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Mating disruption for the control of *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) may contribute to increased effectiveness of natural enemies

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Abstract

BACKGROUND: New directives on sustainable use of pesticides have encouraged research on efficient alternative pest control methods. In the case of the California Red Scale (CRS), *Aonidiella aurantii* (Maskell), this imperative, along with the many difficulties in controlling this pest, have led to the investigation of new approaches. Previously developed mating disruption (MD) dispensers, together with the augmentative releases of the parasitoid *Aphytis melinus* DeBach, are here considered as a combined strategy for use against *A. aurantii*.

RESULTS: Efficacy of MD was demonstrated by a mean reduction of 80% in CRS male catches and a mean fruit damage reduction of 83% compared with the control. A delay in the development of *A. aurantii* instars was observed in the MD plot. This delay increased the period of exposure of the susceptible instars to natural enemies that resulted in higher predation and parasitism levels in the MD plot. Under laboratory conditions, *A. melinus* mating behaviour and effects on *A. aurantii* were not significantly altered in a CRS pheromone-saturated environment.

CONCLUSION: Mating disruption pheromone did not affect the behaviour or level of parasitism by *A. melinus* or the incidence of other generalist predators. Therefore, *A. aurantii* pheromone appears to be compatible with augmentative releases and biological control, making its use a good strategy for CRS management.

Keywords: California Red Scale; pheromone; biological control; *Aphytis melinus*; parasitism; mesoporous dispensers

1 INTRODUCTION

Worldwide, citrus groves are seriously affected by diaspidid pests, specifically by the California Red Scale (CRS), *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). This species is listed as one of the most important citrus pests, causing severe economic damage and crop losses.[1-3] Damage is inflicted when the insects suck the sap from plant organs. In extreme cases, an infestation can even kill the tree;[4] however, given the CRS's preference for fruit[5] and the fact that the presence of scales on fresh fruit considerably reduces market value,[6-8] control methods are addressed to prevent CRS establishment on the fruit.

The CRS life cycle has been extensively studied.[2,9,10] Females can give birth to 100 to 150 active crawlers, which emerge from under the female's scale cover in 1-2 days, depending on the temperature. These crawlers, the only immature instars capable of movement, travel short distances and settle onto twigs, leaves or fruits.[11] In the second instar, females and males begin to develop differently. Adult male emergence coincides with the development of third instar females; the insects then mate and produce the next generation. Virgin females attract males by releasing a pheromone, allowing males either to crawl to nearby females or to fly to other trees.[2] The production of a sex pheromone, a mixture of 3-methyl-6-isopropenyl-9-decen-1-yl acetate (I) and (*Z*)-3-methyl-6-isopropenyl-3,9-decadien-1-yl acetate (II), was demonstrated in CRS years before the description of their chemical structures.[12] Since their description, synthetic sex pheromone traps have been widely employed as a tool for detecting CRS populations.[2,10,13-15]

Currently, integrated control of CRS in citrus is based on the application of pesticides at the pest's point of maximum chemical sensitivity to keep pest populations below economically damaging levels.[3,6,8] Chemical control, however, poses serious problems: (1) it is not always efficient,[8] (2) it can negatively affect natural enemies,[16,17] (3) it can generate chemical resistance,[18,19] (4) it can cause serious marketing problems because of pesticide residues, (5) it can produce important environmental and sustainability problems, and (6) it increases production costs. The EU Directive on sustainable use of pesticides (Directive 2009/128/EC) enforces the implementation of efficient alternative pest control methods. We therefore investigated alternative CRS management methods to ensure sustainability from both the socio-economic and the environmental perspective. To this end, two different approaches to biotechnological control are currently being implemented. First, a new mating disruption mesoporous dispenser for CRS has been developed. The second approach involves the augmentation and conservation of the most effective natural enemy of *A. aurantii*: the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae).

Semiochemical-based pest management programs have increasingly been used as environmentally friendly methods to control major insect pests.[20] Such programs include the use of sex pheromones for monitoring, mass trapping and mating disruption techniques. Mating disruption is an effective tool to control infestations of Lepidoptera species.[21-25] In the late 1980s, some researchers attempted to effect CRS mating disruption using rubber pheromone dispensers, but the effectiveness of the technique was not clearly demonstrated;[26,27] however, a new mating disruption mesoporous dispenser for CRS has proved to be the first effective mating disruption treatment against a diaspidid pest.[28,29]

The ectoparasitoids *Aphytis chrysomphali* Mercet (Hymenoptera: Aphelinidae) and *A. melinus* are the principal natural enemies of CRS in the Mediterranean basin.[7,30-33] The level of natural parasitism on *A. aurantii* is rarely higher than 40%[7,30,31] a value that is unfortunately insufficient to control the pest effectively. Augmentative releases of *A. melinus* are therefore being implemented as an additional control. Apart from the parasitoids, a set of endemic *A. aurantii* predators have also been reported, although their importance seems to be low in comparison to the parasitoids.[30,33]

The effectiveness of mating disruption in managing CRS raises the question of possible unintended effects on natural enemies. This study focuses on the most important natural enemy of CRS, the parasitoid *A. melinus*. Additionally, the influence of mating disruption treatment on mortality caused by parasitoids (mainly *A. melinus*) and generalist predators was measured during one season under field conditions. These data will form a basis for considering the combined use of mating disruption, augmentative releases of parasitoids and the conservation of natural enemies.

2 MATERIALS AND METHODS

2.1 Mesoporous dispenser and device

The CRS mating disruption pheromone (MD) was released in the field by installing the mesoporous pheromone dispensers described by Vacas et al.[28] Each dispenser consisted of cylindrical tablets 9 mm in diameter and 10 mm in length. The mean initial load of the dispensers was 69 mg (a.i.) of the CRS sex pheromone. The formulation contained the diastereomeric mixture (3S,6R and 3S,6S) of the 3-methyl-6-isopropenyl-9-decen-1-yl acetate (74% purity). This mixture was supplied by Ecología y Protección Agrícola S.L. (Valencia, Spain).

Dispensers were hung inside 50×90 mm polypropylene (PP) baskets with hangers at the top for attachment to branches; baskets were supplied by Ecología y Protección Agrícola S.L. The pheromone was released through a 6x5 mm mesh.

2.2 Effects of MD on *A. melinus* in the laboratory

In order to evaluate the influence of the *A. aurantii* mating disruption pheromone on *A. melinus*, two experiments were conducted. The two studies addressed, respectively, the effects of MD on *A. melinus* mating behaviour and the effects on the parasitism efficacy of *A. melinus* on *A. aurantii* susceptible instars. Unless otherwise stated, environmental conditions in laboratory experiments were 25 ± 1ºC, 60 ± 10% RH and a photoperiod of 16:8 h (L:D).

2.2.1 Effects on A. melinus *mating behaviour*

Pupae of *A. melinus* reared on *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) on pumpkins were obtained from the commercial mass rearing facility Koppert Biological Systems S.L. (Águilas, Murcia, Spain). Pupae were removed from their hosts and placed individually in 4.5 cm high, 1 cm diameter glass vials. A small drop of honey was provided as a food source, and the vials were sealed with a piece of cotton. The adult parasitoids were sexed after emergence. Adults were kept isolated and held for two days in these vials under the conditions described above. Males and females were then paired to observe their mating behaviour. Each glass vial containing a wasp was lightly tapped to introduce the pairs to new vials without honey. The vials were then sealed with plastic lids. This experiment included two treatments, one with MD (n=20) and the control, without pheromone treatment (n=19). In the MD treatment, a mesoporous pheromone dispenser was adjusted to a 0.9 cm diameter hole in the vial lid; this hole was covered with a mesh in the control treatment. Mating behaviours were

recorded by observation under a binocular stereoscope. Each observation ended when mating took place or after 30 min without mating. Time of pre-copulations and copulations were recorded in seconds. The number of contacts made before copulation and the number of contacts in non-mating couples were also registered.

2.2.2 Effects on A. melinus *parasitism.*

Adults were obtained following the same protocol described above. After the newly emerged parasitoids were sexed, pairs were formed and left undisturbed for two days. Two mating pairs were then introduced per experimental unit. Each experimental unit consisted of a 30x20x23 cm plastic cage with a tight-fitting lid with a gauze-covered 20x8 cm aperture. The two lateral sides of the cage had two concentric mesh-covered circle holes 30 cm in diameter for ventilation.

Two lemons, each containing 35 third-instar *A. aurantii* scales, were introduced to each cage. The infested lemons were obtained from the *A. aurantii* colony at the IVIA.[7] A light streak of honey was provided on the side of the cage as a food source for the parasitoids. Two treatments (MD and control), each with six replicates, were conducted. For the MD treatment a mesoporous pheromone dispenser as described above was placed inside each cage. Couples were removed after three days of exposure. After six days, parasitised scales, scales showing host feeding symptoms and healthy scales were counted.

2.3 Effects of MD on *A. aurantii* and the effectiveness of its natural enemies

2.3.1 Experimental site

The field trial was conducted in a 2 ha commercial grove of 5 year-old clementines *Citrus reticulata* (Blanco) 'Esbal' located in La Pobla de Vallbona (Valencia, Spain)

(UTM: X713444 Y4390392; Z144 m altitude), with trees spaced 6.5 by 4 m. This grove was surrounded by other citrus and olive groves.

In order to test the efficacy of the mating disruption treatment, the citrus grove was equally divided into two plots, as follows: 1 ha mating disruption plot (MD plot), with MD applied at a dose of 26 g ha^{-1}, and a 1 ha control plot. MD devices were hung at least 1.8 m high inside the tree canopy, at a density of one dispenser per tree (about 400 dispensers ha^{-1}). Pheromone dispensers were installed on 24 March 2009 and never replaced.

Both plots received two mineral spray oil treatments at a concentration of 1.5% (Volck® oil emulsion, Agrodan S.A, Madrid, Spain) on 11 March 2009, against mites and scales, and on 2 June 2009, against the peak of first-generation *A. aurantii* crawlers. Additionally, a third oil application was made in the control treatment on 24 September 2009. Oil applications were made with a low-profile air-blast sprayer calibrated to deliver 2500-3000 L ha^{-1} at 150 psi with the tractor driven at 2.5 km h^{-1}.

Augmentative releases of the parasitoid *A. melinus* were conducted during 2009 in both plots. Each plot received 220,000 wasps divided into 11 releases from the end of February until the beginning of July. *A. melinus* adults were directly obtained from the commercial mass rearing facility of Koppert Biological Systems S.L.

2.3.2 Evaluations

In order to evaluate the efficacy of mating disruption, three commercial white sticky pheromone traps (PHEROCON® V Trap), (Trécé, Adair, OK, USA) were placed in each plot, at least 30 m apart. Sticky traps were replaced weekly, and the PHEROCON® monitoring lures (Trécé, Oklahoma, USA) were replaced every 42 days. The numbers of CRS male and *A. melinus* trapped in the plot treated with pheromone and the plot

without pheromone dispensers were compared. Fruit damage was assessed on 22 September 2009, just before harvest. Twenty trees per plot were randomly selected and 10 fruits per tree (8 outer and 2 inner) were evaluated for crop damage. The number of scales on each fruit was recorded and the percentage of fruit with >3, >5 and >10 scales was calculated.

Three shoot samplings were conducted to estimate the CRS population structure on 4 May, 6 August and 9 September 2009. *A. aurantii*-infested shoots were randomly collected from each plot and taken to the laboratory, where leaves and twigs were removed. Shoots of approximately 10 cm were examined under a binocular stereoscope. From each shoot, irrespective of the number of scales present, only 10 individuals were checked to standardise the sampling. Two hundred and fifty scales were evaluated, and counting continued until 60 mature females were counted or until 500 scales were examined. The number of *A. aurantii* healthy scales, parasitised scales, scales showing host feeding symptoms and preyed-upon scales were recorded. Additionally, on 9 September 2009, fruits were sampled in each plot following the methodology previously described. Numbers of parasitoid pupae and exuviae were also determined.

2.4 Data analysis

In the *A. melinus* mating behaviour experiment, significance was assessed using Student's t-test ($P<0.05$). Where appropriate, *P*-values were calculated using the Fisher exact probability test. In the experiment evaluating the effects of MD on parasitism under laboratory conditions, differences between the two treatments assayed were analysed using Student's t-test ($P<0.05$).

In the field trial, to ascertain the percent reduction in males captured in pheromone traps between MD and control plots, the mating disruption index (MDI) was calculated

according to the following formula: MDI = (1 - (x/y)) *100, where x is the number of males captured in MD plots and y is the number of males captured in control plots. MDI for each flight was the average of the weekly MDI during the flight period. Fruit damage and CRS male captures were evaluated for significance by a Student's t-test ($P<0.05$) (SPSS, 1999). A paired data t-test was employed to compare the weekly average of *A. melinus* caught in CRS monitoring traps between MD and control plots.

3 RESULTS

3.1 Effects of MD on *A. melinus* in the laboratory

3.1.1 Influence on mating behaviour

There was no significant difference in mating behaviour of *A. melinus* when pairs were subjected to CRS pheromone-saturated environment or to a control treatment (Table 1). In the treatment group, 40% of pairs were able to mate, versus 63.2% in the control group. For the mated pairs, the number of contacts taking place before copulation, the time passed before copulations and the duration of copulations were not significantly different in either environment. Similarly, the number of contacts in non-mating couples was not statistically different for both treatments.

3.1.2 Influence on A. melinus *parasitism*

The CRS pheromone-saturated environment had no measurable effect on the capacity of *A. melinus* to parasitise and feed on *A. aurantii* (Table 2). The mean numbers of parasitised *A. aurantii* third-instar nymphs and *A. melinus* progeny were not significantly different in either environment.

3.2 Effects of MD on *A. aurantii* and the effectiveness of its natural enemies

3.2.1 Effects on A. aurantii.

The population dynamics of CRS in the field are depicted in Figure 1. According to the trap captures in the control treatment, an initial minor flight took place in May, with a maximum of 0.5 males per trap per day (MTD). The second flight began in early June, with up to 2 MTD. Catches from the third flight began on 6 August, reaching the maximum of 12 MTD in the first week of September. A partial fourth flight followed in October at the end of the trial.

Data from the first flight were disregarded, as there was not enough information to study statistical differences between plots. Measurements from the following weeks show that pheromone trap catches of CRS males in the MD plot were low throughout the entire season and differed significantly from catches obtained in the control plot (2^{nd} flight: t_{15}= 3.855, P = 0.002; 3^{rd} flight: t_{22}= 4.419, P< 0.001). These data indicate that a male disruption effect was achieved with the mesoporous dispensers. MDI values calculated for 2^{nd} and 3^{rd} flights confirmed that MD treatment reduced CRS male catches by 73.8% and 87.3% , respectively, compared with the control plot.

The fruit damage assessment showed that the percentage of injured fruit was significantly reduced in the MD plot compared with the control plot (Figure 2) for all of the studied thresholds (>3 scales: t_{39}= 8.178, P < 0.001; >5 scales: t_{39}= 2.885, P= 0.006; >10 scales: t_{39}= 3.286, P= 0.002). Only 2% of fruits in the MD plot had more than 10 scales on their surface, indicating an 87% damage reduction compared with the control plot. If the strictest threshold is considered, MD treatment reduced the number of attacked fruits by 83% when compared with the control plot.

On May 4, 1.5 months after the installation of the mesoporous dispensers in the field, there was no significant difference in the population structure of *A. aurantii* between the

two plots (Figure 3a). However, on August 6, the percentage of first nymphal instars was significantly higher in the MD plot than in the control plot (F= 6.931; P= 0.006), indicating a slight delay in the development of *A. aurantii* instars in the MD plot (Figure 3b). Similarly, the proportion of third-instar nymphs was higher in the control (F= 3.748; P= 0.038). This delay was more evident on the third sampling date, 9 September (Figure 3c), when the number of gravid females was also lower in the MD plot (F= 17.438; P< 0.001). The same pattern was observed in the fruit sampling (Figure 4), in which the proportions of first-instar nymphs were significantly higher (F= 22.965; P< 0.001) and numbers of third-instars and gravid females were significantly lower in the MD plot than in the control plot (F= 8.084; P= 0.003 and F= 4.027; P= 0.035, respectively).

3.2.2 Effects of MD on natural enemies.

Differences in causes of *A. aurantii* mortality were observed for the MD plot in comparison to the control treatment (Table 3). Parasitism and predation were significantly higher in the MD plot, resulting in a decrease in the number of healthy scales found. A similar trend was observed in the fruit, although the differences were not significant. *A. melinus* was the only parasitoid species observed (n= 201).

Catches of *A. melinus* in CRS monitoring traps of MD plot were not significantly different from those obtained in the control plot (paired data t-test: t= 0.89, P= 0.39) (Figure 5).

4 DISCUSSION

An integrated strategy for managing CRS is essential because of the variety of difficulties involved in *A. aurantii* control. Methods such as MD that are based on pheromones appear to be good alternatives to conventional insecticide sprays,[28,29] but attention must be paid to potential effects on beneficial insects. This study provides information on the effectiveness and ecological safety of the combined use of MD, augmentative releases of parasitoids and conservation of natural enemies.

Since the discovery of the CRS sex pheromone, *A. aurantii* has become a subject of studies on the application of pheromone-based techniques, first in monitoring and detection, [10, 14, 34] and later in MD research.[26-29] This work further confirms the efficacy of MD in controlling CRS: the main male flights were reduced by 73% and 87% when compared with the population recorded in a control plot. This inhibition ultimately resulted in 83% damage reduction using a fruit damage threshold of more than three surface scales. The mortality caused by parasitoids (mainly *A. melinus*) and generalist predators in the MD plot was the same as or higher than that in the control plot, suggesting that pheromones do not affect the behaviour of beneficial insects under field conditions.

The hypothesis that the CRS sex pheromone may attract *Aphytis* sp. was first raised by Sternlicht in 1973,[35] after finding positive responses both in laboratory and field tests. Later tests by Morgan and Hare found no evidence of host-independent orientation of *Aphytis* sp. towards the female sex pheromone of CRS.[36] This finding is consistent with the results of the field trial reported here, in which *A. melinus* catches were not influenced by the CRS MD environment. Likewise, complementary laboratory tests showed that a CRS pheromone-saturated environment had no influence on *A. melinus* mating behaviour and efficacy. Nevertheless, from our field experiment it is not possible to conclude whether long-range effects of CRS sex pheromone might influence

(positively or negatively) mate finding in *A. melinus*. In any case, Bernal and Luck found that *A. melinus* males searching on substrates rely on a pheromone trail to locate mates,[37] a mechanism that is not vulnerable to interference from the A. *aurantii* sex pheromone

The objective of this work was to determine the influence of the CRS MD environment on the CRS instars susceptible to *A. melinus*, rather than to evaluate the role of the pheromone as a kairomone for *Aphytis* sp. For this reason, the population structure of *A. aurantii* was measured in both MD and control plots. As seen in the results, MD treatment delayed the development of immature *A. aurantii*, resulting in prolonged vulnerability to natural enemies. Accordingly, stage-specific mortality from natural enemies increased in the treated environment. This shift in the relative abundance of CRS immature stages in the field could partly explain the increase in the number of parasitised and preyed-upon *A. aurantii*. These conclusions should encourage future research on this pheromone's effects on the *A. aurantii* life cycle; which may have practical implications for the control of this pest.

In conclusion, this work confirms the compatibility of MD and biological control techniques. Such techniques could prove to be useful tools in Integrated Pest Management programs relying on pesticide-free and environmentally friendly control methods.

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REFERENCES

1 Talhouk AS, Citrus pests throughout the world, in *Citrus*, ed by Häfliger E, Ciba-Geigy, Basel, Switzerland, pp 23-27 (1971).

2 University of California (UC), *Integrated pest management for Citrus*, Division of Agriculture and Natural Resources, Oakland, USA, p. 144 (1991).

3 Tena A and García-Marí F, Current situation of citrus pests and diseases in the Mediterranean Basin. *IOBC/WPRS Bulletin* **In press** (2010).

4 Beardsley JW and Gonzalez RH, Biology and Ecology of Armored Scales. *Annu Rev Entomol* **20**:47-73 (1975).

5 Rodrigo ME, Garcia-Mari F, Rodriguez-Reina JM and Olmeda T, Colonization of growing fruit by the armored scales *Lepidosaphes beckii*, *Parlatoria pergandii* and *Aonidiella aurantii* (Hom., Diaspididae). *J Appl Entomol* **128**:569-575 (2004).

6 Jacas JA and Urbaneja A, Biological control in citrus in Spain: From classical to conservation biological control, in *Integrated management of arthropod pests and insect borne diseases*, ed by Ciancio A and Mukerji KG, Springer, Dordrecht, The Netherlands, pp 61-72 (2010).

7 Pina T, *Control biológico del piojo rojo de California Aonidiella aurantii (Maskell) (Hemiptera: Diaspididae) y estrategias reproductivas de su principal enemigo natural Aphytis chrysomphali Mercet (Hymenoptera: Aphelinidae)*, ed. by Departament de Zoologia, Universitat de Valencia, Spain, 384pp (2007).

8 Pekas A, *Factors affecting the biological control of California red scale Aonidiella aurantii (Hemiptera: Diaspididae) by Aphytis (Hymenoptera; Aphelinidae) in eastern Spain citrus: host size, ant activity, and adult parasitoid food sources*, ed by. Departamento de Ecosistemas Agroforestales, Universidad Politécnica de Valencia, Spain, 167pp. (2011).

9 Tashiro H and Moffitt C, Reproduction in California Red Scale *Aonidiella Aurantii* .2. Mating behavior and postinsemination female changes. *Ann Entomol Soc Am* **61**:1014-1020 (1968).

10 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).

11 Bodenheimer FS, *Citrus Entomology in the Middle East*, ed. by Junk W, The Hague, The Netherlands, 663 pp. (1951).

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

13 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).

14 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).

15 Smith D, Beattie GAC and Broadley R, *Citrus pests and their natural enemies. Integrated pest management in Australia*, State of Queensland, DPI & HRDC, Brisbane (Australia), 272 pp. (1997).

16 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).

17 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).

18 Grafton-Cardwell EE, Ouyang Y, Striggow RA, Christiansen JA and Black CS, Role of esterase enzymes in monitoring for resistance of California red scale (Homoptera: Diaspididae), to organophosphate and carbamate insecticides. *J Econ Entomol* **97**:606-613 (2004).

19 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).

20 El Sayed AM, Suckling DM, Wearing CH and Byers JA, Potential of mass trapping for long-term pest management and eradication of invasive species. *J Econ Entomol* **99:**1550-1564 (2006).

21 Kehat M, Anshelevich L, Harel M and Dunkelblum E, Control of the Codling Moth (*Cydia pomonella*) in apple and pear orchards in Israel by mating disruption. *Phytoparasitica* **23:**285-296 (1995).

22 Gordon D, Zahavi T, Anshelevich L, Harel M, Ovadia S, Dunkelblum E, *et al*, Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae): Effect of pheromone formulations and concentrations. *J Econ Entomol* **98:**135-142 (2005).

23 Lykouressis D, Perdikis D, Samartzis D, Fantinou A and Toutouzas S, Management of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by mating disruption in cotton fields. *Crop Prot* **24:**177-183 (2005).

24 Harari AR, Zahavi T, Gordon D, Anshelevich L, Harel M, Ovadia S, *et al*, Pest management programmes in vineyards using male mating disruption. *Pest Manag Sci* **63:**769-775 (2007).

25 Alfaro C, Navarro-Llopis V and Primo J, Optimization of pheromone dispenser density for managing the rice striped stem borer, *Chilo suppressalis* (Walker), by mating disruption. *Crop Prot* **28:**567-572 (2009).

26 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).

27 Hefetz A, Kronengerg S, Peleg BA and Bar-zakay I, Mating disruption of the California Red Scale *Aonidiella aurantii* (Homoptera: Diaspididae). *Proc 6th Int Citrus Congress, Tel Aviv (Israel)* **3:**1121-1127 (1988).

28 Vacas S, Alfaro C, Navarro-Llopis V and Primo J, Mating disruption of California red scale, *Aonidiella aurantii* Maskell (Homoptera: Diaspididae), using biodegradable mesoporous pheromone dispensers. *Pest Manag Sci* **66**:745-751 (2010).

29 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. *Bull Entomol Res* **99**:415-423 (2009).

30 30 Vanaclocha P, Urbaneja A and Verdu MJ, Mortalidad natural del piojo rojo de California, *Aonidiella aurantii*, en cítricos de la Comunidad Valenciana y sus parasitoides asociados. *Bol San Veg Plagas* **35**:59-72 (2009).

31 Pekas A, Aguilar A, Tena A and Garcia-Mari F, Influence of host size on parasitism by *Aphytis chrysomphali* and *A. melinus* (Hymenoptera: Aphelinidae) in Mediterranean populations of California red scale *Aonidiella aurantii* (Hemiptera: Diaspididae). *Biol Control* **55**:132-140 (2010).

32 Sorribas J, Rodriguez R and Garcia-Mari F, Parasitoid competitive displacement and coexistence in citrus agroecosystems: linking species distribution with climate. *Ecological Applications* **20**:1101-1113 (2010).

33 Sorribas J and Garcia-Mari F, Comparative efficacy of different combinations of natural enemies for the biological control of California red scale in citrus groves. *Biol Control* **55**:42-48 (2010).

34 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).

35 Sternlicht M, Parasitic wasps attracted by the sex pheromone of their coccid host. *BioControl* **18:**339-342 (1973).

36 Morgan DJW and Hare JD, Volatile cues used by the parasitoid, *Aphytis melinus*, for host location: California red scale revisited. *Entomol Exp Appl* **88:**235-245 (1998).

37 Bernal JS and Luck RF, Mate finding via a trail sex pheromone by *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) males. *J Insect Behav* **20:**515-525 (2007).

Table 1. Effect of California red scale pheromone on *Aphytis melinus* mating behaviour

Behavioural variable	**MD**	**Control**	**Statistical test**
Successful matings (%)	40 (n=20) a	63.2 (n=19) a	F= 2.038 P = 0.205
Contacts before copulation[a]	2.4 ± 0.6 a	2.7 ± 0.7 a	t_{18}= 0.33; P = 0.75
Contacts in non-mating couples	9.8 ± 2.8 a	15.3 ± 2.2 a	t_{17}= 1.33.; P = 0.20
Time before mating (s)	6.6 ± 3.1 a	8.92 ± 2.1 a	t_{18}= 0.63; P = 0.54
Duration of mating (s)	3.9 ± 0.6 a	3.6 ± 0.3 a	t_{18}= 0.51; P = 0.61

Within a row, means followed by the same letter are not significantly different from each other (P<0.05).

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Table 2. Effect of California red scale pheromone on *Aphytis melinus* feeding and parasitisation of *Aonidiella aurantii*

Variable	MD	Control	Statistical test
No. offered	68.7 ± 1.4 a	67.1 ± 1.0 a	t_{13}= 0.940; *P*=0.365
No. Alive	44.3 ± 6.0 a	47.0 ± 6.0 a	t_{13}= 0.301; *P*=0.768
No. Parasitism	8.3 ± 3.8 a	4.2 ± 2.0 a	t_{13}= 1.042; *P*=0.317
No. Host feeding	4.0 ± 1.0 a	5.2 ± 1.6 a	t_{13}= 0.557; *P*=0.587
No. Death	12.0 ± 2.9 a	10.6 ± 4.0 a	t_{13}= 0.267; *P*=0.794
A. melinus Progeny	12.0 ± 6.0 a	6.6 ± 3.1 a	t_{13}= 0.892; *P*=0.389

Mean ± SE of living, parasitised, fed-on and dead third-instar nymphs of *A. aurantii* and mean ± SE of *A. melinus* progeny, when 70 susceptible instars of *A. aurantii* were offered to two pairs of *A. melinus* over 3 days in the MD- saturated environment and control treatments. Within a row, means followed by the same letter are not significantly different from each other ($P<0.05$).

Table 3. Percentage of mortality caused by the natural enemies of *A. aurantii*, as found on shoots and fruits under field conditions

Mortality factor		MD		Control		
		Number/n	**Percentage**	**Number/n**	**Percentage**	***P* value**
Shoot	Alive	218/335	65.1 b	141/182	77.5 a	0.002
	Parasitised	87/335	26.0 b	34/182	18.7 a	0.036
	Host feeding	1/335	0.3 a	0/182	0.0 a	0.647
	Preyed-upon	29/335	8.7 b	7/182	3.8 a	0.027
Fruit	Alive	115/168	68.5 a	135/179	75.4 a	0.093
	Parasitised	51/168	30.4 a	42/179	23.5 a	0.092
	Host feeding	0/168	0.0 a	2/179	1.1 a	0.265
	Preyed-upon	2/168	1.2 a	0/179	0.0 a	0.234

For each mortality factor and treatment, values followed by the same letter are not significantly different (*P*-values calculated using the Fisher exact probability test, $P<0.05$).

Figure 1. Male CRS catches per trap per day (MTD) in monitoring sticky traps, for the mating disruption plot (MD) and the control plot receiving oil sprays.

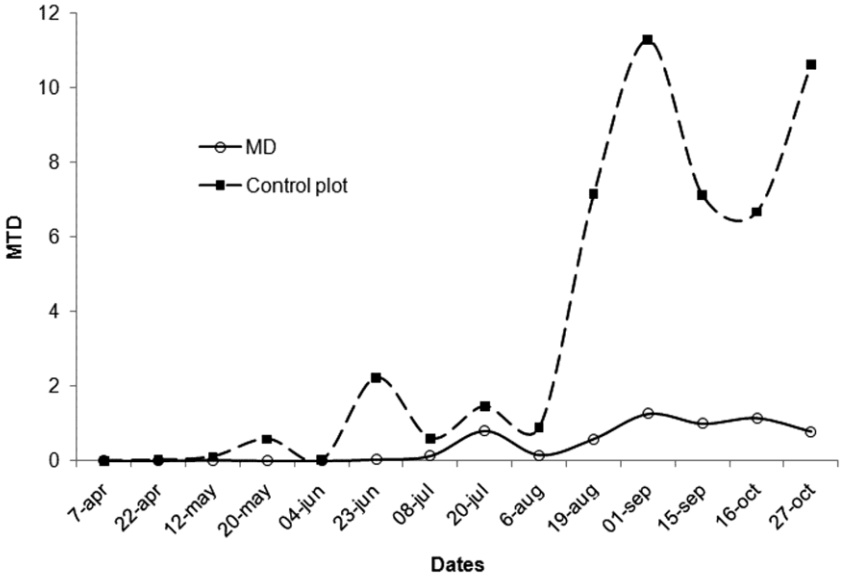

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Figure 2. Mean percentage of damaged fruit assessed just before harvest, according to three damage thresholds (> 3,> 5 or >10 scales per fruit) observed in MD and control plots. Bars labelled with the same letter for each threshold do not differ significantly (Student's t-test, $P<0.05$).

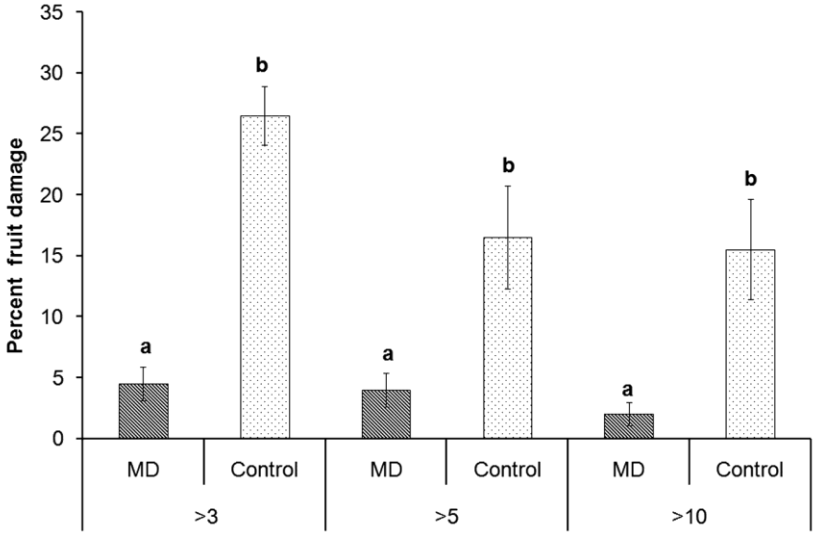

Figure 3. Population structure of *A. aurantii* on shoots observed in different months: a) 4 May, b) 6 August and c) 9 September. The stages recorded were nymphal instars 1, 2 and 3 (N1, N2 and N3), gravid females (GF), gravid females with crawlers (GFC), male prepupae (PPM) and male pupae (PM). Bars labelled with the same letter for each stage are not significantly different (Fisher exact probability test, $P<0.05$).

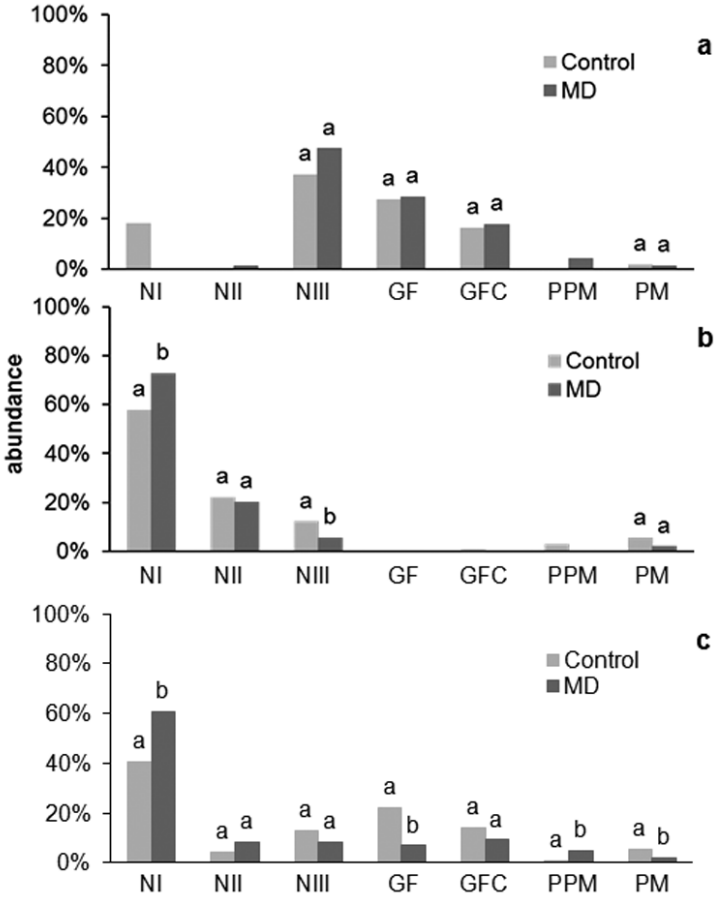

Figure 4. Population structure of *A. aurantii* on fruit, observed September 9. The stages recorded were nymphal instars 1, 2 and 3 (N1, N2 and N3), gravid females (GF), gravid females with crawlers (GFC), male prepupae (PPM) and male pupae (PM). Bars labelled with the same letter for each stage are not significantly different (Fisher exact probability test, $P<0.05$).

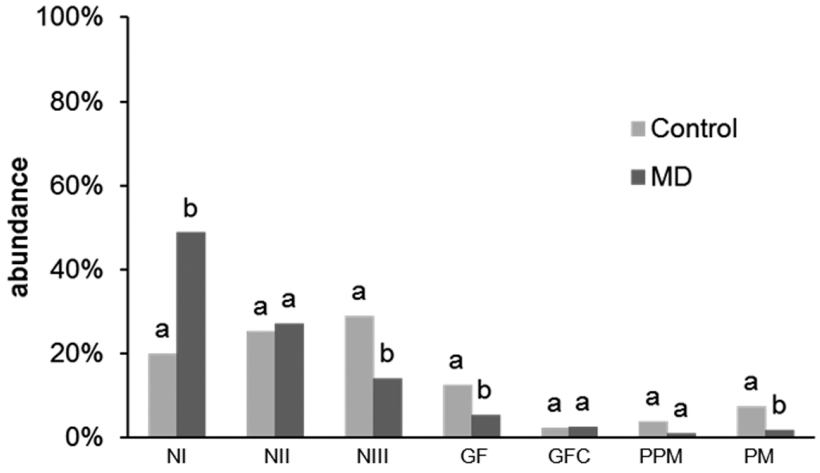

Figure 5. *Aphytis melinus* catches per trap per day (AmTD) in CRS monitoring sticky traps, for the mating disruption plot (MD) and the control plot.