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

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1   **Effects of delayed mating on the reproductive biology of the vine mealybug, *Planococcus***

2   ***ficus* (Hemiptera: Pseudococcidae)**

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10

11 **Abstract**

12

13 The effect of increasing mating delay on the reproductive performance and population growth

14 rates of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), was

15 investigated under laboratory conditions. Virgin females were mated at 1, 3, 5, 7, 14, 21 and

16 28 days after emergence and reproductive and life table parameters were estimated. The pre-

17 oviposition period (number of days between mating and the onset of oviposition) significantly

18 decreased in females mated within 7 days, whereas females mated at older ages showed

19 equivalent pre-oviposition periods (< 4 days). The length of the oviposition period did not

20 vary with increasing age at mating. Female longevity significantly increased in females mated

21 at 21 and 28 days, as a consequence of a longer pre-reproductive period. Fecundity and sex

22 ratio were not affected by the female age at mating, whereas fertility was higher in mealybugs

23 mated at older ages. Additional field observations highlighted that young and old virgin

24 females were equally able to attract males, as both mated on the same day as the field release.

25 Mating delay also affected the life table parameters of *P. ficus*, as the intrinsic and finite rates

26 of increase did not differ in mealybugs mated within 7 days and significantly decreased in

27 females mated at older ages. The mean generation time and the population doubling time

28 were overall similar in females mated at 1-7 days, and increased significantly in females that

29 experienced longer mating delays. In terms of the mating disruption control of *P. ficus*, our

30 findings indicate that this method would be effective if mating is delayed > 7 days, as shorter

31 delays in mating did not reduce the population growth rates.

32

35

36   **Introduction**

37

38   The vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), is found

39   throughout Central and South America, California, the Mediterranean basin, South Africa and

40   the Middle East. It completes a variable number of generations per year, depending on the

41   climatic conditions. In the Mediterranean basin, the annual generations of *P. ficus* range from

42   three to four in Italy (Duso, 1989; Lentini *et al.*, 2008) to six in Tunisia (Mansour *et al.*,

43   2009). In contrast, four to six generations per year have been reported in California (Millar *et*

44   *al.*, 2002) and five to six generations in South Africa (Walton & Pringle, 2004).

45   This pest causes increasing yield losses in wine and table grape vineyards (Daane *et al.*,

46   2012). The conventional control strategy against *P. ficus* consists of multiple applications of

47   insecticides in spring-summer, in order to reduce the population density and avoid fruit

48   damage. However, the chemical control of *P. ficus* is often of limited effectiveness, as

49   mealybugs reside above all underneath leaves, under bark and in roots, being thus protected

50   from contact insecticides (Walton *et al.*, 2004).

51   The identification and synthesis of the *P. ficus* sex pheromone (Hinkens *et al.*, 2001) have

52   facilitated the development of monitoring (Millar *et al.*, 2002; Walton *et al.*, 2004) and mating

53   disruption control strategies. The pheromone-mediated control of *P. ficus* has been

54   successfully applied in California and the Mediterranean basin using a microencapsulated

55   formulation and reservoir dispensers (Walton *et al.*, 2006; Cocco *et al.*, 2014; Sharon *et al.*,

56   2016).

57   High sex pheromone concentrations influence insect mating behaviour in several ways,

58   including the reduced responsiveness of males to pheromone stimuli due to adaptation or

59   habituation, the delayed or diminished response of males due to the continuous presence of

60   pheromones, false trails by synthetic pheromone sources that divert males from calling

61 females, and modifications in the ratios of components of the natural sex pheromone blend

62 which lead to a sensory imbalance effect (Bartell, 1982; Barclay & Judd, 1995; Cardè &

63 Minks, 1995). Irrespective of the modes of action, the effectiveness of mating disruption can

64 be evaluated by assessing damage reduction or variations in the reproductive and

65 demographic parameters of natural pest populations or sentinel females artificially deployed

66 in the field (Baker & Heath, 2005). Studies on insect demography show that the rate of

67 population increase is affected not only by fecundity and fertility but also by the age at first

68 mating (Carey, 1993). Therefore, any factor causing a delay in mating has a significant

69 influence on population dynamics.

70 The effects of delayed mating on the reproductive output have been studied in several

71 lepidopteran species, generally highlighting a higher longevity and pre-oviposition period and

72 a lower fecundity and fertility with increasing female age at mating (Proshold, 1996;

73 Fadamiro & Baker, 1999; Jones & Aihara-Sasaki, 2001; Torres-Vila *et al.*, 2002; Jiao *et al.*,

74 2006). However, the delay of mating differentially affects the biological parameters of the

75 investigated species, thereby altering their fitness (Mori & Evenden, 2013). The decrease of

76 the target pest population density can also be due to a delay of mating encounters rather than

77 the suppression of matings, as observed in the European corn borer, *Ostrinia nubilalis*

78 (Hübner) (Lepidoptera: Crambidae), subjected to mating disruption (Fadamiro *et al.*, 1999).

79 Delayed mating has also been reported to affect the reproductive output of females in other

80 orders of insects. In the oriental beetle, *Anomala orientalis* (Waterhouse) (Coleoptera:

81 Scarabaeidae), the fecundity gradually decreased with increasing female age at mating

82 (Wenninger & Averill, 2006). Similarly, the solenopsis mealybug, *Phenacoccus solenopsis*

83 Tinsley (Hemiptera: Pseudococcidae), mated 30 days after emergence, exhibited a decreased

84 fecundity and pre-oviposition period compared to females mated at the age of two days

85 (Huang *et al.*, 2013).

86   The influence of delayed mating on the reproductive biology of *P. ficus* has not previously

87   been explored. In the congeneric citrus mealybug, *Planococcus citri* (Risso) (Hemiptera:

88   Pseudococcidae), delayed mating and high temperature modified the sex ratio and fecundity

89   (Nelson-Reese, 1961; Ross *et al.*, 2010). To our knowledge, no specific studies have been

90   carried out on the factors influencing the *P. ficus* sex ratio. On the other hand, the

91   reproductive biology of *P. ficus* has been studied in detail by Waterworth et al. (2011), who

92   demonstrated that it cannot reproduce parthenogenetically, even though virgin females are

93   capable of laying unviable eggs. The same authors also reported a very long lifespan of

94   unmated females (94 days), whereas males lived approximately 4.5 days and mated multiple

95   times per day.

96   A deeper understanding of the reproductive biology of *P. ficus* is required in order to evaluate

97   the influence of mating disruption on its populations. Laboratory and field experiments were

98   thus carried out to investigate the effects of increasing delays in mating on reproductive and

99   demographic parameters of *P. ficus*.

100

101   **Materials and methods**

102

103   *Insect rearing and source of adults*

104   The stock colony of *P. ficus* originated from ovipositing females collected in infested

105   vineyards in north-western Sardinia (Italy). It was maintained on sprouted potato tubers (cv.

106   Spunta) in constant darkness at 22-25 °C and 60-70% RH. Species-specific identification was

107   initially confirmed by molecular analysis (Demontis *et al.*, 2007) and is routinely tested

108   approximately every six months to rule out the potential contamination of *P. citri*, which is

109   morphologically very similar to *P. ficus*.

110 Males and females used in the experiments were obtained from eggs of the same age. Thus,

111 approximately 80 mated females at the onset of oviposition were isolated in plastic cylinders

112 (8 cm tall, 6 cm in diameter) sealed with a double layer of paper napkins and a rubber band.

113 At daily intervals, the oviposited eggs were gently moved with a soft sable-hair brush to

114 sprouted potatoes placed inside Tupperware containers (13.5 × 13.5 × 6.5 cm) covered with

115 ventilated lids. Containers were marked with the date of egg collection and stored in a growth

116 chamber kept at 25 °C, 50% RH, 12L:12D photoperiod. Since the preimaginal development

117 time was approximately 30 days (see Results), mealybugs were observed daily after 20 days

118 under a dissecting microscope equipped with an ocular micrometer in order to separate

119 immature males and females. Mature third-instar nymphs, approximately 1.2 mm long, were

120 isolated on a sprouted potato (weight range 25-60 g) inside a plastic cylinder (8 cm tall, 6 cm

121 in diameter), whereas male prepupae were moved to paper towel strips and placed in plastic

122 vials (5.5 cm tall, 1.2 cm in diameter). Vials and cylinders were kept in the growth chamber

123 and checked daily until male and female emergence, the dates of which were recorded. Adult

124 mealybugs from each stock colony were randomly assigned to the different treatments.

125

126 *Delayed mating experiment*

127 In order to evaluate the influence of delayed mating on the reproductive output of *P. ficus*, the

128 following reproductive parameters were investigated: i) length of the pre-oviposition period,

129 from mating to the onset of oviposition; ii) length of the pre-reproductive period, from adult

130 emergence to the onset of oviposition, which also corresponds to the sum of the female age at

131 mating and the pre-oviposition period; iii) length of the oviposition period; and iv) total

132 longevity of females. In addition, the following were also recorded: daily fecundity, i.e. the

133 number of eggs oviposited by each female; fertility, calculated as the percentage of viable

134 eggs over the total oviposited eggs; sex ratio of offspring, calculated as the percentage of

135 female offspring; and survival. Experiments were carried out in the above-described growth

136 chamber at 25 °C, which is the optimal developmental temperature of *P. ficus* (Walton &

137 Pringle, 2005).

138 In order to allow mating, virgin females at ages 1, 3, 5, 7, 14, 21 and 28 days were

139 individually paired at the beginning of the photophase with two 3-day-old virgin males inside

140 the plastic cylinders and were observed until mating occurred. At least 25 females were tested

141 for each age at mating. After mating, females were checked daily until the onset of

142 oviposition, after which eggs were collected daily with a sable-hair brush until female death

143 and were gently placed inside plastic cylinders (4.0 tall, 3.5 cm in diameter). Dates of mating,

144 beginning and end of oviposition, and death of each female were recorded.

145 The offspring sex ratio was preliminarily determined in 10 females mated 1, 7 and 28 days

146 after emergence following the method described by Ross et al. (2010) for *P. citri* eggs. Since

147 there was no significant difference in the sex ratio among the tested treatments (see Results),

148 the sex ratio of other treatments was not evaluated. Therefore, the mean value (59% female

149 offspring) was used in the calculation of the life table parameters.

150 Fertility was assessed in 10 females mated 1, 7, 14, 21 and 28 days after emergence by

151 collecting the oviposited eggs with a sable-hair brush every 2 days. Eggs were placed in

152 cardboards inside sealed plastic cylinders (4.5 tall, 3.5 cm in diameter) and stored for two

153 months under natural laboratory conditions. Fertility was determined as the percentage of egg

154 eclosion, calculated as [first-instar nymphs / (first-instar nymphs + unhatched eggs)] ×100.

155 The ability of *P. ficus* females of different ages to attract and mate with wild males was

156 investigated in field observations. Thus, 20 females at ages 1, 7 and 28 days were reared in

157 the laboratory as described previously, and released in a naturally-infested vineyard. Females

158 were placed individually on a sprouted potato inside a plastic cylinder (8 cm tall, 6 cm in

159 diameter), which had 4 holes on the upper part of the wall (1.5 cm in diameter) to allow male

160 access. The positions of the three treatments were alternated in the central five rows of a 0.5-

161 ha plot in a commercial vineyard (18 years old, cv. Carignano) (for further details about the

162 experimental vineyard, see Muscas *et al.*, 2017) and spaced approximately 3 m along and

163 within rows. Cylinders were sheltered from direct sunlight and rain by laminated cardboard,

164 and hung from trellis wires inside the canopy. In order to prevent ants and wild females from

165 entering the cylinders, the support wires were coated with insect trapping glue. Each female

166 was checked daily in order to estimate the length of the pre-oviposition period, given by the

167 time interval from the field release to the beginning of oviposition. Mating was assumed to

168 occur on the same day as the release. Females accidentally injured during the daily checks

169 were excluded from the analysis.

170

171 *Data analysis*

172 Reproductive parameters (fecundity, fertility, sex ratio, pre-oviposition period, pre-

173 reproductive period, and oviposition period) and female longevity were compared among

174 treatments by a generalized linear mixed model, setting normal and binominal distributions

175 for numerical and percentage data, respectively. Significantly different means were separated

176 by Tukey's multiple comparison test ($P < 0.05$) (PROC GLMM, SAS Institute, 2008).

177 Life tables for females mated at different ages were built from age-specific fecundity (m_x) and

178 survival (l_x) rates, which were calculated from reproductive and survivorship data. The

179 following population growth parameters were estimated (Carey, 1993; Maia *et al.*, 2000):

180 net reproductive rate, $R_0 = \sum(l_x m_x)$;

181 intrinsic rate of increase (r_m), obtained by the iterative solution of the equation $\sum e^{-r_m x} l_x m_x = 1$;

182 finite rate of increase, $\lambda = e^{r_m}$;

183 mean generation time, $T_G = ln R_0 / r_m$;

184 population doubling time, $DT = ln2 / r_m$.

185   The mean values of the life table parameters were estimated with the jackknife method using

186   a Microsoft Excel 2007 spreadsheet (Microsoft, Redmond, WA, USA), which generates

187   pseudo-values of the investigated parameter through a subsampling replication technique

188   (Meyer *et al.*, 1986). Jackknife pseudo-values were compared using analyses of variance (Bari

189   *et al.*, 2015), followed by Tukey's tests to separate means ($P < 0.05$) (PROC GLMM, SAS

190   Institute, 2008).

191

192   **Results**

193

194   The preimaginal development time did not vary significantly among female groups ($F_{6,160} =$

195   1.88, $P = 0.189$), ranging from 29.36 ± 0.41 to 30.71 ± 0.35 days (mean $\pm$ SE, data not

196   shown). The duration of the pre-oviposition period decreased significantly in females mated

197   within 7 days, whereas 14-, 21- and 28-day-old mated females exhibited a non-significantly

198   different pre-oviposition period (< 4 days) ($F_{6,160} = 41.95$, $P < 0.001$) (Table 1). The pre-

199   reproductive period, also on account of being the female age at mating plus the pre-

200   oviposition period, showed a significantly increasing pattern with increased mating delay

201   ($F_{6,160} = 614.78$, $P < 0.001$) (Table 1). The length of the pre-reproductive period increased by

202   about two days from 1- to 7-day-old females and then increased proportionally by about 14

203   days in females mated from 14 to 28 days. The oviposition period ranged from 13.04 to 16.33

204   days revealing a slight, yet not significant, decrease in females mated 14, 21 and 28 days after

205   emergence ($F_{6,160} = 1.42$, $P = 0.212$). Females mated at 21 and 28 days lived significantly

206   longer than those mated at younger ages ($F_{6,160} = 29.83$, $P < 0.001$), with the total lifetime

207   being modified above all by differences in the pre-reproductive period among treatments

208   (Table 1).

209 Females mated within 7 days showed similar daily oviposition patterns, as peaks were

210 reached 5-7 days after the beginning of oviposition and ranged from 27.54 to 33.78 eggs/day

211 (Fig. 1). In contrast, females mated at older ages reached higher peaks (range 50.08-63.68

212 eggs/day) in a shorter time (4-5 days). Only 1-day-old females laid fewer than 300 eggs,

213 whereas the mean fecundity ranged from 302.5 to 355.6 eggs in females mated at older ages.

214 However, no significant difference was revealed among groups ($F_{6,160} = 1.41$, $P = 0.212$).

215 The fertility of the investigated groups (females mated at 1, 7, 14, 21 and 28 days) was

216 87.51% and 85.86% in females mated at 1 and 7 days of age, respectively, whereas it was

217 above 90% in the other groups (range 90.74-94.00%) (Fig. 2). However, a significant

218 difference appeared only between females mated at 1 and 7 days compared with those mated

219 at 28 days ($F_{4,45} = 4.60$, $P = 0.010$), suggesting that older females had higher reproductive

220 performance than young mated females. The percentage of female offspring did not vary

221 significantly among the investigated ages at mating, showing a predominance of female

222 offspring (range = 57.3-62.6%) ($F_{2,27} = 0.90$, $P = 0.419$).

223 The effects of the delayed mating on reproductive traits of *P. ficus* also affected the life table

224 parameters of cohorts originating from females mated at different ages. The net reproductive

225 rate was significantly higher in females mated at 5 and 21 days post-emergence, whereas

226 those mated at 1 day showed the lowest rate ($F_{6,160} = 879.58$; $P < 0.001$) (Table 2). The

227 intrinsic and finite rates of increase did not differ significantly among 1- to 7-day-old groups,

228 whereas it decreased significantly in the other treatments (r_m: $F_{6,160} = 600.37$; $P < 0.001$; λ:

229 $F_{6,160} = 776.01$; $P < 0.001$). The mean generation time ranged from 43.50 to 46.93 days in

230 females mated at 1-7 days, showing a significant increase in females mated at older ages (14-

231 28 days) ($F_{6,160} = 660.28$; $P < 0.001$). The doubling time exhibited a pattern similar to T_G,

232 with values of the same magnitude in females mated at 1-7 days and significantly higher

233 values in females mated 14-28 days after emergence ($F_{6,160} = 30.12$; $P < 0.001$).

234   Females released in the field 1 day after emergence showed a pre-oviposition period of 11.67

235   days, which was significantly longer than that of 7- and 28-day-old females (3.60 and 3.84

236   days, respectively) ($F_{2,54}$ = 112.24; P < 0.001) (Fig. 3). The lengths of pre-oviposition periods

237   confirmed the laboratory findings, showing that females of different ages equally attracted

238   males and mated on the same day as the field release.

239

240   **Discussion**

241

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

243   ages and indicates the influence of delayed mating on the population growth potential of this

244   pest. The preimaginal development time of mealybugs reared on sprouted potatoes was about

245   30.5 days at 25 °C, similarly to the findings of Walton & Pringle (2005) in mealybugs reared

246   on fresh grapevine leaves. The significantly longer development time observed by Varikou et

247   al. (2010) in *P. ficus* immatures developed on grape leaf discs (43.3 days at 25 °C) is likely

248   due to the different rearing substrate. Because the development times of *P. ficus* on sprouted

249   tubers and fresh grape leaves were similar, it is likely that phloem-sap feeders, such as

250   mealybugs, have a feeding preference for fresh substrate over excised plant organs. In

251   addition, the grape cultivar and possible genetic differences among the mealybug populations

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

253   *Planococcus ficus* females can mate shortly after emergence (Waterworth *et al.*, 2011),

254   although a variable time interval before the beginning of oviposition is required for ovarian

255   maturation. Time intervals between mating and the beginning of oviposition (pre-oviposition

256   period) decreased in females mated within 7 days and were almost equivalent in older

257   females. This trend has also been observed in *Ph. solenopsis*: females mated at 30 days

258   showed a significantly shorter pre-oviposition period than that of females mated 2 days after

259   emergence (Huang *et al.*, 2013), thus suggesting that newly-emerged females have a longer

260   pre-oviposition period than older females. This phenomenon can be explained by the egg

261   production and oviposition within the female reproductive tract (Bloch Qazi *et al.*, 2003).

262   Oocytes are firstly generated within the ovaries (oogenesis) and then released in the oviducts

263   (ovulation), through which eggs move down into the common oviduct, where they are

264   fertilized and finally laid. Virgin females retain oocytes in the ovaries and ovulation starts

265   after mating. In *P. citri*, meiotic ovarioles become visible 6 days after female emergence

266   (Nelson-Rees, 1961), which means that meiosis initiates after at least 6 days even if females

267   mate on emergence. Subsequent ovulation, fertilization and oviposition may require a fairly

268   constant period of time (about 3-4 days). Therefore, in our opinion, *P. ficus* virgin females

269   mated at age ≥ 7 days have almost completed the oogenesis of a portion of eggs, and exhibit a

270   nearly constant and slightly longer pre-oviposition period than 3 days.

271   The progressive decrease of the pre-oviposition period in females mated within 7 days

272   affected the length of the pre-reproductive period. In fact, in our study, the pre-reproductive

273   period increased slightly in females mated within 7 days, whereas, in contrast, it increased

274   proportionally with the mating delay in females mated at 14, 21 and 28 days. A pre-

275   reproductive period similar to *P. ficus* females mated at 1 day has been found in both the

276   passionvine mealybug, *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae), and *P.*

277   *citri*. These species showed a pre-reproductive period of 10.2 and 12.3 days when reared at 25

278   °C on sprouted potato and grapevine, respectively (Francis *et al.*, 2012; da Silva *et al.*, 2014).

279   However, no information is available on the pre-reproductive period in mealybugs mated at

280   older ages.

281   The reproductive performance of *P. ficus* females mated at increasing ages differed

282   considerably from that of other insects. A delayed mating of 4-6 days in some lepidopteran

283   species has led to a significant reduction of fecundity and fertility (Vickers, 1997; Jones &

284   Aihara-Sasaki, 2001; Walker & Allen, 2011), whereas *P. ficus* has not been found to be

285   negatively affected by the age at mating. In fact, in our experiment, *P. ficus* fecundity was

286   267.4 eggs in 1-day-old females, ranging instead from 302.5 to 355.6 eggs in females mated

287   at older ages. No studies have been carried out on *P. ficus* egg production. However, it is

288   reasonable to assume that *P. ficus* oogenesis is similar to *P. citri*, for which Nelson-Rees

289   (1961) observed the highest fecundity in 10-20-day-old females and the decay of ovarioles

290   and egg resorption in 30-day-old virgin females. The same author demonstrated that *P. citri* is

291   a capital breeder, as starved newly-emerged females had a reduced body size and

292   undeveloped ovarioles.

293   The different performance of mealybugs relative to other systematic groups could be due to

294   their different evolution. In fact, many lepidopteran species enter in diapause during the

295   winter months and have a short adult lifespan and reproductive period. For instance, the

296   autumn gum moth, *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae), exhibits

297   continuous ovulation after adult emergence and both fecundity and fertility are linearly related

298   to longevity (Walker & Allen, 2010). Unmated *M. privata* females live about 10 days;

299   therefore, a mating delay of 7 days reduces the time available for oviposition by 70% (Walker

300   & Allen, 2011). In contrast, *P. ficus* does not enter into diapause but slows down its life cycle,

301   overwintering mainly as mated adult females and preserving the reproductive potential for

302   several months (Lentini *et al.*, 2008). In fact, overwintering females are usually larger and

303   have higher fecundity than summer females (A. Lentini, personal observation). The minimum

304   development threshold temperature of insects is lower than the reproductive threshold

305   (Gilbert & Raworth, 1996). Therefore, mealybug adult females at low temperatures can feed

306   and increase their 'energy reserve' in order to produce a high number of eggs under

307   favourable climatic conditions.

308   Contrary to findings in Lepidoptera, the fertility of *P. ficus* females did not decrease with age,

309   in fact females mated at 28 days showed the highest fertility values. Similarly, the fertility of

310   *A. orientalis* was not affected by the age at mating, although about 50% of females mated at

311   older ages did not lay fertile eggs (Wenninger & Averill, 2006).

312   Age of mating did not affect the offspring sex ratio, which was about 59% female regardless

313   of the mating delay. The mean sex ratio observed is in accordance with our previous

314   observations carried out in over 11,000 *P. ficus* eggs (female percentage = 60.3%) (Cocco *et*

315   *al.*, 2015). To date, no studies have reported variations in the *P. ficus* sex ratio due to the age

316   of females at mating, whereas research carried out on *P. citri* provided contrasting results.

317   Ross et al. (2011) observed nearly 50% of female eggs in young mated females and about

318   60% in females mated after 30 days. On the other hand, Nelson-Rees (1961) found a

319   prevalence of female offspring among females mated within 20 days and a majority of male

320   offspring among 30-day-old females. Sex ratio in *P. citri* and, by extension, in *P. ficus* is

321   likely influenced not only by female age but also by environmental factors such as food

322   quality, feeding frequency, and population density (Ross *et al.*, 2010, 2011). In addition,

323   climatic conditions may play a role in biasing the sex ratio, as Walton & Pringle (2005)

324   observed a lower proportion of females at 18 °C and 30 °C under laboratory conditions. This

325   could be an adaptive response to adverse environmental conditions in order to ensure higher

326   genetic variability, as reported in some mite species (Margolies & Wrensch, 1996; Rencken &

327   Pringle, 1998).

328   The findings of our field experiment were in accordance with our laboratory results. In fact,

329   mating delay did not impair the calling behaviour of *P. ficus* females and their mating ability,

330   as females of different ages mated on the same day as the field release. Similarly, the female

331   age at mating in the European grapevine moth, *Lobesia botrana* Den. & Schiff. (Lepidoptera:

332   Tortricidae) did not alter the calling activity of females and their receptivity (Torres-Vila *et*

333   *al.*, 2002).

334   Female mating at different ages affected all the investigated demographic parameters. The

335   intrinsic and the finite rates of increase showed a decreasing pattern in females mated at 14-28

336   days, whereas the population doubling time increased when mating was delayed for more than

337   14 days. These patterns indicate a lower growth potential of *P. ficus* populations at increasing

338   ages at mating. Increasing mating delay also led to a lengthening of the mean time required to

339   complete a generation. The net reproductive rate did not show a clear pattern and it is not

340   clear why females mated at 5 and 21 days exhibited the highest values. However, overall the

341   demographic parameters showed a consistent pattern with the finding that a mating delay > 7

342   days reduces the population growth potential.

343   Biological and reproductive parameters of *P. ficus* are affected by several factors, including

344   the host species (Ahmed & Abd-Rabou, 2010), grapevine cultivar (Morandi Filho *et al.*, 2008;

345   Gonzalez Luna & La Rossa, 2016), climatic conditions (Cocco *et al.*, 2017), nitrogen

346   fertilization (Cocco *et al.*, 2015) and vineyard floor management (Muscas *et al.*, 2017). The

347   main aim of this study was to highlight differences in the reproductive output of *P. ficus*

348   females mated at increasing ages. Therefore, life table values may vary under different

349   conditions, but nonetheless they provide an insight into the influence of the female age at

350   mating on the reproductive output and indicate the distinct response of *P. ficus* females to

351   delayed mating. The demographic parameters observed by Gonzalez Luna & La Rossa (2016)

352   on *P. ficus* reared in two-year-old grapevines were comparable with those observed during

353   our experiment in females mated at 1-3 days, which are the natural mating conditions in the

354   field.

355   Changes in the reproductive output of *P. ficus* due to delayed mating could affect pheromone-

356   mediated pest control strategies, such as mating disruption. Mating disruption against *P. ficus*

357 would be effective if mating is suppressed or delayed for more than 7 days. In fact, delayed

358 mating did not reduce mealybug fecundity but increased the development time of *P. ficus*

359 populations, thereby reducing the number of generations per year. The effectiveness of mating

360 disruption is influenced by the persistence of the synthetic pheromone in the vineyard. Our

361 ongoing studies investigating the influence of mating disruption on the reproductive traits of

362 *P. ficus* in the field have indicated a significant reduction of ovipositing females as well as the

363 lengthening of the pre-oviposition period in the pheromone-treated plots throughout the

364 season (A. Cocco, unpublished results). The mating disruption control technique applied to

365 lepidopteran pests is potentially more effective, because a simple mating delay of a few days,

366 even without mating suppression, greatly affects female fecundity, thus reducing the

367 population growth potential (Vickers, 1997; Jones & Aihara-Sasaki, 2001; Walker & Allen,

368 2011). For instance in the koa seedworm, *Cryptophlebia illepida* (Butler) (Lepidoptera:

369 Tortricidae), a mating delay of 6 days reduced the population growth rate by about 20%

370 (Jones & Aihara-Sasaki, 2001), whereas in *P. ficus* a similar growth rate reduction was

371 obtained with a mating delay $\geq$ 3 weeks. In vineyards protected by mating disruption, a higher

372 percentage of unmated *P. ficus* females, a lower percentage of ovipositing females and a

373 lower fecundity were observed, although the contribution of mating delay on the reduction of

374 the pest population density was not estimated (Walton *et al.*, 2006; Cocco *et al.*, 2014). In

375 order to better understand the influence of the reproductive traits on the effectiveness of

376 mating disruption to control *P. ficus*, further field studies are ongoing to quantify the length of

377 the mating delay caused by the control method adopted.

378

379 **Acknowledgements**

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384 **References**

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538

539   **Tables**

540

541   Table 1. Effect of delayed mating on oviposition behaviour and lifetime (mean ± SE) of

542   *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
	$F_{6,160} = 41.95$ $P < 0.001$	$F_{6,160} = 614.78$ $P < 0.001$	$F_{6,160} = 1.42$ $P = 0.212$	$F_{6,160} = 29.83$ $P < 0.001$

543

544   Means within columns followed by the same letter are not significantly different by Tukey's

545   test ($P < 0.05$).

546

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

548 mating.

549

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 $P < 0.001$

550

551 Means within columns followed by the same letter are not significantly different by Tukey's

552 test ($P < 0.05$).
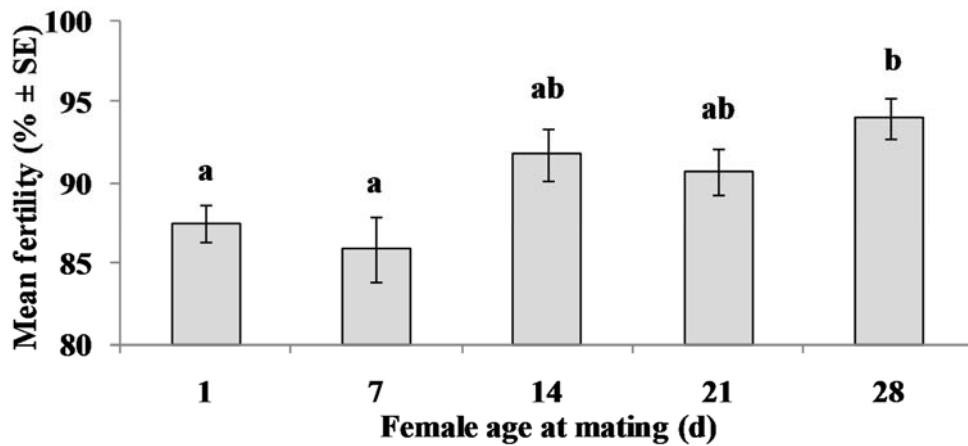
553

554 **Figure**
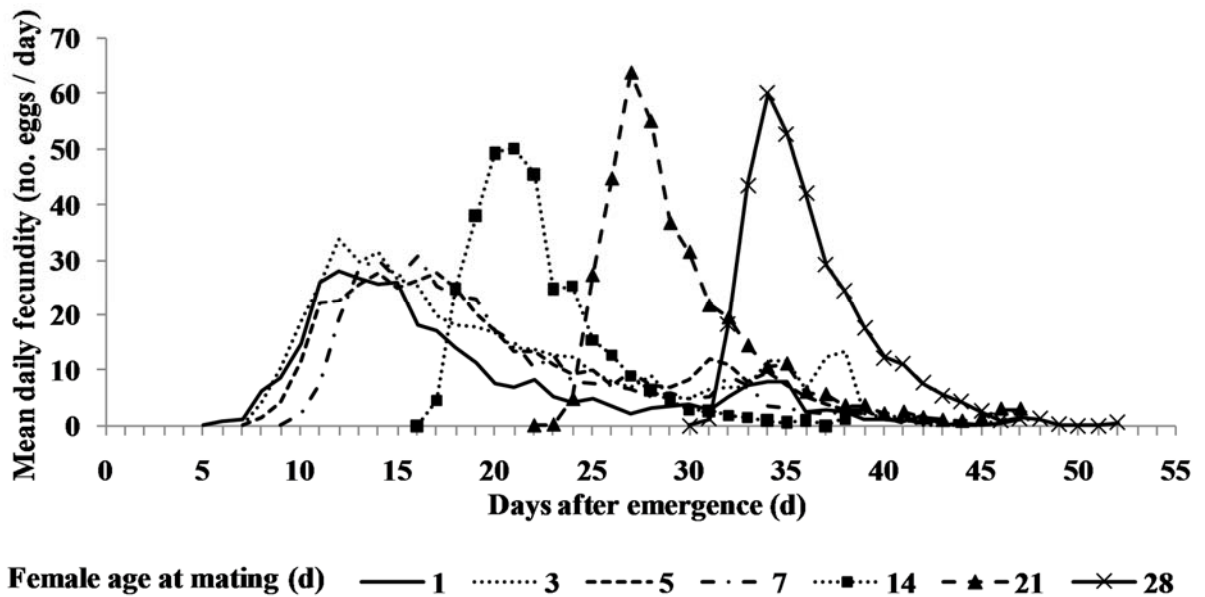
555
556   s

557

558 Fig. 1. Daily oviposition patterns of *Planococcus ficus* females mated at increasing ages (1, 3,

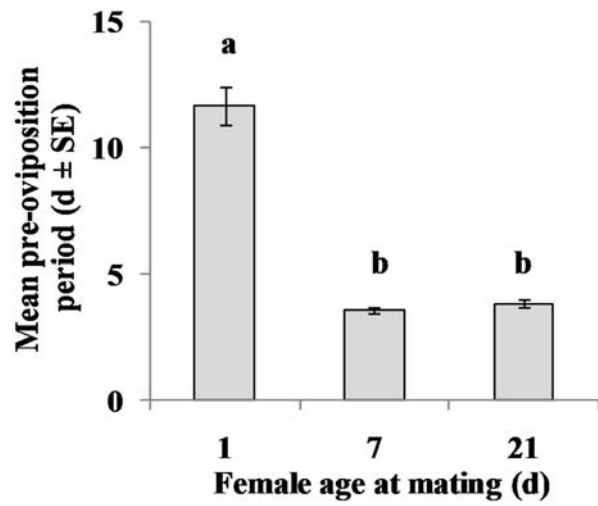559 5, 7, 14, 21 and 28 days after emergence).

560

561

562 Fig. 2. Fertility (mean ± SE) of *Planococcus ficus* females for different ages at mating.

563 Different letters above columns indicate significant differences (Tukey's test, $P < 0.05$). Note

564 that the *y*-axis starts at 80%.

565

566

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

568   at different ages.

569