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Augmentative biological control of *Bemisia tabaci* biotype "Q" in Spanish greenhouse pepper production using *Eretmocerus* spp.

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Abstract

Eretmocerus mundus Mercet is indigenous to the Mediterranean basin and the most abundant parasitoid attacking *Bemisia tabaci* Gennadius on the southern coast of Spain. However, *E. mundus* was not available commercially until 2002 and the North American *Eretmocerus eremicus* Rose and Zolnerowich had been used instead to control whiteflies in greenhouse vegetables, including sweet pepper in Campo de Cartagena (Murcia). The ability of these two *Eretmocerus* species to control *B. tabaci* on pepper by augmentation was compared with weekly releases of *E. mundus* and *E. eremicus,* alone, and in 1:1 combination (three treatments) initiated early in the winter crop cycle in 12 commercial greenhouses. *E. mundus* rapidly displaced *E. eremicus* in greenhouses where both were released, and eventually, even where only *E. eremicus* was released, indicating that a significant portion of the *E. mundus* population entered the greenhouses from outside. Nevertheless, parasitism rates were greater in greenhouses where *E. mundus* was released, especially early in the trial. Whitefly populations were lower compared to where *E. eremicus* was released alone, presumably in response to parasitism. Thus, higher incidence of parasitism and superior control of *B. tabaci* with *E. mundus* confirmed the value of early season augmentation with this parasitoid as opposed to *E. eremicus* under conditions of this test.
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Keywords: *Bemisia*; *Eretmocerus*; Biological control; Protected horticulture; Spain

1. Introduction

Sweet pepper is a major horticultural crop in Spain, with almost 1 million metric tons produced in 2000 (M.A.P.A, 2000). Almost 14% of this production came from 1,500 ha in the province of Murcia, principally greenhouses of "Campo Cartagena" on the southern Mediterranean coast. Planting typically occurs in late fall and harvest ends in late summer. The principal pest of this crop throughout the region is the western flower thrips, *Frankliniella occidentalis* (Pergande), primarily due to its role as a vector of tomato spotted wilt virus (TSWV). The sweetpotato whitefly *Bemisia tabaci* (Gennadius) Biotype "Q" (Guirao et al., 1997; Simón, 2002) is an important secondary pest that debilitates the crop through sap removal and downgrades fruit quality through the deposition of honeydew and subsequent buildup of sooty mold.

There has been a general acceptance of biologically based integrated pest management in of Campo Cartagena during recent years (Monserrat et al., 1998). Thrips have been controlled with the mite *Neoseiulus* (*Amblyseius*) *cucumeris* (Oudemans) and the pirate bug, *Orius laevigatus* (Fieber), while *Eretmocerus eremicus* Rose and Zolnerowich has supplanted *Encarsia formosa* Gahan for whitefly control, although one or two supplemental treatments with a biorational insecticide such as buprofezin were often needed.

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E. eremicus is a North American species reared commercially on greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), but equally adapted to attack *B. tabaci* (Greenberg et al., 2002). It has been evaluated extensively for control of *B. argentifolii* Bellows and Perring (= *B. tabaci* biotype "B") in poinsettia production (Hoddle et al., 1999; Van Driesch et al., 2001), but at weekly release rates well above those used commercially (www.koppert.nl) that still require supplemental applications of buprofezin (Van Driesche et al., 2000).

Studies conducted following releases of *E. eremicus* in greenhouses of Campo Cartagena showed that an indigenous species, *Eretmocerus mundus* Mercet, was recovered from *B. tabaci* on pepper in progressively greater proportions until total displacement of *E. eremicus* (van der Blom, 2002). Similar results were obtained in commercial tomato production, also on the southern Mediterranean coast of Spain (Stansly et al., 2004). One obvious factor favoring *E. mundus* in Spain is the likely movement of wasps into the greenhouse from the exterior. However, *E. mundus* may also be better adapted to *B. tabaci*, as evidenced by it high intrinsic rate of increase on this host ($r_m > 0.2$, Stansly et al., 2002) and ability to out-compete *E. eremicus* on *B. tabaci* in cage studies (López, 2002).

Both *Eretmocerus* species are biparental and endo-ectoparasitic on their whitefly hosts. However, *E. mundus* is relatively maladapted to *T. vaporiarorum* (Greenberg et al., 2002), requiring the costly installation of rearing systems based on *B. tabaci* to provide the parasitoid commercially. In the present study, we evaluated control of *B. tabaci* on pepper following releases of *E. mundus*, *E. eremicus* or a 1:1 mixture of the two species in commercial greenhouses. Ideally, we would have also included an untreated control, but this was not feasible, given the high value of the crops. This apparent shortcoming actually did not detract from the overall objective of the study: to compare the efficacy of the two species to control *B. tabaci* in pepper and thereby justify or not the investment required in bringing *E. mundus* into full-scale commercial production.

2. Materials and methods

2.1. Greenhouses

Twelve greenhouses in the Campo Cartagena were chosen for the study, three on each of two farms and two sets of three on another farm to make 4 blocks (Table 1). Each greenhouse was considered an experimental unit in a randomized complete block design with three treatments and four replicates. The three greenhouses within each replicate were all adjacent. Crops were planted in December 2002 (Table 1) and all management practices were uniform within replicates.

2.2. Parasitoid releases

Each greenhouse of each block was randomly designated for treatment with *E. mundus* (Bemipar™), *E. eremicus* (Ercal 3000™) or a 1:1 mixture of the two (Bemimix™, Koppert Biological Systems, Águilas (Murcia) Spain, all at the same release rate. Bemipar and Bemimix came in bottles of 500 viable pupae that were diluted in sawdust and dispensed in the field using cardboard "D-Boxes"™. Each D-Box was provided with $\frac{1}{8}$ of the pupae in a bottle to yield approximately 60 adult parasitoids per $6 \times 6 \times 7\,cm^3$ box. Ercal came as pupae glued in a narrow strip to a 6×4 cm paper card also calibrated to release 60 adult parasitoids each. The number of viable pupae was based on an expected emergence rate of 70% for Bemipar and Bemimix and 60% for Ercal according to the supplier. Cards or D-boxes were hung on plants and were distributed uniformly throughout the greenhouse. Actual parasitoid emergence was monitored by weekly examination of all pupae from six to eight D-boxes using a stereoscopic microscope to estimate the proportion containing parasitoid emergence holes.

Timing and rate of parasitoid release was determined as a function of whitefly populations and criteria of the grower, who reserved the right to all final treatment decisions. Releases were initiated in all the greenhouses during the 3rd or 4th week of 2002 at rates of from 0.25 to 1 parasitoid/m^2, continued weekly or biweekly and all

Table 1
Location within the Campo Cartagena, greenhouse areas (3 per location), heating capabilities, planting week, pepper cultivar planted, expected number of parasitoids released and number of releases made in 12 greenhouses. Actual number of parasitoids released was estimated at 6, 5, and 8% above the expected emergence rates for Ercal (*E. eremicus*), Bemipar (*E. mundus*) and Bemimix (1:1 mixture of two), respectively

Location	Area	Heating	Planting	Cultivar	Parasitoids/m^2 (Number of releases)		
	(m^2 per greenhouse)		(Week of 2001)		Ercal	Bemimix	Bemipar
El Romero	6000	No	46	Herminio	6.5 (17)	6.5 (17)	6 (16)
El Mirador	2000	Yes	47	Cornago	4.5 (11)	3.5 (10)	3 (9)
San Cayetano1	5000	No	48	Herminio + Pilar	3.5 (6)	5 (8)	3.5 (5)
San Cayetano2	3500	Yes	51	Ginés	3.25 (5)	3.25 (5)	3.25 (5)

terminating by week 21. In addition, the grower at El Romero had released *E. eremicus* at $0.25/m^2$ in all three greenhouses the last week of December 2001. Somewhat more parasitoids were ultimately released in greenhouses receiving *E. eremicus* ($4.44 \pm 0.80/m^2$ mean ± SE) compared to greenhouses receiving *E. mundus* ($3.94 \pm 0.70\,m^2$, Table 1). The most ($4.56 \pm 0.77/m^2$) parasitoids were released in the greenhouses receiving the mixture, largely due to a heavy whitefly infestation at the corresponding San Cayetano1 greenhouse.

2.3. Sampling

Whitefly and parasitoid populations were monitored weekly. Movement of adults was evaluated from week 3 to week 8 by counting captures on eight yellow sticky traps (Hombio BVBA, Sint-Katelijne Waver, Belgium) distributed uniformly and placed at canopy height in each greenhouse. A fresh $20 \times 12\text{-cm}^2$ surface was exposed every week. Resting adults in each greenhouse were monitored on the same day every week from weeks 3 to 27 on 3 randomly selected plants in each of 16 zones obtained by dividing the greenhouse into 16 rectangular areas of equal dimension. Whitefly adults and nymphs were counted on each plant from 3 leaves, one each from the 3rd node, 6th node, and lower canopy. Each leaf was carefully inverted and the all whiteflies counted. Data from individual sample dates and from each sample location among and within greenhouses were averaged over 2 successive weeks and multiplied by 7 to obtain a value for whitefly-days.

Nymphs containing a parasitoid pupa were noted separately. The proportion of parasitized nymphs to total nymphs was used to evaluate incidence of parasitism throughout the trial. Beginning week 16, a separate count of unparasitized 4th instar nymphs and pharate adults was included to provide an additional estimate of apparent parasitism. Parasitism was evaluated in the laboratory on an additional sample composed of a variable number of leaves containing late instar nymphs, and selected at random throughout the greenhouse beginning 5 weeks after the first releases. Whitefly nymphs were classified using a stereoscopic microscope as parasitized or unparasitized. Parasitized nymphs were recognized by displacement of mycetomes, or later, the presence of a parasitoid pupa and lack of whitefly wingbuds. Three or four leaves separated by a piece of paper towel were then placed in a paper envelope ($17 \times 22.5\,cm^2$) from which a lower corner had been cut out to receive a 1.5 mm polypropylene snap cap Eppendorf-type centrifuge tube. The inside of the tube had been smeared with a mixture of honey, glycerol (10%) and a small amount of methylcellulose to attract and hold emerging parasitoids and whiteflies. Envelopes were sealed with cellophane tape and held, vertical, tube upright, and separated in a controlled temperature cabinet ($25 \pm 2\,°C$, $75 \pm 5\%$ RH, 16:8 h L:D) for 3 weeks to allow parasitiods to emerge. All parasitoids and whiteflies found inside or outside the tube, stuck to the cellophane tape or loose within the envelope were counted and preserved in 65% EtOH + 5% glycerol. Parasitoid adults were mounted on microscope slides directly into Hoyers mounting medium and identified at 100 and 400× (Polaszek et al., 1992; Schauff et al., 1996; Rose and Zolnerowich, 1997; Zolnerowich and Rose, 1998).

2.4. Analysis

Whitefly days accumulated over the 25 week sample period were subjected to one-way analysis of variance with mean separation using LSD in the event of a significant F ($P < 0.05$). Treatment effects on proportion of 4th instar nymphs parasitized over weeks 19 to 28, 2002 (when the trial was terminated) were analyzed using a repeated measures analysis, considering each week as a subplot in a split plot design, with the replicate × treatment interaction serving as error term (Freund et al., 1986). Treatment effects on proportion parasitized from leaf samples were analyzed over all collection dates using a one-way analysis of variance. All proportions were arc sine square root transformed prior to analysis, although back transformed data (expressed as percentages) are given in tables and figures.

3. Results

3.1. Whitefly populations

Only modest numbers of whitefly adults were observed on sticky traps, rising from a low of 0.28 ± 0.16 on week 3 to 0.65 ± 0.28 on week 8. This was interpreted to mean that the ingress of whiteflies into the greenhouses was moderate. No significant season long treatment effects on sticky trap captures were observed ($F = 2.2$, df $= 1, 6$, $P = 0.19$) indicating similar pest pressure over treatments.

Numbers of whiteflies on plants were also relatively low, averaging 0.45 ± 0.02/leaf (Mean ± SE) adults and 3.02 ± 0.09 nymphs/leaf over all weeks and treatments. An exception was the greenhouse receiving the mixed species treatment at San Cayetano1 where the initial numbers of adults (0.38 ± 0.26) and nymphs (8.38 ± 4.23) were an order of magnitude above the 0.03 ± 0.01, and 1.01 ± 0.18 observed in the remaining two greenhouses in the block. However, whitefly population density in the mixed treatment greenhouse at San Cayetano1 eventually fell below the global average.

Treatment effects on whitefly-days over all weeks were significant for both nymphs and adults ($F = 22.7$ and 17.2, $P < 0.0001$, df $= 2, 564$ and 2,372 respectively,

Table 2). The highest adult whitefly-days were seen in greenhouses receiving *E. eremicus*, the least in greenhouses receiving *E. mundus*, and an intermediate value observed in greenhouses receiving the mixture (Table 2). Nymph-days were greater in greenhouses receiving *E. eremicus* alone compared to those receiving *E. mundus* alone, although the most were observed in the mixed release treatment. However, when the greenhouse with high initial infestation receiving mixed releases at San Caytano1 was dropped from the analysis, the mixed treatment again fell in between the two single species release treatments for nymph-days (Table 2).

No trends were seen in numbers of whitefly nymphs among treatments for 7 weeks, after which fewer nymphs were seen until week 22 in the four greenhouses where *E. mundus* was released alone (Fig. 1a). Most nymphs were seen from week 17 onward in the four greenhouses where *E. eremicus* was released alone, with a maximum of almost 12 nymphs per leaf on week 21, about twice the number seen in greenhouses where *E. mundus* was released alone. No trends in numbers of whitefly adults were seen until week 18, when more adults were observed in greenhouses where *E. eremicus* was released alone (Fig. 1b).

3.2. Parasitoid emergence and incidence of parasitism

Parasitoid emergence was estimated at 66, 75, and 78 ± 2% for Ercal, Bemipar, and Bemimix, respectively, or 5%, and 8% and 6% above the expected emergence rates of 70% and 70% and 60%, respectively. Thus, the actual release rates were slightly above expected rates.

No parasitized whiteflies were encountered during the course of routine sampling until week 7, or 4 weeks after the first releases. Incidence of parasitism with respect to all nymphs quickly increased to over 30% in greenhouses were *E. mundus* was released alone or in combination, but not until week 19 where *E. eremicus* was released alone (Fig. 2a). Overall incidence of parasitism with respect to 4th instar nymphs averaged 85.4 ± 9.8% in all treatments (Fig. 2b). Lowest levels were observed in greenhouses receiving only *E. eremicus*, and highest levels the mixture ($F = 7.3$, df = 2, 5, $P < 0.033$, Table 2). These results largely agreed with those obtained by microscopic examination of immature stages and emergence from leaf samples in the laboratory, except that differences between the *E. mundus* and mixture treatments were not significant (Table 3). The basic agreement among these different measures of percentage parasitism indicated that all reflected relative incidence of parasitism.

3.3. Relative abundance of parasitoid species

E. mundus constituted 91.1 ± 2.9% of parasitoids emerging from weekly leaf samples in greenhouses receiving the 1:1 mixture of *E. mundus* and *E. eremicus*

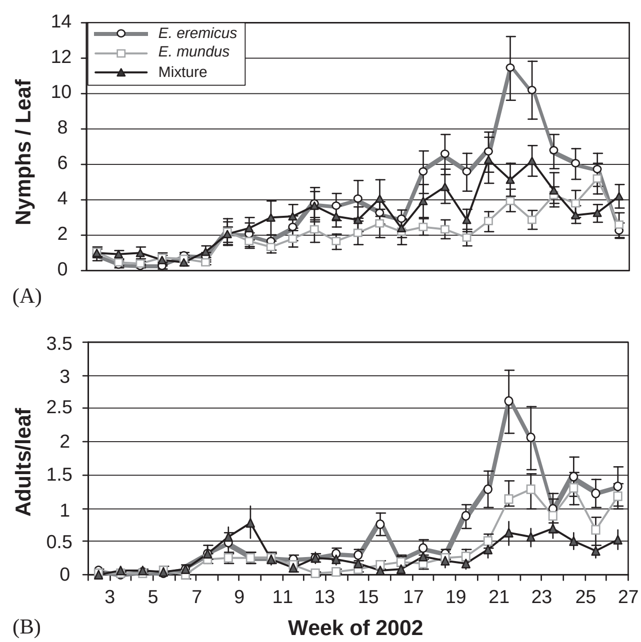

Fig. 1. Mean (SE) number of whitefly (A) nymphs, or (B) adults per pepper leaf in 11 greenhouses receiving augmentative releases of *E. mundus*, *E. eremicus* or a 1:1 mixture.

Table 2
Number (Mean ± SE) of whitefly × days for adults and nymphs per pepper leaf over 25 weeks and incidence of parasitized pupae (%) over 12 weeks in 11 greenhouses, Campo de Cartagena Spain, Spring 2002

Treatment	Whiteflies days per leaf			Incidence parasitism (%)
	Adults (Mean ± SE)	Nymphs(Mean ± SE)		(Mean ± SE)
		All greenhouses	Outlier removed[2]	
E. mundus	20.6 ± 1.5 c[1]	100.7 ± 9.6 c	100.7 ± 9.6 c	83.2 ± 2.2 b
Mixture	28.1 ± 2.6 b	245.1 ± 28.2 a	144.7 ± 13.6 b	90.1 ± 1.7 a
E. eremicus	36.2 ± 2.2 a[2]	183.2 ± 15.6 b	183.2 ± 15.6 a	75.3 ± 1.9 c

[1]Means in the same column followed by the same letter are not significantly different (LSD $P < 0.05$).
[2]Outlier was mixed release treatment that had high initial counts of whitefly nymphs, presumably due to transplantation of infested seedlings.

($N = 79$). All the rest were *E. eremicus* except for 0.2% *Encarsia lutea* (Masi) and one *Encarsia formosa* (Gahan) individual. The greatest proportion of *E. eremicus* emerging from greenhouses receiving the mixture (60.3%) was seen in week 14, after which few *E. eremicus* were seen (Fig. 3a). Even where only *E. eremicus* was released, *E. mundus* constituted 56.7 ± 8.8% of emerging parasitoids, and predominated on week 14 and from week 18 onward (Fig. 3b). A few (2.1%) *E. eremicus* originating from releases made in six greenhouses before the experiment was initiated emerged from samples collected in greenhouses where only *E. mundus* was released during the experiment, (Fig. 3c).

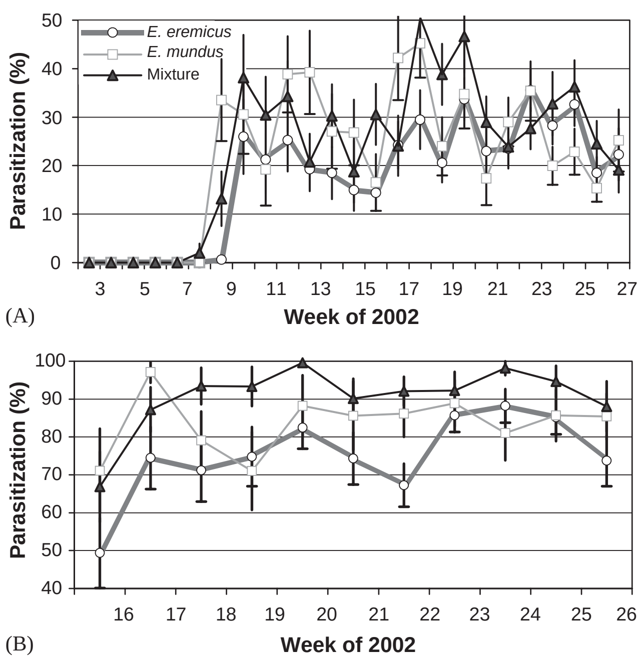

Fig. 2. In field determination of percentage parasitized 4th instar nymphs by release species treatment with respect to (A) number of all whitefly nymphs, and (B) number of whitefly 4th instar + pharate adults.

4. Discussion

No economic threshold has been determined for *B. tabaci* in pepper, but no direct damage from whitefly was observed during the course of this trial, nor were supplemental insecticide treatments for whitefly deemed necessary by either consultants or growers. In the past,

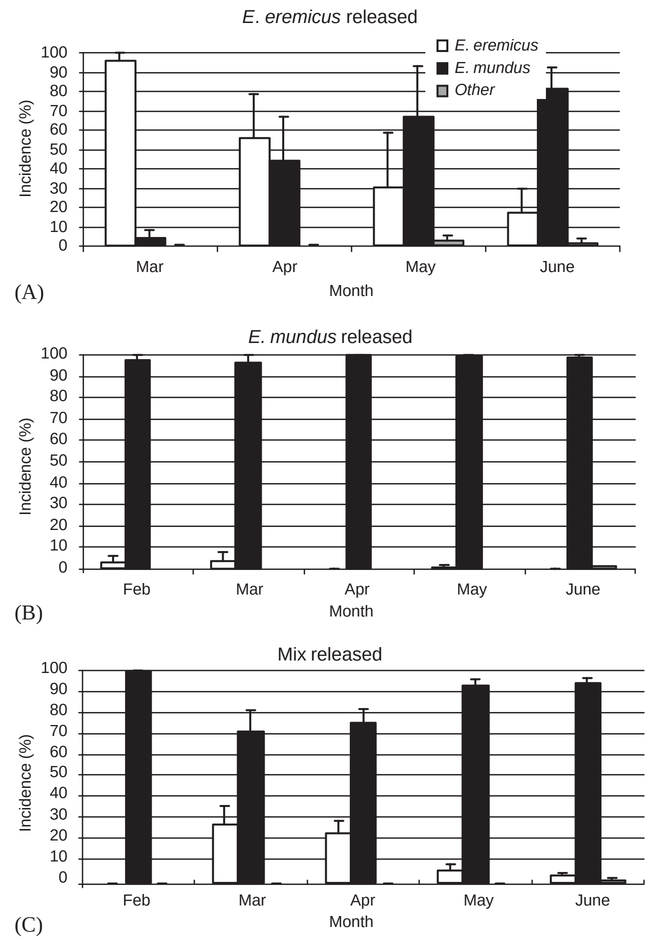

Fig. 3. Mean (SE) relative incidence of parasitoid species emerging from pepper leaf samples taken in greenhouses, grouped by released species: (A) *E. eremicus*, (B) *E. mundus*, and (C) mixture.

Table 3
Overall percentage (mean ± SE) parasitism on leaf samples collected weekly, as estimated by 5 indices: (1) parasitized nymphs prior to parasitoid pupation compared to young nymphs (eyes not yet red, $n = 89$ samples), (2) parasitoid pupae or pharate adults compared to red-eyed nymphs and whitefly pharate adults ($n = 93$), (3) all parasitized whiteflies compared to all whitefly nymphs and pharate adults ($n = 93$), (4) exuviae from which a parasitoid has emerged compared to pupae from which a whitefly has emerged ($n = 83$), and (5) emerged whitefly adults compared to emerged parasitoid adults ($n = 89$)

Treatment	Parasitoid larvae by young whitefly nymphs	Parasitoid pupae by red-eyed nymphs + parate adults	All parasitized whiteflies by all unparasitized whitefies	Whitefly parasitoid exuviae by whitefly exuviae	Parasitoid adults by whitefly adults
E. eremicus	40.0 ± 5.0 b[1]	84.5 ± 3.2 b	64.0 ± 3.9 b	34.6 ± 5.9 b	85.9 ± 4.2 b
E. mundus	56.2 ± 4.6 a	93.6 ± 1.8 a	78.7 ± 2.9 a	52.4 ± 6.5 a	96.3 ± 1.2 a
Mix	56.1 ± 3.8 a	93.0 ± 1.4 a	73.8 ± 2.9 a	37.2 ± 5.3 ab	95.6 ± 1.0 a

[1]Means in the same column followed by the same letter are not significantly different (LSD $P < 0.05$).

when *E. eremicus* was the best parasitoid commercially available for control of *B. tabaci*, it was often necessary to include chemical treatments with biorational products such as insecticidal soaps, oils, or insect growth regulators (Monserrat et al., 1998). Such treatments are no longer typical using the present management plan based on releases *E. mundus* initiated at the beginning of the crop (Urbaneja et al., 2003; Lara and Urbaneja, 2002; Urbaneja et al., 2002). *E. mundus* has also performed well in other Spanish greenhouse crops including tomato and green beans (Stansly et al., 2004; Téllez et al., 2003).

An untreated control would have been useful to gauge the effect of augmentation in the absence of any other control measures, but this is usually impossible in a commercial trial of such magnitude. Nevertheless, high rates of parasitism observed together with the absence of any other control measures suggested that biological control was an important factor in maintaining whiteflies to levels considered tolerable by local growers and practitioners. Furthermore, higher incidence of parasitization and significantly fewer whiteflies observed where *E. mundus* was released indicated that these releases improved control compared to releases of *E. eremicus*. Had an untreated control been included, the differences could only have been greater.

Higher rates of parasitism were observed where *E. mundus* was released compared to *E. eremicus* alone, especially early in the season (see Fig. 2). The observed trend agreed with results from a laboratory study in which the two parasitoids were released in caged pepper and tomato alone and in a 1:1 combination (Urbaneja and Stansly, 2004). The latter treatment from this study resulted in a 9:1 ratio in favor of *E. mundus* after only one generation, demonstrating that competitive exclusion could have contributed to the displacement of *E. eremicus* by *E. mundus* observed in the field. The apparent competitive advantage of *E. mundus* over *E. eremicus* may be due to differences in intrinsic rates of increase between the two congeners using *B. tabaci* as a host. R_m for *E. mundus* was found to exceed 0.2 at 25 °C on *B. tabaci* biotype Q in both tomato and pepper (Stansly et al., 2002) compared to 0.055 on sweet potato and 0.096 on cotton for *E. eremicus* on *B. tabaci* biotype "B" at 28 °C, (Headrick et al., 1999).

Whitefly numbers were lower in greenhouses receiving *E. mundus* compared to those receiving *E. eremicus* or even the mixture of the two, although we did not observe a higher incidence of parasitism in the *E. mundus* treatment compared to the mixed treatment. A likely explanation is that the critical differences in parasitism between these two treatments occurred so early in the season that they could not be evaluated successfully due to low whitefly populations. This emphasizes the importance of releasing natural enemies early, possibly even before the target pest is detected.

Release rates were low in this trial compared to published studies conducted on poinsettia (Hoddle et al., 1999; Van Driesch et al., 2001). Higher release rates might have been necessary had there been less immigration of parasitoids or more of whiteflies, or during a warmer time of year when whitefly populations could increase more rapidly. Nevertheless, the measurable improvement in control obtained through releases of *E. mundus* compared to releases of *E. eremicus* were indicative of the suppression obtainable when modest augmentations of this parasitoid are initiated early in the crop cycle.

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Further reading

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