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Density and phenology of the invasive mealybug *Delottococcus aberiae* on citrus: implications for integrated pest management

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Author contribution statement

AS and AT conceived and designed the research. VM, JP and AT participated in data collection. VM, AT and AS analyzed the data. All authors wrote, read and approved the manuscript.

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Abstract

Delottococcus aberiae De Lotto (Hemiptera: Pseudococcidae) is a new invasive citrus pest in Spain. It causes severe fruit distortions and, as a new invasive mealybug, there is a lack of information about its biology. This research aims to examine the seasonal trend of *D. aberiae* in citrus, using several sampling methods, as a first step to develop an integrated pest management program. Ten citrus orchards from Eastern Spain were periodically sampled during three years using absolute (plant material) and relative (corrugated cardboard band traps and sticky traps) sampling methods. The three sampling methods showed that *D. aberiae* completes multiple generations per year, two of them being clearly defined and resulting in high populations. *D. aberiae* peaked between May and June, damaging the developing fruit. Corrugated cardboard band traps were able to detect prepupa and pupa male instars and gravid females, providing a quantitative measurement of *D. aberiae* density at its first population peak. The use of corrugated cardboard band traps is recommended to monitor population levels and sticky traps to determine male flight periods, representing simple sampling techniques to monitor *D. aberiae*. These results will improve the sampling protocols and allow for the development of an integrated pest management program.

Keywords: corrugated and sticky traps, life cycle, sampling protocols, *D. aberiae*, citrus

Key message

- *Delottococcus aberiae* is a new invasive citrus pest in Europe and its biology is unknown.
- This work aims to study the density of developmental stages of *D. aberiae* throughout the year.
- Different sampling methods showed that *D. aberiae* completes several generations. Two of them are clearly defined and result in high population levels.
- These results are the first seasonal population trend of *D. aberiae* in citrus and may serve as a basis for an integrated pest management program.

Introduction

The globalisation process and the increase in the international trade of ornamental and crop plants has led to an exponential rise in the introduction and establishment of alien and invasive insects in Europe (Pellizzari and Germain 2010; Pellizzari and Porcelli 2014; Roques et al. 2009). Mealybugs (Hemiptera: Pseudococcidae) are the second most diverse family of scale insects (Coccoidea), comprising around 2,000 species distributed worldwide and including many agricultural and ornamental pests which can cause substantial damage (Ben-Dov 1994; García-Morales et al. 2016; Hardy et al. 2008). Due to their small size and cryptic behavior, many mealybug species live in hidden habitats and are often unnoticed during quarantine inspections. Therefore, they are easily introduced into new areas through international plant trade. Once in a new territory their high fecundity favours rapid invasion, constituting an ecological and economic threat to many agricultural and ornamental ecosystems (Hulme et al. 2008; Kenis et al. 2009; Mansour et al. 2017; Miller et al. 2002; Pellizzari and Germain 2010; Pimentel et al. 2001).

In Europe, mealybugs represent the third most numerous family of alien insects; since the 1990s, several species have been recorded as new invaders in the Mediterranean Basin, some examples are *Dysmicoccus brevipes* (Cockerell) (Suma et al. 2015), *Paracoccus marginatus* Williams & Granara de Willink (Mendel et al. 2016), *Phenacoccus defectus* Ferris (Mazzeo et al. 2014), *Phenacoccus solani* Ferris (Mazzeo et al. 1999), *Pseudococcus comstocki* (Kuwana) (Pellizzari 2005) or *Phenacoccus peruvianus* Granara de Willink (Beltrà et al. 2010). Most of these mealybugs have established in anthropogenic habitats, such as cultivated agricultural lands, urban environments, nurseries or greenhouses (Pellizzari and Germain 2010; Roques et al. 2009). The Mediterranean Basin is one of the largest areas of citrus production and one of the leading exporting regions in the world (Lacirignola and D'Onghia 2009). In this area, six alien mealybug species have been reported as citrus pests, these being of different origins and with different histories of invasion (Blumberg et al. 1999; Franco et al. 2000): *Planococcus citri* (Risso), *Pseudococcus cryptus* (Hempel), *Pseudococcus longispinus* (Targioni-Tozzetti), *Pseudococcus calceolariae* (Fernald), *Pseudococcus viburni* (Signoret) and *Nipaecoccus viridis* (Newstead). Among them, *P. citri*, is the most damaging mealybug in Mediterranean citrus, having a wide distribution as a result of international trade (Franco et al. 2004).

Delottococcus aberiae (De Lotto) (Hemiptera: Pseudococcidae) is a mealybug of Southern African origin. It has been reported as a species that feeds on different tropical and subtropical crops, such as citrus, coffee, guava, pear or olive (De Lotto 1961; Miller and Giliomee 2011). In South African citrus orchards it is considered a secondary pest that can go unnoticed for years (Hattingh et al. 1998; Miller and Giliomee 2011). In 2009, nevertheless, *D. aberiae* was detected as an invasive species in Eastern Spain, with serious damages in citrus (Beltrà et al. 2013a), being identification confirmed by molecular and taxonomic techniques (Beltrà et al. 2015; Beltrà et al. 2012). Like other mealybug species, reduces plant vigour and excretes honeydew that promotes the growth of sooty mold fungi and interferes with plant photosynthesis (Franco et al. 2000). However, when *D. aberiae* develops on young citrus fruits causes severe distortions and fruit size reduction, leading to significant crop losses and representing a threat to Mediterranean citrus production (Beltrà et al. 2013a; Soto et al. 2016a). Since its establishment in Spain, different assays have revealed the absence of effective natural enemies to control *D. aberiae* outbreaks (Beltrà et al. 2013a; Soto et al. 2016b; Tena et al. 2017). Therefore, the management of the pest relies on the use of broad-spectrum insecticides, such as chlorpyrifos (Tena et al. 2014), which interferes with the biological control of other citrus pests (Franco et al. 2009; Tena and García-Marí 2011) .

Monitoring protocols improve pest detection, provide information regarding their seasonal occurrence and determine the expected susceptible periods. This information avoids unnecessary spraying and forms the basis of any integrated pest management (IPM) program (De Villiers and Pringle 2007; Gonzalez 1971). Sampling and monitoring mealybugs are processes based on different techniques which have improved their control in agricultural and ornamental ecosystems (Geiger and Daane 2001; Martínez-Ferrer et al. 2006; Mudavanhu et al. 2011; Walton et al. 2004; Waterworth et al. 2011). However, for most mealybug species, sampling consist of laborious and time consuming visual examination of plant material, searching for live insects and counting all life stages (Geiger and Daane 2001; Grimes and Cone

1985; Walton et al. 2004; Waterworth et al. 2011). Alternative monitoring techniques, mainly based on the use of different trap designs, have been developed to determine the mealybug's seasonal occurrence, being the most common ones corrugated cardboard bands and sticky traps (Beltrà and Soto 2012; Goolsby et al. 2002; Millar et al. 2002; Roltsch et al. 2006; Walton et al. 2004).

Corrugated cardboard band traps represent a non-destructive sampling method to monitor mealybug population densities (DeBach 1949; Furness 1976; Goolsby et al. 2002). The bands are wrapped around the trunk or main branches of the trees and serve as a refuge for gravid females to lay their eggs, or for second male instars to make their cocoon and develop into adults males (Beltrà and Soto 2012). This first method has been tested with positive results to sample *P. viburni* (Mudavanhu 2009), *P. longispinus* (DeBach 1949; Furness 1976) or *Maconellicoccus hirsutus* (Green) (Goolsby et al. 2002; Roltsch et al. 2006). Sticky traps are used to monitor some flying pests, including the winged adult males of different mealybugs (Grout and Richards 1991; Samways 1988; Sun et al. 2002). These traps are generally baited with sex pheromones to increase male captures and monitor their seasonal flight periods (Millar et al. 2002; Moreno et al. 1984; Mudavanhu et al. 2011; Walton et al. 2004). This second method has proven useful when monitoring species such as *P. calceolariae, P. citri, P. comstocki* or *M. hirsutus* (Moreno et al. 1984; Moreno et al. 1972; Rotundo and Tremblay 1975; Serrano et al. 2001), and two types of lures may be used to attract the males: live virgin females or synthetic sex pheromones (Meyerdirk et al. 2001).

D. aberiae is up to now a significant citrus pest only in Spain. Due to its recent designation as an invasive species, little is known about the biology and behavior of the insect in this crop. The main objectives of this work are: (i) to determine the seasonal trend of *D. aberiae* throughout the year, by absolute sampling methods (visual examination of plant material) and (ii) to compare the obtained results with relative sampling procedures (corrugated cardboard band traps and sticky traps) in order to identify simpler monitoring methods to establish *D. aberiae* density. These results will be used to improve its control within the existing IPM programs for citrus in Spain.

Materials and Methods

Survey sites

Ten commercial citrus orchards, which presented visual evidence of more than 50% of damaged fruits during previous seasons (400 fruits were sampled randomly in each orchard), were sampled in different areas of Eastern Spain from March 2014 to November 2016. Orchards sampled were carefully selected to avoid mixture with other mealybug species and to ensure that they contained almost exclusively *D. aberiae* populations. They ranged from 0.16 to 2 ha, five of them included sweet orange trees (*Citrus sinensis* (L.) Osbeck; Lane late, Navelina and Sanguinelli varieties) and the other five clementine mandarin trees (*Citrus reticulata* Blanco; Oroval and Clemenules varieties).

Absolute sampling protocol. Plant material

In each of the ten orchards, eight to ten trees were marked and sampled regularly between 2014 and 2016. In 2014 and 2015, samplings were done weekly, during the periods of most rapid mealybug development (March-August), and twice a month or monthly during the rest of the year, depending on population levels; in 2016 samplings were carried out at monthly intervals. No insecticide sprays were applied to the trees during the sampling period. For each sampling date, and at each sampling site, four 20-cm long twigs per marked tree, each one from a different cardinal orientation, were collected randomly from the middle and outer part of the canopy. A minimum of five orchards, fifty trees and two hundred twigs were always sampled simultaneously at each sampling date. Each twig included its leaves, flowers and fruits when these organs were available. Samples were bagged and transported to the laboratory inside a portable cooler. All the material was processed within the next 24 h. Each mealybug present on each twig, on four leaves per twig and on one to eight flowers or fruits (depending on their availability during the year) was counted under a stereomicroscope (Nikon SMZ645). Leaves, flowers and fruits to be examined from each twig were randomly selected. The sex and instar of each mealybug were also recorded. To separate between developmental stages, a laboratory colony of *D. aberiae* was

established at Universitat Politècnica de València (UPV) in 2013, using specimens collected from a clementine orchard located in Quart de les Valls (Valencia, Spain). Previous to starting field samplings, a laboratory assay was done. In this assay, direct observations were carried out, every 24h, in search of successful development from one instar to the following one, being the passage recognized by the presence of exuviae. Afterwards, 20 mealybugs of each instar, obtained from the laboratory colony and successfully molted, were measured (Martínez-Blay et al., in prep.). The following body length ranges were obtained and used to separate instars: first nymphal instar (0.40-0.69 mm), second nymphal instar (0.70-0.98 mm), third nymphal instar (0.99-1.40 mm) and females (>1.41 mm), in the latter case separating young from gravid females. Thus, for routine samplings, mealybugs were separated by measuring them with a stereomicroscope fitted with an ocular micrometer. Males and females of the first and second nymphal instars were pooled together as sex cannot be distinguished until the end of the second instar (Beltrà et al. 2013b; Gullan and Martin 2009).

Relative sampling methods. Traps

In the present study two types of traps were used to capture mealybugs: corrugated cardboard band traps and adapted sticky traps. Both types of traps were placed in five of the ten sites surveyed. Traps were sampled with the same periodicity as plant material. In 2014 and 2015, samplings were done weekly, twice a month or monthly, depending on population levels, and in 2016 samplings were carried out at monthly intervals. No insecticide sprays were applied to these trees during the sampling period.

Corrugated cardboard band traps were placed in five marked trees (in each of the sampled orchards). Four corrugated cardboard bands, of approximately 20 cm wide each, were placed in each tree: one around the trunk and three around the main branches. Traps were opened in the field at each sampling date, and the mealybugs were counted and separated into the following categories: nymphs (first, second and third instars), young females, gravid females and male cocoons (pre-pupa and pupa developmental stages together). After counting, each cardboard band was cleaned, with the help of a small brush, to remove all the present mealybugs and wrapped around the trunk and branches again.

In addition, two sticky traps were placed on two trees in each orchard (different from the ones used for corrugated cardboard band traps) at approximately 1.5 m above the ground in the southern external part of the canopy. Live virgin females were used as a bait to conform a special sticky trap, adapted from the ones previously used in similar studies (Grimes and Cone 1985; Meyerdirk and Newell 1979; Meyerdirk et al. 1981; Meyerdirk et al. 2001; Moreno et al. 1972; Serrano et al. 2001). Therefore, from this point on, these will be referred to as sticky sex pheromone traps. Each trap consisted of a 0.5 L plastic bottle containing one or two lemons and ten new virgin females. Each bottle had a modified lid, consisting of a fine mesh cloth, to allow ventilation and dispersion of the female sex pheromone to attract adult males. Females were obtained from the laboratory established colony. A yellow sticky card, 20 × 12.5 cm (ECONEX S.L.) was attached to each bottle with two plastic clothes pins. At each sampling date, virgin females were replaced with new ones and all yellow sticky cards were changed and transported to the laboratory, where the male mealybugs were counted under a dissecting microscope (Nikon SMZ645). To confirm that males counted were *D. aberiae*, at least ten males (fewer if 10 were not present) were removed from each trap and mounted following the procedure describe by Beardsley (1960). A drop of lemon extract was used to remove the males from the sticky surface of the traps. Afterwards, they were compared, based on the morphology of their genitalia (Afifi 1968; Beardsley 1960; Tremblay et al. 1977), with other mealybug species present in citrus in Eastern Spain (*P. citri*, *P. longispinus* and *P. viburni*) and with *D. aberiae* males obtained from our established laboratory colony. It was possible to separate *D. aberiae* from the rest of species taken into account the anal pair of filaments and the form of the genital capsule and the genital style (Martínez-Blay et al., in prep.).

Data analysis

Data from the seasonal monitoring of *D. aberiae*, by both absolute and relative sampling methods, are presented graphically to show the seasonal abundance trends of the pest. The number of mealybug generations per year was determined by plotting the percentage of each developmental stage per sample

unit over time. To compare differences in population abundance between the years 2014 and 2015, the mean number of mealybugs capture from March to December, per sample unit was calculated. Data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. As data were normally distributed but with unequal variances, an unequal variance t-test (Welch's t-test) was performed to compare means between the two years. An analysis of covariance test (ANCOVA) was made to check the potential effect of the year and the average number of mealybugs captured on traps at the first peak (corrugated cardboard band traps or sticky sex pheromone traps) on the average number of mealybugs per orchard and sample unit at the first *D. aberiae* population peak. Depending on the influence of the factor year, the relationship between the average number of mealybugs per sample unit and the average number of mealybugs per trap at the first peak was plotted and compared, using regression analysis, pooling all data together or separating data by year (Fig. 4). Data collected during 2016 were excluded from all the analysis because samplings were performed much less frequently than in 2014 and 2015. All statistical analyses were conducted using IBM SPSS version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Seasonal trend of *D. aberiae* by absolute sampling methods

The development of *D. aberiae* showed a similar trend over the three-year periods of study. Mealybugs completed multiple generations during the year, as illustrated by first nymph instars or crawler peaks (Fig. 1). Two of these generations were clearly defined every year. The first one was recorded in spring, coinciding with a peak of crawlers between mid-May and early June, with a percentage of crawlers, of the total population, of 89.40 ± 4.04 %, 87.09 ± 4.88 % and 59.46 ± 4.16 % in 2014, 2015 and 2016 respectively. The second one was recorded in summer, between mid-July and mid-August, with percentages of 74.75 ± 3.69 %, 73.54 ± 9.91 % and 66.49 ± 9.86 % each consecutive studied year. These two main generations were those which resulted in a high population density of the pest. The rest of the crawler peaks were not so well defined, probably due to overlapping generations and the low population density after August (Fig. 2).

The density of *D. aberiae* populations started to increase rapidly in April and May, leading to a first population peak in spring, at the end of May-beginning of June, and a second one in summer, between July and August. Both peaks occurred at the crawler emergence periods (Fig. 2). The spring peak population density reached an average (mean ± standard error, SE) of 6.92 ± 0.30, 1.21 ± 0.07 and 0.69± 0.05 mealybugs per sample unit in 2014, 2015 and 2016. In summer, *D. aberiae* population density recorded a mean value, respectively for each year, of 4.55 ± 0.29, 2.85 ± 0.23 and 1.77± 0.13 mealybugs. Afterwards, the population decreased and was almost undetectable in autumn and winter (Fig. 2). Populations were more abundant in 2014 (mean ± SE: 1.86 ± 0.08 mealybugs per sample unit) than in 2015 (0.56 ± 0.03) (t = 16.04, df = 444, P < 0.001).

Seasonal trend of *D. aberiae* by relative sampling methods

Corrugated cardboard band traps caught mainly gravid females and immature, prepupa and pupa, male instars (nymphs and young females were trapped at very low levels and are not represented on Fig. 3), whereas sticky sex pheromone traps attracted adult males. Corrugated cardboard band traps captured immature male stages over the three-year study, captures being much more abundant in the year 2014. Two peaks for these male instars could be observed each year. The first one was recorded at the end of March-beginning of April, with 40.55 ± 2.51, 12.01 ± 1.42 and 8.79 ± 0.73 males per trap (mean ± SE) in 2014, 2015 and 2016 respectively. The second maximum was reached at the end of May-beginning of June, with an average of 69.58 ± 5.65, 6.15 ± 1.21 and 9.10 ± 0.91 males per year. Gravid females were very abundant in corrugated cardboard band traps during certain periods of the year, especially in 2014. Two peaks of females with egg sacs were detected each year. The first one was reached at the end of April-beginning of May, with 67.84 ± 3.84, 22.40 ± 2.11 and 10.31 ± 0.97 females per trap (mean ± SE)

in 2014, 2015 and 2016. The second one was recorded at the end of June, with an average of 107.64 ± 8.53, 9.42 ± 0.95 and 25.02 ± 1.79 females each consecutive studied year. During the rest of the year, female populations in corrugated cardboard band traps remained at undetectable levels.

Delottococcus aberiae was the only mealybug species collected and identified in the sticky sex pheromone traps. Two main peaks of captures were recorded both years of the study, corresponding with two distinct flights. The first one occurred at the end of March-beginning of April, with an average (mean ± SE) of 66.40 ± 7.9, 48.14 ± 14.41 and 9.33 ± 2.40 males in 2014, 2015 and 2016 respectively. The second one was between the end May and the beginning of June, with 41.20 ± 5.75, 17.57 ± 5.49 and 42.33 ± 9.10 males per trap in 2014, 2015 and 2016. The number of males out of those periods decreased considerably; sticky sex pheromone traps were only able to detect small increases in mealybugs between July and December, and at the beginning of the year, but captures were always below the average of 5 males per trap (Fig. 3). Males and females were captured successively over time in the traps (Fig. 3): firstly, males in the stages of prepupa and pupa were detected in corrugated cardboard band traps; secondly, adult males were found in sticky sex pheromone traps and finally gravid females were captured in corrugated cardboard band traps.

ANCOVA tests showed a significant relationship between the average number of mealybugs per sample unit, at the first *D. aberiae* population peak (end of May), and the average number of *D. aberiae* males caught in sticky sex pheromone traps for each orchard (F = 9.94; df = 1, 9; P = 0.02) and the average number of gravid females (F = 39.99; df = 1, 9; $P < 0.001$) and immature male instars (F = 12.81; df = 1, 9; P = 0.01) captured in corrugated cardboard band traps. This relationship differed significantly between years for male captures in sticky sex pheromone traps (F = 52.35; df = 1, 9; $P < 0.001$) but not for gravid females (F = 1.13; df = 1, 9; P = 0.32) or immature male instars in corrugated cardboard band traps (F = 52.35; df = 1, 9; P = 0.08). Thus, the total average number of *D. aberiae* per plant sample unit and orchard at the first *D. aberiae* population peak was regressed, considering data from both years together, in comparison with the average number of gravid females ($y = 0.18x - 2.49$; df = 1,9; F = 273.72; $P < 0.001$; $r^2 = 0.97$) and immature male instars ($y = 0.25x - 1.45$; F = 62.69; df = 1,9; $P < 0.001$; $r^2 = 0.89$) per corrugated cardboard band trap and orchard, showing a significant and positive relationship (Fig. 4). Besides, the total average number of *D. aberiae* per plant sample unit and orchard at the first *D. aberiae* population peak was regressed in comparison with the average number of adult males per sticky sex pheromone trap and orchard, but for each year independently (Fig. 4) (2014: $y = 0.12x + 1.99$; F = 43.53; df = 1,4; P = 0.08; $r^2 = 0.94$ / 2015: $y = 0.02x + 0.63$; F = 56.88; df = 1,4; P = 0.01; $r^2 = 0.95$).

Discussion

The main purpose of the current study was to determine the seasonal trend of the new invasive pest *D. aberiae*, on citrus, as a basis to design sampling protocols and improve its control. Our results reveal that *D. aberiae* density increased in spring, reaching its first significant maximum during May and June, coinciding with fruit development. High population levels developed on fruits until the end of August, when populations decreased and remained at very low levels for the rest of the year. These results are the first quantitative description of *D. aberiae* biology on any crop. The rapid decrease at the end of the summer, and significant differences in mealybug abundance between years, might be a consequence of different biotic and abiotic factors, such as climate, the action of natural enemies or the quality of the feeding substrate. The high temperatures and low humidity that frequently occur during summer, in countries with Mediterranean climate, may cause high mortality in mealybugs, especially of first instars (Bartlett and Clancy 1972; Browning 1959; Furness 1976). The population levels of *D. aberiae* were lower in 2015 than in 2014. In 2015, unusually low temperatures and heavy rains occurred at the end of March, followed by a period of very high temperatures with low humidity levels in April (Benavites data, IVIA SIAR's Weather Net, http://riegos.ivia.es/datos-meteorologicos). The combination of these two consecutive climatic factors might have negatively affected *D. aberiae* in May, as populations did not increase as much as in May of 2014. Moreover, this decrease occurred in all the sampled orchards. The effect of the natural enemies cannot explain this reduction as native and naturalized parasitoid species do

not develop on *D. aberiae* (Tena et al. 2017). The predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) attacks *D. aberiae*, but always after May (Pérez-Rodríguez et al. in preparation). This predator is abundant in June and peaks at the beginning of August, contributing to the decline of mealybug populations at the end of summer and fall. Besides, in the year 2016, sampling was carried out only in five orchards, which already had low levels; this factor might also have contributed to the fact that population levels were even lower than in 2015.

However, the most limiting factor of mealybug populations feeding in citrus trees, during the second half of summer, seems to be the quality of the feeding substrate. As eurymeric species, mealybugs are able to feed on different organs of the host plant (Kozár 1989), but not all the organs constitute a food source of equal quality. Therefore, it is expected that the development and fecundity of the mealybugs vary according to the organ in which they are located (Franco et al. 2000). One of the factors affecting mealybug's distribution, in the different plant parts, is the phenology of the host. Franco (1994) suggested that immature feeding stages of mealybugs on citrus tend to settle at the major carbohydrate sinks of the host plant in each phenological period and Haviland et al. (2012) showed that feeding location of *Ferrisia gilli* Gullan corresponded with carbohydrate allocation in pistachio trees. Most mealybug species are phloem feeders (McKenzie 1967) and their populations follow the movement of plant nutrients. The developing fruit in citrus is a strong sink of carbohydrates, giving better conditions, in terms of food quality, for the development of mealybugs (e. g. higher fecundity) (Franco 1994). Thus, during the period of fruit set and development in citrus, mealybugs tend to aggregate and concentrate on fruits and *D. aberiae* is not an exception. However, in August the physiology of the citrus tree changes and fruits lose their intensive flow of nutrients (Agustí 2003; Franco 1994). This supposes a decrease in the food quality of fruits and, therefore, in female's fecundity, these factors influencing the reduction of population levels.

In this study, absolute sampling methods showed that *D. aberiae* completed several generations per year, remaining active even during winter. Regarding the number of generations, two were clearly defined each year due to a concentrated and homogeneous crawler emergence (Fig. 1): the first one took place in spring and the second in summer, those two generations being the only ones capable of causing fruit distortion and size reduction during fruit development (Martínez-Blay in prep.) The other peaks of crawlers were heterogeneous and varied between years. These generations did not increase *D. aberiae* density and tended to overlap between them (Fig. 2). These overlapping generations resulted in the mix of developmental stages present at the end of the year. Apparently, at least three more generations may occur depending on the year and the environmental conditions: one between January and February, another one between August and October and one more between October and December. Of these, the generation between August and October is the most remarkable, being frequently observed and better defined than the others (Fig. 1). Afterwards, populations remain at very low levels. Similar studies carried out in the Mediterranean Basin with other mealybug species of agronomic and ornamental importance, such as *Phenacoccus madeirensis* Green (Longo et al. 1995), *P. peruvianus* (Beltrà et al. 2013b), *P. citri* (Martínez-Ferrer et al. 2003; Santorini 1977) or *P. viburni* (Panis 1986), showed a similar pattern with several, usually overlapping, generations throughout the year. The overlap of development stages has relevant implications for mealybug management. The host stage can influence the efficiency of natural enemies, especially parasitoids, and must be taken into account when designing future biological control strategies of mealybugs (Beltrà et al. 2013c; Islam and Copland 1997; Jervis et al. 2005). If chemical control is required to manage population outbreaks, we suggest monitoring just after petal fall period, before fruits are damaged (Martínez-Blay in prep.), when most of the individuals are in the first instar.

Monitoring *D. aberiae* populations by absolute sampling methods is a laborious and time-consuming process because it is necessary to count live insects present on plant material. In the present work, results based on plant material were compared with those obtained by simpler monitoring methods such as corrugated cardboard band traps and sticky traps. The two most harmful generations of *D. aberiae* were also detected by these relative sampling methods (Fig. 3). Corrugated cardboard band traps were able to detect immature male instars and gravid females because these instars tend to use the bands as a shelter to develop into male adults or to lay their eggs, respectively. Moreover, these relative levels of *D. aberiae* were highly correlated with mealybug levels in the canopy at the first population peak (Fig. 4).

Interestingly, this peak is also correlated with fruit damage at harvest (Pérez-Rodríguez et al., submitted). Therefore, corrugated cardboard band traps represent a suitable and simple sampling method to detect and quantify *D. aberiae* during this damaging period. This technique has been used in several biological control programs to monitor population densities of mealybugs and also to evaluate the impact of their natural enemies, mainly predators (Browning 1959; DeBach 1949; Furness 1976; Goolsby et al. 2002).

Our results indicate that *D. aberiae* virgin females use a sex pheromone to attract males, as a large number of them were captured. Sticky traps, baited with virgin females, provided evidence of two important flights, confirming the two main generations of *D. aberiae*, one between March and May and another between June and July (Fig. 3), matching subsequently periods of adult females producing egg sacs. The double peak of male captures in 2015 (March-April) has been considered to be part of the same flight and may be a consequence of the unusually low temperatures and heavy rains that occurred at the end of March and beginning of April. Mechanical action of rain drops and lower than expected temperatures, may have killed part of the population (especially young instars) and delayed the development of new males. Like corrugated cardboard band traps, sticky sex pheromone traps provided a quantitative measurement of *D. aberiae* density at its first population peak. However, and contrary to the former, there were significant differences between the sampled years, likely due to the effect of adverse conditions on male flights. Therefore, we would recommend the use of corrugated cardboard band traps to monitor population levels and sticky sex pheromone traps to determine flight periods. In fact, sticky sex pheromone traps are commonly used to monitor flight population peak periods (Suckling 2000; Way and van Emden 2000). Field trapping of males using sticky sex pheromone traps, with virgin females, has been carried out previously with good results for other mealybug species, including *M. hirsutus* (Serrano et al. 2001), *P. citri* (Moreno et al. 1984; Rotundo and Tremblay 1975), *P. calceolariae* (Rotundo and Tremblay 1975), *P. comstocki* (Meyerdirk and Newell 1979; Meyerdirk et al. 1981; Moreno et al. 1972) and *Pseudococcus maritimus* (Ehrhorn) (Grimes and Cone 1985). More recently, synthetic pheromones have been developed and tested for many mealybugs species such as *M. hirsutus* (Hall et al. 2008), *P. citri* (Martínez-Ferrer et al. 2003; Waterworth et al. 2011), *P. ficus* (Millar et al. 2002; Walton et al. 2004), *P. longispinus* (Waterworth et al. 2011), *P. viburni* (Mudavanhu et al. 2011; Waterworth et al. 2011) or *P. maritimus* (Bahder et al. 2013). Identification of the female sex pheromone would allow for the use of pheromone traps to monitor *D. aberiae* in IPM Schemes.

We have shown that *D. aberiae* completes multiple generations per year, two of them being clearly defined and resulting in high populations. Moreover, *D. aberiae* peaks between May and June and causes damage to developing fruit. Corrugated cardboard band traps and sticky pheromone traps are able to identify peak periods of *D. aberiae* populations; corrugated traps provide a quantitative measurement of *D. aberiae* density and are recommended to monitor population levels while sticky traps can be used to determine male flight periods. Both systems represent simple monitoring techniques to detect mealybug population outbreaks. These results are the first description of *D. aberiae* seasonal trend in citrus and may serve to improve the sampling protocols and develop an integrated pest management program.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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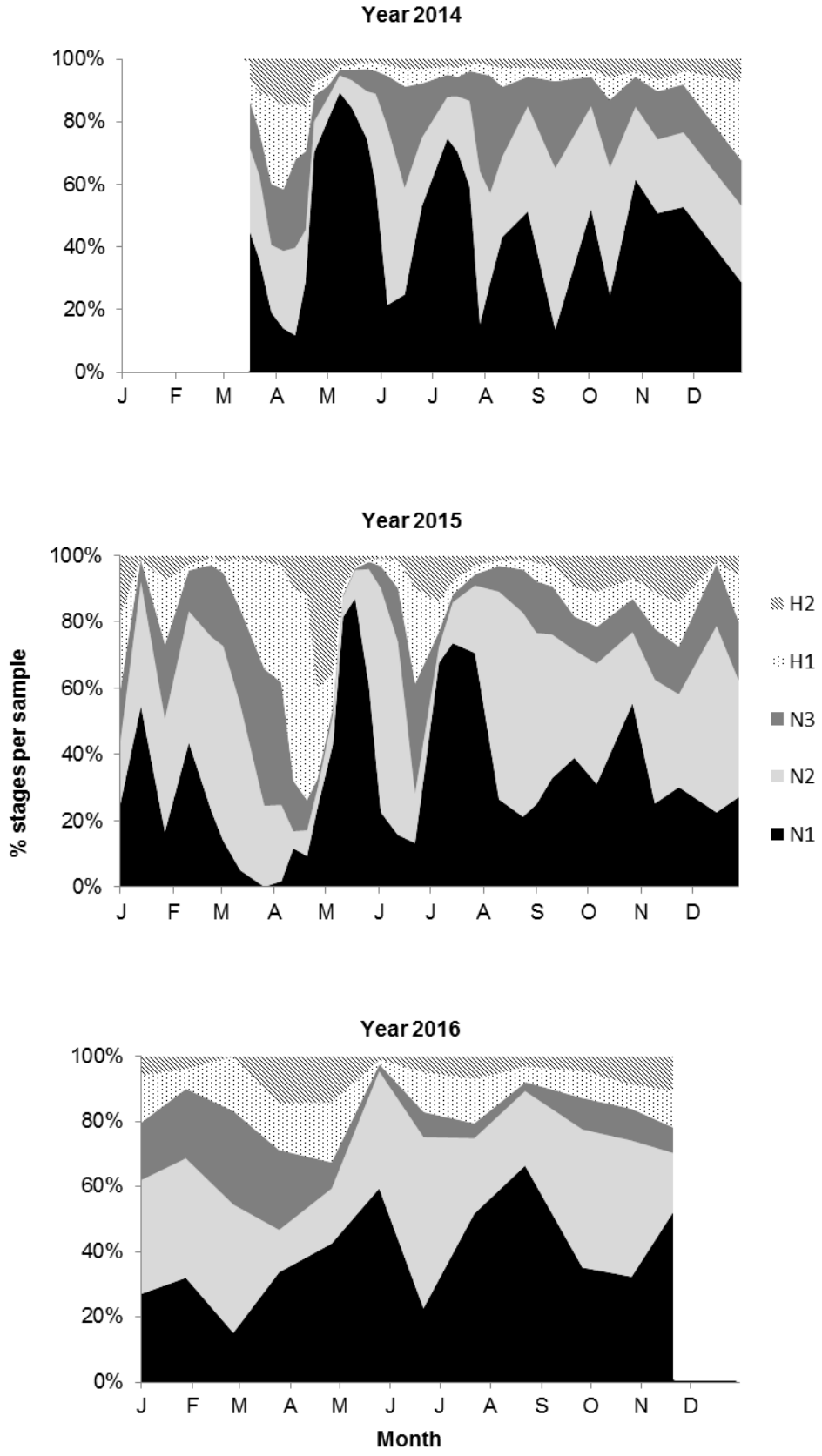
Figure legends

Fig. 1 Seasonal relative abundance of *D. aberiae* developmental stages in ten citrus orchards in Eastern Spain. Percentage of each developmental stage per sample unit and date is represented for the years 2014, 2015 and 2016 (N1 = first nymphal instars, N2 = second nymphal instars, N3 = third nymphal instars, H1 = young females, H2 = gravid females)

Fig. 2 Seasonal trend of *D. aberiae* populations in ten citrus orchards in Eastern Spain. Mean number of mealybugs ± SE collected per sample unit (total number and first instars). Above each graph the length of the flowering period (F), petal fall period (PF) and fruit developing period (FD) is presented

Fig. 3 Seasonal trend of *D. aberiae*, captured with two types of traps, during the years 2014, 2015 and 2016 in five citrus orchards in Eastern Spain. Presented as mean number of mealybugs ± SE captured in corrugated cardboard band traps (gravid females and immature male instars) and in sticky sex pheromone traps (adult males). Note that y-axis scales are different for 2014 and 2015-2016

Fig. 4 Relationship between the mean number of *D. aberiae* per plant sample unit and the mean number of individuals collected in different traps at the first population peak. a) Average number of *D. aberiae* per plant sample unit correlated with average number of gravid females ($y = 0.18x - 2.49$; $df = 1,9$; $F = 273.72$; $P < 0.001$; $r^2 = 0.97$) and immature males ($y = 0.25x - 1.45$; $F = 62.69$; $df = 1,9$; $P < 0.001$; $r^2 = 0.89$) per corrugated cardboard band trap. b) Average number of *D. aberiae* per plant sample unit correlated with the average number of adult males per sticky sex pheromone trap and year (2014: $y = 0.12x + 1.99$; $F = 43.53$; $df = 1,4$; $P = 0.01$; $r^2 = 0.94$ / 2015: $y = 0.02x + 0.63$; $F = 56.88$; $df = 1,4$; $P = 0.01$; $r^2 = 0.95$)


Year 2014
100%
80%
60%
40%
20%
0%
J F M A M J J A S O N D
Year 2015
% stages per sample
H2
H1
N3
N2
N1
Year 2016
Month

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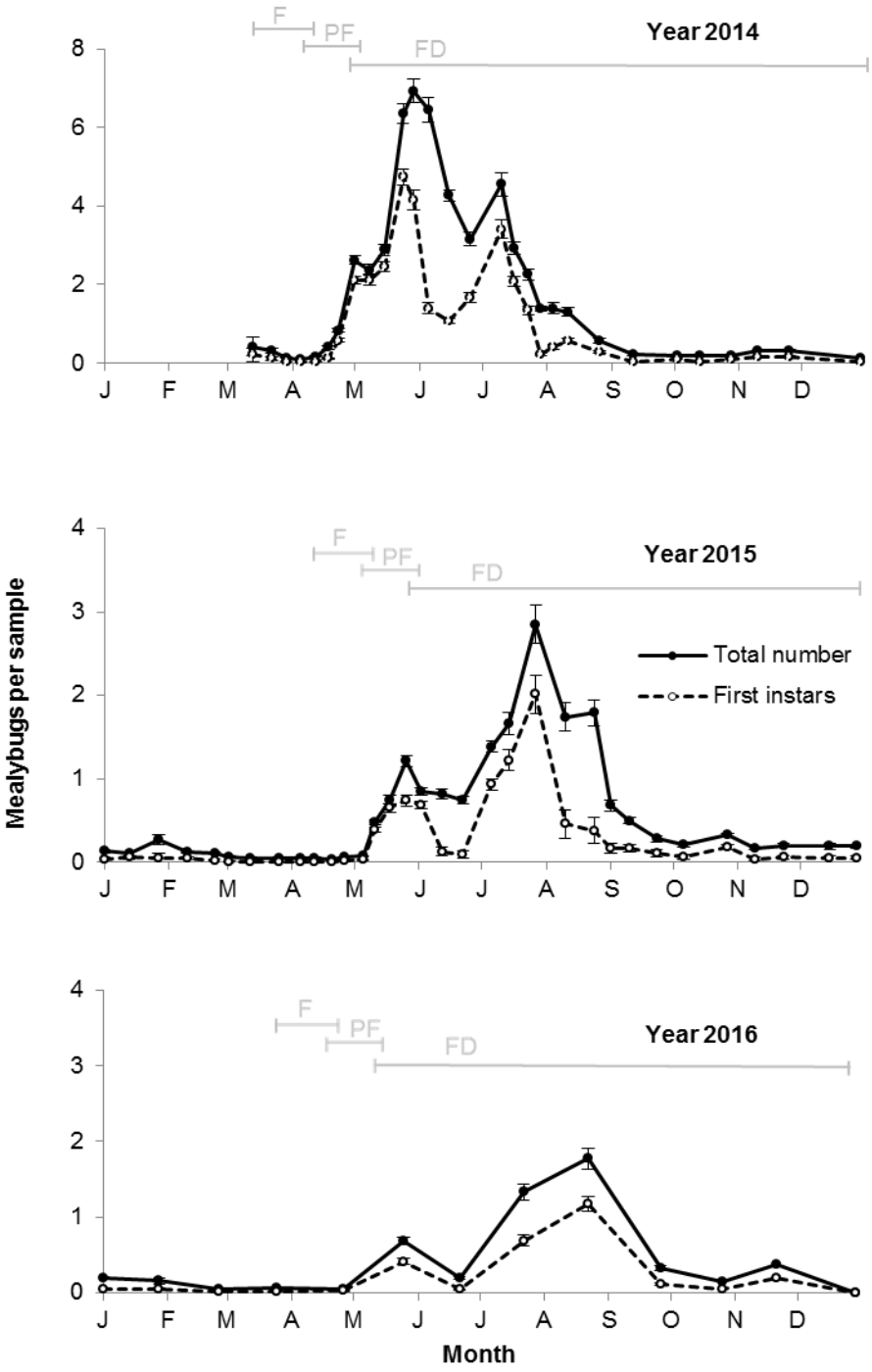
658 Fig. 1



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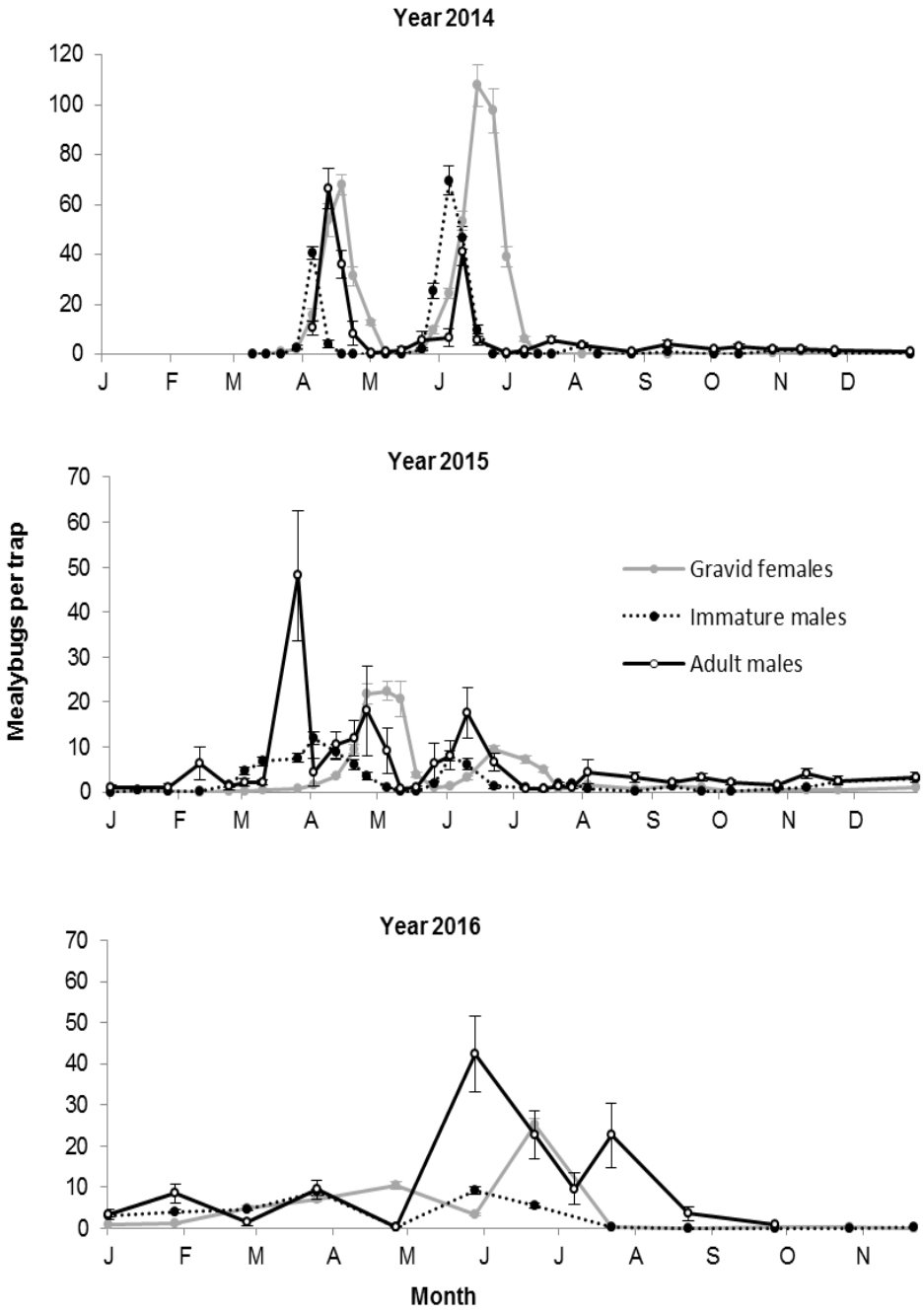

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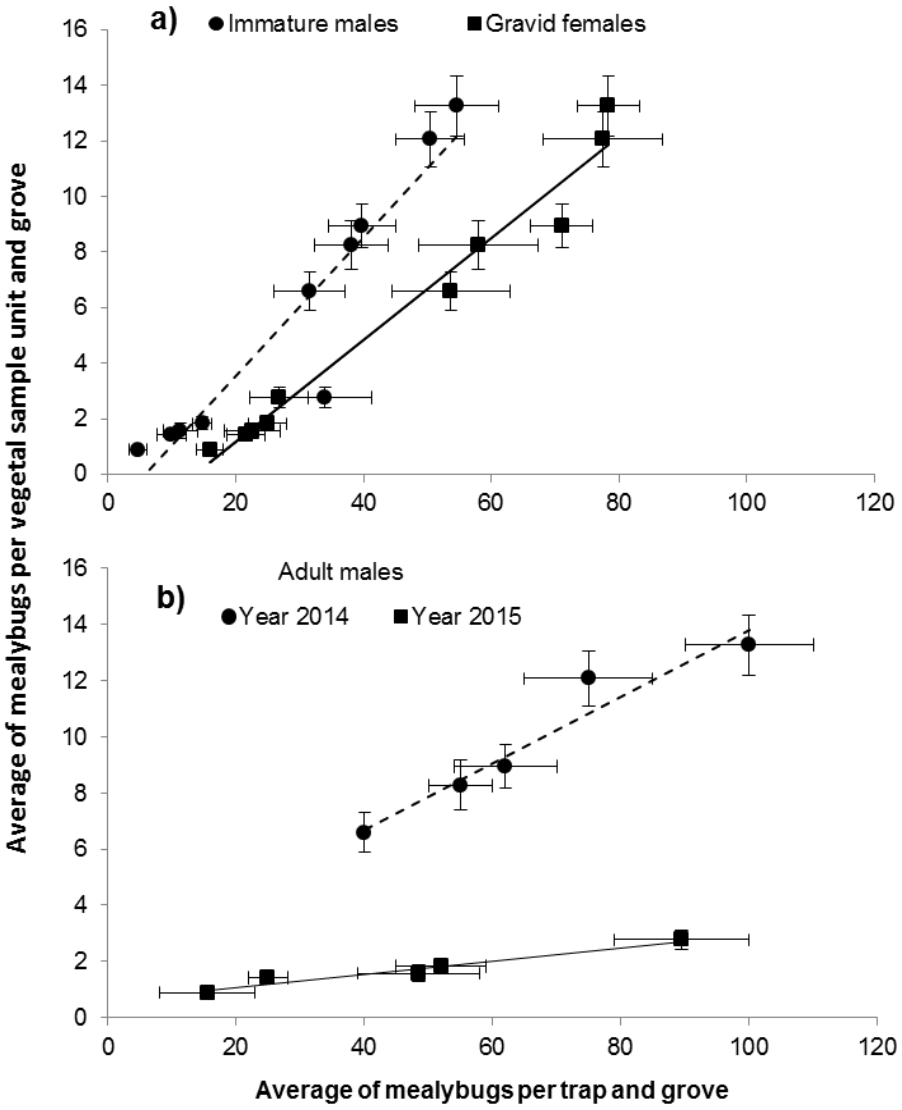

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