

# Studies on the Biology of *Hypogeococcus pungens* (sensu stricto) (Hemiptera: Pseudococcidae) in Argentina to Aid the Identification of the Mealybug Pest of Cactaceae in Puerto Rico

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## Abstract

*Hypogeococcus pungens* Granara de Willink, sensu stricto, is a serious pest of cacti in Puerto Rico threatening many Caribbean islands. A classical biological control program for *H. pungens* was initiated for Puerto Rico in 2010 with a survey for natural enemies of *H. pungens* in its native range of Argentina. Biological differences were observed between populations of *H. pungens* sampled on Amaranthaceae and Cactaceae. Molecular studies suggested that *H. pungens* populations from different host plant families are likely a complex of species. Our objective was to study the biology of *H. pungens* sensu stricto on specimens collected in the same locality and host plant as the holotype [Tucumán Province, Argentina; *Alternanthera pungens* Kunth (Amaranthaceae)]. We were interested in the reproductive biology of females, longevity and survival of adults, the effect of temperature on the development, and nymph performance (survival and development) on five Cactaceae species. We found that *H. pungens* s.s. showed marked biological differences from the populations collected on Cactaceae and exported to Australia for the biological control of the cactus *Harrisia* spp. The main differences were the presence of deuterotoky parthenogenesis and the fact that *H. pungens* did not attack Cactaceae in the laboratory. Our results provide biological evidence that *H. pungens* is a species complex. We propose that the population introduced to Australia is neither *Hypogeococcus festerianus* Lizer y Trelles nor *H. pungens*, but an undescribed species with three circuli, and that the *Hypogeococcus* pest of cacti in Puerto Rico is not *H. pungens*.

**Key words:** Biological control; species complex; host race; *Alternanthera*, cacti

*Hypogeococcus pungens* Granara de Willink (Hemiptera: Pseudococcidae) is native to South America and was described from specimens collected on *Alternanthera pungens* Kunth (Amaranthaceae) in Tucumán Province, Argentina (Granara de Willink 1981). The host range of this mealybug includes species of Cactaceae, Amaranthaceae, and Portulacaceae (Ben-Dov 1994, Claps and de Haro 2001, Hodges and Hodges 2009). Nymphs and females suck photosynthates with their stylet-sheath mouthparts (Fausto-Cisneros 1995). As a result, plants suffer direct and indirect damages (Milonas and Kózar 2008), often manifest as

malformations and galls in cacti (McFadyen 1979, Carrera-Martínez et al. 2015).

The biology of *H. pungens* is not well known. The only observations were made by McFadyen (1979) on a population collected on Cactaceae for the biological control of invasive cacti in Australia. They are bisexual and ovoviviparous. Potential fecundity ranges between 80 and 100 eggs, preoviposition period is around 20 d, and females lay 2–4 nymphs per day over 35 d in contrast to other mealybugs, such as *Dactylopius* spp. on *Opuntia* spp, *H. pungens* causes no immediate damage to mature tissue (McFadyen 1979).

*Hypogeococcus pungens* is an effective biological control agent of invasive cacti in Australia and South Africa (Julien and Griffiths 1999). In 1975, this species was released in Australia to control *Harrisia martinii* (Labouret), *Harrisia tortuosa* (Forbes) Britton and Rose, and *Harrisia bonplandii* (Parmentier) Britton and Rose. The mealybug established immediately, and caused severe damage and control on all the target weeds, except for *Acanthocereus tetragonus* (L.) Hummelinck, in which the control was partial (Julien and Griffiths 1999, McFadyen 2012). In 1983, *H. pungens* was released in South Africa to control *Cereus jamacaru* de Candolle and *H. martinii* (Julien and Griffiths 1999, Zimmermann and Pérez Sandi y Cuen 2010). In both countries, the control was successful, and since *H. pungens* was released, it has not been found in other family than Cactaceae (Zimmermann and Pérez Sandi y Cuen 2010, McFadyen 2012).

At present, *H. pungens* is a serious pest of native and endemic columnar cacti (species of cacti with cylindrical growth) and epiphytic cacti in Puerto Rico (Zimmermann and Pérez Sandi y Cuen 2010, Carrera-Martínez et al. 2015) and a threat to native cacti in Florida, Barbados, other Caribbean islands, and Hawaii (Williams and Granara de Willink 1992, German-Ramirez et al. 2014). In its native range, this species does not cause the same level of damage, probably due to the regulatory effect of the rich complex of natural enemies (McFadyen 1979, Claps and de Haro 2001).

This species has been reported from South America, Australia, South Africa, Italy, Spain, Saint Thomas in the U.S. Virgin Islands, Barbados, United States (Florida, California, and Hawaii), and Puerto Rico (McFadyen and Tomley 1978, 1981, Tomley and McFadyen 1984, Hosking et al. 1988, Moran and Zimmermann 1991, Halbert 1996, Klein 1999, Mazzeo et al. 2008, Hodges and Hodges 2009, Zimmermann and Pérez Sandi y Cuen 2010, Beltrà and Soto 2011). Although it is not known how *H. pungens* colonized areas where it was not deliberately released, it is suspected that it was introduced via the trade of ornamental cacti (Zimmermann and Pérez Sandi y Cuen 2010).

Due to the extensive damage caused by *H. pungens* on native cacti in Puerto Rico, the United States Department of Agriculture (USDA) and the Fundación para el Estudio de Especies Invasivas (FuEDEI) initiated a classical biological control program. A survey of the natural enemies of *H. pungens* in its native range (Argentina) on Cactaceae and Amaranthaceae was initiated in November 2010 (Triapitsyn et al. 2014). Observations on the biology of *H. pungens* collected and reared on Amaranthaceae showed some differences with those collected and reared on Cactaceae (Aguirre 2012). For example, *H. pungens* feeds mainly on roots of Amaranthaceae and stems, flowers, buds, and fruits of Cactaceae. We decided to establish a colony of *H. pungens* on Cactaceae with populations collected from Amaranthaceae with several techniques but all failed to establish a culture (Aguirre 2012). Molecular studies conducted with specimens identified as *H. pungens* collected on different host plants in several countries suggested the presence of host races or cryptic species (de León et al. 2012).

The objective of this research was to study the biology of *H. pungens* sensu stricto, and gather information needed to solve the taxonomic identity of this species. Reproductive biology (fecundity, pre-reproductive and reproductive periods, and mode of reproduction), adult longevity, and effect of temperature on the development and survival of nymphs were studied using specimens from the type locality and the original host plant (Trancas, Tucumán, Argentina; *A. pungens*). Survival and development of nymphs on five potential Cactaceae host species was also studied.

## Materials and Methods

The studies were conducted at FuEDEI, Hurlingham, Argentina, between January 2011 and March 2013. An *H. pungens* colony was established with specimens collected in Trancas, Tucumán, Argentina (26°14'12.2"S, 65°16'26.4"W), on the recorded host plant of the type specimen, *A. pungens* (Granara de Willink 1981). For the remainder of this article, these *H. pungens* specimens will be referred to as *H. pungens* sensu stricto. The colony was maintained in the laboratory on potted plants of one of its natural host *Alternanthera paronychioides*, Saint-Hilaire (Amaranthaceae). *A. pungens* was not used as the host plant because it was difficult to grow under laboratory conditions.

Potted test plants were established 3 mo before the experiment to standardize the growth of the plants. Plants were collected in Hurlingham, Buenos Aires, Argentina, transported to the laboratory, and transplanted in pots (50 ml) with standard soil (blend of organic materials, sand, and perlite) in a greenhouse under irrigation. All plants were grown at room temperature ( $25 \pm 4^\circ\text{C}$ ), at 60–80% RH with natural photoperiod.

When the potted plants reached 20 cm in height, they were infested by transferring gravid females placed individually on leaves and stems. To deny access to undesirable mealybug specimens (mealybugs of the same and different species) and natural enemies, each infested plant was placed inside a cylindrical plastic cage (13 cm diameter by 23 cm height). To vent the cages, the lid had a hole (6-cm diameter) covered with a mesh fabric. The cages were maintained in rearing chambers at 25°C, 60–80% RH, and a photoperiod of 16:8 (L:D) h. All experiments were conducted under these conditions except when specified otherwise.

All study (nymphs and adults) was conducted under dissecting microscope (40×), and measurements were made with a 2-mm micrometer.

## Reproductive Biology

### Potential, Realized Fecundity and Sex Ratio

Potential fecundity was estimated by counting the number of mature and immature oocytes produced per female ( $n = 24$ ). Fed and mated females collected from the laboratory colony were fixed in 96% EtOH, approx. 2 wk after their emergence and before they began producing nymphs. Females were not killed and dissected immediately after adult emergence due to the fragility of egg chorion. Female body length was also measured.

Realized fecundity, or the number of nymphs produced per female, was studied on 12 mated gravid females (0–48 h old) reared individually on *A. paronychioides*. Each female was monitored every 3 d to record the number of nymphs produced until female death. The sex ratio was estimated on the offspring of all females which were reared until the third instar when sex could be determined ( $n = 317$  nymphs).

### Pre-Reproductive and Reproductive Periods

Eleven gravid females (48 h old) were transferred individually to *A. paronychioides*. The pre-reproductive period was the number of days between adult emergence and the birth of the first nymph. The reproductive period was the number of days between the first and the last nymphs produced. Mealybugs were checked every 3 d to record the number of nymphs produced until female death.

### Parthenogenesis

In order to verify the occurrence of parthenogenesis in *H. pungens*, first instars were placed individually on a potted *A. paronychioides* plant until the third-instar. Plants with a male nymph were discarded. Plants with a female nymph were kept and checked every other day until adult emergence. Parthenogenesis was confirmed when an unfertilized female produced viable offspring that reached the adult stage. The number of parthenogenetic nymphs produced for each unmated female ( $n = 10$ ) was counted every 3 d. Nymphs were followed until they completed their development. Survival, developmental time, and sex ratio of parthenogenetic offspring were recorded.

### Adult Longevity and Survival

Adult longevity was estimated based on the observations of 12 females and 19 males. Adults (<24 h old and mated) were checked every 2 d (females) or every day (males) until their death. The individuals used in this test were obtained from the first-instar nymphs placed individually on *A. paronychioides* and monitored until they reached adulthood.

### Nymphal Rate of Development and Survival

In order to study the effect of temperature on the development and survival of immature stages, first-instar nymphs were reared at three constant temperatures ( $20 \pm 1$ ,  $27 \pm 1$ , and  $30 \pm 1^\circ\text{C}$ ). For each temperature treatment, a total of 12 nymphs (0–24 h old) were placed on *A. paronychioides*. The nymphs were examined every 3 d until adult emergence. Six replications were carried out for each temperature. The results were modeled and the survival curves were compared.

The lower development threshold ( $z$ ) and the thermal constant ( $K$ ) (number of degree-days needed for full development) were estimated using the temperature summation model, based on the assumption that a straight line was a useful approximation of the relationship between temperature ( $t$ ) and rate of development ( $1/y$ , where  $y$  = time of development) (Andrewartha and Birch 1954). The thermal constant was  $K = y_i(t_i - z)$ , where  $y_i$  represents the number of days required to complete development at temperature  $t_i$ , and  $z$  is the temperature found by extrapolation of the regression, with  $t$  and  $1/y$  as independent and dependent variables, respectively.

### Nymphal Survival and Development on Different Host Plants

Nymphal survival and development were assessed on five cacti hosts (four from Argentina and one from the Caribbean): *Hylocereus undatus* (Haw) Britton and Rose (Zimmermann and Pérez Sandi y Cuen 2010), *Monvillea cavendishii* (Monv.) Britton and Rose (McFadyen 1979), *H. bonplandii*, *Harrisia pomanensis* (F.A.C. Webber ex K. Schum) Britton and Rose (McFadyen 2012), and *Cleistocactus baumannii* (Lemaire) Lemaire (Williams and Granara de Willink 1992, McFadyen 2012) (Table 1). Nymphal survival and development on cacti were compared with those on *A. paronychioides*, used as a control host plant. Nymphal survival and development time were recorded on each host plant.

A single gravid female (<48 h old) was placed individually on a stem section of cacti (15–20-cm long) or in a potted plant of *A. paronychioides*. All plants with the mealybug were introduced

individually in a vented (7-cm diameter covered with mesh fabric) plastic cage of 20-cm diameter by 8-cm height in the case of Cactaceae, or into a cylindrical plastic cage of 13-cm diameter by 23-cm height in the case of Amaranthaceae. For each female–host plant combination, survival was calculated as the number of nymphs that reached the adult stage compared with the initial number of nymphs produced. In addition, the ability to produce viable offspring was tested for the adults ( $F_1$ ) obtained on each host plant species by allowing copulation and monitoring the containers for nymphs ( $F_2$ ) every 3 d for a period of 80 d. Each host plant species treatment was replicated five times.

### Statistical Analyses

A linear regression model was developed between potential fecundity and female body size to determine the percentage of the variation in fecundity that was explained by female size. A Chi-square ( $\chi^2$ ) test was used to determine if the sex ratio of  $F_1$  deviated from 1:1. The survival curves were estimated according to the Kaplan–Meier method (Kaplan and Meier 1958). The survival curves were compared with Cox regression model (Cox 1972). Statistical analyses were performed using Info Stat, R version 2.15.1 (Di Rienzo et al. 2012). The statistical package R Commander of R was used to construct survival curves (Fox 2005). Results were reported as mean  $\pm$  SD.

## Results

### Reproductive Biology

#### Potential, Realized Fecundity, and Sex Ratio

The potential fecundity of *H. pungens* was  $43 \pm 14$  eggs/female, the average body length was  $1.36 \pm 0.28$  mm, and 44% of the variation observed in the potential fecundity of females was explained by their body length ( $F = 17.26$ ,  $df = 23$ ,  $P = 0.0004$ ; Fig. 1). The realized fecundity was  $37 \pm 8$  individuals/female. The sex ratio of *H. pungens* was 1.5:1 ( $\text{♀}:\text{♂}$ ) and was significantly female-biased ( $X^2 = 11.7$ ,  $df = 1$ ,  $P = 0.0006$ ).

#### Pre-Reproductive and Reproductive Periods

The average pre-reproductive period was  $30 \pm 6$  d, and the reproductive period was  $44 \pm 26$  d. At 42 d from the beginning of the reproductive period, 67% of females had produced 100% of the nymphs. The number of nymphs produced every 24 h ranged between 3 and 14.

### Parthenogenesis

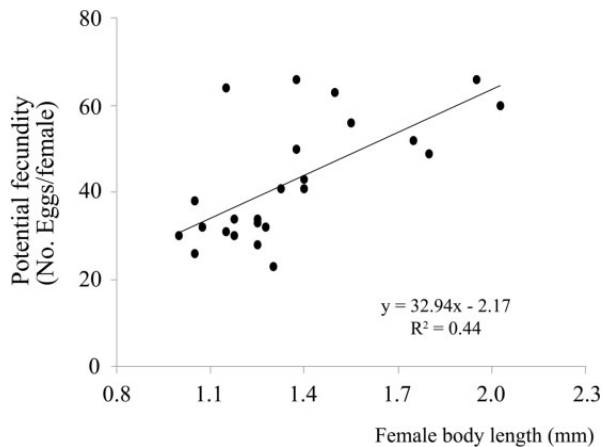
Fifty percent of virgin females of *H. pungens* were able to produce offspring parthenogenetically; females showed deuterotoky parthenogenesis (unfertilized females produced females and males). Survival of the offspring of virgin females was 70% ( $n = 282$ ), and was significantly female-biased ( $5:1 \text{♀}:\text{♂}$ ;  $\chi^2 = 73.8$ ,  $df = 1$ ,  $P = 0.001$ ).

### Adult Longevity and Survival

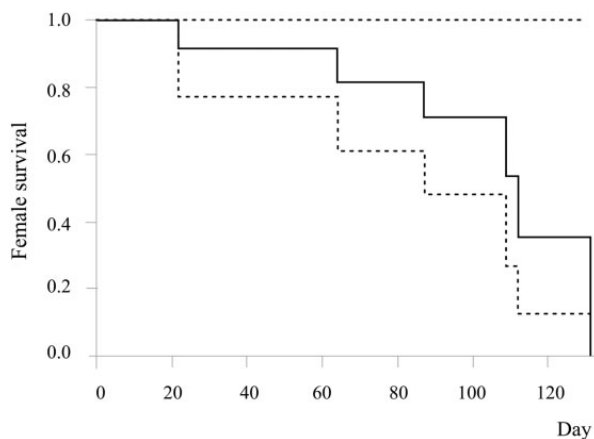
Adult longevity was  $85 \pm 35$  d for females and  $4 \pm 1$  d for males. Females showed type I survival curves, characterized by high survival during early and middle life, followed by a rapid decline toward the end (Fig. 2). In the case of males, a type II curve was observed where a constant mortality rate was observed regardless of age (Fig. 3).

**Table 1.** Nymphal performance of *H. pungens* sensu stricto on its natural host in Argentina, *A. paronychioides* (Amaranthaceae), and five putative hosts of Cactaceae, four native from Argentina, and one from Caribbean.

Host plant	Plant origin	Survival	Development time (days)	F <sub>1</sub> viability	Sex ratio (♀:♂)
<i>A. paronychioides</i>	Argentina	73	29 ± 10	Yes	2.2:1
<i>H. undatus</i>	Caribbean	25	40 ± 18	No	0.6:1
<i>M. cavendishii</i>	Argentina	10	38 ± 9	No	1.4:1
<i>Harrisia bonplandii</i>	Argentina	1	47	No	–
<i>H. pomanensis</i>	Argentina	0	–	–	–
<i>C. baumannii</i>	Argentina	0	–	–	–



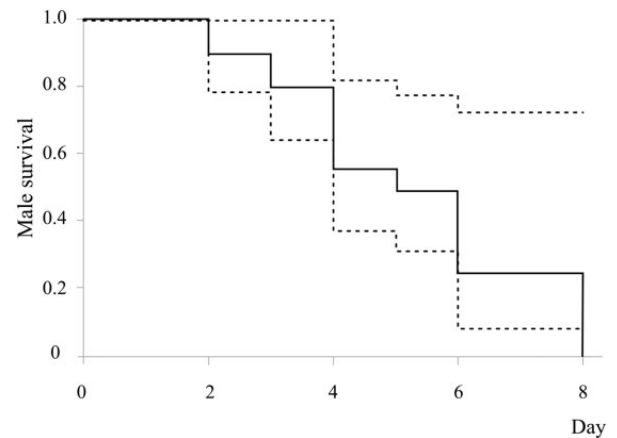
**Fig. 1.** Linear regression of the influence of body length on fecundity of female *H. pungens* sensu stricto ( $Y = 32.94x - 2.17$ ;  $R^2 = 0.44$ ).



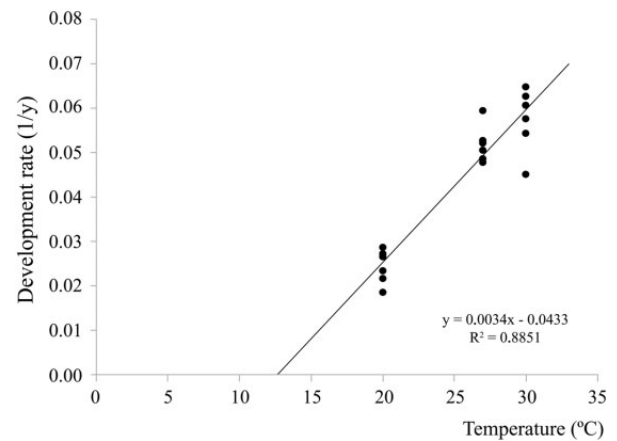
**Fig. 2.** Survivorship curve of *H. pungens* sensu stricto females reared in the laboratory on the host plant *A. paronychioides*. Dashed lines represent 95% confidence levels.

### Nymphal Rate of Development and Survival

The lower developmental threshold of *H. pungens* nymphs was 12.8°C (Fig. 4), and 294 degree-days were needed to complete their development. Nymphs successfully completed their development between 20 and 30°C (Fig. 4). On the other hand, nymph survival was influenced by temperature (Wald Test = 7.04, df = 2,  $P = 0.03$ ). At 20°C nymphal mortality was higher than at 27 or 30°C (Fig. 5). No significant differences in nymphal mortality were observed when reared at 27 or 30°C ( $P > 0.05$ ).



**Fig. 3.** Survivorship curve of *H. pungens* sensu stricto males reared in the laboratory on the host plant *A. paronychioides*. Dashed lines represent 95% confidence levels.



**Fig. 4.** Linear regression of the influence of temperature on development rates of *H. pungens* sensu stricto nymphs ( $Y = 0.0034x - 0.0433$ ;  $R^2 = 0.8851$ ).  $y$ : number of days required to complete development at temperature  $t$ . The  $x$  intercept indicates the lower developmental threshold  $z$ .

### Nymph Survival and Development on Different Host Plants

Nymphal survival and development differed on *H. undatus*, *M. cavendishii*, and *H. bonplandii* (Table 1). On *H. undatus*, 25% of the initial 88 nymphs survived. Three females produced nymphs, but all F<sub>1</sub> offspring died before reaching the adult stage. The survival rate of the 94 nymphs reared on *M. cavendishii* was 10%; only 5 nymphs of the F<sub>1</sub> generation reached the adult stage, but they did not produce viable offspring. All but one nymph reared on *H. bonplandii*



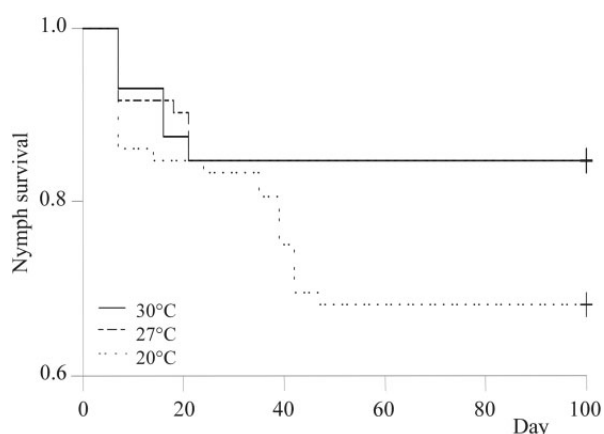


Fig. 5. Survivorship curve of *H. pungens* sensu stricto nymphs reared at three constant temperatures: 20, 27, and 30°C.

died between the first and second instar, living on average  $12 \pm 3$  d (2–30 d). Only one nymph reached the adult stage and it failed to produce offspring. When nymphs were reared on *H. pomanensis*, none of the 102 tested individuals were able to complete development; they lived an average of  $14 \pm 8$  d (3–43 d) and died before reaching the third-instar stage. All 60 nymphs reared on *C. baumannii* died between the first and second instar, and lived  $29 \pm 22$  d (7–85 d). No nymphs reared on *C. baumannii* reached adulthood, although some nymphs lived for 85 d. The only host plant on which nymphs produced viable  $F_1$  offspring was *A. paronychioides*. Of the 159 tested nymphs, 73% survived and reached the adult stage, requiring  $29 \pm 10$  d to complete development (18–49 d).

## Discussion

Our study showed marked differences in biological parameters between populations of *H. pungens* collected on Amaranthaceae and the populations of *H. pungens* studied by McFadyen (1979) on Cactaceae (discussed below).  $F_1$  of *H. pungens* sensu stricto (collected on Amaranthaceae in Argentina), even when they completed development to adulthood, did not produce viable second generation on Cactaceae under laboratory conditions. In addition to the differences in host range demonstrated in this study, “*H. pungens*” populations in Argentina and Puerto Rico also are different in the type of damage and the part of the plant attacked. None of the three cactus-attacking *Hypogeococcus* species in Argentina produce galls (M.B.A. & G.A.L., personal observation), whereas the mealybug pest of cacti in Puerto Rico produce galls (Carrera-Martínez et al. 2015). This suggests that the *Hypogeococcus* pest of cacti in Puerto Rico may be a different species than the “*H. pungens*” in Argentina.

In much of the literature, reports of *H. pungens* were likely misidentified *Hypogeococcus festerianus* (Lizer y Trelles) (Williams 1973, McFadyen 1979, McFadyen and Tomley 1981, Hamon 1984, Suss and Trematerra 1986, Hodges and Hodges 2009). *H. festerianus*, a valid species, is also native to Argentina and restricted to Cactaceae. These two taxa can be clearly distinguished taxonomically; *H. pungens* has three circuli, whereas *H. festerianus* has only one circulus, and *H. festerianus* has dorsal conical setae on the head and thorax, which are absent in *H. pungens* (Williams and Granara de Willink 1992).

According to McFadyen (1979, 2012), the population of *Hypogeococcus* collected from *Harrisia* spp. and *C. baumannii* in the Provinces of Chaco and Formosa, Argentina, and introduced to

Australia for control of *Harrisia* spp., was originally identified as *H. festerianus*, although later was identified as *H. pungens* (Williams and Granara de Willink 1992). The population introduced to Australia developed only on Cactoidea, and has never been found on any other group of plants in the wild, although both Amaranthaceae and Portulacaceae are common in Australia (McFadyen 2012).

Comparing the biology of *H. pungens* sensu stricto, as determined in this study, with that of the population in Australia, strong differences in fecundity, female body length, mode of reproduction, rate of development, and host range were identified. The potential fecundity of females of *H. pungens* sensu stricto was lower than that reported for the population in Australia (McFadyen 1979). Asexual reproduction is common in Pseudococcidae (Miller and Kosztarab 1979, Gavrilov and Trapeznikova 2007), and this study is the first report of parthenogenesis in *H. pungens* sensu stricto. The populations of *H. pungens* on Cactaceae in Australia are not parthenogenic (McFadyen 1979). Females of *H. pungens* sensu stricto showed a facultative parthenogenesis by deuterotoky (Gavrilov and Kuznetsova 2007). In this type of reproduction, unfertilized females produce females and males; eggs may develop either with or without fertilization. Individuals that develop from unfertilized eggs restore diploidy by fusion of the first haploid cleavage nuclei, resulting in complete homozygosity (Nur 1971, Gavrilov and Kuznetsova 2007, Ross et al. 2010). The biased sex ratio found in parthenogenetic reproduction is in agreement with Gavrilov and Trapeznikova (2007) who argued that in species with obligatory sexual reproduction the sex ratio must be close to 1:1, and that deviations from this ratio can point to the probable existence of a parthenogenetic reproduction.

Developmental rate of nymphs of *H. pungens* sensu stricto was greater than that reported by McFadyen (2012) for the nymphs of *H. pungens* breeding on Cactaceae. Nymphs of *H. pungens* sensu stricto did not complete their development below 12.8°C, and required 294 degree-days to complete development. Nymphs of *H. pungens* from Cactaceae have a lower temperature development threshold of 15.3°C, and need 424 degree-days to reach the adult stage (McFadyen 2012). Based on this data, *H. pungens* sensu stricto would have more generations per year than the populations studied by McFadyen (2012) from Cactaceae. Understanding the effects of temperature on the life history of a pest has important implications for its management because it affects the mass rearing of the pest and its natural enemies, and helps in selecting the most appropriate biological control agents, i.e., natural enemies with high intrinsic rates of increase relative to those of the target pest (Huffaker et al. 1976, Chong et al. 2003).

This study found that *H. pungens* sensu stricto did not complete its development and produce viable offspring on Cactaceae. The results of this study reinforced the idea of de León et al. (2012) that *H. pungens* is a complex of species. Our results, strongly supports the idea of McFadyen (2012) that the population introduced in Australia is not *H. pungens*, but we disagree that is *H. festerianus* because the species of *Hypogeococcus* introduced into Australia for biological control purposes has three circuli (*H. festerianus* has one circulus). We propose that it is a new species that share the three circuli with *H. pungens*, and the specificity on cactus with *H. festerianus*. At present, additional biological, taxonomic, and molecular studies are being conducted with populations of *H. pungens* on different host plants and from different locations, including Argentina, Australia, and Caribbean Islands to clarify the identities of *H. pungens* complex components, in particular the pest species of Puerto Rico.

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