EVALUATION OF HOST PLANTS AND A MERIDIC DIET FOR REARING MACONELLICOCCUS HIRSUTUS (HEMIPTERA: PSEUDOCOCCIDAE) AND ITS PARASITOID ANAGYRUS KAMALI (HYMENOPTERA: ENCYRTIDAE)

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EVALUATION OF HOST PLANTS AND A MERIDIC DIET FOR REARING *MACONELLICOCCUS HIRSUTUS* (HEMIPTERA: PSEUDOCOCCIDAE) AND ITS PARASITOID *ANAGYRUS KAMALI* (HYMENOPTERA: ENCYRTIDAE)

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ABSTRACT

Biological control programs of *Maconellicoccus hirsutus* (Green), in the Caribbean have relied on Japanese pumpkins and sprouted potatoes as hosts for rearing both the mealybug and its parasitoids. However, seasonal shortages of these substrates have necessitated that others be found with equal or better qualities for sustaining large mealybug populations. In this paper, we report experiments comparing mass-rearing *M. hirsutus* on acorn squash (*Cucurbita pepo* L. var. 'Turbinata'), chayote (*Sechium edule* [Jacques]), and prickly pear, *Opuntia ficus-indica* [L.]) with Japanese pumpkin and sprouted potato. In addition, a simple meridic diet based on canned pumpkins was developed and compared. Acorn squash produced large quantities of females (up to 1,300 per squash) with a life cycle and reproductive potential equal to that of mealybugs reared on Japanese pumpkin. Parasitoids reared on these mealybugs developed normally and had a female-biased sex ratio similar to those reared on mealybugs on Japanese pumpkins or potato sprouts. Development of *M. hirsutus* reared on chayote and prickly pear was delayed by 1.5-5.0 days compared to that of mealybugs reared on Japanese pumpkins. Mealybugs on these substrates produced parasitoids with prolonged developmental times and male-biased sex ratios. On diet, development and reproduction of *M. hirsutus* was possible only for 3 to 4 consecutive generations. Mealybugs with longer developmental time, lower survival, and smaller ovisacs with lower percentage eclosion were obtained. Parasitoids reared from these mealybugs did not possess desirable characteristics for biological control. The developmental rate of adult parasitoids increased linearly with that of female hosts depending on the quality of the rearing substrate for the mealybugs.

Key Words: Pink hibiscus mealybug, artificial diet, invasive species, biological control

RESUMEN

Desde que invadió el área del Caribe, la cochinilla rosada del hibisco, *Maconellicoccus hirsutus* (Green) se ha criado masivamente en calabaza japónica o en papa en germinación. Sin embargo, la calabaza japónica no se puede producir a lo largo de todo el año en la región del caribe por lo cual se necesitan alternativas para la producción del insecto con el propósito de producir parasitoides para su control biológico. En este artículo presentamos resultados de experimentos en los que comparamos la crianza masiva de la cochinilla rosada en calabacín (*Cucurbita pepo* L. cv. 'turbinata'), Chayote (*Sechium edule* [Jacques]) y en nopal *Opuntia ficus-indica* (L.) con los substratos tradicionales, calabaza japónica y plántulas de papa. También probamos una dieta merídica con base en puree de calabaza y sacarosa. Se produjeron mas de 1,300 hembras de cochinilla por cada calabacín, con un tiempo de desarrollo y reproducción iguales a los de las cochinillas obtenidas en los substratos tradicionales. Los parasitoides que emergieron de estas cochinillas se desarrollaron normalmente y presentaron una relación de sexos similar a los obtenidos de papa y calabaza japónica. Las cochinillas que se criaron en chayote y nopal tuvieron un desarrollo 1.5 a 5.0 d más lento y produjeron parasitoides con tiempo de desarrollo prolongado y relación de sexos sesgada hacia los machos. A pesar que el tiempo de desarrollo de las cochinillas fue más prolongado y la supervivencia y fertilidad fueron menores, fue posible producir la cochinilla rosada durante varias generaciones completas nuestra simple dieta artificial, aunque los parasitoides que se obtuvieron de ellas no presentaron buenas características para el programa de control biológico. Las tasas de desarrollo de ambos sexos del parasitoide fueron más lentas cuando las cochinillas donde se criaron se obtuvieron de substratos de menor calidad.

Translation provided by author.

The pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) continues to invade new areas of the Caribbean, Central, and North America (Sagarra & Peterkin 1999, Michaud & Evans 2000) although successful biological control programs in the region appear to have slowed its spread. *M. hirsutus* was unknown in the Western Hemisphere (with the exception of Hawaii) prior to its

discovery on Grenada in 1994 and on Trinidad, St. Kitts and Nevis in 1995 (Williams 1996). After colonizing the Eastern Caribbean (Cross & Noyes 1998), the U.S. Virgin Islands, and Puerto Rico, *M. hirsutus* has spread to the Americas including California (Anonymous 1999), Mexico, Central America, and Guyana in South America (Pollard 1998). The economic risk of invasion to U.S. agriculture has been estimated at $750 million per year (Moffitt 1999) due mainly to its wide host range that includes over 125 plant species (Ghose 1972). The largest risk would be to ornamental crops, followed by vegetables, citrus, grapes, and avocados. Biological control programs for the U.S. could be implemented for as little as $500,000 per year during the initial 3 years of invasion (Moffitt 1999).

Biological control programs have been established in the Eastern Caribbean region with the introduction of the coccinellids, *Cryptolaemus montrouzieri* Mulsant and *Scymnus coccivora* Ramkrisna, and the encyrtid parasitoid, *Anagyrus kamali* Moursi (Cross & Noyes 1998). The U.S. Department of Agriculture introduced the encyrtid *Gyranusoidea indica* Schaffe, Alam & Agarwal to the area, including the U.S. Virgin Islands and Puerto Rico (Anonymous 1997). These two parasitoid species have been continuously produced since 1997 by USDA-APHIS with support from USDA-ARS and local Departments of Agriculture in laboratories on St. Thomas and Puerto Rico (Anonymous 1997, Serrano et al. 2001).

To mass-produce parasitoids, *M. hirsutus* is reared on the fruits of various species of cucurbits (Babu & Azam 1987a,b, Babu & Azam 1989, Mani & Thontadarya 1989) or on potatoes (*Solanum tuberosum* L.) (Sagarra & Vincent 1999). Of these, the preferred host has been Japanese pumpkin (*Cucurbita moschata* Duchesne cv. 'Chirimen') due to its ribbed rinds and characteristic warted surface, which provide large settling areas for mealybugs (Meyerdirk and Newell 1979). However, seasonal shortages of produce, and difficulties in maintaining a continuous supply of etiolated sprouts of potato, threaten production and increase costs. We looked at alternate rearing hosts that are available year-round. Acorn squash (*Cucurbita pepo* L. cv. 'Turbinata') and chayote fruits (*Sechium edule* Jacques [Schwartz]) are relatively inexpensive and available throughout the year in supermarkets on St. Croix. These fruits have skins that resemble that of Japanese pumpkins. Natural infestations of pink hibiscus mealybug were observed on prickly pear *Opuntia ficus-indica* (L.) Miller on St. Croix. The flattened stem segments of this cactus, known as cladodes or pads (Mizrahi et al. 1997), have traditionally been used to mass-produce mealybug species such as *Dactylopius coccus* Costa or *D. opuntiae* Cockerell for industrial production of dyes (Mizrahi et al. 1997).

These 3 hosts were compared with pumpkins and sprouted potatoes as well as with a simple meridic diet that permitted growth and reproduction of the mealybug. To examine the effect of host quality on parasitoid development, we reared *A. kamali* from mealybugs produced on each rearing substrate and the diet.

Materials and Methods

Field Production of Japanese Pumpkins

Japanese pumpkins were grown at the USDA-ARS Research Station on St. Croix, U.S. Virgin Islands. From August 1997 to September 2000, monthly plantings of 0.28 ha were made with the intent of maintaining a continuous supply of pumpkins. Seeds (American Takii, Salinas, CA) were hand-sown every 1.2 m in 61 m rows with 2.0 m between rows. Pumpkins were fertilized with 680 kg·ha^{-1} of 15-10-10 Green Crop® (Ochoa Fertilizer, Guánica, PR) at 15 and 30 d after planting (dap). In addition, foliar applications of 6.1 kg·ha^{-1} of Nutri-Leaf® (Miller Chemical Fertilizer Corp., Hanover, PA) were made at 45 and 60 dap. Drip irrigation was provided when necessary. Pumpkins suitable for mealybug rearing (>500 g) were hand-harvested at 90-110 dap.

Production of *M. hirsutus*

Japanese pumpkins infested with female *M. hirsutus* and ovisacs were incubated at 27 ± 1°C in a crawler collection box made with a modified oven (Isotemp incubator, model 655G, Fisher Scientific, Pittsburgh, PA). Pumpkins were distributed around a piece of white paper on the top shelf of the oven. A microscope illuminator was vertically inserted through the exhaust port on top of the oven and directed towards the piece of paper. The lamp was adjusted to 6 V at a distance of 18 cm above the paper, to produce a round spot of light 4 cm in diameter. Upon emergence, crawlers were attracted to and concentrated in the spot of light. The sheet of paper with crawlers was taken out and replaced daily. Twenty-four-h-old crawlers were distributed over the surface of fresh Japanese pumpkins using a camel's hair brush to maintain age-specific colonies. Infested pumpkins were maintained on 30 × 45 cm trays in an incubator at 27 ± 2°C and 70 ± 10% RH in the dark.

For comparison of rearing substrates, medium to large-size Japanese pumpkins (300-700 g) were brought to the laboratory, washed by soaking for a minimum of 3 h in tap water, and air-dried overnight. Freshly harvested pumpkins were used for all experiments. Sprouted potatoes were produced by placing 20 medium-size seed potatoes (Ryan Potato Company, East Grand Forks, MN) on a tray with a 2 cm layer of Pro-Mix PGX® (Pre-

mier Horticulture, Inc., Red Hill, PA) for several days in a dark room at 25 ± 2°C and 80 ± 10% RH to induce etiolation. Once sprouts reached about 5 cm in length, potatoes were cleaned and transferred to trays for infestation with *M. hirsutus*. Acorn squash (Big Chuy & Sons, Inc., Nogales, AZ) were purchased from St. Croix supermarkets. Green-skinned fruits that weighed about 450 to 600 g were used. Small chayote gourds, approximately 250 g each, were also obtained from local supermarkets. Uninfested, spineless pads of prickly pear were collected from plants found at the USDA-ARS Research Station. All fruits and cladodes were washed and initially infested with crawlers as previously described. After the first generation was completed, the cycle was repeated using only crawlers produced on the same host to maintain a stock of at least three generations of *M. hirsutus* on each host.

Depending on their size, 6 to 8 Japanese pumpkins, acorn squash or prickly pear pads, and 10-12 chayotes or sprouted potatoes were infested per tray per generation. Observations were made on the production of mature females (with ovisacs) on each host and the 'shelf life' of infested hosts, i.e., their ability to sustain at least one generation of *M. hirsutus* before physically degrading.

A meridic diet based on canned pumpkin and sucrose was also tested. Thirty g of canned pumpkin (Libby's® Solid Pack Pumpkin, Nestlé Food Company, Glendale, CA) were dissolved in 100 ml of distilled water by stirring for 3 h. At the end of the stirring period, 30 g of sucrose and 0.1 g of methyl-parabenzoic acid were added and the pH was adjusted to 7.5 by adding potassium hydroxide. The medium was poured into the tops of 5 cm diam plastic petri dishes, in a laminar flow hood to avoid microbial contamination. Parafilm M® (American Can Co., Greenwich, CT) was stretched over each dish and wrinkled to provide a rough surface for mealybug settling. Crawlers or early second instar nymphs were placed on the parafilm with a camel's hair brush. The petri dishes were closed, inverted, and placed in the growth chamber at the same environmental conditions described previously.

Development and Reproduction of *M. hirsutus*

After 3 *M. hirsutus* generations on each rearing substrate, a cohort of 100 second-instar nymphs was removed from each and placed in groups of 10 on fresh original substrate, including the artificial diet. Each replicate of 10 nymphs on a substrate was placed in a 20.4 × 20.4 cm PVC tube covered at one end with a fine mesh organdy cloth for ventilation. The other end of the tubes was attached with silicone to flat trays (60 × 30 cm) and placed in a rearing room at 26 ± 2°C, 60 ± 10% R.H. and complete darkness. Each tube cage constituted a replicate. Tube cages were checked daily under a magnifying lens. When male nymphs reach the fourth instar, they possess noticeable wing pads (Ghose 1971) and make a waxy 'cocoon' while remaining above a cluster of females. When these 'pupae' were observed, the number of days to this stage was recorded. The number of days to the last molt for the remaining female nymphs (pre-ovisac females) was also recorded to calculate female developmental time. If not enough males were observed inside the tube cages, five 6-h-old males from age-specific colonies on their respective host were added to each tube cage to insure enough males for fertilization of all available females. When ovisacs were noted, tube cages were opened and the number of live females counted to calculate percentage survival. After this, 30 ovisacs were taken from females on each rearing substrate. Each ovisac was isolated in a gelatin capsule (replicate) and incubated at 27 ± 1°C. Gelatin capsules were checked daily. When crawlers were first noticed, the number of days to eclosion was recorded for each ovisac. Two weeks later, all gelatin capsules were placed in a freezer at -5°C for 24 h. Crawlers and unhatched eggs were counted under a stereoscope for each ovisac to estimate its individual percent eclosion.

Data for developmental time for males and females, as well as the number of eggs per ovisac, days to eclosion, and percent eclosion were analyzed by ANOVA and Ryan-Einot-Gabriel-Welsch Multiple *F* test (SAS Institute 1999). Percentage data were transformed to arcsine and days to eclosion were transformed to ln (x) to stabilize the variance.

Effect of Host Quality on Parasitoid Development

The parasitoid *A. kamali* was reared for one generation on *M. hirsutus* obtained from each host described above. One hundred third instar female *M. hirsutus* nymphs from colonies reared for at least 3 generations on each substrate or 2 generations on artificial diet were transferred with a camel's hair brush onto a fresh substrate, and placed in tube cages in a rearing room at 26 ± 2°C, 60 ± 10% R.H. and a photoperiod of 12:12 (L:D). Five tube cages (replicates) were used for each rearing substrate. The following morning, 20 *A. kamali* adults were released in each tube cage and allowed to forage and oviposit for 24 h. Honey streaks and a water-soaked cotton ball were provided as additional food for the parasitoids. After this period, all adult parasitoids were removed from the cage using a vacuum aspirator. Tube cages were checked daily thereafter for emergence of adult parasitoids. Developmental time and gender of each adult parasitoid were recorded and analyzed by anova and Ryan-Einot-Gabriel-Welsch Multiple *F* test (SAS Institute 1999). Parasitoid sex ratios (F/M) were transformed to arcsine. Sex ratios from each substrate were tested

against a 1:1 (unbiased) sex ratio using an unpaired t-test. To study the relationship between the quality of *M. hirsutus* on the developmental rate of the parasitoid, linear regression models (SAS Institute 1999) were fitted to the developmental rate (day^{-1}) of female and male parasitoids and the developmental rate of female hosts produced on each rearing substrate.

RESULTS

Field Production of Japanese Pumpkins

Production peaked during September of each year, but was also high from May through August of the three-year period (Fig. 1). A noticeable decrease in the number and size of pumpkins harvested began in October and continued until April. Production of acceptable size fruits (>500 g) increased again during May. This repetitive pattern was probably caused by variations in environmental factors such as photoperiod or temperature.

Production of *M. hirsutus*

Japanese pumpkins produced between 700 and 1500 mature *M. hirsutus* females per pumpkin per generation. Individual pumpkins regularly produced up to 2 complete *M. hirsutus* generations (>60 d) before deteriorating. Sprouted potatoes produced between 150 and 350 females per generation over 40 d. Each acorn squash sustained between 700 and 1,300 adult female mealybugs and lasted about 50 d or one generation. Chayote fruits lasted only for one generation of *M. hirsutus* (30 d) before sprouting or decaying. Once sprouted, infested chayotes decayed within a few days. Some chayotes started to decay as early as 15 to 20 d after infestation. Prickly pear pads lasted for >60 d and sustained between 90 and 100 *M. hirsutus* females per pad per generation. Complete development from the crawler stage to reproductive females was observed on the meridic diet. Between 30 and 50 females were obtained per petri dish containing 100 ml of diet. The diet typically lasted 7-14 d, before microbial contamination was apparent.

Development and Reproduction of *M. hirsutus*

M. hirsutus males developed faster to the fourth instar (pupa) on Japanese pumpkins than on any other host tested (Table 1). On acorn squash and sprouted potatoes, it took males on average 0.8 to 1.4 d longer than on Japanese pumpkins to molt to the 'pupal' stage. On chayote males reached the fourth instar 2.2 d later than on Japanese pumpkins. On prickly pear and diet, males developed 4.5 and 5.1 d later, respectively,

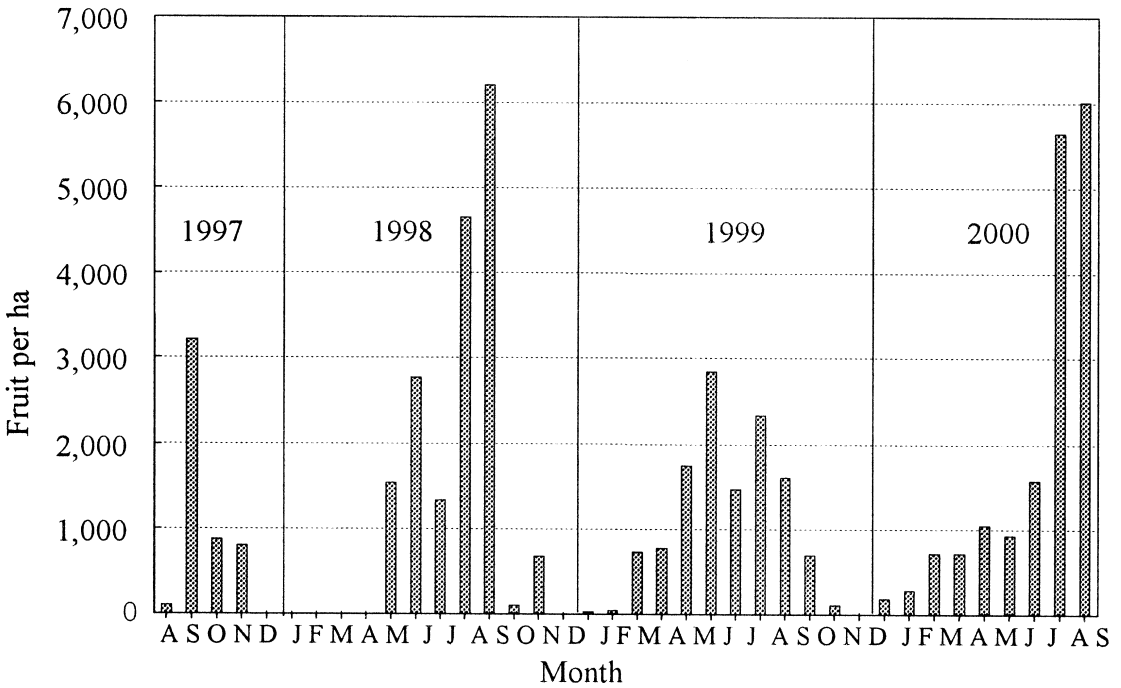

Fig. 1. Monthly production of Japanese pumpkins suitable for mealybug rearing (<500 g) per hectare from 1997 to 2000 at the USDA, ARS, research station on St. Croix, U.S. Virgin Islands.

TABLE 1. MEAN (± STD. DEVIATION) DEVELOPMENT TIME FROM CRAWLER TO MALE PUPAE AND ADULT FEMALE, AND PERCENT SURVIVAL OF *M. HIRSUTUS* AT THE END OF THE FOURTH GENERATION ON FIVE PLANT SUBSTRATES OR A MERIDIC DIET.

Host	Development time (d)		Percent
	Male Pupa[1]	Adult Female[2]	
Japanese Pumpkin	16.2 ± 1.2 a	29.6 ± 1.5 a	98.0 ± 4.2 a
Sprouted Potato	17.4 ± 1.5 b	30.1 ± 1.0 a	97.0 ± 9.5 a
Acorn Squash	17.0 ± 0.8 b	29.5 ± 0.9 a	95.0 ± 5.3 a
Chayote	18.4 ± 1.1 c	31.6 ± 1.6 b	92.0 ± 6.3 a
Prickly pear	20.7 ± 1.6 d	35.3 ± 2.2 c	82.0 ± 10.3 b
Meridic diet	21.3 ± 1.6 d	36.9 ± 2.1 d	72.0 ± 9.2 c

Means within columns followed by the same letter are not significantly different by Ryan-Einot-Gabriel-Welsch Multiple F test ($\alpha = 0.05$) after a significant ANOVA.
[1]$F = 50.8$; df = 5, 52; $P < 0.01$.
[2]$F = 123.2$; df = 5, 54; $P < 0.01$.
[3]$F = 17.3$; df = 5, 54 $P < 0.01$.

than on Japanese pumpkins. The developmental time for females was similar when reared on Japanese pumpkins, sprouted potatoes, and acorn squash, at roughly 30 d. Female development time was significantly slowed by 1.6 d on chayote, 5.3 d on prickly pear pads, and 6.9 d on the diet (Table 1). Survival to the adult stage (>90%) was significantly higher on Japanese pumpkins, sprouted potatoes, acorn squash, and chayote than on prickly pear pads (81.3%). It dropped to 73.2% on the diet (Table 1).

Females reared on all substrates, including the artificial diet, produced 1 ovisac. Shortly after producing the ovisac, females become inactive and die. The number of eggs per ovisac varied depending on the substrate. Females reared on Japanese pumpkin and acorn squash produced significantly more eggs per ovisac than females reared on the other substrates evaluated (Table 2). Females reared on sprouted potatoes and chayote had significantly more eggs per ovisac than those from the diet. However, the number of eggs per ovisac from females obtained on prickly pear pads and chayote was not significantly different. Females reared on the meridic diet produced the smallest ovisacs, with 36.4% fewer eggs than ovisacs produced on Japanese pumpkins (Table 2).

The rearing substrate affected the incubation time of *M. hirsutus* eggs (Table 2). Eggs from mealybugs reared on Japanese pumpkin and acorn squash incubated faster than those from the other rearing substrates. Eggs from mealybugs reared on diet developed a full day later than those from Japanese pumpkin and acorn squash. The highest percentage eclosion was observed for ovisacs from females reared on Japanese pumpkin, but it was not significantly different from the percentage eclosion of ovisacs from females reared on acorn squash and sprouted potato. The lowest percentages of eclosion were obtained from females reared on prickly pear and diet (Table 2).

TABLE 2. NUMBER OF EGGS, DAYS TO ECLOSION AND PERCENT ECLOSION FOR OVISACS OBTAINED FROM FEMALES OF *M. HIRSUTUS* REARED ON FIVE SUBSTRATES OR A MERIDIC DIET. DATA ARE MEANS ± STD. DEVIATION FROM A SAMPLE OF 30 OVISACS FROM EACH REARING MATERIAL.

Host	No. Eggs per Ovisac[1]	Days to Eclosion[2]	% Eclosion per Ovisac[3]
Japanese pumpkin	162.5 ± 39.2 a	4.6 ± 0.6 a	93.2 ± 7.7 a
Acorn squash	148.2 ± 39.6 a	4.8 ± 0.5 a	92.3 ± 4.8 ab
Sprouted potato	124.3 ± 46.9 b	5.3 ± 0.7 b	91.2 ± 8.0 ab
Chayote	106.8 ± 27.5 bc	5.4 ± 0.8 b	87.5 ± 7.2b
Prickly pear	84.4 ± 25.9 c	5.4 ± 0.9 b	76.0 ± 11.5 c
Meridic diet	59.2 ± 43.1 d	5.8 ± 1.1 b	69.8 ± 17.0 c

Means within columns followed by the same letter are not significantly different according to Ryan-Einot-Gabriel-Welsch Multiple F test ($\alpha = 0.05$) after a significant ANOVA.
[1]$F = 31.7$; df = 5, 174; $P < 0.01$.
[2]$F = 9.2$; df = 5, 174; $P < 0.01$.
[3]$F = 30.7$; df = 5, 174; $P < 0.01$.

Effect of Host Quality on Parasitoid Development

There was a significant effect of both rearing substrate ($F = 130.8$; df = 11, 5; $P < 0.01$) and gender of parasitoid ($F = 67.0$; df = 11, 1; $P < 0.01$) on development time of *A. kamali*, with no interaction between the two factors ($F = 2.1$; df = 11, 5; $P = 0.08$) (Fig. 2). The developmental times of female *A. kamali* from mealybugs reared on Japanese pumpkin, sprouted potato, and acorn squash were similar at 20.6-20.7 d (Table 3). Developmental time was significantly delayed by 4.3 and 7.8 d for female parasitoids that emerged from mealybugs reared on chayote and prickly pear, respectively. When the mealybug host was obtained from artificial diet, the developmental time of female parasitoids was 13.4 d longer than those obtained from mealybugs reared on either Japanese pumpkin or acorn squash. The developmental time for male parasitoids followed a similar pattern. The fastest development, between 18.6 and 18.9 d, occurred on *M. hirsutus* obtained from Japanese pumpkin, sprouted potato, and acorn squash. Male parasitoid development was delayed by 5.0-5.7 d when reared on mealybugs from chayote and prickly pear compared with males reared on mealybugs obtained on Japanese pumpkin. On mealybugs reared on artificial diet, male *A. kamali* took almost 29 d to develop, 10 d longer than on mealybugs from Japanese pumpkin or acorn squash. Males developed on average 2 d earlier than females on mealybugs obtained from Japanese pumpkin, sprouted potato, and acorn squash.

Percentage emergence of adult *A. kamali* was between 59 and 62% when the mealybug was reared on Japanese pumpkin, sprouted potato or acorn squash. On mealybugs reared on chayote, prickly pear and diet, the average emergence of adult parasitoids was significantly reduced to between one half and one-third of the emergence on Japanese pumpkin, sprouted potato or acorn squash (Table 3).

The sex ratio was significantly female-biased when parasitoids emerged from *M. hirsutus* reared on Japanese pumpkin ($P > t = 0.02$), sprouted potato ($P > t = 0.01$) and acorn squash ($P > t = 0.01$). It was significantly male-biased on mealybugs from chayote ($P > t = 0.01$), prickly pear ($P > t = 0.02$), and diet ($P > t = 0.02$). The sex ratio of parasitoids obtained from mealybugs produced on the artificial diet was 4.5 times more

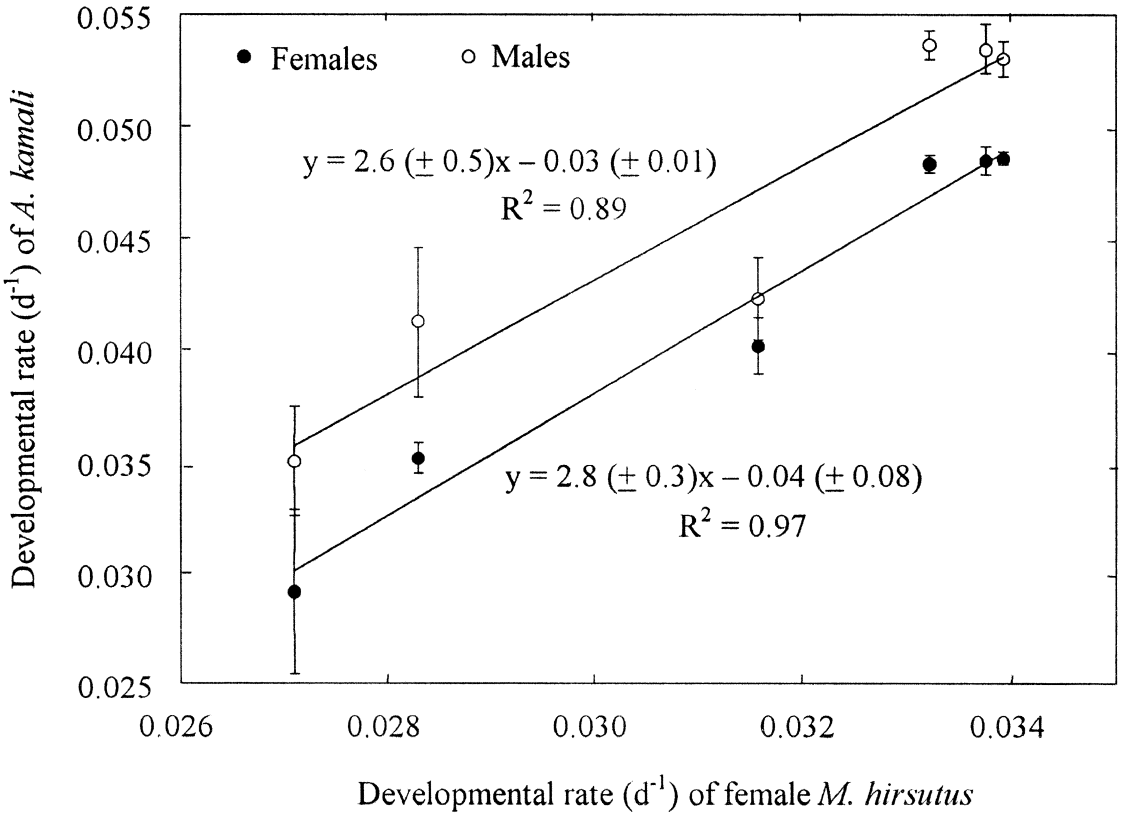

Fig. 2. Relationship between developmental rates of the parasitoid *Anagyrus kamali* and developmental rate of females of the host *Maconellicoccus hirsutus* produced on six rearing substrates.

TABLE 3. MEAN (± STD. DEVIATION, $N = 30$) DEVELOPMENTAL TIME, ADULT EMERGENCE AND SEX RATIO OF *ANAGYRUS KAMALI* DEVELOPED FROM *M. HIRSUTUS* OBTAINED FROM DIFFERENT REARING MATERIALS.

Host mealybug from	Developmental Time (d)		Adult Emergence[3] (%)	Sex Ratio
	Females[1]	Males[2]		
Japanese Pumpkin	20.6 ± 0.3 a	18.7 ± 0.4 a	61.6 ± 3.0 a	1.8 ± 0.2 a
Sprouted Potato	20.7 ± 0.2 a	18.6 ± 0.2 a	60.8 ± 12.1 a	1.7 ± 0.0 a
Acorn Squash	20.6 ± 0.1 a	18.9 ± 0.3 a	58.8 ± 10.4 a	1.7 ± 0.0 a
Chayote	24.9 ± 0.8 b	23.7 ± 1.1 b	29.8 ± 6.4 b	0.9 ± 0.2 b
Prickly pear	28.4 ± 0.6 b	24.4 ± 2.0 b	32.0 ± 7.9 b	0.9 ± 0.2 b
Meridic Diet	34.8 ± 5.2 c	28.7 ± 2.1 c	18.8 ± 6.3 b	0.4 ± 0.1 b

Means within columns followed by the same letter are not significantly different by Ryan-Einot-Gabriel-Welsch Multiple F test ($\alpha = 0.05$) after a significant ANOVA.

[1] $F = 35.17$; df = 5, 24; $P < 0.01$

[2] $F = 51.60$; df = 5,24; $P < 0.01$.

[3] $F = 26.46$; df = 5,24; $P < 0.01$.

[4] $F = 13.19$; df = 5, 24; $P < 0.01$.

male-biased than that of parasitoids from mealybugs reared on Japanese pumpkin (Table 3).

There was a significant linear relationship between the developmental rate of female *M. hirsutus* and that of *A. kamali* females ($F = 116.1$; df = 1,4; $P > 0.01$) and males ($F = 31.0$; df = 1,4; $P < 0.01$) (Fig. 2). When *M. hirsutus* females were obtained from lower quality rearing substrates, i.e., those that induced fewer offspring and slower development, significantly slower developmental rates were obtained for both sexes of the parasitoid. Rearing substrates that favored faster development of *M. hirsutus* were more likely to produce *A. kamali* with faster developmental rates.

DISCUSSION

Development of *M. hirsutus* was completed on all host plants tested and the artificial diet. Optimal development, survival, and reproduction occurred not only on the traditionally used substrates, Japanese pumpkin and sprouted potato, but also on acorn squash. Several other species of mealybugs, including the Comstock mealybug *Pseudococcus comstocki* (Kuwana), citrus mealybug *Planococcus citri* (Risso), and spherical mealybug *Nipaecoccus viridis* (Newstead), have been successfully reared on Japanese pumpkin (Meyerdirk & Newel 1979, Chandler et al. 1980, Meyerdirk et al. 1988) and sprouted potatoes (Gothilf & Beck 1966). *M. hirsutus* has been routinely produced on Japanese pumpkins for research purposes and for mass-producing parasitoids for biological control at the USDA, ARS Research Station on St. Croix since 1998 (Serrano et al. 2001). Acorn squash, reported here for the first time for mass rearing *M. hirsutus*, produced large numbers of insects with the same developmental time and survival as those produced on the two traditionally used hosts. Although prickly pear is a host plant frequently found with natural infestations of *M. hirsutus* on St. Croix, it did not present adequate characteristics for mass-producing mealybugs under our rearing conditions. Chayote has not yet been reported as a naturally infested host. Mealybugs could complete their life cycle and reproduce on these gourds. However, early decay of the fruits soon after infestation severely reduced its suitability for mass-rearing mealybugs.

It is noteworthy that reproduction and survival for several generations was obtained on the crude meridic diet. However, mealybug survival was lower and development prolonged on this diet. We do not consider it suitable for mass production of mealybugs without further refinements. This medium differs from artificial diets previously developed for mealybugs (Gothilf & Beck 1966, Calatayud et al. 1998) in that crude materials were used. The diet included solid particles from canned pumpkin material that may play a role in stylet penetration and the ingestion processes, i.e., by providing an anchor for salivary sheaths as in normal intercellular probing (Calatayud et al. 1994). Particles in the diet interfered with filter sterilization. Even with the addition of preservatives, the medium was prone to bacterial contamination. Nonetheless, results obtained with this method are consistent with those obtained on chemically defined diets. Gothilf & Beck (1966) found longer developmental times and fewer eggs per ovisac for female *P. citri* on an artificial diet versus sprouted potatoes. Calatayud et al. (1998) found reduced survival and a longer developmental time for cassava mealybug (*Phenacoccus manihoti* Matilé-Ferrero) reared on a defined diet when compared with mealybugs reared directly on cassava plants.

Developmental time, survival, and sex ratio of *A. kamali* obtained in this study are similar to results from other authors (Sagarra & Vincent 1999) when mealybug colonies were maintained

on sprouted potato. Although not an exhaustive evaluation of parasitoid quality, the developmental time and sex ratio of parasitoids obtained from mealybugs reared on acorn squash were within the ranges that have been previously published under similar environmental conditions (Sagarra & Vincent 1999) and were not significantly different from those occurring on the commonly used host materials.

The quality of the mealybug host affected the quality of parasitoids. Prolonged developmental time, lower survival, and a male-biased sex ratio were obtained for *A. kamali* reared on *M. hirsutus* from chayote and prickly pear. When reared on mealybugs raised on the artificial diet, female *A. kamali* took 14 d longer to develop compared with *A. kamali* reared on mealybugs on Japanese pumpkin. These parasitoids would not be considered for field releases for biological control. No evaluations were made, however, of adult parasitoid longevity or reproductive potential.

An ideal host for mass rearing *M. hirsutus* for production of parasitoids for biological control programs should have a large surface area for mealybug settling, long 'shelf life' after infestation, and produce adults within a short developmental time (~30 d). Adult females should be able to produce large ovisacs with high percent eclosion. In addition, these hosts should provide mealybugs that allow a short developmental time for A. *kamali* and a female-biased sex ratio. Acorn squash, along with Japanese pumpkin and sprouted potato, appear to satisfy these requirements. During periods of low production of Japanese pumpkins since 1998, parasitoids produced from *M. hirsutus* reared on acorn squash at the USDA, APHIS, PPQ insectary on St. Thomas, U.S. Virgin Islands have been mass-released for biological control of pink hibiscus mealybug in the U.S. Virgin Islands and Puerto Rico (USDA 1999).

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