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GALL FORMATION ON THE ENDANGERED CACTUS, *LEPTOCEREUS QUADRICOSTATUS* CAUSED BY THE INVASIVE MEALYBUG, *HYPOGEOCOCCUS PUNGENS* (HEMIPTERA: PSEUDOCOCCIDAE)

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Abstract: The introduction of Harrisia cactus mealybug (HCM), *Hypogeococcus pungens*, in Puerto Rico causes concern due to its damaging effects to the structure of cacti communities, eliminating species, and severely compromising plant growth and reproduction of susceptible native species. HCM is a polyphagous soft scaly insect considered an aggressive pest outside of its native range of South America. In Puerto Rico, three native species of cacti have been observed to be heavily infested with this invasive HCM, the natives *Pilosocereus royenii*, *Melocactus intortus*, and the endangered *Leptocereus quadricostatus*. Recent studies have shown that HCM affects the growth and survival of *P. royenii*, but limited information is available about the other affected species. To understand more about the threat of HCM, greenhouse experiments were designed to evaluate the pest colonization and to describe the development of galls on the columnar cactus *L. quadricostatus*. The experimental design consisted of two groups of *L. quadricostatus*, infested and non-infested, the infested treatment received twenty crawlers and six females of HCM. The first signs of successful infestation were observed at 27 days after the initial pest transfer, while the first signs of gall formation were observed at 97 days. The initial infestation process was best explained by an exponential growth model ($R2=0.93$, $F=125.4$, $df=1,9$, p-value < 0.05). This stage can be identified by the formation of a powdery wax-like white spot on the areole of the cactus. Also four types of gall structure were observed. To our knowledge this is the first time that the full infestation process and gall development, by HCM has been replicated under controlled conditions. These results provide a better understanding of the interaction between HCM and the endangered host plant, and will help to develop more effective management strategies.

Keywords: Endangered Cactus Species, *Hypogeococcus pungens*, Invasive Species, Mealybug.

Introduction

The introduction of species into new localities and their ecological consequences on native species and local communities are of main concern to conservation biology. Harrisia cactus mealybug (HCM), *Hypogeococcus pungens,* is a polyphagous gall-inducing small scaly insect considered an invasive aggressive pest outside of its possible native range of South America. *Hypogeococcus pungens,* was first detected in San Juan, Puerto Rico fifteen years ago on an ornamental plant, *Portulaca oleracea* (Family: Portulacacea) and in plant material from Guánica in 2005 (Segarra-Cardona et al., 2010). HCM is native to some parts of South America that include Argentina (Williams & Granara de Willink. 1992; Claps & De Haro 2001), Chile, Brazil, Peru and Paraguay (Zimmerman et al. 2010). This species has been introduced and used before as biological control

agent for populations of introduced cacti species in Australia (Tomley & McFadyen 1985) and South Africa (Moran & Zimmermann 1991; Paterson et al., 2011).

The introduction of HCM, *H. pungens*, in Puerto Rico causes concern due to its permanent negative effects to the structure of cacti communities, eliminating susceptible native cacti species (Segarra-Cardona et al. 2010). In Puerto Rico, three important species of cacti have been observed heavily infested with the invasive HCM, the natives *Pilosocereus royenii*, (Fig. 1A), *Melocactus intortus* (Fig. 1B), and the endangered (Quevedo et al. 1990, Gann & Taylor 2013) *Leptocereus quadricostatus* (Fig. 1C). HCM is known to induce morphological grown abnormalities, known as galls, in its host cacti species in Puerto Rico. Recent studies have shown that HCM affects the growth and survival of *P. royenii*, but little is known about the other affected species (Rodrigues et al., 2012). *Leptocereus quadricostatus* ((Bello) Britton & Rose) is a columnar cactus species (Cactaceae) found in the dry forest of the southern coast of Puerto Rico and Anegada, at the northernmost part of the British Virgin Islands (BVI) (Monsegur 2009). There is limited information on the biology of this species. Due to its limited distribution and small population size this cactus is currently listed as endangered by the International Union for Conservation of Nature (IUCN; Gann & Taylor 2013). The two main objectives of this study were: 1) Evaluate the infestation and the population growth of Harrisia cactus mealybug on *Leptocereus quadricostatus*; and 2) Characterize symptoms and gall development induced by Harrisia cactus mealybug infestation.

Figure 1. Native cacti species *Pilosocereus royenii*, *Melocactus intortus* and *Leptocereus quadricostatus* infested with *Hypogeococcus pungens* in Guánica, PR.

Material and Methods

A total of ten potted plants (10L pots) of *Leptocereus quadricostatus* were infested with six (6) adult females and twenty (20) crawlers of *Hypogeococcus pungens* (HCM) from colonies originally collected at USFW Reserve in Cabo Rojo, PR. Equal numbers of plants were maintained without receiving the infestation of the pest, serving as controls for the experiment. All plants were maintained under the same environmental conditions in a greenhouse at the Center for Excellence in Quarantine & Invasive Species (CEQIS) at the Botanical Garden of the University of Puerto Rico. All plants received standardized fertilization and irrigation. The temperature in the green house was monitored using a Hobo Pro v2 temp /RH data logger (Onset Corp., MA, USA). The plants were monitored for development of symptoms and population dynamics of the HCM. Observation about first signs of infestation, establishment of colonies and symptoms were recorded. To access population growth at the stage of establishment, the number of all colonies

per plant were recorded for a period of 3.5 months. In that period the number of galls were also recorded. The development of galls was closely followed, documenting them with pictures and size measurements. A regression analysis was performed in R (R Core Team. 2015) to determine the adjusted model that most closely fit the colony population development.

Result and Discussion

Development of HCM on *Leptocereus quadricostatus*

The experiment, carried out under greenhouse conditions, was able to successfully reproduce the infestation of HCM on *L. quadricostatus*. The temperature in the greenhouse averaged 31.1 ± 4.8°C, with maximum and minimum temperatures of 45.2°C and 23.9 °C. HCM colonies were established and observed on the host cactus areoles (at the base of the spines) at 27 days from an initial infestation event. We observed that each initial colony had an average of 0.67 ± 0.6 (n=6) reproductive females per colony with a maximum of 3 reproductive females per colony. An exponential growth model best explains the initial colonization stage by HCM on *L. quadricostatus*. (R^2=0.93, F=125.4, df=1,9, p-value < 0.05; Fig. 2).

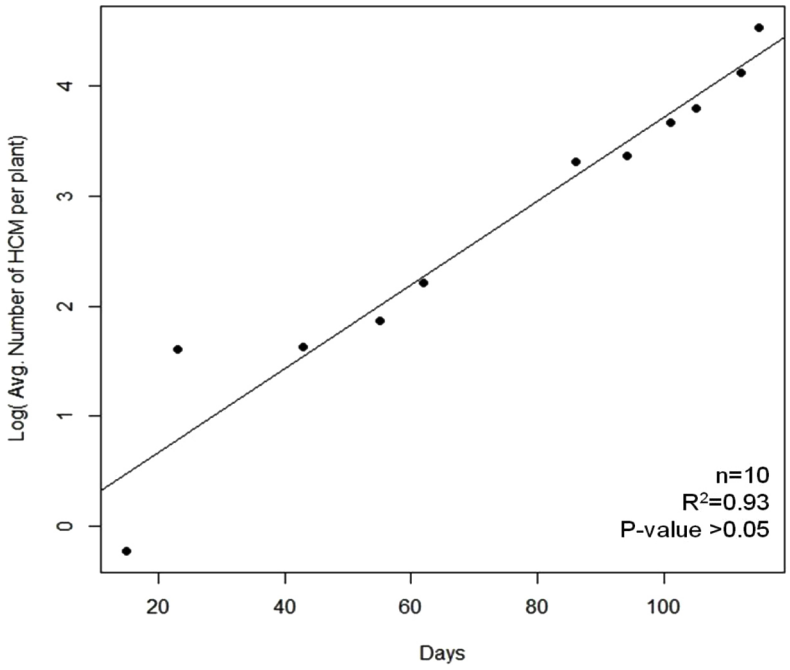

Figure 2. Initial colonization stage by HCM on *Leptocereus quadricostatus*. The colonization process is best explained by an exponential growth model. (R^2=0.93, F=125.4, df=1,9, p-value < 0.05).

In this study in a controlled environment, the only limitation for HCM to continue growth was the progressive reduction of availability of proper sites for establishment of the colonies (areole availability). HCM colonization takes place in the cactus areoles, if there aren't new areoles

available there cannot be new establishment or colony formation. This same pattern has been observed in naturally occurring infested plants of *L. quadricostatus*. Despite the high HCM colony densities observed in this study, *L. quadricostatus* produced flowers in more than one individual in both the infested and non-infested plants.

Symptoms caused by HCM infestation on *Leptocereus quadricostatus*

Infested individuals of *L. quadricostatus* developed two main initial conspicuous visual symptoms. First, were observed the formation of a powdery wax-like white spot on the areole of the cactus resulting from the mealybug secretions (Fig. 3A). This symptom represents the visual cue of a new colony on an infested plant. In addition, in some cases this white spot is not evident and instead we observed a curling (bending) of the cactus branch (this also occurred in areoles with white spot). This curling pattern was usually observed at one of the sides of the secondary and tertiary branches or close to the apex of the cactus (Fig. 3B).This type of branch deformity was never observed in non-infested cacti plants.

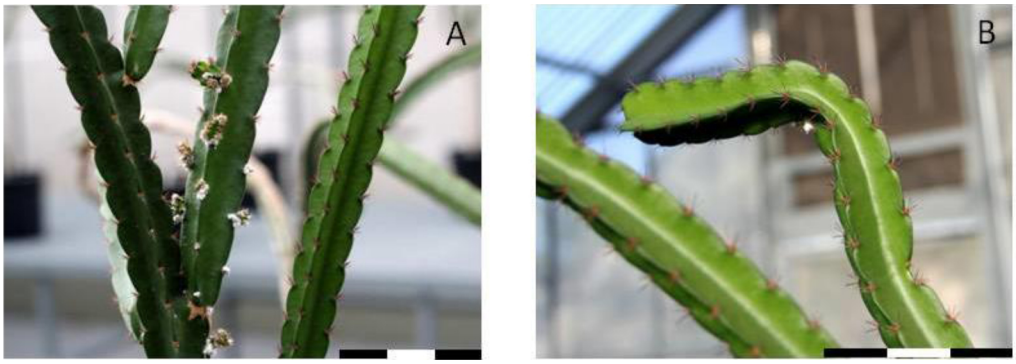

Figure 3. Initial infestation stage symptom. The initial stage of colonization by HCM on *Leptocereus quadricostatus* is characterized by a powdery wax-like white spot on the areole (A) or/and bending of the branch (B). (Scale bar = 3 cm).

After this initial infestation stage, we observed other morphological growth changes and abnormalities on the cactus host. The infested areole starts to develop new vegetative growth that looks like a new ramification bud (Fig 3A). This bud later, due the persistent feeding of HCM, becomes something different, a gall. The initial stage of gall formation seems uniform for all the plants. A gall is the result of abnormal growth of plant tissue promoted by a parasite, in this case HCM. We first observed this type of abnormality at 97 days from the initial infestation event. These galls were observed at the apex and branch sides, but the ones that continued further development were localized mostly on secondary and tertiary branches, which were younger, less lignified tissues and still actively growing.

Although there is a lot of variability in the morphology of the galls observed in *L. quadricostatus*, four types of gall structure were commonly observed.

1. The gall stays as small-deformed bud with minimal growth and with one or little new ramification. Most of the cases the small-deformed bud is densely covered with the powdery wax-like material secreted by the mealybug. In many cases this is observed in primary branches (Fig .4-1A, 4-1B).

2. The bud grows multiple small ramifications that are densely packed forming a type of rounded tumor or gall. The density of spines in the areole is higher than in non-infested cacti. (Fig. 4-2A, 4-2B).
3. The bud elongates as a thin and deformed "branch" which later starts to show multiple branching at the apex of the new branch while gall growth develop similar to type 2 galls (Fig. 4-3A, 4-3B).
4. As a result of the initial curling of the branch, the branch continues to curl. The infested areole that initiated the curling develops a bud that results in abnormal growth of branches resulting in galls similar the type 2 galls. This gall is localized in a protected area inside the curl (Fig. 4-4A, 4-4B).

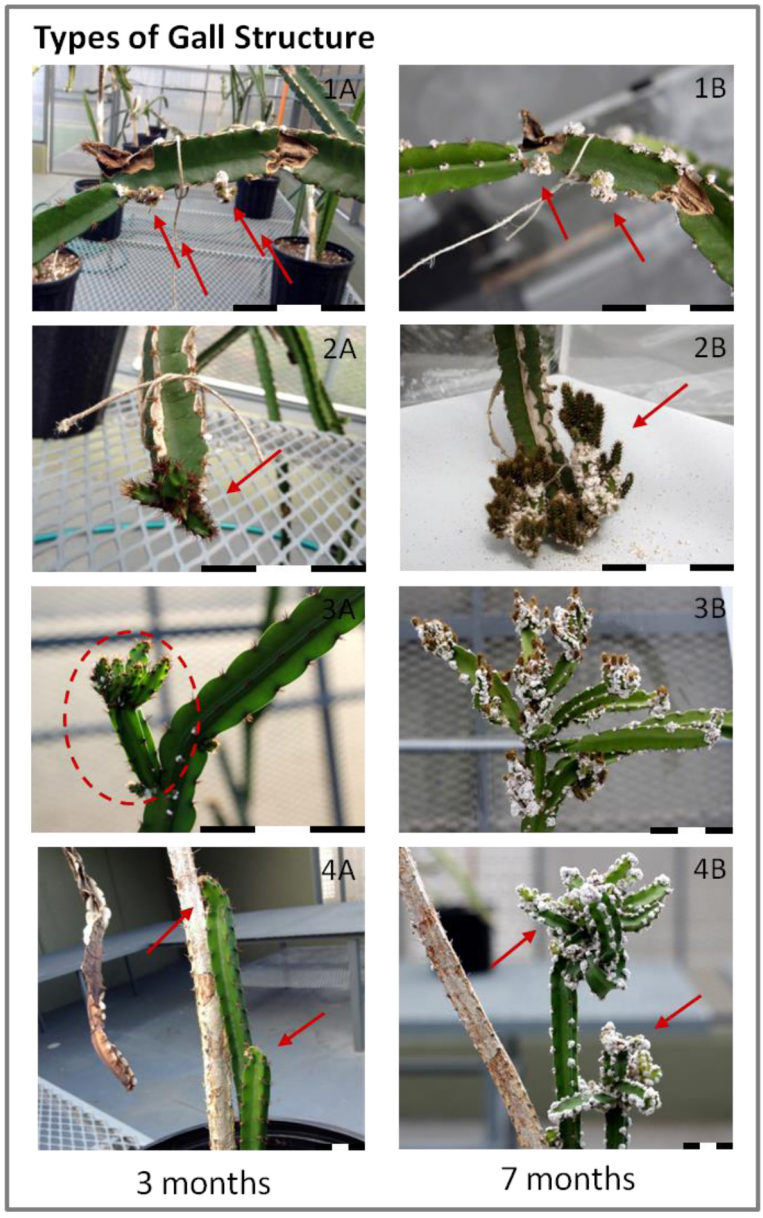

Figure 4. Gall development during initial colonization stages by HCM on *Leptocereus quadricostatus*. (Scale bar = 3 cm).

Although this is the first time the HMC infestation has been reproduced under control condition, gall induction has been documented in naturally occurring populations in other studies and in other cacti species (Segarra-Carmona 2010, Zimmerman 2010). It is also important to mention that that it seems that HCM feeding on plant extremities probably provokes hormonal imbalance and loss of apical dominance, which induces development of new lateral branches that will serve as new colonization sites for the pest insect.

Conclusions

The establishment of HCM under greenhouse conditions on *L. quadricostatus* was successful, inducing gall formation that were similar to some observed in naturally occurring plants infested by the pest. In addition, this plant could be easily propagated in a greenhouse and making it a viable alternative approach to rear abundant population of the pest insect, which could serve to study the pest-host interaction as well as natural enemies efficiencies for biological control. HCM colony establishment occurred in a relatively short time (1.5 month). We were able to identify various morphological alterations cause by the pest insect on the plant. Also we observed what it seems to form four (4) different types of gall development. These results provide a better understanding of the interaction between HCM and the endangered host plant *L. quadricostatus*, and may help develop more effective management strategies for the pest insect, as we continue to investigate tri-trophic interactions between the pest insect, host plant, and potential biological control agents.

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