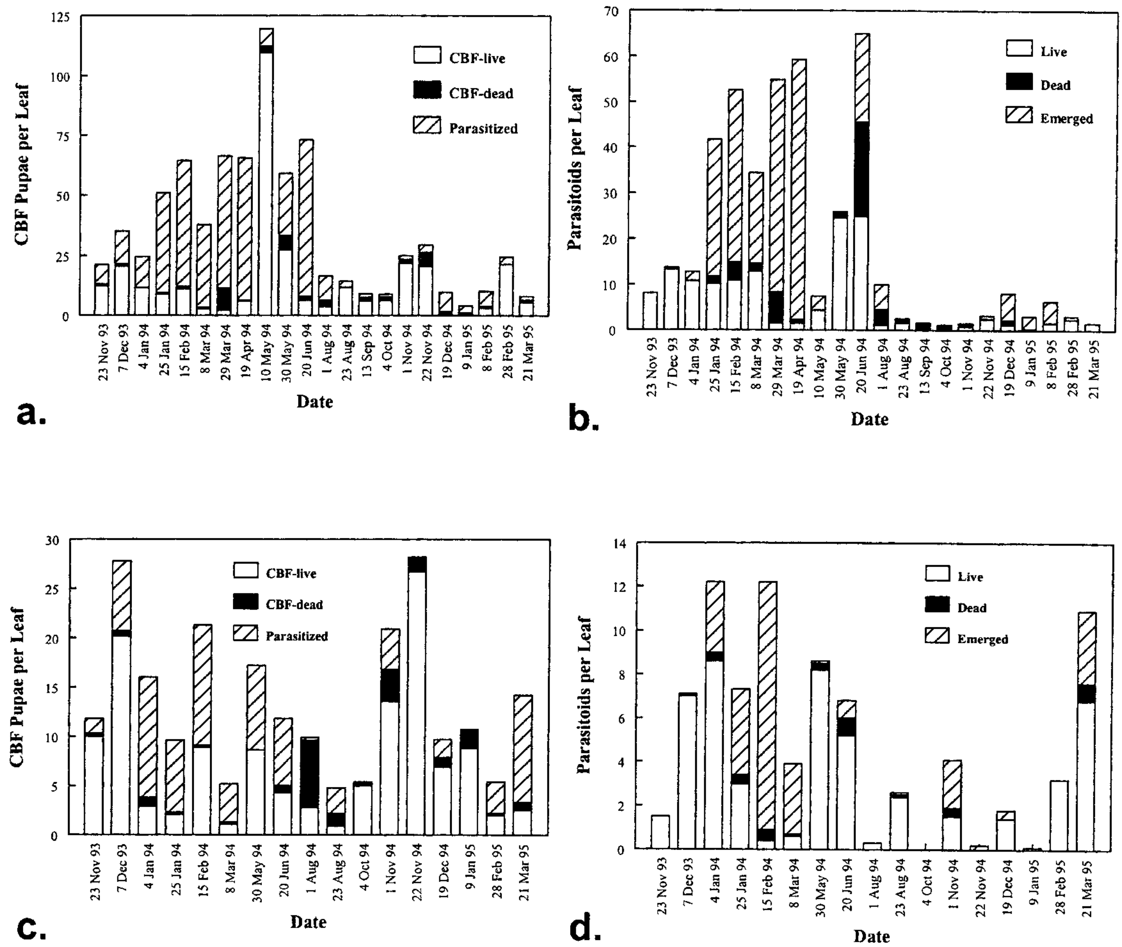
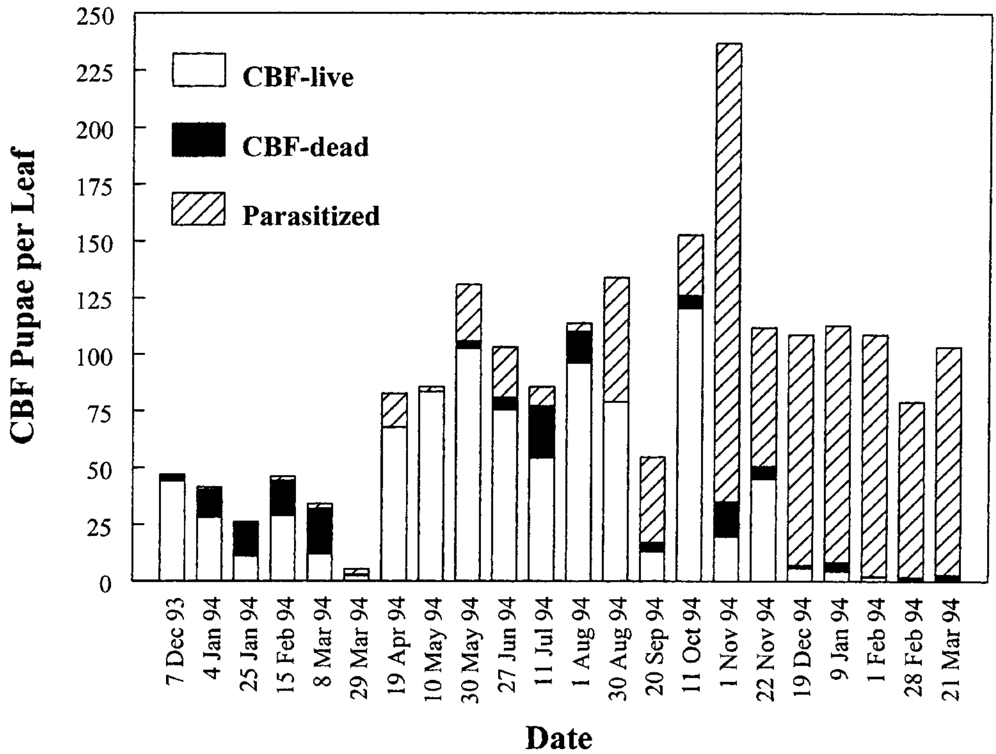


Fig. 3. Population densities of citrus blackfly and two parasitoid species in a grapefruit (a, b) and orange (c, d) grove, Bayview, Texas. Bars correspond to live, dead, or parasitized citrus blackfly per leaf (a, c), or live, dead, or emerged parasitoids per leaf (b, d).

In the Mercedes grove, results suggested increasing populations of *E. opulenta* and population suppression of citrus blackfly. Several reports have documented population regulation by this parasitoid, even within groves under pest management chemical applications (Cherry & Pastor 1980; Swezey & Cano Vasquez 1991). *Encarsia opulenta* has been shown to be able to competitively displace populations of other *Encarsia* species and *A. hesperidum* because of its ability to maintain a stable interaction with its host under low host populations due to density-dependent searching of adult parasitoids (Summy et al. 1983, 1985). In a laboratory study, *E. opulenta* females showed preferences for citrus blackfly that were previously parasitized by *A. hesperidum* (Dowell et al. 1981), although *A. hesperidum* larvae can avoid predation by *E. opulenta* larvae by "hiding" in the midgut (Flanders 1969).

This report suggests that citrus blackfly populations were reduced in groves selected for parasitoid augmentation. Parasitoid populations in these groves increased temporally either due to our reestablishment program or due to the increase of naturally-occurring populations that survived the freeze in residential and commercial citrus trees. Since we did not determine if parasitism was attributed to naturally-occurring or released individuals, the role of our augmentation program on parasitoid reestablishment cannot be identified explicitly. Only carefully planned experiments comparing parasitoid populations in "control" and "treated" groves with similar residential and commercial citrus habitats will provide this information. This type of experimentation has not been accomplished on a large scale in studies involving citrus blackfly biological control because of grower, citrus industry, and logistical demands.

a.



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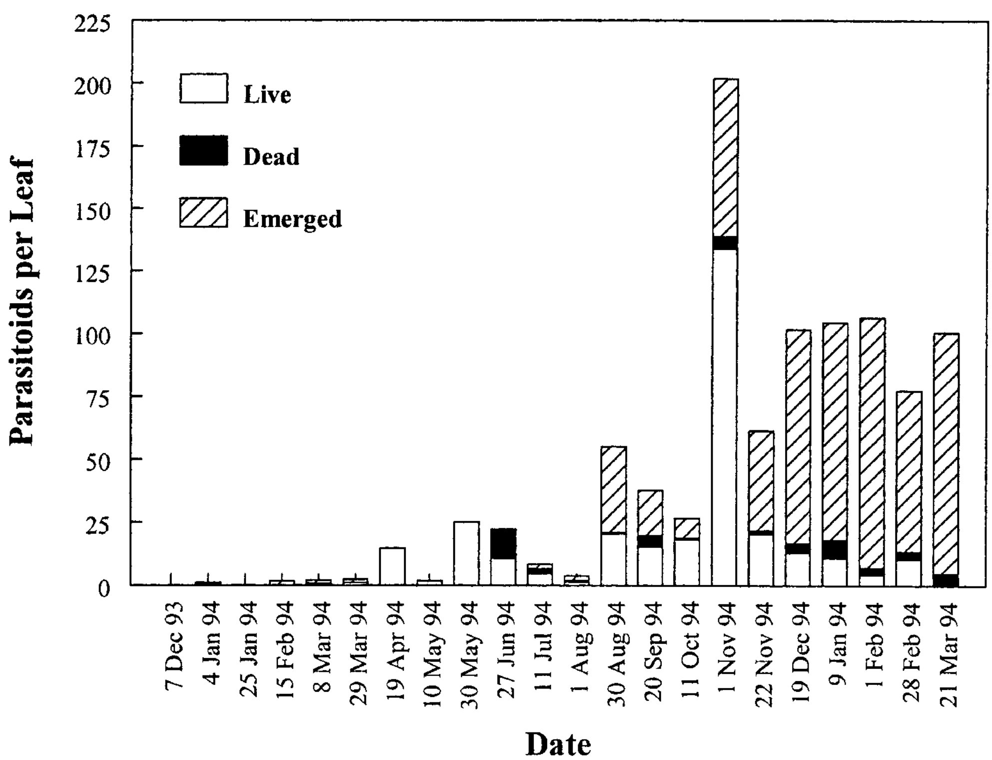

Fig. 4. Population densities of citrus blackfly and two parasitoid species, in a citrus grove, Mercedes, Texas. Bars correspond to live, dead, or parasitized citrus blackfly per leaf (a), or live, dead, or emerged parasitoids per leaf (b).

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