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EFFECTS OF FOUR HOST PLANTS ON BIOLOGICAL PARAMETERS OF *MACONELLICOCCUS HIRSUTUS* GREEN (*HOMOPTERA*: *PSEUDOCOCCIDAE*) AND EFFICACY OF ANAGYRUS KAMALI MOURSI (*HYMENOPTERA*: *ENCYRTIDAE*)

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Abstract: An evaluation of the effect of four host plants on biological parameters of *Maconellicoccus hirsutus* and efficacy of *Anagyrus kamali* was conducted in the laboratory. *Hibiscus rosa-sinensis* and *Hibiscus sabdariffa* produced *M. hirsutus* with life cycles which were 1–2 days shorter than those of *M. hirsutus* reared on *Solanum tuberosum, and Cucurbita pepo* L. Egg to adult *M. hirsutus* survival was lowest in *C. pepo* (8.8%) and highest for *H. sabdariffa* (21.8%). *C. pepo*, *H. rosa-sinensis* and *H. sabdariffa* produced a more female biased *M. hirsutus* sex ratio from single ovisacs, than *S. tuberosum*. Although adult *M. hirsutus* females exhibited variation in size and longevity on the host plants tested, total fecundity was not significantly different. Life cycle of both sexes and offspring sex ratio of *A. kamali* emerging from *M. hirsutus* cultured on the four host plants were statistically similar. *M. hirsutus* infested *H. rosa-sinensis* and *H. sabdariffa* produced *A. kamali* with significantly higher fecundity (post emergence adult counts) and lower longevity compared to the other host plants. Females with largest femur (Mean length = 0.2950 ± 0.0053 mm) were produced by *M. hirsutus* infested *H. rosa-sinensis*. *A. kamali* efficacy measured by percent parasitization and percent adult eclosion was statistically similar for all *M. hirsutus* infested host plants tested.

Key words: *Maconellicoccus hirsutus, Anagyrus kamali, Hibiscus,* biological parameters, parasitoid

INTRODUCTION

The Pink Mealybug, *Maconellicoccus hirsutus* Green (*Homoptera: Pseudococcidae*) was accidentally introduced into Trinidad in 1994 from the neighboring island of Grenada soon became a major pest of ornamentals, fruit and forest trees and food crops (Pollard 1995). *M. hirsutus* has been described as a polyphagous pest which is very pro-

lific and often difficult to control (Mani 1989). In an effort to manage the pest by biological control, the parasitoid *Anagyrus kamali* Moursi (*Hymenoptera: Encyrtidae*) was imported mass reared on a variety of *M. hirsutus* infested host plants and released. *A. kamali* is a solitary endoparasitoid and besides attacking *M. hirsutus,* has a limited host range. Apart from biological evaluations conducted by Moursi (1948), Cross and Noyes (1996), Sagarra and Vincent (1999), Persad and Khan (2000, 2002), little has been published on the *M. hirsutus – A. kamali* complex. Vinson and Barbosa (1987) and Yang and Sadof (1997) reported variation in plant characteristics as having influence on the size of arthropod herbivores and hence affecting their parasitoids since host size dictated the amount of nutrients available for a developing larva. This paper tests this hypothesis on the *M. hirsutus – A. kamali* complex and examines the effect of selected host plants on life cycle, egg to adult survival, offspring sex ratio, fecundity, longevity and size of *M. hirsutus* and subsequent effects on the *A. kamali* complex.

The evaluations were carried out for *M. hirsutus* infested fruits of *Curcubita pepo* L., sprouted tubers of *Solanum tuberosum* L. and plants of *Hibiscus rosa-sinensis* L. and *Hibiscus sabdariffa* L., all of which are used to culture *A. kamali* in Trinidad. Biological parameters of *A. kamali* emerging from each *M. hirsutus* infested host plant type was also similarly assessed along with parasitoid efficacy, estimated as percentage parasitization, percentage adult eclosion and total fecundity based on post emergence adult counts of *A. kamali*. The main objective was to comparatively evaluate the performance of host plants currently used in mass rearing *A. kamali* in an effort to create standards for, and optimize parasitoid production in Trinidad and the rest of the Caribbean.

MATERIALS AND METHODS

Determination of the effect of host plant on life cycle, egg to adult survival, offspring sex ratio, fecundity, longevity and size of adult females of *M. hirsutus*

Four separate cultures of *M. hirsutus* were maintained on *C. pepo* fruits (8–10 cm diameter), sprouted *S. tuberosum* tubers (4–6 cm) and potted *H. rosa-sinensis* and *H. sabdariffa* plants (20–23 cm in height) in the laboratory at 12 L: 12 D and at 27.0°C ± 3.0°C and 58.0 ± 3.0% RH. The plants were treated with Blaukorn® (12N: $12P_2O_3$: $17K_2O_4$: 2MgO) fertilizer at 2g/l weekly and were watered on alternate days. An uninfested sprouted *S. tuberosum* tuber was infested with a 24-hour-old *M. hirsutus* ovisac and placed in a 20 x 20 x 20 cm organza (40 mesh/cm^2) bag cage. The ovisac was collected after it was produced and had been separated from a randomly chosen *M. hirsutus* female on a laboratory *S. tuberosum* culture. The ovisac was carefully teased open with blunt probes under a stereomicroscope. The number of eggs present in each ovisac was recorded and the eggs were then refolded into the fine cottony ovisac before inoculation. An uninfested *C. pepo* fruit and an uninfested plant each of *H. rosa-sinensis* and *H. sabdariffa* were similarly infested with an ovisac each from their respective cultures. Care was taken to ensure that a *M. hirsutus* female of similar age was used in all trials, the area around her was cleared of other females; the chosen ovisac was always the first one produced by that female. After inoculation, each host plant was housed individually in a 30 x 30 x 30 cm organza (40 mesh/cm^2) bag cage.

Observations were made daily and the total egg to adult developmental period of *M. hirsutus* on each host plant was recorded. Adult males *M. hirsutus* were winged and were collected by aspirator after two days of emergence; this allowed time for

mating to occur. The number of males collected in each treatment was recorded. The number of teneral (not yet gravid) adult females emerging from each host plant was also recorded and an offspring sex ratio was determined for all host plants tested. Five randomly selected adult females from each treatment were kept on the host plant; all others were removed. These adults were observed daily and the total number of eggs produced by each was recorded. The longevity of each selected adult *M. hirsutus* female was recorded and on dying was immersed in 70% ethanol, mounted in a drop of glycerine and femur length of the first leg was measured using an eyepiece graticule on a Euromex™ microscope. The femur length was chosen as an indication of the size as it remains unchanged for the duration of the adult life of mealybugs (Watson and Williams 1997).

Ten replicates of each test host evaluation were conducted under the same conditions at which the insects were cultured. These biological parameters were thus evaluated for a total of 50 adult *M. hirsutus* females when cultured on each host type. One Way Analysis of Variance (ANOVA) and the Least Significant Difference (LSD Mean Separation Test) at a rejection level of 0.05 were used to compare the data from all host treatments (Statistix® Version 1.0).

Determination of the effect of *M. hirsutus* infested host plants on life cycle, offspring sex ratio, total fecundity, longevity, size and efficacy of *A. kamali*

Ten replicates of each of the four host plants were infested from respective cultures with *M. hirsutus* 1st instar crawlers and reared to the 3rd instar stage identified according to Ghose (1971). All but 40 *M. hirsutus* females were removed from each host plant. This number provided an excess of hosts as *A. kamali* females generally produce a maximum of 20–25 eggs per day when ovipositing optimally at 48 hours post adult eclosion (Persad and Khan 2002). Third instar *M. hirsutus* nymphs were chosen as Sagarra and Vincent (1999) reported that this stage was readily chosen for oviposition and had relatively low encapsulation. Also as *M. hirsutus* host size affected biological attributes of *A. kamali*, using only third instar *M. hirsutus* helped to standardize the comparison across host plant types. Each of ten replicates of the four host plants was placed in separate organza 30 x 30 x 30 cm bag cages at 12 L: 12 D. One pair of mated, 48-hour old *A. kamali* adults originating from respective cultures, was introduced into each test cage for 24 hours. Honey streaks and a moist cotton wool pad were provided on the cage top as an additional food source for the parasitoids.

After nine days mummies from each treatment were collected and stored separately in size 00 gelatin capsules in the laboratory until emergence. The emerging adults were visually sexed: females are chestnut brown and larger while males are smaller and dark. Two pairs of adults emerging from each cage in all host plant treatments were randomly selected, kept in separate pairs in vials and fed on honey and water for 48 hours. One pair was introduced into a test cage containing 40, 3rd instar *M. hirsutus* nymphs on the respective host plant and was left for 24 hours at 12 L: 12 D. Two days later all surviving *M. hirsutus* were dissected in a drop of 0.8% NaCl solution under a stereomicroscope. *A. kamali* eggs present were by then bigger and could be easily seen, the number present in each individual *M. hirsutus* was recorded. This gave an indication of *A. kamali* percent parasitization of *M. hirsutus* for each host plant; any dead *M. hirsutus* was excluded from the count.

The second pair was introduced into a test cage with a similarly infested host plant. In the second evaluation, 20 additional 3rd instar *M. hirsutus* were added to each treatment daily, to ensure an excess of host until the death of each female. On dying, each female was mounted in a drop of glycerine and the hind femur length was measured using an Euromex™ microscope.

The total number of mummies produced by each female tested was considered an index of fecundity, the emerging adults were sexed and an offspring sex ratio was determined. Percentage adult eclosion was calculated for each treatment from the number of adults emerging out of the number of mummies obtained. All data were analyzed as in the preceding section.

RESULTS

Determination of the effect of host plant on biological parameters of *M. hirsutus*

Table 1 indicates that host plant type had significant effects on all of the biological parameters of *M. hirsutus* investigated except total fecundity. The life cycle of adult female *M. hirsutus* when cultured on *H. sabdariffa* (Mean ± SE = 23.50 ± 0.24 days) was significantly different ($p < 0.05$) to life cycles of *M. hirsutus* cultured on *S. tuberosum* and *C. pepo* (Mean ± SE = 25.38 ± 0.26 days and 25.86 ± 0.46 days respectively); *H. rosa-sinensis* was homogenous with both groups (Mean ± SE = 24.24 ± 0.29 days). *M. hirsutus* cultured on *H. sabdariffa* had significantly ($p < 0.05$) higher percent egg to adult survival (Mean ± SE = 21.8 ± 0.73%) compared to *S. tuberosum*, and *C. pepo* (Mean ± SE = 18.4 ± 0.62% and 8.8 ± 0.42% respectively); egg to adult survival on *H. rosa-sinensis* (Mean ± SE = 24.24 ± 0.29%), while significantly greater than survival on *C. pepo* was homogenous with the other two host plants.

Table. 1. Effect of four host plants on biological parameters of *Maconellicoccus hirsutus* at 27.0 ± 3.0°C and 58.0 ± 3.0% RH. Values represented as Mean ± SE

Biological parameter	Host plant			
	Solanum tuberosum	*Curcubita pepo*	*Hibiscus rosa sinesis*	*Hibiscus sabdariffa*
Life cycle [days] ♀	25.38 ± 0.26 a	25.86 ± 0.46 ab	24.24 ± 0.29 ac	23.50 ± 0.24 c
Egg – adult survival [%]	18.40 ± 0.62 a	8.80 ± 0.42 b	19.60 ± 0.59 ac	21.80 ± 0.73 c
Offspring sex ratio ♂:♀	1.74 ± 0.07 a	1.31 ± 0.06 b	1.29 ± 0.02 b	1.22 ± 0.03 b
Total fecundity	206.76 ± 25.06 a	250.46 ± 11.39 b	241.26 ± 15.08 b	219.32 ± 10.86 a
Longevity [days] [adult ♀]	21.41 ± 1.95 a	28.60 ± 0.70 b	17.42 ± 0.79 a	22.44 ± 0.92 a
Femur length [mm] ♀	0.163 ± 0.004 a	0.211 ± 0.008 b	0.205 ± 0.005 b	0.201 ± 0.003 ab

Means followed by the same letter are not significantly different from each other at 5% in any row (ANOVA, 39df and LSD mean separation test)

The offspring (♂:♀) sex ratio of *M. hirsutus* emerging from *S. tuberosum* was significantly ($p < 0.05$) higher compared to the other hosts, which were not significantly ($p > 0.05$) different from each other. Female *M. hirsutus* lived for significantly ($p < 0.05$)

longer periods on *C. pepo* (Mean ± SE = 28.60 ± 0.70 days) compared to the other hosts, which were homogenous with each other.

H. rosa-sinensis, H. sabdariffa and *C. pepo* produced adult *M. hirsutus* females with significantly ($p < 0.05$) greater femur lengths (Mean ± SE = 0.2049 ± 0.005 mm; 0.2013 ± 0.003 mm and 0.2110 ± 0.008 mm, respectively) than those cultured on sprouted *S. tuberosum* (Mean ± SE = 0.1628 ± 0.004 mm).

Determination of the effect of four different *M. hirsutus* infested host plants on biological parameters and efficacy of *A. kamali*

The life cycle of both sexes of *A. kamali*, offspring sex ratio, length of femur in males, percent parasitization and percent eclosion of the emergent adults were not significantly ($p > 0.05$) affected by the *M. hirsutus*-host plant complex (Table 2).

The total fecundity of *A. kamali* produced from *M. hirsutus* infested *S. tuberosum* and *C. pepo* (Mean ± SE = 15.80 ± 0.72 and 18.00 ± 1.61, respectively) were homogenous and significantly ($p < 0.05$) lower than the total fecundity of *A. kamali* emerging from *M. hirsutus* infested *H. rosa-sinensis* and *H. sabdariffa* (Mean ± SE = 24.80 ± 1.88 and 24.22 ± 1.72, respectively).

Table 2 Effect of four host plants on biological parameters of *Anagyrus kamali* produced from 3rd instar *Maconellicoccus hirsutus* at 27.0 ± 3.0°C and 58.0 ± 3.0% RH. Values represented as Mean ± SE

Biological parameter	Host plant			
	Solanum tuberosum	*Curcubita pepo*	*Hibiscus rosa-sinesis*	*Hibiscus sabdariffa*
Life cycle [days] ♀	17.14 ± 0.42 ns	17.38 ± 0.27 ns	17.82 ± 0.16 ns	17.78 ± 0.27 ns
Life cycle [days] ♂	18.64 ± 0.41 ns	18.50 ± 0.36 ns	17.80 ± 0.26 ns	17.44 ± 0.16 ns
Offspring sex ratio ♂:♀	0.58 ± 0.06 ns	0.52 ± 0.02 ns	0.69 ± 0.06 ns	0.56 ± 0.02 ns
Total fecundity	15.80 ± 0.72 a	18.00 ± 1.61 a	24.80 ± 1.88 b	24.22 ± 1.72 b
Longevity [days] [adult ♂]	17.80 ± 0.90 a	14.60 ± 0.48 a	10.41 ± 0.91 b	9.60 ± 1.06 b
Longevity [days] [adult ♀]	17.28 ± 0.94 a	17.43 ± 1.33 a	10.10 ± 0.73 b	9.42 ± 0.57 b
Femur length [mm] ♂	0.199 ± 0.009 ns	0.191 ± 0.006 ns	0.211 ± 0.004 ns	0.183 ± 0.006 ns
Femur length [mm] ♀	0.215 ± 0.002 a	0.231 ± 0.001 ab	0.295 ± 0.005 c	0.250 ± 0.007 b
Parasitization [%]	78.0 ± 8.48	74.0 ± 12.14	82.0 ± 11.98	80.0 ± 10.23
Adult eclosion [%]	67.22 ± 5.21	62.28 ± 6.11	77.72 ± 5.24	75.35±3.31

Means followed by the same letter are not significantly different from each other at 5% in any row (ANOVA, 39df and LSD mean separation test)
ns – not significant

Longevity of both sexes of *A. kamali* were significantly ($p < 0.05$) lower when cultured on *M. hirsutus* infested *H. rosa-sinensis* (Mean ± SE (♂) = 10.41 ± 0.91 days; Mean ± SE (♀) = 10.10 ± 0.73 days) and *H. sabdariffa* (Mean ± SE (♂) = 9.60 ± 1.06 days; Mean ± SE (♀) = 9.42 ± 0.57 days) compared to *A. kamali* cultured on *M. hirsutus* infested

S. tuberosum (Mean ± SE (♂) =14.80 ± 0.90 days; Mean ± SE (♀) =17.28 ± 0.94 days) and *C. pepo* (Mean ± SE (♂) =14.60 ± 0.48 days; Mean ± SE (♀) =17.43 ± 1.33 days) which were homogenous.

Adult female *A. kamali* had significantly ($p < 0.05$) greater femur lengths when produced on *M. hirsutus* infested *H. rosa-sinensis* (Mean ± SE = 0.2950 ± 0.005 mm); females obtained from cultures of *C. pepo* and *H. sabdariffa* had homogenous femur lengths (Mean ± SE = 0.2314 ± 0.001 mm and 0.2498 ± .007 mm respectively). *M. hirsutus* infested *S. tuberosum* produced *A. kamali* with femur lengths (Mean ± SE = 0.2149 ± 0.002 mm) which were homogenous with those of *A. kamali* cultured on *H. sabdariffa.*

DISCUSSION

Several researchers have reported on variation in biological parameters in herbivores on different hosts, Woets and van Lenteren (1976) attributed differences in whitefly populations on different host plants as a combination of the effect of the host plant on pest fecundity, lifespan and development rate. Growth, development, and fecundity of citrus mealybug, *Planococcus citri* (Risso), differed substantially when fed on red, yellow, or green- leafed *Coleus blumei* (Bentham) (Yang and Sadof 1997).

The data presented in Table 1 indicates that *M. hirsutus* developmental periods were longer for *M. hirsutus* when cultured on *C. pepo* > *S. tuberosum* > *H. rosa-sinensis* > *H. sabdarifa. M. hirsutus* egg to adult survival was higher for *H. sabdarifa* > *H. rosa-sinensis* > *S. tuberosum* > *C. pepo* while greater female biased sex ratios and female size occurred on *C. pepo* > *H. rosa-sinensis* > *H. sabdarifa* > *S. tuberosum.* Adult *M. hirsutus* females lived longer on *C. pepo* > *H. sabdarifa* > *S. tuberosum* > *H. rosa-sinensis.*

Favorable *M. hirsutus* host plants for a mass rearing program for *A. kamali* should ideally have a high mealybug egg to adult survival rate, a low development period for *M. hirsutus*, produce more females that are more fecund, larger and longer lived. Of the four host plants evaluated, *C. pepo* > *H. sabdariffa* > *H. rosa-sinensis* > *S. tuberosum* seem to satisfy most of the required characteristics. Eventual choice of one host plant over another however may reside on a range of additional factors, which may include the effects of host plant on the biological parameters of *A. kamali.*

Yang and Sadof (1997) found that host plants could alter parasitoid life history, these effects might arise from plant-mediated changes on host mealybug size. Life cycle, offspring sex ratio and size of male *A. kamali* were similar when produced from all *M. hirsutus* infested host plants tested. Total fecundity and female size however were higher in *A. kamali* produced on *H. rosa-sinensis* and *H. sabdariffa* compared to *A. kamali* produced from *M. hirsutus* infested *S. tuberosum* and *C. pepo*. *M. hirsutus* infested *H. rosa-sinensis* and *H. sabdariffa* however, produced *A. kamali* with decreased longevity in both sexes. An increased oviposition rate usually results in decrease in longevity in females as more energy is utilized in intense egg production (Persad and Khan 2002). Decreased longevity however, was observed in males, which may suggest an additional host plant effect on longevity of the parasitoid.

Various authors (eg. Smith 1957; Woets and van Lenteren 1976; Cambell and Duffey 1979) have shown that plant features such as leaf surface topography and toxic secondary plant substances could affect parasitoid efficacy. Additionally, Laster (1974) and Vinson (1981) reported on the importance of host habitat in influencing the

behavior of parasitoids, which might elect not to parasitize a preferred host larva if it occurs on a non-preferred plant species.

In the *A. kamali* – *M. hirsutus* complex, *A. kamali* produced from the *M. hirsutus* infested host plants evaluated showed no statistically significant variation in efficacy. Percent parasitization and percent adult *A. kamali* eclosion were similar for the four *M. hirsutus* infested host plants tested. The data obtained in this study thus suggests that in addition to efficacy, there is no clear advantage in life cycle and offspring sex ratio for *A. kamali* progeny in choosing one of the four evaluated host plants in preference to another.

Total fecundity and size of the adult female thus appear to be the determining factors in selection of a favorable host plant. *M. hirsutus* infested *H. rosa-sinensis* and *H. sabdariffa* produce *A. kamali* females which are larger and produce more eggs. These are however green plants that may occupy more space and need sunlight or artificial light and have to be fertilized and regularly watered when utilized in a rearing program. These requirements may be measured against those of *S. tuberosum* and *C. pepo* which by contrast are not as labor intensive to maintain and may produce longer lived *A. kamali* adults, which may be needed for cases of low *M. hirsutus* infestations which have patchy distributions, allowing more time for host location. The former host plants may best be employed in cases of densely occurring *M. hirsutus* field infestations where optimum *A. kamali* reproductive ability is needed. Based on the quantity and availability of resources coupled with the type of infestation targeted; a rearing program for *A. kamali* on these four *M. hirsutus* infested host plants may thus be formulated.

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POLISH SUMMARY

WPŁYW CZTERECH RÓŻNYCH ROŚLIN ŻYWICIELSKICH NA PARAMETRY BIOLOGICZNE *MACONELLICOCCUS HIRSUTUS* GREEN (*HOMOPTERA: PSEUDOCOCCIDAE*) I SKUTECZNOŚĆ *ANAGYRUS KAMALI* MOURSI (HOMOPTERA: ENCYRTIDAE)

Przeprowadzono laboratoryjną ocenę wpływu czterech różnych roślin żywicielskich na parametry biologiczne *Maconellicoccus hirsutus* i skuteczność *Anagyrus kamali*. *M. hirsutus* hodowane na roślinach *Hibiscus rosa-sinensis* i *Hibiscus sabdariffa* miały cykl życia o 1–2 dni krótszy niż wyhodowanych na *Solanum tuberosum* oraz *Cucurbita pepo*. Stosunek przetrwania jaj do dorosłych *M. hirsutus* był najniższy w przypadku hodowli na *C. pepo* (8,8%), a najwyższy dla hodowli na *H. sabdariffa* (21,8%). Na roślinach *C. pepo*, *H. rosa-sinensis* i *H. sabdariffa* rodziło się więcej osobników płci żeńskiej niż na *Solanum tuberosum*.

Chociaż dorosłe samice *M. hirsutus* różniły się pod względem wielkości i długości życia na testowanych roślinach, ich ogólna płodność istotnie się nie różniła. Cykl życia obu płci i stosunek płci potomstwa *A. kamali* hodowanych na wszystkich czterech roślinach żywicielskich były statystycznie podobne. Na roślinach *H. rosa-sinensis* i *H. sabdariffa* zasiedlanych przez *M. hirsutus* stwierdzono jego większą płodność i krótszą żywotność w stosunku do innych roślin żywicielskich. Samice *M. hirsutus* wyhodowane na *H. rosa-sinensis* były większe. Skuteczność *A. kamali* mierzona w procentach pasożytowania i w procentach pojawiania się dorosłych osobników była statystycznie podobna dla wszystkich badanych roślin zasiedlonych przez *M. hirsutus*.