Received: 5 June 2009 Revised: 15 January 2010 Accepted: 15 January 2010 Published online in Wiley Interscience: 15 March 2010

(www.interscience.wiley.com) DOI 10.1002/ps.1937

# Mating disruption of California red scale, *Aonidiella aurantii* Maskell (Homoptera: Diaspididae), using biodegradable mesoporous pheromone dispensers

Sandra Vacas, Cristina Alfaro,* Vicente Navarro-Llopis and Jaime Primo

## Abstract

**BACKGROUND: The control of California red scale, *Aonidiella aurantii* (Maskell), has encountered many difficulties, which has raised interest in alternative control methods. Up to now, the *A. aurantii* sex pheromone has been used only for monitoring. In a previous work the authors described a biodegradable mesoporous pheromone dispenser for mating disruption. To verify the efficacy of these dispensers, three field trials were conducted, and the results are shown in this paper.**

**RESULTS: The study of the release profile of these dispensers revealed a mean pheromone emission value of 269 µg day$^{-1}$ and levels of residual pheromone of 10% at the end of 250 days. During the second flight, an *A. aurantii* male catch reduction of 98% was achieved in the mating disruption plot of trial 1, 93.5% in trial 2 and 76.7% in trial 3. During the third flight, reductions were 94.1, 82.9 and 68.1% in trials 1, 2 and 3 respectively. Considering damaged fruit with more than five scales, reductions of about 80 and 60% were obtained in the mating disruption plots of trials 2 and 3, respectively, compared with an untreated plot, and a reduction of about 70% in trial 1 compared with an oil-treated plot.**

**CONCLUSION: Mating disruption has been found to be an efficient technique to control this pest, working equally well to a correctly sprayed oil treatment. Further studies are needed to improve the determination of the time of dispenser application and evaluate the effects of the pheromone on natural enemies.**


**Keywords:** mating disruption; *Aonidiella aurantii*; California red scale; pheromone

## 1 INTRODUCTION

Worldwide, citrus orchards are greatly affected by diaspidid pests, especially *Aonidiella aurantii* (Maskell), known as California red scale (CRS), which is listed as the most important species causing economic damage and crop losses, and the life cycle of which has been extensively studied.[1-4] Adult male emergence coincides with the development of third-instar females (virgin females),[1-3] which then mate and produce the next generation. Virgin females attract males by releasing a pheromone, and then males may crawl to nearby females or fly to other trees.[3,4] The number of generations of CRS that could develop in citrus ranges from three to five, and this is influenced by temperature.[2,5] Under the climatic conditions of Spanish citrus areas, CRS shows three complete generations with three male flights, the first of which takes place between mid-April and mid-May, the second between mid-June and late July and the third from mid-August to late-September, with little variation between regions. Cosmetic damage caused by this armoured scale leads to downgrading or rejection of the fruit at the packing house. Moreover, heavy scale infestations may lead to yellowing of leaves, defoliation, branch dieback and possible tree death.[6]

The economic importance of this armoured scale is due to the fruit damage and the cost of the management tools to defeat it, as well as the difficulty in efficiently applying insecticide treatments. Traditional chemical control of CRS, including fumigation with hydrocyanic acid (HCN)[7,8] at the beginning of the last century, and subsequently the use of organophosphate and carbamate insecticides,[9-11] has been affected by the development of resistance to insecticides. Consequently, growers have had to rely on new integrated and biological control programmes. The use of mineral oils appeared to be a good alternative to conventional pesticides, these having low residual toxicity for beneficial insects. However, they can potentially be phytotoxic,[6,12] requiring certain precautions to avoid negative effects and to ensure the efficacy of the spray. Moreover, oil treatments require an accurate determination of the timing of the treatment, to be applied when the target pest is in its most vulnerable first-instar stage.[3] The use of insect growth regulators (IGRs) such as buprofezin[13-15] and pyriproxyfen[16-19] provided an important alternative to replace traditional insecticides. These IGRs were classified as reduced-risk insecticides, but their role in the conservation of some natural

* Correspondence to: Cristina Alfaro, Centro de Ecología Química Agrícola-Universidad Politécnica de Valencia, Edificio 6C, Camino de Vera s/n 46022, Valencia, Spain. E-mail: calfaro@ceqa.upv.es

Centro de Ecología Química Agrícola-Instituto Agroforestal del Mediterráneo, Universidad Politécnica de Valencia, Valencia, Spain

 

enemy groups was dubious.[17,20,21] Augmentative releases of the aphelinid parasitoid *Aphytis melinus* DeBach are competitive with conventional insecticide treatments in California,[22] South Africa[23] and Australia.[24] *Aphytis melinus* was introduced into the citrus-growing region of eastern Spain in 1976 from Antibes (France).[25,26] Since then it has been mass reared at the Insectary of the Plant Protection Service in Almazora, (Castellón, Spain) and released in several areas of the Valencian Community (region of eastern Spain). Rodrigo *et al.*[26] reported in 1996 that these parasitoids did not provide an economic level of control of the pest. CRS control by augmentative releases of this parasitoid is currently under study in Spain. Sorribas *et al.*[27] indicated in 2008 that augmentative releases of *Aphytis* sp. could be helpful to naturally occurring parasitism. On the other hand, its effectiveness depends on careful monitoring in order to establish the exact release date, and the use of selective insecticides for other pests that do not affect natural enemies.

The production of sex pheromone was demonstrated in CRS[28] years before the chemical structures were reported by Roelofs *et al.* in 1977.[29] Since then, synthetic sex pheromone traps have been widely employed as a management and detection tool for CRS populations.[2,5,30–34] The CRS sex pheromone was described as 3-methyl-6-isopropenyl-9-decen-1-yl acetate (I) and (*Z*)-3-methyl-6-isopropenyl-3,9-decadien-1-yl acetate (II).[29] All possible geometrical and optical isomers of the two compounds were synthesised and tested by Gieselmann and coworkers in 1980.[35,36] The results showed that only one isomer of each compound was significantly active: (3*S*,6*R*)-I and (3*Z*-6*R*)-II, and the presence of other isomers in the mixture had no effect on trap catches. These findings may lead to the development of new methods of control based on pheromones, such as mating disruption. Some researchers attempted to perform mating disruption for CRS using rubber pheromone dispensers. The results showed a male catch reduction, but the effectiveness of the technique was not clearly demonstrated.[37,38] However, a new mesoporous pheromone dispenser was described in previous work.[39] In the present study, the duration and efficacy of mesoporous mating disruption dispensers to control *A. aurantii* were verified in three citrus orchards in Spain. The mating disruption treatment was compared with untreated plots, oil treatments and the combination of mating disruption + oil spray. This paper describes the first effective mating disruption treatment against a diaspidid pest.

## 2 MATERIALS AND METHODS

### 2.1 Mesoporous dispenser and device

Mesoporous dispensers are cylindrical tablets of 9 mm diameter and 10 mm length, made of a mesoporous material.[40,41] The initial load of dispensers was 50 mg (AI) of the CRS sex pheromone, and the formulation contained the diastereomeric mixture (3*S*,6*R* and 3*S*,6*S*) of 3-methyl-6-isopropenyl-9-decen-1-yl acetate (74% purity). This mixture was supplied by Ecología y Protección Agrícola (Valencia, Spain).

Dispensers were hung inside polypropylene (PP) baskets, supplied by Ecología y Protección Agrícola. The PP baskets were 50 mm wide and 90 mm long. The pheromone was released through a 6 × 5 mm mesh. The pheromone basket had a hanger at the top for attachment to branches.

### 2.2 Experimental design

Three mating disruption trials were conducted in citrus orchards over 10 years old, one trial located in Rio Tinto (Huelva, Spain) and two trials in Picasent (Valencia, Spain), to test the efficacy of the mesoporous mating disruption dispensers. To choose the orchards, the population level of *A. aurantii* during the previous season was monitored on the basis of the flight of males. The maximum of the male catches in Picasent was approximately 1100 males per trap per week, and around 400 males per trap per week in Rio Tinto. For the mating disruption treatment, devices were hung at a height of about 2 m, inside the tree canopy, at a density of one dispenser per tree.

Oil treatments were timed for the presence of crawlers. The plant protection service of the local government carried out the crawler assessments, and the oil treatment timing was defined according to their data. The crawlers were monitored according to the sampling method suggested by the Valencian Community IPM programme. A total of 25 infested branches (2–3 years old) were randomly sampled in each trial, each week from the date of first flight, and taken to the laboratory. Leaves and twigs were removed from those branches and cut into 10 cm long pieces. A total of 100 live scales were identified as first, second and third instars, adult females and adult females with crawlers, using a binocular scope. The oil treatment was applied when first and second instars represented 70% of live scales and more than 90% of adult females had crawlers. The decision to treat the second and third generations was based on the percentage of infested fruit. The treatment threshold was established in 2% of infested fruit according to Valencian Community IPM guidelines. Ten trees and ten fruits per tree (eight outer and two inner) were randomly collected, and the percentage of fruit with more than three scales was recorded. All paraffinic oil (10 g $L^{-1}$) (Argenfrut RV; Gulf Oil Argentina, SA, Argentina) applications were made with an M1500 speed sprayer (Marisan, Valencia, Spain) calibrated to deliver 2500–3500 L $ha^{-1}$ at 150 psi with the tractor driven at 1.55 km $h^{-1}$.

#### 2.2.1 *Trial 1*

This trial was carried out in Rio Tinto (Huelva, Spain) in a Navelina *Citrus sinensis* Osbeck orchard with trees spaced at 7 by 5 m. It is an orchard with a steep slope, with a difference in height of almost 3 m between rows. The orchard was divided by roads into three plots, as follows: (1) 5 ha were treated with the combination of mating disruption and oil spray (MD + oil); (2) the second plot with 10 ha was only treated with an oil spray (oil control plot); (3) inside the MD + oil plot, 0.3 ha was left oil-free and was treated only with mating disruption (MD plot). Separation between plots was approximately 30 m, using roads as boundaries. In this trial, it was not possible to have an untreated plot because of the high cost of the potential crop loss. MD dispensers were applied on 5 March 2008, before the beginning of the first CRS male flight, and they were not replaced throughout the season. Oil sprays were applied on 7 June 2008, timed for the presence of crawlers. After assessing the fruit, no more oil treatments were needed.

#### 2.2.2 *Trials 2 and 3*

In Picasent, two trials were carried out in an organic Clemenules *Citrus reticulata* Blanco orchard (trial 2) and a late-maturing Valencia *Citrus sinensis* Osbeck orchard (trial 3) with trees spaced 6 by 4 m. Both trials were designed with four plots, as follows: (1) 1.5 ha MD + oil plot; (2) 1 ha oil control plot; (3) inside the

MD + Oil, 0.15 ha was left oil free as an MD plot; (4) inside the oil control plot, 0.15 ha was left oil free as an untreated plot. MD dispensers were applied on 21 February 2008, before the beginning of the first CRS male flight, and they were not replaced throughout the season. The oil treatment was applied on 25 May, timed for the presence of crawlers. After assessing the fruit, no more oil treatments were needed.

### 2.3 Evaluation of treatment efficacy

In order to evaluate the efficacy of mating disruption, three commercial white sticky pheromone traps (Pherocon® V Trap; Trécé, OK) were placed across the diagonal of each plot, at least 30 m apart. CRS male trap catches between plots treated with pheromone and plots without pheromone dispensers were compared. Sticky traps were replaced weekly, whereas the Pherocon® monitoring lures (Trécé, OK), loaded with 250 µg sex pheromone, were replaced every 42 days.

Male flights could not be monitored in the untreated plots because of their small area in trials 2 and 3. Oil plots were therefore considered as control plots to compare male catches between plots with and without pheromone dispensers (mating disruption plot versus oil plot). Traps in control plots should catch males, while in pheromone-treated plots an inhibition of male catches should be observed. The absence of trap catches during mating disruption treatment is a good indication of technique effectiveness, but crop damage assessment provides the ultimate proof.[42] To know the percentage reduction in males captured in pheromone traps between MD and control plots, the mating disruption index (MDI) was calculated according to the following formula:

$$\text{MDI} = (1 - (x/y)) \times 100$$

where $x$ is the number of males captured in MD plots and $y$ is the number of males captured in control plots. The MDI for each flight was the average of the weekly MDIs during the flight period.

Damage was assessed on 10 November 2008 in trial 1, on 11 September 2008 in trial 2 and on 6 November 2008 in trial 3. Ten trees per plot were randomly selected and evaluated for crop damage assessment. Forty fruits per tree were evaluated, with ten fruits per orientation. The treatment threshold published in the Valencian Community IPM guidelines is three scales per fruit. However, fruit with up to five scales is generally accepted by the market (market threshold). As the objective was to test the mating disruption treatment efficacy to obtain marketable fruit, this threshold of five scales per fruit was employed to assess the fruit damage. However, the percentage of fruit with 1–5 scales was also recorded to perform a sensitivity analysis. Treatment efficacy results were given as a percentage of damaged fruit. In trial 1, MD efficacy was compared with the efficacy of an oil treatment, and also relative to an untreated region of the orchard in trials 2 and 3.

Degree-days (DD) were calculated according the following formula:

$$\text{DD} = [(T_{\max} + T_{\min})/2] - T_{\text{critical}}$$

where $T_{\max}$ and $T_{\min}$ are the maximum and minimum temperatures of the day respectively, and $T_{\text{critical}}$ was considered to be 11.7 °C.[2] Temperature data were provided by the agroclimate stations of each location.

### 2.4 Pheromone release profiles

In parallel with the field trials, 40 additional dispensers were simultaneously aged over 250 days in a citrus orchard in Picasent, 500 m away from trials 2 and 3. The dispensers were aged from 21 February to 2 November 2008. Residual pheromone content was extracted at different ageing times (0, 7, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 250 days), and then quantified by gas chromatography using a flame ionisation detector (GC/FID). Three dispensers were taken from the field and analysed in the laboratory for each ageing time period. These dispensers were extracted at 40 °C with methanol + dichloromethane (3 + 2 by volume).

Red scale pheromone content was measured by GC/FID analyses (Clarus® 500 gas chromatograph; PerkinElmer, Wellesley, MA) of the extracts using 1-pentanol as internal standard. All injections were made onto a ZB-5 MS (30 × 0.25 mm × 0.25 µm) column, 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 amounts of pheromone and the responses were connected by fitting a linear regression model, $y = a + bx$, where $y$ is the ratio between pheromone and 1-pentanol responses and $x$ is the amount of pheromone remaining in the dispensers.

Pheromone release for each dispenser type was represented by fitting an exponential model, $y = ae^{bx}$, where $y$ is the remaining pheromone load and $x$ represents the ageing days.

### 2.5 Statistical analysis

To normalise the distributions and homogenise variance, male catches in pheromone-baited traps, per trap per week, were transformed by $\log(N + 1)$ before analysis of variance (ANOVA) using data from the period belonging to the second flight (from 25 June to 13 August in trial 1 and from 19 June to 14 August in trials 2 and 3) and third flight (from 20 August to 5 November in trial 1 and from 21 August to 8 October in trials 2 and 3). An LSD test at $P = 0.05$ was used to assess the significance of differences in male captures among plots treated with pheromone and those without.

In order to test for significant differences in percentages of fruit injured between treatments, a one-way ANOVA model was employed with square-root-transformed data (LSD test at $P = 0.05$).

The Statgraphics 5.1 package was used for all the statistical analyses.[43]

## 3 RESULTS

### 3.1 Efficacy trials

#### 3.1.1 *Male catches*

A slight first flight took place in both locations during April (Figs 1 to 3), from 23 April to 15 May in trial 1 (maximum 38 males per trap per week) and from 20 March to 24 April in trials 2 and 3 (maximum nine males per trap per week). The second flight began in mid-June (19 and 25 June respectively), with the maximum number of males caught in mid-July. The first male catches corresponding to the third flight were obtained at the end of August, and third flight ended at the beginning of October in all locations. Male catches from 14 October were considered to be a partial fourth flight, as only 194 °C DDs were accumulated up to December, and 593 °C DDs are needed for the development of one generation.[3]

Pheromone trap catches of CRS males in mating disruption plots were low throughout the entire season and differed significantly with catches obtained in their respective control plots, according to the statistical values of Table 1. Thus, a male disruption effect was achieved with the mesoporous dispensers, obtaining MDI



  

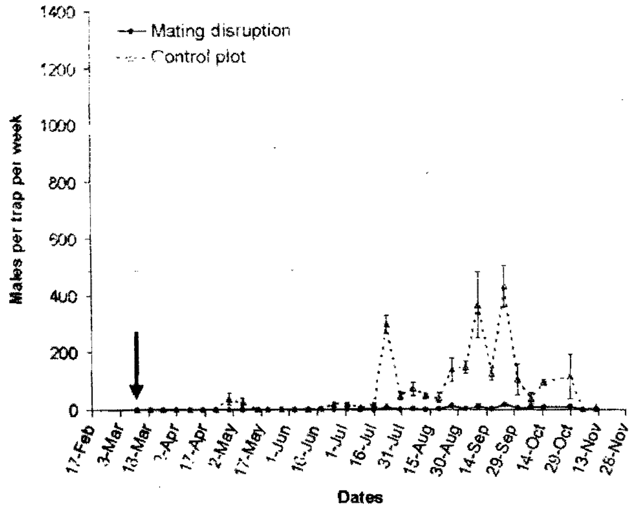


**Figure 1.** Male CRS catches per trap per week, in monitoring sticky traps, for mating-disruption-treated plots and control plots in trial 1 (Rio Tinto, var. Navelina). The oil plot (without pheromone dispensers) was considered as the control plot. The arrow indicates the date of dispenser application.

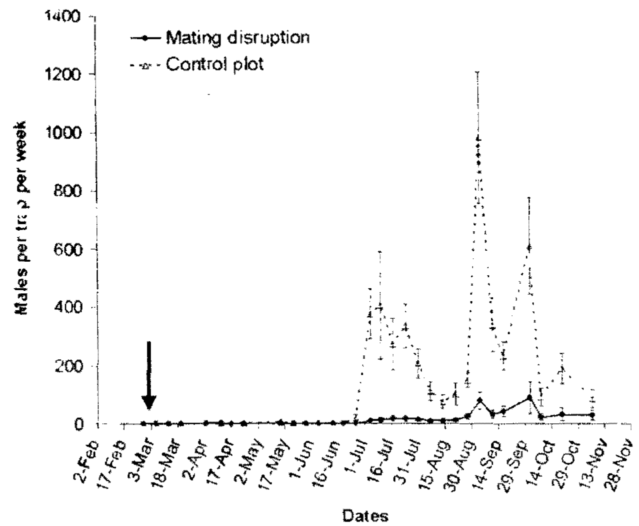


**Figure 2.** Male CRS catches per trap per week, in monitoring sticky traps, for mating-disruption-treated plots and control plots in trial 2 (Picasent, var. Clemenules). The oil plot (without pheromone dispensers) was considered as the control plot. The arrow indicates the date of dispenser application.

values ranging from 98.1 to 94.1% in trial 1, from 93.5 to 82.9% in trial 2 and from 76.7 to 68.1% in trial 3 for the two main flights.

From 15 August to 20 October (third male flight), mean male catches per trap and week were increased in the trial 2 and 3 MD plots in comparison with the trial 1 MD plot. Moreover, the MDI (Table 1) was significantly lower in trials 2 and 3 than in trial 1 during the third flight ($F = 10.60$; df = 2, 22; $P < 0.01$). Both results suggested a loss in disruption during this period in trials 2 and 3.

### 3.1.2 *Fruit damage*

In trial 1, the efficacy of the mating disruption treatment was checked relative to the efficacy of an oil treatment (Fig. 4). No significant differences were found between treatments for 1–5 scales ($F = 1.58$; df = 2, 26; $P = 0.226$), but the percentage of

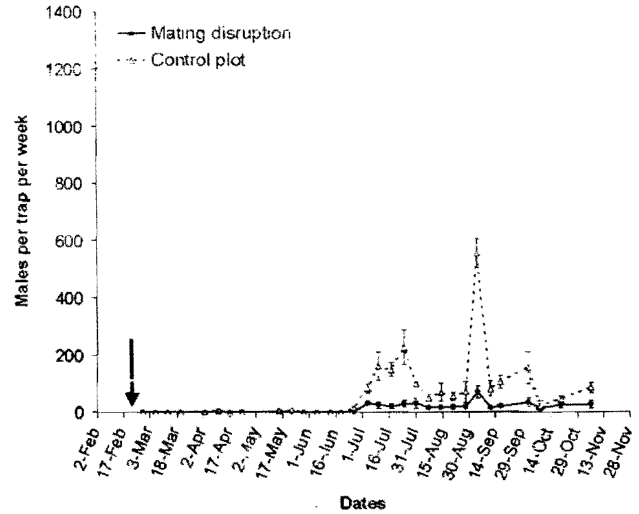


**Figure 3.** Male CRS catches per trap per week, in monitoring sticky traps, for mating-disruption-treated plots and control plots in trial 3 (Picasent, var. Valencia). The oil plot (without pheromone dispensers) was considered as the control plot. The arrow indicates the date of dispenser application.

fruit with over five scales was significantly reduced in the MD and MD + oil plots (less than 7% damaged fruit), compared with 20% scale-infested fruit in the oil control plot, with no significant differences between the MD and MD + oil plots ($F = 12.31$; df = 2, 26; $P < 0.001$). Focusing on trial 2 results (Fig. 5), significant differences were observed between the percentage of fruit with 1–5 scales ($F = 9.06$; df = 3, 41; $P < 0.001$), but, for over five scales per fruit, damage was highly reduced in the MD plot (less than 10% damage), compared with 45% of fruit with over five scales in the untreated plot. In trial 2 there were no significant differences between the MD and oil control plots. The best results for this early variety of citrus were obtained with the combination of mating disruption and oil treatment, applied in the first generation ($F = 24.18$; df = 3, 41; $P < 0.001$). In trial 3 (Fig. 6), significant differences in fruit injured with 1–5 scales were observed between the combination MD + oil and the untreated plot ($F = 1.97$; df = 3, 36; $P = 0.138$). In addition, damage assessment in trial 3 showed that any of these treatments was effective in reducing damage compared with the untreated plot ($F = 8.79$; df = 3, 36; $P < 0.001$) when the threshold of more than five scales was set, although assessment was performed long before harvest and this time may have allowed the development of a new CRS generation on fruit.

## 3.2 Pheromone release profiles

Figure 7 shows the residual pheromone versus time for the mesoporous dispensers. The residual pheromone load fits the exponential model $y = 49.668e^{-0.0087x}$ ($R^2 = 0.98$). This study also showed that the mesoporous dispenser emitted approximately 90% of its pheromone load during the test period. The low content of residual pheromone in the dispenser is a key parameter for the cost of the treatment.

The mean amount of pheromone emitted from this dispenser was 269 μg day$^{-1}$. This value is consistent with data published by the authors' group, determining the minimum mean release value (>250 μg day$^{-1}$) to obtain a disruption effect in CRS males.[39] This mesoporous dispenser has a regular pheromone release during the first 150 days, which decreases significantly from that time on.

**Table 1.** Mean (± SE) males per trap per day, mating disruption index (MDI) and statistical parameters obtained by analysis of variance (ANOVA) to assess the significance of differences in total male captures among plots treated with pheromone and those without, during second and third flights. Means in a row followed by the same letter are not significantly different (ANOVA test, $P > 0.05$). MDIs explain the mean percentage reduction in male catches per flight for each trial

| | | MD plot, mean (± SE) | Control plot, mean (± SE) | MDI | *F* | df | *P* |
|---|---|---|---|---|---|---|---|
| Second flight | Trial 1 | 1.36(±0.51) a | 52.50(±12.98)b | 98.1 | 56.20 | 1, 82 | <0.001 |
| | Trial 2 | 9.35(±1.08) a | 180.13(±29.11) b | 93.5 | 36.65 | 1, 68 | <0.001 |
| | Trial 3 | 17.69(±2.08) a | 85.57(±13.76) b | 76.7 | 26.51 | 1, 67 | <0.001 |
| Third flight | Trial 1 | 6.82(±1.38) a | 144.09(±23.66) b | 94.1 | 53.89 | 1, 75 | <0.001 |
| | Trial 2 | 40.36(±4.39) a | 308.96(±58.11) b | 82.9 | 25.70 | 1, 61 | <0.001 |
| | Trial 3 | 27.81(±2.91) a | 114.92(±31.50) b | 68.1 | 7.45 | 1, 60 | 0.008 |

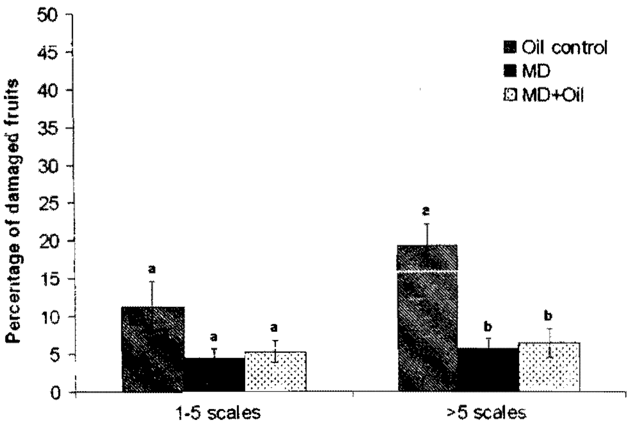


**Figure 4.** Mean percentage of damaged fruits observed in the different plots – oil control, mating disruption (MD) and MD + oil treatment – for trial 1 (Rio Tinto, var. Navelina). Bars labelled with the same letter do not differ significantly (ANOVA test, $P > 0.05$).

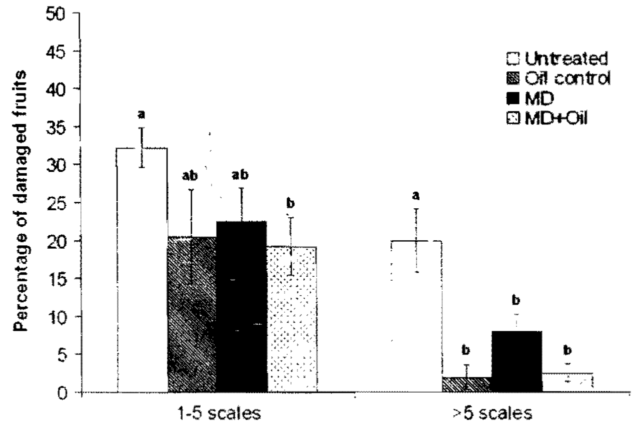


**Figure 6.** Mean percentage of damaged fruits observed in the different plots – untreated, oil control, mating disruption (MD) and MD + oil treatment – for trial 3 (Picasent, var. Valencia). Bars labelled with the same letter do not differ significantly (ANOVA test, $P > 0.05$).

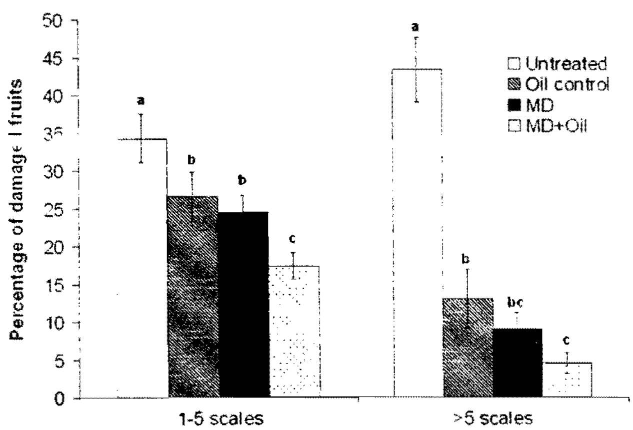


**Figure 5.** Mean percentage of damaged fruits observed in the different plots – untreated, oil control, mating disruption (MD) and MD + oil treatment – for trial 2 (Picasent, var. Clemenules). Bars labelled with the same letter do not differ significantly (ANOVA test, $P > 0.05$).

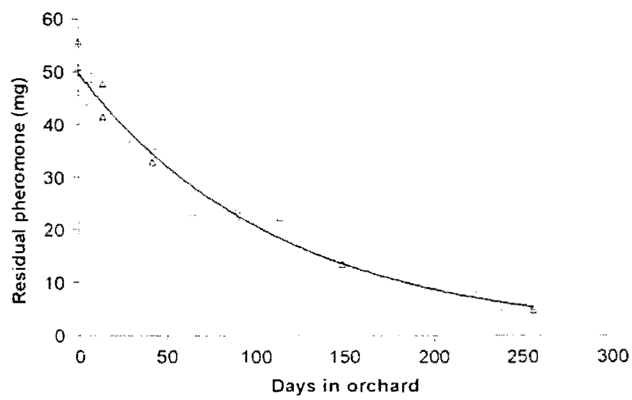


**Figure 7.** Relationship between the amount of pheromone remaining in the mesoporous dispensers (mg) and the corresponding days of field exposure. Significance of the exponential model was $R^2 = 0.98$.

## 4 DISCUSSION

The level of male CRS captures in the trial 2 and 3 MD plots increased significantly in September in comparison with trial 1, with lower MDI values during the third flight (September–October) in trials 2 and 3. This could be due to the pheromone application, which was dispensed 2 weeks earlier in trials 2 and 3. Comparing this increase in captures with the pheromone release profile, this period coincides with the end of the lifespan of these dispensers, which is 6 months (mean pheromone release rate of <250 µg day$^{-1}$).[39] These results indicated that, after 5 months of field application, the disruption effect decreased because the emission of pheromone was not high enough to disrupt the CRS third flight. Not considering the pheromone release profile, this increase in captures could be attributed to higher density of scales at that time of year. However, results of damage assessment showed that the percentage of damaged fruit was significantly lower in the MD plot compared with an untreated plot. This means that the disruption effect took place, and therefore a higher density of scales was not likely before September. Additional trials are needed to adjust the timing of dispenser application and promote pheromone release until the CRS generational cycles are completed. This research could alter the date of application of the dispensers or suggest a higher pheromone dosage. However, increase in the pheromone load has some drawbacks, because the pheromone represents approximately 95% of the price of the dispenser.

The results in trials 2 and 3 showed that any of the control methods employed in these trials was effective against CRS compared with an untreated plot. It has been found that mating disruption treatment alone could reduce damage by *A. aurantii* in fruit by 80 and 60% in trials 2 and 3 respectively. Also, trials 2 and 3 demonstrated that mating disruption worked equally well to a correctly sprayed oil treatment. Correct timing of the oil application, a good calibration of the sprayer and a good coverage of all the above-ground parts of the tree are key factors to ensure the efficacy of the treatment. The fruit injury obtained in the oil plot of trial 1 was significantly higher than in the MD plot, which was not the case in trials 2 and 3. As the timing of the oil application was well defined in accordance with the number of crawlers, the low efficacy of oil treatment in trial 1 could be explained by the particular slope of the Rio Tinto orchard.

CRS is widely distributed, and, although the host susceptibility is related to the number of oil glands in the leaves,[44,45] all citrus varieties are sensitive to its attack. Accordingly, the present trials showed satisfactory results for mid-season varieties during the lifespan of the pheromone dispensers and the three CRS generations that generally take place in Spain. For late-season varieties, like Valencia oranges, it is possible that the first generation of the following year could affect the non-harvested fruit, so it should be treated with a new application of pheromone dispensers before the first flight or other effective treatment.

The polypropylene device employed was a prototype to conduct the trials. The final device should be made of a biodegradable material, which could be left in the field without threatening the environment and could be resistant to weather conditions for almost 7 months.

The pheromone device is still in development; however, it has been estimated that the cost of this treatment will be approximately €200 ha$^{-1}$, which is economically competitive with a conventional oil spray (€266 ha$^{-1}$, including oil and speed sprayer). In addition, in a mating disruption treatment, an accurate determination of the moment of application is not necessary, while for an oil treatment it is essential and assumes an added cost that is often not considered.

As well as oil sprays, the majority of growers have adopted the use of IGRs as a part of integrated pest management programmes. The effect of buprofezin and pyriproxyfen on life stages of natural enemies has been extensively studied, and they appear to be compatible with augmentative releases of *A. melinus*,[46] but they are incompatible with other agents like *Rodolia cardinalis* (Mulsant).[20] It must be added that European Directives regulating the use of insecticides are becoming more and more severe. In fact, the Commission Decision of the European Communities (2008/771/EC) of 30 September 2008, concerning the non-inclusion of buprofezin in Annex I to Council Directive 91/414/EEC, stated that authorisations for plant protection products containing buprofezin were to be withdrawn by 30 March 2009. So mating disruption could be a good alternative to settle this matter.

In conclusion, CRS mating disruption achieved control equal to conventional oil sprays and could provide growers with a method for controlling a key citrus pest without using insecticides. Mating disruption could also be highly conducive to conservation of natural enemies. However, it is necessary to evaluate the possible effect of a high concentration of CRS sex pheromone on the behaviour of *A. melinus* and other parasitoids and predators of *A. aurantii*, as well as the influence of the pheromone on natural enemies of other pests. In this way, the influence of mating disruption treatment on the behaviour of some CRS parasitoids is being studied. In addition, it should be considered that the reduction in a wide range of insecticide treatments, owing to the implementation of mating disruption as an *A. aurantii* control method, could potentially increase secondary pest populations. The mating disruption technique could replace the use of oil spray for CRS control, but these oil treatments could occasionally be necessary for the control of other scale pests.

## ACKNOWLEDGEMENTS

The authors are grateful to Ernesto Machancoses and Vicente Morató (Picasent) and to Antonio Caballero from Rio Tinto Fruits for facilitating study orchards and assisting with the collection of trapping data. This research has been supported by Ministerio de Ciencia y Tecnología project number AGL 2009-10725.

## REFERENCES

1 Tashiro H and Beavers JB, Growth and development of California Red Scale *Aonidiella aurantii*. *Ann Entomol Soc Am* **61**:1009–1014 (1968).
2 Kennett CE and Hoffmann RW, Seasonal development of the California Red Scale (Homoptera: Diaspididae) in San Joaquin Valley citrus based on degree-day accumulation. *J Econ Entomol* **78**:73–79 (1985).
3 *Integrated Pest Management for Citrus*. University of California, Berkeley, CA, 144 pp. (1991).
4 Koteja J, Life history, in *Armored Scale Insects. Their Biology, Natural Enemies and Control. Volume A*, ed. by Rosen D. Elsevier, Amsterdam, The Netherlands, pp. 243–254 (1990).
5 Grout TG, Du Toit WJ, Hofmeyr JH and Richards GI, California Red Scale (Homoptera: Diaspididae) phenology on citrus in South Africa. *J Econ Entomol* **82**:793–798 (1989).
6 Grafton-Cardwell EE and Reagan CA, Selective use of insecticides for control of Armored Scale (Homoptera: Diaspididae) in San-Joaquin Valley California citrus. *J Econ Entomol* **88**:1717–1725 (1995).
7 Yust HR, Nelson HD and Busbey RL, Comparative susceptibility of two strains of California red scale to HCN, with special reference to the inheritance of resistance. *J Econ Entomol* **36**:744–749 (1943).
8 Yust HR, Busbey RL and Nelson HD, Influence of decreasing, constant, and increasing concentrations on results of fumigation of the California red scale with HCN. *J Econ Entomol* **36**:875–878 (1943).
9 Collins PJ, Lambkin TM and Bodnaruk P, Suspected resistance to methidation in *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) from Queensland. *J Aust Ent Soc* **33**:325–326 (1994).
10 Grafton-Cardwell EE and Vehrs SLC, Monitoring for organophosphate-resistant and carbamate-resistant Armored Scale (Homoptera: Diaspididae) in San-Joaquin Valley citrus. *J Econ Entomol* **88**:495–504 (1995).
11 Grafton-Cardwell EE, Ouyang YL and Salse J, Insecticide resistance and esterase enzyme variation in the California red scale (Homoptera: Diaspididae). *J Econ Entomol* **91**:812–819 (1998).
12 Tan BL, Sarafis V, Beattie GAC, White R, Darley EM and Spooner-Hart R, Localization and movement of mineral oil in plants by fluorescence and confocal microscopy. *J Exp Bot* **56**:2755–2763 (2005).
13 Yarom I, Blumberg D and Ishaaya I, Effects of buprofezin on California Red Scale (Homoptera: Diaspididae) and Mediterranean Black Scale (Homoptera: Coccidae). *J Econ Entomol* **81**:1581–1585 (1988).
14 Grout TG and Richards GI, Effect of buprofezin applications at different phenological times on California Red Scale (Homoptera: Diaspididae). *J Econ Entomol* **84**:1802–1805 (1991).

  

15 Ishaaya I, Mendel Z and Blumberg D, Effect of buprofezin on California Red Scale, *Aonidiella aurantii* (Maskell), in a citrus orchard. *Isr J Entomol* **25**:67–71 (1992).

16 Eliahu M, Blumberg D, Horowitz AR and Ishaaya I, Effect of pyriproxyfen on developing stages and embryogenesis of California red scale (CRS), *Aonidiella aurantii*. *Pest Manag Sci* **63**:743–746 (2007).

17 Grafton-Cardwell EE, Lee JE, Stewart JR and Olsen KD, Role of two insect growth regulators in integrated pest management of citrus scales. *J Econ Entomol* **99**:733–744 (2006).

18 Rill S, Grafton-Cardwell EE and Morse JG, Effects of pyriproxyfen on California red scale (Hemiptera: Diaspididae) development and reproduction. *J Econ Entomol* **100**:1435–1443 (2007).

19 Alfaro F, Esquiva M and Cuenca F, Estudio del comportamiento de dos reguladores de crecimiento contra piojo rojo de california, *Aonidiella aurantii* Maskell. 1ª parte. *Levante Agrícola* **3**:406–411 (1999).

20 Grafton-Cardwell EE and Gu P, Conserving vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), in citrus: a continuing challenge as new insecticides gain registration. *J Econ Entomol* **96**:1388–1398 (2003).

21 Lauziere I and Elzen G, Effect of formulated insecticides on *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) and its parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). *J Entomol Sci* **42**:11–19 (2007).

22 Moreno DS and Luck RF, Augmentative releases of *Aphytis melinus* (Hymenoptera: Aphelinidae) to suppress California Red Scale (Homoptera: Diaspididae) in southern California lemon orchards. *J Econ Entomol* **85**:1112–1119 (1992).

23 Bedford ECG, Problems which we face in bringing red scale, *Aonidiella aurantii* (Maskell), under biological control in citrus in South Africa. *Proc Internat Soc Citriculture* **1**:485–492 (1996).

24 Furness GO, Buchanan GA and George RS and Richardson NL, A history of the biological and integrated control of red scale, *Aonidiella aurantii*, on citrus in the Lower Murray Valley of Australia. *Entomophaga* **29**:199–212 (1983).

25 Pina T and Verdú MJ, El piojo rojo de California, *Aonidiella aurantii* (Maskell), y sus parasitoides en cítricos de la Comunidad Valenciana. *Bol San Veg Plagas* **33**:357–368 (2007).

26 Rodrigo E, Troncho P and Garcia-Mari F, Parasitoids (Hym: Aphelinidae) of three scale insects (Hom: Diaspididae) in a citrus grove in Valencia, Spain. *Entomophaga* **41**:77–94 (1996).

27 Sorribas JJ, Rodriguez R, Rodrigo E and Garcia-Mari F, Niveles de parasitismo y especies de parasitoides del piojo rojo de California *Aonidiella aurantii* (Hemiptera: Diaspididae) en cítricos de la Comunidad Valenciana. *Bol San Veg Plagas* **34**:201–210 (2008).

28 Tashiro H and Chambers DL, Reproduction in the California Red Scale, *Aonidiella aurantii* (Homoptera: Diaspididae). I. Discovery and extraction of a female sex pheromone. *Ann Entomol Soc Am* **60**:1166–1170 (1967).

29 Roelofs WL, Gieselmann MJ, Carde AM, Tashiro H, Moreno DS, Henrick CA, *et al.*, Sex-pheromone of California Red Scale, *Aonidiella aurantii*. *Nature* **267**:698–699 (1977).

30 Moreno DS, Rice RE and Carman GE, Specificity of the sex pheromones of female Yellow Scales and California Red Scales. *J Econ Entomol* **65**:698–701 (1972).

31 Gardner PD, Ervin RT, Moreno DS and Baritelle JL, California Red Scale (Homoptera: Diaspididae) – cost-analysis of a pheromone monitoring program. *J Econ Entomol* **76**:601–604 (1983).

32 Moreno DS and Kennett CE, Predictive year-end California Red Scale (Homoptera: Diaspididae) orange fruit infestations based on catches of males in the San-Joaquin Valley. *J Econ Entomol* **78**:1–9 (1985).

33 Samways MJ, Comparative monitoring of Red Scale *Aonidiella aurantii* (Mask) (Hom, Diaspididae) and its *Aphytis* spp (Hym, Aphelinidae) parasitoids. *J Appl Entomol* **105**:483–489 (1988).

34 Grout TG and Richards GI, Value of pheromone traps for predicting infestations of Red Scale, *Aonidiella aurantii* (Maskell) (Hom, Diaspididae), limited by natural enemy activity and insecticides used to control citrus thrips, *Scirtothrips aurantii* Faure (Thys, Thripidae). *J Appl Entomol* **111**:20–27 (1991).

35 Tashiro H, Gieselmann MJ and Roelofs WL, Residual activity of a California Red Scale synthetic pheromone component. *Environ Entomol* **8**:931–934 (1979).

36 Gieselmann MJ, Henrick CA, Anderson RJ, Moreno DS and Roelofs WL, Responses of male California Red Scale to sex-pheromone isomers. *J Insect Physiol* **26**:179–182 (1980).

37 Barzakay I, Hefetz A, Sternlicht M, Peleg BA, Gokkes M, Singer G, *et al.*, Further field trials on management of the California Red Scale, *Aonidiella aurantii*, by mating disruption with its sex-pheromone. *Phytoparasitica* **14**:160–161 (1986).

38 Hefetz A, Kronengerg S, Peleg BA and Bar-zakay I, Proceedings of the Sixth International Citrus Congress, Mating disruption of the California Red Scale. *Aonidiella aurantii* (Homoptera: Diaspididae). **3**:1121–1127 (1988).

39 Vacas S, Alfaro C, Navarro-Llopis V and Primo J, The first account of the mating disruption technique for the control of California Red Scale *Aonidiella aurantii* Maskell (Homoptera: Diaspididae) using new biodegradable dispensers. *B Entomol Res* **99**:415–423 (2009).

40 Corma A, Munoz Pallares J and Primo-Yufera E, Production of semiochemical emitters having a controlled emission speed which are based on inorganic molecular sieves. World Patent WO9944420 (1999).

41 Corma A, Munoz Pallares J and Primo-Yufera E, Emitter of semiochemical substances supported on a sepiolite, preparation process and applications. World Patent WO0002448 (2000).

42 Howse PE, Pheromones and behaviour, in *Insect Pheromones and Their Use in Pest Management*, ed. by Howse PE, Stevens I and Jones O. Chapman and Hall, London, UK, pp. 1–130 (1998).

43 *Statgraphics Plus 5.1 Data Analysis Software*. Statpoint Inc., Warrenton, VA (2000).

44 McClure MS, Patterns of host specificity, in *Armored Scale Insects. Their Biology, Natural Enemies and Control. Volume A*, ed. by Rosen D. Elsevier, Amsterdam, The Netherlands, pp. 301–3[illegible] (1990).

45 Asplanato G and Garcia-Mari F, Distribucion del piojo rojo de California *Aonidiella aurantii* (Maskell) (Homoptera: *Diaspididae*) en arboles de naranjo. *Bol San Veg Plagas* **24**:637–646 (1998).

46 Rill SM, Grafton-Cardwell EE and Morse JG, Effects of two insect growth regulators and a neonicotinoid on various life stages of *Aphytis melinus* (Hymenoptera: Aphelinidae). *Biocontrol* **53**:579–587 (2008).






  