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Abstract

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

Introduction

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

Delottococcus aberiae De Lotto (Hemiptera: Pseudococcidae) is the latest invasive mealybug pest introduced in Mediterranean citrus. The genus *Delottococcus* is common in South Africa and recent studies have demonstrated that invasive populations of *D. aberiae* are native to Limpopo province (NE within South Africa) where citrus is irregularly distributed (Paul 2006, Beltrà et al. 2015). There, *D. aberiae* is also found in wild olive trees (homogeneously distributed at low densities) and on the roots of the flowering shrub *Chrysanthemoides monilifera* (L.) T. Norl (Miller and Giliomee 2011). This mealybug is not considered a pest in South Africa, remaining unnoticeable over decades (Hatting et al. 1998, Miller and Giliomee 2011). Contrarily, since the first individuals of *D. aberiae* were discovered in the core center of the main citrus producing area in the Mediterranean basin (northern part of Valencia province) in 2009 (Beltrà et al. 2013a), damage became noticeable on citrus fruit. Distinctively to other species of citrus mealybugs, *D. aberiae* causes severe direct effects such as distortions and size reduction in fruit which fosters a high depreciation of its commercial value (Beltrà et al. 2013a). This mealybug it also triggers indirect effects arisen from the excretion of honeydew, mainly due to the growth of sooty mold fungi *Capnodium citri* Berk (Capnodiales: Capnodiaceae). In addition, it can give shelter to other secondary pests such as pyralid moths, mites or scale insects. Due to all these negative effects, in those orchards with the presence of *D. aberiae*, the integrated pest management has been partially disrupted by the urgency of the farmers to apply chemical treatments against this new pest, which in most cases are not compatible with a system based on conservation biological control (Franco et al. 2004, Wajnberg et al. 2004, Jacas and Urbaneja, 2010).

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

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

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

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

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

Materials and methods

Sampled orchards

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

Sampling procedure

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

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

Aggregation indices

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

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

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

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

Sampling protocol

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

Binomial sampling

This sampling method estimates densities from occupied and unoccupied organs by the insect. It is used when insect populations show a high aggregation pattern and when there is a correlation between the proportion of sample units infested with the insect (p) and their mean number per sample unit (m). It also enables one to make decisions at less cost than with the enumerative sampling in IPM (Wilson and Room 1983).

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

$$p = 1 - \exp\left[-m \ln\left(a\, m^{b-1}\right) / \left(a\, m^{b-1} - 1\right)\right]$$

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

$$N = D^{-2} (1-p_0)\, p_0^{-(2/k)-1} \left[k \left(p_0^{-1/k} - 1\right)\right]^{-2}$$

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

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

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

Enumerative sampling

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

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

Economic injury levels

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

EIL = C / VIDK

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

EIL= C / VIDK = C / VbK

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

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

Results

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

Aggregation index

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

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

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

Enumerative sampling plan

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

Binomial sampling plan

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

Economic injury level

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

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

$V = 0, 22$ € kg^{-1} x 30 000 kg ha^{-1} =6 600 € ha^{-1}

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

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

Sample size

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

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

Discussion

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

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

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

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

Here we propose a binomial sampling of 275 fruits randomly selected per orchard with a precision of D = 0.30. According to our results, the enumerative sampling needs a lower number of fruits and provides more accurate results but it is more time-consuming. Fruits have to be collected and examined with a stereomicroscope to count the number of *D. aberiae* nymphs under the sepals. All this process could last around six hours considering that the citrus producer has a stereomicroscope in the sampled orchard. By contrast, binomial sampling does not require fruit harvest and *D. aberiae* presence can be determined with a magnifying glass. Moreover, the reduced visibility of first instars is balanced by their high aggregation patterns. Taking all into consideration, the binomial sampling process could last around fifteen minutes and two hours (considering that 30 sec are necessary to sample a fruit). Monitoring techniques based on direct observations of fruit and counting individuals have been widely used in IPM of other mealybug species (Cavalloro 1983, Ripollés 1990, Barbagallo et al. 1993, Roltsch et al. 2006, Mgocheki and Addison 2009). However, the use of plant material is a laborious and time consuming task compared to alternative monitoring techniques based on passive sampling (Geiger and Daane 2001, Waterworth et al. 2011). In this sense, we have recently shown that *D. aberiae* density on plant is highly related with pest level in corrugated cardboard bands in trunks (Martínez-Blay et al. submitted). Further research might determine whether this technique can be used as a sampling method making it simpler and less time-consuming. In fact, these techniques have already been used in several biological control programs in order to monitor population densities of mealybugs and also to evaluate the impact of their natural enemies, mainly predators (Browning 1959, Furness 1976, Goolsby et al. 2002).

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

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

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

Sample unit	Life stage	Generation	Samples (n)	a	b	SE(b)	R^2	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[1]
		Total	104	1.109	1.01	0.02	96.42	-0,722[1]
	All instars	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

[1] Indicates t-value for slope = 1 (P > 0.05).

Figure legends

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

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

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

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

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

Supplemental material

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