Effects of delayed mating on the reproductive biology of the vine mealybug, *Planococcus ficus* (Hemiptera: Pseudococcidae)

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Abstract

The effect of increasing mating delay on the reproductive performance and population growth rates of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), was investigated under laboratory conditions. Virgin females were mated at 1, 3, 5, 7, 14, 21 and 28 days after emergence and reproductive and life table parameters were estimated. The pre-oviposition period (number of days between mating and the onset of oviposition) significantly decreased in females mated within 7 days, whereas females mated at older ages showed equivalent pre-oviposition periods (<4 days). The length of the oviposition period did not vary with increasing age at mating. Female longevity significantly increased in females mated at 21 and 28 days, as a consequence of a longer pre-reproductive period. Fecundity and sex ratio were not affected by the female age at mating, whereas fertility was higher in mealybugs mated at older ages. Additional field observations highlighted that young and old virgin females were equally able to attract males, as both mated on the same day as the field release. Mating delay also affected the life table parameters of *P. ficus*, as the intrinsic and finite rates of increase did not differ in mealybugs mated within 7 days and significantly decreased in females mated at older ages. The mean generation time and the population doubling time were overall similar in females mated at 1–7 days, and increased significantly in females that experienced longer mating delays. In terms of the mating disruption control of *P. ficus*, our findings indicate that this method would be effective if mating is delayed >7 days, as shorter delays in mating did not reduce the population growth rates.

Keywords: mating delay, fecundity, fertility, life history, population growth parameters, mating disruption

Introduction

The vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), is found throughout Central and South America, California, the Mediterranean basin, South Africa and the Middle East. It completes a variable number of generations per year, depending on the climatic conditions. In the Mediterranean basin, the annual generations of *P. ficus* range from three to four in Italy (Duso, 1989; Lentini *et al.*, 2008) to six in Tunisia (Mansour *et al.*, 2009). In contrast, four to six generations per year have been reported in California (Millar *et al.*, 2002) and five to six generations in South Africa (Walton & Pringle, 2004).

This pest causes increasing yield losses in wine and table grape vineyards (Daane *et al.*, 2012). The conventional control strategy against *P. ficus* consists of multiple applications of insecticides in spring–summer, in order to reduce the population density and avoid fruit damage. However, the chemical

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control of *P. ficus* is often of limited effectiveness, as mealybugs reside above all underneath leaves, under bark and in roots, being thus protected from contact insecticides (Walton *et al.*, 2004).

The identification and synthesis of the *P. ficus* sex pheromone (Hinkens *et al.*, 2001) have facilitated the development of monitoring (Millar *et al.*, 2002; Walton *et al.*, 2004) and mating disruption control strategies. The pheromone-mediated control of *P. ficus* has been successfully applied in California and the Mediterranean basin using a microencapsulated formulation and reservoir dispensers (Walton *et al.*, 2006; Cocco *et al.*, 2014; Sharon *et al.*, 2016).

High sex pheromone concentrations influence insect mating behaviour in several ways, including the reduced responsiveness of males to pheromone stimuli due to adaptation or habituation, the delayed or diminished response of males due to the continuous presence of pheromones, false trails by synthetic pheromone sources that divert males from calling females, and modifications in the ratios of components of the natural sex pheromone blend, which lead to a sensory imbalance effect (Bartell, 1982; Barclay & Judd, 1995; Cardè & Minks, 1995). Irrespective of the modes of action, the effectiveness of mating disruption can be evaluated by assessing damage reduction or variations in the reproductive and demographic parameters of natural pest populations or sentinel females artificially deployed in the field (Baker & Heath, 2005). Studies on insect demography show that the rate of population increase is affected not only by fecundity and fertility but also by the age at first mating (Carey, 1993). Therefore, any factor causing a delay in mating has a significant influence on population dynamics.

The effects of delayed mating on the reproductive output have been studied in several lepidopteran species, generally highlighting a higher longevity and pre-oviposition period and a lower fecundity and fertility with increasing female age at mating (Proshold, 1996; Fadamiro & Baker, 1999; Jones & Aihara-Sasaki, 2001; Torres-Vila *et al.*, 2002; Jiao *et al.*, 2006). However, the delay of mating differentially affects the biological parameters of the investigated species, thereby altering their fitness (Mori & Evenden, 2013). The decrease of the target pest population density can also be due to a delay of mating encounters rather than the suppression of matings, as observed in the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), subjected to mating disruption (Fadamiro *et al.*, 1999).

Delayed mating has also been reported to affect the reproductive output of females in other orders of insects. In the oriental beetle, *Anomala orientalis* (Waterhouse) (Coleoptera: Scarabaeidae), the fecundity gradually decreased with increasing female age at mating (Wenninger & Averill, 2006). Similarly, the solenopsis mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), mated 30 days after emergence, exhibited a decreased fecundity and pre-oviposition period compared with females mated at the age of 2 days (Huang *et al.*, 2013).

The influence of delayed mating on the reproductive biology of *P. ficus* has not previously been explored. In the congeneric citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), delayed mating and high temperature modified the sex ratio and fecundity (Nelson-Rees, 1961; Ross *et al.*, 2010). To our knowledge, no specific studies have been carried out on the factors influencing the *P. ficus* sex ratio. On the other hand, the reproductive biology of *P. ficus* has been studied in detail by Waterworth *et al.* (2011), who demonstrated that it

cannot reproduce parthenogenetically, even though virgin females are capable of laying unviable eggs. The same authors also reported a very long lifespan of unmated females (94 days), whereas males lived approximately 4.5 days and mated multiple times per day.

A deeper understanding of the reproductive biology of *P. ficus* is required in order to evaluate the influence of mating disruption on its populations. Laboratory and field experiments were thus carried out to investigate the effects of increasing delays in mating on reproductive and demographic parameters of *P. ficus*.

Materials and methods

Insect rearing and source of adults

The stock colony of *P. ficus* originated from ovipositing females collected in infested vineyards in north-western Sardinia (Italy). It was maintained on sprouted potato tubers (cv. Spunta) in constant darkness at 22–25 °C and 60–70% RH. Species-specific identification was initially confirmed by molecular analysis (Demontis *et al.*, 2007) and is routinely tested approximately every 6 months to rule out the potential contamination of *P. citri*, which is morphologically very similar to *P. ficus*.

Males and females used in the experiments were obtained from eggs of the same age. Thus, approximately 80 mated females at the onset of oviposition were isolated in plastic cylinders (8 cm tall, 6 cm in diameter) sealed with a double layer of paper napkins and a rubber band. At daily intervals, the oviposited eggs were gently moved with a soft sable-hair brush to sprouted potatoes placed inside Tupperware containers ($13.5 \times 13.5 \times 6.5$ cm^3) covered with ventilated lids. Containers were marked with the date of egg collection and stored in a growth chamber kept at 25 °C, 50% RH, 12L:12D photoperiod. Since the preimaginal development time was approximately 30 days (see the 'Results' section), mealybugs were observed daily after 20 days under a dissecting microscope equipped with an ocular micrometer in order to separate immature males and females. Mature third-instar nymphs, approximately 1.2 mm long, were isolated on a sprouted potato (weight range 25–60 g) inside a plastic cylinder (8 cm tall, 6 cm in diameter), whereas male prepupae were moved to paper towel strips and placed in plastic vials (5.5 cm tall, 1.2 cm in diameter). Vials and cylinders were kept in the growth chamber and checked daily until male and female emergence, the dates of which were recorded. Adult mealybugs from each stock colony were randomly assigned to the different treatments.

Delayed mating experiment

In order to evaluate the influence of delayed mating on the reproductive output of *P. ficus*, the following reproductive parameters were investigated: (i) length of the pre-oviposition period, from mating to the onset of oviposition; (ii) length of the pre-reproductive period, from adult emergence to the onset of oviposition, which also corresponds to the sum of the female age at mating and the pre-oviposition period; (iii) length of the oviposition period; and (iv) total longevity of females. In addition, the following were also recorded: daily fecundity, i.e. the number of eggs oviposited by each female; fertility, calculated as the percentage of viable eggs over the total oviposited eggs; sex ratio of offspring, calculated as the

percentage of female offspring; and survival. Experiments were carried out in the above-described growth chamber at 25 °C, which is the optimal developmental temperature of *P. ficus* (Walton & Pringle, 2005).

In order to allow mating, virgin females at ages 1, 3, 5, 7, 14, 21 and 28 days were individually paired at the beginning of the photophase with two 3-day-old virgin males inside the plastic cylinders and were observed until mating occurred. At least 25 females were tested for each age at mating. After mating, females were checked daily until the onset of oviposition, after which eggs were collected daily with a sable-hair brush until female death and were gently placed inside plastic cylinders (4.0 tall, 3.5 cm in diameter). Dates of mating, beginning and end of oviposition and death of each female were recorded.

The offspring sex ratio was preliminarily determined in ten females mated 1, 7 and 28 days after emergence following the method described by Ross *et al.* (2010) for *P. citri* eggs. Since there was no significant difference in the sex ratio among the tested treatments (see the 'Results' section), the sex ratio of other treatments was not evaluated. Therefore, the mean value (59% female offspring) was used in the calculation of the life table parameters.

Fertility was assessed in ten females mated 1, 7, 14, 21 and 28 days after emergence by collecting the oviposited eggs with a sable-hair brush every 2 days. Eggs were placed in cardboards inside sealed plastic cylinders (4.5 tall, 3.5 cm in diameter) and stored for 2 months under natural laboratory conditions. Fertility was determined as the percentage of egg eclosion, calculated as [first-instar nymphs /(first-instar nymphs + unhatched eggs)] × 100.

The ability of *P. ficus* females of different ages to attract and mate with wild males was investigated in field observations. Thus, 20 females at ages 1, 7 and 28 days were reared in the laboratory as described previously, and released in a naturally-infested vineyard. Females were placed individually on a sprouted potato inside a plastic cylinder (8 cm tall, 6 cm in diameter), which had four holes on the upper part of the wall (1.5 cm in diameter) to allow male access. The positions of the three treatments were alternated in the central five rows of a 0.5-ha plot in a commercial vineyard (18 years old, cv. Carignano) (for further details about the experimental vineyard, see Muscas *et al.*, 2017) and spaced approximately 3 m along and within rows. Cylinders were sheltered from direct sunlight and rain by laminated cardboard, and hung from trellis wires inside the canopy. In order to prevent ants and wild females from entering the cylinders, the support wires were coated with insect trapping glue. Each female was checked daily in order to estimate the length of the pre-oviposition period, given by the time interval from the field release to the beginning of oviposition. Mating was assumed to occur on the same day as the release. Females accidentally injured during the daily checks were excluded from the analysis.

Data analysis

Reproductive parameters (fecundity, fertility, sex ratio, pre-oviposition period, pre-reproductive period and oviposition period) and female longevity were compared among treatments by a generalized linear mixed model, setting normal and binominal distributions for numerical and percentage data, respectively. Significantly different means were separated by Tukey's multiple comparison test ($P < 0.05$) (PROC GLMM, SAS Institute, 2008).

Life tables for females mated at different ages were built from age-specific fecundity (m_x) and survival (l_x) rates, which were calculated from reproductive and survivorship data. The following population growth parameters were estimated (Carey, 1993; Maia *et al.*, 2000):

Net reproductive rate, $R_0 = \sum(l_x m_x)$;
Intrinsic rate of increase (r_m), obtained by the iterative solution of the equation $\sum e^{-r_m x} l_x m_x = 1$;
Finite rate of increase, $\lambda = e^{r_m}$;
Mean generation time, $T_G = \ln R_0 / r_m$;
Population doubling time, $DT = \ln 2 / r_m$.

The mean values of the life table parameters were estimated with the jackknife method using a Microsoft Excel 2007 spreadsheet (Microsoft, Redmond, WA, USA), which generates pseudo-values of the investigated parameter through a subsampling replication technique (Meyer *et al.*, 1986). Jackknife pseudo-values were compared using analyses of variance (Bari *et al.*, 2015), followed by Tukey's tests to separate means ($P < 0.05$) (PROC GLMM, SAS Institute, 2008).

Results

The preimaginal development time did not vary significantly among female groups ($F_{6,160} = 1.88$, $P = 0.189$), ranging from 29.36 ± 0.41 to 30.71 ± 0.35 days (mean ± SE, data not shown). The duration of the pre-oviposition period decreased significantly in females mated within 7 days, whereas 14-, 21- and 28-day-old mated females exhibited a non-significantly different pre-oviposition period (<4 days) ($F_{6,160} = 41.95$, $P < 0.001$) (table 1). The pre-reproductive period, also on account of being the female age at mating plus the pre-oviposition period, showed a significantly increasing pattern with increased mating delay ($F_{6,160} = 614.78$, $P < 0.001$) (table 1). The length of the pre-reproductive period increased by about 2 days from 1- to 7-day-old females, and then increased proportionally by about 14 days in females mated from 14 to 28 days. The oviposition period ranged from 13.04 to 16.33 days revealing a slight, yet not significant, decrease in females mated 14, 21 and 28 days after emergence ($F_{6,160} = 1.42$, $P = 0.212$). Females mated at 21 and 28 days lived significantly longer than those mated at younger ages ($F_{6,160} = 29.83$, $P < 0.001$), with the total lifetime being modified above all by differences in the pre-reproductive period among treatments (table 1).

Females mated within 7 days showed similar daily oviposition patterns, as peaks were reached 5–7 days after the beginning of oviposition and ranged from 27.54 to 33.78 eggs day^{-1} (fig. 1). In contrast, females mated at older ages reached higher peaks (range 50.08–63.68 eggs day^{-1}) in a shorter time (4–5 days). Only 1-day-old females laid fewer than 300 eggs, whereas the mean fecundity ranged from 302.5 to 355.6 eggs in females mated at older ages. However, no significant difference was revealed among groups ($F_{6,160} = 1.41$, $P = 0.212$).

The fertility of the investigated groups (females mated at 1, 7, 14, 21 and 28 days) was 87.51 and 85.86% in females mated at 1 and 7 days of age, respectively, whereas it was above 90% in the other groups (range 90.74–94.00%) (fig. 2). However, a significant difference appeared only between females mated at 1 and 7 days compared with those mated at 28 days ($F_{4,45} = 4.60$, $P = 0.010$), suggesting that older females had higher reproductive performance than young-mated females. The percentage of female offspring did not vary significantly among the

Table 1. Effect of delayed mating on oviposition behaviour and lifetime (mean ± SE) of *Planococcus ficus* females.

Female age at mating (*d*)	Pre-oviposition period (*d*)	Pre-reproductive period (*d*)	Oviposition period (*d*)	Total lifetime (*d*)
1	9.18 ± 0.39a	10.14 ± 0.40a	14.92 ± 1.21a	56.96 ± 1.43a
3	7.06 ± 0.42b	10.56 ± 0.42ab	15.44 ± 1.82a	56.42 ± 1.90a
5	6.27 ± 0.52bc	11.78 ± 0.52bc	16.33 ± 1.40a	60.27 ± 2.08a
7	5.04 ± 0.42cd	12.34 ± 0.43c	14.80 ± 1.24a	57.42 ± 1.27a
14	3.52 ± 0.19e	17.92 ± 0.20d	13.20 ± 0.49a	61.38 ± 0.77a
21	3.38 ± 0.11e	24.82 ± 0.12e	13.20 ± 0.70a	68.60 ± 1.00b
28	3.66 ± 0.12ed	32.08 ± 0.11f	13.04 ± 0.50a	76.16 ± 0.77c
	$F_{6,160} = 41.95$	$F_{6,160} = 614.78$	$F_{6,160} = 1.42$	$F_{6,160} = 29.83$
	$P < 0.001$	$P < 0.001$	$P = 0.212$	$P < 0.001$

Means within columns followed by the same letter are not significantly different by Tukey's test ($P < 0.05$).
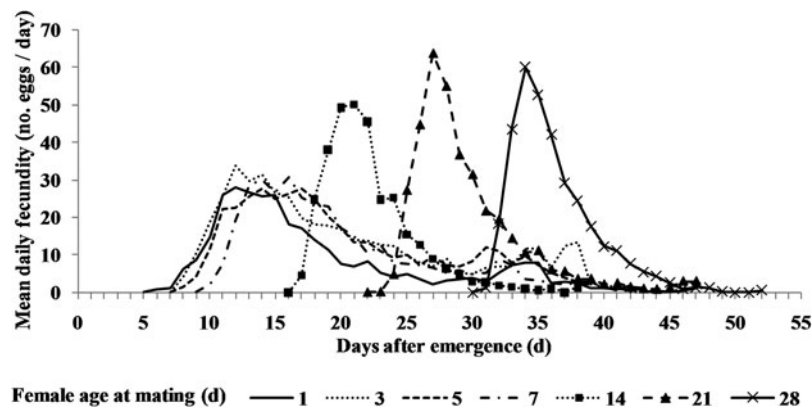

Fig. 1. Daily oviposition patterns of *Planococcus ficus* females mated at increasing ages (1, 3, 5, 7, 14, 21 and 28 days after emergence).

investigated ages at mating, showing a predominance of female offspring (range = 57.3–62.6%) ($F_{2,27} = 0.90$, $P = 0.419$).

The effects of the delayed mating on reproductive traits of *P. ficus* also affected the life table parameters of cohorts originating from females mated at different ages. The net reproductive rate was significantly higher in females mated at 5 and 21 days post-emergence, whereas those mated at 1 day showed the lowest rate ($F_{6,160} = 879.58$; $P < 0.001$) (table 2). The intrinsic and finite rates of increase did not differ significantly among 1- to 7-day-old groups, whereas it decreased significantly in the other treatments (r_m: $F_{6,160} = 600.37$; $P < 0.001$; λ: $F_{6,160} = 776.01$; $P < 0.001$). The mean generation time ranged from 43.50 to 46.93 days in females mated at 1–7 days, showing a significant increase in females mated at older ages (14–28 days) ($F_{6,160} = 660.28$; $P < 0.001$). The doubling time exhibited a pattern similar to T_G, with values of the same magnitude in females mated at 1–7 days and significantly higher values in females mated 14–28 days after emergence ($F_{6,160} = 30.12$; $P < 0.001$).

Females released in the field 1 day after emergence showed a pre-oviposition period of 11.67 days, which was significantly longer than that of 7- and 28-day-old females (3.60 and 3.84 days, respectively) ($F_{2,54} = 112.24$; $P < 0.001$) (fig. 3). The lengths of pre-oviposition periods confirmed the laboratory findings, showing that females of different ages equally attracted males and mated on the same day as the field release.

Discussion

The present study illustrates the reproductive traits of *P. ficus* females mated at increasing ages and indicates the
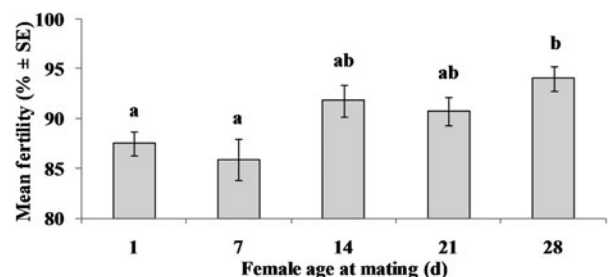

Fig. 2. Fertility (mean ± SE) of *Planococcus ficus* females for different ages at mating. Different letters above columns indicate significant differences (Tukey's test, $P < 0.05$). Note that the *y*-axis starts at 80%.

influence of delayed mating on the population growth potential of this pest. The preimaginal development time of mealybugs reared on sprouted potatoes was about 30.5 days at 25 °C, similarly to the findings of Walton & Pringle (2005) in mealybugs reared on fresh grapevine leaves. The significantly longer development time observed by Varikou *et al.* (2010) in *P. ficus* immatures developed on grape leaf discs (43.3 days at 25 °C) is likely due to the different rearing substrate. Because the development times of *P. ficus* on sprouted tubers and fresh grape leaves were similar, it is likely that phloem-sap feeders, such as mealybugs, have a feeding preference for fresh substrate over excised plant organs. In addition, the grape cultivar and possible genetic differences among the mealybug

Table 2. Life table parameters (mean ± SE) of *Planococcus ficus* in relation to female age at mating.

Female age at mating (d)	Net reproductive rate (R_0) (♀/♀)	Intrinsic rate of increase (r_m) (♀/♀/d)	Finite rate of increase (λ) (♀/♀/d)	Mean generation time (T_G) (d)	Population doubling time (DT) (d)
1	140.23 ± 0.42a	0.114 ± 0.001a	1.120 ± 0.002a	43.50 ± 0.33a	6.10 ± 0.06a
3	175.44 ± 1.00cd	0.115 ± 0.002a	1.121 ± 0.002a	44.94 ± 0.55b	6.03 ± 0.11a
5	190.45 ± 0.66e	0.112 ± 0.002a	1.119 ± 0.001a	46.93 ± 0.74c	6.20 ± 0.10a
7	170.77 ± 0.58b	0.113 ± 0.001a	1.120 ± 0.001a	45.50 ± 0.79bc	6.14 ± 0.08a
14	172.88 ± 0.48bc	0.105 ± 0.002b	1.110 ± 0.002b	49.19 ± 0.31d	6.62 ± 0.07b
21	193.63 ± 0.61f	0.091 ± 0.001c	1.095 ± 0.002c	57.85 ± 0.68e	7.62 ± 0.04c
28	177.11 ± 0.50d	0.080 ± 0.002d	1.084 ± 0.002d	64.43 ± 0.52f	8.63 ± 0.06d
	$F_{6,160} = 879.58$ $P < 0.001$	$F_{6,160} = 600.37$ $P < 0.001$	$F_{6,160} = 776.01$ $P < 0.001$	$F_{6,160} = 660.28$ $P < 0.001$	$F_{6,160} = 30.12$ $P < 0.001$

Means within columns followed by the same letter are not significantly different by Tukey's test ($P < 0.05$).
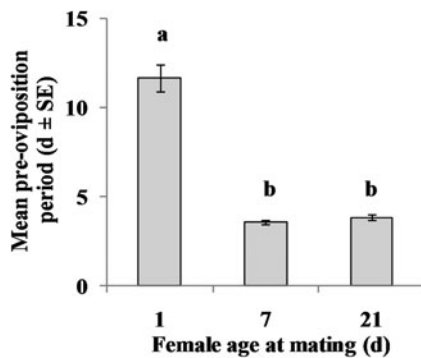

Fig. 3. Length of the pre-oviposition period of *Planococcus ficus* females released in the field at different ages.

populations has been shown to affect the preimaginal development time (Varikou *et al.*, 2010).

P. ficus females can mate shortly after emergence (Waterworth *et al.*, 2011), although a variable time interval before the beginning of oviposition is required for ovarian maturation. Time intervals between mating and the beginning of oviposition (pre-oviposition period) decreased in females mated within 7 days and were almost equivalent in older females. This trend has also been observed in *Ph. solenopsis*: females mated at 30 days showed a significantly shorter pre-oviposition period than that of females mated 2 days after emergence (Huang *et al.*, 2013), thus suggesting that newly-emerged females have a longer pre-oviposition period than older females. This phenomenon can be explained by the egg production and oviposition within the female reproductive tract (Bloch Qazi *et al.*, 2003). Oocytes are firstly generated within the ovaries (oogenesis) and then released in the oviducts (ovulation), through which eggs move down into the common oviduct, where they are fertilized and finally laid. Virgin females retain oocytes in the ovaries and ovulation starts after mating. In *P. citri*, meiotic ovarioles become visible 6 days after female emergence (Nelson-Rees, 1961), which means that meiosis initiates after at least 6 days even if females mate on emergence. Subsequent ovulation, fertilization and oviposition may require a fairly constant period of time (about 3–4 days). Therefore, in our opinion, *P. ficus* virgin females mated at age ≥7 days have almost completed the oogenesis of a portion of eggs, and exhibit a nearly constant and slightly longer pre-oviposition period than 3 days.

The progressive decrease of the pre-oviposition period in females mated within 7 days affected the length of the pre-reproductive period. In fact, in our study, the pre-reproductive period increased slightly in females mated within 7 days, whereas, in contrast, it increased proportionally with the mating delay in females mated at 14, 21 and 28 days. A pre-reproductive period similar to *P. ficus* females mated at 1 day has been found in both the passionvine mealybug, *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) and *P. citri*. These species showed a pre-reproductive period of 10.2 and 12.3 days when reared at 25 °C on sprouted potato and grapevine, respectively (Francis *et al.*, 2012; da Silva *et al.*, 2014). However, no information is available on the pre-reproductive period in mealybugs mated at older ages.

The reproductive performance of *P. ficus* females mated at increasing ages differed considerably from that of other insects. A delayed mating of 4–6 days in some lepidopteran species has led to a significant reduction of fecundity and fertility (Vickers, 1997; Jones & Aihara-Sasaki, 2001; Walker & Allen, 2011), whereas *P. ficus* has not been found to be negatively affected by the age at mating. In fact, in our experiment, *P. ficus* fecundity was 267.4 eggs in 1-day-old females, ranging instead from 302.5 to 355.6 eggs in females mated at older ages. No studies have been carried out on *P. ficus* egg production. However, it is reasonable to assume that *P. ficus* oogenesis is similar to *P. citri*, for which Nelson-Rees (1961) observed the highest fecundity in 10–20-day-old females and the decay of ovarioles and egg resorption in 30-day-old virgin females. The same author demonstrated that *P. citri* is a capital breeder, as starved newly-emerged females had a reduced body size and undeveloped ovarioles.

The different performance of mealybugs relative to other systematic groups could be due to their different evolution. In fact, many lepidopteran species enter in diapause during the winter months and have a short adult lifespan and reproductive period. For instance, the autumn gum moth, *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae), exhibits continuous ovulation after adult emergence and both fecundity and fertility are linearly related to longevity (Walker & Allen, 2010). Unmated *M. privata* females live about 10 days; therefore, a mating delay of 7 days reduces the time available for oviposition by 70% (Walker & Allen, 2011). In contrast, *P. ficus* does not enter into diapause but slows down its life cycle, overwintering mainly as mated adult females and preserving the reproductive potential for several months (Lentini *et al.*, 2008). In fact, overwintering females are usually larger and have higher fecundity than summer females (A. Lentini, personal observation). The minimum development threshold temperature of insects is lower than the reproductive threshold (Gilbert & Raworth, 1996). Therefore, mealybug adult females at low temperatures

can feed and increase their 'energy reserve' in order to produce a high number of eggs under favourable climatic conditions.

Contrary to findings in Lepidoptera, the fertility of *P. ficus* females did not decrease with age, in fact females mated at 28 days showed the highest fertility values. Similarly, the fertility of *A. orientalis* was not affected by the age at mating, although about 50% of females mated at older ages did not lay fertile eggs (Wenninger & Averill, 2006).

Age of mating did not affect the offspring sex ratio, which was about 59% female regardless of the mating delay. The mean sex ratio observed is in accordance with our previous observations carried out in over 11,000 *P. ficus* eggs (female percentage = 60.3%) (Cocco *et al*., 2015). To date, no studies have reported variations in the *P. ficus* sex ratio due to the age of females at mating, whereas research carried out on *P. citri* provided contrasting results. Ross *et al*. (2011) observed nearly 50% of female eggs in young-mated females and about 60% in females mated after 30 days. On the other hand, Nelson-Rees (1961) found a prevalence of female offspring among females mated within 20 days and a majority of male offspring among 30-day-old females. Sex ratio in *P. citri* and, by extension, in *P. ficus* is likely influenced not only by female age, but also by environmental factors such as food quality, feeding frequency and population density (Ross *et al*., 2010, 2011). In addition, climatic conditions may play a role in biasing the sex ratio, as Walton & Pringle (2005) observed a lower proportion of females at 18 and 30 °C under laboratory conditions. This could be an adaptive response to adverse environmental conditions in order to ensure higher genetic variability, as reported in some mite species (Margolies & Wrensch, 1996; Rencken & Pringle, 1998).

The findings of our field experiment were in accordance with our laboratory results. In fact, mating delay did not impair the calling behaviour of *P. ficus* females and their mating ability, as females of different ages mated on the same day as the field release. Similarly, the female age at mating in the European grapevine moth, *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae) did not alter the calling activity of females and their receptivity (Torres-Vila *et al*., 2002).

Female mating at different ages affected all the investigated demographic parameters. The intrinsic and the finite rates of increase showed a decreasing pattern in females mated at 14–28 days, whereas the population doubling time increased when mating was delayed for more than 14 days. These patterns indicate a lower growth potential of *P. ficus* populations at increasing ages at mating. Increasing mating delay also led to a lengthening of the mean time required to complete a generation. The net reproductive rate did not show a clear pattern and it is not clear why females mated at 5 and 21 days exhibited the highest values. However, overall the demographic parameters showed a consistent pattern with the finding that a mating delay >7 days reduces the population growth potential.

Biological and reproductive parameters of *P. ficus* are affected by several factors, including the host species (Ahmed & Abd-Rabou, 2010), grapevine cultivar (Morandi Filho *et al*., 2008; Gonzalez Luna & La Rossa, 2016), climatic conditions (Cocco *et al*., 2017), nitrogen fertilization (Cocco *et al*., 2015) and vineyard floor management (Muscas *et al*., 2017). The main aim of this study was to highlight differences in the reproductive output of *P. ficus* females mated at increasing ages. Therefore, life table values may vary under different conditions, but nonetheless they provide an insight into the influence of the female age at mating on the reproductive output and indicate the distinct response of *P. ficus* females to

delayed mating. The demographic parameters observed by Gonzalez Luna & La Rossa (2016) on *P. ficus* reared in 2-year-old grapevines were comparable with those observed during our experiment in females mated at 1–3 days, which are the natural mating conditions in the field.

Changes in the reproductive output of *P. ficus* due to delayed mating could affect pheromone-mediated pest control strategies, such as mating disruption. Mating disruption against *P. ficus* would be effective if mating is suppressed or delayed for more than 7 days. In fact, delayed mating did not reduce mealybug fecundity, but increased the development time of *P. ficus* populations, thereby reducing the number of generations per year. The effectiveness of mating disruption is influenced by the persistence of the synthetic pheromone in the vineyard. Our ongoing studies investigating the influence of mating disruption on the reproductive traits of *P. ficus* in the field have indicated a significant reduction of ovipositing females as well as the lengthening of the pre-oviposition period in the pheromone-treated plots throughout the season (A. Cocco, unpublished results). The mating disruption control technique applied to lepidopteran pests is potentially more effective, because a simple mating delay of a few days, even without mating suppression, greatly affects female fecundity, thus reducing the population growth potential (Vickers, 1997; Jones & Aihara-Sasaki, 2001; Walker & Allen, 2011). For instance in the koa seedworm, *Cryptophlebia illepida* (Butler) (Lepidoptera: Tortricidae), a mating delay of 6 days reduced the population growth rate by about 20% (Jones & Aihara-Sasaki, 2001), whereas in *P. ficus* a similar growth rate reduction was obtained with a mating delay ≥3 weeks. In vineyards protected by mating disruption, a higher percentage of unmated *P. ficus* females, a lower percentage of ovipositing females and a lower fecundity were observed, although the contribution of mating delay on the reduction of the pest population density was not estimated (Walton *et al*., 2006; Cocco *et al*., 2014). In order to better understand the influence of the reproductive traits on the effectiveness of mating disruption to control *P. ficus*, further field studies are ongoing to quantify the length of the mating delay caused by the control method adopted.

Acknowledgements

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