

Abstract

 Two different experiments were carried out to evaluate three insecticides. In the first one, the effect of two insecticides, methomyl and indoxacarb, on pupae and 26 adults of the whitefly Bemisia tabaci (Gennadius) parasitoid Eretmocerus mundus Mercet was evaluated under laboratory and greenhouse conditions, using sweet pepper (Capsicum annuum L.) plants. In the second experiment, oxamyl was tested to study its effect on the ability of E. mundus to parasitize and control B. tabaci in sweet pepper plants, using a greenhouse cage evaluation. Methomyl and indoxacarb caused low mortality of E. mundus pupae (17.6 and 7.8% respectively), although methomyl mortality was significantly higher. Methomyl produced 100% mortality on E. mundus adults with fresh and 24 hour-old residues on leaves, significantly higher than the mortality produced by indoxacarb (values ranged from 43.9 to 34.4%). The harmful effect of methomyl persisted for a long time (up to 60 days). The results of the experiment with oxamyl showed that E. mundus controlled whitefly population, without significant interaction between the presence of the parasitoid and insecticide on whitefly mortality. Whitefly mortality in the presence of the parasitoid was 87.8%, significantly higher 40 than the mortality in the absence of E . mundus (59.3%). Oxamyl did not produce a significant effect on the emergence of E. mundus adults. Application of the products in IPM programs is discussed.

 Keywords: Eretmocerus mundus, Bemisia tabaci, indoxacarb, oxamyl, methomyl, sweet pepper, parasitism.

Introduction

 Eretmocerus mundus Mercet (Hymenoptera: Aphelinidae), a parasitoid of the tobacco, cotton or sweet potato whitefly Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), has a widespread distribution worldwide. This species is recognized as one of the most important natural enemies of B. tabaci, and has generated a lot of interest in countries where B. tabaci is a problem. The use of heavy chemical control against this pest is also recognized to be involved in many of the outbreaks of B. tabaci around the world, for different reasons: development of insecticide resistance, negative effects on natural enemies, alteration of behaviour and biology of the pest.

 Outbreaks of this whitefly in different crops and countries have stimulated the search for different biological control agents around the world. The most active country in this respect is the U.S.A., where many parasitoid and predator species 62 have been tested. One of the most promising species is E . mundus (Goolsby et al., 1998; Hoelmer et al., 1999; Kirk et al., 2000), especially the strain obtained from southeast Spain. The first time this species was collected in Murcia (Spain) on cotton, in 1991-92, it exhibited a good tolerance to the different pesticides applied regularly to the crop (Kirk et al., 2000).

 Current pest control includes the use of pesticides, combined or not with natural enemies. There are many reports and studies that present evidence of natural enemies of B. tabaci that can remain active in different crops after the use of some pesticides (Gerling, 1996; Gerling and Naranjo, 1998; Simmons and Jackson, 2000), especially parasitoids, such as E. mundus. Possible explanations for this ability of parasitoids to remain in treated plots include the protection that immature stages can obtain inside the host, as well as the type of compound used, the incomplete coverage of the canopy or the timing of the application.

 The presence of E. mundus in commercial plots treated routinely with pesticides has also been reported by other authors (Rodriguez-Rodriguez et al. 1994;

 Gonzalez-Zamora et al., 1996) in southeast Spain. They also studied the parasitism on B. tabaci and Trialeurodes vaporariorum (West.) by different species and the ability of E. mundus to control B. tabaci populations in sweet pepper, melon, and tomato. In summary, these papers show the ability of different whitefly parasitoids to establish and maintain a high level of parasitism in plots, even when they are treated with pesticides. Different pesticides are considered to be selective to this and other parasitoids and can be used together to obtain control of the pest population below injury levels (Koppert Biological Systems, 2003).

 Other papers have studied the combination of pesticides and parasitoids (with special interest in Eretmocerus spp.) to control whitefly populations in different conditions. For example, Birnie and Denholm (1992) used an extended laboratory trial to test the capacity of E. mundus to control B. tabaci populations on cotton with the application of cypermethrin, showing the ability of the parasitoid population to recover after only one application of the compound. Devine et al. (2000) revealed the potential of piperonyl butoxide to improve the level of E. mundus parasitism on B. tabaci, by slowing the development of the whitefly, increasing the parasitism in the treated whitefly population by 7-8%. Van Driesche et al. (2001) demonstrated the possibility of using an insect growth regulator (buprofezin) in combination with Eretmocerus eremicus Rose and Zolnerowich to 100 control T. vaporariorum and B. tabaci in poinsettias in commercial greenhouses.

 The aim of this work was to study the effect of three insecticides on different development stages (pupa and adult) of E. mundus, and the capacity of E. mundus to control B. tabaci populations together with one of the insecticides, presenting evidence of the capacity of this species to be used together with insecticides. Two of these insecticides (methomyl and oxamyl) are currently used in southeast Spain to control different pests, and the third (indoxacarb) has been introduced recently to control caterpillars in different crops, including those with B. tabaci infestation. Indoxacarb is a selective product that is compared with the other two products, non-selective insecticides. These two insecticides can be applied in different ways in order to confer selectivity. Results are discussed considering the possibilities of using these products in a IPM program.

Material and Methods

 Two different experiments were carried out to test the insecticides: in the first one, methomyl (chemical name: S-methyl-N-[(methylcarbamoyl)oxy] 118 thioacetimidate) (commercial product Lannate[®] 20 L, Du Pont Iberica S.L.), and indoxacarb (chemical name: (S)-7-chloro-3-[methoxycarbonyl-(4-trifluoromethoxy- phenyl)-carbamoyl]-2,5-dihydro-indenol 1,2 -e[1,3,4]oxadiazine-4a(3H)-carboxylic 121 acid methyl ester) (commercial product Steward® 30 WG, Du Pont Iberica S.L.) were tested on E. mundus pupae and adults; in the second one, oxamyl (chemical name: methyl-N',N'-dimethyl-N[(methylcarbamoyl)oxy]-1-thio-oxamimidate) 124 (commercial product Vydate® 10L, Du Pont Iberica S.L.) was tested to determine its effect on the parasitism and capacity of E. mundus to control a population of 126 B. tabaci, using a greenhouse cage evaluation.

128 A colony of B. tabaci was created from adults captured in the Seville province (southwest Spain), from cotton and aubergine plants, and kept in an insect rearing room, feeding on sweet pepper (Capsicum annuum L.) plants (cv. Largo Italiano, Semillas Batlle S.A., C/Matadero 10, E-10100-Miajadas (Caceres, Spain)). The biotype of B. tabaci was not determined. Adults of E. mundus were collected in the regions of Almeria (southeast Spain) and Seville, on sweet pepper and cotton plants, and reared on sweet pepper plants infested with B. tabaci nymphs. The rearing room was kept at 26±2 ºC with a photoperiod of 16:8 (Light:Dark).

Experiment 1: Effect of methomyl and indoxacarb on Eretmocerus mundus pupae and adults

 Four young sweet pepper plants were selected for each treatment (i.e. product), with 4-5 leaves each, to assess the effect of insecticides on E. mundus pupae. They were infested with adults of B. tabaci, which were allowed to lay eggs for 48-72 hours and then removed from the plants. Development of the offspring was 144 followed until 2^{nd} -3rd instar nymphs were present, then females of E . mundus were introduced into the cages for oviposition, together with several males. The 146 plants were kept in the rearing room until the parasitoid pupa was observed inside the whitefly pupal case: the form of the pupa was evident and the eyes took on a cherry colour (Garrido et al., 1982; Garrido, 1992). At that moment, a minimum of twenty-five pupae per plant were marked with an indelible pen, and the plants were put in a plastic greenhouse. The total number of pupae used in each treatment was 115, 107, and 114 for indoxacarb, methomyl and the control, respectively. The products were applied in that moment, using a trigger-operated hand sprayer, at the recommended field rates: methomyl, 0.4 g a.i./l; indoxacarb, 0.0375 g a.i./l until run-off. A group of four plants was treated with water, as control.

 Counts were done every 3-4 days with the help of a 5x magnifying hand lens, counting dead and live pupae and pupal cases from where an insect had emerged. The experiment finished when all the adults had emerged.

161 The effect of the insecticides on E . mundus adults was evaluated by applying the products and water at the same rates with the trigger-operated hand sprayer on several sweet pepper plants with developed leaves. The products were allowed to dry and then two types of cages were mounted: with fresh residues and with twenty-four hour old residues. The methodology used was described in Jones et al. (1995).

 The experimental units (cages) consisted of 5-6 newly emerged adults of E. mundus, confined to a Petri dish of 55 mm diameter and 14 mm height. The Petri dishes were modified by replacing most of the bottom with organdy cloth (subsequently inverted to become the top). The new bottom half of the Petri dish was used as a template to cut out a circular section of sprayed leaf. The leaf discs were fitted in the inner surface of a dish lid (now the bottom), with the bottom leaf surface facing upwards. A cotton cloth moistened with water and honey was put inside the cage. The parasitoids were collected from the rearing units and briefly chilled before being introduced in the experimental units. The ventilated dish bottom was replaced, becoming the top. Dish halves were secured using a pair of clips, and the cages were put in a laboratory room. Six replicates per treatment (with a total of 32-36 individuals per treatment) were used with the fresh residues leaves, and eight replicates per treatment (with a total of 53-61 individuals per treatment) with the leaves with twenty-four hour old residues. The adults were kept in the cages for twenty-four hours, then opened and the adults counted, separating dead and live insects.

 A similar test was performed to study methomyl persistence in sweet pepper plant leaves. Different groups of plants were selected, and in each group one of the plants was treated with water and the others with methomyl at the previous rate. The plants were allowed to dry and after different time periods, ranging from seven to sixty-two days after treatment, cages were mounted with leaf discs from plants treated with methomyl and water. The number of adults introduced in each cage varied between eight and thirteen. The adults were introduced in the cages as explained above, and after twenty-four hours the cages were opened and the number of dead and live adults counted. Treated plants and cages were kept in laboratory conditions.

Experiment 2: Effect of oxamyl on the capacity of Eretmocerus mundus to control Bemisia tabaci

 The experimental design studied two levels (presence and absence) of two factors (parasitoid and insecticide). There were, therefore, four treatments: 1) application of oxamyl, 2) application of oxamyl and introduction of E. mundus, 3) introduction of E. mundus, and 4) no application of oxamyl and no introduction of E. mundus (control). Each treatment was replicated four times, using one sweet pepper plant 204 per treatment and replicate, making a total of 16 plants.

 The plants were infested with B. tabaci adults in the rearing room, allowed to lay 207 eggs for three days only on one leaf and then removed. The plants were kept in the rearing room and, after nine days, first and second instar whitefly nymphs were present on the leaves. The number of nymphs per leaf ranged from 132 to 198. Plants were then maintained in a greenhouse inside a metallic structure covered with organdy for the rest of the experiment.

 Oxamyl was applied at a rate of 0.028 g a.i./plant (equivalent to 500 g a.i./ha, with 18,000 plants/ha) in treatments 1) and 2). It was applied on a weekly basis, a maximum of eight times, beginning the same day the plants had been put in the greenhouse. The oxamyl was diluted in 100 ml of water per plant. The pH of the broth was adjusted to 4.5-5.5 with phosphoric acid. A drip irrigation system was used to water the plants, simulating the normal irrigation management of this crop, and the broth was injected with a previous-pressure sprayer in the irrigation system.

 E. mundus was introduced in treatments 2) and 3) at a total rate of 12 to 18 females per replicate, following the recommended ratio proposed by Jones et al. (1999). Adult parasitoids were placed in the cages in two to four separate introductions, beginning on the first day that the plants were put in the greenhouse.

 Plants were evaluated every 3-4 days during the first two weeks, and then weekly until all the adults of B. tabaci and E. mundus emerged. The number of living whitefly nymphs and pupae, the number of parasitized whitefly nymphs and 231 parasitoid pupae, and the number of pupal cases from where an adult (whitefly or parasitoid) had emerged were counted. This experiment included only one generation of the whitefly B. tabaci and the parasitoid E. mundus.

 Analysis of variance was performed on mortality and parasitism (Statistical Graphics Corporation, 1999) in both experiments, with the transformation of 237 z=arcsin \sqrt{p} , where p is mortality or parasitism. A 2x2 factorial analysis was applied

238 in experiment 2. If treatments were significant at $P < 0.05$, then differences between means were determined using the LSD test at 95% confidence level. Abbot's formula (Abbot, 1925) was used to correct the mortality in experiment 1.

242 Voucher specimens of the parasitoid are maintained by the first author in the Department collection (Departamento de Ciencias Agroforestales, Universidad de Sevilla).

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- **Results**
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Experiment 1: Effect of methomyl and indoxacarb on Eretmocerus mundus pupae and adults

 Methomyl significantly increased the mortality of E. mundus pupae parasitizing B. 252 tabaci (Figure 1) seven days after application $(F=4.7, d.f.=2, 9, P=0.04)$, while indoxacarb was not significantly different from the control. Mortality increased three days later to 17.6 % in methomyl, greater than indoxacarb and the control, 255 with 7.8 and 5.1% mortality respectively ($F=8.20$, d.f. $=2$, 9, P=0.009).

 Both insecticides significantly increased the mortality of E. mundus adults (Figure 2), though methomyl was more harmful (100 % mortality in both cases, with fresh and 24 hour-old residues in leaves) than indoxacarb (43.9% and 34.4%, respectively), with F=59.8, d.f.=2, 6, P<0.0001, and F=84.60, d.f.=2, 9, P<0.0001, for fresh and 24 hour-old residues respectively.

 The harmful effect of methomyl on E. mundus adults lasted up to sixty days after application (Figure 3A), when the mortality was still significantly higher than the control treated with water. The mortality was corrected with the Abbot's formula 266 and adjusted to a curve (Figure 3B; $R^2 = 0.88$; P<0.01). At least 55 days were required for adult mortality after treatment with methomyl to drop to 50%.

Experiment 2: Effect of oxamyl on the capacity of Eretmocerus mundus to control Bemisia tabaci

272 The percentage mortality of B . tabaci nymphs treated with oxamyl was not significantly different from untreated nymphs (F=0.05, d.f.=1, 12, P=0.83). The addition of E. mundus produced a significant effect on the mortality of B. tabaci 275 nymphs (F=6.90, d.f.=1, 12, P=0.02). No interaction was observed between the 276 two factors studied $(F=2.69, d.f.=1, 12, P=0.13)$.

 The results of whitefly mortality in the four treatments are shown in Figure 4A. The highest mortality was obtained with the introduction of E. mundus without application of oxamyl (95.7% at the end of the study), whereas the mortality decreased to 79.9% when the plants were treated with the insecticide. The natural mortality of the whiteflies was 51.3% and the mortality in the treatment with oxamyl reached 67.3%. There is no significant difference between the four 284 treatments (F=3.21, d.f.=3, 12, P=0.06), but the P-value is very near to the limit of P=0.05. Although not completely justified, Tukey's HSD test only showed differences between treatment 3 (introduction of E. mundus, 95.7% mortality) and treatment 4 (control, 51.3% mortality).

 Levels of whitefly mortality in the treatments with and without E. mundus are shown in Figure 4B. The values at the end of the study were 87.8 and 59.3% respectively, which differ significantly (F=6.90, d.f.=1, 12, P=0.02).

 The number of fourth instar nymphs with signs of parasitization was also recorded in the two treatments where E. mundus was used (Figure 5A). This was very similar in the treatments with and without oxamyl, although without oxamyl the proportion of parasitized nymphs was always higher. In any case, the standard error bars superimpose in most of the counting dates, indicating that there was no significant difference between the two treatments.

 Finally, the proportion of parasitoids that emerged at the end of the experiment from whitefly pupal cases was higher in plants not treated with oxamyl (Figure 5B), but not significantly different from treated plants (24.7% and 10.6%, respectively; F=4.11, d.f.=1, 6, P=0.09). The final value was measured in terms of the proportion of the initial nymphal population.

Discussion

 The results reveal that indoxacarb did not significantly increase E. mundus pupal mortality, while methomyl presented a low mortality. A similar pattern has been found with different insecticides tested on E. mundus pupae. Gonzalez-Zamora et al. (1997) found that only three of thirteen insecticides tested were included in the category of moderately harmful (between 80 and 98% mortality, Abbott's corrected mortality), according to the I.O.B.C. classification. On the other hand, Jones et al. (1998), testing six insecticides on E. mundus pupae, found that three of them produced a mortality higher than 90 %, and the other three of around or below 50%. The fact that the insect develops inside the pupal case of the whitefly can help to explain the low mortality produced by some of the insecticides tested. The timing of application of the compounds, which is related to the development stage of the parasitoid, and their ways of action can also help to explain the different effect on the parasitoid, as discussed by Gerling and Sinai (1994) and Jones et al. (1998). Gerling and Sinai (1994) observed that buprofezin was toxic to the eggs and young larvae of Eretmocerus sp., but harmless to the parasitoid pupae, and Jones et al. (1998) also found differential mortalities of the products they tested depending on the stage of the parasitoid, as young larva or parasitoid pupa. In the present work, the compounds were applied when pupae were easily observed, and all the individuals were in the same stage, in order to have an homogeneous population.

 This result, however, cannot be generalized for all parasitoids and products. Garrido et al. (1982) found high mortality (97.2%) on Cales noacki Howard pupae (Hymenoptera: Aphelinidae) parasitizing Aleurothrixus floccosus Maskell

 (Hemiptera: Aleyrodidae) treated with methomyl. In other parasitoids such as Aphidius colemani Vierek (Hymenoptera: Aphidiidae) parasitizing Myzus persicae (Sulzer) (Hemiptera: Aphididae), methomyl showed no effect on the emergence of adults from inside the mummies (DuPont, interim report, 1999). Indoxacarb also showed the same low toxicity with other parasitoids, such as aphid parasitoids developing inside the mummy (Dinter and Wiles, 2000).

 The two compounds tested with E. mundus adults showed a different pattern (Figure 2). Indoxacarb produced a low mortality with fresh and 24 hour-old residues (43.9 and 34.4% respectively), whereas methomyl produced 100% mortality in both cases. Indoxacarb has been tested on different beneficial 343 arthropods (Dinter and Wiles, 2000), and proved harmless to Typhlodromus pyri 344 Scheuten (Acari: Phytoseiidae), Episyrphus balteatus De Geer (Diptera: Syrphidae), Orius laevigatus (Fieber) (Hemiptera: Anthocoridae) and Aleochara bilineata Gyllenhal (Coleoptera: Staphylinidae), but harmful to parasitic wasps (A. colemani) under worst-case laboratory conditions (the same conditions as in the present study). In extended and semi-field trials it proved to be compatible with the presence of different aphid parasitoids. The conclusion of the authors was that this product could be included in integrated pest management programs. Our results indicate a low impact of this product on both E. mundus adult and pupa.

 On the other hand, our results reveal that methomyl is a very toxic compound for E. mundus adults, although this result may be different with other insects. Methomyl produced high mortality in A. colemani adults, under worst-case laboratory conditions after 24 hour exposure to fresh residues, although this effect lasted around two weeks when the mortality dropped to 25-50% (DuPont, interim report, 1999). In laboratory and field conditions, methomyl showed partial and short-term effects on foliage- and surface-dwelling predators, such as larvae of Chrysoperla carnea (Stephens) and Coccinella septempunctata L., in which the mortality was comparable to the control 4 days after the treatment (Dinter and Kratz, 2000).

 The harmful effect of methomyl residues on adults of E. mundus clearly shows a long lasting negative effect on this parasitoid (Figure 3). About 55 days were necessary for adult mortality to decline below 50%. This means that adults emerging from pupae onto treated foliage will not survive, considering that the time spent on pupa is about 6 to 15 days at 25 ºC (Foltyn and Gerlin, 1985; Sharaf and Batta, 1985). On the other hand, it is necessary to consider the effects of light intensity and crop growth on product degradation and dilution of the residues. In commercial crops, the time to reach the 50% mortality could be shorter for these reasons and, also, parasitoids may not come into contact with treated foliage, simply because their hosts are present on younger growth, or leaves treated early in the crop have senesced or been removed. Anyway, 55-56 days (or 8 weeks) is the minimum time some companies recommend to wait before introducing parasitoids such as E. eremicus (and other species) after a treatment with methomyl (Koppert Biological Systems, 2003).

 The second experiment, with oxamyl, showed interesting results: (1) the control that E. mundus can exert on a B. tabaci population, and (2) the absence of a significant interaction between the parasitoid and the insecticide.

 In this trial, the presence of E. mundus produced a mortality of whitefly nymphs of 87.8%, significantly different from the absence of the parasitoid. The whitefly mortality data alone cannot tell us the fate of the population, because experiment 2 only included one generation of the whitefly and parasitoid. Different authors (Birnie and Denholm, 1992; Simmons and Minkenberg, 1994; Goolsby et al., 1998; Heinz and Parrella, 1998) have shown the ability of E. mundus, and other related species, to control whitefly populations in extended laboratory and semi-field experiments. These authors kept whiteflies in cages and released adult parasitoids, albeit in different proportions than in the present work, and generally for more than one generation.

 The high whitefly mortality when the parasitoid is present is caused by active parasitism and the feeding activity of the adult parasitoids. This last component

 can be very important, as Heinz and Parrella (1998) showed for different adult parasitoids, including E. mundus. The final result is that the whitefly population reaches low numbers, significantly lower than the control in which no parasitoid is added.

 Oxamyl exerted little control on the B. tabaci population, compared with the untreated control in the conditions of the experiment, but it must be noted that nymphs were already installed on the leaves when the product was applied. Oxamyl is a product that can control whitefly populations (and other sucking pests) on a long-term basis, as Cabello et al. (1997) demonstrated. These authors found significant differences between treated and untreated plots after 56 days 407 from the beginning of the treatments, whereas in our case we only studied one generation of the population of B. tabaci and E. mundus.

 The absence of a significant interaction between the two factors (parasitoid and insecticide), measured in terms of whitefly mortality, was also observed. The combination of oxamyl and a whitefly parasitoid was also studied by Helyer et al. (1984) using Encarsia formosa Gahan to control T. vaporiarorum in tomato, finding that the level of parasitism was 90% 145 days after treatment with oxamyl, although they only applied the product once and only introduced the parasitized pupae of E. formosa after 56 days. In the present study, oxamyl did not produce a significant effect on the proportion of final emergence of E. mundus 418 adults, although the P value obtained (0.09) was near to the limit of P= 0.05. The evolution of parasitized nymphs over the sampling dates was rather similar in the treated and untreated plots.

 Finally, our results suggest that indoxacarb could be used in integrated pest management programs for crops where E. mundus is present. Oxamyl could also 424 be considered, although the effect on the parasitoid population in the longer term should be studied. Methomyl is very toxic to E. mundus adults, and the use of this product in crops where this parasitoid is an important agent to control B. tabaci should be avoided, especially when whitefly is a key pest. In other cases, aspects such as the crop, the type of pest or pests to control, the timing of pesticide application and location on the plant, or the relation between pests and natural enemies present in the crop should be studied to use or reject this product.

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Figure captions

 Figure 1. Percentage mortality of Eretmocerus mundus pupae parasitizing 560 Bemisia tabaci treated with methomyl (\Box) , indoxacarb $(+)$, and control (\Box) . Treatments with different letters in the same day are significantly different with 95% of confidence, using the LSD test. Vertical bars indicate the standard error of the mean.

 Figure 2. Percentage mortality of Eretmocerus mundus adults treated with methomyl, indoxacarb, and the control, with fresh and 24-hour old residues. Treatments with different letters are significantly different with 95% of confidence, using the LSD test. Vertical bars indicate the standard error of the mean.

 Figure 3. Percentage mortality of Eretmocerus mundus adults treated with methomyl over a period of 60 days, **(A)** compared with the control treated with 573 water; methomyl (\blacksquare) , water (\blacktriangledown) ; and (\blacksquare) mortality corrected using Abbot's 574 formula and with an adjusted curve (R^2 =0.88, P<0.01). Vertical bars indicate the standard error of the mean.

 Figure 4. Cumulative percentage mortality of Bemisia tabaci larvae in the 578 experiment with oxamyl, **(A)** considering the four treatments, oxamyl (\square) , 579 oxamyl plus Eretmocerus mundus (∇) , E. mundus (∇) , and control $(+)$; and **(B)** 580 considering the treatments with E. mundus (\blacksquare) , and without parasitoids $(+)$. Vertical bars indicate the standard error of the mean.

 Figure 5. Effect of oxamyl on Eretmocerus mundus parasitizing Bemisia tabaci larvae. **(A)** Percentage evolution of parasitized whitefly larvae treated with 585 oxamyl (\blacksquare) and water (\star) ; and **(B)** Cumulative percentage emergence of 586 Eretmocerus mundus adults from Bemisia tabaci larvae treated with oxamyl (\blacksquare) 587 and water (\triangle) . Vertical bars indicate the standard error of the mean.

