Effect of Host Plants on the Development, Survivorship, and Reproduction of Pseudococcus viburni (Hemiptera: Pseudococcidae)

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Effect of host plants on the development, survivorship, and reproduction of *Pseudococcus viburni* (Hemiptera: Pseudococcidae)

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Abstract

The obscure mealybug *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae) is one of the most important damaging mealybugs in vineyards worldwide and in South American temperate fruit production. The life history of *P. viburni* on 3 fruit species was studied under laboratory conditions. Leaves of apple *Malus domestica* (Borkh.), persimmon *Diospyros kaki* L., and grapevine *Vitis labrusca* L., were used as host plants. Mealybugs were able to develop, survive, and reproduce on all 3 hosts; however, persimmon and grape leaves were most suitable for *P. viburni* development and reproduction. The cumulative development time (nymph to adulthood) ranged from about 35 d on persimmon and grape leaves to 40.87 ± 0.53 d on apple leaves for females, and from 30.68 ± 0.44 d on persimmon to about 34 d on apple and grape leaves for males. The obscure mealybug exhibited biparental oviparous reproduction, and the fecundity significantly decreased on apple leaves from a mean of 88 eggs per female on persimmon and grape leaves to 30.61 ± 2.9 eggs on apple leaves. The preoviposition period was shortest on persimmon (17.57 ± 0.59 d), and the reproductive period was longest on grape (31.77 ± 0.92 d). The longevity of mated females was higher on grape (54.84 ± 1.23 d) than on apple and persimmon (about 43 d). These results suggest that all 3 hosts can support the development and reproduction of *P. viburni*, but that persimmon and grape leaves are more suitable hosts.

Key Words: Biology, Coccoidea, mealybugs, obscure mealybug

Resumen

La cochinilla oscura, *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae,) es una de las cochinillas más perjudiciales en los viñedos de todo el mundo y en la producción sudamericana de frutas templadas. Se estudio la historia de vida de *P. viburni* sobre 3 especies de frutos bajo condiciones de laboratorio. Se usaron hojas de manzana *Malus domestica* (Borkh.), caqui *Diospyros kaki* L. y vid *Vitis labrusca* L., como plantas hospederas. Las cochinillas podrian desarrollarse, sobrevivir y reproducirse sobre los 3 hospederos; sin embargo, el caqui y las hojas de uva fueron las más adecuadas para el desarrollo y la reproducción de *P. viburni*. El tiempo de desarrollo acumulado (ninfa a la edad adulta) varió entre aproximadamente 35 dias en hojas de caqui y de uva a 40,87 ± 0,53 dias sobre hojas de manzana para las hembras y de 30,68 ± 0,44 d en hojas de caqui a aproximadamente 34 dias en hojas de manzana y de uva para los machos. La cochinilla oscura exhibió la reproducción biparental ovipara y la fecundidad disminuyó significativamente en las hojas de manzana de un promedio de 88 huevos por hembra en las hojas de caqui y de uva a 30,61 ± 2,9 huevos en hojas de manzana. El período de preoviposición fue más corto en el caqui (17,57 ± 0,59 dias), y el período reproductivo fue más prolongado en la uva (31,77 ± 0,92 dias). La longevidad de las hembras apareadas fue mayor en la uva (54,84 ± 1,23 d) que en la manzana y el caqui (aproximadamente 43 días). Estos resultados sugieren que los 3 hospederos pueden apoyar el desarrollo y la reproducción de *P. viburni,* pero que el caqui y las hojas de uva son los hospederos más adecuados.

Palabras Clave: Biología, Coccoidea, cochinillas, cochinilla oscura

Mealybugs (Hemiptera: Pseudococcidae) are sap-sucking insects in a family with about 2,000 species (Garcia Morales et al. 2017). Some of them are important crop pests, particularly in vineyards, orchards, and ornamentals, where they can cause severe damage due to honeydew secretion, promoting the growth of sooty mold, which decreases photosynthesis and reduces the marketability of fruit (Franco et al. 2009; Daane et al. 2012), and their ability to transmit viruses, such as grapevine leafroll-associated virus (GLRaV-3) (Golino et al. 2002; Naidu et al. 2014).

Temperate fruit production is an important activity in southern Brazil, totaling nearly US $300 million annually (Fachinello et al. 2011), as well as in Argentina, Chile, and Uruguay (Retamales & Sepúlveda 2011). In southern Brazil, mealybug importance has increased during the last decade, especially in vineyards and on persimmon trees. In apples, pears, and persimmons, nymphs and adult females develop on leaves, trunks, and fruits — on the calyx and underneath sepals — and in grapes they can feed on stems, leaves, and berries, causing severe aesthetic damage (Ciampolini et al. 2002; Dapoto et al. 2011; Daane et al. 2012).

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The obscure mealybug *Pseudococcus viburni* (Signoret) is a polyphagous insect recorded from 236 host-plant genera in 89 families (Garcia Morales et al. 2017). Different evidences suggest that *P. viburni* originated in southern South America (Charles 2011; Correa et al. 2015). It is a pest of several temperate fruits, including apples, pears, and grapevines (Ciampolini et al. 2002; Iracheta 2004; Dapoto et al. 2011). In vineyards it is a key pest in California (Daane et al. 2008), Chile (Correa et al. 2012), and Brazil (Pacheco Da Silva et al. 2014), mainly because *P. viburni* is known to transmit GLRaV-3 (Golino et al. 2002).

The life cycle of female mealybugs consists of an egg, often laid inside a filamentous ovisac, 3 nymphal stages, and a wingless neotenic adult stage (Franco et al. 2009; Daane et al. 2012). The life cycle of the male includes 4 nymphal stages, of which the 3rd instar is considered the prepupal stage and the 4th instar the pupal stage; followed by the fully developed adult (Borges da Silva et al. 2009; Franco et al. 2009). Reproduction can occur sexually, parthenogenetically, or by facultative parthenogenesis (Borges da Silva et al. 2010; Waterworth et al. 2011).

Despite the economic importance of *P. viburni*, knowledge of its life history remains fragmented. Knowledge of the pest bio-ecology is mandatory for successful pest management. Here, we present the results of a study of the development time, longevity, fecundity, fertility, and survivorship of *P. viburni* reared on leaves of apple *Malus domestica* (Borkh.), persimmon *Diospyros kaki* L., and grape *Vitis labrusca* L.

Materials and Methods

SAMPLE COLLECTION AND INSECT REARING

A colony of *P. viburni* was initiated in Oct 2012 with nymphs, adult females, and ovisacs collected from infested "fruits" of organically managed strawberry *Fragaria x ananassa* Duch. 'Aromas' and 'Albion' fields located in Farroupilha City, Rio Grande do Sul, Brazil. Thousands of new insects were continually added to the colony until the end of the experiments. Identifications of adult females (from the colony and experiments) were confirmed as *P. viburni* by the first author and Jean-François Germain from ANSES, Laboratoire de la Santé des Végétaux, Montferrier-sur-Lez, France, based on the key of Williams & Granara de Willink (1992).

The colony was maintained in the laboratory on sprouted potatoes *Solanum tuberosum* L. in a controlled environment room at 25 ± 1 °C, 70 ± 10% RH and complete darkness. Potatoes were stored in a cold chamber at 4 ± 1 °C in complete darkness until the start of sprouting. When needed, potatoes were transferred to incubators at 25 ± 1 °C in complete darkness to stimulate complete sprouting. Each week, newly sprouted potatoes were infested passively by allowing crawlers to settle. Infested potatoes (3–5) were kept in 1 L plastic containers closed with a lid with a ventilation hole approximately 10 cm in diam and covered with voile to prevent crawlers from escaping. The bottoms of the containers were covered with paper towels to absorb honeydew. Crawlers used in this experiment were obtained from newly developed ovisacs which were transferred from the rearing area to glass tubes (5 cm ht × 1 cm diam) closed with Parafilm® and checked daily until the eggs hatched (after hatching nymphs tend to cluster under the ovisac, which is easily seen through the glass).

HOST PLANTS

Experiments were conducted in the Laboratory of Entomology of Embrapa Uva e Vinho, Bento Gonçalves, Brazil, during the 2013–2014 growing year, in controlled environment rooms at a temperature of 25 ± 1 °C, 12:12 h L:D cycle and 70 ± 10% relative humidity (RH). The study was conducted with leaves from adult plants of apple *Malus domestica* 'Fuji Suprema' (Rosaceae), persimmon *Diospyros kaki* 'Fuyu' (Ebenaceae), and grape *Vitis labrusca* 'Niágara Rosada' (Vitaceae). Leaves were collected from experimental fields free of insecticide applications at Embrapa Uva e Vinho Station. Leaves were washed in a solution of water and sodium hypochlorite (10 ml per L) for 2 min and dried with a paper towel before they were offered to the mealybugs.

DEVELOPMENT AND SURVIVORSHIP

Neonate crawlers were transferred with a fine paintbrush into Petri dishes (6 cm diam × 1.5 cm ht) containing a leaf disk (3.5 cm diam) cut from the host plant and placed on a thin layer (15 ml) of water-agarose (agar 1.5% and 0.15% methylparaben) in order to maintain the turgidity of the leaves. Petri dishes were closed with Parafilm®. The leaf disk was cut to keep at least 1 large vein in the center of the disk. The scales were placed on the upper side of the leaf, and most of them moved to the underside. For the 1st instar, 30 crawlers were placed in each Petri dish and kept together until the first molt. After molting, each mealybug was transferred to its own leaf disk (Petri dish). There were about 200 nymphs (replicates) for each host-plant treatment. Weekly (or before when the leaves started to show symptoms of degradation) leaf disks were changed and the mealybugs transferred to new leaves to ensure a supply of fresh food. In order to avoid damage to mouthparts, the mealybugs were transferred with a paintbrush only when they were moving. When they were settled, the old leaf disk was cut around the mealybug (about 1 cm^2) and placed on the fresh leaf. Mealybugs were observed daily under a stereomicroscope and ecdysis was confirmed by the presence of exuviae near nymphs. Developmental time for each instar was recorded by checking daily for the presence of exuviae. For males, the duration of development of the prepupal and pupal stages was determined by checking for exuviae pushed to the end of the tubular tests (Chong et al. 2003).

In each treatment, the life cycle was monitored by recording the means for development duration calculated for each instar as follows: the number of d from egg hatching until emergence, the survival rate of each instar, recorded by counting the number of individuals that successfully molted to the next instar, and the number of emerging adult males and females.

LONGEVITY AND REPRODUCTION

The influence of the host plant on the fecundity, reproductive period, and adult longevity of *P. viburni* was studied with adult mealybugs collected from the development and survivorship experiment. Newly emerged virgin females were placed in individual Petri dishes with a leaf disk (as described previously) to assess reproduction. Twenty females were held alone to assess the non-mating treatment, and 30 were provided with 2 to 4 newly emerged males reared from each host plant to ensure fertilization. The fecundity, fertility, preoviposition, and oviposition times were recorded for all ovipositing females for each host plant. Females were checked every day for signs of oviposition. When ovisac production started, the presence of eggs in the wax was checked. The number of eggs was recorded every 2 d, removing part of the ovisac from the Petri dish at each count. The ovisacs removed were transferred to new Petri dishes until eggs hatched, and fertility was calculated by the percentage of viable eggs. Females that did not reproduce were excluded from the data analyses. Adult longevity was recorded as the duration between adult emergence and death, includ-

ing females that did not reproduce. Mealybugs were considered dead if they did not move after being touched with a fine-tip brush. The proportion of females was used as a secondary sex ratio, and was determined by dividing the number of adult females by the total number of adults on each host plant.

STATISTICAL ANALYSIS

The effects of the host plant on development time, survivorship, longevity, and reproduction of *P. viburni* were determined with 1-way analyses of variance (ANOVA), followed by Tukey test to separate means at a significant threshold of 0.05. The homogeneity of variances of the data was examined by Levene's test. The analyses were undertaken with SPSS Statistics v. 22.

Results

DEVELOPMENT AND SURVIVORSHIP

All stages of development of *P. viburni* are shown in Fig. 1. Development of *P. viburni* was significantly influenced by the host plant (Table 1). The 1st instar development period was shortest on persimmon and longest on grape leaves for both females and males. In contrast, the female 2nd nymphal developmental period was shortest on grape leaves (9.84 ± 0.28 d) (Table 1). The 3rd instar development period was 3 to 4 d longer for *P. viburni* developing on apple leaves compared to the other treatments. The cumulative development time for the female increased on apple leaves (40.87 ± 0.53 d) and was similar on persimmon and grapevine leaves (Table 1). The incubation period did not differ among the host plants.

The host plant significantly influenced survivorship of 2nd instar females (Table 2). Total mortality of *P. viburni* nymphs was low on all host species, with the highest mortality rate (nymph to adult) on apple leaves (30%), but differences among treatments were not significant (Table 2).

LONGEVITY AND REPRODUCTION

Pseudococcus viburni reproduced sexually. Virgin females did not lay eggs on any of the three host species, although some produced empty ovisacs. About 90% of the females offered males produced eggs (91% on persimmon, 89% on grapevine, and 88% on apple leaves). The host plant strongly affected the fecundity, preoviposition time, and reproduction time (Table 3). Females feeding on apple leaves produced significantly fewer eggs (30.61 ± 2.92 eggs per female; range 5–64), while on persimmon females laid a mean of 93.58 ± 6.87 eggs (range 21–201), and on grape, 83.90 ± 6.81 eggs (range 19–178) (Table 3). No significant difference was found in the fertility of eggs. The preoviposition period was shorter on persimmon (17.57 ± 0.59 d) than on apple and grape leaves, and the reproduction period was highest on grape leaves (31.77 ± 0.92 d) (Table 3). Longevity was significantly affected by host species. The longevity of mated females was higher on grapevine than on apple and persimmon leaves. Longevity was significantly longer in unmated females on apple and grape leaves than on persimmon leaves, and the longevity of males on persimmon was higher than on grape and apple leaves (Table 3).

Discussion

Different host plants significantly affected the biology of *P. viburni*. The species was able to reproduce on all host plants studied; however, persimmon and grape leaves were more suitable for its development and reproduction. Several characteristics of the host plants, such as morphology, nutritional values, and secondary chemical compounds, may have affected the biological responses of *P. viburni*. However, the effects of these factors upon development of this species remain speculative. Chemical and morphological characteristics of the plants, as well as nitrogen levels, are known to be related to insect development (Cocco et al. 2014; Schoonhoven et al. 2005).

Our study showed that at 25 °C a female *P. viburni* can complete its life cycle in about 42 d on persimmon and grape leaves and 50 d on apple leaves, with about 9 d for incubation. The present results are congruent with previous studies on 'Kripps red' apple-potted plants with 45 to 47 d for female development (egg to adult) at 25 °C (Mudavanhu 2009). After hatching the nymphs remained in the ovisac for 3 to 4 d before starting to disperse. Second- and 3rd instars and adult females tend to remain in place, feeding near a leaf vein, and moving only when disturbed or after molting. The life cycle of *Pseudococcus* (Hemiptera: Pseudococcidae) species can be longer compared to the main mealybug pest of vineyards, *Planococcus ficus* (Signoret), which consequently affects the number of generations in a year. In vineyards, *Pseudococcus maritimus* Ehrhorn is predicted to have only 2 generations per year (Geiger & Daane 2001), while *P. ficus* can complete 5 to 6 generations (Walton & Pringle 2005) depending on the temperature. Our results suggest that at 25 °C *P. viburni* could complete 3 to 4 generations in a year, which corroborates findings in California vineyards and pear orchards in Argentina (Dapoto et al. 2011; Daane et al. 2008), but more studies must be performed to evaluate the effect of temperature on *P. viburni* biology. Knowledge of the biology of *P. viburni* is helpful in planning the number and the period of chemical applications, the use of pheromone traps, and timing of release of natural enemies. Management of mealybugs often relies on the applications of insecticides targeting immature stages due to their higher susceptibility than later stages (Daane et al, 2012). Similarly, natural enemies, especially parasitoids, are selective as to optimal host stage (Cadée & Van Alphen 1997), and must be released in the field in the period corresponding to the preferred mealybug developmental stages.

The reproduction of *P. viburni* is exclusively biparental, and unmated females produced empty and poorly formed ovisacs but no eggs. Previous studies reported that 54 to 93% of unmated females could produce ovisacs without eggs (Borges da Silva et al. 2010; Waterworth et al. 2011), or sometimes with infertile eggs (15% of unmated females) (Borges da Silva et al. 2010).

Fecundity was strongly affected in mealybugs fed on apple leaves. On persimmon and grape leaves, the mean number of eggs laid per female was about 88, reaching a maximum of 201 eggs. Other studies of *P. viburni* fecundity varied greatly from about 70 to a maximum of 300 eggs on sprouted potatoes (Iracheta 2004; Bettencourt & Simões 2008), and in apple-potted plants females laid 240 eggs (Mudavanhu 2009). Grimes & Cone (1985) suggested that the number of copulations can stimulate egg production, but Waterworth et al. (2011) observed that the number of matings does not affect the total *P. viburni* fecundity. The result observed here is congruent with field observations of females collected directly from commercial strawberry fields that laid a mean of 76.42 eggs per ovisac, ranging from 51 to 112 eggs (unpublished data).

The longevity of mated females was only about half that of unmated females, and this was expected given that oviposition involves a high metabolic cost for females (Grimes & Cone 1985; Waterworth & Millar 2012). Waterworth et al. (2011) observed similar longevity for unmated *P. viburni* females on sprouted potatoes (105.4 ± 2.7 d). The life span of adult males was about 3 d, also observed on sprouted potatoes by Iracheta (2004).

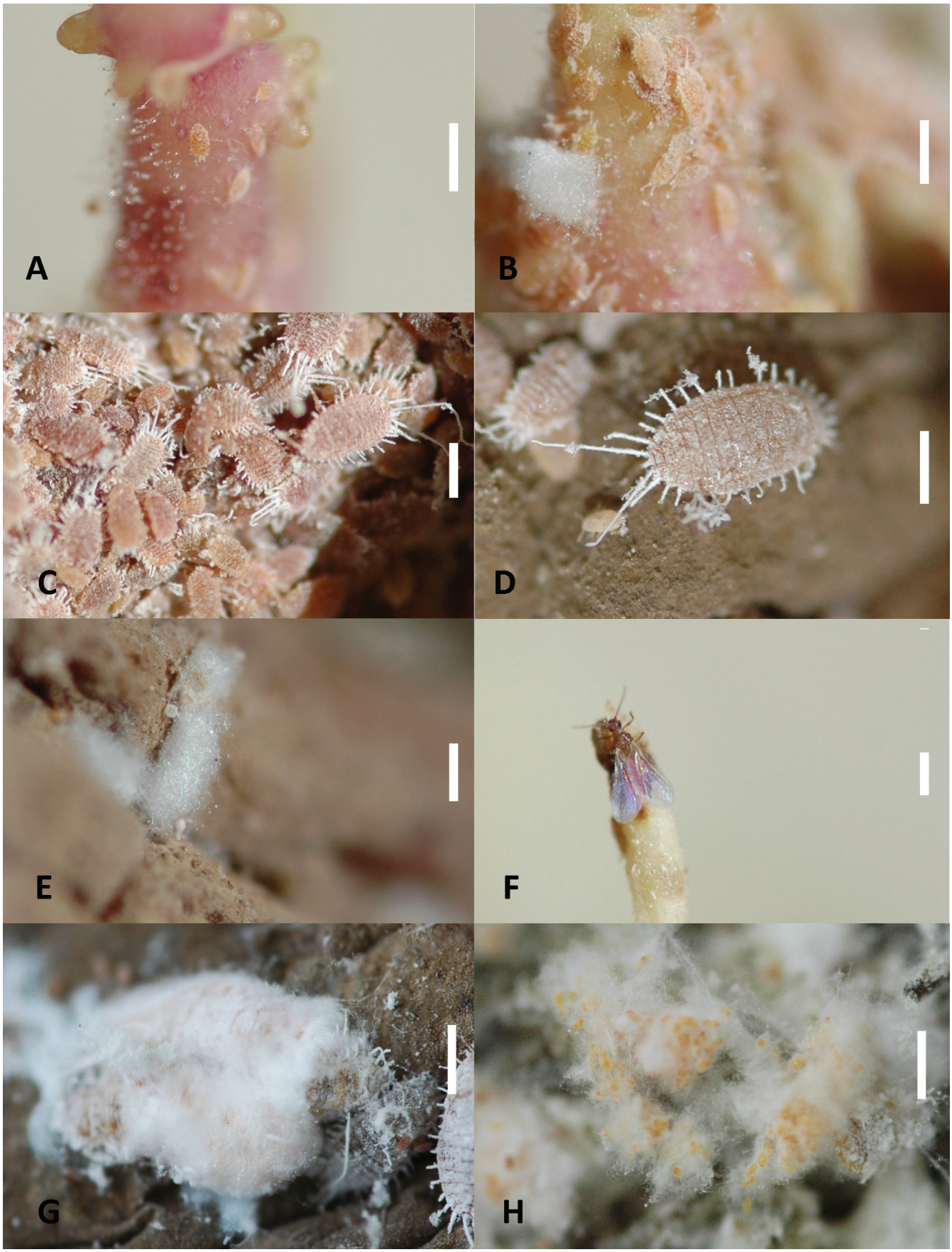

Fig. 1. Life cycle of *Pseudococcus viburni* (Signoret). (A) 1st instar; (B) 2nd instar; (C) 3rd instar female; (D) adult female; (E) adult male tests; (F) adult male; (G) ovisac; (H) eggs inside an ovisac. Scale: 1 mm.

Table 1. Developmental period (Mean ± SE in days) of *Pseudococcus viburni* on persimmon, apple, and grape leaves, at 25 ± 1 °C, 12:12 h L:D and 70 ± 10% RH.

Host plant	Incubation period	Female 1st instar	Male 1st instar	Female 2nd instar	Male 2nd instar	Female 3rd instar	Male Pre-pupa	Male pupa	Nymph to adult Female	Nymph to adult Male
Diospyros kaki	8.06 ± 0.63	11.88 ± 0.14a[a]	11.53 ± 0.18a	11.04 ± 0.27a	12 ± 0.43	11.80 ± 0.34a	3.16 ± 0.20a	4.48 ± 0.14	34.43 ± 0.60a	30.68 ± 0.44a
Malus domestica	10.40 ± 0.92	12.82 ± 0.21b	12.46 ± 0.34ab	11.55 ± 0.35a	13.41 ± 0.68	16.28 ± 0.39b	3.32 ± 1.19a	4.54 ± 0.25	40.87 ± 0.53b	34.58 ± 0.83b
Vitis labrusca	7.60 ± 0.68	14.29 ± 0.26c	13.32 ± 0.31b	9.84 ± 0.28b	11.93 ± 042	12.92 ± 0.32a	7.82 ± 0.22b	4.44 ± 0.10	36.02 ± 0.50a	33.60 ± 0.64b
F	2.323	34.15	11.63	6.67	2.09	44.81	171.36	0.277	36.57	11.31
df_1	2	2	2	2	2	2	2	2	2	2
df_2	31	392	148	302	137	286	124	116	309	134
P	0.115	< 0.0001	< 0.0001	< 0.001	0.127	< 0.0001	< 0.0001	0.758	< 0.0001	< 0.0001

[a]Means followed by the same letter are not significantly different (P < 0.05) according to Tukey test.

Table 2. Sex ratio and survivorship (%) of *Pseudococcus viburni* on persimmon, apple, and grape leaves, at 25 ± 1 °C, 12:12 h L:D and 70 ± 10% RH.

Host plant	Sex ratio	1st instar	2nd instar	Female 3rd instar	Male pre-pupa	Male pupa	Nymph to adult Female	Nymph to adult Male
Diospyros kaki	0.70 ± 0.18	97.61 ± 1.17	93.62 ± 2.32a[a]	95.07 ± 0.18	97.22 ± 0.19	92.86 ± 0.31a	83.33 ± 4.41	87.61 ± 3.46
Malus domestica	0.76 ± 0.09	95.11 ± 1.52	81.67 ± 3.96b	95.08 ± 0.19	95.35 ± 0.32	90.24 ± 0.32a	69.37 ± 4.22	81.73 ± 8.64
Vitis labrusca	0.63 ± 0.15	96.38 ± 0.77	90.32 ± 3.13ab	95.04 ± 0.21	100.00 ± 0.00	100.00 ± 0.00a	74.55 ± 4.94	97.33 ± 2.66
F	2.218	0.930	7.00	0.001	1.162	2.515	1.995	3.012
df_1	2	2	2	2	2	2	2	2
df_2	331	620	596	360	166	162	447	170
P	0.110	0.395	0.001	0.999	0.315	0.084	0.137	0.052

[a]Means followed by the same letter are not significantly different (P < 0.05) according to Tukey test.

Table 3. Reproduction characteristics (Means ± SE) and longevity (days) of *Pseudococcus viburni* on persimmon, apple, and grape leaves, at 25 ± 1 °C, 12:12 h L:D and 70 ± 10% RH.

Host plant	Fecundity	Fertility (%)	Preoviposition period (d)	Reproduction period (d)	Longevity		
					Males	Matted females	Unmated females
Diospyros kaki	93.58 ± 6.87a[a]	98.84 ± 0.80	17.57 ± 0.59a	24 ± 1.31b	6.07 ± 0.21a	42.33 ± 1.71a	91 ± 2.32a
Malus domestica	30.61 ± 2.92b	92.46 ± 3.08	23.81 ± 1.57b	22.05 ± 1.10b	3.85 ± 0.45b	44.16 ± 2.31a	100 ± 3.51b
Vitis labrusca	83.90 ± 6.81a	99.11 ± 0.23	22.14 ± 1.09b	31.77 ± 0.92a	4.87 ± 0.22b	54.84 ± 1.227b	103.15 ± 3.64b
F	31.84	5.10	11.154	14.598	14.854	13.80	3.702
df_1	2	2	2	2	2	2	2
df_2	78	78	64	55	61	64	153
P	< 0.0001	0.008	< 0.0001	0.001	0.0001	< 0.0001	0.027

[a]Means followed by the same letter are not significantly different (P < 0.05) according to Tukey test.

Although apple leaves did not give the best conditions for *P. viburni* development and reproduction, the resistance of the cultivar 'Fuji Suprema' may be related to the low fecundity observed here. On the 'Kripps red' cultivar a higher fecundity and shorter pre-oviposition period was observed (Mudavanhu 2009). In apple crops in South Africa *P. viburni* usually prefers hidden locations underneath the bark, cracks, and crevices of trunks and old stems as a site for feeding and development, migrating to the leaves and fruits only in late season (Mudavanhu 2009). To better understand this effect, more studies with other apple cultivars should be done. In southern Brazil, the most important apple production region in the country (Fachinello et al. 2011), mealybugs are not often observed in apple orchards, but there is concern about the emergence of mealybugs pests in this crop due to its importance in neighboring countries, such as Uruguay (Casco Mila 2012). Our results show that 'Fuji Suprema' may not be a suitable host for *P. viburni*, but it can complete its development and reproduce when fed on apple leaves in the laboratory. Thus, we should not discard the hypothesis that *P. viburni* may eventually become a pest of apple.

Besides the importance of *P. viburni* in South American vineyards, the ability of this species to develop and reproduce on apple and persimmon leaves suggests that it is a threat to fruit crops grown in this region. With rapid development and high survival rates, *P. viburni* can cause significant damage to many economically important crops. If the ecological conditions are suitable, a female *P. viburni* can complete its development in about a month, and produce up to 200 eggs. Knowledge of the biology of an insect pest is important for improving programs for pest management, predicting the population emergence and abundance, and for determining the timing of insecticide applications, release of natural enemies, and use of pheromone traps in pest management.

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