Available online at www.sciencedirect.com
SCIENCE DIRECT
Biological Control 37 (2006) 155–166
Biological Control
www.elsevier.com/locate/ybcon

Classical biological control of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), in southern California

William J. Roltsch [a,*], Dale E. Meyerdirk [b], Richard Warkentin [b], Earl R. Andress [c], Karina Carrera [a]

[a] *California Department of Food and Agriculture, Biological Control Program, 3288 Meadowview Road, Sacramento, CA 95832, USA*
[b] *US Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Pest Detection and Management Programs, 4700 River Road, Unit 135 Riverdale, MD 20737, USA*
[c] *US Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, 4151 Hwy 86, Brawley, CA 92227, USA*

Received 9 June 2005; accepted 13 January 2006
Available online 28 February 2006

Abstract

A cooperative classical biological control project against the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), infestation in the low-desert region of California was initiated in the fall of 1999. Subsequently, the parasitoids *Anagyrus kamali* Moursi (Encyrtidae), *Gyranusoidea indica* Shafee, Alam & Agarwal (Encyrtidae) and *Allotropa* sp. nr. *mecrida* (Walker) (Platygastridae) were reared and released for permanent establishment. Population densities of mealybug and percent parasitism were monitored at a number of mulberry tree and carob tree sites for five consecutive years. The population density of *M. hirsutus* within the first year was reduced by approximately 95%. Over the entire 5-year period of the project, the average regional population density of the mealybug exhibited a continued decline. *Anagyrus kamali* was the predominant parasitoid, often parasitizing in excess of 50% of the mid-to-late stage *M. hirsutus* in the first 2 years following the parasitoid's release. Although *Gyranusoidea indica* was rarely found from spring through early fall, it did represent 40% of the parasitoid species composition during winter. By 2005, the platygastrid parasitoid, *Allotropa* sp. nr. *mecrida* did not appear to be established following numerous releases in 2003 and 2004. Hyperparasitism of *A. kamali* by resident species (*Marietta* sp. & *Chartocerus* sp.) was frequently over 35% during 2000. However, hyperparasitism was considerably lower during each successive year, coincident with declining densities of both mealybug and the primary parasitoid host. Field collections of two other species of mealybugs common in Imperial Valley demonstrated that they are not being utilized as alternate non-target hosts by the newly introduced parasitoids.

Keywords: *Maconellicoccus hirsutus*; *Anagyrus kamali*; *Gyranusoidea indica*; *Allotropa mecrida*; Encyrtidae; Platygastridae; Biological control; Parasitism; Hyperparasitism; Non-target impact

1. Introduction

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), has a long history as an important insect pest species, having invaded many regions of the world. The genus *Maconellicoccus* has three described species in southern Asia, identified as that region from the Indian subcontinent to Malaysia (Williams, 1996, 2004). Four species have been described from Australia and one species from Africa. Originally described from specimens collected in India (Green, 1908), Williams (1996, 2004) notes that *M. hirsutus* is native to an area within Southern Asia. Its first widely documented invasion occurred in Egypt in 1912 (Hall, 1926). Subsequently, it became established in many areas in the African continent (Williams, 1986). *M. hirsutus* was first detected in the Western Hemisphere in Hawaii during 1984 (Beardsley, 1985), but never reached pest status, and later became a serious pest in numerous Caribbean islands soon after its presence was confirmed in Grenada in 1994 (Williams, 1996). One of the first biological control agents imported for the control

* Corresponding author. Fax: +1 916 262 2059.
E-mail address: wroltsch@cdfa.ca.gov (W.J. Roltsch).

1049-9644/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2006.01.006

of *M. hirsutus* in the Caribbean was *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) from China, imported by CABI International Institute of Biological Control (IIBC), UK (Kairo et al., 2000). In 1995, the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Pest Quarantine (USDA-APHIS-PPQ) initiated an offshore pest mitigation project utilizing classical biological control, drawing upon the cooperation of other US and international agencies and Caribbean governments. The goal of this project was to utilize biological control to assist neighboring countries in anticipation of this pest's eventual arrival into the United States Mainland. The biological control program in the Caribbean was very successful, reducing population densities of *M. hirsutus* between 92 and 99% (unpublished data, DEM). Briefly, this resulted in identifying and acquiring additional biological control agents, developing and refining rearing procedures, release and monitoring protocols, etc. (Meyerdirk et al., 2002). In addition to the *A. kamali* strain from China, a second strain of this solitary wasp was imported from Hawaii. A second species of parasitoid, *Gyranusoidea indica* Shafee, Alam & Agarwal, was imported from Egypt, Pakistan and Australia and all were used in the USDA, APHIS, PPQ cooperative efforts to control *M. hirsutus* in the Caribbean.

Maconellicoccus hirsutus was first detected on Mainland US in August of 1999 in Imperial Valley, a low desert region in southern California. Population densities of *M. hirsutus* on mulberry (*Morus alba* L.), carob (*Ceratonia siliqua* L.), silk oak (*Grevillea robusta* A. Cunn.), hibiscus (*Hibiscus rosa-sinensis* L.) and orchid tree (*Bauhinia variegata* L.) were very large, commonly exceeding 100 individuals per branch terminal in several urban communities in southern Imperial Valley. Two parasitoid species, *A. kamali* and *G. indica*, were initially shipped from insectaries in St. Thomas, US Virgin Islands and Puerto Rico, with releases commencing in late September 1999. These two insectaries were cooperatively funded and managed by USDA-APHIS and the respective local governments. Subsequently, the two species were then produced locally in El Centro, California with numerous releases commencing in June 2000 to assure rapid and widespread colonization and establishment.

The present study summarizes *M. hirsutus* population densities, parasitism and life history patterns primarily over a 5-year period following initial releases of the biological control agents. In addition, sample techniques were employed to assess hyper-parasitism and non-target species effects.

2. Materials and methods

2.1. Species released and origin

In September 1999 to April 2000, *A. kamali* and *G. indica* were received from the USDA, APHIS, PPQ supported insectaries in the Caribbean for release in Imperial Valley (Table 1). At that time, a mixed culture of *A. kamali* from China and Hawaii was released, along with *G. indica* from Egypt and Pakistan. By June 2000, parasitoids were being produced at an insectary facility in El Centro, CA; setup and operated by the California Department of Food and Agriculture and subsidized in part by USDA, APHIS, PPQ. Parasitoids were reared in the insectary utilizing *M. hirsutus* as the host insect reared on Japanese pumpkins (*Cucurbita moschata* (Duchesne), cv. Chirimen). At this time, the above populations were being reared along with a population of *G. indica* originally collected in Australia.[1] The Australian population was reared for three generations in separate cages and released up to mid-August 2000 before being combined with the *G. indica* from Egypt and Pakistan. Strains of *G. indica* were combined because of space constraints that were likely to result in cross contamination given that all *G. indica* rearing cages were present in the same room. Rearing of these populations continued at the El Centro insectary through 2001 (Table 1). *Allotropa* sp. nr. *mecrida* (Walker) and a population of *A. kamali* were collected in Egypt in 2000 (Gonzalez et al., 2003). In 2002, the Egyptian population of *A. kamali* was reared in the absence of all other populations of *A. kamali* and released. In January of 2003, *A.* sp. nr. *mecrida* was obtained for rearing at the El Centro insectary following the issuing of a USDA-APHIS importation and release permit that had required host range study results.

Populations of *A. kamali* and *G. indica* were in continuous culture for varying lengths of time prior to release, since the various populations were first collected from 1995 to 2000. As noted, several populations were combined at various times to form single cultures. It is estimated that 15 and 13 generations of *A. kamali* and *G. indica*, respectively, occurred in culture each year.

2.2. Releases

Based on surveys in Imperial Valley during the fall of 1999 throughout 2000 by the California Department of Food and Agriculture, it was determined that the *M. hirsutus* infestation occupied an area approximately 40×26 km in size (Weddle and Roltsch, 2000). The infestation was found to be highest in the community of Calexico (Fig. 1). From late September 1999 to April 2000, *A. kamali* and *G. indica* were received for release in Imperial Valley, California, primarily in the communities of Calexico, El Centro, and Imperial (Fig. 1). During this time, 100–300 parasitoids of each species were released one time at each of 16 locations with infested mulberry trees, carob trees or hibiscus.

Parasitoids produced at the El Centro insectary in 2000 enabled many releases to take place. The parasitoids were released at 540 locations predominantly in the communities of El Centro and Calexico in 2000 (Table 1). Each inoculative release of *A. kamali* and *G. indica* typically consisted of

[1] This is a correction to published accounts indicating that the Australian population of *G. indica* was not released in the United States (Goolsby et al., 2002).

Table 1
Annual releases of parasitoids in Imperial Valley, CA, and adjacent Mexicali Valley, Mexico

Species	Year	Origin (strains)	No. released		Source	Collectors/collaborators	Reference
			Imp. Val.	Mexico			
Anagyrus kamali	1999	China	4,500		ST & PR	Anonymous, CABI IIBC	Kairo et al., 2000
		Hawaii				D.E. Meyerdirk	None
	2000	China	167,550		CA	—	
		Hawaii				—	
	2001	China	22,100	45,400	CA	—	
		Hawaii				—	
	2002	Egypt	97,850[a]	38,250	CA	D. Gonzalez, Univ. of Calif.-Riverside, CA; A.H. El-Heneidy, Plant Prot. Inst., Ministry of Agri., Ciro, Egypt	Gonzalez et al., 2003
Gyranusoidea indica	1999	Pakistan	1,900		ST & PR	Anonymous	
		Egypt				E. Helmy & S. Abd-Rabou, Plant Prot. Research Inst., Dokki, Giza, Egypt; D.E. Meyerdirk,	
	2000	Pakistan	231,900		CA, ST & PR	—	
		Egypt				—	
		Australia				J.A.Goolsby,USDA-ARS Aust. Biol. Control Lab., Indooroopilly, OLD, Australia; A.A. Kirk, USDA-ARS, European Biol. Control Lab., Montferrier sur Lez, France	Goolsby et al. (2002)
	2001	Pakistan	39,800	70,075	CA	—	
		Egypt				—	
		Australia				—	
	2002	Pakistan	13,800		CA	—	
		Egypt				—	
		Australia				—	
Allotropa mecrida	2003	Egypt	208,800	88,800	CA	D. Gonzalez, Univ. of Calif.-Riverside, CA; A.H. El-Heneidy, Plant Prot. Inst., Ministry of Agric., Ciro, Egypt	Gonzalez et al., 2003
	2004	Egypt	107,000	26,000	CA	—	

[a] From Feb. 2002 onward, *A. kamali* culture was from Egypt. Source-insectary: ST, St. Thomas,Virgin Islands; PR, Puerto Rico; CA, California.

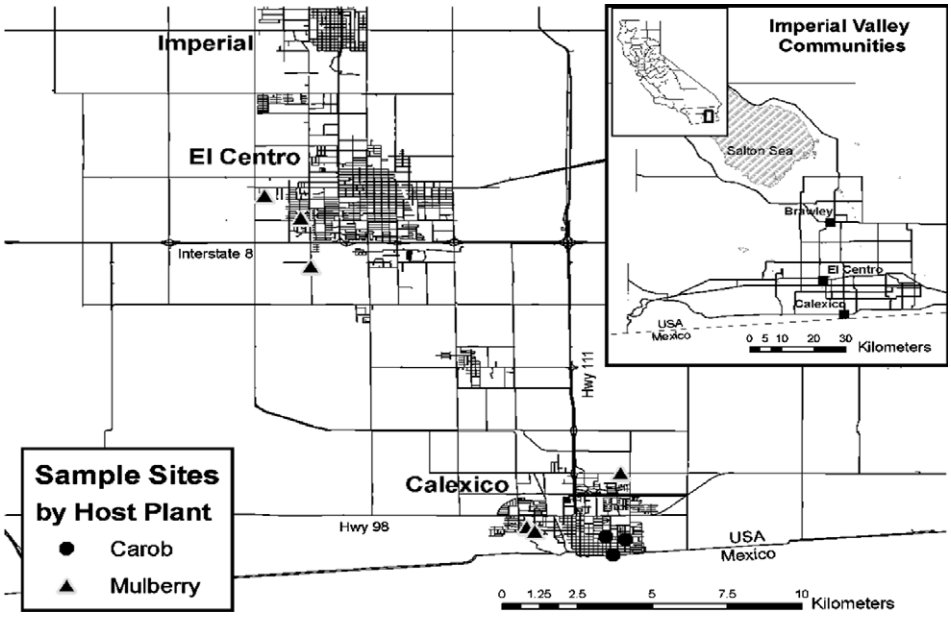

Fig. 1. Locations of long-term sample sites in two communities of Imperial Valley.

400 parasitoids of each species contained in a 6 or 9-dram plastic vial that was wrapped in a white paper towel to minimize exposure to direct sunlight and possible overheating at the time of release. Releases of *A. kamali* and *G. indica* resumed in 2001, however, much of the production in 2001 (i.e., all production from May to November) was supplied

to SAGARPA (Mexican Secretary of Agriculture, Animal Husbandry, Urban Development, Fisheries and Food) in Mexicali, Mexico for release in Mexicali Valley, across the US border from Calexico. In 2002, the previous cultures were eliminated and a population of *A. kamali* from the very warm arid region of southern Egypt was reared and released at 144 sites in Imperial Valley (Table 1). *Allotropa* sp. nr. *mecrida* was reared and released in 2003 and 2004, using methods consistent with those used in previous years, and released in groups of 500–1000 parasitoids per site at nearly 500 sites over this 2-year period.

To minimize conflicts between the need to release parasitoids and monitoring efforts to assess the seasonal activity of *M. hirsutus* and parasitism, parasitoids were released from early to late summer at locations starting in the periphery of the known infested areas of Imperial Valley. By early fall, releases were made as close as ca. 150 m from our long-term monitoring sites. Given the high level of parasitism typically recorded, it is unlikely that the fall releases would have significantly influenced overall parasitoid activity at these study sites.

2.3. Monitoring for M. hirsutus, primary parasitoids, and hyperparasitoids

Study sites for the long-term monitoring of mealybug and parasitoid activity were located in home yards in El Centro and Calexico (Fig. 1). Three sites where releases were made in the fall of 1999 were selected for long-term monitoring, followed by three additional sites identified in January. Each of these six sites contained two to six mulberry trees. In June of 2000, three additional sites containing carob trees were selected. In all, nine sites were used to monitor mealybug and parasitoid activity. One mulberry tree site was lost in October of 2001, without replacement, and one carob tree sample site was destroyed in August of 2002 and replaced in January of 2003 by a site located ca. 400 m away. One carob tree site contained two carob trees, whereas the other two locations each contained a single carob tree. Pesticides were not applied to any of the trees sampled and were not known to have been used within the immediate area.

Mulberry trees are deciduous, with leaf drop in December and bud break occurring in late March to early April in Imperial Valley. In contrast, carob trees are evergreen. Samples were taken seven to eight times each of 5 years through 2004. Samples were taken only twice in 2005 during August and September for the primary purpose of detecting *A.* sp. nr. *mecrida*. A sample consisted of eight branch terminals (≈30 cm in length) randomly selected and cut from the tree or trees at a site. Branch terminals were placed in plastic bags, that were held in an ice cooler and taken to the lab to obtain life stage counts using a 30× stereo microscope. The terminal sample unit included the partially unfurled terminal bud area and adjacent five fully expanded leaves. All egg masses, second and third instars, and adult male and female mealybugs were counted. In addition to the main sample, several terminals observed to be infested with *M. hirsutus* were collected to increase the number of mealybugs available for isolation and quantifying parasitism. Depending on their availability in a sample, a maximum of 100 live late-third and adult female *M. hirsutus* were randomly selected and placed individually in gelatin capsules, placed in a plastic bag, labeled, and held at ambient room temperature (23–26 °C). These methods are similar to those used for studying parasitism of the Comstock mealybug (Meyerdirk et al., 1981). After a minimum of 6 weeks, each specimen was inspected to determine if an adult parasitoid had emerged. Percent parasitism was calculated by dividing the number of parasitized mealybug specimens by the total number of mealybugs placed in gelatin capsules. Beginning in 2002, late second and early third instar mealybugs were also placed in gelatin capsules to assess parasitism. These specimens were held and recorded separately from later stages (late third instar nymphs and adult females). Field-collected mummies of parasitized mealybug were isolated in gelatin capsules to assess hyperparasitism. Hyperparasitism was calculated by dividing the number of mummies from which hyperparasitoids emerged by the number of mummies from which primary or hyperparasitoids emerged. It was assumed that unemerged mummies contained either dead primary or hyperparasitoids in proportion to emerged counts and therefore were not utilized in calculating percent hyperparasitism. On each sample date, arthropod predator density was monitored by collecting four beat-sheet (75 × 75 cm) samples of the host plant foliage at each site (three terminals per sample unit) with four strikes of the stick per sample. This included monitoring for the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera, Coccinellidae). At the onset of the project during fall 1999, the mealybug destroyer was released until late spring of 2000 by Imperial County personnel for control of *M. hirsutus* at select locations, separate from the parasitoid monitoring sites.

To assess parasitoid activity during the winter months, a different sample plan was developed because *M. hirsutus* on deciduous plants such as mulberry moves from the leafless branch terminals late in the fall to the center trunk region of the tree where the rough bark provides protection. Monitoring was implemented to provide details on life stage composition and parasitoid survival and impact during the winter. In fall 2000, five separate mulberry tree sites were selected for monitoring the winter activity. Sites were selected that were found to contain a high level (>30%) of terminals infested with *M. hirsutus*. Information suggesting that a site may be useful for winter monitoring, was made available through an area-wide survey previously conducted by the California Department of Food and Agriculture from April through October of 2000. At each site, five, 5-cm wide corrugated cardboard bands were wrapped around the trunk and or branches that were 10 cm in diameter or greater (DeBach, 1949). This was done in mid-November, at the approximate time when mealybugs were moving from branch terminals on the perimeter of a tree

canopy to the trunk region of the tree. One of the five bands at each site was removed and inspected in the laboratory every 3 weeks. As a result, the fifth band was removed in March. In addition, a single band was placed on a tree each time one of the original five bands was removed. This band was removed and replace at 3-week intervals. This was done to monitor within tree movement of mealybugs throughout the winter to assist interpreting data collected from the sequentially removed bands. Mealybug life stage was recorded and late stage specimens were placed in gelatin capsules to determine parasitism.

2.4. Non-target species impact

In recent years, concern has been raised regarding the potential for unintended non-target impacts by agents used in the classical biological control of insect pests (Hoddle, 2004a,b; Louda et al., 2003; Louda and Stiling, 2004). The level of polyphagy of a newly introduced natural enemy species is one of the elements of this debate. Included in our project was the intent to document whether other species of resident mealybugs were being parasitized by parasitoid species released to control *M. hirsutus*. Collections were made within the known geographic range of *M. hirsutus* in Imperial Valley. Two species were sampled: the native solenopsis mealybug, *Phenacoccus solenopsis* Tinsley, and resident *Ferrisia* species. These species were common in this area on ornamental plants. All samples were obtained from mulberry trees, hibiscus or carob trees. Populations of these mealybug species were found and collected while collecting samples of *M. hirsutus* or while performing other tasks such as making parasitoid releases. In addition, several populations were found as a result of local residents reporting them to the Agricultural Commissioners Office. Branch terminals were removed from infested plants, returned to the laboratory, and mid-to-late instars were removed and held individually in gelatin capsules for parasitoid emergence.

2.5. Species identification

Mealybug species including *M. hirsutus*, *P. solenopsis* and *Ferrisia* sp. were identified by R. Gill, of the California Department of Food and Agriculture. Primary and hyperparasitoid specimens that emerged from *M. hirsutus* nymphs and mummies collected from sample sites in 2000 were submitted for identification to M.W. Gates, Sytematics Entomology Laboratory, Agricultural Research Service, US Department of Agriculture. Vouchers of each parasitoid species were retained by SEL. Vouchers are also held by the California State Collection of Arthropods (CSCA) at the California Department of Food and Agriculture, Sacramento, CA.

2.6. Analysis

Data comparing parasitism of young *M. hirsutus* nymphs (late-second to early third instars) to that of older nymphs (collected as late third instars and adults) were analyzed using Wilcoxon's signed-ranks. For this, percent data underwent an arcsine transformation prior to analysis, and means were back transformed for reporting. Winter-band study data are presented to illustrate the pattern of occurrence and magnitude of life stage abundance over time. In addition, the cumulative distribution of select life stages occurring from late November to mid-March is presented to illustrate the comparative timing of life stage occurrence over the winter period and to present standard errors illustrating the degree of site-to-site variation of a life stage's occurrence within the region of *M. hirsutus* infestation. Each value represents the mean across all sites of the cumulative proportion of a given life stage with respect to the total count of the respective life stage for the duration of the study.

3. Results

3.1. Abundance and parasitism of M. hirsutus on tree terminals

Population densities of *M. hirsutus* on infested mulberry trees averaged 256 mealybugs/terminal (excluding crawlers) in September 1999 at study sites prior to the release of exotic parasitoids (Fig. 2). The mean regional density of *M. hirsutus* declined and remained low for five consecutive years, which corresponded to the broad establishment of *A. kamali*. By 2003, the peak population density was approximately 1% of that in the fall of 1999 (Fig. 2). Parasitism increased progressively through each summer, during which time *M. hirsutus*'s density characteristically increased. An increased level of percent parasitism in direct response to increasing densities of *M. hirsutus* during mid-to-late summer of each year suggests that a density-dependent relationship exists between parasitoid and mealybug host. Specimens of primary parasitoids emerging from *M. hirsutus* from field samples collected in 2000 were confirmed to be *Anagyrus kamali* (33 cnt.) or *G. indica* (5 cnt.).

Similar results were recorded at three study sites consisting of carob trees (Fig. 3). Densities of *M. hirsutus* at these sites when parasitoids were initially released in June 2000 were 120 mealybugs per terminal. At this time, a low level of parasitism (1.8%) by *A. kamali* and *G. indica* was recorded. This indicated that the parasitoid species released at least several hundred meters away in the fall of 1999 on mulberry and hibiscus had already dispersed to some of these locations and had become established.

In 2004, *M. hirsutus* densities were at their lowest to date on both mulberry and carob trees, and *A. kamali* continued to be the predominant parasitoid (Figs. 2 and 3). Due to very low *M. hirsutus* densities in 2003 and 2004, it was not feasible to collect *M. hirsutus* specimens for assessing percent parasitism at many long-term sample sites. Overall percent parasitism for 2003–2004 was lower than in past years, peaking at 30% or less compared to more than 50% in previous years. Presumably, parasitism was lower in response to very low densities of *M. hirsutus*. In 2005, monitoring limited to August and September indicated that *A.* sp. nr *mecrida* had not established.

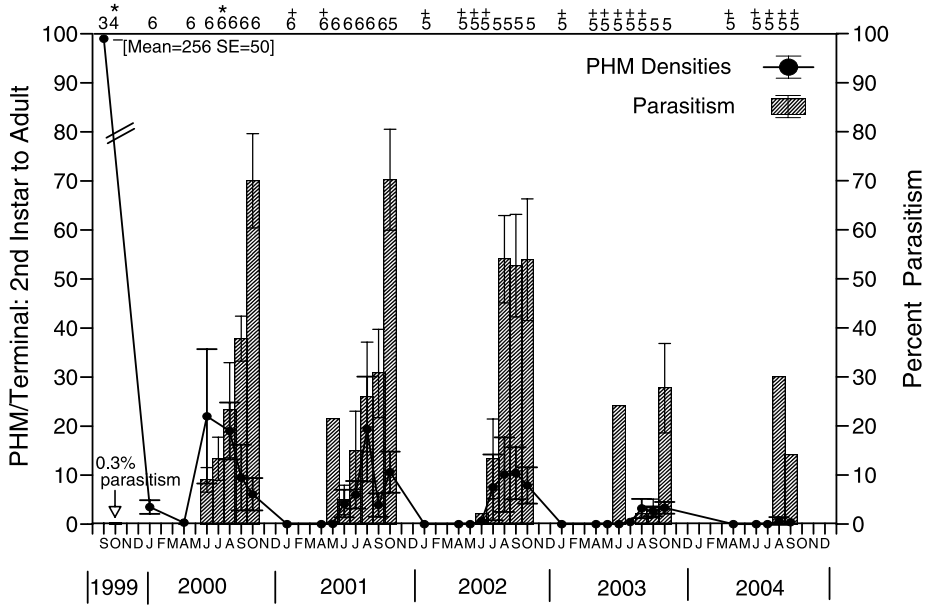

Fig. 2. Density of second instar nymphs to adult *M. hirsutus* per branch terminal and parasitism on mulberry trees in Imperial Valley, California. Mulberry terminal samples with buds only are available in January. Numbers at the top of the graph represent the number of sites sampled by date. [∗ = specimens collected for determining parasitism only, "+" = percent parasitism determined for two or less sites].

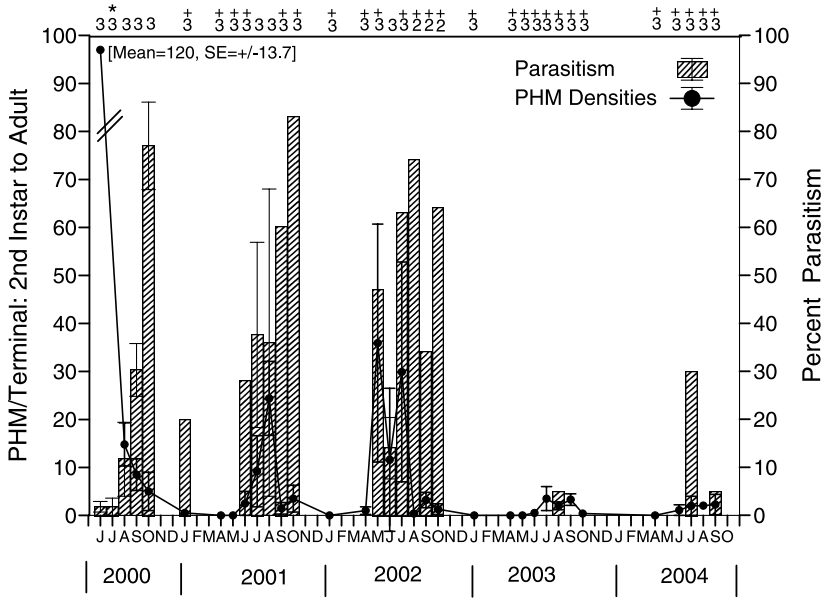

Fig. 3. Density of second instar nymphs to adult *M. hirsutus* per branch terminal and parasitism on carob trees in Imperial Valley, California. Numbers at the top of the graph represent the number of sites sampled by date. [∗ = specimens collected for determining parasitism only, "+" = percent parasitism determined for two or less sites].

Although *G. indica* was also established in Imperial Valley, its numbers on terminal branch samples were typically low during much of the year, particularly during the warmest months from June through September. In 2002 from spring through fall, less than 10% of all parasitoids were *G. indica*, however, *G. indica* represented 21% of the primary parasitoids collected in October. This pattern was characteristic of all years of the study. Regarding potential predator species, almost none were collected in beat-sheet samples taken throughout the study.

Sample numbers of *M. hirsutus* life stages (excluding crawlers) from the fall of 1999 to October of 2000, were sufficient to provide a life stage phenology profile (Fig. 4). A portion of the mealybug population was present on tree terminals in early January, although the majority of *M. hirsutus* typically moves to the central trunk region of a tree during this time of year. In part, its detectability (128 total

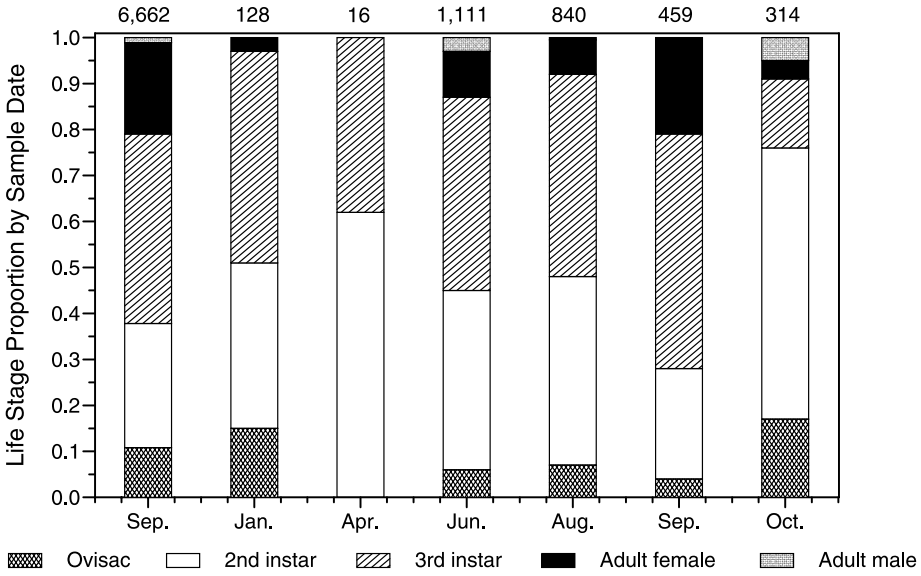

Fig. 4. Proportion of each *M. hirsutus* life stage (excluding crawlers) by sample date. Total sample size by date is stated above each bar.

individuals across 6 sample sites) on mulberry tree terminals in January 2000 was due to the recent peak numbers on mulberry in 1999. Even though this 1-year period provided the greatest numbers of mealybugs in the samples compared to subsequent years, mealybug sample counts were above zero at only 2 sites in April for a total of 16 second and third instar specimens (Fig. 4). Adult female mealybugs were common from June through September, declining through the fall and winter relative to younger life stages. Furthermore, it was indicated that the *M. hirsutus* population during mid-spring primarily consisted of second and third instars from eggs that hatched during the winter and early spring.

In 2002, parasitism of early and late stages of the mealybug was compared. The mean percent parasitism of late-second to early third instars was significantly lower than for those specimens collected as late-third to adult female mealybugs on mulberry and carob trees (mulberry: mean 6.6% vs. 49.8% Wilcoxon's signed-ranks, $S=-52$, $P \leqslant 0.0001$, $n=14$; carob: mean 10.7% vs. 40.8%, Wilcoxon's signed-ranks, $S=-38$, $P \geqslant 0.001$, $n=12$).

Weather data were obtained from a local weather station from September 1999 to September 2000 to investigate the temperature extremes common to the low desert climate of Imperial Valley (University of California's IPM World-wide Web Site [http://www.ipm.ucdavis.edu]). Temperatures exceeded 38 °C on 140 days during this 1-year period. Temperatures exceeded 43 °C on 24 days, whereas daily low temperatures were less than 4.5 °C on 14 days, with freezing temperatures occurring on 2 days.

3.2. Winter population patterns based on tree limb bands

Cardboard band data collected in mid-December, demonstrated that all immature life stages as well as adult female and male *M. hirsutus* are present during the winter months of December and much of January (Figs. 5A and B). By 14 February, very few live *M. hirsutus* life stages were present in all bands. Although ovisacs persisted well into March, the extent of their viability during late winter is in question. Ovisac viability was not assessed until the last sample collected in March. At this time, based on a visual examination noting discolored and dehydrated eggs, approximately 87% of the ovisacs consisted of dead eggs. Based on counts obtained from bands left in the field for only three weeks preceding the 3 January and 24 January collection dates, it was estimated that 7.6% SE ± 3.3 and 7.8% SE ± 6.7, respectively, of the second instar to adult mealybug stages were of recent origin in all bands. That is, the remainder of these life stages in the original set of bands sequentially removed every 3 weeks, had been present since late November when bands were initially installed.

Based on *M. hirsutus* mummy densities in late January, a high level of parasitism was apparent (Figs. 5A and B). Mean parasitism from 14 December to 24 January, based on the collection of late-third instar or adult female mealybugs from bands that had four or more specimens, was high (mean 41%, SE ± 5.6, $n=8$ bands, 139 total specimens). By comparison, parasitism of specimen's collected and isolated as late second and early third instars was much lower (mean 21%, SE ± 8.3, $n=8$ bands, 270 specimens). Species composition of the 117 parasitoid specimens identified over this period consisted of 60% *A. kamali* and 40% *G. indica*. Of 281 parasitoid specimens emerging from specimens collected and isolated as mummies, 69% of the primary parasitoids were *A. kamali*, whereas 31% were *G. indica*. Hyperparasitism was very low during the winter. Hyperparasitoids emerged from only 5 of the 286 mummies (1.7%) from which parasitoids emerged. Four hyperparasitoids were *Chartocerus* sp. (Hymenoptera: Signiphoridae) and one was *Marietta* sp. (Hymenoptera: Aphelinidae).

The moderate degree of standard error associated with the cumulative distribution of each life stage across all five

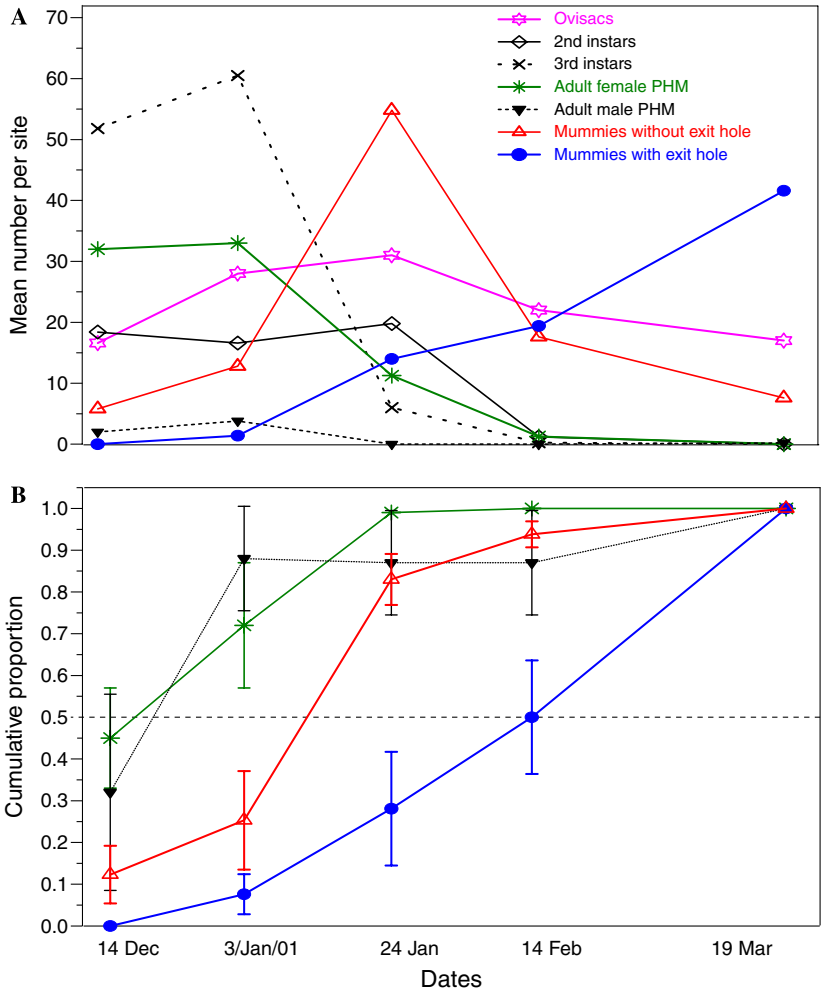

Fig. 5. Presence of *M. hirsutus* life stages and parasitoid pupal stage and exit holes on cardboard bands on mulberry trees during the winter and spring of 2000–2001. (A) Mean life stage count per site at 3-week intervals following 11 Nov. 2000 setup date. (B) Cumulative proportion of select life stages including exit holes by sample date with associated standard error.

sites demonstrated that the pattern of activity was relatively consistent within the geographic area of Imperial Valley (Fig. 5B). It is shown that the point of 50% occurrence for female *M. hirsutus*, mummies (i.e., parasitoid pupae), and exit holes (i.e., emergence of adult parasitoids) are separated sequentially by roughly a one month period, and that nearly 50% of the parasitoids have emerged by approximately mid-February. Upon separately tabulating the cumulative mean counts of mummies and exit holes up to 19 March 2001, the two values were within 80% of one another, indicating that a high proportion of parasitoids successfully emerged by spring. Similarly, 84% of the 246 mummies counted in the bands collected on the last date (19 March 2001) contained exit holes.

3.3. Parasitoid sex ratios

The proportion of *A. kamali* field populations composed of females from branch samples, indicated that the sex ratio does fluctuate, however, populations are typically composed of more than 50% females. Based on 54 separate samples collected predominantly from mulberry trees from 2000 through 2002, each containing a minimum of 5 parasitoids, 81% of the samples consisted of 50% or more females [Mean ± SE, Total *n*: 2000, 58% ± 17.4, 256; 2001, 64% ± 12.1, 274; 2002, 69% ± 10.2, 334]. These samples were obtained from late June through October. Samples collected at other times of the year provided an insufficient number of specimens for estimating sex ratio.

The percentage of female *A. kamali* emerging from late third and adult *M. hirsutus* ($n = 67$) and mummies ($n = 200$) during the winter months from band samples were very close to 50% during December and early January; however, females represented 23 and 30%, respectively, of the mealybug and mummy samples obtained in late January. Mummies were collected in the February sample from which 40% of the emerging *A. kamali* were female.

The number of *G. indica* associated with branch terminal samples was too low to tabulate the proportion of females. From winter band samples however, 47 specimens emerged from mealybugs isolated in gelatin capsules, of which 57% of the specimens across all dates were female. Of *G. indica*

emerging from specimens collected as mummies, 60–80% were females from December through February.

3.4. Hyperparasitism

Two species of Hymenoptera were found to emerge exclusively from *M. hirsutus* collected as mummies and isolated in gelatin capsules. These were confirmed to be hyperparasitoid species identified as *Marietta* sp. (Aphilinidae) (5 cnt.) and *Chartocerus* sp. (Signophoridae) (2 cnt).

The impact of native hyperparasitoid species on newly introduced primary parasitoid species was considerable during 2000, with values at approximately 30% from June to September and exceeding 60% in October (Figs. 6A and B). A *Marietta* sp. was first collected from mulberry tree samples in June 2000 (Fig. 6A). At that time, all records came from one site. Dissected samples confirmed that the primary parasitoid, *A. kamali* was under attack by *Marietta* sp. and to a limited extent by *Chartocerus* species. *Marietta* sp. was common through the remainder of 2000, as represented by the percent of specimens collected as mummies from which hyperparasitoids emerged. Based on samples collected from mulberry, hyperparasitoid attack of *A. kamali* declined after 2000 (Fig. 6A). This was not apparent for samples taken from carob, however, mummy sample counts from carob were typically very small (Fig. 6B). Few mummies were collected in 2003 at most mulberry and carob sample sites for evaluating hyperparasitism because *M. hirsutus* densities were very low (Figs. 2 and 3). *Marietta* sp. was common during one sample date at two locations. From four small samples (25 total specimens) taken during September of 2003, overall hyperparasitism was estimated to be 24%; all *Marietta* species. The relationship between the predominant hyperparasitoid, *Marietta* sp., and its host, *A. kamali* on mulberry from 2000 to 2002 suggests that it was density-dependent, given that hyperparasitism declined over consecutive years as did absolute densities of mealybug and primary parasitoids (Figs. 2 and 6A).

Unlike samples of mummies collected during the winter banding study, mummy samples collected throughout the summer and held were found to have a highly variable rate of successful parasitoid emergence. That is, a number of specimens collected as mummies did not emerge either as primary or hyperparasitoids (Mean non-emergence, ±SD,

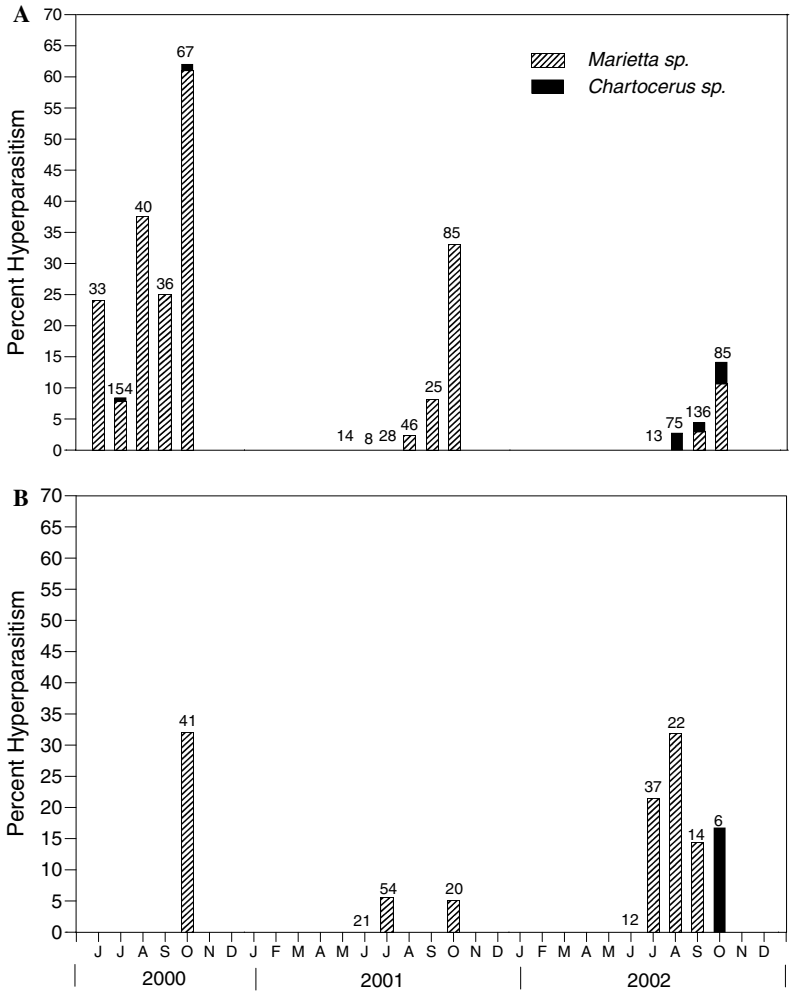

Fig. 6. Percent hyperparasitism of *M. hirsutus* parasitoids on mulberry trees (A) and carob trees (B) in Imperial Valley, CA. Number of primary and secondary parasitoids, all sites combined by date, used to calculate percent hyperparasitism is present above each bar.

2000: 41%, ±24.0; 2001: 28%, ±21.6; 2002: 24%, ±20.5). The cause of mortality is unknown, however, hyperparasitism was nearly absent from the winter banding samples.

3.5. Non-target spp. impact

Two resident species of mealybug were collected from 2000 to 2005 within the known infested geographic area of Imperial Valley to monitor for non-target effects of recently released parasitoids used in the biological control of *M. hirsutus*. In all, 17 separate samples of *P. solenopsis* (5–133 specimens per sample, 743 total specimens) and 12 samples of *Ferrisia* sp. (5–19 specimens per sample, 150 total specimens) were collected from 2000 to 2005. To date, neither *A. kamali*, *G. indica* nor *A*. sp. nr. *mecrida* have been recovered from either mealybug species.

4. Discussion

The two initial parasitoid species released against *M. hirsutus* have become widely established throughout infested areas of Imperial Valley, and one species, *A. kamali*, has had a large impact to date, based on pre- and post-release density samples and estimates of percent parasitism. Although parasitism by *G. indica* is low much of the year, its activity during the fall and winter is elevated and may be responsible for significantly contributing to mealybug mortality during winter. Banding data of percent parasitism and percent successful emergence of parasitoids from mealybug mummies clearly shows that parasitoids successfully pass through the winter and are available to begin attacking *M. hirsutus* during spring.

In addition to having achieved more than a 98% decline of *M. hirsutus* densities since 1999, the successful biological control of this pest has essentially stopped the natural spread of *M. hirsutus* from this isolated desert region to other areas of California. In 1999, there were millions of mealybug crawlers produced per tree, subject to an array of methods of transport, including being windblown or mechanically transferred by vehicles, tree, and shrub trimming equipment, etc. With such extraordinary numbers, spread was likely even with a 99% chance of crawler mortality during dispersal. By reducing the abundance of *M. hirsutus*, it would appear that many such avenues for dispersal became ineffective. Presently, the spread of *M. hirsutus* is largely limited to the transfer of mealybug life stages on plants that are moved, or specifically ovisacs or adult female mealybugs on equipment. In either event, it is likely that parasitoids will be moved as well.

Presently, *M. hirsutus* is occasionally found in high numbers on residential plants. Typically these include isolated single plants or a small hedge, and infestations are of short duration. However, there are several exceptions to this typical condition. A chronically elevated density has been found on mulberry trees at the El Centro Navel Air Facility west of El Centro, CA. Records of modest levels of parasitism (up to 30%) suggests that parasitism is being disrupted. Our experience is that relative estimates of parasitism of 60% are commonly achieved when *M. hirsutus* densities are elevated. In a separate residential home setting, a hibiscus plant continues to support an elevated population of *M. hirsutus*. In part, this appears to be a function of ants tending the mealybugs. However, the problem has persisted at a diminished level even when measures are taken to control ants. The plant has a large amount of old distorted growth that perhaps provides a refuge for mealybugs.

The sex ratio of *A. kamali* is typically skewed in favor of females during much of the year. These results are comparable to those recorded by Sagarra et al. (2000a,b) and Serrano and LaPointe (2002) for parasitoids emerging from *M. hirsutus* in laboratory studies. Winter band samples did indicate that the sex ratio could become male biased at times in the winter, as was demonstrated by the late January sample. The reason for this and the consequences are not clear. In contrast, *G. indica* sex ratios were consistently female biased throughout the winter.

Documenting parasitism (even as a relative measure) is a significant challenge (Van Driesche, 1983 and Van Driesche et al., 1991). Utilizing mealybugs held in gelatin capsules to determine levels of parasitism sacrifices precision; however, with available resources it enabled numerous samples to be taken and evaluated. The collection and isolation of young (late second and early third instars) versus older mealybug stages to assess parasitism, clearly demonstrated that late stages should be used to determine parasitism, since parasitism of young stages was approx. 75% less than that for late third and adult female mealybugs. Whereas mealybug mummies collected to quantify hyperparasitism suggested that non-emergence of specimens that were known to be parasitized was notable, it is also likely that healthy (based on external appearance) late-third to adult female mealybug stages held for emergence provides an underestimate of percent parasitism. That is, it is likely that some parasitized mealybugs die before parasitoid pupation and are recorded as unparasitized. Despite the potential for underestimation, this approach to assessing parasitism seems to be a reasonable relative approach, given that high levels of parasitism (60–90%) were commonly recorded during this study.

Hyperparasitism sample records with levels reaching 60% (predominantly by *Marietta* sp.) were initially of concern. However, in subsequent years, mealybug densities continued to stay low and evidence suggests that the hyperparasitoid, *Marietta* sp., functions in a density-dependent manner. A density-dependent response by the native hyperparasitoid community in Africa was also observed in cassava following the introduction of a parasitoid species to control the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Neuenschwander and Hammond, 1988). Winter band data suggests that *M. hirsutus* supports a very small hyperparasitoid population during the winter months, in that fewer than 2% of the parasitoids were hyperparasitized. To date, control of *M. hirsutus* by *A. kamali* and *G. indica* in the urban environment within the desert southwest has been very effective despite the presence

of hyperparasitoids that appeared to exert a large impact upon *A. kamali* during the initial years of the project. Species of *Marietta* are nearly all hyperparasitic, attacking parasitoids of Homoptera, including Diaspididae, Coccidae, and other families (Krombein et al., 1979; Myartseva and Ruiz-Cancino, 2001). Neither *Marietta* nor *Chartocerus* spp. were collected among parasitoids of either non-target mealybug species. Prior to the introduction of *M. hirsutus*, it is likely they were sustained by primary parasitoids of mealybug species other than *P. solenopsis* and *Ferrisia* sp., or resident scale insect species.

Despite *A.* sp. nr. *mecrida* having come from the hot and arid area of Upper Egypt, there is no evidence to date indicating the establishment of this parasitoid in the Imperial Valley. Based on field survey data, given its seemingly predominant role in attacking *M. hirsutus* in Egypt, coupled with *A. kamali*'s considerably less apparent role in Egypt, this result was unexpected (Awadallah et al., 1999 and Gonzalez et al., 2003).

There has been no indication that the parasitoids introduced to control *M. hirsutus* are parasitizing non-target mealybug species in the region. This is the case, despite records of *A. kamali* attacking *Ferrisia virgata* elsewhere (Noyes and Hayat, 1994). Our findings conform to expectations based on work by Sagarra et al. (2001), indicating that *A. kamali* does not appear to have a wide host range, therefore, would not be considered a host generalist.

Acknowledgments

We thank the US Virgin Islands and Puerto Rico Departments of Agriculture for their assistance in providing parasitoids to this California program. We thank Brian Morse, Josué Vallejo, José Zúñiga and Rubén Aguilar for their technical assistance, and Kris Godfrey, Robert Dowell and two anonymous reviewers for their valuable manuscript review comments. We also thank the Imperial County Agricultural Commissioner's Office for assistance in providing space and facilitating insectary setup and other services, and Danny Hamon, USDA-APHIS-PPQ Western Region, in support of facilities and personnel. This article was co-authored by US Federal employees, however, it does not necessarily reflect US Department of Agriculture policy.

References

Awadallah, K.T., Ibrahim, A.M.A., Atia, A.R., Nada, S.M.A., 1999. Survey of mealybug parasitoids and their associated hyper-parasitoids on certain ornamental host plants at Giza region. Bulletin of the Entomological Society of Egypt 77, 97–101.

Beardsley, J.W., 1985. Notes and exhibitions. *Maconellicoccus hirsutus* (Green). Proceedings of the Hawaiian Entomological Society 25, 27.

DeBach, P., 1949. Population studies of the long-tailed mealybug and its natural enemies on citrus trees in southern California, 1946. Ecology 30, 14–25.

Gonzalez, D., El-Heneidy, A.H., Mousa, S.M., Triapitsyn, S.V, Adly, D., Trjapitzin, V.A., Meyerdirk, D.E., 2003. A survey for pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) and its parasitoids in Egypt, Spain and Morocco. Egyptian Journal of Biological Pest Control 13, 1–5.

Goolsby, J.A., Kirk, A.A., Meyerdirk, D.E., 2002. Seasonal phenology and natural enemies of *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) in Australia. Florida Entomologist 85, 494–498.

Green, E.E., 1908. Remarks on Indian scale insects (Coccidae), part III. With a catalogue of all species hitherto recorded from the Indian continent. Memoirs of Department of Agriculture India (Entomological Series) 2, 15–46.

Hall, W.J., 1926. The hibiscus mealybug (*Phenacoccus hirsutus*, Green) in Egypt in 1925 with notes on the introduction of *Cryptolaemus montrouzieri*, Muls. Ministry of Agriculture, Egypt. Technical and Scientific Service. Bulletin No. 70, 1–15.

Hoddle, M.S., 2004a. The strength of biological control in the battle against invasive pests: a reply. Conservation Biology 18, 61–64.

Hoddle, M.S., 2004b. Restoring balance: using exotic species to control invasive exotic species. Conservation Biology 18, 38–49.

Kairo, M.T.K., Pollard, G.V., Peterkin, D.D., Lopez, V.F., 2000. Biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera; Pseudococcidae) in the Caribbean. Integrated Pest Management Reviews 5, 241–254.

Krombein, K.V., Hurd Jr., P.D., Smith, D.R., Burks, B.D., 1979. Catalog of Hymenoptera in America north of Mexico. vol. 1, Smithsonian Inst. Press, Washington, DC, 1198 pp.

Louda, S.M., Stiling, P., 2004. The double-edged sword of biological control in conservation and restoration. Conservation Biology 18, 50–53.

Louda, S.M., Pemberton, R.W., Johnson, M.T., Follett, P.A., 2003. Nontarget effects-the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annual Review of Entomology 48, 365–396.

Meyerdirk, D.E., Newell, I.M., Warkentin, R.W., 1981. Biological control of Comstock mealybug. Journal of Economic Entomology 74, 79–84.

Meyerdirk, D.E., Warkentin, R., Attavian, B., Gersabeck, E., Francis, A., Adams, J., Francis, G., 2002. Biological control of pink hibiscus mealybug project manual. United States Dept. of Agric., Animal and Plant Health Inspection Service, Plant Protection and Quarantine. 194pp. http://www.aphis.usda.gov/ppq/manuals/pdf_files/phm.pdf.

Myartseva, S.N., Ruiz-Cancino, E., 2001. Mexican species of parasitoid wasps of the genus *Marietta* (Hymenoptera: Aphelinidae). Florida Entomologist 84, 293–297.

Neuenschwander, P., Hammond, W.N.O., 1988. Natural enemy activity following the introduction of *Epidinocarsis lopezi* (Hymenoptera: Encyrtidae) against the cassava mealybug, *Phenacoccus manihoti* (Homoptera: Pseudococcidae), in southwestern Nigeria. Environmental Entomology 17, 894–902.

Noyes, J.S., Hayat, M., 1994. Oriental Mealybug Parasitoids of the *Anagyrini* (Hymenoptera: Encytidae). CAB International, Wallingford, UK. 560 pp.

Sagarra, L.A., Vincent, C., Peters, N.F., Stewart, R.K., 2000a. Effect of host density, temperature, and photoperiod on the fitness of *Anagyrus kamali*, a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus*. Entomologia Experimentalis et Applicata 96, 141–147.

Sagarra, L.A., Vincent, C., Stewart, R.K., 2000b. Mutual interference among female *Anagyrus kamali* Moursi (Hymenoptera): Encyrtidae) and its impact on fecundity, progeny production and sex ratio. Biocontrol Science and Technology 10, 239–244.

Sagarra, L.A., Vincent, C., Stewart, R.K., 2001. Suitability of nine mealybug species (Homoptera: Pseudococcidae) as hosts for the parasitoid *Anagyrus kamali* (Hymenoptera: Encyrtidae). Florida Entomologist 84, 112–116.

Serrano, M.S., LaPointe, S.L., 2002. Evaluation of host plants and a meridic diet for rearing *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) and its parasitoid *Anagyrus kamali* (Hymenoptera: Encyrtidae). Florida Entomologist 85, 417–425.

Van Driesche, R.G., 1983. Meaning of "percent parasitism" in studies of insect parasitoids. Environmental Entomology 12, 1611–1622.

Van Driesche, R.G., Bellows Jr., T.S., Elkinton, J.S., Gould, J.R., Ferro, D.N., 1991. The meaning of percentage parasitism revisited: solutions to the problem of accurately estimating total losses from parasitism. Environmental Entomology 20, 1–7.

Weddle, R., Roltsch,W., 2000. Intensive population monitoring program of pink hibiscus mealybug in Imperial Valley. In: Anonymous. Cooperative pink hibiscus mealybug program annual report for 2000. Calif. Dept. of Food and Agric., Plant Health and Pest Prevention Services, Sacramento, CA, 35pp.

Williams, D.J., 1986. The identity and distribution of the genus *Maconellicoccus* Ezzat (Hemiptera: Pseudococcidae) in Africa. Bulletin of Entomological Research. 76, 351–357.

Williams, D.J., 1996. A brief account of the hibiscus mealybug *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae), a pest of agriculture and horticulture, with descriptions of two related species from southern Asia. Bulletin of Entomological Research 86, 617–628.

Williams, D.J., 2004. Mealybugs of southern Asia. The Natural History Museum, London. 896 pp.