*Corresponding author:

Antonia Soto

Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València (UPV)

Camino de Vera s/n, 46022, València, (Spain)

Phone: +34 963879252, Fax: +34 963877331

E-mail: asoto@eaf.upv.es

Seasonal distribution and movement of the invasive pest *Delottococcus aberiae* (Hemiptera: Pseudococcidae) within citrus tree: implications for its integrated management

V. Martínez-Blay[1], J. Pérez-Rodríguez[2,3], A. Tena[2] and A. Soto[1*]

[1]Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València, Camino de Vera s/n, 46022, València, (Spain)

[2]Instituto Valenciano de Investigaciones Agrarias (IVIA). Centro de Protección Vegetal y Biotecnología. Carretera CV-315, Km 10,7,, 46113 Moncada, València (Spain)

[3]Departament de Zoologia. Facultat de Ciències Biològiques. Universitat de València, Carrer Doctor Moliner s/n. 46100 Burjassot, València (Spain)

Abstract

Delottococcus aberiae (De Lotto) is the most recent species of mealybug introduced to Spain that is impacting citrus. The feeding behavior of *D. aberiae* causes severe direct damage to citrus fruits, distorting their shape and/or causing reduction in size. There is no information available regarding its distribution within the citrus trees. The main objective of this study was to describe the seasonal distribution of *D. aberiae* within citrus trees and its migration patterns on the plants. Ten citrus orchards from eastern Spain were periodically sampled during three years. In each orchard, the mealybug was sampled in different infested strata (canopy, trunk and soil) and canopy structures (flower, fruit, leaf and twig). Results showed that, within the sampled strata, *D. aberiae* was mostly in the canopy. Within the canopy, the feeding location of *D. aberiae* changed throughout the year. *Delottococcus aberiae* overwintered in the twigs and moved to the flowers and fruits in spring. Once there, its populations started to increase exponentially until August. From February to September 5% to 30% of the mealybugs migrated to the trunk and soil. These results will facilitate an early detection of the pest in the areas where it is spreading and improve sampling protocols and pesticide applications.

Keywords: applied entomology, IPM, mealybug, migration

Introduction

Mealybugs (Hemiptera: Pseudococcidae) are considered one of the most significant agricultural pests worldwide, causing substantial economic loss when introduced into new areas without their natural enemies (Miller et al. 2002, García-Morales et al. 2016). These insects are small and have a cryptic lifestyle and, because of their habit of hiding in cracks and difficult places to observe, are often overlooked in agricultural inspections (Roques et al. 2009, Pellizzari and Germain 2010). Within this context, *Delottococcus aberiae* (De Lotto) is the latest invasive mealybug introduced in citrus in Spain. In 2009, *D. aberiae* was found to be causing significant damage in citrus orchards in the region of “Les Valls” (Valencia, eastern Spain) (Beltrà et al. 2012, García-Marí 2012, Beltrà et al. 2013b, Beltrà et al. 2015). Identification was confirmed by molecular and taxonomic techniques (Beltrà et al. 2012, Beltrà et al. 2015) and after an unsuccessful eradication program, based on the extensive application of chemical treatments, with insecticides authorized against mealybugs in Spain, in the initially affected areas, *D. aberiae* became established in the region (Beltrà et al. 2013b). Since then, the mealybug has been spreading, slowly but constantly, towards adjoining areas, becoming a significant pest in eastern Spain (Pérez-Rodríguez et al. 2017, Tena et al. 2017, Martínez-Blay et al. 2018b).

Delottococcus aberiae is native to sub-Saharan Africa, being a common species in South Africa (Miller and Giliomee 2011). Recently, it has been confirmed that the invasive populations in Spain are native to Limpopo province, in Northern South Africa (Beltrà et al. 2015). Like other mealybug species in Mediterranean conditions, such as *Planococcus citri* (Risso), *Pseudococcus longispinus* (Targioni-Tozzetti) or *Pseudococcus viburni* (Signoret), *D. aberiae* completes several generations throughout the year (Franco et al. 2000, Martínez-Ferrer et al. 2003, García-Marí 2012, Pérez-Rodríguez et al. 2017, Martínez-Blay et al. 2018b). Two generations are very clearly defined and result in high population levels between May and July. The rest of the year, apparently, three more overlapping generations may occur, depending on the climatic conditions: one between January and February, another one between August and

October and one more between October and December (Martínez-Blay et al. 2018b). From May to July, nymphs and adults settle and feed on fruitlets. However, unlike other mealybug species present in citrus in Spain, this feeding behavior causes severe direct damage to citrus fruits, distorting its shape (mainly protuberances around fruit calyx) and/or causing size reduction, which depreciates its commercial value (Pérez-Rodríguez et al. 2017, Martínez-Blay et al. 2018a, Martínez-Blay et al. 2018b) (Supp Fig. S1 [online only]). Similarly, other mealybug species also cause direct damage. For example, *Maconellicoccus hirsutus* (Green) causes severe distortions on hibiscus shoots, leaves and flowers, reducing its production and marketability (Meyerdirk et al. 2001, Chong et al. 2015). Direct damage caused by *D. aberiae* has been observed in all citrus cultivated in eastern Spain (sweet oranges, mandarins and hybrids) (Pérez-Rodríguez et al. 2017, Martínez-Blay et al. 2018b). Recently, the damaging period has been established, including the phenological stages between flowering (March-April in eastern Spain conditions) and fruits with a diameter of 25-30 mm (around July in eastern Spain conditions) (Pérez-Rodríguez et al. 2017, Martínez-Blay et al. 2018b). It has also been shown that distortions appear during this period because *D. aberiae* interferes with the fruit cell division process (Martínez-Blay et al. 2018a). Afterwards, at the end of summer, populations decrease and remain at low levels, but active, for the rest of the year (Martínez-Blay et al. 2018b).

The complex of natural enemies of *D. aberiae* in its native area is unknown, and since its introduction in Spain no effective biological control has been found: the existing parasitoids in Spain fail to control this pest (Tena et al. 2017) and the predators, mainly *Cryptolaemus montrouzieri* Mulsant, appear late when the damage to the fruit has already been done (Pérez-Rodríguez et al. 2017). Thus, the management of *D. aberiae* currently depends on the use of broad-spectrum insecticides (Pérez-Rodríguez et al. 2017). However, these applications interfere with the existing biological control of other citrus pests in the Mediterranean Basin (Franco et al. 2009, Tena and Garcia-Marí 2011), making essential the monitoring of the seasonal trend of the pest to avoid unnecessary spraying. Within this context, recent studies have shown that *D. aberiae* presents a clustered distribution on the plants they infest and that fruit damage at harvest is strongly correlated with fruit occupation in spring (Pérez-Rodríguez et al. 2017). Based on these results the Economic Injury Level (EIL) and the Economic Environmental Injury Level (EEIL) for *D. aberiae* have been calculated as 7.1 and 12.1% of occupied fruits in spring, respectively, being recommended to sample 275 fruits (binomial sampling) or 140 fruits (enumerative sampling) between petal fall and July (Pérez-Rodríguez et al. 2017).

Pest monitoring is a fundamental component of any integrated pest management program (IPM). The ability to predict future pest damage, based on early field counts, is valuable and necessary for good control decisions (Kogan 1998), especially in the case of cryptic species that can easily pass unnoticed. Previous research has shown that mealybugs migrate within the plant throughout the season (Geiger and Daane 2001, Haviland et al. 2012, Beltrà et al. 2013a, Wunderlich et al. 2013, Kumar et al. 2014). Thus, it is necessary to change sampling strategies throughout the year to detect and quantify the density levels of the pest in the infested stratum (canopy, trunk and soil) and structure (flower, fruit, leaf and twig) of the plant. This information has improved the control of many mealybug species affecting agricultural and ornamental ecosystems worldwide (Geiger and Daane 2001, Martínez-Ferrer et al. 2006, Mudavanhu et al. 2011, Haviland et al. 2012, Kumar et al. 2014). To date, there is no information available regarding *D. aberiae* distribution patterns within the citrus trees. This makes it difficult to detect early infestations of this mealybug, especially in the absence of typical damage symptoms.

The main objective of this work is to describe the seasonal distribution of *D. aberiae* within citrus trees and its migration patterns on the plants. This information will help to design better sampling protocols, facilitating an early detection of the pest and improving pesticide applications within the existing IPM programs for citrus in Spain.

Materials and Methods

Sampling sites and general sampling protocol

Ten citrus orchards infested with *D. aberiae* located in eastern Spain (region of Les Valls, Valencia) were sampled from March 2014 to December 2016. Five orchards 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). Within each orchard, eight to ten trees were marked and sampled regularly. These trees were not sprayed with pesticides during the sampling period. In 2014 and 2015, sampling was done weekly, during the periods of most rapid mealybug development (March-August), and bimonthly or monthly during the rest of the year. In 2016, to confirm previous results, sampling was done in five orchards at monthly intervals.

Canopy sampling protocol

At each sampling date, four 20-cm long twigs (each one from a cardinal orientation), with its leaves and flowers or fruits, were collected randomly from the canopy of each marked tree per orchard. Samples were bagged individually and transported to the laboratory, being examined under a stereomicroscope (Nikon SMZ645) within the next 24h. Mealybugs present on each twig, on four leaves per twig and on one to eight flowers or fruits (depending on their availability during the year) were counted. Leaves, flowers and fruits were randomly selected within the twigs. All developmental stages counted were pooled together, as data regarding the phenology of *D. aberiae* in the canopy of the tree has recently been published in a companion manuscript (Martínez-Blay et al. 2018b).

Trunk sampling protocol

Trunk samplings consisted of visual counts, during 2 minutes, of all the mealybugs present on the trunk and main branches of the trees (until 60 cm in height). Each mealybug counted was classified in one of the following categories: nymphs (first, second and third instars together), adult females, gravid females and immature males (pre-pupa and pupa).

To determine the direction of migration, the directionality of the movement was also recorded in 2015. That is if the mobile instars (nymphs and adult females) were ascending or descending the trunk. Immobile mealybugs were not considered for this analysis.

Soil sampling protocol

At each sampling date, four orchards and four trees per orchard were sampled from March 2014 to December 2015. From each tree, soil samples were collected at three distances horizontally from the base of the trunk (0-15 cm, 15-30 cm and 30-45 cm) and at each distance one per cardinal direction (North, South, East and West). This is 12 samples per tree and 48 samples per orchard. Each sample was collected from the soil surface and consisted of a circular area with a diameter of 10 cm and 2 cm depth that was bagged and transported to the laboratory. Once there, each sample was placed in a Berlese funnel for 48 hours. Afterwards each Berlese container was checked, under a dissecting microscope (Nikon SMZ645), for the presence of *D. aberiae*. Each mobile mealybug found was counted and classified into one of the following categories: first nymphal instar, second nymphal instar, third nymphal instar, adult females and adult males. Data from the three distances was used to determine the location of *D. aberiae* in the soil. Data from soil samples collected within a distance of 0 to 15 cm, horizontally from the base of the trunk, was used to analyze the seasonal trend of *D. aberiae* in the soil.

Data analysis

Sampling data of the different strata and structures were averaged per tree and afterwards per orchard, with the latter sampling unit used for the graphics and statistical analysis. Results from the samplings carried out in 2014 and 2015 are presented in all the figures. Data from the year 2016 are presented for the figures 1 and 2 (general strata distribution and distribution on tree canopy).

The percentage of mealybugs is represented per unit area (cm^2) to be able to compare the abundance of *D. aberiae* in each sampled structure (flower, fruit, leaf and twig) or stratum (canopy, trunk and soil) (Fig.1 and Fig.2).

The mean percentage of mealybugs per unit area (cm^2) and orchard in the soil between March and July [period in which *D. aberiae* causes damage to fruits and chemical treatments must be applied (Pérez-Rodríguez et al. 2017, Martínez-Blay et al. 2018a)] was compared using analysis of variance (ANOVA). Month was the explanatory variable and two ANOVAs were carried out, separately for 2014 and 2015, being means compared using Tukey tests (Fig. 1). Data were tested for homogeneity of variances using Levene's test. If required, percentage data were subjected to an angular transformation before analysis to satisfy model assumptions regarding homogeneity of variances and to approximate a normal distribution (Kasuya 2004).

The directionality of the movement of the mobile instars present on the trunk was analyzed separately for nymphs and adult females (Fig. 4). The number of nymphs and adult females were first averaged per tree and afterwards per orchard, using the mean per orchard for the statistical analysis. Within each month of the year 2015, t-tests were used to determine whether the mean number of nymphs and adult females ascending or descending the trunk differed significantly from each other. Data were tested for homogeneity of variances using Levene's test. If required, data were log transformed, before the analysis, to satisfy homogeneity of variances and to approximate a normal distribution.

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 with the software Statgraphics Centurion XVI.II (Statpoint Technologies Inc, Warrenton, USA).

Results

Strata distribution

Population density of *D. aberiae* started to increase in March and the maximum was reached in May for the three years of study (Fig. 1). Afterwards, population density began to drop, and from September to February mealybugs remained at low levels. Within the sampled strata, *D. aberiae* was present mostly in the tree canopy (Fig. 1). For each month, more than 70% of the total number of mealybugs found per unit area (cm^2) was located on the canopy during the three years of study. However, from February to September, some of the mealybugs were also detected in the soil and trunk (Fig. 1). During this period, the percentage of *D. aberiae* in soil was always lower than 30% and remained below 5% in the case of the trunk.

From March to July, period in which *D. aberiae* causes damage to fruits, the percentage of *D. aberiae* in the soil differed significantly between months for 2014 and 2015 (ANOVA 2014: F = 7.05, df = 4, 15, P = 0.002; ANOVA 2015: F = 2.08, df = 4, 15, P < 0.001). Means compared using Tukey tests showed that the percentages of *D. aberiae* in the soil were significantly higher in March and April followed by June and July in 2014 and 2015. The lowest percentage was registered in May also in both years (Fig. 1).

Distribution on tree canopy

Within the canopy, the feeding structure of *D. aberiae* changed seasonally, as it is shown by the differences in the percentage of mealybugs found per structure throughout the year (Fig. 2). From November to March (winter), the highest percentage of mealybugs was always on twigs, with more than 60% of the mealybugs distributed on this structure, mainly protected in the insertion of the twigs with the leaves. In March and April, during the flowering period, a small percentage of mealybugs was present in flowers. Fruit set (April-May) and fruit development marked a change in *D. aberiae* feeding location preference (Fig. 2). Thus, from May to August, more than 70% of the mealybugs settled and fed on the fruit, mainly underneath the calyces, coinciding with the period of highest mealybug density in the orchards.

Migration through the trunk

Mobile and immobile instars on trunk

Both, mobile (nymphs and adult females) and immobile instars (immature males and ovipositing females) were present on the trunk from March to August, coinciding with the period of high mealybug density in the canopy of the tree. Mobile and immobile instars peaked together and twice during this period (Fig. 3). The first peak occurred between March and April, and the second from mid-May to July.

Directionality of mobile instars

Adult females migrated mostly from the tree canopy to the soil in two periods, March-April and June-July, as the number of adult females descending was significantly higher than ascending (March: t = -3.95, df = 18, P = 0.001; April: t = -2.54, df = 18, P = 0.02; June: t = -3.26, df = 18, P = 0.004; July: t = -2.86, df = 18, P = 0.01) (Fig 4a). The rest of the year the number of adult females ascending and descending was similar (January: t = -0.90, df = 18, P = 0.38; February: t = -0.41, df = 18, P = 0.07; May: t = -0.60, df = 18, P = 0.56; August: t = -0.90, df = 18, P = 0.38; September: t = -0.45, df = 18, P = 0.66; in October, November and December the number of adult females was null).

Nymphs migrated mostly from the tree canopy to the soil in two periods, March and May-June, as the number of nymphs descending was significantly higher than ascending (March: t = -2.40, df = 18, P = 0.02; May: t = -2.47, df = 18, P = 0.02; June: t = -2.52, df = 18, P = 0.02) (Fig 4b). The rest of the year the number of nymphs ascending and descending was similar (February: t = -0.74, df = 18, P = 0.47; July: t = -0.34, df = 18, P = 0.74; August: t = -0.26, df = 18, P = 0.80; September: t = -1.00, df = 18, P = 0.33; November: t = 0.00, df = 18, P = 1.00; in January, October and December the number of nymphs was null), except in April when the number of nymphs ascending was significantly higher than descending (t = 2.81, df = 18, P = 0.01).

Distribution and seasonal trend on soil

97% of the total number (4567 mealybugs) of *D. aberiae* collected from soil samples were captured within a distance of 0 to 15 cm horizontally from the base of the trunk, 3% from 16 to 30 cm and 0% in samples separated more than 30 cm from the base of the trunk.

Within a distance of 0 to 15 cm horizontally from the base of the trunk, *D. aberiae* was present from March until August in the samples obtained with Berlese funnels (Fig. 5). Second instar nymphs and adult females peaked in March and June; first instar nymphs in April-May (with a maximum in April) and July and adult males in April and June.

Discussion

The present study has shown that within the sampled strata (canopy, trunk, soil), *D. aberiae* was mostly found in the tree canopy. Besides, within the tree canopy, the preferred feeding locations of *D. aberiae* changed seasonally, with a preference for the developing fruit. Distribution patterns in scale insects are the result of its intrinsic and physiological behavior, morphological characteristics of the host-plant tissue and the activity of predators and parasitoids (Nestel et al. 1995). As most mealybug species are phloem feeders, they vary their feeding and settling locations throughout the year, searching the movement of nutrients in their hosts to find the best nutritional conditions for them (McKenzie 1967, Boavida et al. 1992, Geiger and Daane 2001, Cid et al. 2010, Haviland et al. 2012, Wunderlich et al. 2013, Kumar et al. 2014). This behavior results in the migration of mealybugs to different strata of their hosts, adapting to plant phenology (Browning 1959, Furness 1976, Franco 1994, Geiger and Daane 2001, Grasswitz and James 2008, Cid et al. 2010, Haviland et al. 2012, Wunderlich et al. 2013).

Besides, herein we have shown that from November to February (winter), *D. aberiae* remained mainly in the twigs, where mealybugs were usually found in the insertion of the leaves. According to a companion manuscript, these individuals are mostly nymphs of second and third instar during winter (Martínez-Blay et al. 2018b). Afterwards, during the flush and blossom period (from March to April in eastern Spain), *D. aberiae* moved from the twigs to the new shoots and flowers in blooming , where they reached the adult stage (Martínez-Blay et al. 2018b). In March and April, during flowering period, a small percentage of *D. aberiae* was present in flowers. This percentage was much lower than the percentage of mealybugs infesting fruits later on. Fruit set (April-May in eastern Spain conditions) and fruit development marked a significant change in *D. aberiae* feeding location preference. During this period, crawlers from the first generation of the pest (Martínez-Blay et al. 2018b) tended to settle in the new citrus fruitlets in development, mainly in the calyx area. Thus, from May to August most of the mealybugs developed on fruits, this coinciding with the period of highest *D. aberiae* density in the orchards. The developing citrus fruit is the preferred feeding location of mealybugs affecting this crop because it provides very good nutritional conditions for their development (Franco 1994). Our results show that *D. aberiae* tended to search for and settle at the major carbohydrate sinks of the citrus tree. During the three years of this study, the movement of mealybugs from overwintering sites to the shoots and flowers coincided with spring flush and blossom period, when carbohydrates are shift acropetally from the roots to the buds; afterwards, *D. aberiae* aggregated on the developing fruit, a strong sink of carbohydrates (Agustí 2003, Iglesias et al. 2007). The behavior of nymph's migration following the plant nutrients has also been reported for other mealybug species, such as *Ferrisia gilli* Gullan in pistachio trees (Haviland et al. 2012), *P. citri* in citrus (Franco 1994, Martínez-Ferrer et al. 2003) or *Pseudococcus maritimus* (Ehrhorn) in vineyards (Geiger and Daane 2001). *D. aberiae* was not an exception and herein we have described these movements within citrus trees.

Besides looking for food, mealybugs are a group of insects that usually migrate to find protection against bad weather conditions and natural enemies (Gutierrez et al. 2008, Daane et al. 2012, Mani and Shivaraju 2016). Herein, mobile and immobile instars of *D. aberiae* were present and active on the trunk and soil from February to September and during this period the mealybug peaked twice on both strata, simultaneously with the two main peaks in the canopy. Some studies mention that mealybugs might migrate and overwinter in the soil (Bodenheimer 1951, Rotundo et al. 1979, Franco et al. 2000). Our results, however, show that *D. aberiae* did not stay protected in this stratum in the coldest months. Besides, adult females migrated mostly from the tree canopy to the trunk and soil in two periods, March-April and June-July. The peaks of first instars observed in this stratum after the presence of adult females indicate that crawlers emerged from eggs laid by ovipositing females. Results are in agreement with the migration of females to the trunk and soil for ovipositing in protected places previously reported for other citrus mealybug species, such as *P. citri* (Franco 1994, Franco et al. 2000, Martínez-Ferrer et al. 2003, García-Marí 2012).

On the other hand, nymphs migrated mostly from the tree canopy to the trunk and soil in two periods, March and May-June. The migration of nymphs downwards the trunk is not commonly reported in mealybugs but some previous studies have mentioned this descending movement in other mealybug species, reporting that it could be an adaptive strategy to facilitate the mating process between males and females (Browning 1959, Franco 1994, Franco et al. 2000). It seems to be also the strategy followed by *D. aberiae* as adult males were captured after the migration to the soil of second instars at the end of February-beginning of March. On the other hand, nymph migration from the soil to the canopy was only observed in April. This month coincides with fruit set period. Thus, this ascending movement is probably a migration to the new fruitlets of the crawlers emerged in soil in the same way that has been aforementioned within the tree canopy. It is also remarkable that nymphs from the second generation did not ascend back to the canopy in summer. The high temperatures and low humidity of Mediterranean countries may cause high mortality in young mealybugs (Browning 1959, Bartlett and Clancy 1972, Furness 1976, Beltrà et al. 2013a). Therefore, soil could be a drain of *D. aberiae* in summer. Furthermore, the fact that most mealybugs were found horizontally close to the base of the trunk, suggests that *D. aberiae* moves to the soil intentionally depending on the phenology of the plant and the climatic conditions (Browning 1959, Bartlett and Clancy 1972, Furness 1976, Franco et al. 2000, Martínez-Ferrer et al. 2003, Beltrà et al. 2013a); and not because insects fall by chance from the tree canopy.

Finally, it is remarkable that mealybug movement from the canopy to the trunk and soil represent only a relatively small part of *D. aberiae* populations within trees, and therefore is not a general behavior. Apparently, some individuals tend to move to the trunk or soil, whereas most of the mealybugs remain in the canopy. The exact reasons for this behavior are not clear but as mentioned above it seems that there are some adaptive advantages in this variability of behavior, because it has been observed in several mealybug species (Browning 1959, Franco et al. 2000, Geiger and Daane 2001, Martínez-Ferrer et al. 2003, Cid et al. 2010).

Our results show that *D. aberiae* change its distribution patterns due to physiological and behavioral requirements. Chemical control programs against *D. aberiae* are likely to continue until more sustainable approaches, particularly biological control, can be implemented against this mealybug. Until then, these results will improve the management of this pest, which should take into consideration the migration and presence of *D. aberiae* in the soil in spring but not in summer, when crawlers likely die because of warmer and drier conditions. Insecticide applications are currently recommended only if 12% or more of the fruits are is infested by *D. aberiae* after petal fall (Pérez-Rodríguez et al. 2017). Since some mealybugs are present on the trunk and soil during that period, insecticides recommended against mealybugs in citrus in Spain should also soak the trunk and the base of the trunk. However, insecticide application to the soil may originate negative environmental impacts, which should also be taken into consideration. Therefore, possible alternatives to insecticides, such as the use of trunk barriers, or the study of the predatory fauna in the soil of citrus orchards (i.e. predatory mites) in order to evaluate their potential as natural enemies should be taken into account for future studies. Finally, our results will facilitate an early detection of the pest in those areas where *D. aberiae* is spreading in Spain, as technicians will be able to search in the correct plant strata and structure depending on the season.

Aknowledgements

We would like to thank the owners of the orchards for allowing us to use their plantations, especially Plácido Calabuig. The authors thank Debra Westall (UPV) for English corrections. The authors are also grateful to two anonymous reviewers for helpful comments and corrections. This research was supported by two predoctoral grants (FPU to V. Martínez-Blay and Val I+d to J. Pérez-Rodríguez from the Spanish Ministry of Education, Culture and Sport and Generalitat Valenciana, respectively), a national project provided by INIA (Project no. RTA2014-00067) and the European grants FP7-IAPP #324475 ‘Colbics’ and FP7-IRSES #612566 ‘Biomodic’.

References

Agustí, M. 2003. Citricultura, Mundi-Prensa, Madrid, Spain.

Bartlett, B. R., and D. W. Clancy. 1972. The Comstock Mealybug in California and Observations on Some of Its Natural Enemies. J. Econ. Entomol. 65: 1329-1332.

Beltrà, A., A. Soto, and T. Malausa. 2012. Molecular and morphological characterisation of Pseudococcidae surveyed on crops and ornamental plants in Spain. Bull. Entomol. Res. 102: 165-172.

Beltrà, A., F. Garcia-Marí, and A. Soto. 2013a. Seasonal Phenology, Spatial Distribution, and Sampling Plan for the Invasive Mealybug *Phenacoccus peruvianus* (Hemiptera: Pseudococcidae). J. Econ. Entomol. 106: 1486-1494.

Beltrà, A., F. Garcia-Marí, and A. Soto. 2013b. El cotonet de Les Valls, *Delottococcus aberiae*, nueva plaga de los cítricos. Levante Agrícola 419: 348-352.

Beltrà, A., P. Addison, J. A. Ávalos, D. Crochard, F. Garcia-Marí, E. Guerrieri, J. H. Giliomee, T. Malausa, C. Navarro-Campos, F. Palero, and A. Soto. 2015. Guiding Classical Biological Control of an Invasive Mealybug Using Integrative Taxonomy. PLoS ONE 10: e0128685.

Boavida, C., P. Neuenschwander, and F. Schulthess. 1992. Spatial distribution of *Rastrococcus invaden*s Williams (Hom., Pseudococcidae) in mango trees. J. Appl. Entomol. 114: 381-391.

Bodenheimer, F. S. 1951. Citrus Entomology in the Middle East: with special references to Egypt, Iran, Irak, Palestine, Syria, Turkey., Dr W. Junk, The Hague, Netherlands.

Browning, T. O. 1959. The long-tailed mealybug, *Pseudocuccus aonidum* (L.), in South Australia. J. Agric. Res. 10: 322-339.

Cid, M., S. Pereira, C. Cabaleiro, and A. Segura. 2010. Citrus Mealybug (Hemiptera: Pseudococcidae) Movement and Population Dynamics in an Arbor-Trained Vineyard. J. Econ. Entomol. 103: 619-630.

Chong, J. H., L. F. Aristizabal, and S. P. Arthurs. 2015. Biology and Management of *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) on Ornamental Plants. J. Integr. Pest. Manag. 6: 1-14.

Daane, K. M., R. P. P. Almeida, V. A. Bell, J. T. S. Walker, M. Botton, M. Fallahzadeh, M. Mani, J. L. Miano, R. Sforza, V. M. Walton, and T. Zaviezo. 2012. Biology and Management of Mealybugs in Vineyards, pp. 271-307. In N. J. Bostanian, C. Vincent and R. Isaacs (eds.), Arthropod Management in Vineyards: Pests, Approaches, and Future Directions. Springer Netherlands, Dordrecht.

Franco, J. 1994. Citrus Phenology as a Basis to Study the Population Dynamics of the Citrus Mealybug Complex in Portugal, pp. 929-930. In E. Tribulato, A. Gentile and G. Reforgiato (eds.), Proc. Int. Soc. Citric., vol. 3.

Franco, J. C., E. B. Silva, and J. P. Carvalho. 2000. Cochonilhas-algodão (Hemiptera, Pseudococcidae) associadas aos citrinos em Portugal, ISA Press, Lisboa, Portugal.

Franco, J. C., A. Zada, and Z. Mendel. 2009. Novel Approaches for the Management of Mealybug Pests, pp. 233-278. In I. Ishaaya and A. R. Horowitz (eds.), Biorational control of arthropod pests: application and resistance managements. Springer, Netherlands.

Furness, G. 1976. The Dispersal, Age-structure and Natural Enemies of the Long-tailed Mealybug, *Pseudocccus longispinus* (Targioni-Tozzetti), in Relation to Sampling and Control. Aust. J. Zool. 24: 237-247.

García-Marí, F. 2012. Plagas de los cítricos. Gestión integrada en países de clima mediterráneo, Phytoma, Spain.

García-Morales, M., B. D. Denno, D. R. Miller, G. L. Miller, Y. Ben-Dov, and N. B. Hardy. 2016. ScaleNet: a literature-based model of scale insect biology and systematics. Database (Oxford) 2016: 1-5.

Geiger, C. A., and K. M. Daane. 2001. Seasonal Movement and Distribution of the Grape Mealybug (Homoptera: Pseudococcidae): Developing a Sampling Program for San Joaquin Valley Vineyards. J. Econ. Entomol. 94: 291-301.

Grasswitz, T. R., and D. G. James. 2008. Movement of grape mealybug, *Pseudococcus maritimus*, on and between host plants. Entomol. Exp. Appl. 129: 268-275.

Gutierrez, A. P., K. M. Daane, L. Ponti, V. M. Walton, and C. K. Ellis. 2008. Prospective evaluation of the biological control of vine mealybug: refuge effects and climate. J. Appl. Ecol. 45: 524-536.

Haviland, D. R., R. H. Beede, and K. M. Daane. 2012. Seasonal Phenology of *Ferrisia gilli* (Hemiptera: Pseudococcidae) in Commercial Pistachios. J. Econ. Entomol. 105: 1681-1687.

Iglesias, D. J., M. Cercós, J. M. Colmenero-Flores, M. A. Naranjo, G. Ríos, E. Carrera, O. Ruiz-Rivero, I. Lliso, R. Morillon, F. R. Tadeo, and M. Talon. 2007. Physiology of citrus fruiting. Braz. J. Plant. Physiol. 19: 333-362.

Kasuya, E. 2004. Angular transformation – another effect of different sample sizes. Ecol. Res. 19: 165-167.

Kogan, M. 1998. Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annu. Rev. Entomol. 43: 243-270.

Kumar, R., V. S. Nagrare, M. Nitharwal, D. Swami, and Y. G. Prasad. 2014. Within-plant distribution of an invasive mealybug, *Phenacoccus solenopsis*, and associated losses in cotton. Phytoparasitica 42: 311-316.

Mani, M., and C. Shivaraju. 2016. Mealybugs and their Management in Agricultural and Horticultural crops, Springer, New Delhi.

Martínez-Blay, V., M. Benito, and A. Soto. 2018a. Characterization and damage period to fruits caused by the invasive pest *Delottococcus aberiae* De Lotto (Hemiptera: Pseudococcidae). Integrated Control in Citrus Fruit Crops. IOBC-WPRS Bull. 132: 7-15.

Martínez-Blay, V., J. Pérez-Rodríguez, A. Tena, and A. Soto. 2018b. Density and phenology of the invasive mealybug *Delottococcus aberiae* on citrus: implications for integrated pest management. J. Pest. Sci. 91: 625-637.

Martínez-Ferrer, M. T., F. García-Marí, and J. L. Ripollés. 2003. Population dynamics of *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) in citrus groves in Spain. Integrated Control in Citrus Fruit Crops. IOBC-WPRS Bull. 26: 149-161.

Martínez-Ferrer, M. T., J. L. Ripollés, and F. Garcia-Marí. 2006. Enumerative and Binomial Sampling Plans for Citrus Mealybug (Homoptera: Pseudococcidae) in Citrus Groves. J. Econ. Entomol. 99: 993-1001.

McKenzie, H. L. 1967. Mealybugs of California, with taxonomy, biology, and control of North American species (Homoptera: Coccoidea: Pseudococcidae). Cambridge University Press, Berkeley, CA, USA.

Meyerdirk, D. E., R. Warkentin, B. Attavien, E. Gersabeck, A. Fracis, M. Adams, and G. Francis. 2001. Biological Control of Pink Hibiscus Mealybug Project Manual, pp. 194. United States Department of Agriculture (USDA), Washington, USA.

Miller, D. R., and J. H. Giliomee. 2011. Systematic revision of the mealybug genus *Delottococcus* Cox & Ben-Dov (Hemiptera: Pseudococcidae). Afr. Entomol. 19: 614-640.

Miller, D. R., G. L. Miller, and G. W. Watson. 2002. Invasive species of mealybugs (Hemiptera: Pseudococcidae) and their threat to U.S. agriculture. Proc. Entomol. Soc. Wash. 104: 825-836.

Mudavanhu, P., P. Addison, and L. Pringle Ken. 2011. Monitoring and action threshold determination for the obscure mealybug *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae) using pheromone-baited traps. Crop. Prot. 30: 919-924.

Nestel, D., H. Cohen, N. Saphir, M. Klein, and Z. Mendel. 1995. Spatial Distribution of Scale Insects: Comparative Study Using Taylor's Power Law. Environ. Entomol. 24: 506-512.

Pellizzari, G., and J.-F. Germain. 2010. Scales (Hemiptera, Superfamily Coccoidea), Chapter 9.3. BioRisk 4: 475-510.

Pérez-Rodríguez, J., V. Martínez-Blay, A. Soto, J. Selfa, C. Monzó, A. Urbaneja, and A. Tena. 2017. Aggregation Patterns, Sampling Plan, and Economic Injury Levels for the New Citrus Pest *Delottococcus aberiae* (Hemiptera: Pseudococcidae). J. Econ. Entomol. 110: 2699-2706.

Roques, A., W. Rabitsch, J.-Y. Rasplus, C. Lopez-Vaamonde, W. Nentwig, and M. Kenis. 2009. Alien Terrestrial Invertebrates of Europe, pp. 63-79. In DAISIE (ed.), Handbook of Alien Species in Europe, vol. 3. Springer Netherlands.

Rotundo, G., E. Tremblay, and R. Giacometti. 1979. Final results of mass captures of the citrophilous mealybug males (*Pseudococcus calceolariae* Mask.) (Homoptera Coccoidea) in a citrus grove. Boll. Lab. Entomol. Agr. Portici. 36: 266-274.

Tena, A., and F. Garcia-Marí. 2011. Current situation of citrus pests and diseases in the Mediterranean basin. Integrated Control in Citrus Fruit Crops IOBC/WPRS Bull. 62: 365-378.

Tena, A., J. García-Bellón, and A. Urbaneja. 2017. Native and naturalized mealybug parasitoids fail to control the new citrus mealybug pest *Delottococcus aberiae*. J. Pest. Sci. 90: 659-667.

Wunderlich, L. R., M. L. Cooper, and K. M. Daane. 2013. Seasonal Biology of *Ferrisia gilli* (Hemiptera: Pseudococcidae) in California Sierra Foothill Vineyards. J. Econ. Entomol. 106: 1716-1725.

Figure legends

Fig. 1 Strata distribution of *D. aberiae* in ten citrus orchards in eastern Spain. Percentage of mealybugs per strata (canopy, trunk and soil) (primary Y axis) compared to the number of mealybugs per unit area (cm^2) (secondary Y axis) is represented per month for the years 2014, 2015 and 2016. Different letters, on the left of soil percentages, indicate that those proportions differed significantly between them (ANOVA 2014: $F = 7.05$, df = 4, 15, $P = 0.002$; ANOVA 2015: $F = 2.08$, df = 4, 15, $P = 0.01$), being means compared by Tukey tests ($P<0.05$).

Fig. 2 Distribution of *D. aberiae* within the tree canopy in ten citrus orchards in eastern Spain. Percentage of mealybugs per structure (flower, fruit, leaf or twig) (primary Y axis) compared to the number of mealybugs per unit area (cm^2) in the canopy (secondary Y axis) is represented per month for the years 2014, 2015 and 2016.

Fig. 3 Seasonal trend of mobile (nymphs and adult females) and immobile instars (immature males and ovipositing females) of *D. aberiae* on trunk in ten citrus orchards in eastern Spain in 2014 and 2015. Mean number of mealybugs counted visually in the orchards, during 2 minutes, is represented. Vertical bars represent the positive standard error (+SE). Note that y-axis scales are different for each year.

Fig. 4 Directionality of the mobile instars on the trunk during the 2-minutes visual samplings. The monthly mean number of mealybugs (± SE), ascending or descending, is represented for the year 2015, separating between (a) adult females and (b) nymphs. Within each month, different letters, on the left of each bar, indicate that the mean number of mealybugs ascending or descending differed significantly between them (t-tests).

Fig. 5 Seasonal trend of *D. aberiae* in soil in ten citrus orchards in eastern Spain in 2014 and 2015. Mean number of mobile instars (nymphs, adult females and adult males) captured by Berlese funnels is represented. Vertical bars represent the positive standard error (+SE). Note that y-axis scales are different for 2014 and 2015.

Supp Fig. S1 [online only]) Adult female of the mealybug *D. aberiae* (left) and direct damage caused by *D. aberiae* (right)

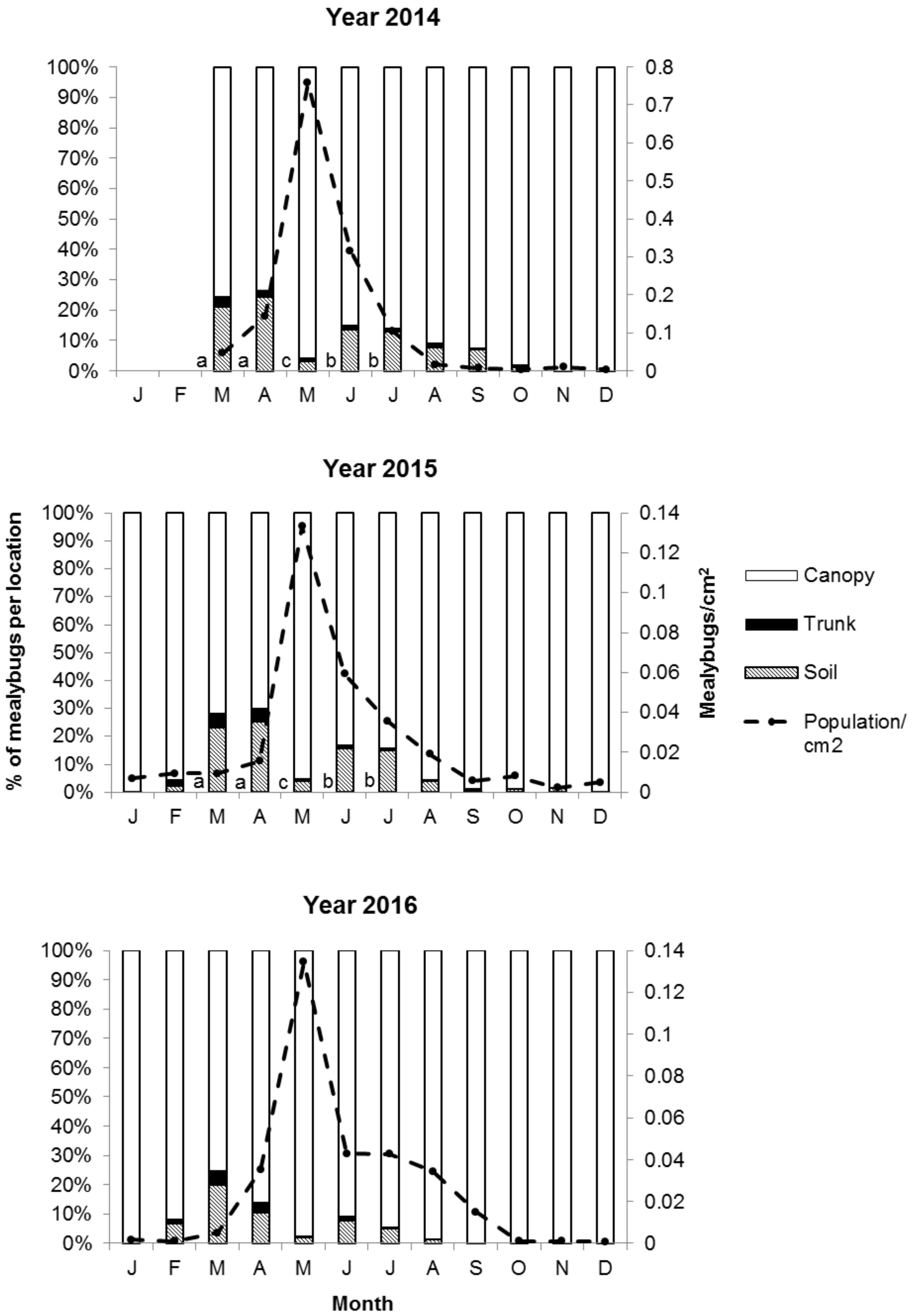
Year 2014
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a a c b b
J F M A M J J A S O N D
Year 2015
% of mealybugs per location
0.14
0.12
0.1
0.08
0.06
0.04
0.02
Mealybugs/cm²
a a c b b
Canopy
Trunk
Soil
Population/ cm2
Year 2016
Month

Fig. 1

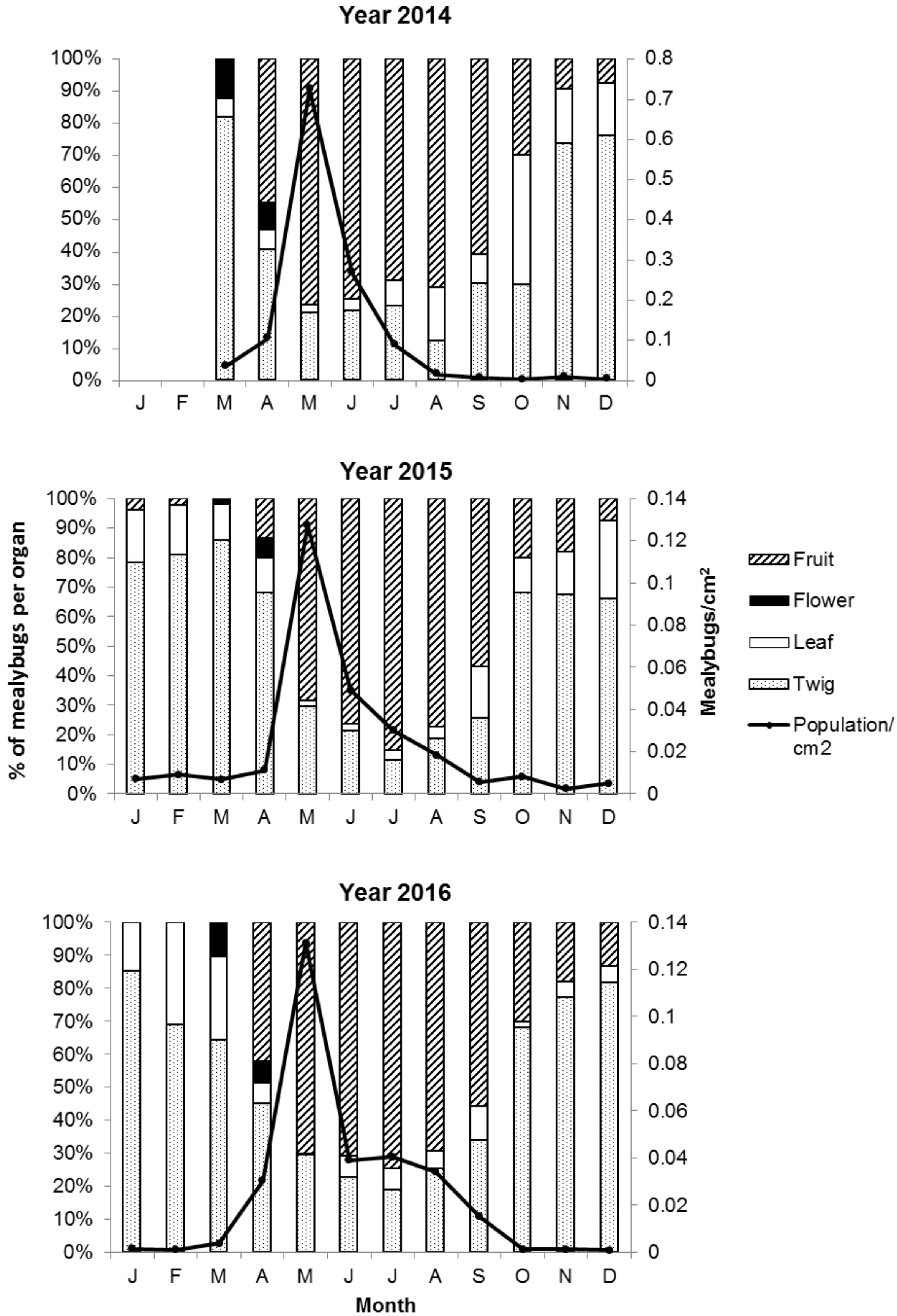
Year 2014
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
J F M A M J J A S O N D
Year 2015
% of mealybugs per organ
0.14
0.12
0.1
0.08
0.06
0.04
0.02
Mealybugs/cm²
Fruit
Flower
Leaf
Twig
Population/ cm2
Year 2016
Month

Fig. 2

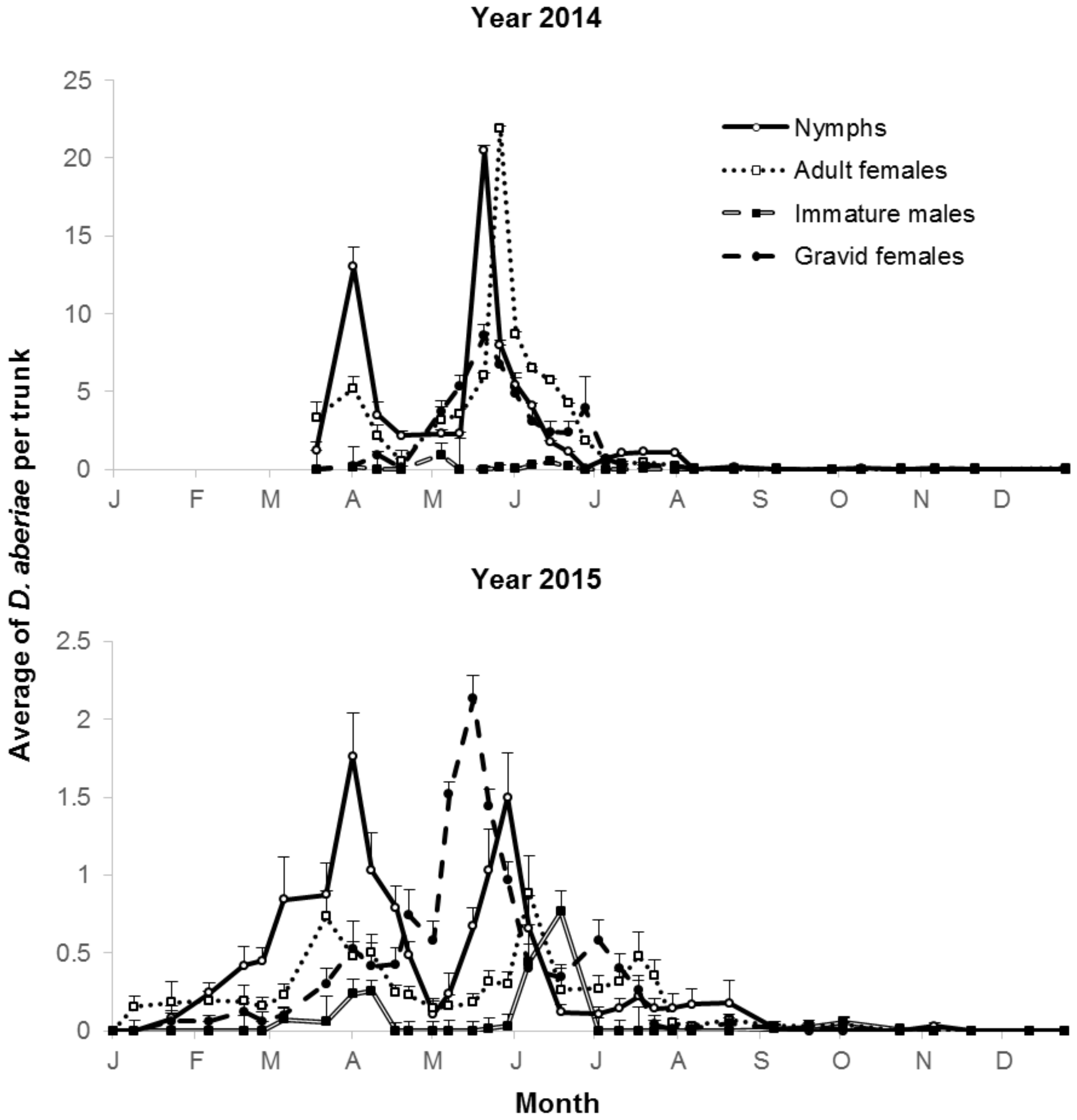
Year 2014
25
20
15
10
5
0
Nymphs
Adult females
Immature males
Gravid females
J
F
M
A
M
J
J
A
S
O
N
D
Year 2015
2.5
2
1.5
1
0.5
0
Average of D. aberiae per trunk
Month

Fig. 3

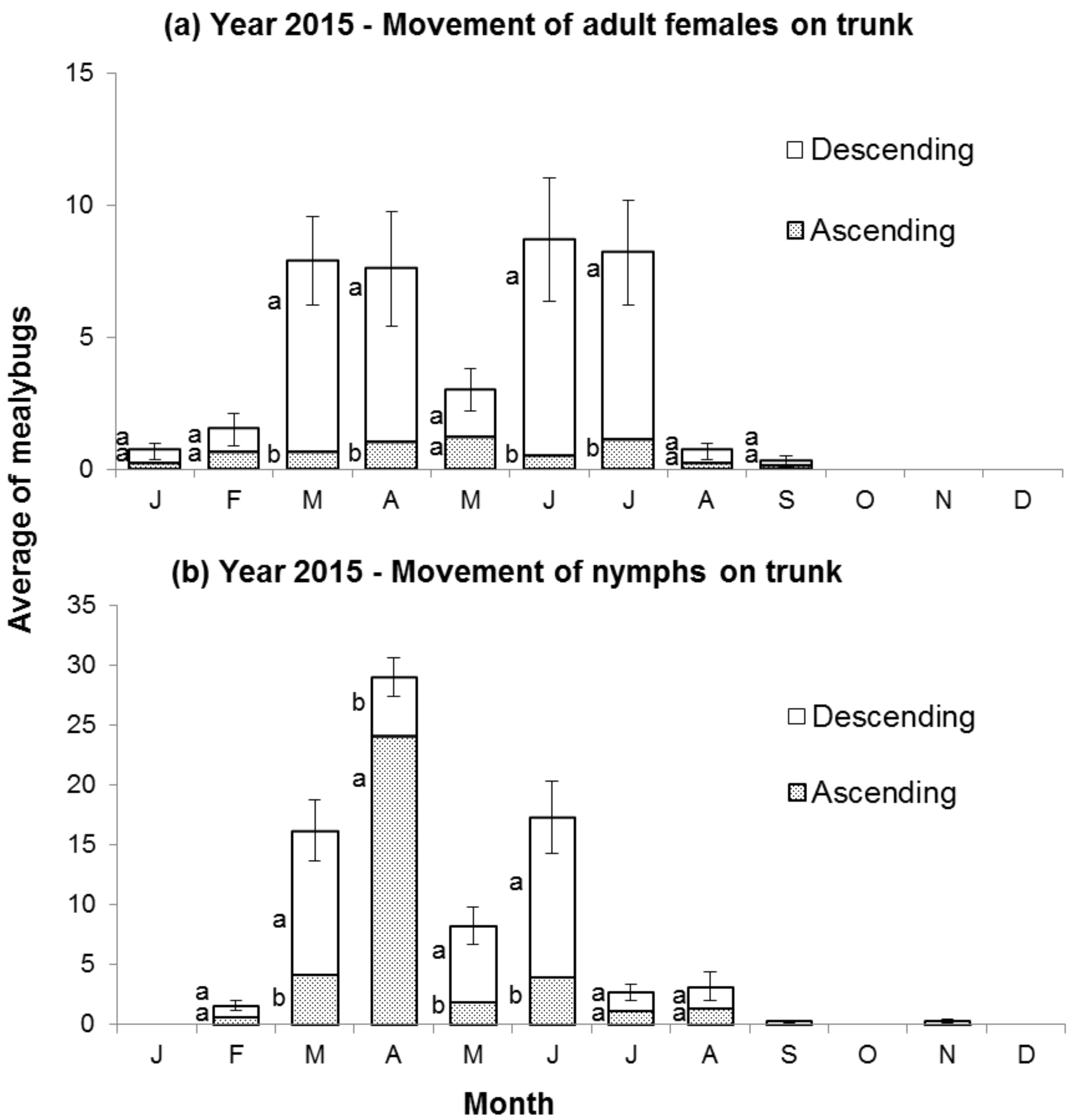
(a) Year 2015 - Movement of adult females on trunk
(b) Year 2015 - Movement of nymphs on trunk
Average of mealybugs
Descending
Ascending
J F M A M J J A S O N D
Month

Fig. 4

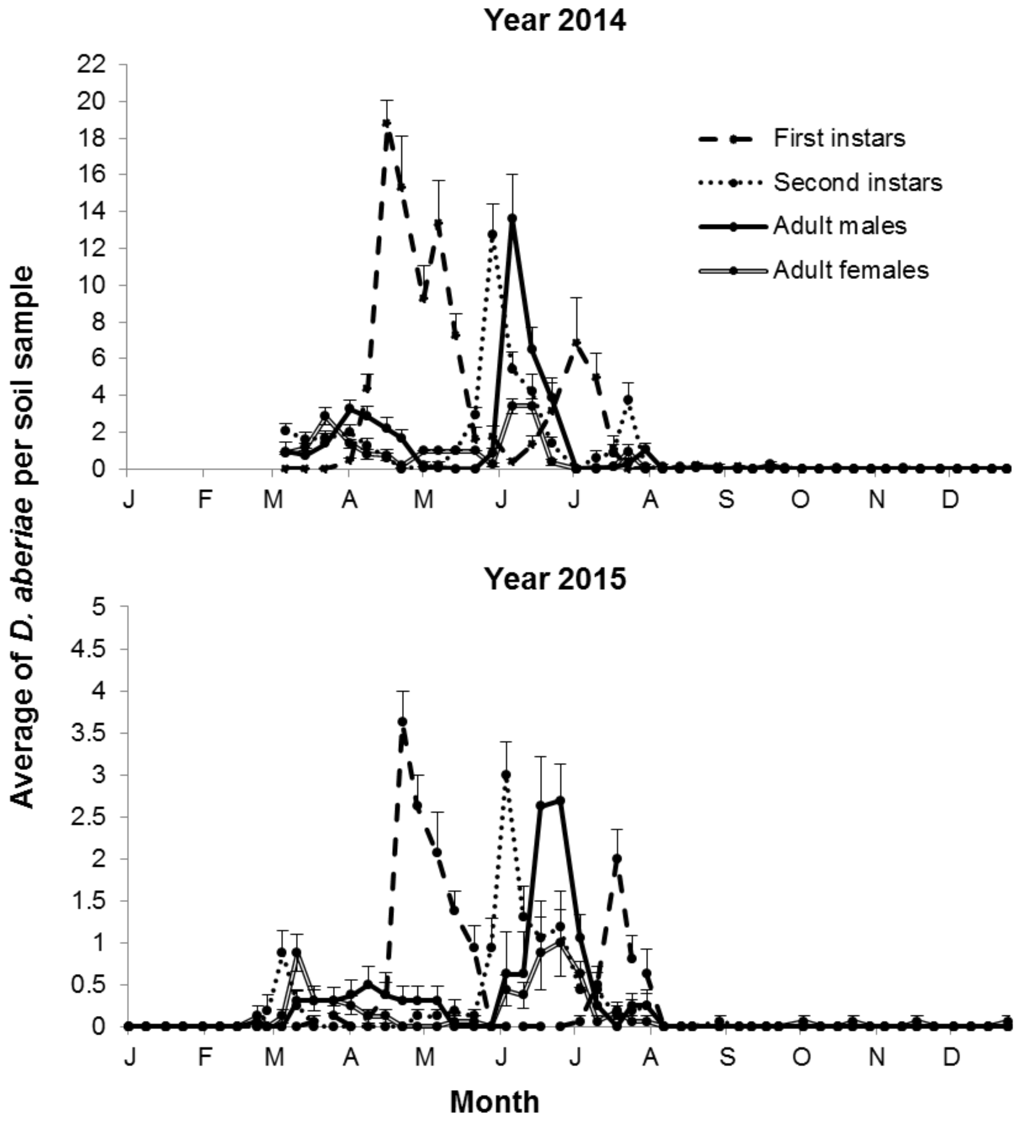
Year 2014
First instars
Second instars
Adult males
Adult females
Year 2015
Average of D. aberiae per soil sample
Month
J F M A M J J A S O N D

Fig. 5

Supp Fig. S1 [online only])