SUITABILITY OF NINE MEALYBUG SPECIES (HOMOPTERA: PSEUDOCOCCIDAE) AS HOSTS FOR THE PARASITOID *ANAGYRUS KAMALI* (HYMENOPTERA: ENCYRTIDAE)

L. A. SAGARRA[1,2], C. VINCENT[3] AND R. K. STEWART[4]
Department of Natural Resource Sciences, Macdonald Campus of McGill University
Sainte-Anne-de-Bellevue, Qc, Canada H9X 3V9

[1]Pernod-Ricard Trinidad Ltd., Orange Grove Estate, Tacarigua, Trinidad & Tobago

[2]Present address: 7 rue de Plaisance, 94130 Nogent sur Marne, France

[3]Horticultural Research and Development Center, Agriculture and Agri-Food Canada
430 Gouin Blvd., Saint-Jean-sur-Richelieu, Qc, Canada J3B 3E6

[4]Department of Natural Resource Sciences, Macdonald Campus of McGill University
Sainte-Anne-de-Bellevue, Qc, Canada H9X 3V9

ABSTRACT

The parasitoid *Anagyrus kamali* Moursi [Hymenoptera: Encyrtidae] has been recently introduced into the Caribbean as a biological control agent against the Hibiscus Mealybug, *Maconellicoccus hirsutus* Green [Homoptera: Pseudococcidae]. In order to understand host/parasitoid ecological interactions and optimize the mass-production system of this parasitoid, eight mealybug species (*Planococcus citri* (Risso), *Planococcus halli* Ezzat & McConnel, *Dysmicoccus brevipes* (Cockerell), *Pseudococcus elisae* Borchsenius, *Saccharococcus sacchari* (Cockerell), *Puto barberii* (Cockerell), *Nipaecoccus nipae* (Newstead), *Plotococcus neotropicus* (Williams & Granara de Willink)) common to Trinidad were tested to determine their potential as alternative hosts for the parasitoid. Susceptibility, preference and suitability tests were conducted. In addition to *M. hirsutus* (4.5 ± 2.04 hosts parasitized per female parasitoid in 30 min), *Planococcus citri* (1.1 ± 1.23 hosts parasitized) and *Planoccocus halli* (0.8 ± 1.41 hosts parasitized) were the only species parasitized. However, the parasitoid did not complete its development in the latter two hosts. Out of nine mealybug species, *M. hirsutus* was the only suitable host for the complete development of *A. kamali* progeny. This level of host specificity by *A. kamali* may prevent adverse effect to other Caribbean mealybug species.

Key Words: *Maconellicoccus hirsutus*, hibiscus mealybug, parasitoid, Pseudococcidae, host species selection

RESUMEN

El parasitoido *Anagyrus kamali* Moursi [Hymenoptera: Encyrtidae] fue introducido en el Caribe para el control biologico de la cochinilla rosada, *Maconellicoccus hirsutus* Green [Homoptera: Pseudococcidae]. Para mejorar nuestro conocimiento del systema plaga/parasitoido y optimizar la produccion del parasitoido, ocho especies de cochinillas (*Planococcus citri* (Risso), *Planococcus halli* Ezzat & McConnel, *Dysmicoccus brevipes* (Cockerell), *Pseudococcus elisae* Borchsenius, *Saccharococcus sacchari* (Cockerell), *Puto barberii* (Cockerell), *Nipaecoccus nipae* (Newstead), *Plotococcus neotropicus* (Williams & Granara de Willink)) de Trinidad fueron probadas para determinar sus potencial como hospedantes alternativos del parasitoido. *M. hirsutus* (4.5 ± 2.04 hospedantes parasitados por parasitoido en 30 min), *Planococcus citri* (1.1 ± 1.23 hospedantes parasitados) y *Planoccocus halli* (0.8 ± 1.41 hospedantes parasitados) fueron las unicas especies parasitadas, sin embargo, el parasitoido nunca completo su desarrollo en las dos ultimas especies. *M. hirsutus* fue la unica especies en la cual el parasitoido pudiera completar su desarrollo. Por eso, la introduccion de *A. kamali* en el Caribe no deberia afectar la biodiversidad locale.

Since its accidental introduction into the island of Grenada in 1994, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae), commonly named the hibiscus or pink mealybug, has been inexorably spreading through the Caribbean and is now present on 18 Islands (International Institute of Entomology 1997). *M. hirsutus* has high reproductive potential (384-540 eggs laid per fe-

male (Mani 1989)) and injects its saliva at the point of feeding, causing severe distortion of leaves, new shoots and fruit (Williams 1996). Because of its wide host range (ca. 125 host species (Malviaceae, etc.), Mani 1989) and its rapid geographic expansion on agricultural land, home gardens and forest areas, biological control appears to be the best strategy to reduce *M. hirsutus* populations.

The solitary endoparasitoid *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) was imported from China by CABI Bioscience, for biological control of *M. hirsutus* in the Caribbean (Sagarra & Peterkin 2000). The preferred host of *A. kamali* is *M. hirsutus* (Moursi 1948), but it was also reported utilizing five other mealybug species in Jordan and India (Noyes & Hayat 1994), including *Nipaecoccus viridis* (Newstead) (Meyerdirk et al. 1988), and *Ferisia virgata* (Cockerell) (Cross & Noyes 1996). These mealybug species were found to be parasitized by other *Anagyrus* species that were subsequently considered as synonymous to *A. kamali*. However their suitability for the development of *A. kamali* has not been formally determined.

It is important to ensure that introduction of a newly introduced natural enemy will minimally disrupt local mealybug biodiversity. On the other hand, the impact of an insect parasitoid on target pest populations may be enhanced by the availability of alternative hosts in or around the crop especially in periods of host shortage (Powell 1986). It has been suggested that alternative hosts can help to improve synchrony between parasitoids and their pest hosts, improve parasitoid distribution and reduce intraspecific competition in the parasitoid population (van den Bosch & Telford 1964).

An understanding of host/parasitoid interactions is essential to determine if other co-existing mealybug species could be used by *A. kamali* as alternative hosts. Our objective was to determine the susceptibility, preference, and suitability of eight common mealybug species in Trinidad (i.e., *Planococcus citri* (Risso), *Planococcus halli* Ezzat & McConnel, *Dysmicoccus brevipes* (Cockerell), *Pseudococcus elisae* Borchsenius, *Saccharococcus sacchari* (Cockerell), *Puto barberii* (Cockerell), *Nipaecoccus nipae* (Newstead), *Plotococcus neotropicus* (Williams & Granara de Willink) and *M. hirsutus* to parasitization by *A. kamali*. The latter mealybug species are pantropical and have been present in Trinidad for more than a hundred year (Records from CABI insect collection).

Materials and Methods

Rearing of *M. hirsutus*

Mealybugs were reared on sprouted potatoes in nylon mesh cages (32 potatoes per cage) supported on steel wire frames (48 × 48 × 68cm). The cultures were maintained in the dark at 27 ± 2°C. Each week, 64 potatoes were individually infested with 20 adult female mealybugs having well-formed ovisacs. Weekly infestations ensured a continuous supply of different *M. hirsutus* nymphal instars. Three weeks after infestation, sprouted potatoes had *M. hirsutus* populations that consisted predominantly of second and third instar mealybugs. Adult females with ovisacs were available after 4-6 weeks. Size was used to distinguish *M. hirsutus* stages (Ghose 1971).

Rearing of Other Mealybug Species

Planococcus citri, *P. halli*, *Dysmicoccus brevipes*, and *Pseudococcus elisae* were reared on sprouted potatoes in plastic boxes with a mesh cover (6 potatoes per box). The potato sprouts were individually infested with 20 adult female mealybugs of each species. *Saccharococcus sacchari* were reared on young sugar cane seedlings individually infested with 5 adults. *Puto barberi* were reared on young Bougainvillea seedlings individually infested with 30 adult mealybugs. *Nipaecoccus nipae* were reared on young coconut trees, and leaves were individually infested with 30 adults. *Plotococcus neotropicus* were reared on young Guava seedlings individually infested with 30 adult mealybugs. Four weeks after initial infestation, third instars and young adults of each species were collected and used for the experiments. Mealybug identification was confirmed by J. Etienne from INRA, Guadeloupe, and D. Matile-Ferrero, Musée d'Histoire Naturelle in Paris, France.

Rearing of *A. kamali*

Each week, 100 *A. kamali* adult females were released into two cages each containing 32 infested sprouted potatoes supporting three-week old populations of *M. hirsutus* (after Sagarra and Vincent, 1999). Colonies were maintained at 27 ± 2°C, 60 ± 10% RH, under a photoperiod of LD 12:12. Light was provided by two fluorescent lamps (70W) suspended 30cm above the cages. Emerged parasitoids were collected after 20-25 days. Two-day old, mated females were used for experiments. Female parasitoids were considered as experienced because they had been exposed to different stages of mealybugs in the rearing cages for a maximum of 12 hours after emergence.

In all experiments with the nine mealybug species we used third instars and early adult females (preovisac). All experiments were conducted in the laboratory at 27 ± 2°C, 60 ± 10% RH.

Host Species Susceptibility (No-Choice Experiments)

Ten mealybugs from each species were collected and placed in groups in separate Petri

dishes. One female parasitoid was introduced into each dish. The parasitoid and the mealybugs were observed continuously for 30 min to record number of encounters, and host probing with the ovipositor. Twenty groups of each species were exposed to different individual *A. kamali* females in this manner. The parasitoids were removed after 30 min. All exposed mealybugs were dissected in a drop of ethanol (70%), and the number of parasitoid eggs in each individual host was recorded.

The number of encounters, ovipositor probing, hosts parasitized by each *A. kamali* female, the total number of eggs laid per parasitoid female, and the number of parasitoid eggs per accepted host were used as the criteria for determining host species susceptibility. Paired Chi-square frequency analysis was used to determine if the number of encounters, ovipositor probing, hosts parasitized by each *A. kamali* female, total number of eggs laid per parasitoid female, and number of parasitoid eggs per accepted host was dependent on the mealybug species at $p = 0.05$ (Systat 7.0 for Windows 95).

Host Species Preference (Two-Choice Experiments)

In two-choice experiments, five *M. hirsutus* were placed in a Petri dish with five specimens of one of the eight other mealybugs species, and a single female parasitoid was introduced into each experimental arena. The parasitoid and mealybugs were observed continuously for 30 min to record the number of encounters, and probing with the ovipositor for each mealybug species. The parasitoid was then removed. We dissected each host in a drop of ethanol (70%), and the number of eggs in each host was recorded for each species. Twenty batches of each of the eight host combinations were exposed to individual *A. kamali* females in this manner.

The number of encounters, probing, hosts parasitized per parasitoid, the total number of parasitoid eggs laid per replicate, and the number of parasitoid eggs per accepted host were used as the criteria for determining preference between *M. hirsutus* and the other host species. Paired Chi-square frequency analysis was done to determine if the number of encounters, ovipositor probing, hosts parasitized by each *A. kamali* female, total number of eggs laid per parasitoid female, and number of parasitoid eggs per accepted host was dependent on the mealybug species at $p = 0.05$ (Systat 7.0 for Windows 95).

Host Species Suitability

Thirty individuals of each mealybug species were transferred onto sprouted potatoes and placed in a cage made of a transparent plastic cylinder (20×12 cm diameter) with the top covered with nylon mesh. Three adult female parasitoids were introduced into the cage for a period of 24h. The mealybugs were observed on a daily basis to record parasitoid emergence. Parasitoid progeny was collected, sexed and the size (i.e., fitness) of emerged parasitoids was estimated by measuring the length of the left hind tibia (Ghose 1971). The total body length (base of head to tip of abdomen) and the length of the hind tibia were measured for 35 adult *A. kamali* and fitted to a linear regression model (Systat 7.0 for Windows 95). The coefficient of determination (r^2) indicated that 84.2% of the variation in the body length of the wasps can be explained by the length of the hind tibia of the parasitoid in a linear relationship, therefore providing an accurate and rapid means of assessing of the overall size of the parasitoid. The host suitability experiment was replicated five times for each mealybug species. The criteria used to determine host suitability were the number of emerged parasitoids per replicate, the secondary sex ratio (number of males divided by total progeny number, as suggested by Godfray 1994; Van Alphen & Jervis 1996), the development time and the size of the parasitoids.

RESULTS

Host Species Susceptibility (No-Choice Experiment)

In no-choice experiments, *A. kamali*'s encounter rate, ovipositor probing, number of hosts parasitized, and number of eggs oviposited was significantly higher on *M. hirsutus*, compared to the other mealybug species (Table 1). Among the eight other mealybug species, the parasitoid's encounter rate for *P. citri*, *P. halli*, and *P. elisae* were significantly ($P < 0.05$) higher than for *D. brevipes*, *N. nipe*, *P. barberi*, *P. neotropicus*, and *S. sacchari*. The latter species did not induce searching responses by *A. kamali* females, which remained on the Petri dish cover during most of the 30 min. One species, *P. barberi*, defended itself against parasitoid encounter by violent torsions of its abdomen. The number of ovipositor probings of mealybugs was not significantly different ($P < 0.05$) among the eight tested hosts. Probing of *M. hirsutus* was significantly ($P < 0.05$) greater than for the other mealybug species. *D. brevipes*, *N. nipe*, *P. barberi*, *P. neotropicus*, and *S. sacchari* were not probed, whereas few individuals of *P. citri*, *P. halli*, and *P. elisae* were probed.

Planococcus citri, and *P. halli* were the only species, other than *M. hirsutus*, that were parasitized by *A. kamali*, but at a significantly ($P < 0.05$) lower level than *M. hirsutus* (Table 1).

In all three attacked species, superparasitism occurred, the number of eggs oviposited being greater than the number of hosts parasitized. Signs of egg encapsulation were observed in these three species.

TABLE 1. ENCOUNTER, PROBING AND OVIPOSITION OF *A. KAMALI* ON NINE SPECIES OF PSEUDOCOCCIDAE (NO-CHOICE TEST). FOR EACH SPECIES, TEN INDIVIDUALS WERE EXPOSED TO ONE FEMALE PARASITOID FOR 30 MIN (N = 20).

HMB species	Average (±SD)			
	Hosts encountered	Ovipositor probing	Hosts parasitized	Eggs oviposited
Maconellicoccus hirsutus	23.9 ± 12.45 a*	13.4 ± 11.05 a*	4.5 ± 2.04 a*	6.4 ± 3.65 a*
Planococcus citri	10.7 ± 6.20 b	3.3 ± 3.55 b	1.1 ± 1.23 b	1.2 ± 1.47 b
Planococcus halli	8.1 ± 5.99 b	1.3 ± 2.49 b	0.8 ± 1.41 bc	1.0 ± 1.91 b
Dysmicoccus brevipes	0.5 ± 0.83 c	0.0 b	0.0 c	0.0 b
Pseudococcus elisae	7.7 ± 5.93 b	0.4 ± 1.05 b	0.0 c	0.0 b
Saccharococcus sacchari	0.2 ± 0.49 c	0.0 b	0.0 c	0.0 b
Puto barberi	2.0 ± 1.91 c	0.0 b	0.0 c	0.0 b
Nipaecoccus nipe	0.2 ± 0.41 c	0.0 b	0.0 c	0.0 b
Plotococcus neotropicus	1.8 ± 1.07 c	0.0 b	0.0 c	0.0 b

*Within columns, pairs of means followed by the same letters are not significantly different (Chi-square test, $P < 0.05$).

Host Species Preference (Two-Choice Experiments)

In choice experiments, *M. hirsutus* was significantly preferred to all other mealybug species in terms of encounter rate, ovipositor probing, number of hosts parasitized, and number of eggs oviposited. *Dysmicoccus brevipes*, *N. nipe*, *P. barberi*, *P. elisae*, *P. neotropicus*, and *S. sacchari* were not utilized by female parasitoids, and were not parasitized when exposed to *A. kamali* simultaneously with *M. hirsutus*. Low numbers of *P. citri*, and *P. halli* were parasitized (0.3 ± 0.39, and 0.1 ± 0.21 hosts parasitized respectively) when compared to *M. hirsutus* (3.9 ± 0.95 hosts parasitized) in the 30 min assay. This level of parasitism in *P. citri*, and *P. halli* was not significantly different ($P = 0.05$) from the other six mealybug species tested.

Host Species Suitability

Of the nine mealybug species tested, successful development of parasitoid progeny occurred only in parasitized *M. hirsutus*, with an average progeny emergence of 14 ± 1.6 individuals out of the 30 hosts exposed to the three female parasitoids. The average progeny sex-ratio was 0.49 ± 0.107.

DISCUSSION

Of the eight tested mealybug species, *D. brevipes*, *N. nipe*, and *S. sacchari* did not induce searching behavior by *A. kamali*. *Puto barberi*, and *P. neotropicus* induced searching behavior by *A. kamali,* but host rejection occurred after antennation, prior to ovipositor probing. *Pseudococcus elisae* was rejected after being probed with the ovipositor. *Planococcus citri* and *P. halli* were recognized by *A. kamali* as potential hosts and parasitized, but these two host species were not suitable for parasitoid progeny development. *M. hirsutus* was the most suitable host, allowing the complete development of *A. kamali*. The parasitoid discriminated among different host species and selected the most suitable host for the development and survival of its progeny.

A. kamali has been recorded as a parasitoid of *M. hirsutus* (Moursi 1948), but also on several Pseudococcidae host species like *Pseudococcus sp.* on *Citrus limonium* (Agarwal 1965) and cocoa (Shafee et al. 1975), *Ferrisia virgata* (Cockerell) and *Ferrisia spp.* on an avenue tree (Subba Rao & Rai 1970), and *Nipaecoccus viridis* (Newstead) on Citrus (Meyerdirk et al. 1988). *A. kamali* has also been recorded from *Nipaecoccus spp.* on *Acacia sp.* (Shafee et al. 1975). Except for *Ferrisia virgata*, none of these mealybug species are present in Trinidad. In addition no emergence of the parasitoid from this host was obtained in laboratory experiments (M. Hoy 1999, pers. comm.). The members of the genera *Pseudococcus* and *Nipaecoccus* reported from Trinidad (i.e., *Nipaecoccus nipe* and *Pseudococcus elisae*) were not susceptible to parasitization by *A. kamali*.

The absence of alternative hosts for *A. kamali* development in Trinidad might reduce the efficiency of the parasitoid as a biological control agent since other alternative host species will not be available for *A. kamali* when *M. hirsutus* is at low densities. However, this is an advantage from a point of view of preservation of native mealybug biodiversity, as the introduction of this new natural enemy should not disturb indigenous species because *A. kamali* is relatively specific to the HMB pest, and therefore not competing with indigenous species of natural enemies.

From a mass production perspective, it was observed that contamination of *M. hirsutus* cultures with *Planococcus* sp. led to a decrease of the parasitoid progeny emergence (L. Sagarra, unpublished data). This can be explained by the fact that parasitoid eggs allocated to *Planococcus* will not develop into adults. Parasitization of *P. citri*

and *P. halli* by *A. kamali* will require keeping the *M. hirsutus* cultures for *A. kamali* mass-production free from these two other mealybug species.

ACKNOWLEDGMENTS

We thank M. A. Hoy, University of Florida at Gainesville, and M. Kairo, CABI Bioscience, Trinidad, W.I., for commenting on an early version of the manuscript, and N. Peters for technical assistance. This is contribution no 335/2000.09.04R of the Horticulture Research and Development Research Center, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Qc, Canada.

REFERENCES CITED

AGARWAL, M. M. 1965. Taxonomy of encyrtid parasites (Hymenoptera: Chalcidoidea) of Indian Coccidea. Acta Hymenopterologica 2: 37-97.

CROSS, A. E., AND J. S. NOYES. 1996. Dossier on *Anagyrus kamali* Moursi, Biological Control Agent for the Pink Mealybug, *Maconellicoccus hirsutus*, in Trinidad and Tobago. IIBC—Ascot, UK. 16 pp.

GHOSE, S. K. 1971. Morphology of various instars of both sexes of the mealybug *Maconellicoccus hirsutus* (Green) (Pseudococcidae: Hemiptera). Indian Journal of Agricultural Sciences 41: 602-611.

GODFRAY, H. C. J. 1994. Parasitoids, behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ, 473 pp.

INTERNATIONAL INSTITUTE OF ENTOMOLOGY. 1997. Distribution map of pest, series A (Agricultural) map #100 (2nd revision) *Maconellicoccus hirsutus*.

MANI, M. 1989. A review of the pink mealybug-*Maconellicoccus hirsutus* (Green). Insect Science and its Applications 10: 157-167.

MEYERDIRK, D. E., S. KHASIMUDDIN, AND M. BASHIR. 1988. Importation, colonization and establishment of *Anagyrus indicusi* [Hym.: Encyrtidae] on *Nipaecoccus viridis* [Hom.: Pseudococcidae] in Jordan. Entomophaga 33: 229-237.

MOURSI, A. A. 1948. *Anagyrus kamali* Moursi, a parasite of the Hibiscus Mealybug, Phenacoccus hirsutus Green. Bulletin de la Société Fouad 1st d'Entomologie 32: 9-16.

NOYES, J. S., AND M. HAYAT. 1994. Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae). CAB International, Wallingford, UK.

POWELL, W. 1986. Enhancing parasitoid activity in crops. pp. 319-340. *In* J. Waage and D. Greathead (eds.). Insect parasitoids. Academic Press, London, 389 pp.

SAGARRA, L. A, AND D. D. PETERKIN. 2000. Invasion of the Caribbean by the hibiscus mealybug, *Maconellicoccus hirsutus* Green [Homoptera: Encyrtidae]. Phytoprotection 80: 103-113.

SAGARRA, L. A., AND C. VINCENT. 1999. Influence of Host Stage on Oviposition, Development, Sex Ratio, and Survival of *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), a parasitoid of the Hibiscus Mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). Biol. Control 15: 51-56.

SHAFEE, S. A., M. ALAM, AND M. M. AGARWAL. 1975. Taxonomic survey of encyrtid parasites (Hymenoptera: Encyrtidae) in India. Aligarh Muslim University Publications (Zoological Series) on India Insect Types 10: 1-125.

SUBBA RAO, B. R., AND P. S. RAI. 1970. Studies on the Indian species of the genus *Anagyrus* Howard, 1896 (Hymenoptera: Encyrtidae). Beitrage zur Entomologie 20: 85-96.

VAN ALPHEN, J. J. M., AND M. A. JERVIS, 1996. Foraging behaviour. p. 36. in M. A. Jervis and N. Kidd. Insect natural enemies: Practical approaches to their study and evaluation. Chapman & Hall, London.

VAN DEN BOSCH, R., AND A. D. TELFORD. 1964. Environmental modification and biological control. pp. 459-488. *In* P. DeBach (ed.). Biological control of insect pests and weeds. London, Chapman & Hall. 844 pp.

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. Bull. Entomol. Res. 86: 617-628.