Biological control of the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in Guam

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Summary
The papaya mealybug (PM), *Paracoccus marginatus*, a pest in the Central America and the Caribbean was noted to have established on Guam in April 2002 and was causing serious damage to papaya, *Plumeria* spp., *Hibiscus* spp. and other plants. The parasitoids *Anagyrus loecki*, *Pseudleptomastix mexicana* and *Acerophagous papayae* totalling 46 200 individuals were introduced from Puerto Rico and field released in Guam from June to October, 2002. A reduction of over 99% of papaya mealybug was observed about a year after the introduction of these parasitoids. This has reduced risk of introduction of this mealybug to neighbouring islands in the Pacific Region. A biological control system now appears successful and is available for implementation if necessary in other islands in the Pacific region.

Keywords: Papaya mealybug, *Paracoccus marginatus*, Hemiptera, Pseudococcidae, *Anagyrus loecki*, *Pseudleptomastix mexicana*, *Acerophagous papayae*, Hymenoptera, Encyrtidae, biological control, Guam.

Introduction
The papaya mealybug (PM), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) is native to Mexico and/or Central America (Miller *et al.* 1999) and was first described in 1992 (Williams and Granara de Willink 1992) and re-described by Miller and Miller (2002). It was first reported in St. Martin in the Caribbean in 1995 and since then has spread to 13 countries in the Caribbean, Florida in the US, and three countries each in Central and South America by 2000 (Miller *et al.* 1999, Matile-Ferrero *et al.* 2000, Kauffman *et al.* 2001). In the early part of 2002, heavy infestations of this mealybug were observed on papaya (*Carica papaya* L. (Caricaceae)) in Guam. In April 2002, it was identified as *P. marginatus* by G.W. Watson, Natural History Museum, London, England. It has a wide host range of over 60 species of plants including economically important plants such as *Annona squamosa*, *Carica papaya*, *Hibiscus rosa-sinensis*, *Ipomoea* spp., *Manihot esculenta* and *Solanum melongena* (Meyerdirk and Kauffman 2001).

Heavy infestations of the PM on papaya have been noted along the veins and the midribs of the older leaves and all areas of the tender leaves and fruits. Severely affected older leaves turn yellow and dry up. Tender leaves become crinkled. Terminal shoots become bunched and distorted. Heavy mealybug populations produce a large volume of honey dew, which causes black sooty mould to cover the infected fruits and vegetation. The infested leaves become distorted in *Plumeria* spp. (Apocynaceae) plants. On *Hibiscus* spp. (Malvaceae) the tender shoots covered by mealybugs become scorched.

Adult females are yellow in colour covered by a white waxy secretion and have no longitudinal depressions; short waxy filaments around the body; short caudal filaments; a body fluid which is yellow, and specimens in alcohol turn bluish-black (Meyerdirk and Kauffman 2001).

The recent establishment of PM in Guam was a serious concern for the Pacific Islands including Hawaii, Commonwealth of the Northern Marianas and Federated States of Micronesia. It became established in the Republic of Palau in April 2003 (Anon. 2003). The serious damage caused by PM to papaya, *Plumeria* spp., *Hibiscus* spp. and other plants in Guam warranted immediate attention as in the case of the countries in the Caribbean and Florida (Meyerdirk 1999, 2000).

Since the establishment of PM in Guam, farmers and home gardeners have been using some chemical and cultural control methods to control PM without much success. Some homeowners cut papaya trees in their yards and the commercial growers abandoned papaya cultivation.

In 1999 the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) and cooperators in Mexico collected the parasitoids of PM, *Apoanagyrus* nr. *californicus* Compere, *Anagyrus loecki* Noyes, *Acerophagous papayae* Noyes and Schauff *Pseudaphycus* sp. and *Pseudleptomastix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) (Noyes and Schauff 2003). All five species were screened for purity through quarantine at the USDA, ARS Beneficial Insects Laboratory in Newark, Delaware. An environmental assessment (Meyerdirk, 1999, 2000) was completed on all five species and specimens were shipped to San Juan, Puerto Rico, where they were cultured and mass produced in a cooperative effort with the Puerto Rico Department of Agriculture and USDA, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ). These parasitoids have been released in the Dominican Republic, Puerto Rico, and Florida.

This paper documents the introduction and establishment of the parasitoids of PM in Guam that appears to be resulting in complete biological control. This has considerably reduced the chances of PM spread to neighbouring islands.

Materials and methods
Plumeria spp. and *Hibiscus* spp. were selected as the study plants to monitor the population densities of PM and the newly released exotic parasitoids. *Plumeria* spp. samples included four mature leaves on four terminal shoots selected at random, totalling 16 leaves per tree for mealybug density counts. Only the lower surface of the leaves was examined under a dissecting microscope. Mealybugs were counted on only one lateral half of each leaf from the midrib to the leaf's edge for samples with high densities. This was later adjusted at the end of the count by multiplying by two for a complete leaf estimate. Mechanical counters were used to tally the total number of mealybugs per stage of development. Stages counted included: egg masses as single individual units with eggs alone, egg masses with eggs and crawlers, second and third instars, adult male and female mealybugs, mummies with and without exit holes. The second and third instar and adult male and female stages were totalled per leaf, and all 16 leaves used to average those stages per leaf per study site. One tree represented one study site. A total of eight study sites, each with one *Plumeria* tree, served as the source of counts for this study. Data collected on the number of egg masses, 2nd and 3rd instars and adult males and females of PM per whole *Plumeria* spp. leaf sample were converted to total number of mealybugs per 100 cm^{-2} of the leaf.

Additional *Plumeria* spp. leaves were collected from each study site that showed signs of the PM infestation in order to isolate a total of 100 individual mealybugs per site. Late second and third instars and adult female PM were individually collected for the percent parasitization records. This was conducted on a monthly schedule with, mealybugs removed from the leaf samples and individually encapsulated in clear gelatin capsules (size 0). These capsules were labelled and placed in Ziploc plastic bags for 30 days in the laboratory in an air conditioned room (25°C). After the 30 day period, each capsule was examined to determine if the mealybug was parasitized and a parasitoid emerged from the mealybug. Emerged parasitoids were counted and identified to species.

In addition, four study sites were selected using PM infested *Hibiscus* shrubs. A total of four terminal shoots 15 cm long were randomly selected from each sampled plant. Additional terminal shoot samples were collected that showed evidence of PM in order to encapsulate 100 individual mealybugs similar to the procedure mentioned for the *Plumeria* spp. samples above. The four 15 cm terminal shoots were used as separate sample units counting all egg masses with eggs only and egg masses with eggs and crawlers, second and third instars, and adult female and male stages, and mummies with and without exit holes.

Exotic parasitoids were originally imported from Southern Mexico in 1999 by USDA, ARS and screened through the quarantine facility at USDA, ARS Beneficial Insects Research Laboratories in Newark, Delaware. The primary parasitoids were later shipped to the Puerto Rico Department of Agriculture in San Juan, Puerto Rico for mass production and field release under appropriate permits. Three species of parasitoids (*A. loecki*, *A. papayae* and *P. mexicana*) were shipped to Guam between June 24 and October 17, 2002. A total of 200–400 individual parasitoids of each species were released at each study site on each test plant initially on June 27, 2002. A second final release was made on July 25, 2002, because the study sites were hit by a typhoon 'Chataan' on July 6, 2002.

Additional parasitoids were released on PM at 27 different locations across the Island of Guam during the period of three months (August 10 until October 17, 2002) (Table 1) to ensure establishment over a wide geographical area. Further spread would have resulted from dispersal.

The sampling sites, numbering one to eight, in Guam are shown in Figure 1. The survey for population density estimations of the PM before the release of the parasitoids was carried out on June 6, 2002. The parasitoids *A. loecki*, *A. papayae* and *P. mexicana* were first released on June

Table 1. Details of parasitoids released at different locations in Guam.

Date	Village	Parasitoids released		
		A. loecki	*P. mexicana*	*A. papayae*
June 27, 2002	Yigo	100	200	100
June 27, 2002	Pagat	100	200	100
June 27, 2002	Hagatña (Legislature)	100	100	100
June 27, 2002	Hagatña (Lock up)	100	100	100
June 27, 2002	Piti	200	200	200
June 27, 2002	Tumon	130	200	130
June 27, 2002	Tiyan	140	200	140
June 27, 2002	UOG	130	200	130
July 25, 2002	Yigo	100	400	200
July 25, 2002	Tumon	200	400	200
July 25, 2002	Pagat	100	400	200
July 25, 2002	UOG	200	400	200
July 25, 2002	Hagatña (Legislature)	200	400	200
July 25, 2002	Hagatña (Lock up)	0	400	0
July 25, 2002	Tiyan	200	400	200
July 25, 2002	Piti	200	400	200
August 9, 2002	Tiyan	100	200	100
August 9, 2002	Talafofo (Golf CT)	200	300	200
August 9, 2002	Hagat	400	400	200
August 15, 2002	Chalan Pago	400	200	400
August 15, 2002	Talafofo	200	200	400
August 15, 2002	Inarajan	400	200	400
August 15, 2002	Malesso	200	200	400
August 15, 2002	Hagatña	200	200	400
August 22, 2002	Hagatña (skinner plaza)	400	400	400
August 22, 2002	Inarajan (AES)	200	600	200
August 22, 2002	Pago Bay Estates	200	400	200
August 29, 2002	Chalan Pago-Olivia	200	200	200
August 29, 2002	Tumon (Hilton)	400	400	800
August 29, 2002	Dededo (Mall)	400	600	1000
September 6, 2002	Dededo (Mall)	200	1200	1600
September 6, 2002	Barrigada	200	1000	1400
September 6, 2002	Yona	400	1000	1400
September 20, 2002	Barrigada	200	800	400
September 20, 2002	Hagatña Heights	200	1000	400
September 26, 2002	Dededo	100	600	200
September 26, 2002	Yigo	0	1000	400
September 26, 2002	Yona	0	400	400
October 3, 2002	Yigo	200	600	1000
October 3, 2002	Tiyan	200	400	1000
October 3, 2002	Tumon	0	1200	0
October 10, 2002	Yigo	0	0	400
October 10, 2002	Yona	200	200	200
October 10, 2002	Mangilao	0	0	200
Total		8800	19900	17500

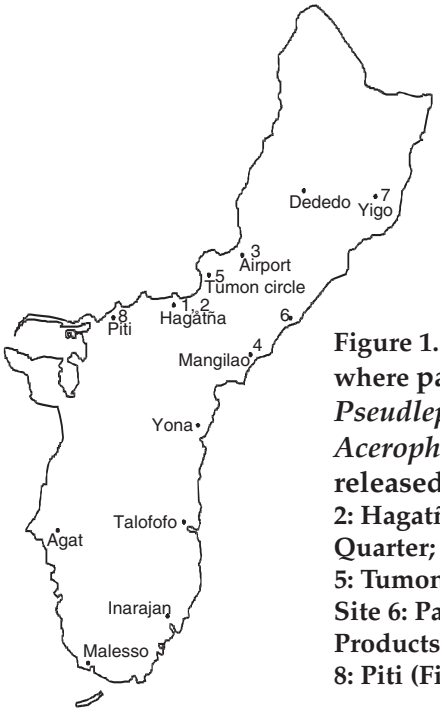

Figure 1. Areas in the island of Guam where parasitoids (*Anagyrus loecki*, *Pseudleptomastix mexicana* and *Acerophagus papayae*) have been released. Site 1: Hagatña Legislature; Site 2: Hagatña Lockup; Site 3: Airport Living Quarter; Site 4: UOG Theatre Building; Site 5: Tumon Circle Upper Level (San Victores); Site 6: Pagat Mangilao (Hawaiian Rock Products); Site 7: Yigo Baptist Church; Site 8: Piti (Fish Eye Park).

27, 2002 and continued until October 10, 2002 (Table 1). A total of 46 200 parasitoids were released. Monthly sampling to determine the establishment of the introduced parasitoids, the percentage of parasitism and quarterly sampling of PM populations were originally planned for one year, however, the typhoons named Chataan on July 6, 2002 and Pongsona on December 8, 2002 severely damaged some on the plants used for sampling and also disrupted our data collection. The first population assessment of PM after release of the parasitoids was on September 25, 2002 and the second on July 28, 2003 (Table 2).

Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae), a pre-existing mealybug predator in Guam, was also sampled during papaya mealybug density counts by using a beating sheet (53 × 53 cm). A total of four taps on *Plumeria* spp. terminal shoots were used to knock off *C. montrouzieri* adults and larvae, which were then counted in each terminal shoot

Table 2. Mean number of *Paracoccus marginatus* population per 100 cm^{-2} leaf area of *Plumeria* spp. before and after release of parasitoids (*Anagyrus loecki*, *Pseudleptomastix mexicana* and *Acerophagus papayae*) at various locations in Guam.

Location	June, 2002 (1) Sept, 2002 (2) Aug, 2003 (3)	Egg mass		Second instar		Third instar		Adult ♀		Adult ♂	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Site 1	1	2.1	3.2	184.2	133.9	38.6	26.9	4.8	3.8	4.4	4.7
	2	0.1	0.2	6.4	10.6	13.9	20.4	0.7	1.2	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Site 2	1	31.7	26.5	249.9	228.8	75.6	78.4	5.7	11.7	3.2	2.7
	2	17.8	14.3	47.4	47.5	35.1	22.1	13.5	13.6	0.7	1.4
	3	0.0	0.0	3.7	5.2	2.1	4.2	0.2	0.3	0.0	0.0
Site 3	1	5.2	3.9	59.0	39.8	9.4	8.2	0.6	1.0	0.6	1.1
	2	2.7	2.9	51.5	24.5	50.0	37.8	4.8	3.6	0.1	0.2
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Site 4	1	35.6	42.3	116.2	97.9	57.5	53.1	14.7	12.7	3.2	4.9
	2	2.2	2.7	39.3	36.3	20.0	20.5	1.6	2.6	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Site 5	1	3.6	4.7	112.8	67.5	17.7	13.5	4.9	3.3	2.7	3.3
	2	0.0	0.0	1.2	2.5	0.6	1.2	0.0	0.2	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Site 6	1	2.9	3.1	147.5	171.9	16.3	19.3	5.4	5.3	3.4	4.1
	2	3.9	5.6	14.2	23.0	16.1	14.0	2.5	2.1	0.1	0.2
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Site 7	1	30.5	37.7	173.0	207.4	61.5	55.1	3.9	4.1	4.5	4.2
	2	0.7	0.6	1.8	1.8	2.8	2.1	0.7	1.0	0.1	0.5
	3	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.3	0.0	0.0
Site 8	1	8.0	8.2	370.1	206.6	74.7	48.5	12.8	11.7	7.1	5.8
	2	10.0	11.3	41.8	30.4	34.2	23.6	7.9	8.4	0.6	1.1
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Site 1: Hagatña Legislature; Site 2: Hagatña Lockup; Site 3: Airport Living Quarter; Site 4: UOG Theatre Building; Site 5: Tumon Circle Upper Level (San Victores); Site 6: Pagat Mangilao (Hawaiian Rock Products); Site 7: Yigo Baptist Church; Site 8: Piti (Fish Eye Park).

of each *Plumeria* spp. tree at random. *Hibiscus* spp. was also sampled in the same manner at four different randomly selected sections of each test shrub. Notes were taken on other predators observed at each study site.

Results

Papaya mealybug population densities on *Plumeria* spp. for the eight sampling sites are given in Table 2. The reduction in population density of various stages of PM over a period of one year from the time of release of the parasitoids is shown in Figure 2. The population density of PM on *Hibiscus* spp. plants before and after release of the parasitoids is given in Table 3. The percent parasitism by all the three parasitoids from July to October 2002 is shown in Figure 3. By September 2002, there was an average PM population density reduction of 80% over six sites, 79.2% at eight sites, 61.4% at seven sites, 74.6% at six sites and 94.4% at eight sites in the population of egg mass, second instar, third instar and adult female and male, respectively on *Plumeria* spp. There was an increase in population at two sites for egg mass, one site for third instar and two sites for adult female. The percent parasitism increased to 45% by October 2002. The estimations of parasitism and PM population density counts were disrupted by the two major typhoons that hit Guam in the middle and later parts of 2002. A year after introduction of the parasitoids the PM population density reductions were 100% egg mass, 99.5% second instar, 99.8% third instar and 100% adults of both sexes in the sampling sites. Similarly on *Hibiscus* spp. the PM population density reductions of various mealybug stages three months after the release of parasitoids were 92.8% egg mass, 95.6% second instar, 96.6% third instar, 97.7% adult female and 100% adult male. There was 100% reduction of all stages a year after release of the parasitoids.

Discussion

Survey of PM on Guam before the release of the parasitoids showed that there were no local parasitoids that had shifted to attacking this mealybug. A few coccinellids such as *C. montrouzieri* and *Chilocorus nigritus* (Fabricius) (Coleoptera: Coccinellidae) were however, found feeding on it (data not shown). The populations of polyphagous predators fluctuate irregularly in time and space as their numbers are influenced by other hosts (Bokonon-Ganta and Neuenschwander 1995). These, therefore, were not apparently capable of suppressing the populations of PM. Parasitoids of PM imported from Puerto Rico were released within two months of confirmation of the establishment of PM on Guam. Establishment of the parasitoids was confirmed within a month of release at the sample sites, releases were continued at other geographical locations across the Island until October 2002 in an effort to establish the parasitoids throughout Guam and to suppress the PM population as quickly as possible. By August 2003 the population of PM declined to a level which was hard to find in the field. Almost all papaya, *Plumeria* spp. and *Hibiscus* spp. plants recovered and no symptoms of damage were noted at that time. In early 2002, farmers on Guam gave up growing papaya as they were unable to control the PM with conventional insecticides. A year after the introduction of the parasitoids, papaya cultivation has become common and homeowners are now relieved of their worries over the deformation of leaves and shoots of *Plumeria* spp. and *Hibiscus* spp. plants. Although this mealybug has recently established itself in the Republic of Palau, possibly from Guam, its threat of invading other islands in the Western Pacific such as the Commonwealth of the Northern Mariana Islands, Federated States of Micronesia,

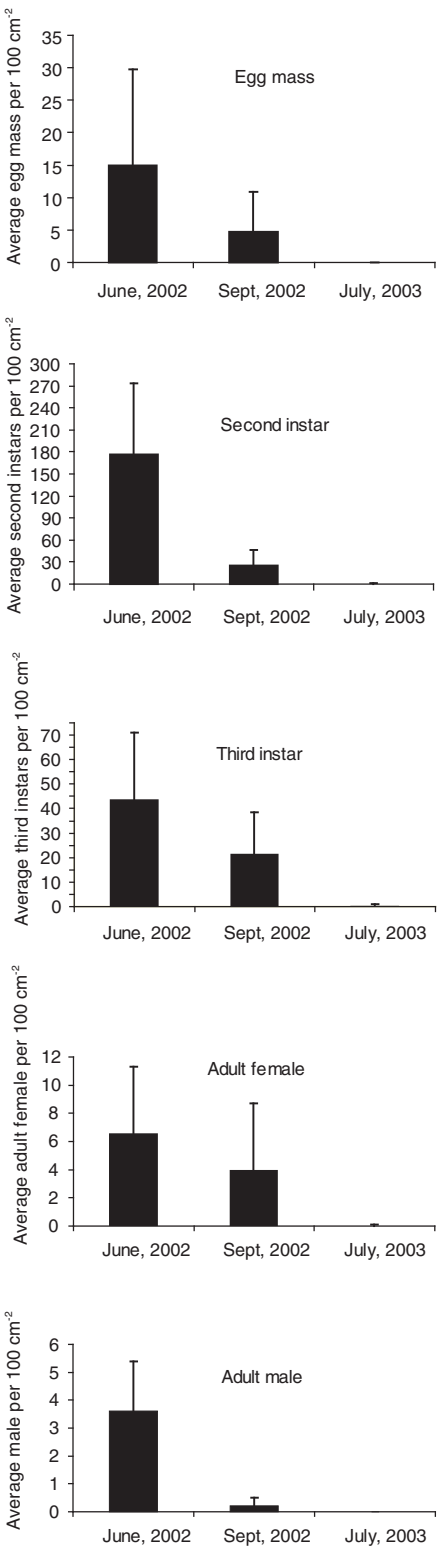

Figure 2. Average number (±SD) of various stages of *Paracoccus marginatus* population per 100 cm^{-2} leaf area of *Plumeria* spp. before and after release of parasitoids (*Anagyrus loecki*, *Pseudleptomastix mexicana* and *Acerophagus papayae*).

Table 3. Mean number of *Paracoccus marginatus* population per shoot of *Hibiscus* spp. before and after release of parasitoids (*Anagyrus loecki*, *Pseudleptomastix mexicana* and *Acerophagus papayae*) in Guam.

	Egg mass		Second instar		Third instar		Adult ♀		Adult ♂	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
June, 2002	19.4	35.3	85.7	79.4	44.2	74.3	8.9	13.0	0.8	1.0
September, 2002	1.4	2.8	3.8	5.0	1.5	2.0	0.2	0.3	0.0	0.0
August, 2003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Each value is mean of four locations (Hagatña Legislature, Airport Living Quarter, UOG Theatre Building, Tumon Circle Upper Level (San Victores).

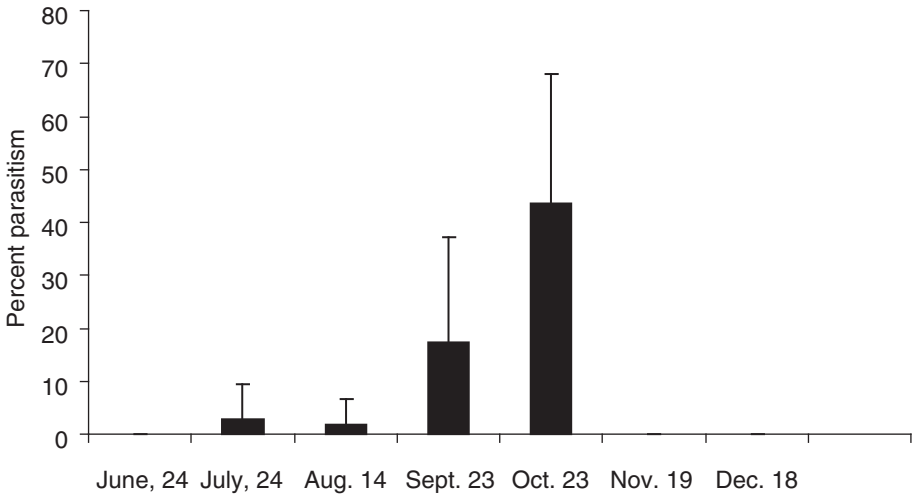

Figure 3. Percent of parasitism (±SD) in *Paracoccus marginatus* by three introduced parasitoids in 2002 (*Anagyrus loecki*, *Pseudleptomastix mexicana*, and *Acerophagus papayae*).

and the State of Hawaii are considerably reduced by suppressing its population in Guam. A program for biological control of PM in Palau is presently in progress.

Similar results have been noted in the Dominican Republic and Puerto Rico, obtaining about 97% reduction in PM populations a year after the introduction of the parasitoids (Kauffman *et al.* 2001, Meyerdirk and Kauffman 2001). The program in Guam has become one of the classic examples of the technology transfer of a classical biological control program from the Caribbean to the Pacific.

Similar successes in classical biological control programs on hemipterans in recent years are cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in Africa (Neuenschwander 2001), mango mealybug, *Rastrococcus invadens* (Williams) (Hemiptera: Pseudococcidae) in West Africa (Bokonon-Ganta and Neuenschwander 1995, Pitan *et al.* 2000), red coconut scale, *Furcaspis oceanica* Lindinger (Hemiptera: Diaspididae) in Saipan and Guam (Muniappan *et al.* 2003) and pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) in the Caribbean (Kairo *et al.* 2000).

Acknowledgements

We thank USDA, APHIS, PPQ for providing funding support for this research, Puerto Rico Department of Agriculture for shipping the parasitoids and Ms. Carol Russell, USDA, APHIS, PPQ, Hawaii for taking care of the administrative transactions of this project. This article was co-authored by a US Federal employee. However, it does not necessarily reflect US Department of Agriculture policy. Guam Agriculture Experiment Station Publication No. 460.

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