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Prospects for biological control of *Bemisia tabaci* (Homoptera, Aleyrodidae) in greenhouse tomatoes of southern Spain

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Abstract

The whitefly *Bemisia tabaci* Gennadius is the key pest of protected tomato production in Spain. The predominant form is biotype "Q", an efficient vector of tomato yellow leafcurl virus (TYLCV), which is the principal cause of damage. Although management has relied primarily on chemical control, factors such as overlapping crop cycles, insecticide resistance and public pressure have spurred development of alternative management tactics. These include TYLCV-tolerant varieties and pest exclusion methods that, along with more selective insecticides, have created a more compatible environment for biological control. Here we describe trials of an integrated pest management (IPM) system conducted during the fall season in 12 commercial greenhouses throughout the production area compared with 7 greenhouses utilizing only chemical control (termed "conventional"). Each IPM greenhouse was divided into 4 equal sections, two receiving weekly releases of the indigenous *Eretmocerus mundus* Mercet and two receiving the exotic *Eretmocerus eremicus* Rose & Zolnerowich. Fewer and more selective pesticides were used in IPM greenhouses compared to conventional greenhouses. Early use of broad-spectrum insecticides in IPM greenhouses appeared to be counterproductive in that establishment of parasitioids was delayed with no real gain in control. Incidence of parasitized whiteflies in IPM greenhouses averaged around 50%, with *E. mundus* predominating, compared to less than 3% in conventional greenhouses originating from immigrating *E. mundus*. Whiteflies were on average more numerous on plants in IPM greenhouses although there were exceptions. Also, whitefly populations in IPM greenhouses tended to decrease as the crop matured, in contrast to conventional greenhouses. Biological control was most successful where TYLCV-resistant cultivars and exclusion strategies (insect netting) reduced whitefly populations and the risk of virus disease. Continued acceptance of these tactics, and increasing public demand should create a favorable climate for increased implementation of biologically based pest management in protected tomato culture.

Keywords: *Bemisia tabaci*; *Eretmocerus mundus*; *Eretmocerus eremicus*; Biological control; Tomato; Spain

1. Introduction

Spain is a major tomato producer, harvesting 3.7 million tons from 147,000 acres in 1998. Almost 40% of this production consisted of fresh market tomatoes grown in greenhouses on the southern Mediterranean coast in the communities of Andalucía and Murcia. Approximately 58% of these tomatoes were exported, primarily to northern Europe.

Transplanting in greenhouses begins in late summer, with a possible additional planting in late winter. Harvesting begins in October, peaks in March, but continues through early summer. The best prices usually occur in winter when there is little competition from greenhouses in northern Europe or elsewhere. Spanish production methods are steadily improving, principally

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through upgrading of greenhouses from traditional low roofed structures (little more than grape arbors covered with polyethylene film) to large, high-roofed, multiple units often provided with automatically controlled heating and ventilation, and with vents and doors often fitted with pest-excluding screen. Nevertheless, they generally lag behind their counterparts in Northern Europe who generally grow in fully equipped glass greenhouses and use biological control through most if not all of the season. Therefore, the annual shift south for sources of tomatoes and other vegetables may result in products of lesser quality, subjected to greater pesticide use.

In response to customer demand, buyers are in turn pressuring growers to reduce pesticide use and provide some produce using Integrated pest management (IPM) methods that include only natural enemies and selective chemicals. Fortunately, most tomato growers in the region are already conditioned to use selective insecticides because of the almost universal practice of bumblebee pollination. Nevertheless, biological control has advanced more quickly against the western flower thrips *Frankliniela occidentalis* (Pergande) in greenhouse pepper. This was especially true in the northern part of the region around Cartagena, where pepper is planted in late fall or early winter. This system allows sufficient time early in the crop cycle for establishment of the mite *Amblyseis cucumeris* (Oudemans) (Acari: Phytoseiidae) and the minute pirate bug *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Van der Blom et al., 1997). Biological control in tomato presents a number of additional challenges.

As in most of the hot regions of the world where tomatoes are intensively cultivated, the whitefly *Bemisia tabaci* Gennadius is a key pest, due primarily to its role as a virus vector. In southern Spain this includes two types of tomato yellow leaf curl virus (TYLCV), Israeli and Sardinian (begonmoviridae = geminivirus) , as well as tomato chlorosis virus (ToC), a crinivirus (Closteroviridae), (Navas-Castillo et al., 2000). Biotype ''B'' = *B. argentifolii* has been detected in southern Spain but has been completely or largely displaced by the native biotype ''Q'' (Guirao et al., 1997; Simón, 2002). One consequence of this predominance may be rapid spread of TYLCV, given that biotype ''Q'' has been shown to be the more efficient transmitter of the virus (Sánchez-Campos et al., 1999). Cultural practices in Spain also lend themselves to whitefly and virus problems. Overlapping crop cycles assure high pest and inoculum levels and heavy pesticide use against captive pest populations. Resistance against imidacloprid and other neonicotinoids has already been documented (Cahill et al., 1996; Elbert and Nauen, 2000).

Biological control is directed at immature stages and cannot control the spread of TYLCV by the adult. Virus spread must first be controlled through a combination of tactics such as TYLCV-resistant cultivars, whitefly excluding structures, late planting to reduce migration from the previous season's crop, and selective insecticides. Integration of biological control with these methods would decrease selection pressure against insecticides, reduce environmental and health risks associated with heavy insecticide use, and increase consumer acceptance and market value of the product.

Commercially available options for biological control of *B. tabaci* include *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae). This North American species is mass reared on the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) but is equally adapted to both whitefly species as host (Greenberg et al., 2002). However, *E. eremicus* released to control *B. tabaci* on greenhouse tomato and pepper is typically displaced by an indigenous cogenitor, *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae), immigrating from outside (Van der Blom, 2002). *E. mundus* is maladapted to *T. vaporiarorum* (Greeenberg et al., 2002) necessitating rearing systems based on *B. tabaci*. The objective of the present study was to test the feasibility of biologically based IPM in greenhouse tomato using *E. mundus* for control of *B. tabaci* under a variety of commercial conditions in southern Spain.

2. Materials and methods

2.1. Greenhouses

A total of 19 greenhouses were selected for the study in four provinces of the southern Mediterranean coast of Spain: Murcia (Águilas, Mazarrón), Almería (La Cañada, El Ejido) Granada (Motril), and the Canary Islands (Tenerife, Las Palmas, Fig. 1, Table 1). All greenhouses were covered with polyethylene film except

Fig. 1. Map of Spain showing location of study sites.

Table 1
Identification number, location and management system of greenhouse, its size and type, tolerance or susceptibility of tomato variety to TYLCV of tomato variety, planting date, duration of study, release rates (no./m^2 or kg/ha) of natural enemies and number of releases (applications) made

Greenhouse IPM (I), conventional (C)	Size (m^2)	Type[a]	Cultivar	Planting date	Duration (weeks)	*E. eremicus* no./m^2 (releases)	*E. mundus* no./m^2 (releases)	*M. caliginosus* no./m^2 (releases)	D. isaea no./m^2 (releases)	*P. persimilis* no./m^2 (releases)
1 Ágilas1(I)	3600	L–P–M +	Tolerant	15-Aug	17	16.7 (9)	11.7 (7)	0.61 (1)	1 (6)	19.4 (11)
2 Águilas1(C)	9000	L–P–M + +	Tolerant	15-Aug	17	None	None	None	None	None
3 Ágilas2 (I)	2700	L–P–S +	Tolerant	28-Jul	16	3 (9)	9 (3)	None	None	None
4 Canarias1 (I)	20,000	6 × 6+	Tolerant	9-Sep	20	10.5 (5)	10.5 (5)	None	None	None
5 Canarias2(C)	3000	12 × 14+ +	Susceptible	22-Sep	13	None	None	None	None	None
6 Canarias3 (C)	4000	6 × 10+	Susceptible	18-Sep	21	None	None	None	None	None
7 El Ejido (I)	8000	L–P–M + +	Susceptible	20-Aug	22	18.75 (8)	18.8 (8)	0.1 (1)	None	None
8 El Ejido (C)	8000	L–P–M + +	Susceptible	20-Aug	17	None	None	None	None	None
9 La Cañada1(I)	25,000	H–A–M + + +	Susceptible	15-Aug	28	10.2 (4)	10.2 (4)	None	None	None
10 La Cañada1(C)	22,000	H–A–M + + +	Susceptible	30-Aug	26	None	None	0.5 (3)	1 (5)	None
11 La Cañada2 (I)	20,000	H–A–M + + +	Susceptible	23-Aug	22	19.5 (8)	19.5 (8)	1 (2)	0.675 (5)	None
12 La Cañada2 (C)	20,000	H–A–M + + +	Susceptible	30-Aug	22	None	None	None	None	None
13 Mazarrón1 (I)	1000	H–A–S + + +	Tolerant	26-Sep	23	10 (4)	10 (4)	0.5 (1)	0.75 (3)	None
14 Mazarrón2 (I)	2500	L–P–S +	Tolerant	15-Sep	21	33 (5)	33(5)	None	0.3 (3)	3.75 (3)
15 Motríl1 (I)	7000	H–A–M + + +	Susceptible	14-Aug	23	6 (4)	6 (4)	None	None	None
16 Motríl2 (I)	10,000	H–A–M + + +	Susceptible	14-Aug	29	6 (4)	6 (4)	None	None	None
17 Motríl2 (C)	10,000	H–A–M + + +	Susceptible	16-Aug	29	None	None	None	None	None
18 Motríl3a (I)	2500	L–P–S +	Susceptible	7-Aug	21	8 (5)	8 (5)999	None	None	None
19 Motríl3b (I)	3000	L–P–S +	Susceptible	8-Aug	20	15 (5)	15 (5)	None	None	None

[a] Greenhouse types: height (L = low, H = high), pitch (F = flat, P = pitched, A = Arched), units (S = single, M = multiple), exclusion (poor +, fair + +, good + + +).

those in the Canary Islands, which were covered with polyethylene mesh screen. Size ranged from 0.1 to 2.5 ha and design was classified as (1) high or low roofed, (2) flat ("parral"), pitched capilla or arch-roofed (túnel), and (3) either single or multiple unit. Effectiveness of pest exclusion was rated as poor, good or excellent based on installation of screened vents and double doors and sticky card captures.

2.2. Pest management

Natural enemies were released to control whiteflies and other pests in 12 of the greenhouses designated "IPM". The 7 remaining greenhouses relied totally on insecticidal control and were termed "conventional". In five cases, an IPM and conventional greenhouse shared the same location, grower/operator, and growing conditions for paired comparison. Each greenhouse was divided into 4 equal-sized sectors for sampling purposes. In addition, sectors in IPM greenhouses were used for two replicates each of two treatments in a Latin square design. *E. eremicus* (Ercal, Koppert Biological Systems, Berkel en Rodenrijs Holland) (reared on *T. vaporariorum*) was released in 2 of the sectors and *E. mundus* (Bemipar, Koppert Biological Systems S.A., Águilas (Murcia), Spain) (reared on *B. tabaci*) was released in the remaining two. Release rates and timing (based on weekly counts of whiteflies) were made at the discretion of the Koppert consultant responsible for the greenhouse, subject to the grower's approval.

Additional natural enemy species that may have been released included *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) for whiteflies, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) for leafminers, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) for spider mites. Cooperating growers purchased these biological control agents from Koppert Biological Systems, Águilas (Murcia) Spain. Decisions on pesticide applications in conventional greenhouses were made by the grower and in the IPM greenhouses jointly by the grower and Koppert consultant.

2.3. Pesticide impact

Pesticide applications were noted and their likely impact on natural enemies assessed according to the Koppert Side Effects Guide (Anonymous, 2002). In this guide, effects of pesticides are rated for pupae or nymphs and adults of each natural enemy as: (1) harmless (reduction in control capacity $< 25\%$), (2) slightly harmful (25–50% reduction in control capacity), (3) moderately harmful (50–75% reduction in control capacity), or (4) very harmful ($>75\%$ reduction in control capacity). A third rating for persistence in weeks is given as a single number or as a range. The impact of each application in the greenhouse was measured as the sum of the ratings (1–4) for pupae and adults of *E. eremicus* or the closest other species given (usually *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), and the mean number of weeks of residual effect. The sum of these 3 numbers varied between 2 and 18 (Table 2). For example, the side effects of abamectin on *E. eremicus* were rated as 1 for pupae, 4 for adults and 3 for weeks of persistence, giving a total rating of 8 for each application. For the fungicide dinocap, values for *E. eremicus* are incomplete but are given for *E. formosa* as 1, 4 and 1 (effects on pupae, adults and persistence respectively), giving an overall rating of 6. These impact ratings were summed for each greenhouse and then divided by the number of weeks of monitoring to give an index of incompatibility (II) during the period of study.

2.4. Monitoring

Each of the 4 sectors of each greenhouse was monitored weekly for pests and diseases by the assigned consultant. Eight yellow and eight blue sticky traps (Hombio BVBA, Sint-Katelijne Waver, Belgium) were placed at canopy height in each sector, exposing a single fresh 20 × 12 cm surface every week. Whiteflies (yellow card) and thrips (blue card) were counted up to 25, or classified as medium (26–100) or high (100+). Adult whiteflies and nymphs on an upper, middle and lower leaf of 8 plants in each sector were counted until 10 per leaf or classified as medium (11–25) or high (25+). Larvae of Lepidoptera and several species of beneficial insects including adults of Aphelinidae (*Encarsia* spp., *Eretmocerus* spp.) and predaceous Miridae (*M. caliginosus*, *Nesidiocoris* [*Cyrtopeltis*] *tenuis* [Reuter]) were also counted on these leaves. Infestations of spider mites (*Tetranychus urticae* Koch), leafminers (*Liriomyza bryoniae* [Kaltenbach] and *L. trifolii* [Burgess]), and thrips (*F. occidentalis*) were estimated as light, medium or heavy according to the consultant's criteria. The presence of foliar or vascular diseases was noted. Production data, when available, were supplied by the grower.

The incidence of parasitism was estimated from samples taken between mid-October and February. Leaves with late 4th instar whitefly nymphs (wingbuds visible, heretofore referred to as "pupae") were collected at random by sector, placed in plastic bags, and transported to the laboratory in an insulated cooler. Whitefly pupae and exuviae were classified to species and as parasitized (presence of parasitoid pupa) or not parasitized (presence of whitefly wingbuds) using a stereoscopic microscope. Leaves were then placed in a paper envelope (17 × 22.5 cm) 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

Table 2
Pesticides used in greenhouses under study and side effects ratings of: (1) harmless, (2) slightly harmful, (3) moderately harmful, or (4) very harmful (Anonymous, 2002) summed for pupae and adults *E. eremicus* or the closest other species plus mean number of weeks of residual effect

Broad-spectrum insecticide	Side effect rating	Selective insecticide (w) targeted whitefly	Rating	Fungicide	Side effect rating
Asephate	15	Abamectine	8	Benomyl	2
Bifentrin	18	Amitraz	8	Bromopropylate	2
Chlorpyrifos	17	Azadiractin (w)	4	Captan	2
Cypermethrin	18	*B. thuringiensis*	2	Carbendazim	3
Deltametrin	18	Buprofezin (w)	3	Clorothalonil	2
Endosulfan	15	Cyromazine.	2	Copper	2
Fenitrotion	14	Fenbutatin Oxide	2	Cymanazil	5
Fenpropatrin	18	Flufenoxuron	8	Cymoxanil	3
Imidacloprid (foliar)	10	Heptenofos	8	Cyproconazol	3
Malathion	18	Hexithiozox	3	Cyprodinil	2
Methomil	16	Imidacloprid (soil) (w)	5	Dimethomorph	3
Oxamil (foliar)	18	Lufenuron	4	Dinocap	6
Tau Fluvalinate	14	Oxamil (soil) (w)	5	Fludioxonil	2
Tralometrine	16	HMO[a](w)	4	Folpet	2
		Potassium Soap (w)	4	Iprodion	2
		Pymetrozine (w)	3	Mancozeb	2
		Pyridaben (w)	8	Metalaxil	3
		Pyriproxyfen (w)	5	Metiram	6
		Tebufenpyrad	3	Myclobutanil	2
		Teflubenzuron	2	Nuarimol	3
				Nuarimol	3
				Procimidon	2
				Procloraz	2
				Propamocarb	2
				Pyrimethanil	2
				Sulfur	7
				Thiofanate methyl	2
				Tiram.	4
				Triadimenol	2
				Vinclozolin	2
				Zineb	2

[a] Horticultural mineral oil.

honey, glycerol (10%) and a small amount of methylcellulose to attract and hold emerging parasitoids and whiteflies. The envelope was sealed with cellophane tape and held, tube upright, in a controlled temperature cabinet (25±2°C, 75±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 lose within the envelope were counted and preserved in 65% EtOH and 5% glycerol. Whitefly parasitoids ($n = 750$ total) 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).

2.5. *Analysis*

Mean incidence of each parameter was compared between IPM and conventional greenhouses over all sample weeks using a one-way analysis of variance with the greenhouse × week interaction as the error term (SAS Institute, 2000). One-way analysis of variance was also used to evaluate whitefly and incidence of parasitism within IPM greenhouses between sectors receiving *E. mundus* or *E. eremicus*. Consistency of sex ratio with the 1:1 null hypothesis was tested using chi-square (Sokal and Rohlf, 1981). Correlation analysis was used to look for relationships between pesticide use and incidence of pests. Data are reported as mean ± standard error throughout.

3. Results

3.1. *Pesticide use*

Whitefly and TYLCV inoculum pressure was greatest in the early season due to migration from senescing summer crops and rapid insect reproduction caused by high temperatures. Most growers either planted TYLCV-resistant cultivars or used broad-spectrum insecticides early in the season (September–October).

Only one did both. IPM growers were more likely to use resistant cultivars, tended to spray less often and used products that were more selective when they did spray (Table 3).

Frequency of pesticide applications varied from 5 in 23 weeks to 27 in 41 weeks (Table 3), or a mean (±SE) of 0.69±0.11 applications per week in conventional greenhouses and 0.49±0.64 per week in IPM greenhouses. Total number of products employed in these applications ranged from 9 to 74 (40.0±9.8 and 20.4±2.5 per greenhouse, conventional and IPM respectively). Broad-spectrum insecticides were applied in 5 or 42% of the IPM greenhouses and 5 or 71% of conventional greenhouses, mostly for whitefly control early in the cropping season. All but one of these 10 greenhouses was planted to TYLCV-susceptible cultivars. The most frequently used pesticide in this category was methomyl, followed by endosulfan and tralomethrin (Table 4). The remaining 9 greenhouses where no broad-spectrum insecticides were used either contained tolerant varieties or were tightly screened against whiteflies. The one exception to this pattern was a conventionally managed greenhouse (Águilas_1) where mixtures of 3 or 4 products were sprayed once or twice weekly even though a resistant cultivar was planted.

Nine of the 20 selective insecticides used also targeted whiteflies early season (14.6±5.6 conventional; 8.6±1.4, IPM). Pymetrozine was the most frequently used of these, followed by buprofezin, horticultural mineral oil, oxymyl applied through drip irrigation and pyriproxyfen (Table 4). Other selective pesticides targeted spider mites, Noctuidae, and agromyzid leafminers. Fungicides (16.9±3.3, 9.6±1.4 IPM, conventional) were applied mostly late season (November–January) when cool, humid conditions in the greenhouses were especially conducive to fungal disease.

The index of incompatibility (II) in IPM greeenhouses varied from 1.1 to 8.7 for a mean of 4.5±0.7 and in conventionally managed greenhouses from 1.1 to 35.7 for a mean of 11.2±4.4 ($F=3.8$, df=1,17, $P<0.067$). Differences in II between IPM and conventional greenhouses among the 5 paired comparisons ranged from 30 (Águilas_1) to 0 (Cañada_2).

3.2. Whitefly species and incidence of parasitism

Bemisia tabaci was the most frequent whitefly observed and the only one seen in the most southerly mainland locations of Motríl and El Ejido. *Trialeurodes vaporarirum* was most often observed during the cooler months in Águilas, and to a lesser extent in the Canary Islands, Mazarrón and rarely in La Cañada. Only 29 of 1885 pupae observed in leaf samples were *T. vaporiariorum*, reflecting in part the fact that almost 2/3 of the samples came from Motríl.

Incidence of parasitized pupae from leaf samples taken in IPM greenhouses where *Eretmocerus* spp. were released was estimated at 50.7±3.7% ($n=108$), compared to 2.2±0.97% ($n=16$) in conventionally managed greenhouses. The corresponding numbers for emerged adult parasitoids were 41.6±3.5% ($n=113$) and 0.73±0.46% ($n=20$) respectively. In IPM greenhouses, no differences were observed by either measure between sectors where *E. mundus* or *E. eremicus* was released ($F=1.39$, $P=0.27$, df=1,10 and $F=0.22$, $P=0.65$, df=1.11 respectively). Of 570 *Eretmocerus* spp. adults that emerged from IPM greenhouse leaf samples, 85.0±3.7% ($n=113$) were *E. mundus*. Overall, the percentage of *E. mundus* rose from 47.5±20.6% ($n=4$) during the last 2 weeks of October to 100% the last 2 weeks of January. However there were no differences in incidence of parasitism over all dates between sectors regardless of which *Eretmocerus* species was released ($F=0.001$, $P<0.98$, df=1,10).

Sex ratio of *E. mundus* favored females by 1.39:1 which deviated significantly from 1:1 ($X^2=7.67$, $P<0.01$, df=1). Sex ratio of the 52 *E. eremicus* that emerged from leaf samples also was skewed 1.48:1 towards females ($X^2=4.81$, $P<0.05$, df=1). We found 122 *Encarsia sophia* (Girault & Dodd) = *Encarsia transvena* (Timberlake), mostly from one location in Motril late in the season. The sex ratio of this heteronomous hyperparasitoid was strongly male biased (0.37:1, $X^2=12.9$, $P<0.01$, df=1) indicating that many *Eretmocerus* spp. were being parasitized. No other aphelinids were observed from samples taken on the mainland, although one small sample from Tenerife contained 2 female *Encarsia lutea* (Masi).

3.3. Pest and natural enemy incidence: IPM vs conventional

Numbers of adults captured on sticky traps were not different between IPM and conventional greenhouses. Thus, movement of whiteflies (and presumable ingress into greenhouses) was not affected overall by the pest management system employed ($F=0.09$, df=1,23, $P<0.77$, Table 5). Nevertheless, almost twice as many whitefly adults and 3 times as many nymphs were observed overall on plants in IPM greenhouses compared to conventional greenhouses ($F=36.1$ and 35.1 respectively, df=1,23, $P<0.001$). Although scouts had difficulty discerning parasitized whitefly nymphs or seeing adult whitefly parasitoids, they did observe significantly more in IPM greenhouses compared to conventional greenhouses ($F=26.7$ and 21.8, respectively, df=1,23, $P<0.0001$). Numbers of the whitefly predator *Nesidiocoris tenuis* were also greater in IPM greenhouses ($F=8.04$, df=1,23, $P<0.009$). No significant differences were observed in numbers of other pests

Table 3
Duration of study, number of pesticide applications and products included, and the number of those classified as broad-spectrum insecticides (score of 9 or more), selective insecticides/acaracides or fungicides, sum of side effect ratings for all pesticides used, and index of incompatibility of pesticide regime with *Eretmocerus* spp. or similar parastitoids

Greenhouse IPM (I), conventional (C)	Cultivar tolerance to TYLCV	Production (kg/m^2)	Duration (w)	Applications (no.)	Products (no.)	Broad spectrum	Selective	fungicides	Sum of side effect ratings[a]	Index[b] of incompatibility
Águilas_1(I)	Tolerant	8.7	17	12	28	0	17	11	77	4.5
Águilas_1(C)	Tolerant	8.9	19	22	71	41	7	23	679	35.7
Ágilas_2 (I)	Tolerant		16	6	14	0	7	7	48	3.00
Cañada_1(I)	Susceptible		28	9	25	4	3	18	116	4.14
Cañada_1(C)	Susceptible		26	10	26	5	2	19	134	5.2
Cañada_2 (I)	Susceptible		22	7	9	0	2	7	25	1.14
Cañada_2 (C)	Susceptible		22	7	9	0	2	7	25	1.14
Canarias_1 (I)	Tolerant		20	6	20	0	5	15	64	4.00
Canarias_2(C)	Susceptible		33	13	38	2	15	21	160	4.8
Canarias_3 (C)	Susceptible		23	25	74	2	45	27	287	13.7
El Ejido (I)	Susceptible	4.0	17	7	17	0	11	6	68	3.09
El Ejido (C)	Susceptible	3.9	17	7	14	0	11	3	95	5.6
Mazarrón_2 (I)	Tolerant		21	10	16	0	10	6	56	2.67
Mazarrón_1 (I)	Tolerant		23	5	10	0	2	8	26	1.13
Motríl_1 (I)	Susceptible	14.9	23	19	24	5	11	8	155	6.7
Motríl_2 (I)	Susceptible	12.9	29	27	41	7	16	18	253	8.7
Motríl_2 (C)	Susceptible	10.3	29	32	48	10	20	18	377	13.0
Motríl_3a (I)	Susceptible	4.6	11	21	21	6	9	6	151	7.2
Motríl_3b (I)	Susceptible	5.4	10	20	20	5	10	5	149	7.5

[a] Sum of ratings from Table 2 for all pesticides used.
[b] Score/weeks.

Table 4
Number of applications of broad-spectrum and selective insecticides directed at whitefly per greenhouse in 19 greenhouses

Broad spectrum	Applications mean±SE	(max)	Selective	Applications mean±SE	(max)
Methomil	1.37±0.89	(17)	Pymetrozine	1.42±0.68	(10)
Endosulfan	0.79±0.47	(8)	Buprofezin	0.79±0.36	(5)
Tralometrine	0.63±0.29	(3)	HMO[a]	0.74±0.37	(5)
Tau Fluvalinate	0.37±0.26	(4)	Oxamyl (injected)	0.74±0.32	(5)
Malathion	0.26±0.26	(5)	Soap	0.74±0.26	(4)
Methamidophos	0.21±0.14	(2)	Pyriproxyfen	0.63±0.29	(5)
Fenitrotion	0.16±0.16	(3)	Pyridaben	0.58±0.19	(3)
Fenpropatrin	0.16±0.16	(3)	Azadiractin	0.37±0.22	(3)
Asephate	0.11±0.07	(1)	Imidacloprid	0.11±0.07	(1)
Bifentrin	0.11±0.07	(1)			
Deltametrin	0.11±0.07	(1)			
Cypermethrin	0.05±0.05	(1)			
Heptenofos	0.05±0.05	(1)			

[a] Horticultural mineral oil.

Table 5
Mean incidence of whiteflies, parasitoids, *N. tenuis*, leafmines spider mites and larvae of Lepidoptera observed by scouts in IPM and conventional greenhouses

	Conventional	IPM
Adults whiteflies (no./trap/wk)	30.2±1.52a[a]	28.4±1.01a
Adult whiteflies (no./leaf)	0.29±0.011b	0.54±0.014a
Whitefly nymphs (no./leaf)	0.39±0.02b	0.99±0.03a
Parasitized "pupae" (%)	0.90±0.08b	3.7±0.13a
Eretmocerus sp. (adults/leaf)	0.0003±0.0002b	0.0083±0.0008a
N. tenuis (no./leaf)	0.004±0.001b	0.053±0.003a

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

monitored, including thrips, leaf miners, spider mites and caterpillars (data not shown).

Comparisons between individual IPM-conventional greenhouse pairs illustrated some effects not evident in the overall comparison. Similar pesticide regimes were used in IPM and conventional greenhouses in 4 of the 5 locations (Table 3) including La Cañada_2, where low counts on yellow sticky traps reflected tight construction and screening (Table 6). Numbers of whiteflies were also low on plants at this location, although more adults were observed in the IPM greenhouse. The apparent failure of parasitoids to reduce the whitefly population in the IPM greenhouse may have been due to relatively low incidence of both whiteflies and parasitism 28.8±10.3% ($N=9$).

At nearby La Cañada_1 the grower used broad-spectrum insecticides early in the crop cycle to control whiteflies and virus on his TYLCV-susceptible tomatoes (Table 3). Almost 5 times fewer whiteflies were seen on traps in the conventional greenhouse compared to the IPM greenhouse where the common entrance to both greenhouses was located (Table 6). As a likely result, more whiteflies were also seen on plants in the IPM greenhouse. However, parasitism rose to over 40% in the IPM greenhouse and whitefly populations tended to decrease toward the end of the crop cycle, finishing the season at 0.60±0.087 nymphs per leaf, well below the overall mean of 2.3±0.18 nymphs per leaf. This decrease was not seen in the conventional greenhouse where whitefly populations were maintained at near the mean of 0.58±0.08 nymphs per leaf toward the end of the growing season.

Although similar pesticide regimes were also used in both greenhouses at El Ejido, only selective materials were applied. These consisted of the insect growth regulators (IGR)s azadiractin, buprofezin and pyriporxyfen as well as pyridaben and insecticidal soap. However, seven more such sprays were applied in the conventional greenhouse than in the IPM greenhouse, a difference of 1.5 incompatibility units (Table 3). Both greenhouses were initially screened against whiteflies, but the netting was removed in week 44 to increase ventilation and reduce fungal disease, causing an influx of whitefly adults mainly into the IPM greenhouse as indicated by higher trap counts (Table 6). Consequently, more than twice as many nymphs were seen in the IPM greenhouse compared to the conventional greenhouse although numbers of adults on plants were similar. Apparently, the effect of extra IGR insecticides on whitefly nymphs in the conventional greenhouse coupled with fewer immigrating whiteflies outweighed the additional parasitism occurring in the IPM greenhouse.

Broad-spectrum insecticides were extensively used on the TYLCV-susceptible tomatoes in both greenhouses at Motril 2, especially early in the season (II = 8.7 and 13.7, IPM and conventional greenhouses respectively, Table 3). Incidence of parasitized whitefly "pupae" was low and similar in both greenhouses: 2.2±2.2%, $n=9$ and 3.9±2.0%, $n=5$ IPM and conventional respectively). Whitefly numbers were similar in both.

Table 6
Mean ± SE number of adult whiteflies per yellow sticky trap, *B. tabaci* adults and nymphs per leaf from in field monitoring and percent parasitized whitefly "pupae" from leaf samples

Greenhouse	Management system	*B. tabaci*			
		Whiteflies/Trap/wk	Adults/leaf	Nymphs/leaf	Parasitized pupae (%)
La Cañada_1	IPM	7.9 ± 1.2	0.17 ± 0.01	0.18 ± 0.02	28.8 ± 10.3
	Conventional	8.6 ± 1.6	0.097 ± 0.002	0.11 ± 0.03	2.14 ± 2.14
La Cañada_2	IPM	7.5 ± 2.9	0.23 ± 0.02	2.27 ± 0.18	41.1 ± 11.6
	Conventional	1.6 ± 0.4	0.12 ± 0.03	0.58 ± 0.08	0 ± 0
El Ejido	IPM	50.6 ± 4.8	0.89 ± 0.05	2.02 ± 0.19	51.3 ± 12.6
	Conventional	40.4 ± 5.0	0.68 ± 0.04	1.076 ± 0.14	5.45 ± 2.2
Motrĩl_2	IPM	14.9 ± 1.7	0.2 ± 0.02	0.26 ± 0.04	2.22 ± 0
	Conventional	20.6 ± 2.27	0.24 ± 0.03	0.26 ± 0.03	3.9 ± 2.04
Águilas_1	IPM	21.5 ± 2.9	0.26 ± 0.02	0.20 ± 0.03	43.3 ± 12.9
	Conventional	14.3 ± 2.4	0.21 ± 0.02	0.14 ± 0.02	0 ± 0

Only at Águilas_1 was there a great disparity in pesticide use between the IPM and conventional greenhouse even though a resistant cultivar was planted in both (Table 3). The grower ordered mixtures of broad-spectrum insecticides to be applied once or twice a week against whiteflies in the conventional greenhouse, accumulating an incompatibility index of 35.7 (Table 3). In contrast, only selective pesticides were used in the IPM greenhouse, resulting in an incompatibility index of only 4.5. Although captures on sticky traps were 50% higher in the IPM greenhouse (Table 6, Fig. 2a), probably because of wind damage to the plastic roof, there was little difference in numbers of adults or nymphs of *B. tabaci* overall. Furthermore, almost 5 times fewer *B. tabaci* adults plus nymphs were observed on leaves in the IPM greenhouse (0.083 ± 0.0, Fig. 2b) compared to the conventional greenhouse at the end of the crop cycle (Fig. 2b). The decline of the whitefly population in the IPM greenhouse may have been due to the effect of parasitism which averaged 43% (Table 6). In contrast to *B. tabaci*, greenhouse whitefly was eliminated in the conventional greenhouse but rose toward the end of the crop cycle in the IPM greenhouse, probably because *E. mundus* is not effective against this species (Greenberg et al., 2002) (Fig. 2c).

3.4. Additional greenhouses

Experiences in the remaining greenhouses receiving *Eretmocerus* spp. further illustrated the role of pest exclusion in determining whitefly numbers, of virus resistance in influencing pesticide use, and of the interplay of these factors on the effectiveness of biological control. Trap captures at Aguilas_2 averaged 88 per week (Table 7), higher than in any greenhouse monitored. Although only insecticidal soap was used, fewer adults and nymphs were seen on plants than in many greenhouses where trap counts were lower and broad-spectrum insecticides used. Thus, considerable control could be attributed to parasitism that averaged 50% and the mirid predator *N. tenuis* that was found on an average of 1 every 2 leaves (Table 7). Nevertheless, losses due to TYLCV would probably have been great had not a resistant variety been used.

Mazarrón_2 was a small, open-sided greenhouse where whitefly captures reached a peak of 80 per trap in week 38. However, these numbers were not sustained (mean 16.95 ± 2.18) and numbers on plants remained low (Table 7). Index of incompatibility was low (2.67, Table 3), with only imidacloprid applied at planting, and pyridaben and buprofezin sprayed 2 weeks later. Incidence of parasitized whitefly "pupae" was only 30% (Table 7), but samples were probably taken too early, just after the release period (weeks 45 and 47). Thus, there was little other than parasitism to explain declining whitefly numbers over the course of the season.

TYLCV-susceptible cherry tomatoes typical of the region were grown at Motríl_1 and broad-spectrum insecticides were freely used early in the crop cycle (II = 6.74, Table 3). As a likely result, releases of *Eretmocerus* spp. resulted in little (3.57 ± 3.57%) parasitism being observed. Although the greenhouse was tightly constructed and whitefly counts on plants were generally low (Table 7), an early influx in week 41 resulted in 30 whiteflies per trap and a high incidence of TYLCV, requiring over 700 infected plants to be removed by week 45.

Both IPM greenhouses at Motríl_3 were open-sided allowing free entry of whiteflies and both were treated extensively with broad-spectrum insecticides early in the

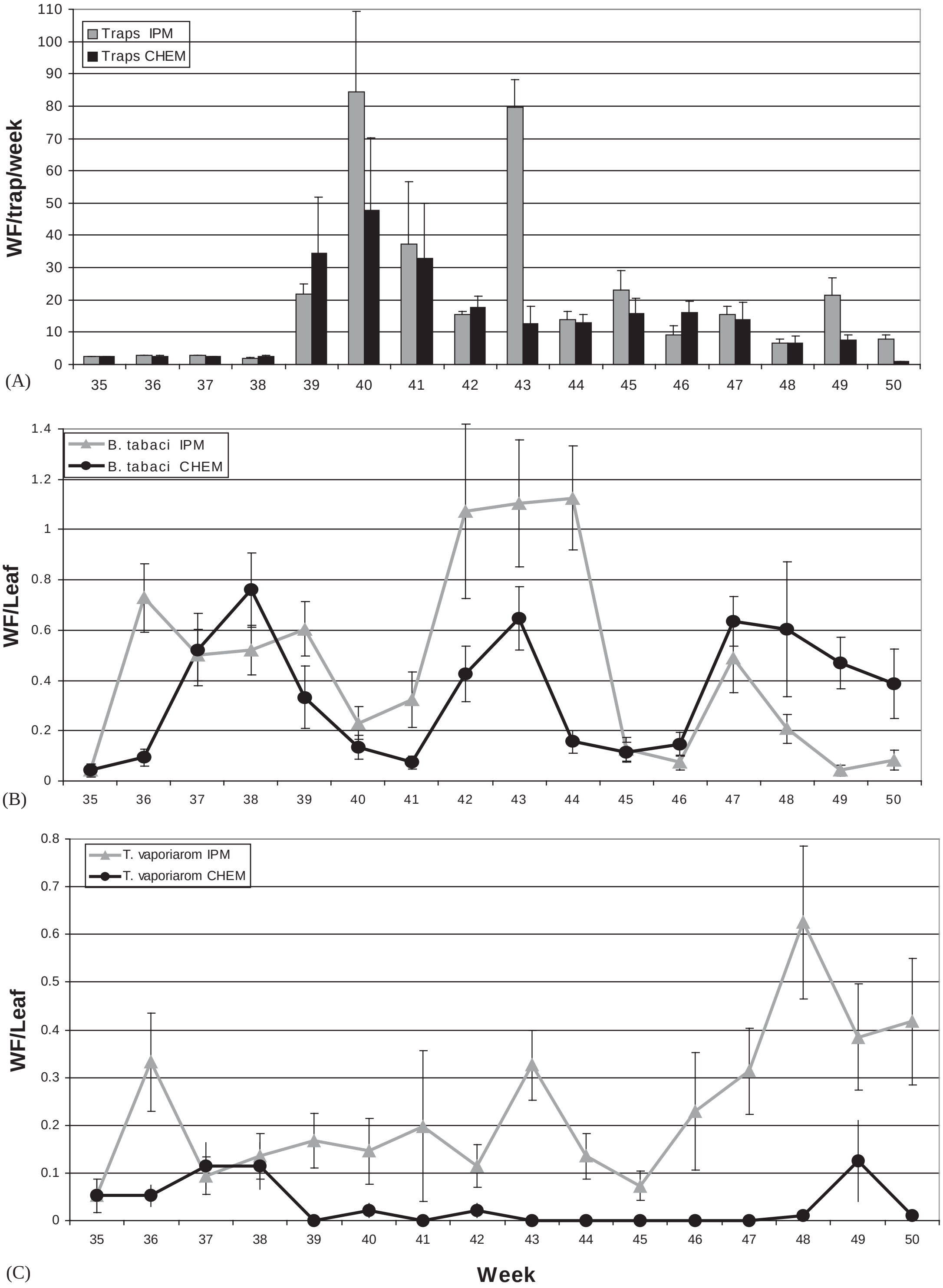

Fig. 2. (A) Mean (SE) number of whiteflies per trap per week, (B) mean (SE) number of *B. tabaci* nymphs per tomato leaf, and (C) mean number of *T. vaporiariorum* nymphs and pupae per tomato leaf in the IPM and conventionally managed greenhouses at Águilas_1 IPM Sep.–Dec. 2001.

crop cycle, preventing early establishment of parasitoids. Only horticultural mineral oil was used later but whitefly numbers remained high on sticky traps and plants, despite an ultimately high incidence of parasitism (Table 7). Extensive hyperparasitism, (17% of all parasitoids observed were *E. sophia* males) may also have contributed to the failure of biological control at this location.

The greenhouse Canarias_1 was planted to a TYLCV-resistant cultivar and covered with coarse mesh screen that did not exclude whiteflies. Pesticide use was limited to fungicides and selective insecticides, none directed

Table 7
Mean ± SE number of adult whiteflies per yellow sticky trap, number of *B. tabaci* adults and nymphs per leaf, parasitized "pupae" (%), and number of *Nesidiocorus tenius* per leaf in 5 IPM greenhouses and 2 conventional greenhouses

Greenhouse	Management	*B. tabaci (no.)*				*N. tenuis*
		Adults/trap/wk	Adults/leaf	Nymphs/leaf	Parasitized[a] (%)	No./leaf
Aguilas_2	IPM	88.0 ± 4.11	1.04 ± 0.07	1.66 ± 0.16	50.44 ± 7.05	0.48 ± 0.03
Mazarron_2	IPM	17.0 ± 2.18	0.42 ± 0.03	0.33 ± 0.06	30.2 ± 13.9	0
Motríl_1	IPM	6.7 ± 0.80	0.09 ± 0.01	0.11 ± 0.02	3.6 ± 3.6	0
Motríl_3	IPM	60.5 ± 2.68	1.23 ± 0.06	1.96 ± 0.09	79.3 ± 4.3	0
Canarias_1	Conventional	37.1 ± 3.8	0.25 ± 0.02	1.5 ± 0.12	84.6 ± 5.8	0.15 ± 0.09
Canarias_2	Conventional	76.2 ± 8.3	0.06 ± 0.01	0.17 ± 0.03	NA	0.02 ± 0.01
Canarias_3	Conventional	69.1 ± 8.5	0.83 ± 0.09	0.62 ± 0.08	NA	0.01 ± 0.00

[a] % parasitized from leaf samples.

against whiteflies. Whitefly numbers peaked at 73 per sticky trap followed by a peak of *B. tabaci* nymphs to 3.9 ± 0.78 per leaf 2 weeks later. Incidence of parasitized *B. tabaci* pupae also rose to 84.6% ± 5.8 ($n = 8$) and the predator *N. tenuis* appeared spontaneously. In contrast, pesticide use was intense in the two conventional screenhouses in the Canaries (Table 3). Trap catches and numbers of adults observed on leaves were greater in the conventionally managed greenhouse, although there were more nymphs overall in the IPM greenhouse (Table 7). Again, whitefly numbers tended to increase over the season in the conventional greenhouses but declined in the IPM greenhouse.

4. Discussion

E. mundus from either adjacent plots and/or outside the greenhouse replaced *E. eremicus* in the IPM greenhouses where both were released. These IPM greenhouses ranged in size from 25,000 to 1000 m^2 for an average of 8775 m^2. When divided into fourths, the average plot size would have been 2194 m^2. Assuming a square plot, the average distance to a neighboring plot would be equal to $\frac{1}{2}$ the square root of the area or 23.4 m. There are no published reports on movement of *E. mundus* although *E. eremicus* has been observed to fly an average of 35 minutes and to move at least 10 m in the open field (Byrne and Bellamy, 1999). Thus, considerable dispersal among plots could have occurred. Additional evidence for dispersal of *E. mundus* within the greenhouse came from the two adjacent greenhouses at Cañada_1, both of which covered 2 ha, were tightly sealed against insects and received the same minimal pesticide regime (II = 1.14). Incidence of parasitism was only 2% in the conventional greenhouse compared to 29% in the IPM greenhouse where only 7 of 73 parasitoids emerging from leaf samples were *E. eremicus*. These observations of apparent competitive superiority of *E. mundus* over *E. eremicus* are in accord with single generation cage studies on pepper and tomato (López, 2002), and probably relate to differences in suitability of *B. tabaci* as a host (Greenberg et al., 2000).

Fewer and more selective pesticides were used in IPM greenhouses than in conventional greenhouses included in this study, although there were some exceptions. Where the index of incompatibility exceeded 5 as it did in the "IPM" greenhouses of Motríl and Cañada_1, little benefit from biological control of whitefly could be demonstrated. The strategy in these greenhouses was to utilize broad-spectrum insecticides early to bring down whitefly populations to a low level before initiating biological control. However, residues from these materials typically persist 8–12 weeks (Anonymous, 2002). Consequently, *E. mundus* either did not establish or established too late to provide adequate control. The residual secondary effect on parasitoids is probably shorter for whiteflies than for *E. mundus,* allowing the pest to build up in the crop. Thus, "pesticide first" strategy using broad-spectrum chemistry was not compatible with biological control using *E. mundus*.

In contrast, incidence of parasitism was moderate to high and whitefly populations on plants remained moderate to low in IPM greenhouses where the index of incompatibility was less than 5, (Águilas_1 and 2, Mazerón2, El Ejido, Canarias_1), in spite of often high levels of whitefly immigration. Predation by *N. tenuis* provided additional whitefly control at Águilas_2 and Canaria_1 where no broad-spectrum insecticides were used. The observed tendency of whitefly populations to decline in the latter half of the season in IPM greenhouses (in contrast to conventional greenhouse) was consistent with an increasing influence of natural enemies over the crop cycle. Factors contributing to this increasing influence could include absence of interference from insecticides late in the crop and a higher inherent rate of increase exhibited by *E. mundus* compared to *B. tabaci* (Stansly et al., 2002a; Urbaneja et al., 2003). Early establishment of natural enemies in the absence of interfering insecticides was the best biological control strategy.

The ability of *E. mundus* to control whiteflies in Spain has been in demonstrated in commercial-scale trials in pepper and green beans (Calvo et al., 2002; Urbaneja et al., 2002; Téllez et al., 2003). Parasitoid-induced mortality of whiteflies above 80% was commonly observed, and populations were controlled with no recourse to insecticides. *E. mundus* appears to be equally well adapted to tomato as to pepper (Stansly et al., 2002a; Urbaneja et al., 2003) although higher release rates appear to be necessary in tomato due to more rapid whitefly population growth on that crop (Stansly et al., 2002b). Unfortunately, some growers in this study had little faith in the resistant cultivars they were using for the first time, and their exclusion methods gave inconsistent results. Increased adoption of such tactics and consequently of biological control should be favored in the future by improved technology and continued movement towards fewer and more selective insecticides.

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