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ORIGINAL PAPER

Deployment of mating disruption dispensers before and after first seasonal male flights for the control of *Aonidiella aurantii* in citrus

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Abstract The rejection of citrus fruit caused by infestations of the California red scale (CRS), *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), raises concerns about its management. This fact has led to the introduction of new integrated control methods in citrus orchards, including the implementation of techniques based on pheromones. Previous works described efficient mating disruption pheromone dispensers to control *A. aurantii* in the Mediterranean region. The main aims of the present study were to adjust the timing of dispenser applications and study the importance of controlling the early first generation of *A. aurantii* by testing two different application dates: before and after the first CRS male flight. The efficacy of the different mating disruption strategies was tested during 2010 in an experimental orchard and these results were confirmed during 2011 in a commercial citrus farm. Results showed that every mating disruption strategy achieved significantly lower male captures in monitoring pheromone traps compared with untreated plots, as well as mean fruit infestation reductions of about 80 %. The control of the first CRS generation is not essential for achieving a good efficacy as demonstrated in two locations with different pest pressure. The late application of MD dispensers before the second CRS male flight has proven to be effective, suggesting a new advantageous way to apply mating disruption.

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Key message

Mating disruption (MD) of *Aonidiella aurantii* proved successful in citrus orchards. The importance of controlling the first generation of *A. aurantii* has been investigated by checking the efficacy of MD applied before and after the first generation. We concluded that the control of the first generation is not essential and the application of MD before the second male flight has proven to effectively reduce fruit infestation. This late deployment allows a reduction in the required quantity of pheromone, thus increasing the economic viability of MD in citrus crops.

Introduction

Infestations of California red scale (CRS), *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), pose a serious problem for citrus growers, as CRS may lead to a reduction in tree vigor and the downgrading or commercial rejection of fruits. The economic importance of this armored scale is due to its presence on the surface of the fruits, as a cosmetic damage, and the cost of the strategies needed to control it, even more intense in fresh citrus market.

Since CRS control has been affected by the development of resistances to insecticides (Yust et al. 1943; Collins et al. 1994; Grafton-Cardwell and Vehrs 1995; Levitin and Cohen 1998), the development and introduction of new integrated and biological control programs were essential

for citrus crops. The use of mineral and vegetable oils (University of California 1991; Grafton-Cardwell and Reagan 1995; Rongai et al. 2008) and insect growth regulators (Yarom et al. 1988; Grout and Richards 1991a; Grafton-Cardwell et al. 2006; Eliahu et al. 2007; Rill et al. 2007) appeared as good alternatives to conventional pesticides. These products have provided good control results but they can be harmful to beneficial arthropods (Grafton-Cardwell and Gu 2003; Grafton-Cardwell et al. 2006; Desneux et al. 2007; Vanaclocha et al. 2013). The main enemies of CRS are species of the *Aphytis* parasitoids (Hymenoptera: Aphelinidae) (DeBach 1959; DeBach and Argyriou 1967). Specifically, *Aphytis melinus* (DeBach) is the most successful agent but the control of CRS by augmentative releases of this parasitoid is still under study in Spain (Sorribas et al. 2008, 2012; Pekas et al. 2010; Tena et al. 2013). Although in some regions the augmentative biocontrol of CRS through *A. melinus* has achieved good results (Avidov et al. 1970; McLaren and Buchanan 1973; Furness et al. 1983; Moreno and Luck 1992; Bedford 1996), the current cosmetic thresholds in citrus fresh fruit market makes the *A. melinus* success quite improbable in the short term.

Integrated pest management programs include the implementation of control methods based on pheromones. Tashiro and Chambers (1967) demonstrated the production of a sex pheromone in CRS, whose chemical structures were reported by Roelofs et al. (1977), as 3-methyl-6-isopropenyl-9-decen-1-yl acetate and (*Z*)-3-methyl-6-isopropenyl-3,9-decadien-1-yl acetate. Since then, synthetic sex pheromone traps have been widely employed as a detection and monitoring tool for CRS populations (Kennett and Hoffmann 1985; Moreno and Kennett 1985; Grout et al. 1989; Grout and Richards 1991b). The efficacy of mating disruption (MD) technique against CRS was not clearly demonstrated in the first experiments using rubber pheromone dispensers (Barzakay et al. 1986; Hefetz et al. 1988). However, by studying different pheromone doses, Vacas et al. (2009) described a new mesoporous dispenser capable of interfering with normal *A. aurantii* chemical communication. The efficacy of these mesoporous dispensers was further verified, when CRS male catches and fruit infestation were significantly reduced by applying doses of about 40 g pheromone/ha for 6 months (Vacas et al. 2010). Moreover, the pheromone environment in MD-treated orchards resulted harmless for the performance of *A. melinus* in field and laboratory trials (Vacas et al. 2011; Vanaclocha et al. 2012). By means of all these studies, it was found that CRS mating disruption achieved control at least equal to conventional oil sprays, providing growers with an advantageous control tool. It is commonly known that oil sprays need careful planning and application for a satisfactory control level. On the other hand, the deployment of MD dispensers does not need replacement or qualified hand labor, and only a minimum flight monitoring is needed to determine the moment of application; all these are important advantages to take into account. However, research had shown the need for additional trials to adjust the timing of dispenser application to cover all the CRS generational cycles. In Spain, CRS shows between three and four complete generations with four male flights; the first taking place from mid-April to mid-May, the second from mid-June to mid-July, the third from end-August to September and a late fourth flight during October to beginning of November. Following this dynamic, if all the generations must be affected, mating disruption should be maintained for 8 months what would mean a large amount of pheromone, making this control method unaffordable. However, several authors have stated that the first flight of *A. aurantii* males is not correlated with fruit infestation and abundance of the following flights (Moreno and Kennett 1985; Hernández-Penadés et al. 2002; Campos-Rivela et al. 2012). Thus, the importance of controlling the early first generation of *A. aurantii* has been investigated and the efficacy of MD applied before and after the development of the CRS first generation has been examined both in small plots and in a commercial large size orchard.

Materials and methods

Mesoporous dispenser and device

The pheromone dispensers applied in the mating disruption (MD) treatments are based on the technology of inorganic molecular sieves (Corma et al. 1999, 2000). Pheromone is impregnated in a natural clay mineral matrix called sepiolite. Its structure, with a high specific surface area, confers to the dispenser good properties for the adsorption and release of organic molecules. Besides the pheromone, formulations include different additives to give consistency and protect the dispenser against humidity. The impregnated material is then compressed in a cylindrical mold by means of a hydraulic press. The manufacturing process has been licensed to Ecologia y Protección Agrícola S.L. (Valencia, Spain) who manufactured the dispensers for these trials. Currently, these MD dispensers are commercialized since 2013 by Syngenta Agro (Madrid, Spain) under the name Dardo®.

Dispensers contained 70 mg (a.i.) of the CRS sex pheromone as the diastereomeric mixture (3S,6R and 3S,6S) of the 3-methyl-6-isopropenyl-9-decen-1-yl acetate with 75 % chemical purity; the remaining 25 % belongs to the by-product 3-methyl-6-isopropylidene-9-decen-1-yl acetate, without pheromonal activity. Dispensers were

attached to tree branches inside polypropylene baskets of 50 mm wide and 90 mm long with a hanger at the top (Ecología y Protección Agrícola SL, Valencia, Spain). Pheromone is released through the 6 × 5 mm grid walls of the basket.

Experimental design—trial 2010

The field trial was conducted in a 3 ha mandarin (*Citrus reticulata* × *sinensis*; var. Ortanique) experimental orchard located in Denia (Alicante, Spain; UTM: X243500 Y4303900) under Mediterranean climate conditions (mean temperature = 19.9 °C, mean relative humidity = 71.2 %). Trees were 20 years old and spaced 6 m by 4 m. The trial was designed with 11 plots: nine plots of 0.3 ha alternately arranged to test three different procedures for the application of mating disruption and two plots of 0.1 ha as reference untreated plots. Pheromone-treated plots were at least 50 m apart, whereas untreated plots were separated 60 m from any MD plot. Pheromone dispensers were applied on 29 March 2010 in three plots before the appearance of the first CRS flight (MD-I treatment). Another three plots had dispenser application on 28 May 2010, before the CRS second flight (MD-II treatment). Timings of dispensers' deployment were determined according to general population dynamics in the study area and degree-day accumulation (DD = $[(T_{max} + T_{min})/2] - T_{critical}$; being $T_{critical} = 11.7$ °C) (Kennett and Hoffmann 1985). Finally, the combination of MD-II application with an oil treatment against nymphs of the first generation was tested in another three plots (MD-II + Oil), where dispensers were also placed on 28 May 2010 and oil treatments were applied on 28 May in only these three plots. Pheromone dispensers in every plot were placed at a density of one per tree (420 dispensers ha^{-1}) and were not replaced during the experiment.

Experimental design—trial 2011

A new field trial was conducted in a commercial citrus farm, with larger plots, to confirm the result obtained in the previous trial 2010. MD timings were tested in a 10 ha orange (*Citrus sinensis* Osbeck, var. Navelina) orchard located in Nerva (Huelva, Spain; UTM: X717288 Y4177107) under semi-continental climate conditions (mean temperature = 21.6 °C, mean relative humidity = 56.6 %). Trees were 25 years old and spaced 7 m by 3.5 m. In this case, plots with the same MD timing were contiguous as mating disruption is more efficient when applied in large areas. Thus, pheromone dispensers were applied on 7 April 2011 in a whole 1 ha as MD-I treatment, while for MD-II treatment, 1.5 ha had dispenser application on 6 June 2011. Timings were determined according to population dynamics and degree-day accumulation. MD pheromone dispensers in both strategies were placed at a density of 410 dispensers ha^{-1} and were not replaced during the experiment. A third 0.3 ha plot was left without treatments as untreated plot, which was 100 m apart from the pheromone-treated areas.

Oil sprays

Given that fruit infestation was over 20 % in the previous season, oil treatments in the corresponding plots were timed for the presence of crawlers, which were monitored according to the sampling method suggested by the Valencian regional IPM program (DOCV 2008). A total of 25 infested branches (2–3 years old) were randomly sampled each week from the date of the first flight and taken to the laboratory. Leaves and twigs were removed from the branches and cut into 10 cm long pieces. Using a binocular scope, a total of 100 live scales were identified as first, second and third instars, adult females, and adult females with crawlers. The oil treatment was applied when first and second instars represented 70 % of live scales and more than 90 % of adult females had crawlers. Paraffinic oil (15 g l^{-1}) (Argenfrut RV; GulfOil Argentina SA, Argentina) applications were made with an airblast sprayer calibrated to deliver 3,500 l ha^{-1} at 150 psi with the tractor driven at 1.55 km h^{-1}.

Evaluation of treatment efficacy

The efficacy of the different strategies was evaluated according to the CRS male flight inhibition and the fruit infestation assessment. Both parameters were studied in the center of each plot to avoid possible edge effects due to pheromone drift between contiguous treated areas, as buffer areas are considered to be 15 m from the plot borders (Vacas et al. 2009). One commercial white sticky pheromone trap (Pherocon® V Trap; Trécé Inc., Adair, OK) was placed in the center of each plot in trial 2010 to compare male catches between the different control strategies. In the case of trial 2011, captures where also evaluated in triplicate, as in the center of three subplots within each above-described area. All the monitoring traps were revised every 7 or 15 days, from April to November, and the recorded captures in each trap were divided by the corresponding number of days (7 or 15) to obtain the number of males captured per trap and day (MTD). Pherocon® monitoring lures (Trécé Inc., Adair, OK), loaded with 250 μg sex pheromone, were replaced every 42 days.

To measure the inhibition of male catches that occurred in pheromone-treated plots, the mating disruption index (MDI) for each strategy was calculated as an indicator of the treatment efficacy using the following formula: $MDI = (1-(x/y)) \times 100$, where x is the number of males

captured in MD plots and y is the number of males captured in untreated plots.

Fruit was evaluated for scale infestation of Ortanique mandarins on 21 September 2010 and 29 September 2011 in Navelina oranges. Forty fruit per tree (10 fruits per direction: north, east, south, and west) were evaluated on trees located on the center of each plot (250–300 fruits/ha) in trial 2010 and in the center of three subplots within each area in trial 2011 (250–300 fruits/ha). A fruit was considered to be scale infested when it had more than three scales on its surface, as suggested by the treatment threshold published in the Valencian regional IPM guidelines (DOCV 2008). The percentage of fruit with more than 10 scales was also recorded to perform a sensitivity analysis.

Field data analysis

Generalized linear model (GLM) techniques assuming negative binomial error variance were employed to compare the number of CRS males captured in the different treated plots. Models were constructed with MTD data as the dependent variable and treatment, time (week of the study period) and their interaction as the explanatory variables. Given that the MD-II application of dispensers was not carried out until 28 May in trial 2010 and 6 June in trial 2011, we constructed different models for data from the first male flight and data from the rest of flights (second and third). In this way, we evaluate the initial disruption level in MD-I plots during the first flight when MD-II was not yet installed (from April to beginning of June), and also the significance of the male captures inhibition during the second and third flights (from mid-June to October) for both trials.

The significance of the explanatory variables was assessed by backward elimination from the model. When significant effects were found, the *glht* function in the multcomp package (Hothorn et al. 2008) was used to perform Tukey HSD tests for post-hoc pairwise comparisons.

Likewise, we used GLM techniques assuming negative binomial error variance to assess scale infestation differences between the different treatments. For both trials, models were constructed with the percentages of scale-infested fruit in the trees inspected at the end of the trial as the dependent variable and treatment as the explanatory variable. All statistical analyses were conducted with R (R Development Core Team 2012)

Pheromone release profile

The pheromone release profile of the mesoporous dispensers was studied during the trials. Additional dispensers were aged under field conditions in a nearby area, 500 m away from trial orchard, in order to extract their residual pheromone content at different aging times (from 0 to 250 days of field exposure). Three dispensers were taken per aging time, to be extracted at 40 °C for 2 h, with magnetic agitation, in 15 ml of dichloromethane/methanol (2/3, v/v) as solvent. After 1 h of extraction, 0.5 ml of the internal standard 1-dodecanol (20 mg ml^{-1}) was added to the extracts. The extracts were centrifuged (3,000 rpm for 8 min) and then filtered with syringe filters before chromatographic analysis. Pheromone was then quantified by gas chromatography with flame ionization detector (GC/FID; Clarus® 500 gas chromatograph; PerkinElmer Inc., Wellesley, MA). All injections were made onto a ZB-5 ms (30 × 0.25 mm × 0.25 μm) column (Phenomenex Inc., Torrance, CA), held at 160 °C for 5 min, and then programmed at 2 °C min^{-1} up to 180 °C, where it was held for 1 min, and then programmed at 45 °C min^{-1} up to 250 °C. The carrier gas was helium at 1.2 ml min^{-1}. The pheromone amount was estimated by means of a calibration curve which was previously built by preparing standard solutions with the following concentrations: 10.0, 5.0, 2.0, 1.0, and 0.4 mg ml^{-1} of pheromone and 1 mg ml^{-1} of the internal standard. Calibration curve was described by the equation $y = a + bx$, where y is the FID peak area ratio of the pheromone and the internal standard ($area_{ph}/area_{IS}$) and x is the known amount of pheromone.

Simple regression was used to determine the evolution of the residual pheromone load (mg) according to aging time for the dispenser employed. The quantified residual pheromone contents were employed in a polynomial regression as the dependent variable, to study the significance of the linear and quadratic effect of time (days and $days^2$) on pheromone emission and check whether the pheromone emission was constant during the time under study. In this case, the residual pheromone load decrease at a constant level and the mean emission rate is given by the slope of the fitted linear model. Statgraphics Centurion XVI v16.1 software (StatPoint Technologies Inc., Warrenton, VA) was used for these analyses.

Results

Trial 2010

Population dynamics of *A. aurantii* in the area of Denia can be observed by the data obtained with traps located in the untreated plots (Fig. 1). The first flight took place during May with a maximum of 6.93 CRS males per trap and day (MTD). The second flight began at the end of June with the maximum number of males captured in mid-July. Male captures of *A. aurantii* increased from the first week of August. They reached a maximum on 31 August and began

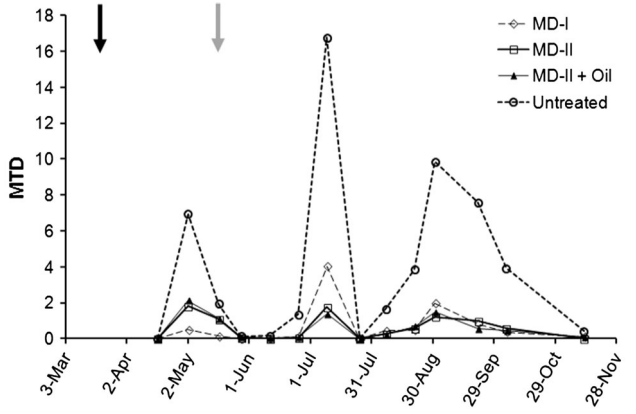

Fig. 1 Population dynamics of *Aonidiella aurantii* in trial 2010 (Alicante, Spain), shown as males per trap per day (MTD) captured on the different plots: dispenser application before the first CRS male flight (MD-I), application before the second flight (MD-II), the combination of MD-II application with an oil treatment, and the untreated plots. *Black arrow* indicates dispenser application in MD-I strategy (29 March 2010).The *grey arrow* points out pheromone application in MD-II strategy (28 May 2010) and oil application in the May + Oil plots

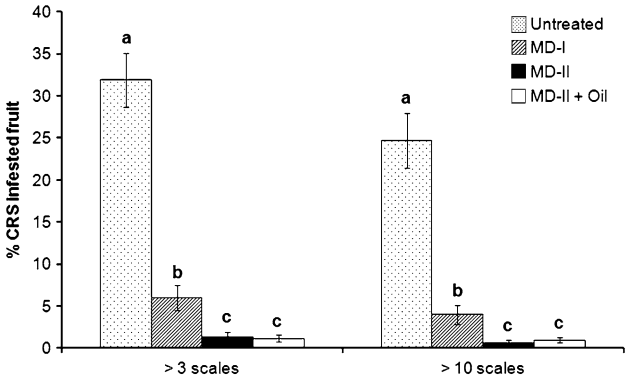

Fig. 2 Mean percentage of scale-infested fruits observed in the different plots of trial 2010: untreated, MD-I, MD-II, and the combination of MD-II with oil spray. *Bars* labeled with the same letter do not differ significantly (Tukey HSD tests, $p > 0.05$)

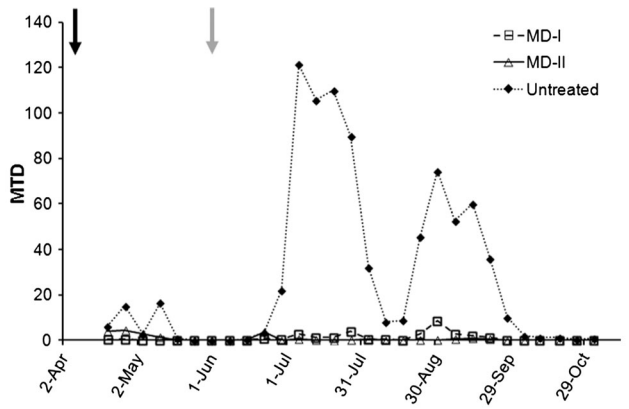

Fig. 3 Population dynamics of *Aonidiella aurantii* in trial 2011 (Huelva, Spain), shown as males per trap per day (MTD) captured on the different plots: dispenser application before the first CRS male flight (MD-I), application before the second flight (MD-II), and the untreated plot. *Black arrow* indicates dispenser application in MD-I strategy (7 April 2011) and the *grey arrow* points out pheromone application in MD-II strategy (6 June 2011)

to decrease slowly up to the beginning of November, when only 0.4 MTD was registered in the untreated plots.

Male catches in plots treated with pheromone remained low throughout the entire season, and only slight peaks were registered according with the three described male flights (Fig. 1). The effect of time factor on male catches during the first flight was statistically significant ($F_{1,16} = 5.30$, $p = 0.035$) according to the natural population dynamics. Yet more crucial, the treatment applied significantly affected male captures ($F_{3,16} = 6.67$, $p = 0.004$) as follows. When first flight was taking place (April to beginning of June), MD was already installed on MD-I plots and captures were 94.2 % inhibited relative to the untreated plots ($p < 0.001$). Meanwhile, MD was not yet established in MD-II strategies but mean initial population levels were lower compared to the untreated plots although not significantly different (MD-II: $p = 0.226$; MD-II + Oil: $p = 0.310$) (Tukey test; adjusted P values with single step method). Considering, catches from the most abundant flights (mid-June to October), time factor resulted statistically significant ($F_{7,75} = 27.89$, $p < 0.001$), as well as the effect of treatment ($F_{3,75} = 84.75$, $p < 0.001$). Compared to the untreated, male catches were significantly lower in the MD-I, MD-II, and MD-II + Oil plots ($p < 0.001$). Thus, communication disruption occurred during this period with the three tested mating disruption strategies, resulting in average male flight inhibitions of 81.5 % with the MD-I timing, 87.7 % in plots with MD-II, and 88.9 % with the combined strategy MD-II + Oil (without significant differences among them, $p > 0.2$).

Regarding fruit damage assessment, 31 % of fruit were scale infested with more than three scales on the surface when no treatment was applied in the orchard (untreated plot in Fig. 2). Results of every MD treatment differed significantly from the absence of treatments for both infestation levels recorded (more than three scales: $F_{3,76} = 20.98$, $p < 0.001$; more than 10 scales: $F_{3,76} = 22.97$, $p < 0.001$). All of the mating disruption deployments achieved a reduction in scale-infested fruit compared to the untreated plot: 81 % damage reduction for MD-I ($p = 0.006$); 95 % for MD-II; and 96 % by the combination MD-II + Oil ($p < 0.001$). Although CRS fruit infestation was significantly reduced by MD-I application, it differed significantly from the MD-II timing ($p = 0.003$) and MD-II + Oil strategy ($p < 0.001$) (Fig. 2). Fruit infestation observed in MD-I plots did not

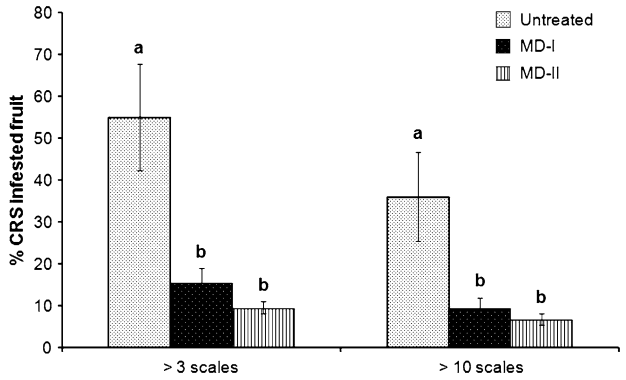

Fig. 4 Mean percentage of scale-infested fruits observed in the different plots of trial 2011: MD-I, MD-II, and the untreated plot. *Bars* labeled with the same letter do not differ significantly (Tukey HSD tests, $p > 0.05$)

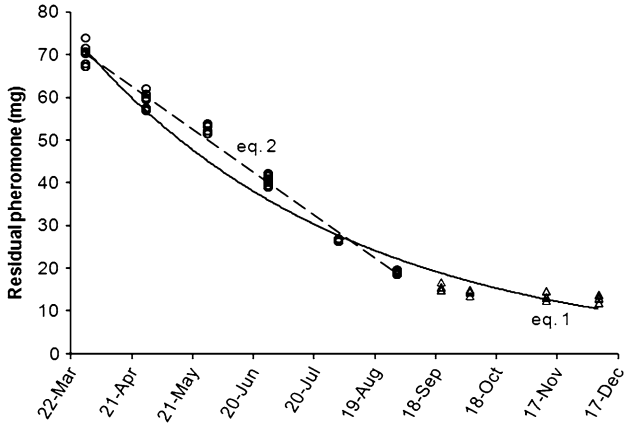

Fig. 5 Evolution of the remaining load of pheromone (mg) on the mesoporous dispensers versus time (days in orchard). The complete release profile was fitted to an exponential model (Eq. 1, $R^2 = 0.95$), although pheromone release rate was constant until 154 days of field exposure and fitted a linear model (Eq. 2, $R^2 = 0.99$). The x-axis represents the dates corresponding to aging time

exceed 6 %, whereas less than 1.5 % of fruit were found to be scale infested in the MD-II plots.

Trial 2011

The male population level was significantly higher in Nerva regarding to level of captures obtained in Denia ($F_{1,7} = 9.19$, $p = 0.02$). In the area of Nerva, the first male flight peaked during April and the first weeks of May (Fig. 3) with a maximum of 16.57 MTD in the untreated plot. The first catches belonging to the second flight were obtained on 22 June, while the third flight peaked on 31 August.

Male catches in plots treated with pheromone remained low throughout the entire season with statistical differences regarding to the untreated plot (Fig. 3). Time had a significant effect on catches during the entire period of study (first flight: $F_{9,18} = 21.78$, $p < 0.001$; rest of flights: $F_{18,36} = 9.05$, $p < 0.001$), according to natural population dynamics. The effect of treatment factor was significant (first flight: $F_{2,18} = 64.66$, $p < 0.001$; rest of flights: $F_{2,36} = 248.55$, $p < 0.001$). Considering catches from the first flight, MD-I achieved a significant mean male flight reduction ($p < 0.001$) of 97.3 % relative to the control. Given that MD-II was not yet installed when the first flight took place, mean male captures during April and May in MD-II plots were significantly higher compared to MD-I ($P < 0.001$). When multiple comparison was performed with data from the most abundant flights (July–October), CRS male captures were 96.2 % and 99.1 % reduced, relative to control, with MD-I and MD-II, respectively (with significant differences between MD strategies, $p < 0.001$).

Damage assessment revealed that pheromone treatments had a significant effect on CRS fruit infestation (more than three scales: $F_{2,67} = 27.16$, $p < 0.001$; more than 10 scales: $F_{2,67} = 16.33$, $p < 0.001$). Damage was significantly lower in both pheromone-treated plots, regarding to the untreated which had 55 % fruit with more than three scales ($p < 0.001$). The percentage of fruit with more than three scales was 71.8 % reduced with MD-I treatment, while this reduction achieved 82.7 % with the MD-II application, although they were not significantly different ($p = 0.145$) (Fig. 4).

Pheromone release profile

Pheromone release profile of mesoporous dispensers is depicted in Fig. 5. The complete model was fitted to an exponential model (solid line in Fig. 5, Eq. 1) resulting in $R^2 = 0.95$.

$$y = 71.322 \times e^{-0.008x} \quad (1)$$

However, statistical analysis showed that the curvature of this model was due to data from the last 3 months of the dispenser lifespan. Polynomial regression of data from 0 to 154 days of aging (end March to August), evaluated the significance of the quadratic (days^2) and linear (days) effects of time and confirmed the absence of curvature (quadratic effect not significant: $p = 0.31$; linear effect: $p < 0.001$). Thus, the release profile was fitted to the line given by Eq. 2 (discontinuous line in Fig. 5), resulting $R^2 = 0.99$. This means that emission of mesoporous dispensers is assumed to be constant from 0 to 154 days (until August), and the mean release rate given by the slope of the linear model is 334 μg day^{-1}. From this date on, emission level decreased below 100 μg day^{-1} during the last months of field exposure.

$$y = 70.241 - 0.334x \quad (2)$$

Discussion

The efficacy of the mating disruption technique against CRS infestations was previously demonstrated by Vacas and coworkers (Vacas et al. 2009, 2010, 2011); nevertheless, timing of dispensers' deployment needed to be adjusted for an optimal practical application. In the present work, the efficacy of the late application of MD dispensers, before the second CRS male flight (MD-II), has been demonstrated in two trials carried out in 2010 and 2011, in two different locations of Spain, with different climates, male population levels and citrus varieties.

CRS is able to develop from three to five generations per year, mainly influenced by temperature (Kennett and Hoffmann 1985; Grout et al. 1989). Under the climatic conditions of Spanish citrus areas, CRS shows three complete generations and a possible fourth generation in some areas and during warmer autumns. Generally, the first male flight takes place between mid-April and mid-May, and is usually not too abundant. In the present work, the importance of controlling the first generation of *A. aurantii* was investigated in 2010 by the application of mating disruption at two different times of deployment: before (MD-I) and after (MD-II) the occurrence of the first male flight. According to CRS male flight monitoring in trial 2010, the first flight was significantly inhibited in MD-I plots. Once MD was also installed in MD-II and MD-II + Oil plots, catches were maintained at low levels throughout the study and both MD timings achieved more than 80 % reduction of CRS male catches. This disorientation effect was confirmed in trial 2011 in larger commercial plots with higher male population levels, where both MD strategies achieved mean flight inhibition of about 97 %.

In the trial carried out in 2010, the scale infestation assessment revealed that the MD-II results were significantly better than the MD-I strategy, despite reducing damage by around 80 %. This could be explained by the release profile and lifespan of the pheromone dispensers. According to the results, flight inhibition in MD-I plots was not significantly different from MD-II plots during the third flight but MD-II achieved significantly greater reduction of fruit infestation. Extraction and quantification of the pheromone remaining in the aged dispensers showed that release rate was constant for 5 months and equal to 334 μg day^{-1}, and thereafter it decreased. This reduction in release rate is not due to climate factors but to dispenser formulation itself. The release profile of this kind of dispensers is highly temperature independent (Domínguez-Ruiz et al. 2008) and release rate is lower as closer the pheromone load to the residual content that remains retained in the dispenser. If the mesoporous dispensers are applied in March (MD-I), this five-month period with proper pheromone emission would protect the crop only until the end of August, without covering the entire third flight of CRS males. Mean pheromone emission during September in MD-I plots was 130 μg day^{-1}, clearly under the optimum release level of 250 μg day^{-1} suggested by Vacas et al. (2009). We think that this lower emission rate could still have a disorientation effect of males toward traps, but could be insufficient to disrupt the short-range attraction and mating of males. It has been described for moth pests that the amount of pheromone needed to disrupt male orientation to traps is lower than the amount needed to disrupt mating (Ioratti et al. 2011). In this way, when dispensers are applied on May (MD-II), pheromone emission is maintained at suitable levels to protect the crop against the entire third flight.

The present work confirms that the late deployment of dispensers is at least as efficient as the deployment before the first flight. Therefore, it has been observed with different infestation levels that the control of the CRS first generation is not crucial and damage can be controlled by establishing MD before the second flight with mesoporous dispensers releasing 334 μg day^{-1} constantly for at least 5 months. This could be related with the fact highlighted by several authors who stated that the first flight is not correlated with the abundance of the following flights (Moreno and Kennett 1985; Hernández-Penadés et al. 2002; Campos-Rivela et al. 2012). Thus, the first flight is not a good predictor of infestation later in the season, probably because survival and activity of the first generation is highly affected by more unstable weather conditions. However, mating disruption of the first emerging moths is crucial for the development of the subsequent generations throughout the season in Lepidoptera species. Several authors demonstrated that early pheromone applications prevent mid-season increases in Lepidoptera populations (Staten et al. 1987; Kehat et al. 1995; Lykouressis et al. 2005); these populations being responsible for high yield losses. By contrast, we have observed that the control of the first CRS generation is not so essential for achieving a good efficacy. Moreover, this first generation does not usually colonize the fruit and the second annual crawler generation, which takes place in the summer, is generally considered to be mainly responsible for the infestation of fruit (Rodrigo et al. 1996).

It has been demonstrated that the late application of dispensers allows a reduction in the required quantity of pheromone, thus increasing the economic viability of CRS mating disruption. Currently, available dispensers are not able to release pheromone at a suitable level during all the CRS male flights. The development of pheromone dispensers with higher load and longer lifespan would not be cost-effective, as the current cost of pheromone synthesis is

a limiting factor for MD implementation. At the moment, pheromone represents about 90 % of the value of the dispenser (Ecología y Protección Agrícola, pers. comm.) and this technique is already in the upper limit of the costs affordable by the growers (300 € ha^{-1} for the MD treatment versus 200–250 € ha^{-1} for two oil sprays). Therefore, with the dispensers available in the market, the recommended strategy will be the application of mating disruption before the second CRS male flight.

Author Contribution

VN and JP conceived and designed research. SV, CA and VN conducted experiments. SV analyzed data. SV and VN wrote the manuscript. All authors read and approved the manuscript.

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