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Monitoring *Pseudococcus calceolariae* (Hemiptera: Pseudococcidae) in Fruit Crops Using Pheromone-Baited Traps

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ABSTRACT The citrophilus mealybug, *Pseudococcus calceolariae* (Maskell), is an important pest of fruit crops in many regions of the world. Recently, its sex pheromone has been identified and synthesized. We carried out field experiments with the goal of developing monitoring protocols for *P. calceolariae* using pheromone-baited traps. Traps checked hourly for 24 hours showed a distinct diel pattern of male flight, between 18:00 and 21:00 h. The presence of unnatural stereoisomers did not affect trap captures, with isomeric mixtures capturing similar amounts of males as the biological active isomer. Dose of isomeric mixture pheromone (0–100 µg) had a nonlinear effect on male captures, with 10, 30, and 50 µg capturing similar amounts. The effective range of pheromone traps was determined by placing traps at different distances (15, 40, and 80 m) from an infested blueberry field, loaded with 0, 1 and 25 µg of the pheromone. For all distances, 25 µg dose captured more males, and was highly attractive up to 40 m. There was a significant effect of lure age on male captures (0–150 d), with similar amount of males captured up to 90-day-old lure, and lower captures in the 150-day-old lure compared with fresh ones. We found significant positive correlations between *P. calceolariae* males caught in pheromone traps with female abundance and fruit infestation at harvest. Our results show the usefulness of *P. calceolariae* pheromones for monitoring at field level and provide information for the design of monitoring protocols.

KEY WORDS mealybug, monitoring, pheromone trap, *Pseudococcus calceolariae*

The citrophilus mealybug, *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae), is a pest thought to be native from Australia and now causing economic damage in many continents and countries around the world, such as Australia, New Zealand, the United States, South Africa, Italy and Chile (Compere and Smith 1932, Williams 1985, Daane et al. 2012, Ben-Dov 2014). Nevertheless, it has not been reported for other regions, particularly in many countries of South and Central America and Asia (Williams and Granara de Willink 1992, Ben-Dov 2014), where it is an insect of quarantine status. *P. calceolariae* is a polyphagous insect, reported in many plant species belonging to 49 genera (Ben-Dov 2014), and the host plant can greatly influence its development and fecundity in laboratory conditions (Zaviezo et al, 2010). The citrophilus mealybug is responsible for economic losses particularly in fruit crops such as citrus, avocados, grapes, apples, and berries, among others (Ripa and Larral 2008, Charles et al. 2010, González 2011, Daane et al. 2012, Dreistadt 2012).

The economic damage in *P. calceolariae*, as in many other mealybug species, can be related to a combination of causes. First, owing to their feeding habit of sucking phloem from the host plant, severe infestations can lead to weakening, lower yield, defoliation, and even death of the plant (Charles 1982, Walton and Pringle 2004). Second, in fresh fruits and vegetables, there is a cosmetic or quality damage when they are found in or on the marketable product, and also when honeydew or sooty molds develop on it (Daane et al. 2011, González 2011). In fresh exports, mealybugs represent important quarantine pests, and *P. calceolariae* is currently a restriction for the commercialization of fresh fruits in many countries of America and Asia (González 2011, Daane et al. 2012). In wine grapes, the most important economic damage is associated with the role that mealybugs have in the transmission of plant pathogenic viruses, with *P. calceolariae* being a vector of grapevine leafroll-associated viruses (GLR-aV), which greatly impacts yield and wine quality (Golino et al. 2002, Walton and Pringle 2004, Charles et al. 2009). Because of its economic importance, control methods have been developed since the early 1900s, when successful biological control programs against *P. calceolariae* were implemented (Compere and Smith 1932, Ripa and Larral 2008, Charles et al. 2010). Nevertheless, nowadays in some regions or crops, chemical control is still needed (González 2011, Daane et al. 2012).

Monitoring mealybug populations for determining the need and optimal timing of pesticide applications has usually been done by visual inspections, which is a

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laborious task that requires trained personnel (Geiger and Daane 2001, Daane et al. 2012). Thus, the development of an alternative, economically attractive technique for monitoring mealybug populations is highly desirable. Pheromones have been shown to have a high potential to be incorporated in mealybug management programs as a monitoring tool (Walton et al. 2004, 2006; Mudavanhu et al. 2011; Waterworth et al. 2011; Roda et al. 2012). The pheromone of *P. calceolariae* produced by adult females has recently been identified as (1*R*,3*R*)-chrysanthemyl (*R*)-2-acetoxy-3-methylbutanoate (El-Sayed et al. 2010, Unelius et al. 2011). With the synthetic pheromone available, sensitive and selective monitoring tools for this species using pheromone-baited traps can now be developed.

The objective of this study was to investigate key parameters regarding the use of pheromone-baited traps for monitoring *P. calceolariae* in the field. Particularly, we determined the dial flight activity of males in the field, the effect of non-natural stereoisomers, pheromone dose, range of traps, and aging of baits on the capture of males in pheromone-baited traps under field conditions. Furthermore, we correlated male captures with female abundance from visual inspections and fruit damage at harvest.

Materials and Methods

Pheromone-Baited Traps. The isomeric mixtures of the sex pheromone of *P. calceolariae* were synthesized by esterification of chrysanthemol (40:60 mixture of *cis* and *trans* isomers) with either racemic 2-acetoxy-3-methylbutanoic acid (8-compound mixture) or (*R*)-2-acetoxy-3-methylbutanoic acid (4-compound mixture) as previously described (El-Sayed et al. 2010). The pure (R,R,R) isomer was produced by reduction of (1R,3R)-chrysanthemic acid and subsequent esterification with (R)-2-acetoxy-3-methylbutanoic acid (Unelius et al. 2011). For the preparation of lures, synthetic compounds were dissolved in hexane, and white rubber septa (Sigma-Aldrich, St. Louis, MO) were loaded with 100 µL of the respective solutions. Septa treated with 100 µL hexane were used as control. For field trials, septa were placed inside Delta traps (Pherocon II B, Trécé Inc., Adair, OK).

Field Trials. Field tests were carried out in fruit orchards known to be infested with citrophilus mealybugs. Unless otherwise indicated, completely randomized block designs were used, with a distance between traps of at least 20 m within each block and at least 30 m between blocks. Traps were hung at canopy level. All figures are presented in daily captures (number of males by trap^{-1} by day^{-1}) to have a common unit, because the number of days traps were deployed varied between trials.

Daily Flight Pattern. Three traps (30 µg dose) were placed in an apple orchard in summer (21 February 2013), and the number of males captured was counted every hour from 06.00 pm until 05.00 pm of the next day. Three delta traps with hexane were used as a control. To analyze the diel pattern of male flight, a circular statistical analysis was used, which is appropriate to data that are collected in a circular interval scale, like time of day, where there is no true zero point and designation of high or low values is arbitrary (Zar 1999). To determine if observations of male flight were uniformly distributed during the 24-h period, a Rayleigh uniformity test was run (Fisher 1993, Zar 1999) using Oriana software (Rockware, 2010).

Effect of Non-Natural Stereoisomers. Chrysanthemyl 2-acetoxy-3-methylbutanoate has three chiral carbon atoms (Fig. 1A), leading to eight possible stereoisomers, but only one is produced by females, showing (*R*,*R*,*R*) configuration (Fig. 1B). This trial was carried out in a raspberry orchard (var. Heritage) near Nogales (Valparaíso Region, Chile), from 15 to 18 January 2010. Three replicates of each of the following treatments were used: 100 µg of the eight-compound isomeric mixture (containing 17 µg of the (*R*,*R*,*R*) isomer), 50 µg of the four-compound isomeric mixture with defined R configuration at the chiral carbon in the acid moiety (containing 17 µg of the (*R*,*R*,*R*) isomer), and 17 µg of the (*R*,*R*,*R*) isomer. Differences between treatments were analyzed by ANOVA, and means were separated by Tukey's HSD test ($\alpha = 0.05$).

Dose. Having determined that the non-natural stereoisomers did not affect trap captures, the eight-compound isomeric mixture was used for this and the following experiments. The doses indicated hereafter refer to the total amount of isomeric mixture, which contained 15–20% of the natural (*R*,*R*,*R*) isomer. To determine the effect of the dose on trap captures, lures with doses of 0, 1, 10, 30, 50, and 100 µg were

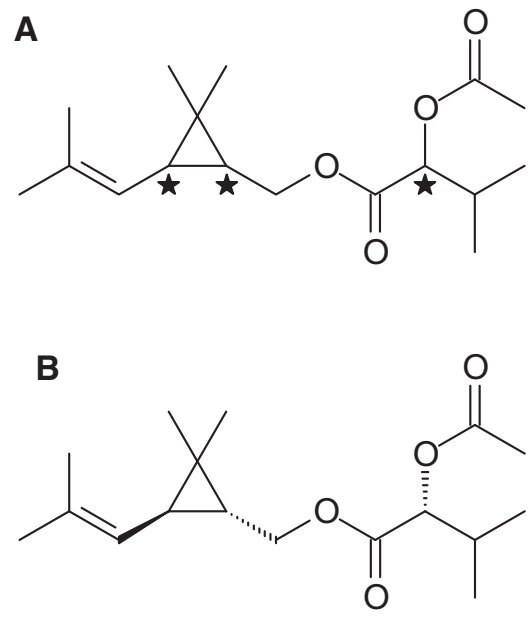

Fig. 1. (A) Molecular structure of chrysanthemyl 2-acetoxy-3-methylbutanoate; stars show the position of the three chiral carbon atoms. (B) (*R*,*R*,*R*) configuration, corresponding to the stereoisomer produced by *P. calceolariae* females.

deployed in traps (three replicates per treatment), which were placed in orchards with two different levels of pest infestation located near San Fernando (Central Chile), in February 2013. The low infestation was an apple (var. Royal Gala) orchard and the high infestation was a pear orchard. Trials were set up in a randomized block design, with blocks being parallel to the road. The numbers of males captured were counted after four days in apples and seven days in pears. Differences between dose treatments for each crop were analyzed independently by ANOVA, including the block effect, and means were separated by Tukey's HSD test ($\alpha = 0.05$). Data were transformed into $\sqrt{(x + 0.5)}$ before the analyses, to control for heterogeneity of variances.

Effective Trap Range and Dose. Three distances (15, 40, and 80 m) were evaluated to determine the effective trap range. For each distance, lures with 0, 1, and 25 µg of pheromone were placed outside a blueberry orchard (Valparaíso Region, Chile), where only very sparse shrubs and herbs were present. The experiment was carried out for one week for each distance, during three consecutive weeks to avoid interference from the closer traps, starting on 24 March 2014. Three replicates for each dose were used. Because trials for each distance were carried out at different dates, to control for different insect abundance or flight activity at the different dates, in each trial, we set two traps (lures of 30 µg) in the interior of the orchard at a similar distance from the edge. Males were counted at the end of each of the 7-day period, and the effect of dose was analyzed by ANOVA for each distance separately, and difference among treatments was tested by Tukey's HSD test ($\alpha = 0.05$). Data were transformed into log $(x + 1)$ before the analyses. Because the captures in the interior reference traps varied between dates and distances, the proportion of males trapped in each of the exterior traps in any given distance in relation to the corresponding interior traps was calculated. These data were then arcsine$\sqrt{(x)}$ transformed, and analyzed by two-way ANOVA, with distance and dose as the independent variables.

Effect of Lure Age. Eighteen rubber septa were loaded with 30 µg of the pheromone. Three septa were placed in the field protected from direct light and the remaining lures were kept frozen at −20°C. Every 30 d, three traps were taken out of the fridge and placed outdoors. After 150 d, the aged lures were collected, and together with the remaining lures ("fresh"), were deployed in delta traps in an apple (var. Royal Gala) orchard near San Fernando, Chile. Because the area available for this experiment was limited, each replicate remained for seven days in the field. The experiment was carried out during three consecutive weeks starting on 22 April 2014, each week corresponding to a block. In each week, new lures and traps were deployed and the position of the treatments was rerandomized. Males were counted for the 7-d period and transformed into log (x) before the analyses. Data were analyzed by ANOVA, including the block effect, and means were separated by Tukey's HSD test ($\alpha = 0.05$).

Correlation Between Male Captures Versus Population Visual Count and Fruit Infestation. Two organically managed apple orchards near San Fernando, Chile, that differed in their levels of infestation were selected. In each orchard, six plots were set at least 30 m apart, and in the center of each one, a pheromone trap (50 µg dose) was placed in spring (October 2013), recording male captures every other week until the fall (May 2014). Lures were not replaced during the season. Concurrently, *P. calceolariae* females (adults and third-instar nymphs) were counted in 12 plants surrounding a pheromone trap (October 2013–April 2014), by visually inspecting each plant for 5 minutes, following a procedure described by Geiger and Daane (2001). At harvest time (February–March 2014), 10 fruits from each monitored plant (120 fruits per plot, 720 fruit per orchard) were taken to the laboratory and carefully inspected for mealybugs, by cutting the apples in half. Mean number of *P. calceolariae* females per fruit and proportion of infested fruit (with ≥1 mealybug) were calculated. For regression analysis, mean season-long trap captures (N males by trap^{-1} by day^{-1}) were plotted against mean season-long female counts (N females by plant^{-1}), mean females per fruit at harvest, and proportion of infested fruits. Regression coefficients were calculated using JMP ver. 11 (Cary, NC), for each orchard separately ($n = 6$) and also for the combination of all 12 plots.

Results

Daily Flight Pattern. Traps checked every hour for 24 h showed a distinct diel pattern of male flight (Rayleigh uniformity test: $Z = 123$; $P < 0.0001$). Males were caught only between 18:00 and 21:00 h (Fig. 2), 19:04 h ± 00:07 h being the mean vector time (±SEM). Control traps caught no males.

Effect of Non-Natural Stereoisomers. The attractiveness of isomeric mixtures as compared with the pure natural (R,R,R) isomer was evaluated. More than 500 males per trap per day were captured by traps with the pure or a mixture of stereoisomers, while only around 20 males per trap per day were captured in the control traps ($F = 6.05$; df = 3, 11; $P = 0.018$; Fig. 3). Captures in traps baited with the pure (*R*,*R*,*R*) stereoisomer were similar to captures in traps containing the isomeric mixtures ($F = 0.32$; df = 2, 8; $P = 0.74$, for ANOVA without the control treatment).

Dose. The attractiveness of the pheromone at different doses, in a range from 1 to 100 µg, was evaluated. There was a significant effect of dose in the number of males trapped in apple and pear orchards, respectively (apples: $F = 53.32$; df = 5, 17; $P < 0.0001$; Fig. 4A; pears: $F = 3.75$; df = 5, 17; $P = 0.04$; Fig. 4B). In the apple orchard, the control trap captured the lowest number of males per day (only one male in seven days for the total number of traps), followed by the 1 µg dose treatment. Treatments with 10, 30, and 50 µg captured similar amount of males, but significantly more than the 1 µg dose. The 100 µg dose captured the highest amount of males (40 males per trap per day approximately), more than 10 times of the 1 µg

dose, and about double of the amount captured in the intermediate doses. In the pear orchard, the pattern of captures in relation to dose was similar to the apple orchard, but in this crop, captures were, on average, over six times the captures of apples (average across treatment: 108 males by trap^{-1} by day^{-1} in pears versus 16 males by trap^{-1} by day^{-1} in apples). The large error bars in pear were owing to the strong block effect ($F = 21.95$; df = 2, 17; $P = 0.0002$), with Block 1 capturing less than half of Block 2, and almost seven times less than Block 3.

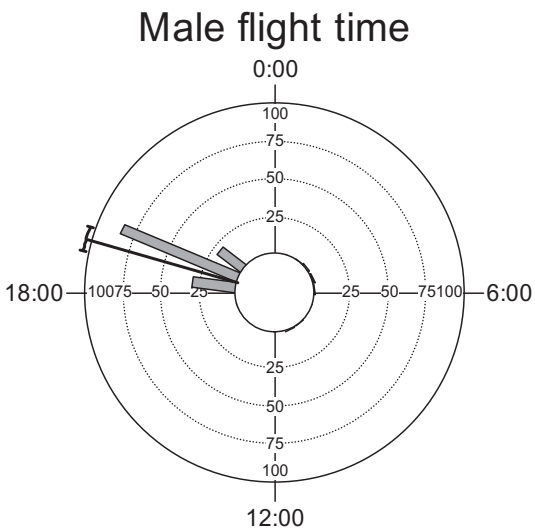

Fig. 2. Diel activity patterns of male *P. calceolariae* flight. Bars in the circular graph represent the frequency of occurrence by time of day, with 00:00 as midnight. Numbers on axis indicate numbers of males caught in three pheromone traps during the 24 h period. The line running from the center of the diagram to the outer edge and arc represent the mean time vector (μ) and 95% confidence interval, respectively.

Effective Trap Range and Dose. The effective range of pheromone traps was determined by placing traps outside an infested blueberry orchard, where only sparse non-host vegetation was present. For the three distances tested, there was a significant effect of dose (15 m: $F = 5.44$; df = 1, 8; $P = 0.04$; 40 m: $F = 58.22$; df = 1, 8; $P = 0.0001$; 80 m: $F = 5.70$; df = 1, 8; $P = 0.04$), with the 25 μg dose capturing significantly more than the control at all the distances (Fig. 5). Captures in the interior traps for the same dates and distances from the edge varied from 3.8 to 17.7 and 22.8 males per trap per day in the 15, 40, and 80-m trials, respectively, showing a lower abundance and/or activity at the time of the 15-m distance trial. Analysis of the number of males caught in the exterior traps in relation to the interior traps for the same distance showed an overall significant effect of dose ($F = 34.8$; df = 2, 26; $P < 0.0001$), with the 25 μg dose capturing a significantly larger proportion of males than the 0 and 1 μg dose treatments. There was also a distance effect ($F = 9.27$; df = 2, 26; $P = 0.002$), with the 40 m distance capturing significantly a larger proportion of males when compared with the respective interior traps, than the 15- or 80-m treatments.

Effect of Lure Age. There was a significant effect of lure age on male captures ($F = 5.02$; df = 4, 14; $P = 0.02$; Fig. 6). The largest male captures were in the fresh lures (aged 0 d) and the 90-d-old lures, and the lowest in the lures aged for 150 d (the "oldest" lures). The 30- and 60-d-old lures had intermediate captures. There was also a significant block effect ($F = 17.67$; df = 2, 14; $P = 0.001$), which in this trial represented consecutive weeks. Male trap captures increased over time, with the second week capturing, on average, twice the males of week 1, and the third week three times more of what was captured on week 1.

Correlation Between Male Captures Versus Population Visual Count and Fruit Infestation. Traps in the apple orchards captured males throughout

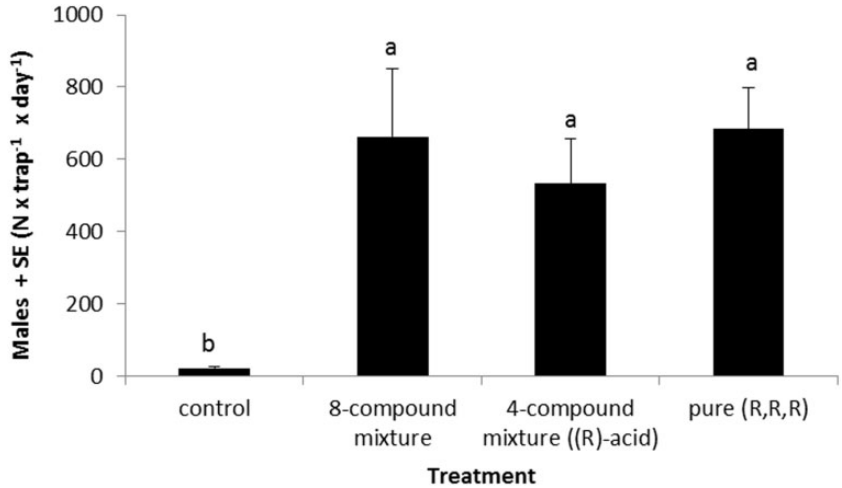

Fig. 3. Capture of *P. calceolariae* males (males by trap^{-1} by day^{-1}) in traps baited with lures of isomeric and pure pheromone. Values of columns with a similar letter do not differ significantly (one-way ANOVA, Tukey's test, $\alpha = 0.05$).

the season, with peak populations in January and, in the high-abundance orchard, another peak by mid-April. In total, 12,928 and 49,962 males were counted throughout the season in the low- and high-abundance orchard, respectively. Female populations from visual counts were found through the monitoring period (October to April), with peak populations by the end of December in the low-abundance orchard and by the end of January in the high-abundance orchard. A total of 1,124 and 2,443 females were found in visual counts in total through the season in the low- and high-abundance orchards, respectively. At harvest, a total of 163 and 311 females were found on or in apples (0.23 and 0.43 females per fruit) in the low- and high-abundance orchards, respectively. Male trap captures significantly related with female populations from visual

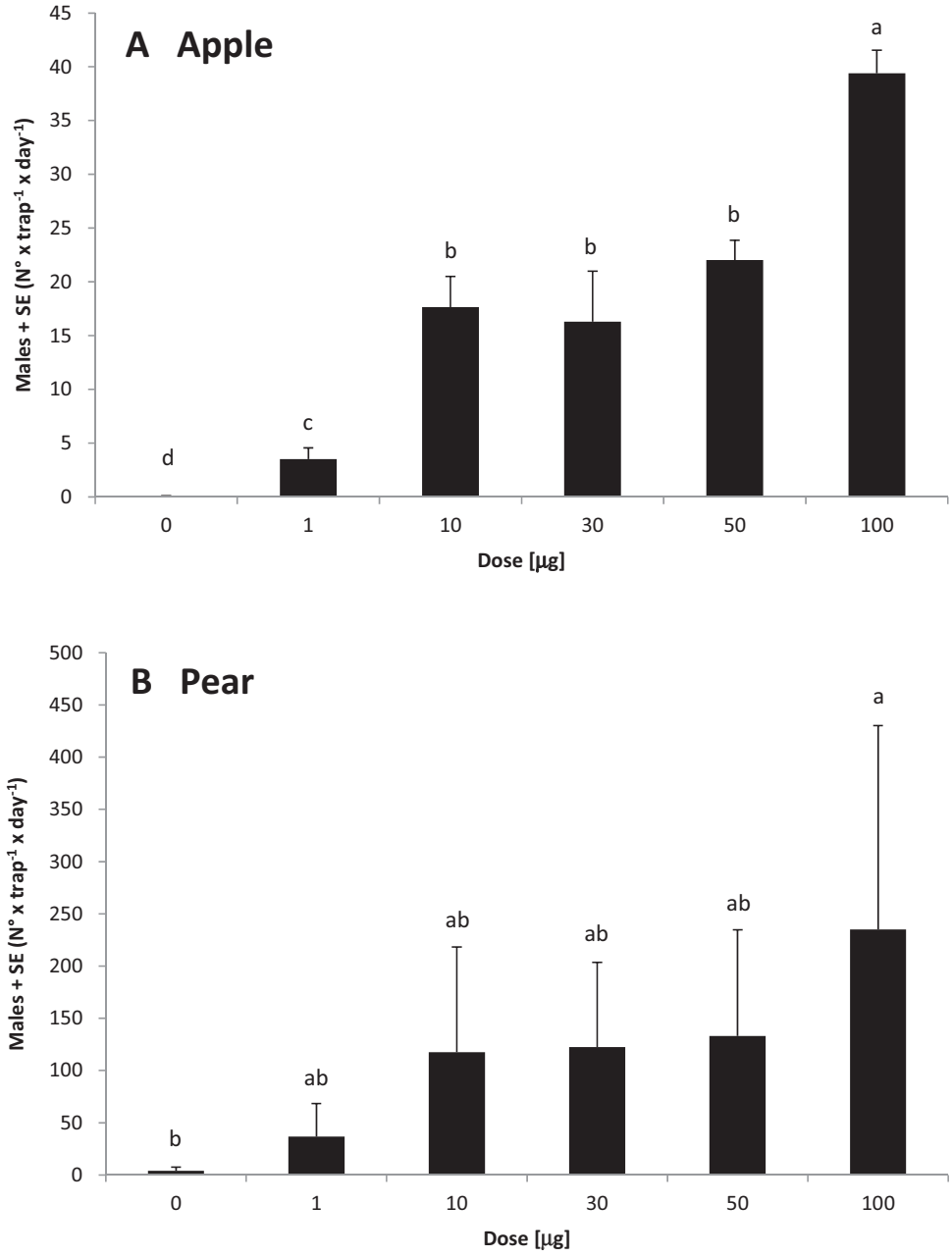

Fig. 4. Capture of *P. calceolariae* males (males by trap^{-1} by day^{-1}) in traps baited with different doses of isomeric chrysanthemyl-2-acetoxy-3-methylbutanoate. Assays were conducted in February 2013, in (A) apple, and (B) pear orchard differing in infestation levels. Means of males by trap^{-1} by day^{-1} followed by different letters are significantly different (one-way ANOVA, Tukey's test, $\alpha = 0.05$).

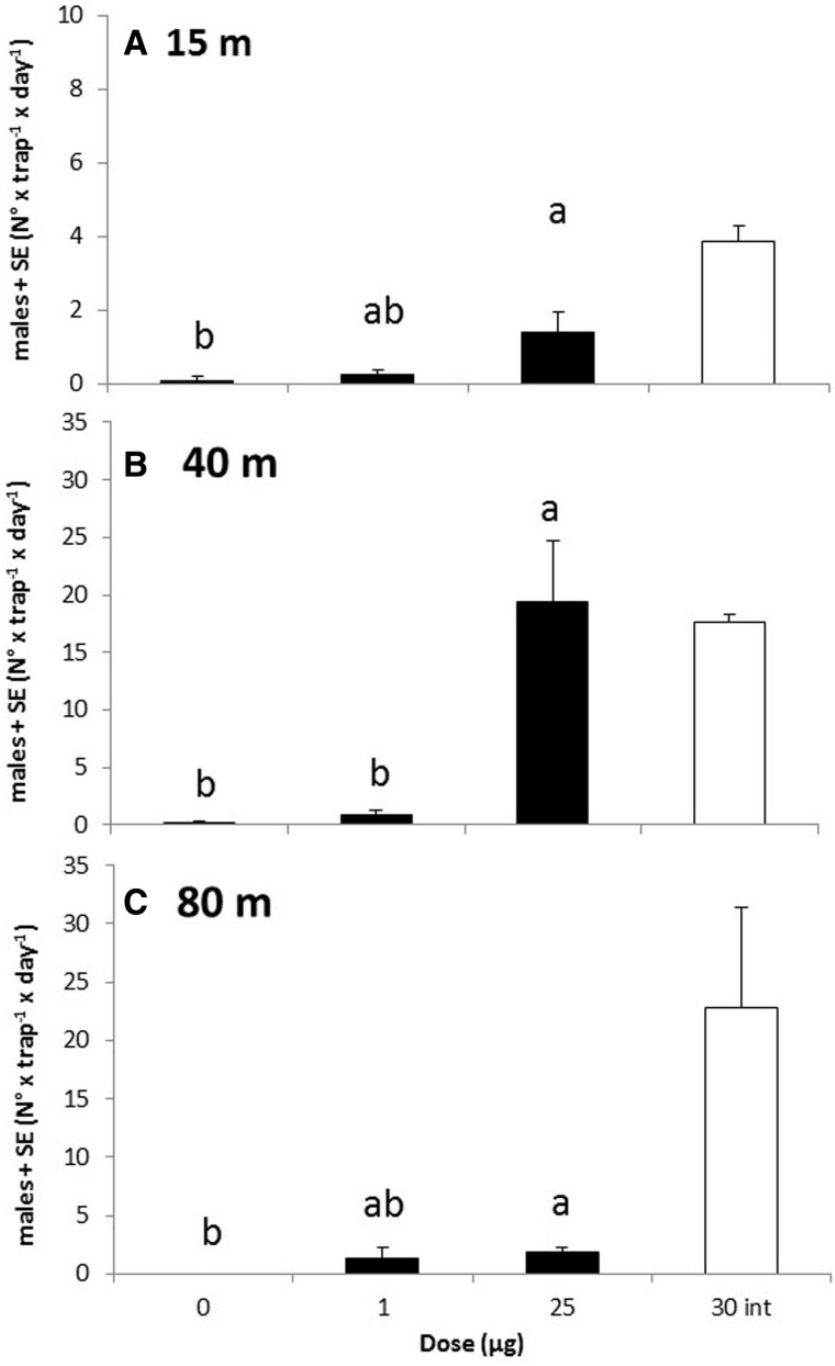

Fig. 5. Capture of *P. calceolariae* males (males by trap^{-1} by day^{-1}) placed at three different distances from a blueberry-infested orchard border. Black bars correspond to traps outside the orchard and white bars to traps at the interior of the orchard (lure with 30 µg). Captures in the outside traps were compared for each distance by one-way ANOVA, followed by Tukey's test. Significant differences between means are indicated by different letters above the bars ($\alpha = 005$).

counts in both orchards (low-abundance orchard: adjusted $R^2 = 0.71$; $P = 0.023$; high-abundance orchard: adjusted $R^2 = 0.58$; $P = 0.047$) and when all traps were combined (Fig. 7A). At harvest, in the low-abundance orchard, mean male trap captures did not relate with females per fruit nor with fruit infestation ($P = 0.83$ and 0.95, respectively). In the high-abundance orchard, male trap captures showed a trend with females per fruit (adjusted $R^2 = 0.50$; $P = 0.070$) and were significantly correlated with proportion of infested fruit

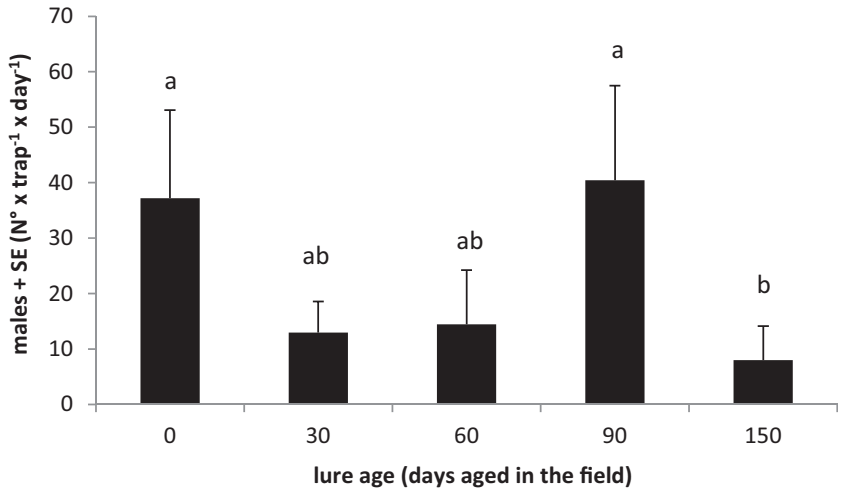

Fig. 6. Response of *P. calceolariae* males to pheromone lures with different ages (0, 30, 60, 90, 150 d). The assay was carried out during three consecutive weeks in April 2014 in an apple orchard. Significant differences between capture of *P. calceolariae* (males by trap^{-1} by day^{-1}) in traps with lures with different pheromone residue are indicated by different letters (one-way ANOVA, Tukey's test, $\alpha = 0.05$).

(adjusted $R^2 = 0.62$; $P = 0.039$). When all traps were combined, significant relations were found for females per fruit (Fig. 7B) and fruit infestation (Fig. 7C).

Discussion

The combined results of the field trials presented in this research are important in providing a baseline data for the design of monitoring protocols for *P. calceolariae* using pheromone traps in fruit orchards and for the development of pheromone-based control strategies.

P. calceolariae male flight activity in the field, as indicated by pheromone traps, only occurred at dusk, between 18:00 and 21:00 h. This contrasts with the field activity reported before for this species, where short periods of flight activity were found at sunrise and sunset (Rotundo and Tremblay 1976 cited by Silva et al. 2013). It also contrasts with the activity period found for other mealybug species under laboratory conditions (Mendel et al., 2012), where there was either no effect of time of the day (morning = 8:00–10:00, midday = 12:00–14:00, and early afternoon = 16:00–18:00) in male attraction toward pheromones for *Nipaecoccus viridis* (Newstead), *Planococcus citri* (Risso), and *Pseudococcus cryptus* Hempel, or a decrease in attraction toward the afternoon in *Planococcus ficus* Signoret (but note that in this experiment sunrise, sunset, and nighttime were not tested). In a later experiment that comprised the whole day, it was found that males of *Pl. citri* and *Pl. ficus* displayed maximal flight activity in the morning, between 06:00 and 08:00 h (Levi-Zada et al. 2014), which was in agreement with the flight activity reported earlier in the field for these species (Moreno et al. 1984, Zada et al. 2008, Silva et al. 2009). Given the different patterns found in male flight among mealybug species, it would be interesting to study a wider range of species from different genera for a 24-h period. Knowing the flight activity period is important when designing pheromone-based control strategies, like mating disruption or mass trapping, because a device that can release the pheromones only at the time of the day that males are active can substantially lower the amount of pheromone needed and, consequently, the cost of the control strategy.

The presence of unnatural stereoisomers did not affect trap captures, indicating that male *P. calceolariae* are indifferent to them. This is very useful, as the isomeric mixture can be used in practical applications and hence there is no need to develop stereospecific syntheses for the pheromone, which are usually much more expensive. Like in *P. calceolariae*, males of many mealybug species appear to be insensitive to unnatural stereoisomers; however, in at least two species, the presence of unnatural stereoisomers negatively affected the trap captures in field trials. For the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), the presence of the unnatural isomers resulted in lower trap captures (Zhang and Amalin 2005, Zhang et al. 2006), as was the case for the passionvine mealybug, *Planococcus minor* (Maskell), where the presence of the unnatural geometric isomer had an antagonistic effect in field trials (Ho et al. 2007).

Trials showed an effect of dose and distance between lures in male captures under field conditions. There was a nonlinear effect of dose on trap captures, with lower amount of males captured in the 1 µg dose, similar captures for the 10, 30, and 50 µg doses, and larger captures at the 100 µg dose, that differed significantly only in the lower-abundance trial. These results suggest that for monitoring *P. calceolariae* at the orchard level, 10 µg of the isomeric mixture would be the more appropriate dose, but probably when surveying larger areas with the aim of detecting early infestations, traps loaded with a 100 µg dose could perform better. Comparable studies with other species have been carried out, finding variable effects of dose on male captures.

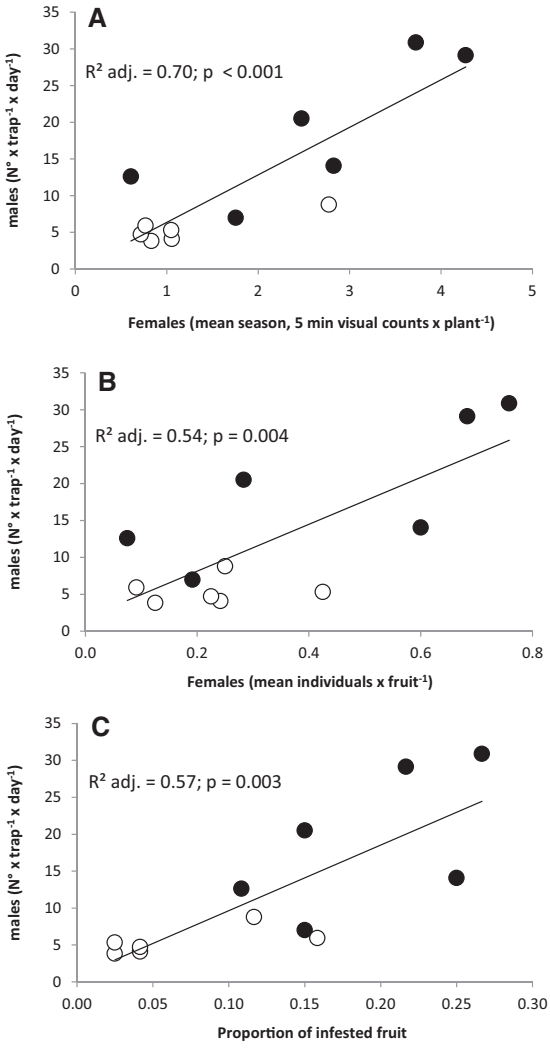

Fig. 7. Males caught in pheromone-baited traps (males by trap^{-1} by day^{-1}) regressed against (A) female mealybugs observed during 5 minutes (females by plant^{-1}) in apple trees, (B) mean number of females per apple at harvest, and (C) proportion of infested apples at harvest. White circles are from the lowest-abundance orchard and black circles from the highest-abundance orchard. Regression coefficients shown are for the combined traps of both orchards.

Similar results to ours were found for *Pseudococcus longispinus* (Targioni Tozzetti) and *Pseudococcus viburni* (Signoret) in ornamental plants in California (Waterworth et al. 2011). Waterworth et al. (2011) considered six doses (from 1 to 320 µg plus a control), and found that for both species, 1 µg traps caught the least (but much more than the controls), with a trend of increased captures with dose, but nonsignificant differences for the range 10–320 µg. Similarly, Zada et al. (2004) found a positive effect of dose for *Pl. citri* in citrus orchards in Israel when comparing 25 with 100 µg in high-infestation situations (approximately 60 to 70 males by trap^{-1} by day^{-1}), and when comparing six different doses (from 25 and 800 µg plus a control) in lower populations (approximately 7 to 30 males by trap^{-1} by day^{-1}). In this last experiment, significant differences were found only between the 25 µg versus the highest doses, with the range of 50–800 µg being similar. On the other hand, Millar et al. (2002) found no effect of lure dose (five doses between 10 and 1,000 µg) for *Pl. ficus* in California vineyards. It is interesting to note that in this trial, captures ranged from 155 to 200 in a 16-d period, equivalent to 9.7 to 15.5 males by trap^{-1} by day^{-1}, which is considerably lower to what we trapped in our highest dose tested (100 µg), suggesting that maybe the lack of dose effect in *Pl. ficus* could be related to its low abundance in the vineyards used. Finally, Zhang and Amalin (2005) tested four different doses (0.1, 1, 10, and 100 µg) for *Maconellicoccus hirsutus* (Green) in ornamental plants in Florida, and found that the highest captures (approximately 130 males by trap^{-1} by day^{-1}) were with the intermediate doses, compared with the captures with the lowest and highest doses (60 and 30 males by trap^{-1} by day^{-1}, respectively). In this case, the low captures at the highest dose could have been a result of interference between traps, as they were placed only 1 m apart. All these results, along with ours, suggest that there is often a nonlinear relationship between male captures and dose, and that the lowest possible dose has to be determined for each species.

Dose also had an effect when estimating the effective trap range and male flight capabilities. We found that 25 µg lures were highly attractive to males over a distance of at least 40 m, and that 1 µg lures were only slightly attractive even at 15 m from the orchard, the shortest distance tested. It is also interesting to note that at 80 m distance, although absolute numbers were low, trap captures were still significantly increased as compared with control traps, indicating that some *P. calceolariae* males are capable of distant flights, which agrees to what has been found in other mealybugs, such as *Pl. ficus* (Millar et al. 2002, Walton et al. 2004). The effective trap range and dose used are important considerations when setting up traps for detection and/or monitoring, as traps should be placed at certain distances from each other to avoid mutual influence. Equally important is that male mealybugs can be attracted from the vegetation surrounding the orchard if traps are placed too close to the edges of the field, giving the wrong impression of large abundances in the field when considerable amount of males can be coming from outside (Walton et al. 2004). According to our results, the effective trap range for *P. calceolariae* pheromone with a dose of 25 µg is somewhere between 40 and 60 m, which is similar to what was found for *Pl. ficus* when using 100 µg lures (Walton et al, 2004). Thus, in the field, *P. calceolariae* traps should be placed at distances of 100 m or more from each other to exclude interferences between them. Bahder et al. (2013) when studying optimal trap density for *Pseudococcus maritimus* (Ehrhorn) in vineyards in Washington State found no difference in season-long captures when using densities of one, four, or eight traps per 12.14 ha, suggesting no interference in captures at a density of one trap per 1.5 ha.

In relation to lure longevity, our results show that traps with a dose of 30 µg of pheromone remain active after being aged for 150 d (21 wk) under field conditions. No progressive loss of activity with increasing lure age was found, at least up to 90 days (approximately 21 wk). Additionally, in our monitoring trials, lures were deployed for the whole season (6 mo approximately) with no detectable loss of attraction until the first rain in winter (May), suggesting they might have an activity of up to 24 wk or more (approximately 170 d). Long periods of pheromone activity in the field have been also found in other mealybug species: 17 wk for *Pl. minor* at 25 µg dose (Roda et al. 2012); 19 wk and 21 wk at 25 µg dose for *P. viburni* and *P. longispinus*, respectively (Waterworth et al. 2011); at least 12 wk at 100 µg or at least 24 wk at 200 µg dose for *Pl. ficus* (Millar et al. 2002, Walton et al. 2004, Zada et al. 2008); and at least 24 wk at 200 µg dose for *Pl. citri* (Zada et al. 2004), but only 4 wk when using a commercial formulation (Roda et al. 2012). In the case of *M. hirsutus*, lures with 1 and 10 µg doses were active for at least 21 wk, and had 0.18 and 1.39 µg of pheromone, respectively, remaining in the septa after this period. The long-lasting activity of mealybug pheromones in the field is very appealing for regular monitoring by farmers, because there is no need for changing the lures, which lowers the cost and makes its management easier. Nevertheless, traps still need to be serviced regularly, because counting males in the field is challenging, particularly when large amounts are trapped. For this reason, traps with removable floors can be very attractive for routine field monitoring (Vitullo et al. 2007), but they have not been used in most of the trials carried out with mealybugs so far, and thus guidelines would need to be adapted or validated for this type of traps.

Good correlations between males caught in pheromone traps and female population abundance or crop damage have been found for other mealybug species in relevant crops, such as *Pl. ficus* in grapes in California (Millar et al. 2002) and South Africa (Walton et al. 2004), *P. viburni* in apples in South Africa (Mudavanhu et al. 2011), *P. longispinus* in ornamental nurseries in California (Waterworth et al. 2011), and *P. maritimus* in grapes in Washington (Bahder et al. 2013). Our results in apple orchards in central Chile also show significant positive correlations between *P. calceolariae* males caught in pheromone traps and abundance of females according to visual counts, and also with females per fruit and fruit infestation at harvest. It has been considered that a 2% infestation level in apples would be a reasonable threshold for treatment. When using the linear regression estimated with all 12 plots, a 2% infestation gives a negative value for male captures. A linear regression forced to begin at zero gives 1.9 males by trap^{-1} by day^{-1} as a threshold. Because the data tend to level off at low female abundance (Figure 7C), an exponential function gave a better fit ($y = 3.6722*e^{5.39232*x}$; $R^2 = 0.70$), in which case the threshold abundance for a 2% infestation is 4.1 males by trap^{-1} by day^{-1}. This value is much higher than the one calculated for *P. viburni* in apples in South Africa, which was 2.5 males in 14 d equivalent to 0.18 males by trap^{-1} by day^{-1} (Mudavanhu et al. 2011). This great difference in thresholds found could be owing to several reasons, including the methodology used to derive the relations between infestation and males captures, differential sensitivity of the pheromones for each species, different pattern in fruit colonization for the species, different population growth potential of the species, and different population growth potential owing to climate or mortality factors in each region. Regardless of the reason, or combination of reasons, the different threshold values estimated for *P. viburni* in South Africa and *P. calceolariae* in Chile point out the importance of carrying out experiments in the local agroecosystems.

Overall, our experiments provide important information for the design of pheromone-baited trap monitoring protocols for *P. calceolariae* in apples. Our results also show the usefulness of this technique as a tool for decision making at field level in comparison with visual inspection, which is a laborious and time-consuming task. Future studies should be carried out for other relevant species and crops, including a variety of regions to incorporate significant aspects that may influence the relationship between male captures in traps and relevant economic damage.

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