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EXPRESSION OF FEEDING SYMPTOMS FROM PINK HIBISCUS MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) BY COMMERCIALY IMPORTANT CULTIVARS OF HIBISCUS

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ABSTRACT

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), is a highly polyphagous pest that invaded southern Florida in 2002 and is now widely established throughout most of the state. Although *Hibiscus rosa-sinensis* L. is a preferred and economically important host of *M. hirsutus*, the susceptibility and expression of feeding symptoms by different cultivars have not been evaluated. Cultivars of *H. rosa-sinensis* were infested with *M. hirsutus* and evaluated daily for 40 d for the onset and percentage of terminals expressing feeding symptoms. Under different initial densities of *M. hirsutus*, the cultivar 'President' showed no difference in the latency to expression of feeding symptoms, which occurred between 7 and 15 d after infestation, but did show significant differences between initial density and percentage of terminals expressing feeding symptoms from 10 d onward. When infested with 20 females, 80% of 'President' terminals exhibited symptoms 30 d after infestation. Four other cultivars initially infested with 10 female *M. hirsutus* showed significant differences in the onset and severity of feeding symptoms. All plants of the cultivars 'Florida Sunset' and 'Joanne' expressed damage symptoms at 12 ± 2 SE d and 10 ± 1 d, respectively, following infestation. Only a single plant of the cultivars 'Double Red' and 'Snow Queen' showed such symptoms at 19 and 30 d after infestation, respectively. Significant differences between cultivar and the percentage of terminals expressing feeding symptoms were observed from 20 d onward. Terminals sampled from all plants after 40 d revealed that egg, nymph, and adult female *M. hirsutus* were found on all plants, including those that did not exhibit feeding symptoms. These data have shown that hibiscus cultivars differ in their expression of *M. hirsutus* feeding symptoms, that *M. hirsutus* can reproduce on cultivars of hibiscus that do not express feeding symptoms, and that feeding symptoms are not a reliable indicator of infestation by *M. hirsutus*, highlighting the need for further investigation of the mechanisms underlying differences among cultivars.

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

RESUMEN

La cochinilla rosada del hibisco, *Maconellicoccus hirsutus* (Green), es una plaga altamente polífaga que invadió el sur de la Florida en 2002 y ahora esta extensivamente establecida en el mayor parte del estado. Aunque *Hibiscus rosa-sinensis* L. es el hospedero preferido y económicamente importante de *M. hirsutus*, la susceptibilidad y la expresión de los síntomas debido a su alimentación en diferentes variedades no han sido evaluados. Variedades de *H. rosa-sinensis* con *M. hirsutus* fueron infestadas y evaluadas diariamente por 40 días para la aparición y porcentaje de partes terminales que expresaron síntomas de la alimentación. Bajo diferentes densidades iniciales de *M. hirsutus*, la variedad 'President' no mostraron una diferencia en la latencia de la expresión de los síntomas de la alimentación, que ocurrió entre los 7 y 15 días después de la ingestación, pero mostraron diferencias significativas entre la densidad inicial y el porcentaje de los terminales que expresaron síntomas de alimentación de los 10 días en adelante. Cuando las plantas fueron infestadas con 20 hembras, 80% de los terminales de la variedad 'President' mostraron síntomas 30 días después de la ingestación. Cuatro otras variedades que fueron infestados con 10 hembras de *M. hirsutus* mostraron diferencias significativas en la aparición y severidad de las síntomas de la alimentación. Todas las plantas de las variedades 'Florida Sunset' y 'Joanne' expresaron síntomas de daño a los 12 ± 2 SE días y 10 ± 1 d, respectivamente, después de la ingestación. Solamente una planta en cada una de las variedades 'Double Red' y 'Snow Queen' mostró es-

tos síntomas a los 19 y 30 días después de la ingestación, respectivamente. Se observaron diferencias significativas entre las variedades y el porcentaje de los terminales que expresaron síntomas de la alimentación de los 20 días en adelante. El muestreo reveló que se encontraron huevos, ninfas y hembras adultas de *M. hirsutus* en los terminales en todas las plantas después de 40 días, incluyendo aquellas plantas que no mostraron síntomas de daño por la alimentación. Estos datos han mostrado que las variedades de hibisco varían en su expresión de los síntomas provocados por la alimentación de *M. hirsutus*, que *M. hirsutus* puede reproducirse en variedades de hibisco que no expresan síntomas por la alimentación, que los síntomas de la alimentación no son indicadores confiables para evaluar la ingestación de *M. hirsutus*, y destaca la necesidad de hacer más investigaciones de los mecanismos que causan las diferencias entre las variedades.

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is a highly polyphagous pest that invaded southern Florida in 2002 (Amalin et al. 2003), Louisiana in 2006 (Anonymous 2006), Texas in 2007 (Borgan & Ludwig 2007), and Georgia in 2008 (Horton 2008). It feeds in phloem tissue and injects saliva that can result in malformed leaf and shoot growth, stunting, and plant death (USDA 2001). Many economically important agricultural and horticultural crops and common native plants that are potential hosts of *M. hirsutus* occur in Florida (Hodges & Hodges 2005) and throughout the southern USA. Reported hosts include more than 300 plant species in 74 families (USDA 2001; Borgan & Ludwig 2007) and damaging populations have been recorded on numerous fruits, vegetables, and ornamentals (Hall 1921; Hall 1926; Ghose 1972; Persad 1995; Chang & Miller 1996).

Variations among *H. rosa-sinensis* cultivars in their susceptibility to *M. hirsutus* or their expression of feeding symptoms may have important ramifications for management programs. In response to the arrival of *M. hirsutus* in Florida, a biological control program involving the release of 2 encyrtid parasitoids, *Anagyrus kamali* (Moursi) and *Gyranusoidea indica* (Shafee, Alam and Agarwal), and the coccinellid predator *Cryptolaemus montrouzieri* (Mulsant) was implemented by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. New infestation sites for release of biocontrol agents are based on visual scouting of preferred hosts in the landscape (USDA 2001). While *M. hirsutus* populations have been greatly reduced in Florida through the establishment and actions of biological control agents (Amalin et al. 2003), biological control does not cause local extinction of *M. hirsutus* populations (Ranjan 2004) and the pest has continued to spread in Florida. Sex pheromone traps for pink hibiscus mealybug deployed continuously between 18 May 2007 and 10 Oct 2008 have shown that males continue to be captured at locations in southern Florida where parasitoids were released in 2007 (Vitullo, unpublished data). Given that management decisions for *M. hirsutus* are often based on the results of surveys recording the presence or absence of symptoms, understanding the variations in the expression or se-

verity of these symptoms among cultivars is important. Furthermore, differences in susceptibility among cultivars may be helpful toward sustaining hibiscus production in Florida.

Hibiscus rosa-sinensis L. (Malvaceae), is considered a preferred host of pink hibiscus mealybug (Hall 1921; Mani 1989; Kairo 1998) and 15 *Hibiscus* species have been confirmed with damaging populations (Stibick 1997). Based on the historical importance of hibiscus to ornamental production in south Florida, *M. hirsutus* has had negative impacts on local nurseries. A zero tolerance policy has been in effect since 2004 (FDACS 2005) and numerous quarantine actions have occurred (Ranjan 2004; Gaskalla 2006). Retail distributors of ornamental plants in regions of the US not presently infested by pink hibiscus mealybug have reduced hibiscus orders to mitigate the risk of being sent infested stock (J. Cou, Garden Depot, Miami, FL, personal communication), especially since 2004, when infested hibiscus plants were shipped from a Homestead, FL nursery to 36 States (Hodges & Hodges 2005). Additionally, landscape design firms in southern Florida have reduced the planting of hibiscus since the invasion by *M. hirsutus*, and nurseries in southern Florida have significantly curtailed hibiscus production (J. Cou, personal communication). As with many other host plants, typical feeding symptoms from *M. hirsutus* on hibiscus include leaf curl and shortened internodes, leading to rosetteing or "bunchy top" (USDA 2001). However, during the course of our research in southern Florida, we noticed that certain cultivars of hibiscus growing in residential areas appeared to suffer more damage from pink hibiscus mealybug than others, which may be attributed to symptoms varying among hosts (Stibick 1997; Anonymous 2005), and/or *M. hirsutus* preferring certain hibiscus varieties (Abdel-Moniem et al. 2005).

The following research addresses the response of several commercially important *Hibiscus rosa-sinensis* cultivars to feeding by pink hibiscus mealybug. Specific objectives were to determine the relationship between mealybug density and the expression of feeding symptoms in a susceptible cultivar and the expression of feeding symptoms in 4 cultivars infested with a single density of mealybugs.

MATERIALS AND METHODS

Insects and Test Plants

Experiments were conducted in a greenhouse at 28°C at the University of Florida, Tropical Research and Education Center, Homestead, FL. Greenhouse temperature varied by 1°C with a relative humidity range of 40 to 98%. *Hibiscus rosa-sinensis* plants, approximately 1 m high and 0.66 m wide, were grown in 12-L plastic pots and maintained in accordance with standard horticultural recommendations (Ingram & Rabinowitz 1991). Periodic releases of *Aphidius colemani* Vier. (IPM Laboratories, Inc., Locke, NY) kept aphid levels low in the greenhouse.

Pink hibiscus mealybugs were reared on potatoes in a controlled environmental chamber at 26°C and a 12:12 L:D regime (Serrano et al. 2001). Plants were infested by transferring adult female mealybugs with attached egg sac to the apical bud of terminals with a micro-pin tool.

Expression of Feeding Damage in Relation to Mealybug Density

To evaluate the expression of feeding damage in a susceptible *H. rosa-sinensis* cultivar infested with different numbers of female mealybugs, we placed 16 plants of the cultivar 'President' in the greenhouse on 2 July 2007. Plants were infested with 1, 5, 10, or 20 female mealybugs per plant ($n = 4$ plants per infestation level). Due to constraints on the availability of mealybugs, the 4 replicates were infested on successive weeks, beginning 19 Jul.

The number of terminals on each plant was recorded (range = 14-22 terminals per plant) and plants were evaluated daily for 40 d for the number of terminals exhibiting "bunchy top" feeding symptoms. This experimental duration allowed for the completion of the first infesting generation of mealybugs and the dispersal of the second generation crawlers on each plant (Mani 1989).

Expression of Feeding Damage by *Hibiscus* Cultivars

To evaluate feeding damage expressed by different *H. rosa-sinensis* cultivars, 3 plants of 'Double Red', 'Snow Queen', 'Florida Sunset' and 'Joanne' were placed in the greenhouse on 18 Jan 2008. These cultivars were selected following consultation with Mr. J. Cou (Garden Depot, Miami, FL) and were based on horticultural differences among them and their commercial popularity. 'Double Red' has cordate leaves densely packed along the shoot and red full double blooms. 'Snow Queen' has variegated cordate leaves with a sprawling habit and fringed single red blooms. 'Florida Sunset' has cordate leaves and yellow regular single blooms with an orange eye zone.

'Joanne' has ovate leaves and orange cartwheel overlapped single bloom with a dark orange eye zone. Plants were pruned to have between 17 and 20 terminals, then infested with 10 female mealybugs on 12 Mar and evaluated daily for 40 d for the expression of feeding symptoms.

After 40 d, 6 terminals (15 cm long) were pruned from each plant. Terminals could now be grouped into strata by presence or absence of feeding symptoms, and a stratified random sample was used (Ott & Longnecker 2001). When possible, 3 terminals exhibiting "bunchy top" were removed from each plant, and the remaining terminals that exhibited no feeding symptoms were selected to complete the sample size of 6 terminals. Terminals were examined under a dissecting stereomicroscope at 250X magnification and the number of adult females, nymphs, and egg sacs per terminal was recorded.

Data Analysis and Statistics

The latency to the first expression of feeding symptoms (days) for 'President' plants with different initial infestation levels was analyzed by randomized complete block design ANOVA with 4 replicated blocks (SAS Institute 2001). The latency to the first expression of feeding symptoms was compared by descriptive statistics among hibiscus cultivars initially infested with an equal number of mealybugs because plants that did not become infested could not be included in the analyses.

Repeated measures ANOVA (PROC MIXED, SAS Institute 2001) was carried out to determine effects of initial infestation density, days after infestation and their interaction on severity of feeding symptoms by application of compound symmetry (cs) and first-order autoregressive (ar(1)) models (Ott & Longnecker 2001). Severity of feeding symptoms was then compared among 'President' plants with different initial infestation levels at 10, 20, 30, and 40 d after infestation by a randomized complete block design ANOVA (SAS Institute 2001), and evaluated for block effect. Assessment of the severity of feeding symptoms was based on the percentage of terminals exhibiting feeding symptoms on each plant, analyzed as arcsine square-root transformed percentages (Zar 1999). Severity of feeding symptoms was compared among cultivars infested with the same number of females with the same tests as above. The number of unhatched egg sacs, nymphs, and adult female mealybugs recovered from terminals sampled after 40 d, regardless of feeding symptom, was compared among cultivars by ANOVA (SAS Institute 2001). The number of mealybugs at different developmental stages found on cultivars with varied feeding symptoms was compared with descriptive statistics. Analyses of data from all experiments were considered significant at

<0.05. Prior to ANOVA, tests for normality with PROC UNIVARIATE (SAS Institute 2001) were performed. Means of significant effects in ANOVA were separated by Tukey Honestly Significant Difference (HSD) test.

RESULTS AND DISCUSSION

Infestation of the cultivar 'President' with pink hibiscus mealybug resulted in the expression of "bunchy top" feeding symptoms in all plants within 15 d. Initial infestation levels (mean latency in days \pm SE) of 1 (11.0 ± 1.8), 5 (8.5 ± 1.2), 10 (7.3 ± 0.8), or 20 (7.0 ± 0.6) adult females and their egg sac did not significantly affect the latency to the first terminal exhibiting feeding symptoms ($F = 2.23$; $df = 3,12$; $P = 0.1369$). Low levels of variance are evidence of an insignificant block effect.

There were negligible differences between compound symmetry and first-order autoregressive analysis for all repeated measures comparisons for severity of feeding symptoms and initial infestation density of the 'President' cultivar, days after infestation and their interaction. Compound symmetry analysis on severity of feeding symptoms demonstrated a significant difference for initial infestation density ($F = 50.24$; $df = 3,48$; $P < 0.0001$), day after infestation ($F = 65.65$; $df = 3,48$; $P < 0.0001$) and their interaction ($F = 2.17$; $df = 9,48$; $P = 0.0415$). As such, these data provide a good indication of how the severity of feeding symptoms changes over time under differing initial infestation densities; cumulative plant injury over time was density dependent (Fig. 1). On d 10, plants infested with 1 and 20 females differed sig-

nificantly ($F = 4.98$; $df = 3,12$; $P = 0.0180$). On day 20, plants infested with 10 and 20 females differed from those with 1 female ($F = 9.37$; $df = 3,12$; $P = 0.0018$). Plants infested with 20 females differed from all other plants at day 30 ($F = 12.65$; $df = 3,12$; $P = 0.0005$) and 40 ($F = 11.79$; $df = 3,12$; $P = 0.0007$) (Fig. 1). When day was excluded, severity of feeding symptoms was different for infestation density ($F = 9.98$; $df = 3,48$; $P = 0.0001$), but not for block ($F = 1.14$; $df = 3,48$; $P = 0.3436$), or their interaction ($F = 0.48$; $df = 9,48$; $P = 0.8811$).

Infestation of 4 cultivars with 10 pink hibiscus mealybug females and their egg sac did not result in the expression of bunchy top feeding symptoms on all plants. One of 3 'Double Red' plants exhibited symptoms, first occurring 19 d after infestation and totaled 2 shoots during the trial. One of 3 'Snow Queen' plants exhibited feeding symptoms, with 1 terminal exhibiting bunchy top at 30 d after infestation. All 'Florida Sunset' and 'Joanne' plants exhibited bunchy top, with a mean (\pm SE) number of days to first expression of feeding symptoms of 12 ± 2 and 10 ± 1 , respectively.

Repeated measures comparisons by compound symmetry analysis on severity of feeding symptoms demonstrated a difference for cultivar ($F = 82.73$; $df = 3,32$; $P < 0.0001$), day after infestation ($F = 19.29$; $df = 3,32$; $P < 0.0001$), and their interaction ($F = 3.72$; $df = 9,32$; $P = 0.0028$). As such, these data provide a good indication of the change in severity of feeding symptoms over time for different cultivars (Fig. 2). The severity of feeding symptoms and cultivar at 10 d intervals showed differences from 20 d after infestation onward; 20 d ($F = 28.55$; $df = 3,8$; $P < 0.0001$), 30 d ($F = 25.59$; $df = 3,8$; $P = 0.0002$) and 40 d ($F = 15.51$; $df = 3,8$;

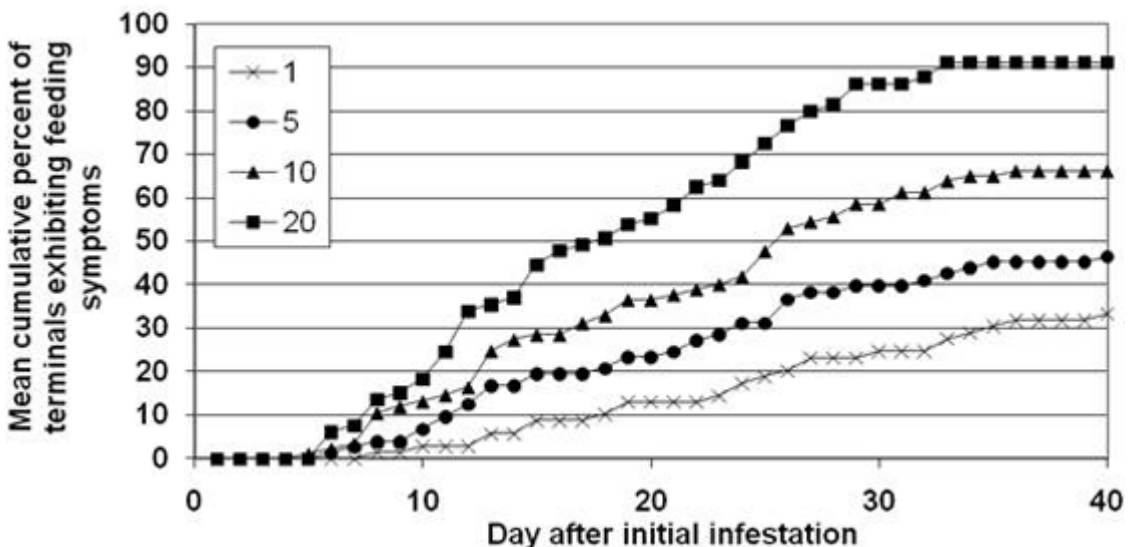


Fig. 1. Mean cumulative percent of terminals of 'President' hibiscus plants exhibiting feeding symptoms in response to different initial infestation densities of *M. hirsutus*.

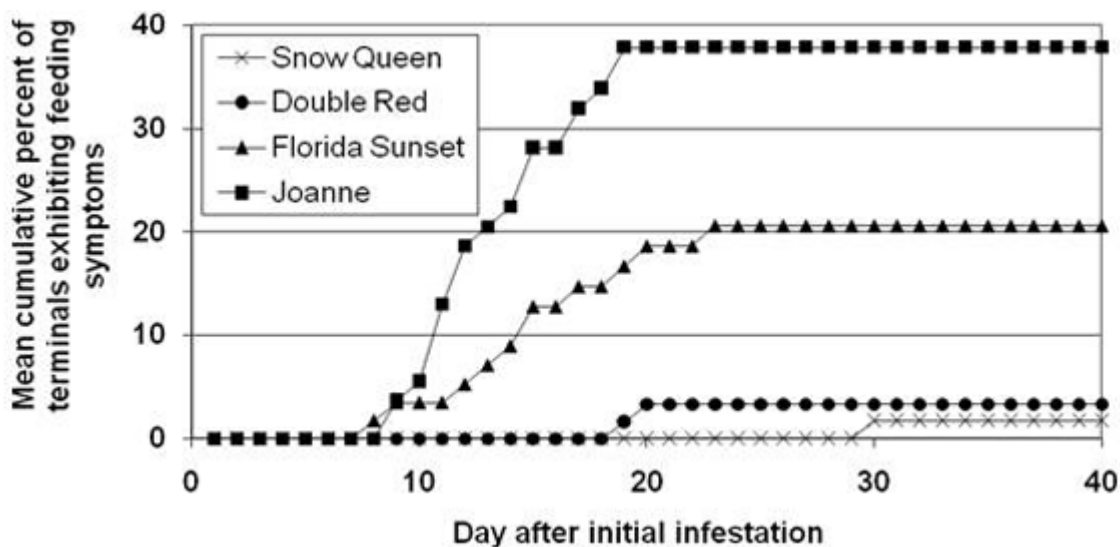


Fig. 2. Mean cumulative percent of terminals of 4 hibiscus cultivars exhibiting feeding symptoms in response to an initial infestation density of 10 female *M. hirsutus*.

$P = 0.0011$). 'Florida Sunset' and 'Joanne' were not significantly different, but were different from 'Snow Queen' and 'Double Red', which did not differ (Fig. 2).

When terminals were removed from cultivars on day 40, *M. hirsutus* egg sacs, nymphs, and adults were found on all cultivars with or without feeding symptoms (Table 1). The number of *M. hirsutus* found on cultivars regardless of feeding symptom resulted in 'Florida Sunset' having significantly more unhatched egg sacs present than 'Double Red' and 'Snow Queen', but not 'Joanne' ($F = 5.94$; $df = 3,68$; $P = 0.0012$). 'Snow Queen' had fewer nymphs than all other cultivars ($F = 10.50$; $df = 3,68$; $P < 0.0001$). 'Florida Sunset' had significantly more adults than 'Snow Queen', but not 'Double Red' or 'Joanne' ($F = 5.67$; $df = 3,68$; $P = 0.0016$). The mean (\pm SE) number of pink hibiscus mealybugs found on terminals without feeding symptoms ($n = 51$) was 8 ± 2 egg sacs, 379 ± 56

nymphs, and 10 ± 2 adults, and terminals with symptoms ($n = 21$), had 44 ± 6 egg sacs, 1279 ± 140 nymphs, and 49 ± 7 adults. The presence of adults and egg sacs at d 40 indicate that *M. hirsutus* was able to reproduce on the hosts, based on lifecycle duration (Mani 1989).

During our research with pheromone traps for assessing populations of *M. hirsutus* in residential areas in south Florida, we noticed that not all hibiscus plants exhibited the typical feeding symptoms associated with pink hibiscus mealybug, despite capturing many males in traps suspended from these hosts (Vitullo, unpublished). It is important for growers and landscape managers to know that the number of pink hibiscus mealybugs it takes to elicit feeding symptoms and the latency from first infestation to the expression of symptoms is not the same for all cultivars. The latency to the first terminal exhibiting feeding symptoms for the 'President' cultivar was not den-

TABLE 1. MEAN \pm SE NUMBER OF PINK HIBISCUS MEALYBUGS AND THEIR DEVELOPMENTAL STAGES FOUND ON TERMINALS OF HIBISCUS CULTIVARS AT D 40, WHEN FEEDING SYMPTOMS WERE PRESENT OR ABSENT.

Cultivar	Presence of feeding symptoms	No. of terminals	Nymph	Egg sac	Adult female
'Double Red'	No	16	620 \pm 93	15 \pm 4	18 \pm 5
'Double Red'	Yes	2	907 \pm 262	23 \pm 7	28 \pm 1
'Snow Queen'	No	17	86 \pm 23	4 \pm 1	5 \pm 1
'Snow Queen'	Yes	1	101	17	17
'Florida Sunset'	No	9	690 \pm 169	8 \pm 4	11 \pm 6
'Florida Sunset'	Yes	9	1421 \pm 160	63 \pm 10	68 \pm 10
'Joanne'	No	9	194 \pm 39	2 \pm 1	2 \pm 1
'Joanne'	Yes	9	1352 \pm 245	34 \pm 7	38 \pm 8

sity dependent. A single female with her egg sack produced "bunchy top" feeding symptoms in 15 d or less.

Timing of management action can be greatly aided with information on differences in the latency to the expression of the first feeding symptoms among different Hibiscus cultivars. For 'President', 'Florida Sunset', and 'Joanne', symptoms occurred in 7 to 15 d following infestation, while in 'Double Red' and 'Snow Queen', symptom expression occurred much later or did not occur at all. There may be different characteristics that confer degrees of susceptibility. Abdel-Moniem et al. (2005) evaluated the vertical distribution of *M. hirsutus* on three *Hibiscus sabdariffa* L. varieties in an Egyptian nursery and found that only 1 in 3 varieties tested was attacked. Although feeding symptoms were not evaluated, they suggested that morphological and physiological characteristics of *H. sabdariffa* may increase its susceptibility to *M. hirsutus*. While much additional research would be necessary to reveal the mechanisms of tolerance or resistance of hibiscus cultivars to pink hibiscus mealybug, traditional breeding for reduced susceptibility to feeding injury may be possible.

Expression of feeding symptoms varies between cultivars, but may be dependent on the growth of the plant. The feeding symptoms of *M. hirsutus* are an inability of the ground meristem of leaf primordia to differentiate into palisade and spongy mesophyll when leaves open (Babu et al. 2004), suggesting that feeding symptoms will only occur during this developmental time. The limiting factor for hibiscus growth is temperature (Ingram & Rabinowitz 1991), suggesting that the temperature in the region and time of year also may have an effect on the appearance of feeding symptoms during hibiscus growth periods. While infestation density had no effect on the latency to first expression of feeding symptoms, cumulative plant injury over time was density dependent. When the cultivar 'President' was infested with a single female and her egg sack, 33% of the terminals exhibited "bunchy top" after 40 d, whereas feeding by twenty females deformed 90% of the terminals. Of the cultivars tested, only 'Joanne' infested with 10 females expressed similar levels of damage as 'President' at d 20. There was little difference in the severity of symptoms from d 20 to 40 for 'Double Red', 'Snow Queen', 'Florida Sunset', and 'Joanne'. Anecdotal observations suggest that newly-hatched crawlers of *M. hirsutus* may walk for considerable distances on a plant before settling to feed (Misra 1920). Feeding symptoms before d 20 were likely caused by the infesting generation. Based on the duration of the *M. hirsutus* lifecycle (Mani 1989), symptoms from d 20 to 40 were likely caused by the second generation. The colonization and resulting feeding symptoms from the second generation would likely depend

on the size and structure of the plant being evaluated.

Many plants have been evaluated and shown to be suitable for the development and reproduction of pink hibiscus mealybug (Serrano & Lapointe 2002), while others expressed damage symptoms but were not suitable for reproduction or development (Kairo et al. 2000). *Maconellicoccus hirsutus* can reproduce on cultivars of hibiscus without producing symptoms, including 'Double Red' and 'Snow Queen'. Given that feeding by an initial density of 1 pink hibiscus mealybug per plant elicited feeding symptoms on 'President' and that feeding by 10 females caused minimal or no damage on 'Snow Queen', it appears that feeding symptoms are not a reliable indicator of the presence or level of infestation of all hibiscus cultivars by *M. hirsutus*.

When making landscape design selections, knowledge of how pink hibiscus mealybug infestations affect different hosts will aid in creating aesthetic injury thresholds. Selection of plants that are tolerant to pink hibiscus mealybug feeding symptoms may make it possible to create low management input landscapes for *M. hirsutus* infested areas, especially in conjunction with bio-control programs.

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