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Evaluation of the local population of *Eretmocerus mundus* (Hymenoptera: Aphelinidae) for biological control of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) in greenhouse peppers in Argentina

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ABSTRACT

Bemisia tabaci biotype B is a key pest in pepper crops in Argentina. The parasitoid *Eretmocerus mundus* is frequently found parasitizing this whitefly in greenhouses without pesticide applications. The present studies were carried out with the objective of evaluating control obtained with different rate and number of parasitoid releases under experimental conditions. Release rate: cages with pepper pots were positioned in an experimental greenhouse and randomly assigned to the release rate treatments (0, 1 and 3 pairs of *E. mundus*/plant/week with a total of three introductions). Number of releases: similar cages were assigned to the number of parasitoid introduction treatments (0, 1, 2 and 3) with the best release rate obtained in the previous trial. In both assays whitefly (adults and nymphs) and parasitoid (parasitized nymphs) population sizes in each cage were monitored weekly for a period of 10 weeks. Results suggested that the introduction of 2 *E. mundus*/plant/week was enough to suppress host population compared to control treatment (peaks of 7.75 adults and 58.75 nymphs/cage and 643.75 adults and 1598 nymphs/cage, respectively) ($p < 0.05$), with 85% of parasitism. *E. mundus* had to be introduced three times to achieve the best pest control (peaks of 1.17 adults and 20.33 nymphs/cage vs. 55.67 adults and 75 nymphs/cage in control treatment) with 84% of parasitism ($p < 0.05$). These results were then validated in a pepper crop under experimental greenhouse conditions. Whitefly population was lower in those greenhouses where *E. mundus* was released compared to control greenhouses (0.15 adults and 0.71 nymphs/4 leaves and 0.73 adults and 1.64 nymphs/4 leaves, respectively), with a peak of 54% of parasitism ($p < 0.05$). We concluded that good suppression of *B. tabaci* could be achieved using *E. mundus* under spring conditions in Argentina.

1. Introduction

The *Bemisia tabaci* (Gennadius) biotype complex is a key pest of many horticultural crops worldwide. Many biological characteristics, including broad host-range, multivoltinism, high reproductive rate, ability to vector plant viruses and a propensity to develop resistance to insecticides have contributed to the difficulty of managing this whitefly (Naranjo, 2001).

In Argentina *B. tabaci* has been found in most of the agricultural production areas. Up to 2000 it was mainly recorded in the northwestern region on cotton, soybean and tobacco (Viscarret, 2000), and it has been spreading into southern areas since then, especially in vegetables crops. In 2004 *B. tabaci* was identified around La Plata, Buenos Aires province, one of the most important horticultural area of Argentina (130,000 ha) (INTA Informa, 2004). The main host plant in this region is pepper (*Capsicum annuum* L., Solanaceae), which occupies 200–250 ha of greenhouses and is the fourth in importance after tomato, lettuce and spinach. This crop is planted in August and the harvest extends from November–December to May–June (Balcaza, 2006). Populations of *B. tabaci* begin to increase in the spring and typically reach outbreak levels during the summer months.

The biotype *B. tabaci* found in Buenos Aires province was B, a biotype capable of causing severe economic damage (Truol et al., 2005). The presence of this aggressive biotype and the high input of pesticides needed to control it have caused a change in pest status from a sporadic to major pest in many vegetable crops, particularly in pepper under greenhouse conditions. The use of pesticides has proven to be inefficient to control it.

The diversity of *B. tabaci* parasitoids in Argentina is not well known. Viscarret (2000) has listed five primary parasitoids (*Eretmocerus* sp., *Encarsia porteri* Mercet, *Encarsia sophia* (Girault & Dodd), *Encarsia pergandiella* Howard and *Encarsia* sp.) and one

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hyperparasitoid (*Signiphora* sp.) The taxonomy, biology and ecology of these wasp species has not been widely studied (Viscarret, 2000; Viscarret and López, 2004).

Recently the parasitoid *Eretmocerus mundus* Mercet has been collected on *B. tabaci* nymphs on pepper and melon (*Cucumis melo* L., Cucurbitaceae) in the North-East, the West and the centre of the country (López and Evans, 2008).

Eretmocerus mundus is native to the Mediterranean region where it is the most abundant parasitoid recovered spontaneously from *B. tabaci* in tomato and pepper grown in greenhouses. It is biparental, and like its congeners, ecto–endoparasitic on the whitefly host. In Spain it has been providing the best control of *B. tabaci*, even displacing *Eretmocerus eremicus* Rose & Zolnerowich, a species commercially available and introduced for whitefly control. Consequently, *E. mundus* has been commercially reared elsewhere since 2002 (Stansly et al., 2005a). The biology and effectiveness of this parasitoid has been mainly studied using *B. tabaci* biotype Q, the predominant biotype in most of the Mediterranean basin (Stansly et al., 2004, 2005a,b; Urbaneja et al., 2007).

Although it is unknown how this Mediterranean species arrived in Argentina, it has been dominating the parasitoid guild associated with *B. tabaci* in the vegetable producing regions since 2002 (Cáceres et al., 2005).

The objective of this work was to evaluate the efficiency of the local population of *E. mundus* as a biological control agent of *B. tabaci* in pepper crops under greenhouse conditions. Evaluations were conducted in three steps, with the first two screenings of different rates and numbers of parasitoid releases in experimental enclosure cages. The best performing rate and number of releases were then validated in a pepper crop under greenhouse conditions.

2. Materials and methods

2.1. Sources and rearing of whitefly and parasitoid

Bemisia tabaci adults were collected from pepper crops in greenhouses in La Plata, Buenos Aires province, in 2005. *E. mundus* was established from parasitized nymphs of *B. tabaci* biotype B on pepper grown in greenhouses in Bella Vista, Corrientes province, Argentina, in 2005. The whitefly and *E. mundus* were maintained on pepper plants.

Voucher specimens were preserved in the Insectario de Investigaciones para Lucha Biológica, Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Buenos Aires province, Argentina.

To obtain parasitoid adults of a uniform age for the experiments, a large number of parasitized nymphs were collected from the parasitoid rearing cage and were carefully placed in glass tubes (1×3 cm). Adults were allowed to emerge, sexed using a stereomicroscope and placed in tubes ((5 females + 5 males)/tube) for mating for 24–48 h. After this period, the pairs to be used were isolated and introduced in the experimental arena.

2.2. Experimental cages

The first two experiments were conducted in a polyethylene greenhouse (16 m by 10 m) located in Castelar. Enclosure cages (0.80 m high by 0.55 m wide by 0.60 m long) covered with fine-mesh cotton organdy were positioned in the greenhouse with the lower edges buried in the soil. Six (6) peppers in pots (15 cm diameter) were planted so they were equidistant from each other in a 2 by 3 array within each cage. Before the start of the experiments, the plants bore 6–8 leaves and were free of whiteflies. Temperature and relative humidity were recorded within one of the cages with a thermohygrometer.

2.3. Experiment 1: release rate

Twelve (12) cages were randomly assigned to 1 of 3 release rates (treatments): 0 (control), 1 and 3 females of *E. mundus*/plant/week (each female was introduced with a male). Whitefly population was established by adding to each cage 6 female and 6 male adults per week for 3 weeks, starting on September 28, 2005. One week after the last introduction, the wasp pairs were added to each cage weekly for 3 weeks (except for the no-release treatment).

From the first parasitoid release, whitefly and parasitoid population sizes in each cage were monitored weekly for a period of 10 weeks. Whitefly nymphs (fourth stage) and adults were counted on each plant by carefully examination of both sides of each leaf. Counting of small nymphs (first to third stage) was excluded because it would have been unreliable under greenhouse conditions and would have taken too much time. Moreover, as fourth nymph mortality is low (about 5%) the number of adults + fourth-instar nymphs was assumed to represent the population size that would develop into a future generation of whiteflies.

Nymphs containing a parasitoid pupae were identified by their characteristic orange color and recorded separately. The proportion of parasitized nymphs to total nymphs was used to measure parasitism throughout the trial.

Because each cage was considered an experimental unit, whitefly and parasitoid numbers were counted on all potted plants in the cage and were summed for each sampled date. A repeated-measures ANOVA was conducted to detect significant differences in whitefly and parasitoid population sizes among treatments. The number of whitefly adults and nymphs per cage were transformed using $\log(x + 1)$ and parasitism was transformed by square root to meet the ANOVA assumptions. Means were separated using the least significant difference (LSD) test at the 0.05 level (Statsoft Inc., 2000). Untransformed values of means are shown in results and figures.

2.4. Experiment 2: number of releases

Results from the release rate study suggested that the introduction of one mated female of *E. mundus*/plant per week was effective in suppressing *B. tabaci* population within the enclosure cage. Therefore, this release rate was used in a new trial, in which 24 cages were randomly assigned to one of the following treatments: 0 (control), 1 release in the first week, 2 releases in two consecutive weeks and 3 *E. mundus* releases in three consecutive weeks.

Whitefly population was established in the same way as in the previous assay, starting on September 25, 2006. One week after the last whitefly introduction, wasps were introduced at the described rate (except for the no-release treatment).

Starting from the first parasitoid release, whitefly and parasitoid population sizes in each cage were monitored as described in Experiment 1. Data was also analysed with a repeated-measures ANOVA. The number of whitefly adults and nymphs per cage were transformed using $\log(x + 1)$ and parasitism was transformed by square root to meet the ANOVA assumptions. Means were separated using the least significant difference (LSD) test at the 0.05 level (Statsoft Inc., 2000). Untransformed values of means are shown in results and figures.

2.5. Experiment 3: greenhouse experiment

Results from the previous assays suggested that it is possible to control an incipient *B. tabaci* population with three introductions of *E. mundus* at a rate of one mated female/plant. In order to test these results under greenhouse condition, an experiment was conducted in six polyethylene greenhouses (10 m by 5 m) located in Castelar,