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## LIFE HISTORY OF PINK HIBISCUS MEALYBUG, *MACONELLYCOCCUS HIRSUTUS* (HEMIPTERA: PSEUDOCOCCIDAE) ON THREE *HIBISCUS ROSA-SINENSIS* CULTIVARS

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### ABSTRACT

The pink hibiscus mealybug, *Maconellicoccus hirsutus* Green, is a widespread invasive pest of *Hibiscus rosa-sinensis* in Florida and elsewhere. We evaluated 3 cultivars of *H. rosa-sinensis* expected to have high ('President'), low ('Double Red'), and intermediate ('Joanne') levels of susceptibility to this pest. We found little evidence that *M. hirsutus* responded differently among the 3 cultivars in terms of survival, development rate, size, or oviposition period in laboratory tests at a permissive temperature. However, higher average fecundity (377 eggs per female) was observed on "President" compared with the other 2 cultivars ( $\approx$  300 eggs/female). The F1 generation developed on all varieties, with similar cumulative survival rates of 71, 74, and 76% on "Double Red", "Joanne" and "President" cultivars, respectively. In greenhouse tests over 9 wk, feeding symptoms of stunted and deformed plant terminals ("bunchy top") were observed in all cultivars but increased more rapidly in "President", especially after the 4th wk post infestation. Significantly higher mealybug populations were also observed on "President" compared with the other 2 cultivars, reaching an average of  $\approx$  50 and 1,400 insect per terminal after the first and second generations, 30 and 60 d post infestation, respectively, on this variety. Although none of the hibiscus varieties tested were resistant to *M. hirsutus* in our studies, selection (or breeding) of additional cultivars tolerant to feeding symptoms may be useful in low management input landscapes for *M. hirsutus* infested areas, especially in conjunction with biocontrol programs.

Key Words: *Maconellicoccus hirsutus*, *Hibiscus rosa-sinensis*, host plant resistance

### RESUMEN

La Cochinilla Rosada del Hibiscus, *Maconellicoccus hirsutus* Green, es un insecto plaga invasor, ampliamente disperso en Florida y otros lugares. Tres cultivares de *H. rosa-sinensis* fueron evaluados, expectantes por alto ('President'), bajo ('Double Red') e intermedio ('Joanne') niveles de susceptibilidad a este insecto plaga. Se encontró poca evidencia en la respuesta de *M. hirsutus* en los tres cultivares en cuanto a la tasa de supervivencia, el periodo de desarrollo, el tamaño y la oviposición en las pruebas de laboratorio a una temperatura constante. Sin embargo, el aumento en el promedio de la fecundidad (377 huevos/hembra) fue observado en el cultivar "President" en contraste con los otros dos cultivares ( $\approx$  300 huevos/hembra). La primera generación F1, se desarrollo en todas los cultivares con similares tasas de supervivencia acumulada, 71, 74 y 76% en "Double Red", "Joanna" y "President" respectivamente. En las evaluaciones de invernadero durante mas de 9 semanas, los síntomas de alimentación, el retraso en el crecimiento y la deformación de los terminales de la planta conocidos como arrosamiento fueron observados en todos los cultivares, sin embargo, en el cultivar "President" fueron mas rápido después de las cuatro semanas posteriores a la infestación. Las poblaciones de la cochinilla rosada fueron significativamente mas altas en el cultivar "President" en comparación con los otros dos cultivares, alcanzando en promedio 50 y 1400 individuos por terminal en la primera y segunda generación, 30 y 60 días después de la infestación respectivamente. Aunque ninguno de los cultivares evaluados mostraron resistencia a *M. hirsutus*, la selección o reproducción de cultivares tolerantes podrían ser útiles en el diseño y manejo de paisajes para área infestadas con *M. hirsutus*, especialmente en conjunto con programas de control biológico.

Translation provided by the authors.

Native to southern Asia (Williams 1996), the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae), is an introduced pest in many parts of the world including Africa, Australia, South and Central America and more recently the Caribbean Islands and North America (Anonymous 2005; Kairo et al. 2000). In North America, the range of *M. hirsutus* has greatly expanded to include California in 1999, Florida in 2002, Louisiana in 2006, Texas in 2007, Georgia in 2008, and South Carolina in 2009 (Anonymous 2005; Bográn & Ludwig 2007; Chong 2009; Horton 2008). An extensive list of plants of economic importance to the United States may be attacked by *M. hirsutus*, with host records extending to 76 families and over 200 genera (Mani 1989; Anonymous 2005). In the southeastern United States *M. hirsutus* is a recognized pest of several horticultural and native woody ornamental plants, with ornamental *Hibiscus rosa-sinensis* L. (Malvaceae) a typical host which is frequently attacked and, in part, after which this species derived its common name.

Like other mealybugs, *M. hirsutus* feeds on phloem tissues, attacking the growing points and young twigs of plants and injecting salivary toxins, which cause characteristic distortion of stems, leaves and fruits. Typical feeding symptoms on hibiscus plants include leaf curl, and shortened internodes which causes resettling or "bunchy top" (Williams 1996). The "bunchy top" injury is permanent, and the terminal distortion will remain until all the leaves mature and drop off. Heavily infested leaves, flowers, and fruits often abscise prematurely, although prior infestation does not usually prevent subsequent normal development of terminals or leaves if the infestation is eliminated. Additional damage may be caused by sooty mold growing on the secreted honeydew, reducing photosynthesis and affecting plant marketability. A case in point, *Hibiscus rosa-sinensis* is an important production and landscape plant in southern Florida; however, its production has decreased due to widespread and severe damage to hibiscus in managed urban landscapes by *M. hirsutus* (Vitullo et al. 2009). After the introduction of *M. hirsutus* into Florida, the Department of Consumer Services, Division of Plant Industry implemented a zero tolerance policy for shipping plants to non-infested areas along with a biological control strategy relying on the release of 2 encyrtid parasitoids (*Anagyrus kamali* Moursi and *Gyranusoidia indica* Shafee, Alam & Agarwal) and the coccinellid, *Cryptolaemus montrouzeri* Mulsant; current status of release efforts and pest surveys are detailed at <http://www.fresh-fromflorida.com/pi/plantinsp/phm.html>.

Despite these control efforts, *M. hirsutus* remains a pest in central and south Florida, suggesting that additional management tactics are needed. Although some systemic insecticides may

be effective against *M. hirsutus*, their use risks disruption to biological control (Williams 1996). Little information is available on host-plant resistance or on methods of cultural control for *M. hirsutus*. Although no fully resistant varieties of *H. rosa-sinensis* have been reported, variations among cultivars in their susceptibility and expression of feeding symptoms to *M. hirsutus* have been noted (Vitullo et al. 2009). However, the influence of hibiscus cultivars on *M. hirsutus*'s life history was not assessed (Vitullo et al. 2009).

Given that pest management decisions may be based on surveys of plant damage due to *M. hirsutus*, understanding the relationship between plant symptom development and pest reproduction among different cultivars is important. The choice of cultivars used in the landscape may similarly influence the reproduction and spread of this pest. Here, we evaluated several commercially important *H. rosa-sinensis* cultivars for potential resistance to *M. hirsutus*. Specific objectives were to quantify the life history (fitness) of *M. hirsutus*, and characterize the expression of feeding symptoms on cultivars that we anticipated might have different levels of susceptibility to this pest.

## MATERIAL AND METHODS

### Source of insects and plants

A colony of *M. hirsutus* that originated from infested hibiscus plants collected in Miami, Florida in 2009 was maintained at the Tropical Research and Education Center (TREC) in Homestead, Florida. Insects were maintained on sprouted potatoes, *Solanum tuberosum* L., in 3.5 L plastic containers maintained at  $27 \pm 2$  °C and 70-80% RH in complete darkness. The following cultivars of *Hibiscus rosa-sinensis* were obtained from a local supplier and grown under greenhouse conditions; 'President' with cordate leaves and red full blooms, 'Joanna' with ovate leaves and orange cartwheel overlapped single bloom with a dark orange eye zone, and 'Double Red' with cordate leaves densely packed along the shoot and red full double blooms.

### Laboratory studies

The development of *M. hirsutus* on the 3 cultivars of *Hibiscus rosa-sinensis* was evaluated in the laboratory. The bioassay unit consisted of a modified Petri-dish (20 × 100 mm) that contained a single young fully expanded leaf. The stem (4 cm long) from the excised leaf protruded through a small hole in the Petri-dish into a cup of water. Fifteen eggs collected from a single female from the laboratory colony within 24 h of oviposition were placed on each leaf using a fine paintbrush. Petri-dishes were checked daily for crawler emergence. Three crawlers that attached

to the leaf were selected randomly for monitoring and the others were removed. The progression of each stadium (crawler, nymphal stages through to adulthood) was recorded daily by the presence of exuviae. The length of each insect stadium was also recorded following each molt using an ocular micrometer. There were 48 replicates per cultivar. Petri dishes were maintained in an incubator at 27 °C, 70-80% relative humidity and 12:12 h L:D photoperiod. The study was conducted twice.

In addition, life history parameters of the F1 generation were tracked to compare the fitness of *M. hirsutus* on each cultivar. For this, eggs collected at the end of the previous insect developmental study were used to infest new leaves of the same cultivar at the rate of 15 eggs per leaf. The survival rates for each stadium and adult sex ratio were recorded from 12 Petri dish replicates for each cultivar. Adults of the F1 generation were also used to compare the longevity and fecundity (including pre- and post oviposition periods) of insects reared on the different cultivars. Adult females were placed on new leaves and eggs were counted and removed daily until the insect died. The study was conducted twice, using 15 and 30 replicate insects for each cultivar, respectively.

#### Greenhouse study

To further evaluate the growth of *M. hirsutus* populations on *H. rosa-sinensis* cultivars, plants of each cultivar in 11.4 liter (3-gal) pots with multi-purpose potting compost were infested and maintained under greenhouse conditions. To standardize the infestation protocol, male and female *M. hirsutus* from the colony were first isolated on cuttings (of the respective cultivars) for 24 h to allow mating and oviposition. Each plant (7 plants per cultivar) was infested with 5 female *M. hirsutus* and 5 egg sacs placed on separate terminals. Plants were evaluated weekly for 9 wk and rated based on the percentage of terminals expressing “bunchy top” feeding symptoms. Mealybug development was evaluated from 3 randomly selected terminals per plant at d 30 and 60 post infesta-

tion. Terminals were removed from the plants and the number of egg sacs, crawlers, nymphs, and adult stages were counted in the laboratory using a dissecting stereomicroscope. This study was conducted between Feb and Apr, 2010.

#### Data Analysis

Cultivars were compared using one-way analysis of variance (ANOVA) and Tukey HSD test procedures at  $P < 0.05$ . Data from the 2 tests were pooled because there were no significant cultivar by test interactions. In the greenhouse tests, cultivar damage symptoms were compared with repeated measures ANOVA, based on 9 weekly time intervals (SPSS for Windows v.17). If needed, data were normalized via  $\log(n + 1)$  or arcsine prior to analysis.

## RESULTS

#### Laboratory tests

The development duration (d) of *M. hirsutus* life stages were similar among the hibiscus cultivars tested (Table 1). The generation time (egg to adult) was similar for both sexes, i.e.  $\approx 26$ -27 d at 27 °C. There was no difference in average *M. hirsutus* size among the cultivars, with the exception of second nymphal stages reared on ‘Double Red’ which were smaller compared with the other two cultivars (Table 2). Adult males were about half as long as adult females. There were no differences in cumulative survival or resulting sex ratio of the F1 generation among the cultivars (Table 3). The reproductive and the longevity parameters of F1 adults were similar among cultivars, although adult females reared on ‘Joanne’ lived significantly ( $\approx 1$  d) shorter and had a reduced preoviposition period compared with the other 2 cultivars (Table 4). The preoviposition period was longer on ‘Double Red’ compared with ‘President’, although there were no cultivar differences in the oviposition periods, at 8-9 d. There was a significant effect of cultivar on fecundity; mealybugs reared

TABLE 1. DEVELOPMENT RATE OF *M. HIRSUTUS* (DAYS) REARED ON THREE HIBISCUS CULTIVARS AT 27 °C.

Cultivar	Life history stage (instar) <sup>1</sup>						Generation time (d)	
	Egg	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup> ♀	3 <sup>rd</sup> ♂	4 <sup>th</sup> ♂ <sup>2</sup>	♂	♀
Double Red	7.43 a	6.73 a	5.04 a	7.62 a	2.57 a	3.90 a	26.8 a	25.8 a
Joanne	7.38 a	6.70 a	5.26 a	7.59 a	2.59 a	3.81 a	26.9 a	25.8 a
President	7.51 a	6.88 a	5.02 a	7.58 a	2.64 a	4.14 a	27.0 a	26.2 a
<i>F</i>	1.58	1.32	2.63	0.06	0.09	1.33	0.45	1.59
<i>df</i>	2,285	2,285	2,272	2,205	2,62	2,59	2,199	2,59
<i>P</i>	0.208	0.270	0.074	0.945	0.909	0.272	0.639	0.214

<sup>1</sup>Means within a column followed by the same letter are not significantly different at  $\alpha = 0.05$  (Tukey's HSD test).

<sup>2</sup>*M. hirsutus* gender cannot be determined until 3rd instar; males have an additional (4th) instar.

TABLE 2. LENGTH (MM) OF DEVELOPMENTAL STADIA OF *M. hirsutus* REARED ON THREE HIBISCUS CULTIVARS AT 27 °C.

Cultivar	Life history stage (instar) <sup>1</sup>					Adult	
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup> ♀	3 <sup>rd</sup> ♂	4 <sup>th</sup> ♂ <sup>2</sup>	♀	♂
Double Red	0.37 a	0.68 b	1.11 a	1.04 a	1.10 a	2.09 a	1.09 a
Joanne	0.36 a	0.71 a	1.10 a	1.05 a	1.09 a	2.09 a	1.10 a
President	0.37 a	0.71 a	1.12 a	1.03 a	1.10 a	2.08 a	1.10 a
<i>F</i>	0.98	6.35	0.11	0.46	0.77	0.08	0.59
df	2,284	2,272	2,205	2,62	2,59	2,199	2,58
<i>P</i>	0.378	0.002	0.893	0.631	0.467	0.923	0.559

<sup>1</sup>Means within a column followed by the same letter are not significantly different at  $\alpha = 0.05$  (Tukey's HSD test).

<sup>2</sup>*M. hirsutus* gender cannot be determined until 3rd instar; males have an additional (4th) instar.

TABLE 3. PERCENTAGE SURVIVORSHIP UNTIL EACH DEVELOPMENTAL STADIUM AND ADULT SEX RATIO OF *M. hirsutus* (F1 GENERATION) REARED ON THREE HIBISCUS CULTIVARS AT 27 °C.

Cultivar	Life history stage (instar) <sup>1</sup>				
	Crawler	Second	Third	Adult	♀ sex ratio
Double Red	93.3 a	81.2 a	73.8 a	70.9 a	77.9 a
Joanne	92.7 a	83.4 a	77.5 a	73.6 a	74.8 a
President	95.8 a	86.9 a	78.5 a	75.7 a	78.5 a
<i>F</i>	2.55	2.00	1.86	1.30	1.78
df	2,68	2,68	2,68	2,68	2,68
<i>P</i>	0.086	0.143	0.163	0.280	0.18

<sup>1</sup>Means within a column followed by the same letter are not significantly different (following arcsine transformation) at  $\alpha = 0.05$  (Tukey's HSD test).

TABLE 4. ADULT LONGEVITY AND REPRODUCTIVE PARAMETERS OF *M. hirsutus* (F1 GENERATION) REARED ON THREE HIBISCUS CULTIVARS AT 27 °C.

Cultivar	Longevity (days) <sup>1</sup>		♀ reproduction (days) <sup>1</sup>		
	♀ <sup>2</sup>	♂	Preoviposition	Oviposition	Fecundity
Double Red	15.7 a	2.7 a	6.8 a	8.1 a	312.4 b
Joanne	14.4 b	2.6 a	5.3 c	8.4 a	309.8 b
President	15.4 a	2.4 a	6.0 b	8.7 a	377.6 a
<i>F</i>	10.06	1.44	14.06	1.28	7.16
df	2,132	2,60	2,132	2,132	2,132
<i>P</i>	0.000	0.25	0.001	0.280	0.001

<sup>1</sup>Mean within a column followed by the same letter are not significantly different at  $\alpha = 0.05$  (Tukey's HSD test).

on 'President' contained  $\approx 22\%$  more eggs in their ovisac compared with the other cultivars (Table 4). Adult males only lived  $\approx 2$ -3 d.

#### Greenhouse test

Symptoms of stunted and deformed plant terminals "bunchy top" were observed in all cultivars during the study (Fig. 1). 'President' and 'Joanne' exhibited symptoms by wk 2, and Double Red by wk 3. Symptoms increased more rapidly in 'President' starting in the 4th wk post infestation.

All terminals in all cultivars were infested by 9 wks post infestation. Repeated measures analysis revealed that cultivar explained a significant amount of the variation in these symptoms ( $F_{2,18} = 6.9$ ,  $P < 0.01$ ), with 'President' being different from the other cultivars ( $P < 0.05$  Tukeys HSD in the repeated measures model).

Mealybug counts conducted mid-way and near the end of the greenhouse study confirmed that all cultivars were reproductive hosts for *M. hirsutus*, which developed through 2 complete generations by the second sample. Significantly more insects



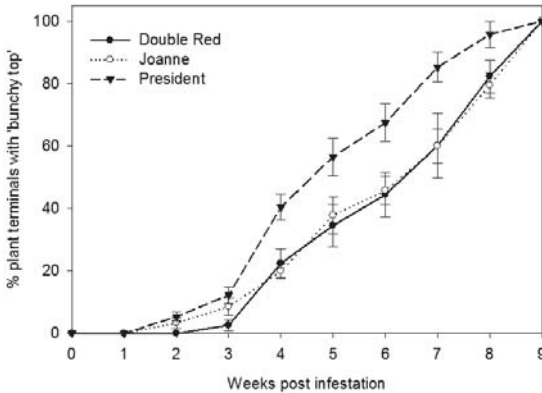


Fig. 1. Cumulative percentage of hibiscus terminals expressing "bunchy top" feeding symptoms among 3 cultivars infested initially with 5 adults and 5 ovisacs of *Maconellicoccus hirsutus*.

(all stages combined) were observed on 'President' after 30 d compared with the other 2 cultivars ( $F_{2,18} = 5.2, P < 0.05$ ), with 50 individuals per terminal on this cultivar (Fig. 2A). Differences were also observed between President and the other cultivars for both nymphs ( $F_{2,18} = 4.0, P < 0.05$ ) and females ( $F_{2,18} = 4.3, P < 0.05$ ). At 60 d post infestation, mealybug numbers increased markedly, reaching >1,300 individuals (all stages) per terminal on 'President' (Fig. 2B). The total number of mealybugs at d 60 was significantly higher on President compared with the other cultivars ( $F_{2,18} = 7.6, P < 0.01$ ), which was due to the higher number of nymphs on this cultivar ( $F_{2,18} = 7.9, P < 0.01$ ). Males were infrequently observed on the plants.

## DISCUSSION

Previous research (Vitullo et al. 2009) showed that several hibiscus cultivars differed in their expression of *M. hirsutus* feeding symptoms; cultivars 'Double Red' and 'Snow Queen' exhibited little or significantly delayed frequency of "bunchy top" compared with 'Florida Sunset' and 'Joanne'. This observation is significant for management decisions, since it suggests that feeding symptoms are not necessarily a reliable indicator of pest presence or pressure. To examine the possibility of plant antibiosis, we further investigated the relationship between life history (fitness) of *M. hirsutus* and "bunchy top" symptom development on 3 hibiscus cultivars expected to have high ('President'), low ('Double Red'), and intermediate ('Joanne') levels of susceptibility.

We found no major differences in the response of *M. hirsutus* among the 3 cultivars in terms of their survival, development rate, size, or oviposition period in laboratory tests at a temperature conducive for mealybug development. Although

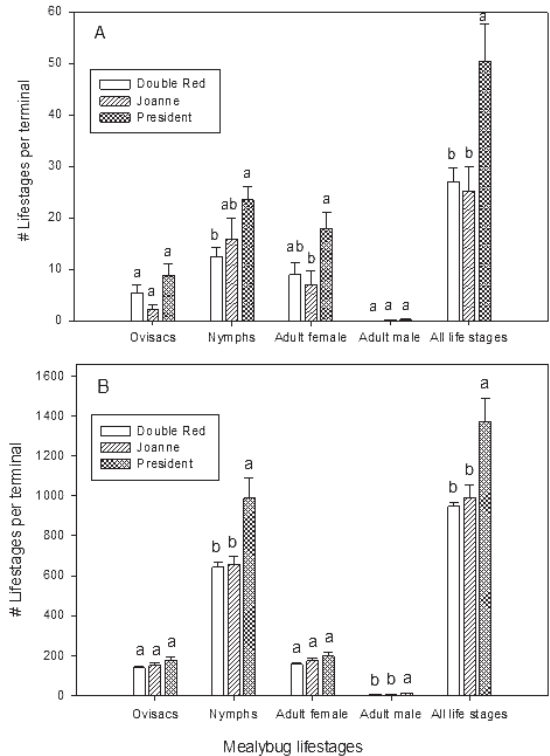


Fig. 2. The number of *Maconellicoccus hirsutus* on 3 hibiscus cultivars at (A) 30 and (B) 60 days post infestation.

there were some differences, for example, a higher fecundity on 'President', the mealybugs readily developed on all varieties, with cumulative survival rates of 71, 74, and 76% on the 'Double Red', 'Joanne' and 'President' cultivars, respectively. Similar findings occurred in the greenhouse, although in this case we noted significantly delayed development of "bunchy top" symptoms and mealybug populations developing on 'Joanne' and 'Double Red' cultivars compared with 'President'.

There are several possible explanations for our findings. "Bunchy top" symptoms of *M. hirsutus* represent a failure of the ground meristem of leaf primordia to differentiate into palisade and spongy mesophyll during leaf expansion (Babu et al. 2004); thus, symptoms may be expected to correlate with plant growth, especially shoot initiation and elongation. Variations among cultivar growth rates or, for example, plants receiving different N, P, K inputs, might similarly be expected to influence development of "bunchy top" symptoms. Abdel-Moniem & Abdel-Wahab (2006) also suggested that different levels of *M. hirsutus* infestation observed among *H. sabdariffa* varieties in an Egyptian nursery might be explained by differences in morphological and physiological characteristics. Characteristics such as leaf trichomes, or cuticular waxes or secondary

plant compounds could affect mealybug development, although this was not assessed in our study. Cumulative plant injury will also correlate with pest density, especially on susceptible varieties (Vitullo et al. 2009).

In practice, various additional factors may correlate with levels of plant resistance. Chong et al. (2008) showed that the intrinsic rate of increase of *M. hirsutus* was optimized at temperatures between 25 and 27 °C, with survival and fecundity significantly reduced at 20 and 30 °C and upper and lower developmental thresholds of 35 and 14.5 °C, respectively. Host plant quality also often limits the developmental rates of herbivorous insects through the availability of nitrogen in the form of amino acids and proteins in host plant tissues (Scriber & Slansky 1981). Host plant type will similarly be important; for example Persad & Khan (2007) showed that egg to adult survival *M. hirsutus* was 21.8% on *H. sabdariffa* compared with only 8.8% on *Cucurbita pepo* L. In addition, there may be tri-trophic responses of natural enemies of *M. hirsutus* responding to different host plant volatiles or other cues.

In conclusion, we observed some differences among hibiscus cultivars in terms of damage symptoms and development of *M. hirsutus* populations, but none exhibited resistance to this pest. Selection (or breeding) of additional plant cultivars tolerant to feeding symptoms of this pest may be useful in low management input landscapes for *M. hirsutus* infested areas, especially in conjunction with biocontrol programs.

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