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Influence of different plants substrates on development and reproduction for laboratory rearing of *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae)

Tania Zaviezo[1], Elizabeth Cadena[1], M. Fernanda Flores[2], and Jan Bergmann[2]
[1]Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Casilla 306-22, Santiago, Chile.
[2]Instituto de Química, Pontificia Universidad Católica de Valparaíso, Avda. Brasil 2950, Valparaíso, Chile.

Abstract

T. Zaviezo, E. Cadena, M. F. Flores, and J. Bergmann. 2010. Influence of different plants substrates on development and reproduction for laboratory rearing of *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae). Cien. Inv. Agr. 37(3): 31-37. The citrophilus mealybug, *Pseudococcus calceolariae*, is a polyphagous pest that has a major impact on fruit crops in central Chile, and is of quarantine importance for many markets. To study many control alternatives, it is important to develop efficient rearing protocols. The objective of this work was to determine mealybug development on three different plant substrates: sprouted potatoes, lemon fruits and Butternut squash. Insects were inoculated on the substrates, maintained at 25°C and in total darkness until completing their development. Every one to 4 days, advancement on the development was checked by counting the exuvia. Mean developmental time for each stage, adult longevity, fecundity and fertility were determined. Preimaginal developmental time was similar for females and males, but it differed among plant substrates. For females, preimaginal development was about nine days shorter on potatoes, as compared to butternut squash or lemons, and for males about 12 days longer in squash than on the other two substrates. The preoviposition period was significantly longer in squash and consequently, a longer generational time resulted. Female adult longevity was similar in all substrates, around 31 days, and for males it was 6 days in potatoes and lemons and 4 days on squash. Female fecundity was similar in potatoes and squash, and lower on lemons. Egg fertility was significantly higher on potatoes. Parthenogenetic reproduction was not observed. Therefore, the three substrates were adequate for rearing *P. calceolariae*, but the shortest developmental time and highest fecundity and fertility were obtained on sprouted potatoes.

Key words: Developmental time, mealybug, rearing substrates.

Introduction

The citrophilus mealybug *Pseudococcus calceolariae* (Maskell) is a polyphagous pest species that has a major impact on fruit crops in central Chile, including avocado (*Persea americana* Mill.), lemon (*Citrus limon* (L.) Burm.f.), orange (*Citrus sinensis* Osbeck), tangerine (*Citrus reticulata* Blanco), and grapefruit (*Citrus paradisi* Macfad.), to name the most important (Artigas, 1994; Ripa and Larral, 2008). *Pseudococcus calceolariae* is a cosmopolitan species, and possible native to east Australia (Williams, 1985; Wil-

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Corresponding author: tzaviezo@uc.cl

liams and Granara de Willink, 1992). It was detected for the first time in Chile around 1925, and its area of distribution in the country ranges now from the extreme north (Arica) to the region of the Araucanía, in southern Chile (Artigas, 1994).

Plant damage is inflicted by *P. calceolariae* due to its piercing-sucking feeding habit; during feeding the mealybugs inject toxic saliva into the host plant, leading to a change in color and malformation of fruits, leaves, or shoots. Furthermore, they excrete a viscous sweet substance, called honeydew, which may cover considerable areas of the plant and is used as a substrate by various fungi, developing as a sooty mold on the leaves and fruits, and thus compromising fruits commercialization. The feeding along with the sooty mold on leaves, which lowers the photosynthetic ability of the plant, finally affects plant growth and fruit production. The most severe economic losses, however, are due to quarantine restrictions imposed by several countries on products infested with mealybugs (Artigas, 1994; Ripa and Larral, 2008).

Mass-rearing of mealybugs is done for several reasons, e.g. to study the developmental biology (e.g. Arai, 1996; Walton and Pringle, 2005; Amarasekare *et al*., 2008), their susceptibility to pesticides, or for molecular studies (e.g. Beuning *et al*., 1998). Because mealybugs are not easily controlled by pesticides, mass-rearing has also been developed to study and implement alternative control methods, like rearing of natural enemies for augmentative biological control (e.g. Roltsch, 2002; Serrano and Lapointe, 2002), or to identify sex pheromone (e.g. Bierl-Leonhardt *et al*., 1981). Several substrates have been used to rear mealybugs in order to have faster development and higher reproduction rate, such as sprouted potatoes (*Solanum tuberosum* L.) (Rotundo *et al*., 1977; Bierl-Leonhardt *et al*., 1981; Chong *et al*., 2008), Japanese pumpkins or butternut squash (*Cucurbita moschata* Duch) (Arai, 2002; Zhang *et al*., 2004; Millar *et al*., 2005), germinated broad beans (*Vicia faba* L.) (Murai *et al*., 2001), lemons and oranges (Wakgari and Giliomee, 2003), or meridic diets based on canned pumpkins (Serrano and Lapointe, 2002). An ideal substrate should favor mealybug development, with high survival and reproduction rate, but it should also be inexpensive, constantly available in the market and easy to manage. Other factors to be considered when choosing the substrate depend of the rearing objectives, for example when it will be used to collect pheromone emitted by mealybugs, adult longevity, high fecundity and fertility, and a low background of plant volatiles are of importance.

In the present work, the developmental time and reproduction parameters of *P. calceolariae* on sprouted potatoes, butternut squash, and lemons were investigated since there is no detailed information on the development of the different stages of this species on these substrates. The first two were included because they are commonly used for rearing mealybugs, and the latter is a natural host plant and its fruit is readily available and easy to handle.

Materials and methods

Insects

Mealybugs were collected in February 2008 from shoots, fruits, leaves, and stems of organically managed *Rubus idaeus* (raspberry cv. Heritage) in Nogales and from shoots, fruits, and leaves of *Citrus limon* (lemon cv. Eureka) and *Citrus reticulate* (mandarin cv. Clementina) in Olmué in the Valparaíso region in central Chile. In the laboratory, adult females and ovisacs were transferred to *Cucurbita moschata* (squash cv. Butternut) and reared for one generation before starting the experiments.

Identification of species and phenotype

Insects were identified using the USDA key for females (Miller *et al*., 2007). For identification of the phenotype, the following characters were determined according to Charles *et al*. (2000): length of tibia plus tarsus of hindlegs, combined length of trochanter and femur of hindlegs, ratio hind tibia:tarsus and number of oral rim tubular ducts. Distances were measured by calibrating digital photographs taken

at a magnification of 20x (Micropublisher 3.3 RTV, Qimaging, Burnaby, BC, Canada) with a known standard (100 um = 252 pixels), using the software Image J 1.40 (http://rsb.info.nih.gov/ij/index.html).

Developmental times and longevity

The plant substrates used were: *Solanum tuberosum* (L.) (sprouted potatoes), fruits of *Citrus limon* (Blanco) (lemon cv. Eureka), and fruits of *Cucurbita moschata* (D.) (squash cv. Butternut). The fruits and potatoes were treated with sodium hypochlorite solution before use. For potatoes and lemons three ovisacs per substrate (n = 8) from three different females were transferred to each substrate with a camel hair brush. For the case of squash, either 4 ovisacs per fruit or newly emerged nymphs were used (n = 5) since establishment directly from eggs was not always successful. Once the insects were transferred, each fruit was maintained individually in sealed plastic containers at 25.2° ± 1° C and 60 ± 5% RH in an environmental chamber (BIOREF, model VV-11, P+L Electrónica S.A., Santiago, Chile) in total darkness, to avoid nymph escape. Temperature and humidity inside the chamber were checked with a data logger HOBO H8 (Onset Computer Corporation).

Mealybug populations were checked every 24 to 96 hours until completion of a generation, and the development was observed by removing the exuviae after each molt. In some cases, microscopic preparations were necessary to distinguish between 3rd instar nymphs and adult females. For each replicate, the average time needed for the population to reach each stadium and complete the preimaginal phase (egg to 3rd instar nymph in females and egg to pupa in males) was recorded, as well as the generational time (egg to oviposition) and the mean longevity of adults. The developmental time on the different substrates was analyzed by ANOVA and means were separated by Tukey test.

Reproduction parameters

To determine the influence of the different substrates on reproductive parameters such as fecundity and egg fertility, twenty females with their ovisac were placed individually on a filter paper (4 cm diameter) within a sealed Petri dish, which was maintained in the same conditions mentioned above (25.2° ± 1° C; 60 ± 5% RH; complete darkness). Individuals were checked twice each day and duration of the oviposition period, total number of eggs and number of eclosed eggs were recorded. To study the possible existence of parthenogenetic reproduction, virgin females were transferred to sprouted potatoes, and were checked daily during 45 days for oviposition.

The effect of rearing substrate on female fecundity and egg fertility was analyzed by ANOVA, and means were separated by Tukey test. Fertility data were transformed to arcsine square of the percentage prior to analysis.

Results and discussion

Identification of species and phenotype

The morphological analysis revealed that the phenotype of *P. calceolariae* used in this study corresponds to the most common one present in Australia and New Zealand, as the four parameters measured were well within the range reported for this phenotype (Cox, 1987; Williams, 1985), and were different from the phenotype described originally as *P. similans* and later synonymized with *P. calceolariae* (Charles *et al*., 2000) (Table 1).

Developmental times and longevity

Preimaginal developmental time was similar for females and males on all substrates (ANOVA, $P > 0.1$ for all substrates), but it differed among plant substrates. For females, preimaginal development was about nine days shorter on potatoes, as compared to butternut squash or lemons, and for males about 12 days longer in squash than on the other two substrates ($P < 0.001$; Table 2). The longer total developmental time for immature on squash can be explained mainly by differences on second instar nymphs, and additionally for males by the longer dura-

Table 1. Comparison of differentiating characters for the population collected in Chile and those from New Zealand and Australia.

Character	*P. calceolariae*[1] (n=10)		*P. calceolariae*[2]		*P. similans*[2]
Combined length of hind trochanter + femur (mm)	0.45 ± 0.01	Au:	0.37 – 0.47	Au:	0.23 – 0.36
		NZ:	0.38 – 0.47	NZ:	0.24 – 0.40
Combined length of tibia + tarsus (mm)	0.44 ± 0.03	Au	0.41 – 0.49	Au	0.30 – 0.40
		NZ:	0.42 – 0.53	NZ:	0.28 – 0.43
Ratio hind tibia: tarsus	2.35 ± 0.27 : 1	Au:	2.14 – 3.4 : 1	Au:	1.5 – 2.3 : 1
Number of oral rim tubular ducts	32 ± 0	NZ:	21 – 55	NZ:	0 – 31

[1]Insects collected in the Valparaíso Region, Chile. Means followed by SEM (standard error mean).
[2]Insects from New Zealand (NZ) and Australia (Au), according to Cox (1987) and Williams (1985).

tion of the pupal stage, which was almost twice than on the other two substrates. Preoviposition period was also significantly longer in squash than on the other substrates and consequently, a longer generational time (preimaginal development plus preoviposition period), which varied between 49 and 71 days at 25 °C, depending on the plant substrate (Table 3). Generational time was eight days shorter on sprouted potatoes than in lemons, but this is not significant (Table 3). Wakgari and Giliomee (2003) for populations from South Africa found similar total generational time for squash and lemons at 27 ºC (36-44 vs 37-44), which differs from our results, where development was slower on squash. Sprouted potatoes were not included in the cited study. Generational times reported for other species reared in different substrates are in general shorter than those obtained for *P. calceolariae* here. This is the case for *Paracoccus marginatus* (24.4 – 25.5 days at 25 ºC, depending on the substrate; Amarasekare et al., 2008), *Planococcus ficus* (28.1 days at 25 ºC, reared on grapevines; Walton and Pringle, 2005), *Maconellicoccus hirsutus* (32.4 days at 25 ºC, reared on hibiscus cuttings; Chong *et al.*, 2008) and *Planococcus kraunhiae* (37.8 days reared on broad bean seeds; Narai and Murai, 2002).

Adult longevity was around 31 days for females and 6 days for males. In the case of females, it was independent of the substrate, but males reared on squash lived less than the ones reared on potatoes (Table 3). Longevity determined for *P. calceolariae* was greater than the ones reported for other species. For example, in *M. hirsutus* female longevity was 21 days and male 2.5 days; when reared between 25 and 30 °C (Chong *et al*., 2008), and it was 25 days for *P. kraunhiae* females, independent of substrate, at 24 °C (Narai and Murai, 2002).

Reproduction

Female fecundity did not differ between potatoes and squash, but was about half on lemons (Table 4, $P \leq 0.001$). Egg fertility, measured as percent of egg eclosion was around 85% for females reared on lemons and squash, and significantly higher on potatoes, around 92% (Table 4, $P \leq 0.001$). These results partially agree with those obtained by Wakgari and Giliomee (2003), who found significantly higher fecundity in squash than in lemons and no differences in fertility. However, their values were much larger (i.e. 417 and 239 eggs per female respectively, with 90% fertility, at 27 ºC) and variable than what was found in this study. Our results further indicate that both reproduction parameters are significantly higher on sprouted potatoes, a substrate which was not evaluated in the mentioned study. Also, higher fecundity values have been reported for *P. ficus* reared on grapevines (297 eggs per female at 24 ºC; Walton and Pringle, 2005) and *M. hirsutus* reared on hibiscus cuttings (300 eggs

Table 2. Mean developmental time (days ± SEM) for each pre-imaginal stadium and cumulative preimaginal developmental time of *P. calceolariae,* reared on three host species at 25 º C and 60% relative humidity.

	Developmental time (days ± SEM)[1]					Pre-imaginal development	
Host	Egg	First	Second	Third female[2]	Pupal male[3]	Female	Male
Lemon	11.6 ± 0.7a	13.1 ± 0.6a	8.0 ± 0.5b	14.6 ± 0.6a	11.3 ± 0.8a	47.4 ± 1.4b	44.0 ± 1.8a
Potatoes	8.8 ± 0.7a	11.9 ± 0.4a	6.4 ± 0.4a	12.8 ± 0.7a	12.3 ± 0.4a	39.8 ± 1.2a	39.4 ± 1.0a
Squash	9.6 ± 1.4a	13.5 ± 1.3a	14.2 ± 0.6b	13.4 ± 0.6a	20.4 ± 1.2b	50.7 ± 2.1b	57.7 ± 3.3b
P	< 0.060	0.31	<0.001	0.12	<0.001	<0.001	<0.001

[1]Means followed by the same letter are not significantly different by ANOVA and Tukey (P ≤ 0.05). SEM = standard error mean.
[2]Females develop to adults by going through egg stage, 1st larval stage, 2nd larval stage and 3rd larval stage.
[3]Males develop to adults by going through egg stage, 1st larval stage, 2nd larval stage, and two pupal stages.

Table 3. Pre-oviposition period, generational time and longevity (days ± SEM) for *P. calceolariae* reared on three plant substrates at 25 º C y 60% relative humidity.

			Longevity	
Host	Preoviposition period	Generational time[1]	Females	Males
Lemon	9.3 ± 0.6a	56.6 ± 1.8a	31.3 ± 1.2a	5.6 ± 0.4ab
Potatoes	8.8 ± 0.8a	48.6 ± 1.7a	29.8 ± 1.4a	6.6 ± 0.5b
Squash	20.4 ± 0.8b	71.1 ± 1.9b	31.1 ± 1.5a	4.4 ± 0.2a
P	<0.001	<0.001	0.69	0.01

Means followed by the same letter are not significantly different by ANOVA and Tukey (P ≤ 0.05). SEM = standard error mean.
[1]from hatch to oviposition.

per female at 25 ºC; Chong *et al*., 2008). Female *P. kraunhiae* reared on broad bean seeds laid an average of 965 eggs per female at 24 ºC (Narai and Murai, 2002). Fertility values reported for other species are lower than those obtained for *P. calceolariae* in this study and include, for example, 72 % for *M. hirsutus* on hibiscus cuttings at 25 ºC (Chong *et al*., 2008) and 82.2 – 83.5 % for *P. marginatus* on different substrates at 25 ºC (Amarasekare *et al*., 2008).

Parthenogenetic reproduction was not observed after a period of 30 days, and virgin females died without having laid eggs. Only the production of one ovisac was observed, but it did not contain any eggs. Parthenogenesis has been described for other species, e. g. *Phenacoccus solanis* (Ferris) (Ben-Dov, 2005a), *Phenacoccus bengalensis* (Pramanik & Ghose) (Mishra *et al*., 1999), or *Ferrisia malvastra* (McDaniel) (Ben-Dov, 2005b).

Table 4. *Pseudococcus calceolariae* reproduction parameters reared on different plant substrates (mean ± SEM) 25 º C and 60% relative humidity.

Host	Fecundity (number of eggs)	Fertility (% hatch)
Lemon	92.8 ± 2.1 b	85 ± 0.5 b
Potatoes	191.3 ± 13.2 a	92 ± 0.7 a
Squash	202.5 ± 8.9 a	86 ± 8.9 b
P	<0.0001	<0.0001

Means followed by the same letter are not significantly different by ANOVA and Tukey (P < 0.05). SEM = standard error mean.

In summary, our results indicate that all three substrates evaluated in this study are adequate for rearing *P. calceolariae*. However, the shortest developmental time and highest female fecundity and egg fertility were obtained when using sprouted potatoes, which is therefore a better rearing substrate than lemons or butternut squash.

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Resumen

T. Zaviezo, E. Cadena, M. F. Flores y J. Bergmann. 2010. Influencia de diferentes sustratos de plantas en el desarrollo y reproducción para la crianza en laboratorio de *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae). Cien. Inv. Agr. 37(3): 31-37. El chanchito blanco citrófilo, *Pseudococcus calceolariae*, es una plaga polífaga de gran impacto en frutales en Chile, y además tienen importancia cuarentenaria en mercados de destino. Para estudiar alternativas de control es importante contar con métodos de crianza eficientes. El objetivo de este trabajo fue determinar el desarrollo de Pseudococcus calceolariae en tres sustratos: papas etioladas, frutos de limón y zapallo Butternut. Los insectos fueron inoculados en los sustratos y mantenidos a 25°C en oscuridad hasta completar su desarrollo. El avance en el desarrollo poblacional fue medido contando los exuvios cada uno a cuatro días. Se determinó el tiempo de desarrollo promedio para cada estado, longevidad de adultos, fertilidad y fecundidad de hembras. El tiempo de desarrollo preimaginal fue similar para hembras y machos, pero difirió entre sustratos. En hembras el desarrollo preimaginal fue nueve días menos en papas en comparación con zapallos y limones. En machos fue 12 días más en zapallos que en los otros sustratos. El período de preoviposición y tiempo generacional fue más largo en zapallos. La longevidad de hembras adultas fue similar en los sustratos, cerca de 31 días, pero para machos fue de seis días en papas y limones y cuatro en zapallo. La fecundidad de las hembras fue mayor en papas y zapallos que en limones. No se observó reproducción por partenogénesis. Por lo tanto, los tres sustratos probados son adecuados para criar *P. calceolariae*, pero en papas etioladas se obtienen los tiempos generacionales más cortos y la mayor fecundidad y fertilidad de hembras.

Palabras claves: Chanchitos blancos, sustratos de crianza, tiempo de desarrollo.

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