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27 **Abstract**

28 *Delottococcus aberiae* De Lotto (Hemiptera: Pseudococcidae) is the latest exotic mealybug  
29 species introduced in citrus in the Mediterranean basin. It causes severe distortion and size  
30 reduction on developing fruits. Due to it is its first interaction with citrus, *D. aberiae* economic  
31 thresholds are still unknown for this crop and the current Integrated Pest Management programs  
32 have been disrupted. The objectives of this study were to determine the aggregation patterns of  
33 *D. aberiae* in citrus, develop an efficient sampling plan to assess its population density and  
34 calculate its Economic and Economic Environmental Injury Levels (EIL and EEIL,  
35 respectively). Twelve and 19 orchards were sampled in 2014 and 2015, respectively. At each  
36 orchard, population densities were monitored fortnightly in leaves, twigs and fruit and fruit  
37 damage was determined at harvest. Our results showed a clumped aggregation of *D. aberiae* in  
38 all organs with no significant differences between generations on fruit. Fruit damage at harvest  
39 was strongly correlated with fruit occupation in spring. Based on these results and using  
40 chlorpyrifos as the insecticide of reference, the EIL and EEIL were calculated as 7.1% and  
41 12.1% of occupied fruit in spring, respectively. With all this, we recommend sampling 275  
42 fruits using a binomial sampling method or alternatively, 140 fruits with an enumerative method  
43 bimonthly between petal fall and July.

44

## 45 **Introduction**

46 The introduction of alien species in Europe has increased over the last decades (Roques et al.  
47 2009, Bellard et al. 2016). One of the main causes is the establishment of the international trade  
48 across continents as well as the globalization process (Meyerson and Mooney 2007, Hulme  
49 2009, MacDonald et al. 2015). In this regard, the number of biological invasive species is  
50 expected to rise in the near future (Pimentel et al. 2005, Mainka and Howard 2010, Sutherland  
51 et al. 2011). The impact of non-native species may trigger not just an environmental damage  
52 upon indigenous species, but also important economic losses, especially in agriculture (Pimentel  
53 et al. 2000, Gaertner et al. 2009, Sujay et al. 2010). Within this context, mealybugs (Hemiptera:  
54 Pseudococcidae) are considered one of the main primary pests all over the world (Williams and  
55 Miller 2002) and represent one of the groups with a major number of alien insects in Europe  
56 (Pellizzari and Germain 2010).

57 *Delottococcus aberiae* De Lotto (Hemiptera: Pseudococcidae) is the latest invasive mealybug  
58 pest introduced in Mediterranean citrus. The genus *Delottococcus* is common in South Africa  
59 and recent studies have demonstrated that invasive populations of *D. aberiae* are native to  
60 Limpopo province (NE within South Africa) where citrus is irregularly distributed (Paul 2006,  
61 Beltrà et al. 2015). There, *D. aberiae* is also found in wild olive trees (homogeneously  
62 distributed at low densities) and on the roots of the flowering shrub *Chrysanthemoides*  
63 *monilifera* (L.) T. Norl (Miller and Giliomee 2011). This mealybug is not considered a pest in  
64 South Africa, remaining unnoticeable over decades (Hatting et al. 1998, Miller and Giliomee  
65 2011). Contrarily, since the first individuals of *D. aberiae* were discovered in the core center of  
66 the main citrus producing area in the Mediterranean basin (northern part of Valencia province)  
67 in 2009 (Beltrà et al. 2013a), damage became noticeable on citrus fruit. Distinctively to other  
68 species of citrus mealybugs, *D. aberiae* causes severe direct effects such as distortions and size  
69 reduction in fruit which fosters a high depreciation of its commercial value (Beltrà et al. 2013a).  
70 This mealybug it also triggers indirect effects arisen from the excretion of honeydew, mainly  
71 due to the growth of sooty mold fungi *Capnodium citri* Berk (Capnodiales: Capnodiaceae). In

72 addition, it can give shelter to other secondary pests such as pyralid moths, mites or scale  
73 insects. Due to all these negative effects, in those orchards with the presence of *D. aberiae*, the  
74 integrated pest management has been partially disrupted by the urgency of the farmers to apply  
75 chemical treatments against this new pest, which in most cases are not compatible with a system  
76 based on conservation biological control (Franco et al. 2004, Wajnberg et al. 2004, Jacas and  
77 Urbaneja, 2010).

78 *Delottococcus aberiae* has many generations throughout the year but contrary to other citrus  
79 species in the Mediterranean growing conditions, it remains active during the winter.  
80 Populations tend to reach the maximum peak between June and July and decrease during the  
81 summer period (Martínez-Blay et al. submitted). Fruit distortion and size reduction occurs from  
82 the flowering period to July and all citrus varieties are sensitive to *D. aberiae* attack (herein and  
83 Martínez-Blay et al. submitted).

84 At the present, due to the lack of effective natural enemies against *D. aberiae* in the  
85 Mediterranean basin, its management relies on the application of broad-spectrum insecticides  
86 such as chlorpyrifos (Tena 2017). For optimal control, insecticides must be applied once fruit  
87 set begins. However, there is no criterion based on pest abundance or damage to determine  
88 whether the application may be necessary or not. The estimation of Economic Injury Levels  
89 (EIL) defined as the lowest population density that will cause economic damage for this pest  
90 would therefore ease the integration of its management in the current Integrated Pest  
91 Management (IPM) strategies of citrus crops. Economic damage begins to occur at the pest  
92 density in which the monetary cost of suppressing pest-caused injury equals the potential loss  
93 resulting from this pest density (Stern et al. 1959, Pedigo et al. 1986, Pedigo and Rice 2009).  
94 However, in IPM, environmental costs must be taken into account, producing sustainable  
95 solutions to pest problems. For this reason, another intervention threshold that incorporates both  
96 economic criteria and environmental risks is preferred: the Economic Environmental Injury  
97 Level (EEIL) (Higley and Wintersteen 1996, Groffman et al. 2006).

98 In order to implement both EIL and EEIL, a methodology of sampling able to accurately  
99 estimate pest populations at the minimum cost is required. The selection of a sampling method  
100 mainly depends on the spatial distribution of the sampled population (Kuno 1991).

101 EILs have been established for other species of pseudococcids in citrus such as *Planococcus*  
102 *citri* (Risso) (Martinez-Ferrer 2006), as well as for *Pseudococcus viburni* (Signoret) in pome  
103 fuits (Mudavanhu et al. 2011). However, due to the recent arrival of *D. aberiae*, EILs have not  
104 yet been developed.

105 Herein, we sampled between 12 and 19 citrus orchards during two consecutive years to  
106 determine the distribution pattern of the new citrus pest *D. aberiae* on canopies. Based on these  
107 results, we developed an accurate sampling plan and established the EIL and EEIL. These  
108 results will be used to integrate this pest within the current IPM program.

109

## 110 **Materials and methods**

### 111 **Sampled orchards**

112 The study was conducted in the region of Les Valls (Valencian Community, eastern Spain). A  
113 total of 12 orchards were sampled in 2014 and 19 in 2015, including eight from the previous  
114 year. Seven orchards belonged to four different varieties of sweet orange, *Citrus sinensis* (L.)  
115 Osbeck: Sanguinelli (three orchards) and Powell Navel (four orchards). Eleven orchards  
116 consisted of *Citrus reticulata* Blanco, including the varieties: Oroval (four orchards), Marisol  
117 (one orchard) and Clemenules (six orchards). The remaining four orchards consisted of the  
118 hybrid varieties Ortanique (three orchards), Moncada and Orri (one orchard each one). Sampled  
119 orchards ranged from 0.4 to 3 ha and all of them were drip-irrigated. The selected orchards were  
120 under several ground cover management strategies (Supp. Table S1). Within each orchard, the  
121 area where the evaluations were done was not sprayed with pesticides.

122

123

124 **Sampling procedure**

125 To determine the dispersion pattern of *D. aberiae* and the sampling protocol, 12 orchards were  
126 sampled in 2014 and 19 in 2015 (Supp. Table S1). In each orchard, between six and ten trees  
127 were sampled bimonthly during the two years of the study. Four 30-cm long twigs with flowers  
128 and/or fruits were collected randomly from the canopy of each tree (each twig belonged to a  
129 different cardinal orientation). It means one twig randomly selected in each orientation. All  
130 samples of a tree were placed in individual plastic bags, enclosed, and transported to the  
131 laboratory. Within the next 24 hours, twigs and four leaves and a maximum of eight flowers or  
132 fruits per twig were examined under a stereomicroscope. Leaves and flowers or fruits were  
133 selected at random within the twig. All post-embryonal development stages of *D. aberiae* were  
134 counted: from first nymph instar to the third (N1, N2, N3), adult males (M) and adult females  
135 (F1) including females with ovisac (F2).

136 To determine the economic injury levels (EIL), the percentage of damaged fruit was assessed at  
137 harvest in the same sampled trees. Orchards where fruit thinning operations were conducted in  
138 the summer were excluded from these analyses. We sampled 40 fruits per tree, ten per  
139 orientation (N, S, E, and W) of the tree canopy. We considered that a fruit was damaged when  
140 distortion and size reduction could trigger commercial depreciation.

141

142

143 **Aggregation indices**

144 In order to calculate the spatial distribution of *D. aberiae*, Taylor's power law (Taylor 1961)  
145 index was determined. This index establishes a correlation between a population's density and  
146 variance by the power function:  $s^2 = a m^b$  where  $s^2$  is the sample variance,  $m$  is the sample mean  
147 density and "a" and "b" are Taylor's coefficients. In order to estimate both coefficients, the  
148 model was fit as a linear regression in logarithms ( $\log s^2 = \log a + b \log m$ ). Coefficient "a" is a  
149 sampling factor that depends on sample's size and it lacks ecological meaning and "b" is the

150 Taylor's aggregation index. Coefficient "b" is regarded as a species characteristic, which  
151 provides a basis for a sampling program and describes the aggregation pattern. When  $b = 0$ , the  
152 population is distributed uniformly,  $b = 1$  indicates a random distribution, and  $b > 1$  is an  
153 indication of a clustered distribution (Taylor 1984).

154 Taylor's coefficient was calculated for each sampled tree organ: trunk, twig, leaf and fruit.  
155 Flowers were not included because of the low number of *D. aberiae* specimens. Aggregation  
156 coefficients were calculated separately for the first (April-May) and second generations (June-  
157 July) of *D. aberiae*. To simplify the analysis and because of the difficulty differentiating some  
158 instars, N1 and N2 were combined and N3 and adult immature females (F1) were also  
159 combined. The gravid females (F2) were analyzed as a separate group.

160 MANCOVAs (multivariate analysis of covariance) were used to determine if aggregation  
161 patterns differed between generations, taking the mean as the quantitative factor and variance as  
162 the dependent variable. These analyses were conducted for each citrus organ (twig, fruit and  
163 leaf) and developmental group. For the following analyses (sampling protocols and EILs), we  
164 examined all possible regressions and selected the sample unit (among leaf, twig and fruit)  
165 where all the instar groups aggregated similarly in both generations.

#### 166 **Sampling protocol**

167 To develop the sampling protocol we only considered the fruits because: i) aggregation in this  
168 organ did not differ between generations and ii) fruit is already sampled during the spring to  
169 determine the population levels of another important citrus pest, *Pezothrips kellyanus* Bagnall  
170 (Thysanoptera: Thripidae) (Navarro-Campos et al. 2012, Planes et al. 2015). Therefore, farmers  
171 can use the same organ to sample both pests.

#### 172 **Binomial sampling**

173 This sampling method estimates densities from occupied and unoccupied organs by the insect. It  
174 is used when insect populations show a high aggregation pattern and when there is a correlation  
175 between the proportion of sample units infested with the insect ( $p$ ) and their mean number per



176 sample unit ( $m$ ). It also enables one to make decisions at less cost than with the enumerative  
177 sampling in IPM (Wilson and Room 1983).

178

179 Wilson and Room's (1983) model relates  $m$  and  $p$  according to Taylor's indices  $a$  and  $b$ :

$$180 \quad p = 1 - \exp [-m \ln (a m^{b-1}) / a m^{b-1} - 1]$$

181 The sample size ( $N$ ) required to estimate *D. aberiae* mean density ( $m$ ) for a fixed precision ( $D$ )  
182 in the binomial sampling was calculated using the expression of variance proposed by Kuno  
183 (1986):

$$184 \quad N = D^{-2} (1-p_0) p_0^{-(2k)-1} [k (p_0^{-1/k} - 1)]^{-2}$$

185 Where  $p_0$  is the proportion of non-occupied sample units and  $k$  was calculated from the mean  
186 and the Taylor's indices by the equation:

$$187 \quad k = m^2 / (am^b - m)$$

188 Although  $D = 0.25$  is the value commonly used in research studies of insects' populations  
189 (Southwood and Henderson 2000)  $D = 0.30$  and  $D = 0.35$  also were considered as our sampling  
190 protocol is designed to be implemented by farmers. For each level of precision, sample size was  
191 calculated at different population means.

192

### 193 **Enumerative sampling**

194 In order to calculate the minimum sample size ( $n$ ) required for a known mean density ( $m$ ) to  
195 achieve prefixed levels of precision ( $D = 0.25$ ,  $D = 0.30$  and  $D = 0.35$ ), Green's method (1970)  
196 was used. It establishes that the standard error ( $s/\sqrt{n}$ ) is a fixed proportion ( $D$ ) of the sample  
197 mean. The variance was substituted by its expression according to Taylor's indices:

$$198 \quad n = a m^{(b-2)} / D^2$$

### 199 **Economic injury levels**

200 The economic injury level (EIL) for *D. aberiae* was calculated using the formula of Pedigo et al.  
201 (1986):

$$202 \text{ EIL} = C / \text{VIDK}$$

203 Where C is the *D. aberiae* management costs per production unit (€ha<sup>-1</sup>), V is the price of the  
204 fruit in origin (€ha<sup>-1</sup>), I is the injury unit per insect per production unit [proportion damaged  
205 fruits / (insect ha<sup>-1</sup>)], D is the damage per injury unit [kg reduction ha<sup>-1</sup>]/proportion fruits  
206 damaged], and K is the proportional reduction in injury with treatment (i.e. the efficacy of the  
207 treatment). I\*D is the yield loss per pest and it is obtained from the slope b of the damage  
208 function:  $y = a + bx$ , where y is the percentage of damaged fruits at harvest, and x is the  
209 percentage of sample units (fruits) occupied by *D. aberiae*. Consequently:

$$210 \text{ EIL} = C / \text{VIDK} = C / VbK$$

211 In the damage function, percentage of damaged fruit was obtained by dividing the number of  
212 damaged fruits by the total number of fruits sampled per tree at harvest. Percentage of  
213 occupation was obtained by dividing the highest number of occupied fruits by the total number  
214 of sampled fruits in each sampling date and then calculating the maximum percentage of  
215 occupied fruit during the season (first and second generation). We considered damaged fruit  
216 those with a size reduction or deformation that completely depreciated them from a commercial  
217 view.

218 The EEIL, which takes into account environmental costs, was calculated by multiplying the EIL  
219 by 1.7 (Higley and Wintersteen 1996).

## 220 **Results**

221 During the sampling period, a total of 6,801 specimens were collected on twigs, 13,714 on  
222 leaves and 87,895 on fruits.

223

224

## 225 **Aggregation index**

226 The aggregation pattern of the first and second instar (analyzed together) on twigs was similar  
227 in both generations ( $F = 3.6$ ;  $df = 1, 133$ ;  $P = 0.06$ ;  $R^2 = 95.12$ ) (Table 1). However, the  
228 aggregation pattern of the rest of the developmental groups on twigs differed between  
229 generations (third instar and adult females:  $F = 4.41$ ;  $df = 1, 135$ ;  $P = 0.04$ ; gravid females and  
230 ovisacs:  $F = 7.58$ ;  $df = 1, 98$ ;  $P = 0.0071$ ). When we pooled all the developmental groups, the  
231 aggregation pattern of *D. aberiae* on twigs was similar in both generations ( $F = 0.71$ ;  $df = 1,$   
232  $168$ ;  $P = 0.4$ ).

233 The aggregation pattern of the first and second instars (analyzed together) on leaves was similar  
234 in both generations ( $F = 1.44$ ;  $df = 1, 137$ ;  $P = 0.23$ ) (Table 1). However, the aggregation  
235 pattern of the rest of the developmental groups on leaves differed between generations (third  
236 instar and adult females:  $F = 7.39$ ;  $df = 1, 114$ ;  $P = 0.01$ ; gravid females and ovisacs:  $F = 12.13$ ;  
237  $df = 1, 123$ ;  $P = 0.0007$ ). When we pooled all the developmental groups, the aggregation pattern  
238 of *D. aberiae* on leaves was similar in both generations ( $F = 0.98$ ;  $df = 1, 175$ ;  $P = 0.32$ ).

239 The aggregation pattern of all the developmental groups on fruits was similar in both  
240 generations (first and second instar:  $F = 0.02$ ;  $df = 1, 146$ ;  $P = 0.9$ ; third instar and adult  
241 females:  $F = 0.71$ ;  $df = 1, 130$ ;  $P = 0.4$ ; gravid females and ovisacs:  $F = 0.03$ ;  $df = 1, 103$ ;  $P =$   
242  $0.87$ ) (Table 1). When we pooled all the developmental groups, the aggregation pattern of *D.*  
243 *aberieae* on fruit was similar in both generations ( $F = 0.07$ ;  $df = 1, 157$ ;  $P = 0.8$ ) (Fig. 1).

## 244 **Enumerative sampling plan**

245 The sample size was calculated for all the instars and generations together as there were not  
246 significant differences between generations. For a mean population level of 0.1 insects per fruit,  
247 which represents a mean population value during the sampling process, 250, 210 and 150 fruits  
248 are required with a  $D = 0.25, 0.30$  and  $0.35$  respectively (Fig. 2).

## 249 **Binomial sampling plan**

250 The model by Wilson and Room (1983) adequately fits the correlation between the number of  
251 *D. aberiae* (insect density) per fruit (sample unit) and the percentage of occupied fruits (Fig. 3).  
252 Using these data and Kuno's method (1986), for a mean density of 0.1 insects per fruit in a  
253 binomial sampling, 470, 330 and 260 fruits are needed for a  $D = 0.25$ , 0.30 and 0.35  
254 respectively (Fig. 4). Compared to the enumerative sampling method, more samples are  
255 required.

## 256 **Economic injury level**

257 *Delotococcus aberiae* management costs ( $C = 285 \text{ €ha}^{-1}$ ) were estimated as the sum of the  
258 product ( $135 \text{ €ha}^{-1}$ ) and application ( $150 \text{ €ha}^{-1}$ ) costs. Most of the applications against this pest  
259 are done with chlorpyrifos and therefore it was selected for this model. The treatment price was  
260 established from published assays with chlorpyrifos ( $96 \text{ g l}^{-1}$  of water) (Coloff *et al.*, 2003; Tena  
261 *et al.*, 2009).

262 Fruit price ( $V$ ) was fixed according to official national statistics about prices on origin for navel  
263 oranges (MARM, 2016; Navarro-Campos *et al.* 2012) as:

$$264 \quad V = 0,22 \text{ €kg}^{-1} \times 30\,000 \text{ kg ha}^{-1} = 6\,600 \text{ €ha}^{-1}$$

265 The efficacy ( $K$ ) of chlorpyrifos was taken as  $K = 0.70$  (Tena *et al.* in prep). The estimated value  
266 of  $b$  was 0.87 (Fig. 5). Consequently,  $\text{EIL} = 285 \text{ €ha}^{-1} / (6\,600 \text{ €ha}^{-1} \times 0.87 \times 0.70) = 7.1 \%$  fruits  
267 infested by *D. aberiae*. All varieties of mandarins and oranges are included as they showed a  
268 similar trend and good fit along the regression line ( $R^2 = 0.85$ ).

269 Higley and Wintersteen (1996) proposed to estimate the EEIL for chlorpyrifos multiplying EIL  
270 x 1.7. As a result,  $\text{EEIL} = 12.1\%$  of fruits occupied by *D. aberiae*. This percentage corresponds  
271 to a 0.24 insects per fruit (Figure 3).

## 272 **Sample size**

273 The number of samples required to achieve the prefixed precision levels ( $D = 0.25$ , 0.30 and  
274 0.35) at the estimated *D. aberiae* density of 0.24 insects per fruit for the obtained EEIL were

275 210, 140 and 105 fruits respectively for the enumerative method and 390, 275 and 200 fruits for  
276 the binomial plan.

277

278

279

## 280 **Discussion**

281 All *Delottococcus aberiae* instars tended to aggregate in fruits, leaves and twigs of citrus trees.  
282 Other mealybugs, like *P. citri* (Nestel et al. 1995, Martínez-Ferrer et al. 2006) also aggregate on  
283 citrus. In addition, other mealybugs are known to aggregate on their hosts, including  
284 *Rastrococcus invadens* Williams on mango leaves (Boavida et al. 1992), *Saccharicoccus*  
285 *sacchari* (Cockerell) on sugarcane stalks (Allsopp 1991), *Pseudococcus maritimus* (Ehrhorn) on  
286 vines (Geiger and Daane 2001) and *Phenacoccus peruvianus* Granara de Willink on ornamental  
287 plants (Beltrà et al. 2013b). Among the different instars of *D. aberiae*, the aggregation index  
288 decreased with mealybug age. *D. aberiae* crawlers (first instar), as occurs in other species when  
289 conditions are favorable (Greathead 1997), settled close to the ovisac after hatching. As they  
290 grew and space became limited on fruits, nymphs tended to disperse during the first and second  
291 generation.

292 Mealybugs are multivoltine under mild Mediterranean conditions. *D. aberiae* has between five  
293 and six generations per year on citrus (Martínez-Blay et al. submitted). In spring, *D. aberiae* has  
294 the two first and homogeneous generations (Martínez-Blay et al. submitted). The aggregation  
295 pattern of the young instars was similar in both generations. This result is in accordance with the  
296 observations of other mealybug species such as *P. citri* also on citrus or *P. peruvianus* on  
297 ornamental plants (Martínez-Ferrer et al. 2006, Beltrà et al. 2013b). Third instar nymphs and  
298 adult females behaved similar in the first and second generation when settled on fruits.  
299 However, this pattern changed when these instars were settled on leaves and twigs, as  
300 individuals of the second generation had higher Taylor's indices than those of the first.

301 Generally, these differences within the same species are explained by environmental variations  
302 such as temperature or the presence of natural enemies (Taylor et al. 1988). In the case of *D.*  
303 *aberieae* in citrus, no effective predator or parasitoid attacks these two generations as parasitoids  
304 do not develop on *D. aberieae* (Tena et al. 2017) and the density of its main predator  
305 *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is very low until June (Pérez-  
306 Rodríguez et al. in prep).

307 According to our data, the EEIL for *D. aberieae* in citrus is 12.58% of infested fruits after petal  
308 fall. Economic thresholds of *D. aberieae* could be obtained because the aggregative pattern on  
309 fruit was similar for both generations. It also is worth mentioning that these values have been  
310 calculated considering only direct damage: fruit reduction and distortion. Indirect damage  
311 produced by honeydew excretion was not considered because they were much lower. For  
312 example in some of our orchards, 90 per cent of the fruit was damaged by *D. aberieae* whereas  
313 sooty mold was scarce. In the case of *P. citri*, the main mealybug pest in citrus worldwide,  
314 economic thresholds were calculated considering indirect damage due to the lack of the direct  
315 ones. Although Cavalloro and Prota (1983) proposed thresholds for *P. citri* between 5% to 15%  
316 of infested fruit in summer, Martínez (2006) established the EEIL in 20% of infested fruit.  
317 Following this study, the IPM of citrus in Spain recommends spraying when 20-30% of fruits  
318 are infested. As expected, these thresholds are much higher than the ones obtained for *D.*  
319 *aberieae*. Finally, it is noticeable that our thresholds are similar to those obtained for *P. kellyanus*  
320 and *Scirtothrips citri* Moulton (Thysanoptera: Thripidae), other citrus pests which cause serious  
321 direct damage on young fruits after petal fall (Navarro-Campos et al. 2012, Planes et al. 2015).  
322 These species produce a scar ring between petal fall and 4-6 weeks later (Planes et al. 2015), the  
323 same period of *D. aberieae*. Therefore, the same sampling plan can be used to sample both citrus  
324 pests and decide whether spraying is necessary.

325 Here we propose a binomial sampling of 275 fruits randomly selected per orchard with a  
326 precision of  $D = 0.30$ . According to our results, the enumerative sampling needs a lower number  
327 of fruits and provides more accurate results but it is more time-consuming. Fruits have to be

328 collected and examined with a stereomicroscope to count the number of *D. aberiae* nymphs  
329 under the sepals. All this process could last around six hours considering that the citrus producer  
330 has a stereomicroscope in the sampled orchard. By contrast, binomial sampling does not require  
331 fruit harvest and *D. aberiae* presence can be determined with a magnifying glass. Moreover, the  
332 reduced visibility of first instars is balanced by their high aggregation patterns. Taking all into  
333 consideration, the binomial sampling process could last around fifteen minutes and two hours  
334 (considering that 30 sec are necessary to sample a fruit). Monitoring techniques based on direct  
335 observations of fruit and counting individuals have been widely used in IPM of other mealybug  
336 species (Cavalloro 1983, Ripollés 1990, Barbagallo et al. 1993, Roltsch et al. 2006, Mgocheki  
337 and Addison 2009). However, the use of plant material is a laborious and time consuming task  
338 compared to alternative monitoring techniques based on passive sampling (Geiger and Daane  
339 2001, Waterworth et al. 2011). In this sense, we have recently shown that *D. aberiae* density on  
340 plant is highly related with pest level in corrugated cardboard bands in trunks (Martínez-Blay et  
341 al. submitted). Further research might determine whether this technique can be used as a  
342 sampling method making it simpler and less time-consuming. In fact, these techniques have  
343 already been used in several biological control programs in order to monitor population  
344 densities of mealybugs and also to evaluate the impact of their natural enemies, mainly  
345 predators (Browning 1959, Furness 1976, Goolsby et al. 2002).

346 Monitoring processes should be carried out fortnightly after petal fall according to our results  
347 and the seasonal trend of *D. aberiae* presented in a companion manuscript (Martínez-Blay et al.  
348 submitted). Sampling should start just after petal fall because spraying is forbidden during the  
349 flowering period. *D. aberiae* density increases exponentially between April and July and fruit  
350 damage is caused mainly during this period. When populations reach the EEIL, four insecticides  
351 are currently recommended against mealybugs in citrus in Spain: chlorpyrifos, chlorpyrifos-  
352 methyl, mineral oils and spirotetramat (Tena 2017). More information is needed to evaluate the  
353 efficacy of these insecticides but it is worth mentioning that some *D. aberiae* adult females  
354 descend to the trunk and soil where they lay their ovisacs in spring (Martínez-Blay et al.

355 submitted). Therefore, the application should reach at least the base of the trunk. After  
356 insecticide application, the monitoring process should continue because *D. aberiae* can reach  
357 the EEIL again as occurs with *P. kellyanus* (Planes et al. 2015). Finally, it should not be  
358 overlooked that fruit of the previous year might have not been harvested during the damaging  
359 period, in some late varieties like Valencia oranges. . Therefore, farmers should be cautious with  
360 insecticide residuals in the fruits of the previous year.

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369

### 370 **References**

- 371 **Allsopp, P. G. 1991.** Binomial sequential sampling of adult *Saccharicoccus sacchari* on  
372 sugarcane. *Entomol. Exp. Appl.* 60: 213–218.
- 373 **Barbagallo, S., S. Longo, C. Rapisarda, and G. Siscaro. 1993.** Status of the biological control  
374 against citrus whiteflies and scale insects in Italy. *Bull. IOBC WPR.* 16: 7-15.
- 375 **Bellard, C., P. Cassey, and T. M. Blackburn. 2016.** Alien species as a driver of recent  
376 extinctions. *Biol. Lett.* 12: 20150623.
- 377 **Beltrà, A., A. Soto, and T. Malausa. 2012.** Molecular and morphological characterisation of  
378 Pseudococcidae surveyed on crops and ornamental plants in Spain. *Bull. Entomol. Res.*  
379 102: 165–172.
- 380 **Beltrà, A., A. Garcia-Marí, and A. Soto. 2013a.** El cotonet de Les Valls, *Delottococcus*  
381 *aberiae*, nueva plaga de los cítricos. *Levante Agrícola* 419: 348-352.
- 382 **Beltrà, A., A. Garcia-Marí, and A. Soto. 2013b.** Seasonal phenology, spatial distribution, and  
383 sampling plan for the invasive mealybug *Phenacoccus peruvianus* (Hemiptera:  
384 Pseudococcidae). *J Econ Entomol.* 106: 1486-1494.
- 385 **Beltrà, A., P. Addison, J. A. Ávalos, D. Crochard, F. Garcia-Marí, E. Guerrieri, J. H.**  
386 **Giliomee, T. Malausa, C. Navarro-Campos, F. Palero, and A. S. Soto. 2015.** Guiding  
387 classical biological control of an invasive mealybug using integrative taxonomy. *PLoS*  
388 *One* 10: 1–14.



- 389 **Boavida, C., P. Neuenschwander, and F. Schulthess. 1992.** Spatial distribution of  
390 *Rastrococcus invadens* Williams (Hom., Pseudococcidae) in mango trees. J. Appl.  
391 Entomol. 114: 381–391.
- 392 **Browning, T. O. 1959.** The long-tailed mealybug, *Pseudococcus adonidum* (L.) in South  
393 Australia. Crop Past. Sci. 10: 322-339.
- 394 **Cavalloro, R., and R. Prota. 1983.** Integrated control in citrus orchards: sampling  
395 methodology and threshold for intervention against the principal phytophagous pests. In:  
396 Proc. EC-Expert’s Meeting “Integrated Control in Citrus: Comparison of Results Achieved  
397 by Applying a Standardized Methodology”.
- 398 **Franco, J. C., A. Russo, P. Suma, E.B. Silva, E. Dunkelblum, and Z. Mendel. 2001.**  
399 Monitoring strategies for the citrus mealybug in citrus orchards. Boll. Zool. Agrar. Bachic.  
400 33: 297-303.
- 401 **Franco, J. C., P. Suma, E. B. Da Silva, D. Blumberg, and Z. Mendel. 2004.** Management  
402 strategies of mealybug pests of citrus in Mediterranean countries. Phytoparasitica 32: 507-  
403 522.
- 404 **Furness, G. O. 1976.** The dispersal, age-structure and natural enemies of the long-tailed  
405 mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti), in relation to sampling and  
406 control. Aust. J. Zool. 24: 237-247.
- 407 **Gaertner, M., A. Den Breeyen, Cang Hui, and D. M. Richardson. 2009.** Impacts of alien  
408 plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis.  
409 Prog. Phys. Geogr. 33: 319–338.
- 410 **Geiger, C. A., and K. M. Daane. 2001.** Seasonal movement and distribution of the grape  
411 mealybug (Homoptera: Pseudococcidae): developing a sample program for San Joaquin  
412 Valley vineyards. J. Econ. Entomol. 94: 291–301.
- 413 **Goolsby, J. A., A.A. Kirk, and D.E. Meyerdirk. 2002.** Seasonal phenology and natural  
414 enemies of *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) in Australia. Fla.  
415 Entomol. 85: 494-498.
- 416 **Greathead, D. J. 1997.** Crawler behaviour and dispersal, pp.339-342. In Ben-Dov, Y.,  
417 Hodgson, C.J. (eds.), Soft scale insects: their biology, natural enemies and control, World  
418 Crop Pests, vol. 7. Elsevier, Amsterdam.
- 419 **Groffman, P. M., J. S. Baron, T. Blett, A. J. Gold, I. Goodman, L. H. Gunderson, B. M.**  
420 **Levinson, M. A. Palmer, H. W. Paerl, G. D. Peterson, N. L. Poff, D. W. Rejeski, J. F.**  
421 **Reynolds, M. G. Turner, K. C. Weathers, and J. Wiens. 2006.** Ecological thresholds:  
422 the key to successful environmental management or an important concept with no practical  
423 application? Ecosystems 9: 1-13.
- 424 **Hatting, V., Cilliers, C.J. and E.C.G. Bedford. 1998.** Citrus mealybugs, pp. 112-120. In  
425 Bedford, E.C.G., M.A. van den Bergh, and E.A. de Villiers (eds.), Citrus Pests in the  
426 Republic of South Africa, 2nd Edition. Institute for Tropical and Subtropical Crops,  
427 Nelspruit. South Africa.
- 428 **Higley, L. G., and W.K. Wintersteen. 1996.** Threshold and environmental quality, pp. 174-  
429 290. In Higley, L. G., and L.P Pedigo (eds.), Economic Thresholds for Integrated Pest  
430 Management. University of Nebraska Press, Lincoln, NE, USA.
- 431 **Hulme, P. E. 2009.** Trade, transport and trouble: managing invasive species pathways in an era  
432 of globalization. J. Appl. Ecol. 46: 10-18.

- 433 **Jacas, J. A., and A. Urbaneja. 2010.** Biological control in citrus in Spain: from classical to  
434 conservation biological control, pp. 61-72. In Ciancio A, Mukerji KG (eds.), Integrated  
435 management of arthropod pests and insect borne diseases, vol. 5. Springer, Netherlands.
- 436 **Kuno, E. 1986.** Evaluation of statistical precision and design of efficient sampling for the  
437 population estimation based on frequency of occurrence. *Res. Popul. Ecol.* 28: 305–319.
- 438 **Kuno, E. 1991.** Sampling and Analysis of Insect Populations. *Annu. Rev. Entomol.* 36: 285–  
439 304.
- 440 **MacDonald, G. K., K. A. Brauman, S. Sun, K. M. Carlson, E. S. Cassidy, J. S. Gerber, and**  
441 **P. C. West. 2015.** Rethinking agricultural trade relationships in an era of globalization.  
442 *Bioscience* 65: 275–289.
- 443 **Mainka, S. A., and G. W. Howard. 2010.** Climate change and invasive species: Double  
444 jeopardy. *Integr. Zool.* 5: 102-111.
- 445 **MARM. 2016** Ministerio de Medio ambiente y Medio Rural y Marino. <http://www.marm.es>.
- 446 **Martínez-Ferrer, M. T., J. L. Ripollés, and F. Garcia-Marí, F. 2006.** Enumerative and  
447 binomial sampling plans for citrus mealybug (Homoptera: Pseudococcidae) in citrus  
448 groves. *J. Econ. Entomol.* 99: 993-1001.
- 449 **Meyerson, L. A., and H. A. Mooney. 2007.** Invasive alien species in an era of globalization.  
450 *Front. Ecol. Environ.* 5: 199-208.
- 451 **Mgocheki, N., and P. Addison. 2009.** Incorporating sampling precision into an action  
452 threshold for monitoring ant (Hymenoptera: Formicidae) population levels in vineyards.  
453 *Crop Prot.* 28: 257-263.
- 454 **Miller, D. R., and J. H. Giliomee. 2011.** Systematic revision of the mealybug genus  
455 *Delottococcus* Cox & Ben-Dov (Hemiptera: Pseudococcidae). *Afr. Entomol.* 19: 614-640..
- 456 **Mudavanhu, P., P. Addison, and L. Pringle Ken. 2011.** Monitoring and action threshold  
457 determination for the obscure mealybug *Pseudococcus viburni* (Signoret) (Hemiptera:  
458 Pseudococcidae) using pheromone-baited traps. *Crop Prot.* 30: 919–924.
- 459 **Navarro-Campos, C., A. Aguilar, and F. Garcia-Marí. 2012.** Aggregation pattern, sampling  
460 plan, and intervention threshold for *Pezothrips kellyanus* in citrus groves. *Entomol. Exp.*  
461 *Appl.* 142: 130–139.
- 462 **Nestel, D., H. Cohen, N. Saphir, M. Klein, and Z. Mendel. 1995.** Spatial distribution of scale  
463 insects: comparative study using Taylor's power law. *Environ. Entomol.* 24: 506-512.
- 464 **Paul, I. 2006.** Modelling the distribution of citrus black spot caused by *Guignardia citricarpa*  
465 Kiely. Doctoral dissertation, University of Pretoria.
- 466 **Pedigo, L. P., S. H. Hutchins, and L. G. Higley. 1986.** Economic injury levels in theory and  
467 practice. *Annu. Rev. Entomol.* 31: 341–368.
- 468 **Pedigo, L. P., and M. E. Rice. 2009.** Entomology and pest management. *Entomol. Pest Manag.*  
469 - 6th Edition. Pearson Prentice Hall, New Jersey. 784.
- 470 **Pellizzari, G., and J.-F. Germain. 2010.** Scales (Hemiptera, Superfamily Coccoidea). Chapter  
471 9.3. BIORISK – Biodivers. Ecosyst. Risk Assess. 4: 475–510.
- 472 **Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000.** Environmental and economic costs  
473 of nonindigenous species in the United States. *Bioscience* 50: 53–65.
- 474 **Pimentel, D., R. Zuniga, and D. Morrison. 2005.** Update on the environmental and economic  
475 costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52: 273-288

- 476 **Planes, L., J. Catalan, J. A. Jaques, A. Urbaneja, and A. Tena. 2015.** *Pezothrips kellyanus*  
 477 (Thysanoptera: Thripidae) nymphs on orange fruit: importance of the second generation  
 478 for its management. *Florida Entomol.* 98: 848–855.
- 479 **Ripollés, J.L. 1990.** Las cochinillas de los agrios. 4º Symposium Nacional de Agroquímicos.  
 480 Sevilla 1990. *Levante Agrícola.* 14: 297-298.
- 481 **Roltsch, W. J., D. E. Meyerdirk, R. Warkentin, E. R. Andress, and K. Carrera. 2006.**  
 482 Classical biological control of the pink hibiscus mealybug, *Maconellicoccus hirsutus*  
 483 (Green), in southern California. *Biol. Control.* 37: 155-166.
- 484 **Roques, A., W. Rabitsch, J. Rasplus, C. Lopez-Vaamonde, W. Nentwig, and M. Kenis.**  
 485 **2009.** Alien Terrestrial Invertebrates of Europe. *Handb. Alien Species Eur.* 63–79.
- 486 **Southwood, T. R. E., and P. A. Henderson. 2000.** *Ecological Methods*, Third Edition,  
 487 Blackwell Sci. Ltd.
- 488 **Stern, V., R. Smith, R. Van Den Bosch, and K. Hagen. 1959.** The Integrated Control  
 489 Concept. *Hilgardia.* 29: 81–100.
- 490 **Sujay, Y. H., H. N. Sattagi, and R. K. Patil. 2010.** Invasive alien insects and their impact on  
 491 agroecosystem. *Karnatka J. Agric. Sci.* 23: 26–34.
- 492 **Sutherland, W. J., S. Bardsley, L. Bennun, M. Clout, I. M. Côté, M. H. Depledge, L. V.**  
 493 **Dicks, A. P. Dobson, L. Fellman, E. Fleishman, D. W. Gibbons, A. J. Impey, J. H.**  
 494 **Lawton, F. Lickorish, D. B. Lindenmayer, T. E. Lovejoy, R. Mac Nally, J. Madgwick,**  
 495 **L. S. Peck, J. Pretty, S. V. Prior, K. H. Redford, J. P. W. Scharlemann, M. Spalding,**  
 496 **and A. R. Watkinson. 2011.** Horizon scan of global conservation issues for 2011. *Trends*  
 497 *Ecol. Evol.* 26: 10-16.
- 498 **Taylor, L. R. 1961.** Aggregation, Variance and the Mean. *Nature* 189: 732–735.
- 499 **Taylor, L. 1984.** Assessing and interpreting the spatial distributions of insect populations.  
 500 *Annu. Rev. Entomol.* 29: 321–357.
- 501 **Taylor, L. R., J.N. Perry, I.P. Woiwod, and R.A.J. Taylor. 1988.** Specificity of the spatial  
 502 power-law exponent in ecology and agriculture. *Nature* 332:721-722
- 503 **Tena, A. 2017.** “Pseudocóccidos”. In “Gestión Integrada de Plagas de Cítricos”, Ed. by  
 504 Urbaneja A., Catalán J., Tena A., Jacas, J. [http://gipcitricos.ivia.es/area/plagas-](http://gipcitricos.ivia.es/area/plagas-principales/pseudococcidos/delottococcus-aberiae)  
 505 [principales/pseudococcidos/delottococcus-aberiae](http://gipcitricos.ivia.es/area/plagas-principales/pseudococcidos/delottococcus-aberiae)
- 506 **Tena A., J. Catalán C. Monzó, J. Jacas and A. Urbaneja. 2009.** Control químico de  
 507 *Pezothrips kellyanus*, nueva plaga de los cítricos, y sus efectos sobre la entomofauna  
 508 auxiliar. *Levante Agrícola* 397: 281–289.
- 509 **Tena, A., J. García-Bellón, and A. Urbaneja. 2017.** Native and naturalized mealybug  
 510 parasitoids fail to control the new citrus mealybug pest *Delottococcus aberiae*. *J. Pest Sci.*  
 511 90: 659-667.
- 512 **Wajnberg, E., B. D. Roitberg, G. Boivin, T. Doctoral, I. Vänninen, H. Koskula, H. Rogg,**  
 513 **GVA, C. Navarro-Campos, F. L. Wäckers, A. Pekas, J. M. DiTomaso, R. A. Van**  
 514 **Steenwyk, R. M. Nowierski, J. L. Vollmer, E. Lane, E. Chilton, P. L. Burch, P. E.**  
 515 **Cowan, K. Zimmerman, C. P. Dionigi, A. Beltra, P. Addison, J. A. Ávalos, D.**  
 516 **Crochard, F. García-Mari, E. Guerrieri, J. H. Giliomee, T. Malausa, C. Navarro-**  
 517 **Campos, F. Palero, A. S. Soto, F. Begum, R. M. Bajracharya, B. K. Sitaula, S.**  
 518 **Sharma, S. Ali, H. Ali, P. Huguier, N. Manier, O. J. Owojori, P. Bauda, P. Pandard,**  
 519 **J. Römbke, S. E. Naranjo, P. C. Ellsworth, G. B. Frisvold, P. F. Hendrix, R. W.**  
 520 **Parmelee, D. A. Crossley, D. C. Coleman, P. Odum, P. M. Groffman, S. Ramroodi, O.**  
 521 **Joharchi, J. Hajizadeh, J. C. J. . J. C. van Lenteren, M. B. Postma-Blaauw, R. G. M.**

- 522 De Goede, J. Bloem, J. H. Faber, L. Brussaard, P. Kardol, W. N. Reynolds, R. J.  
523 Norby, A. T. Classen, F. Beaulieu, J. A. Jacas, G. Mar, M. Jonsson, S. D. Wratten, D.  
524 A. Landis, G. M. Gurr, J. G. Lundgren, M. E. Ellsbury, D. A. Prischmann, R.  
525 Messing, M. Wright, J. . Bale, J. C. J. . J. C. van Lenteren, F. Bigler, T. Collier, R. A.  
526 Van Steenwyk, L. Cole, S. M. Buckland, R. D. Bardgett, T. Collier, R. A. Van  
527 Steenwyk, J. C. J. . J. C. van Lenteren, V. H. P. Bueno, M. S. M. Hoddle, D. P.  
528 Schmitt, O. Berndt, R. Meyhöfer, H. M. Poehling, W. O. C. Symondson, K. D.  
529 Sunderland, M. H. Greenstone, A. Enkegaard, M. A. Sardar, H. F. Brodsgaard, W.  
530 H. Van Der Putten, L. E. M. Vet, J. A. Harvey, F. L. Wäckers, J. J. Obrycki, L. C.  
531 Lewis, D. B. Orr, D. A. Crossley, B. R. Mueller, J. C. Perdue, and F. F. Costa-  
532 comelles J García-mari F Laborda D Roca R Marzal C. 2004. A critical evaluation of  
533 augmentative biological control. Biol. Control 31: 245–256.
- 534 Waterworth, R. A., R.A. Redak, and J.G Millar. 2011. Pheromone-baited traps for  
535 assessment of seasonal activity and population densities of mealybug species (Hemiptera:  
536 Pseudococcidae) in nurseries producing ornamental plants. J.Econ. Entomol. 104: 555-  
537 565.
- 538 Williams, D. J., and D. R. Miller. 2002. Systematic studies on the *Antonina crawi* Cockerell  
539 (Hemiptera: Coccoidea: Pseudococcidae) complex of pest mealybugs. Proc. Entomol. Soc.  
540 Washingt. 104: 896–911.
- 541 Wilson, L. T., and P. M. Room. 1983. Clumping Patterns of Fruit and Arthropods in Cotton,  
542 with implications for binomial sampling. Environ. Entomol. 12: 50–54.
- 543
- 544

545 **Table 1.** Taylor's indices for each sample unit, generation and life instar of *D. aberiae*. (N1=  
 546 first nymphal instar; N2= second nymphal instar; N3= third nymphal instar; H1= adult female;  
 547 H2= gravid adult female).

548

Sample unit	Life stage	Generation	Samples (n)	a	b	SE(b)	R <sup>2</sup>	t-Value for slope=1	
Twigs	N1-N2	1	73	5.714	1.504	0.03	96.61	-18,665	
		2	61	8.091	1.612	0.05	95.14	-16,573	
		Total	134	6.653	1.548	0.03	95.64	-21,512	
	N3-F1	1	76	2.63	1.31	0.03	95.92	-12,167	
		2	60	3.873	1.406	0.03	97.03	-16,22	
	F2-ovisac	1	55	1.517	1.138	0.05	90.82	-3,671	
		2	44	3.055	1.321	0.04	96.39	-11,284	
	All instars	1	89	4.508	1.512	0.03	96.05	-18,668	
		2	80	6.561	1.552	0.03	96.45	-19,957	
		Total	169	5.37	1.525	0.02	95.92	-23,705	
	Leaves	N1-N2	1	72	14.997	1.569	0.04	95.18	-16,655
			2	66	19.953	1.656	0.06	91.93	-13,455
Total			133	16.982	1.6	0.04	93.61	-18,491	
N3-F1		1	62	3.733	1.26	0.03	95.71	-9,772	
		2	53	6.823	1.425	0.05	94.63	-11,787	
F2-ovisac		1	69	1.607	1.091	0.03	94.98	-3,708	
		2	55	3.266	1.248	0.03	96.32	-13,279	
All instars		1	90	11.83	1.567	0.04	94.64	-16,981	
		2	86	15.241	1.627	0.04	93.56	-16,145	
		Total	162	13.459	1.597	0.03	93.9	-20,423	
Fruits		N1-N2	1	56	7.551	1.411	0.05	94.19	-11,213
			2	91	5.546	1.418	0.03	95.51	-15,249
	Total		147	6.252	1.398	0.03	94.87	-16,419	
	N3-F1	1	44	2.761	1.21	0.03	97.53	-9,711	
		2	87	2.506	1.245	0.03	96.34	-11,168	
	Total	131	2.547	1.214	0.02	97.13	-13,135		
		F2-ovisac	1	33	0.925	0.98	0.005	99.93	4,835
			2	71	1.096	0.99	0.03	93.59	0,454 <sup>1</sup>
	All instars	Total	104	1.109	1.01	0.02	96.42	-0,722 <sup>1</sup>	
		1	61	7.079	1.41	0.04	94.75	-12,104	
		2	97	4.677	1.42	0.03	95.78	-16,223	
	Total	158	5.495	1.39	0.03	95.12	-17,094		

549

550 <sup>1</sup> Indicates t-value for slope = 1 (P > 0.05).

551

552 **Figure legends**

553 **Figure 1.** Taylor's power law regression for *Delottococcus aberiae* on developing fruits during  
554 the damaging period (between petal fall and July) ( $R^2 = 95.12$ ).

555 **Figure 2.** Enumerative sampling for *Delottococcus aberiae* in citrus. Number of fruits required  
556 based on the mean number of mealybugs pre fruit based on Green's method (1970) to achieve a  
557 precision level of  $D = 0.25, 0.30$  and  $0.35$ . The vertical line represents the obtained EEIL (0.24  
558 insects per fruit).

559 **Figure 3.** Relationship between the percentage of occupied fruits and the mean population  
560 density of *Delottococcus aberiae* in citrus. Solid line represents Wilson and Room's theoretical  
561 model.

562 **Figure 4.** Binomial sampling for *Delottococcus aberiae* in citrus. Number of fruits required  
563 based on the percentage of occupied fruit based on Kuno's method (1986) to achieve a precision  
564 level of  $D = 0.25, 0.30$  and  $0.35$ . The vertical line represents the obtained EEIL (0.24 insects per  
565 fruit).

566 **Figure 5.** Relationship between the maximum fruit occupation throughout the damaging period  
567 (petal fall until July) and the percentage of damaged fruit by *Delottococcus aberiae* at harvest  
568 ( $R^2 = 0.85$ ;  $P < 0.001$ ;  $n = 28$ ). Each point represents an orchard during one year (the maximum  
569 percentage of occupied fruits throughout the damaging period was considered only if more than  
570 12 fruits were counted per tree).

571

572

573 **Supplemental material**574 **Supp. Table S1.** Sampled sites and years, number of trees sampled per orchard, citrus variety and cover crop.

Sampling year	Locality	Number of trees	Citrus variety	Ground cover	Used for
2014-2015	Algimia	8	Clemenules clementine	wild weed	EIL(2014), aggregation pattern
2015	Almenara	8	Ortanique	poaceae grass	EIL, aggregation pattern
2014-2015	Benifairó de les Valls	8	Clemenules clementine	wild weed	EIL(2014), aggregation pattern
2014	Benifairó de les Valls	6	Oroval clementine	wild weed	EIL, aggregation pattern
2015	Benifairó de les Valls	8	Marisol clementine	poaceae grass	EIL, aggregation pattern
2015	Benifairó de les Valls	10	Sanguinello	bare soil	EIL, aggregation pattern
2015	Benifairó de les Valls	10	Oroval clementine	wild weed	EIL, aggregation pattern
2015	Faura	8	Clemenules clementine	poaceae grass	EIL, aggregation pattern
2014-2015	Quart de les Valls	8	Clemenules clementine	bare soil	EIL, aggregation pattern
2014	Quart de les Valls	8	Powell Navel	poaceae grass	EIL, aggregation pattern
2014-2015	Quart de les Valls	10	Oroval clementine	bare soil	EIL, aggregation pattern
2014-2015	Quart de les Valls	10	Oroval clementine	bare soil	EIL, aggregation pattern
2015	Quart de les Valls	8	Orri	poaceae grass	EIL, aggregation pattern
2015	Quart de les Valls	8	Ortanique	bare soil	EIL, aggregation pattern
2015	Quart de les Valls	8	Powell Navel	poaceae grass	EIL, aggregation pattern
2015	Quart de les Valls	8	Clemenules clementine	poaceae grass	EIL, aggregation pattern
2014-2015	Quartell	10	Powell Navel	wild weed	EIL, aggregation pattern
2014-2015	Quartell	10	Sanguinello	wild weed	EIL(2015), aggregation pattern
2014-2015	Quartell	10	Powell Navel	wild weed	EIL, aggregation pattern
2015	Quartell	8	Sanguinello	wild weed	EIL, aggregation pattern
2015	Quartell	8	Ortanique	poaceae grass	EIL, aggregation pattern
2014	Vall d'Uixó	8	Moncada	bare soil	EIL
2014	Quart de les Valls	8	Clemenules clementine	wild weed	EIL, aggregation pattern

