academicJournals

Vol. 12(23), pp. 3609-3614, 5 June, 2013
DOI: 10.5897/AJB2013.12311
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Review

Practical problems and their solutions in studying the biology of the mealybug *Paracoccus burnerae* (Brain) (Hemiptera: Pseudococcidae)

T. Johnson* and J. H. Giliomee

Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland, 7602 South Africa.

Accepted 5 June, 2013

Mealybugs are among the most widespread and important pests of plants in commercial glasshouses and conservatories. They are also important quarantine pests that hinder international trade of fruits, vegetables and ornamental plants. Studies on their biology are important because they attack a wide range of plants and are generally difficult to control. Some of the many problems generally encountered in studying their biology are: obtaining live specimens of the mealybugs, establishing the culture, keeping them isolated from other greenhouse plants, risks of contamination from natural enemies, other mealybug species and diseases in the greenhouse, feeding the mealybugs for prolonged periods in incubators, discolouration and rotting of rearing substrates, maintaining the right humidity inside the incubators, obtaining enough material from the field for observations over a 12 month period, obtaining their natural enemies from orchards regularly sprayed with insecticides, and combating vandalism in study orchards. Researchers often present impressive results of their studies on the biology of mealybugs and other scale insects without mentioning the problems they encountered and had to solve. In this paper, the practical problems encountered during a study of the biology of the oleander mealybug, *Paracoccus burnerae* (Brain), an endemic pest of citrus in South Africa, are discussed and solutions offered.

Key words: *Paracoccus burnerae*, citrus, natural enemies, development, humidity, insect culture, parasitoids.

INTRODUCTION

The oleander mealybug, *Paracoccus burnerae* (Brain) (Pseudococcidae), is a species mostly found in the southern part of the Afrotropical Region, although, it has also been recorded from India (Ben-Dov et al., 2012). It feeds on a wide variety of plants, but in South Africa, it is mainly known as a pest of citrus. South Africa exports citrus fruits to many countries of the world and some of them, notably the USA, China and South Korea, regard this species as a quarantine pest (Wakgari and Giliomee, 2003; Pieterse et al., 2010). Since very little is known about the ecology of this species, laboratory and field studies were conducted to study its developmental biology and to determine its natural enemies. In this paper, the practical problems experienced during this study are discussed as well as the technologies developed to solve them. This is an aspect seldom addressed by other workers. However, brief references to the technologies used by other workers in studies on the biology of mealybugs are included. Several workers have given accounts on the rearing of mealybugs. Their studies differ largely in their use of lighting and maintenance of humidity levels. Lighting varied from constant

*Corresponding author. E-mail: jhg@sun.ac.za.

Figure 1. Parasitoid emergence boxes containing mealybug infested plants exposed to parasitoids for three weeks. Parasitoids are attracted to a light source shining on the emergence funnels and are collected in a tube attached to each funnel, closed off with cotton wool.

light to constant darkness (Chong et al., 2008; Serrano and Lapointe, 2002) while relative humidity (r.h.) varied from 40.5% (Vennila et al., 2010), 60% (Sagarra et al., 2001; Johnson and Giliomee, 2010; Polat et al., 2008), 65% (Amarasekare et al., 2008), 70% (Goldasteh et al., 2009), 80% (Calatayud et al., 1998), 90% (Walton and Pringle, 2005; Chong and Oetting, 2007) to 92.5% (Vennila et al., 2010). Workers such as Arai (1996), Wakgari and Giliomee (2003), Laflin and Parrella (2004) and Chong et al. (2008) reared mealybugs at constant and varying temperatures.

For the rearing of mealybugs, the natural host plants can be used. Thus, Arai (1996) reared three mealybug species, namely *Pseudococcus citriculus* (Green), *Planococcus citri* (Risso), and *Planococcus kraunhiae* (Kuwana) on citrus, while Wakgari and Giliomee (2003) reared *Planococcus citri*, *Pseudococcus calceolariae* (Maskell) and *Pseudococcus longispinius* (Targioni Tozzetti) on fresh lemons. However, when mass rearing is required, as for biological control strategies, alternative hosts that are easier to maintain are used. Examples of such alternative hosts are the Japanese pumpkin (Serrano and Lapointe, 2002), potato sprouts (Sagarra et al., 2001; Daane et al., 2004; Kontodimas et al., 2004; Karamaouna and Copland, 2009; Zaviezo et al., 2010) and butternut (Daane et al., 2004; Wakgari and Giliomee, 2003; Mudavanhu, 2009). In the past, Japanese pumpkin and potato sprouts were the preferred hosts for rearing the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green). However, seasonal shortages and the difficulties of maintaining continuous supplies led to increased production costs. To reduce the costs of producing and maintaining potato sprouts and Japanese pumpkin, Narai and Murai (2002) developed a new rearing method using broad bean seeds as an alternative food source for the Japanese mealybug *P. kraunhiae* (Kuwana).

In these studies, the methods of previous workers were used where applicable, but new technologies had to be devised to overcome the specific problems encountered.

SPECIMEN COLLECTION

It is difficult to obtain specimens of this species for study purposes because their numbers are kept low in commercial orchards through the application of insecticides. They only become conspicuous on mature fruit during the time when sprays are no longer applied. In order to expedite the study, a laboratory colony was searched for and located. The natural enemies of *P. burnerae* are even more difficult to obtain than their hosts. The chances of finding them in a commercial orchard are remote as the level of parasitism is very low where insecticides are being applied. This problem was solved when a situation was found where a farmer's house and garden were only some 30 m away from his commercial orchards that were known to have had problems with *P. burnerae* infestations. Two or three plants infested with various stages of the mealybug in a greenhouse were placed in the farmer's garden where they were looked after by his gardener. The mealybugs were exposed for two weeks every month over a period of one year (except mid-winter) and brought back to the laboratory for possible parasitoid emergence. Mealybug infested plants were also hid in an abandoned citrus orchard, where they had to be watered during the hot and dry summer months, and returned to the laboratory after two weeks' exposure to parasitoids.

To obtain the parasitoids, parasitized mealybugs are usually kept on twigs or leaves in dark containers and the emerging parasitoids attracted by a light source through a funnel into glass vials, closed off with cotton wool. In this case, the host trees were about 1 m high and therefore, boxes were obtained in which refrigerators were packed from a furniture dealer (Figure 1). A variety of measures were used by other workers to obtain parasitoids of mealybugs. Summy et al. (1986) used an aspirator to collect adult parasitoids from sleeve–cage cultures of various parasitoids and released them onto greenhouse plants infested with *P. citri.* The resulting mummified mealybugs were held for four to five weeks in cardboard containers for adult parasitoid emergence and the relative

abundance of the species determined. Goolsby et al. (2002) used corrugated cardboard bands around the trunks of hibiscus to collect adult females of *M. hirsutus* and placed them in cans, streaked with honey as food, for the parasitoids to emerge. Mudavanhu (2009) collected natural enemies of *Pseudococcus Viburni* (Signoret) by using yellow delta sticky traps baited with the mealybug's pheromone. He also collected live females of *P. viburni* and mummies during the seasonal monitoring process in all study areas and isolated them in individual gelatine capsules for parasitoid emergence. Karamaouna and Copland (2009) first presented adult mealybugs in Petri dishes to freshly emerged female parasitoids which they assumed had mated. When the hosts had mummified, they were transferred into 0.5 ml Eppendorf tubes and kept until parasitoid offspring emerged.

Roltsch (2010) collected parasitoids of *M. hirsutus* using a standard aspirator attached to a vacuum pump station. In order to minimize injury to parasitoids, he used a piece of foam on the bottom of the collecting vial.

ISOLATION OF THE CULTURE

P. burnerae was reared on young citrus seedlings to the greenhouse of the Department of Botany and Zoology at the University of Stellenbosch. Young, 1 m tall, citrus plants in black bags with young, lush growth and no infestation of any kind were acquired from a nursery and replanted into pots. In the greenhouse, they were then infested with mealybugs from the laboratory colony by placing infested leaves on the new plants. Later, it was considered unnecessary to replant them from the black bags, as they were continuously replaced. To prevent the mealybugs from spreading, we placed the pots on brick islands in big plastic buckets with water. The buckets with the plants were placed on a dedicated and separate shelf in the middle of the greenhouse. Towards the end of the study, plants of other projects in the greenhouse did get infested, so they were sprayed with an appropriate insecticide (chlorpyrifos). The plants in the greenhouse were well isolated from potential infectious insects outside, but towards the end of the study, infestations with the greenhouse whitefly; *Trialeurodes vaporariorum* (Westwood), the woolly whitefly, *Aleurothrixus floccosus* (Maskell), the citrus psylla, *Trioza erytreae* (Del Guercio), and an unidentified coccinellid were experienced. The larvae of the latter could be killed by hand, while the others were controlled by treating infested leaves with an insecticide and a local dishwashing liquid from a hand held sprayer. Polat et al. (2008) isolated their culture of *P. citri* by placing an ovisac in a Petri dish with a leaf of each of the four host plants on several layers of damp filter paper.

When the eggs hatched, crawlers were transferred onto fresh leaves in other Petri dishes using a camel hair brush. Madavanhu (2009) used barriers of petroleum jelly around individuals of *P. viburni* on trunks of potted apple trees. Venilla et al. (2010) also isolated their culture of the cotton mealybug, *P. solenopsis* (Tinsley) by rearing individuals on cotton leaves in Petri dishes.

REARING MEASURES

One of the aims was to study the development of *P. burnerae* on citrus at different constant temperatures in incubators (Johnson and Giliomee, 2011a). For the studies at different temperatures to run concurrently, several incubators are required and were at our disposal. Keeping plants in the limited space of the incubators presented a problem. This was solved by obtaining very young seedlings, about 10 cm tall, from a nursery and planting them in small plastic bottles (12 cm high, 6 cm wide) with holes in the bottom for drainage, a method suggested by W. Pieterse (pers. com.). With the plants in small plastic bottles, it was easy to follow the development of each individual mealybug under the stereomicroscope. The mealybugs did very well under a light: darkness regime of 16L: 8D. The seedlings were infested by placing mature females on the leaves for oviposition, removing them later together with excess eggs. The next problem was the lack of humidity control in the incubators. It was clear that the ambient humidity in the incubators was too low for the mealybugs to thrive. Various measures were tried to increase the humidity, and the most successful method was placing the bottles with seedlings on three soaked, brick shaped, so-called oases used by florists on a plastic tray in the incubator. This increased the humidity between 60 and 90%, which was adequate for normal development of the mealybugs.

In addition to rearing *P. burnerae* on citrus, the use of alternative hosts that are long lasting and easy to maintain, and thus more suitable for mass rearing than citrus, was investigated. Sprouting potatoes lasted slightly over three months and were the preferred host for mass rearing of this species at constant temperatures (Johnson and Giliomee, 2011b). The use of these alternative food sources involves the risk of the source being contaminated with other insects and diseases. To prevent contamination, especially by fungi, researchers have devised methods such as soaking of the food source in sodium hypochlorite or bleach and rinsing them with water (Addis, 2005; Daane, 2004; Mudavanhu, 2009). Wakgari and Giliomee (2003), washed butternuts in benomyl wettable powder solution before infestation with mealybugs to prevent rotting and discourage fungal growth. In this study, a broad spectrum fungicide was applied at the two points where pieces of wire were inserted onto the substrate to turn it around easily and without squashing the mealybugs. Karamaouna and Copland (2000) maintained their primary mealybug culture of *P. viburni* on potato sprouts in plastic sandwich boxes with net covered openings for ventilation in a rear-

ing room at 26±1°C, 50 to 65% RH, L16: D8 photoperiod light intensity.

Sagarra et al. (2001) reared *M. hirsutus* on potato sprouts in nylon mesh cages supported on steel wire frames. The mealybug cultures were kept in the dark at 27±2°C. Mudavanhu (2009) kept a stock culture of the mealybug, *P. viburni* on butternuts in wooden rearing bins. Nylon sleeves were used to wrap the butternuts so as to allow the mealybugs to clutch onto the butternuts and enhance colony establishment. The butternuts were placed onto mini wooden stakes in such a way that they were not resting directly on the surface floor of the wooden rearing bin.

This stopped the butternuts from rolling around and squashing the delicate mealybugs during routine maintenance. The mini wooden stakes also allowed the circulation of air around each butternut. The wooden rearing bin had an access lid tightly secured by petroleum jelly to prevent entry of ants and infestation by parasitoids as well as other insects.

The butternuts with their mealybugs were stored and reared at room temperature of 25±1°C, with an average 16L: 8D photoperiod throughout the year. Santa-Cecília et al. (2008) reared *P. citri* in PVC clip cages attached to leaves of coffee plants and on leaf sections placed over an agar film layer.

TRANSFERRING MEALYBUGS ONTO NEW HOSTS

Transferring mealybug eggs or newly hatched crawlers onto a new host is always tricky because of their delicate nature. To overcome this, freshly laid eggs were not transferred from one leaf to another, but rather a female with the ovisac. The ovisac was then opened with a dissecting pin and the eggs spread out on the same leaf. Thereafter, the ovipositing female was removed. Goldasteh et al. (2009) individually transferred newly laid eggs of the citrus mealybug, *P. citri* to detached coleus leaves.

Chong and Oetting (2007) conducted no-choice experiments on the parasitoid of the Madeira mealybug, *Phenacoccus madeirensis* (Green), by transferring experimental mealybugs onto excised coleus leaves that were kept alive by pushing the petioles through holes drilled at the bottom of petri dishes into cups of water.

OBSERVING MEALYBUGS UNDER FIELD CONDITIONS

The development of *P. burnerae* on citrus was also studied under field conditions. That is impossible in commercial orchards where numbers of insects are kept low with insecticides or biological control measures. Permission was obtained from the owner of an abandoned orchard to infest the trees with *P. burnerae.*

Merely placing infested seedlings in the trees did not result in a visible infestation, for reasons that were not clear. It may have been due to the effect of natural enemies, the condition of the neglected trees or climatic conditions.

In order to facilitate establishment of the mealybugs, infested leaves were placed on some 20 young branches from different trees and covered with sleeve cages to exclude natural enemies. This proved successful, but unfortunately the sleeve cages were conspicuous and many of them were vandalized by inhabitants of nearby cottages, farm workers or passersby. The solution to this problem was to increase the number and scatter them widely in the orchard. As a result, a sufficient number remained intact from which data could be collected. Other workers used a variety of methods to study field populations of mealybugs. De Bach (1949) found that populations of the long-tailed mealybug, *P. longispinus*, on citrus could be sampled by wrapping corrugated cardboard bands around the trunk of host trees. The mealybugs would seek shelter here when migrating from leaves and fruit to the trunk.

In studying the distribution of the root mealybug, *Cataenococcus ensete* (Williams and Matile-Ferrero) on the root system of enset plants, Addis (2005) randomly selected and assessed ten three year old plants in farmer's fields. He dug out cubes of soil and roots with a size of 20 × 20 × 20 cm starting from the area adjacent to the corm up to a distance of 80 cm from the corm and up to a depth of 100 cm. The roots were carefully separated from the soil, and roots and mealybug numbers counted for each of the 20 cubes.

Wakgari and Giliomee (2003) studied the phenology of three mealybug species on citrus by taking two-weekly samples of infested plant parts. Sampling units consisted of two infested twigs, 40 cm long and bearing at least two infested fruits. Grasswitz and James (2008) studied the movement of individuals of the grape mealybug, *Pseudococcus maritimus* (Ehrhorn) in a commercial vineyard. They chose pairs of contiguous shoots from a number of adjacent plants, with one shoot being designated as the 'donor' (to be infested), and the other as the 'recipient'. Eggs were placed on the basal pair of leaves of each donor shoot and left to hatch *in situ*. Numbers of live mealybugs on each shoot were recorded every week for four weeks. The corrugated cardboard method previously used by DeBach (1949) was also used by Mudavanhu (2009) as an aid to monitor females of the obscure mealybug, *P. viburni*, on apple trees. The methods used by DeBach (1949) and Wakgari and Giliomee (2003) would only be successful in the case of reasonably high populations, as is usually the case with *P. longispinus* (personal observations) or in situations where no pesticides are used and biocontrol agents ineffective. In the commercial and abandoned citrus orchards in which these studies were conducted, this was never the case.

CONCLUSION

In studying the biology of mealybugs in the laboratory and field, many problems are usually encountered which require innovative solutions from the research workers. This was also the case in this study of the biology of *P. burnerae*. After solving the practical problems experienced, data could be gathered on the development of this species at constant temperatures in the laboratory and at varying temperatures in the field, as well as on its parasitoids. From these data, life tables for *P. burnerae* were produced (Johnson and Giliomee, 2011a).

ACKNOWLEDGEMENTS

We wish to thank the Citrus Research International and the University of Stellenbosch, South Africa for financially supporting this study as well as Mr Piet Smit of Boontjiesrivier, Citrusdal and Blaauwklippen Estates, Stellenbosch for making study sites available.

REFERENCES

Addis T (2005). Biology of enset root mealybug, C*ataenococcus ensete* (Williams and Matile-Ferrero) (Homoptera: Pseudococcidae), and its geographical distribution in southern Ethiopia. M.Sc Thesis. Alemaya University, Ethiopia.

Amarasekare KG, Mannion CM, Osborne LS, Epsky ND (2008). Life history of *Paracoccus marginatus* (Hemiptera: Pseudococcidae) on four host plant species under laboratory conditions. Environ. Entomol. 37:630-635.

Arai T (1996). Temperature-dependent developmental rate of three mealybug species, *Pseudococcus citriculus* (Green), *Planococcus citri* (Risso), and *Planococcus kraunhiae* (Kuwana) (Homoptera: Pseudococcidae) on citrus. Jpn. J. Appl. Entomol. Z. 40:25-34.

Ben-Dov Y, Miller DR, Gibson GAP (2012). A database of the scale insects of the world. http://www.sel.barc.usda.gov/scalenet/scalenet.htm. (Accessed on 26th February 2012).

Calatayud PA, Delobel B, Guillaud J, Rahbe Y (1998). Rearing the cassava mealybug, *Phenacoccus manihoti*, on a defined diet. Entomol. Exp. Appl. 86:325-329.

Chong J-H, Oetting RD (2007). Progeny fitness of the mealybug parasitoid *Anagyrus sp. nov.* nr. *sinope* (Hymenoptera: Encyrtidae) as affected by brood size, sex ratio, and host quality. Fla. Entomol. 90:656-664.

Chong J-H, Roda AL, Mannion CM (2008). Life history of the mealybug, *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae), at constant temperatures. Environ. Entomol. 37:323-332.

Daane KM, Raksha D, Malakar-Kuenen RD, Walton VM (2004). Temperature-dependent development of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) as a parasitoid of the vine mealybug, *Planococcus ficus* (Homoptera: Pseudococcidae). Biol. Control 31:123-132.

DeBach P (1949). Population studies of the long-tailed mealybug and its natural enemies on citrus trees in southern California, 1946. Ecology 1:14-25.

Goldasteh S, Talebi AA, Fathipour Y, Ostovan H, Zamani A, Shoushtari RV (2009). Effect of temperature on life history and population growth parameters of *Planococcus citri* (Homoptera, Pseudococcidae) on coleus [*Solenostemon scutellarioides* (L.) codd.]. Arch. Biol. Sci. 61:329-336.

Goolsby JA, Kirk AA, Meyerdirk DE (2002). Seasonal phenology and natural enemies of *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) in Australia. Fla. Entomol. 85:494-498.

Grasswitz TR, James DG (2008). Movement of grape mealybug, *Pseudococcus maritimus*, on and between host plants. Entomol. Exp. Appl. 129:268-275.

Johnson T, Giliomee JH (2010). Is there a link between developmental rate and occasional dominance of the oleander mealybug, *Paracoccus burnerae* (Brain) (Hemiptera: Pseudococcidae), on citrus in South Africa. Afr. Entomol.18:354-359.

Johnson T, Giliomee JH (2011a). Development of the oleander mealybug, *Paracoccus burnerae* (Brain) (Hemiptera: Pseudococcidae), on citrus at five temperatures. Afr. Entomol. 19:641-649.

Johnson T, Giliomee JH (2011b). Evaluation of citrus, butternut and sprouting potato asmass rearing substrates for the oleander mealybug, *Paracoccus burnerae* (Brain) (Hemiptera:Pseudococcidae. Afr. J. Biotechnol. 10:8320-8344.

Karamaouna F, Copland MJ (2009). Fitness and life history parameters of *Leptomastix epona* and *Pseudaphycus flavidulus*, two parasitoids of the obscure mealybug *Pseudococcus viburni*. BioControl 54:65-76.

Kontodimas DC, Eliopoulos PA, Stathas GJ, Economou LP (2004). Comparative Temperature-Dependent Development of *Nephus includens* (Kirsch) and *Nephus bisignatus* (Boheman) (Coleoptera: Coccinellidae) Preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): Evaluation of a Linear and Various Nonlinear Models Using Specific Criteria. Environ. Entomol. 33:1-11.

Laflin HM, Parrella MP (2004) Developmental biology of citrus mealybug under conditions typical of California rose production. Ann. Entomol. Soc. Am. 97:082-988.

Mudavanhu P (2009). An investigation into the integrated pest management of the obscure mealybug, *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae), in pome fruit orchards in the Western Cape Province, South Africa. M.Sc. Thesis. University of Stellenbosch, South Africa.

Narai Y, Murai T (2002). Individual rearing of the Japanese mealybug, *Planococcus kraunhiae* (Kuwana) (Homoptera: Pseudococcidae) on germinated broad bean seeds. Appl. Entomol. Zool. 37:295-298.

Pieterse W, Muller DL, Jansen van Vuuren B (2010). A molecular identification approach for five species of mealybug (Hemiptera: Pseudococcidae) on citrus fruit exported from South Africa. Afr. Entomol. 18:23-28.

Polat F, Ulgenturk S, Kaydan MB (2008). Developmental biology of citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), on ornamental plants. In: Branco M, Franco JC and Hodgson CJ (eds) (2008) Proceedings of the XI International Symposium on Scale Insect Studies, 24-27 September 2007. ISA Press, Lisbon, Portugal. p. 322.

Roltsch W (2010). Insectary rearing of the pink hibiscus mealy bug and Its parasitoids in a desert environment. http://www.cdfa.ca.gov/phpps/ipc/biocontrol/pdf/insects/phm parasite-production.pdf. (accessed on 15th October 2010)

Sagarra LA, Vincent C, Tewart RK (2001). Suitability of nine mealybug species (Homoptera: Pseudococcidae) as hosts for the parasitoid *Anagyrus kamali* (Hymenoptera: Encyrtidae). Fla. Entomol. 84:112-116.

Santa-Cecília LVC, Prado E, Borges CM, Correa LRB, Sonza B (2008). Methodology for biological studies of mealybugs (Hemiptera: Pseudococcidae). Coffee Sci., Lavras 3:152-155.

Serrano MS, Lapointe SL (2002). Evaluation of Host Plants and a Meridic Diet for Rearing *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) and Its Parasitoid *Anagyrus kamali* (Hymenoptera: Encyrtidae). Fla. Entomol. 85:417-425.

Summy KR, French JV, Hart WG (1986). Citrus Mealybug (Homoptera: Pseudococcidae) on Greenhouse Citrus: Density-dependent Regulation by an Encyrtid Parasite Complex. J. Econ. Entomol. 79:891-895.

Vennila S, Deshmukh AJ, Pinjarkar D, Agarwal M, Ramamurthy VV, Joshi S, Kranthi KR, Bambawale OM (2010). Biology of the mealybug, *Phenacoccus solenopsis* on cotton in the laboratory. J. Insect Sci. 10:115.

Wakgari WM, Giliomee JH (2003). The biology of three mealybug species (Hemiptera: Pseudococcidae) found on citrus in the Western Cape Province, South Africa. Afr. Entomol. 11:173-182.

Walton VM, Pringle KL (2005). Developmental biology of the vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), and its parasitoid *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae). Afr. Entomol.13:143-147.

Zaviezo T, Cadena E, Flores MF, Bergmann J (2010). Influence of different plant substrates on development and reproduction for laboratory rearing of *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae). Cien. Inv. Agr. 37:31-37.