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Trophic relationships between predators, whiteflies and their parasitoids in tomato greenhouses: a molecular approach

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Abstract

The whiteflies *Bemisia tabaci* Gennadius and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) are two of the main pests in tomato crops. Their biological control in Mediterranean IPM systems is based on the predators *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), as well as on the parasitoids *Eretmocerus mundus* (Mercet) and *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae). These natural enemies may interact with each other and their joint use could interfere with the biological control of those whitefly pests. Analysis of predator-prey interactions under field conditions is therefore essential in order to optimize whitefly control. Species-specific polymerase chain reaction (PCR)-primers were designed to detect DNA fragments of these whiteflies and parasitoids within both predator species in tomato greenhouses. We demonstrated that both predators feed on both whitefly species, as well as on both parasitoids under greenhouse conditions. Prey molecular detection was possible where prey abundance was very low or even where predation was not observed under a microscope. Whitefly DNA detection was positively correlated with adult whitefly abundance in the crop. However, a significant relationship was not observed between parasitoid DNA detection and the abundance of parasitoid pupae, even though the predation rate on parasitoids was high. This unidirectional intraguild predation (predators on parasitoids) could potentially reduce their combined impact on their joint prey/host. Prey molecular detection provided improved detection of prey consumption in greenhouse crops, as well as the possibility to identify which prey species were consumed by each predator species present in the greenhouse, offering a blueprint with wider applicability to other food webs.

Keywords: gut-content analysis, species-specific molecular markers, polyphagous predators, parasitism, tomato crops, *Bemisia tabaci*, *Trialeurodes vaporariorum*, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*, *Eretmocerus mundus*, *Encarsia pergandiella*

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Introduction

Biological control in intensive crops is usually based on the inoculation and/or conservation of predator and parasitoid species to control pests that invade the crop (Albajes & Alomar, 1999). In Mediterranean tomato crops, the whiteflies *Bemisia tabaci* Gennadius and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) are two of the main pests. They can cause significant reductions in crop yield by either feeding directly on the plant leaves or by producing honeydew, on which sooty mold develops quickly. This mold hampers photosynthesis and respiration, and renders fruits unmarketable. Also, several viruses that severely affect protected vegetable crops worldwide are transmitted by these whitefly species. In particular, *B. tabaci* transmits the Tomato Yellow Leaf Curl Disease that causes severe crop losses (Gabarra & Besri, 1999; Avilla *et al.*, 2004). In order to control these whitefly species, two polyphagous predators, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), have been shown to reduce whitefly populations in greenhouses (Albajes *et al.*, 2003; Calvo *et al.*, 2009). Until recently, *M. pygmaeus* found on tomato has been misidentified as *M. caliginosus* Wagner (=*M. melanotoma* (Costa)) and is still named as *M. caliginosus* by commercial producers (Martinez-Cascales *et al.*, 2006). *Macrolophus pygmaeus* and *N. tenuis* spontaneously colonize Mediterranean tomato crops when spray applications are reduced, and IPM programs based on conservation of these natural enemies are applied (Castañé *et al.*, 2004).

Parasitoids are also used in biological control of whiteflies in protected crops. Abundant naturally occurring populations of *Eretmocerus mundus* (Mercet) and *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) are also present in IPM tomato crops in northeast Spain (Gabarra *et al.*, 1999; Arnó *et al.*, 2005). *Eretmocerus mundus*, which is commercially available, specifically parasitizes *B. tabaci*, while *E. pergandiella* parasitizes both *T. vaporariorum* and *B. tabaci*. Therefore, it is common to find the two whitefly species, the two whitefly parasitoids and the two predator species, in the same tomato crop. The natural enemies may interact with each other and their joint use could either have an additive, positive synergistic or negative effect on the control of pest species. For this reason, the analysis of this food web under natural field conditions is essential if the mechanics of the interactions are to be understood. Better understanding of trophic relationships and the control capacities of the species involved, acting in concert, should allow better strategies to be devised for the optimization of whitefly control.

In field situations, prey choice by predators is not easily quantified by direct observations and is made much more difficult where cryptic species are involved. Apart from direct observation, predation on whiteflies has usually been estimated by counting the remains of whitefly pupae (Castañe *et al.*, 2004), but this measure is the result of the accumulation of predated whitefly pupae over time. When more than one predator is present in the crop, it is impossible to determine which one has consumed the target prey. An alternative approach is to use microscopic examination of gut contents to identify prey remains in predators, but this is only possible if indigestible solid remains are present in the foregut. Many arthropod predators, including the Hemiptera, are fluid feeders, making this approach impossible. In addition, predation on parasitized whitefly nymphs is even more difficult to evaluate because of the difficulty of finding parasitoid early stages, even by dissection.

In recent years, molecular techniques have facilitated the detection of prey remains within predator gut contents, generally by identifying prey-specific protein or DNA sequences (Symondson, 2002). Currently, the most common way to analyze dietary studies of arthropods is by DNA-based gut content analysis using prey-specific molecular markers, which can provide accurate information about which predator species has fed on a particular target prey (King *et al.*, 2008; Kuusk & Agustí, 2008). This approach has also been successfully used to detect parasitoid DNA within hosts (Agustí *et al.*, 2005; Traugott & Symondson, 2008). Molecular detection of predation and parasitism (reviewed by Gariepy *et al.*, 2007; King *et al.*, 2008) is now a well-proven technology, with increasing numbers of studies conducted in the field (Agustí *et al.*, 2003; Harper *et al.*, 2005; Harwood *et al.*, 2007; Juen & Traugott, 2007; Zhang *et al.*, 2007; Kuusk *et al.*, 2008). However, among all of them, few have been focused on predation of parasitized prey under field conditions (Chacón *et al.*, 2008; Traugott *et al.*, 2011) and none in tomato crops.

It was, therefore, the aim of this study to test the existence and extent of trophic interactions between predators and parasitoids of whiteflies under greenhouse conditions, which could interfere with the biological control of those whitefly pests.

Materials and methods

Insects

Macrolophus pygmaeus and *N. tenuis* were reared at our facilities (IRTA, Cabrils) as described in Agustí & Gabarra (2009a, b). This colony is renewed every year with introductions of new field-collected insects from the same area. They were fed with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs on tobacco. *Ephestia kuehniella* eggs were provided by Biotop (Valbonne, France). *Bemisia tabaci* and *T. vaporariorum* were reared on cabbage and tomato, respectively. *Eretmocerus mundus* was reared on *B. tabaci* on cotton plants. All insects were reared under controlled conditions of 25 ± 2°C, 70 ± 10% RH and L16:D8 photoperiod. The remaining species were obtained from crops near the study site.

Primer design, DNA extraction and amplification

Two pairs of specific primers, Bt2F/Bt3R and Tv1F/Tv1R, were designed for *B. tabaci* and *T. vaporariorum*, respectively, from the mitochondrial cytochrome oxidase I (COI) region. Several sequences from the GenBank database (www.ncbi.nlm.nih.gov) were used as reference sequences to design species-specific primers for the COI gene for *B. tabaci* (AM691052, *B. tabaci*; AF418672 and AF110708, *T. vaporariorum*; AY842502, *Aphis gossypii*; DQ059302, *Helicoverpa armigera*) and *T. vaporariorum* (AY521259, *B. tabaci*; AY521265, *T. vaporariorum*; AY227082, *Aphis gossypii*; AY437834, *H. armigera*). Similarly, GenBank reference sequences for the ITS-1 region were used in the development of specific primers for *E. mundus*, Em2F/Em1R and *E. pergandiella*, Ep1F/Ep1R (AY854061, *B. tabaci*; AY854055, *T. vaporariorum*; AF273635, *E. mundus*; AY615778, *E. pergandiella*). In this case, ITS-1 region was used because of the impossibility of finding enough differences in the COI region to design specifics primers. Sequence alignments were performed using CLUSTALW

Table 1. Prey, parasitoid and predator species tested for cross-amplification using whitefly and parasitoid specific primers described in table 2.

Group	Order	Family	Species tested
Prey	Acari	Tetranychidae	*Tetranychus urticae* Koch
	Diptera	Agromyzidae	*Liriomyza trifolii* (Burgess)
	Homoptera	Aleyrodidae	*Aleyrodes proletella* (Linnaeus)
			Bemisia tabaci Gennadius
			Trialeurodes vaporariorum (Westwood)
		Aphididae	*Aphis gossypii* Glover
			Nasonovia ribisnigri (Mosley)
	Lepidoptera	Noctuidae	*Chrysodeixis chalcites* (Esper)
			Helicoverpa armigera (Hübner)
			Autographa gamma Linnaeus
		Pyralidae	*Ephestia kuehniella* Zeller
	Thysanoptera	Thripidae	*Frankliniella occidentalis* (Pergande)
Predator	Diptera	Cecidomyiidae	*Aphidoletes aphidimyza* (Rondani)
	Hemiptera	Anthocoridae	*Orius laevigatus* (Fieber)
			Orius majusculus (Reuter)
	Heteroptera	Miridae	*Dicyphus tamaninii* Wagner
			Macrolophus pygmaeus (Rambur)
			Nesidiocoris tenuis Reuter
Parasitoid	Hymenoptera	Aphelinidae	*Encarsia formosa* Gahan
			Encarsia pergandiella Howard
			Eretmocerus eremicus Rose and Zolnerowich
			Eretmocerus mundus (Mercet)
		Braconidae	*Aphidius colemani* Viereck
			Habrobracon hebetor (Say)
		Eulophidae	*Diglyphus isaea* (Walker)
		Trichogrammatidae	*Trichogramma evanescens* Westwood

(http://www.ebi.ac.uk/clustalw). AMPLICON software (Jarman, 2004) was used to design *B. tabaci* primers. The remaining primers were designed as described in Agustí *et al.* (2003).

DNA was extracted from individual insects using the DNeasy Tissue Kit (QIAGEN; protocol for animal tissues). Total DNA was eluted in 100 µl of AE buffer provided by the manufacturer and stored at −20°C. Negative controls were added to each DNA extraction set. Samples were amplified in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). Reaction volumes (25 µl) contained 4 µl of resuspended DNA. All pairs of primers were amplified by using 0.65 U of *Taq* DNA polymerase (Invitrogen), 0.25 mM of dNTPs (Promega), 0.4 µM of each primer and 1.5 mM (Em2F/Em1R), 3 mM (Bt2F/Bt3R and Tv1F/Tv1R) or 6 mM (Ep1F/Ep1R) of $MgCl_2$ in 10 × manufacturer's buffer. Samples were amplified for 35 cycles (except Ep1F/Ep1R, 40 cycles) at 94°C for 30 s; 63°C (Tv1F/Tv1R), 62°C (Em2F/Em1R) or 58°C (Bt2F/Bt3R and Ep1F/Ep1R) for 30 s; and 72°C for 40 s. A first cycle of denaturation at 94°C for 3 min and a final extension at 72°C for 5 min was carried out. Target DNA and water were always included as positive and negative controls, respectively. PCR products were separated by electrophoresis in 1.5% agarose gels stained with ethidium bromide and visualized under UV light.

Species specificity

At least ten individuals of each target prey were tested with their respective specific primers to test for intraspecific differences. Ten individuals of the B and Q biotypes of *B. tabaci*, which are the most common in the studied area (Moya *et al.*, 2001) were also analyzed. Specificity of the four pairs of primers was analysed by testing ten individuals of each prey, parasitoid and predator species potentially present in horticultural crops in the study area (table 1). *Ephestia kuehniella* was also tested as it was the prey of the predators during mass rearing.

Field sampling and analysis of field-collected predators

Eight tomato greenhouses located near Barcelona (NE Spain), where IPM programs based on conservation of these natural enemies were applied (Castañé *et al.*, 2004), were sampled in summer (May–October) for mirid predators, whiteflies and whitefly parasitoids. In order to determine their abundance, the number of adult whiteflies, as well as nymphs and adults of each predator species, were surveyed on ten leaflets per plant and on 30 plants per greenhouse. For whitefly pupae and parasitoid abundance, 30 tomato leaflets were collected per greenhouse from those leaves where adult whiteflies were starting to emerge from the pupae. The pupae were classified under a binocular microscope as alive, consumed or parasitized. Consumed whitefly pupae were easily distinguished from incomplete predation or other causes of mortality, because complete consumption by a fluid-feeding mirid bug leaves an empty cuticle without an insect emergence hole (Castañé *et al.*, 2004).

Immediately after *M. pygmaeus* and *N. tenuis* were collected, they were placed at 4°C and then frozen at –20°C when arriving to the lab. In order to avoid false negatives (Sint *et al.*, 2011), each predator was analyzed up to three times if previous times a positive result was not obtained. One predator was considered negative if prey DNA was not detected in the three analyses. Percentages of molecular detection of each prey species were calculated in each greenhouse, as well as

Table 2. Whitefly and parasitoid species-specific primer sequences (5′–3′), amplified fragment sizes and gene targeted.

Target species	Primer	Sequence	Length (bp)	Region
B. tabaci	Bt2 F Bt3R	TTGGTGTCTCAATTTTATATC ATACTCAAAATCCTTCCCGC	158	COI
T. vaporariorum	Tv1 F Tv1R	TCTCACAGGGGTGATTTTG CTGGGAAAGAAGAAGGTTAAAA	187	COI
E. mundus	Em2 F Em1 R	CGTATGCGGATAACAACGC GAACTCGCAAGAGCTCGAAC	345	ITS-1
E. pergandiella	Ep1 F Ep1R	ACGCAAGTCGTACCGATGAG AAACGTAACTTTGACGACGC	314	ITS-1

percentages of multiple prey detection in the same individual predator.

Whitefly pupae and adult abundances were compared with whitefly molecular detection percentages in each greenhouse for both predator species. Abundances of non-parasitized and parasitized pupae were combined because whitefly DNA could also be detected in the parasitized pupae, depending on the parasitoid developmental stage (data not shown). Abundance of parasitized pupae was also compared with parasitoid molecular detection. The number of predators testing positive for whitefly DNA was also compared with the mean numbers of predated whitefly pupae in each greenhouse. The number of positive predators of both species was added, as it was not possible to determine which species had fed on the whitefly pupae when both predator species were present.

Multiple regression analyses were done to evaluate the relationships between molecular detection and the number of available whitefly pupae and adults, parasitized and predated prey (SAS Institute Inc., 2001). The available and predated whitefly data were $\log_n$-transformed.

In order to determine whether prey detection depended on predator species, molecular detection percentages of both whiteflies within each predator species were studied. A two-tailed Fisher exact test was performed (SAS Institute Inc., 2001).

Results

Species specificity

Specific primers that were designed for *B. tabaci*, *T. vaporariorum*, *E. mundus* and *E. pergandiella* (table 2) showed successful amplifications of the target prey in all cases (fig. 1). In the case of *B. tabaci*, B and Q biotypes were both successfully amplified. When the four pairs of primers were tested for cross-amplification against other potential prey (26 species belonging to 14 families; see table 1), only the target prey were detected, thus showing a high degree of primer specificity.

Predation in greenhouses

Predator-prey abundance and prey molecular detection

Predator and prey abundances found in the eight sampled greenhouses are shown in table 3. *Macrolophus pygmaeus* was found in all greenhouses where it was sampled (greenhouse A was not sampled for abundances, although predators were collected for molecular analysis), while *N. tenuis* was found only in four. Predator nymphs were more abundant than adults in all greenhouses, except in greenhouse G for *N. tenuis*.

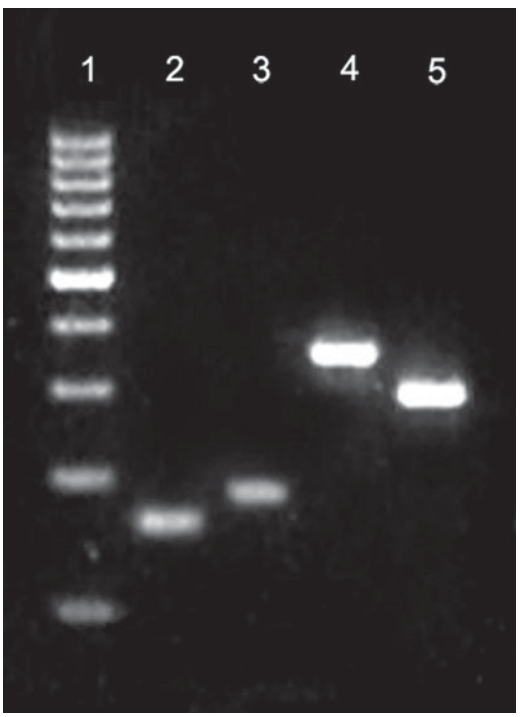

Fig. 1. Amplification products obtained with the specific primers for the four target prey. Lane 2, *Bemisia tabaci* (158 bp); 3, *Trialeurodes vaporariorum* (187 bp); 4, *Eretmocerus mundus* (345 bp); 5, *Encarsia pergandiella* (314 bp). Lane 1, 100 bp DNA ladder.

Trialeurodes vaporariorum adult abundance was higher than *B. tabaci* in four of the sampled greenhouses, while the same was found for *T. vaporariorum* pupae in six of them. The abundance of pupae parasitized by *E. mundus* and *E. pergandiella* was low, except in greenhouse A, where *E. pergandiella* reached a very high level (34.6 ± 8.81 pupae per leaflet).

Percentages of prey detection by PCR in the eight sampled greenhouses are shown in table 4. Predators were collected from 30 plants per greenhouse, but only those greenhouses with more than 14 collected predators were analysed by PCR. Therefore, although low densities of *N. tenuis* and *M. pygmaeus* were observed in greenhouses E and F, respectively, analyses were not conducted. All prey species were detected by PCR in both predator species. Prey was detected in 39% ($n = 142$) of *M. pygmaeus* and in 46% ($n = 61$) of *N. tenuis*. *Bemisia tabaci* and *T. vaporariorum* molecular detection was achieved in all

Table 3. Predator and prey abundances (mean ± SE) in eight tomato greenhouses.

Greenhouse	Predator abundance				Prey abundance					
	M. pygmaeus/plant		*N. tenuis*/plant		Adults/plant		Pupae/leaflet			
	Adults	Nymphs	Adults	Nymphs	Bt	Tv	Bt	Em	Tv	Ep
A	–	–	–	–	–	–	0	0	7.5 ± 1.63	34.6 ± 8.81
B	0.2 ± 0.12	1.2 ± 0.26	0	0	0	3.8 ± 0.93	0.3 ± 0.17	0	4.3 ± 1.00	0.7 ± 0.25
C	0.8 ± 0.21	2.8 ± 0.39	0	0	0	5.9 ± 1.06	0.6 ± 0.39	0	1.7 ± 0.33	0.4 ± 0.15
D	0.1 ± 0.06	0.5 ± 0.15	0	0	1.0 ± 0.23	3.3 ± 0.66	0.6 ± 0.24	0	3.4 ± 0.80	0.1 ± 0.13
E	0.2 ± 0.09	1.2 ± 0.18	0	0.1 ± 0.08	0.1 ± 0.06	0.3 ± 0.09	0.1 ± 0.07	0	0	0
F	0.1 ± 0.06	0.5 ± 0.18	0.2 ± 0.11	0.3 ± 0.10	5.3 ± 0.78	1.1 ± 0.29	3.9 ± 0.97	1.0 ± 0.05	0.5 ± 0.22	0
G	0.1 ± 0.05	0.5 ± 0.18	1.4 ± 0.23	1.1 ± 0.25	1.3 ± 0.27	0.3 ± 0.15	0	0	0.1 ± 0.08	0
H	0.1 ± 0.05	0.4 ± 0.13	0.2 ± 0.11	0.4 ± 0.13	3.9 ± 0.82	1.6 ± 0.30	1.8 ± 0.92	0.1 ± 0.08	2.9 ± 1.04	1.1 ± 0.54

Bt, *B. tabaci*; Tv, *T. vaporariorum*; Em, *B. tabaci* parasitized by *E. mundus*; Ep, *T. vaporariorum* parasitized by *E. pergandiella*.

Table 4. Predated prey observed under microscope (mean ± SE) and percentages of positive *M. pygmaeus* and *N. tenuis* by PCR in eight tomato greenhouses.

Greenhouse	Consumed whitefly pupae per leaflet		Molecular detection									
			M. pygmaeus (%)					*N. tenuis* (%)				
	Bt	Tv	Bt	Em	Tv	Ep	*n*	Bt	Em	Tv	Ep	*n*
A	–	6.0 ± 1.13	–	–	43.5	30.4	23	–	–	–	–	–
B	0	2.9 ± 0.92	18.8	–	37.5	6.3	16	–	–	–	–	–
C	0	2.4 ± 0.52	0.0	–	41.7	0.0	24	–	–	–	–	–
D	0	1.0 ± 0.31	22.2	5.6	44.4	0.0	18	–	–	–	–	–
E	0	2.6 ± 0.63	4.2	–	0.0	–	24	–	–	–	–	–
F	1.4 ± 0.46	0.5 ± 0.24	–	–	–	–	–	72.2	44.4	27.8	–	18
G	0	4.9 ± 1.01	35.7	–	21.4	14.3	14	4.2	–	0.0	0.0	24
H	0.6 ± 0.32	2.0 ± 0.51	20.0	5.0	15.0	0.0	20	37.5	0.0	50.0	18.8	16

Bt, *B. tabaci*; Tv, *T. vaporariorum*; Em, *B. tabaci* parasitized by *E. mundus*; Ep, *T. vaporariorum* parasitized by *E. pergandiella; n*, number of predators analyzed.

greenhouses where they were present, except in two cases: *B. tabaci* in greenhouse C, where only *B. tabaci* pupae were found; and *T. vaporariorum* in greenhouse E, where only adults were found and with the lowest abundance (table 3). However, predated *B. tabaci* pupae were only observed microscopically in two of the seven greenhouses. Therefore, molecular techniques were more likely than microscopy to detect the occurrence of rare predation events, as is shown in the detection of predation by *M. pygmaeus* on *B. tabaci* in four greenhouses where predation was not observed upon microscopic examination. Also, in those greenhouses where both *M. pygmaeus* and *N. tenuis* were present and analysed by PCR (G and H), molecular detection identified which whitefly species were being consumed by each predator species. In addition, *E. mundus* predation was detected by molecular analysis in all greenhouses where it was visually observed (F and H; see table 4), while *E. pergandiella* predation was detected only in three of the five greenhouses where it was documented in visual counts. In two greenhouses, molecular detection of one parasitoid was possible even when they were not observed (*E. pergandiella* in greenhouse G and *E. mundus* in greenhouse D), again reaffirming the utility of molecular techniques in the detection of rare predation events.

Relationship between prey abundance and molecular detection

Detection of whitefly DNA in predators was positively correlated with whitefly adult abundance (both whitefly species together) for both predator species ($r^2 = 0.55$; $P = 0.009$ for *M. pygmaeus* and $r^2 = 0.67$; $P = 0.045$ for *N. tenuis*; fig. 2a, b). Similarly, when whitefly pupae abundances (both whitefly species together) were related to molecular detection of whitefly DNA within predators, a significant positive relationship was found for *N. tenuis* ($r^2 = 0.88$; $P = 0.005$; fig. 2d) but not significant for *M. pygmaeus* ($r^2 = 0.28$; $P = 0.079$; fig. 2c). There was no significant relationship between parasitized whitefly pupae (both parasitoid species together) and detection of parasitoid DNA in *M. pygmaeus* ($r^2 = 0.21$; $P = 0.304$). This relationship was not calculated in *N. tenuis* because parasitoids were only found in two greenhouses together with this predator (see table 3), which was not enough to determine a relationship.

No significant relationship was found between the number of predated whitefly pupae observed under the microscope (table 4) and the whitefly DNA detected in the two predator species ($r^2 = 0.02$; $P = 0.679$).

Multiple prey molecular detection

Up to three different prey species were detected in both predator species (table 5). From the 56 *M. pygmaeus* in which prey were detected, one prey species was detected in 77% of them, two prey species were detected in 21% and three prey species were detected in only 2%. From the 28 *N. tenuis* in which the prey was detected, 46% were positive for one prey

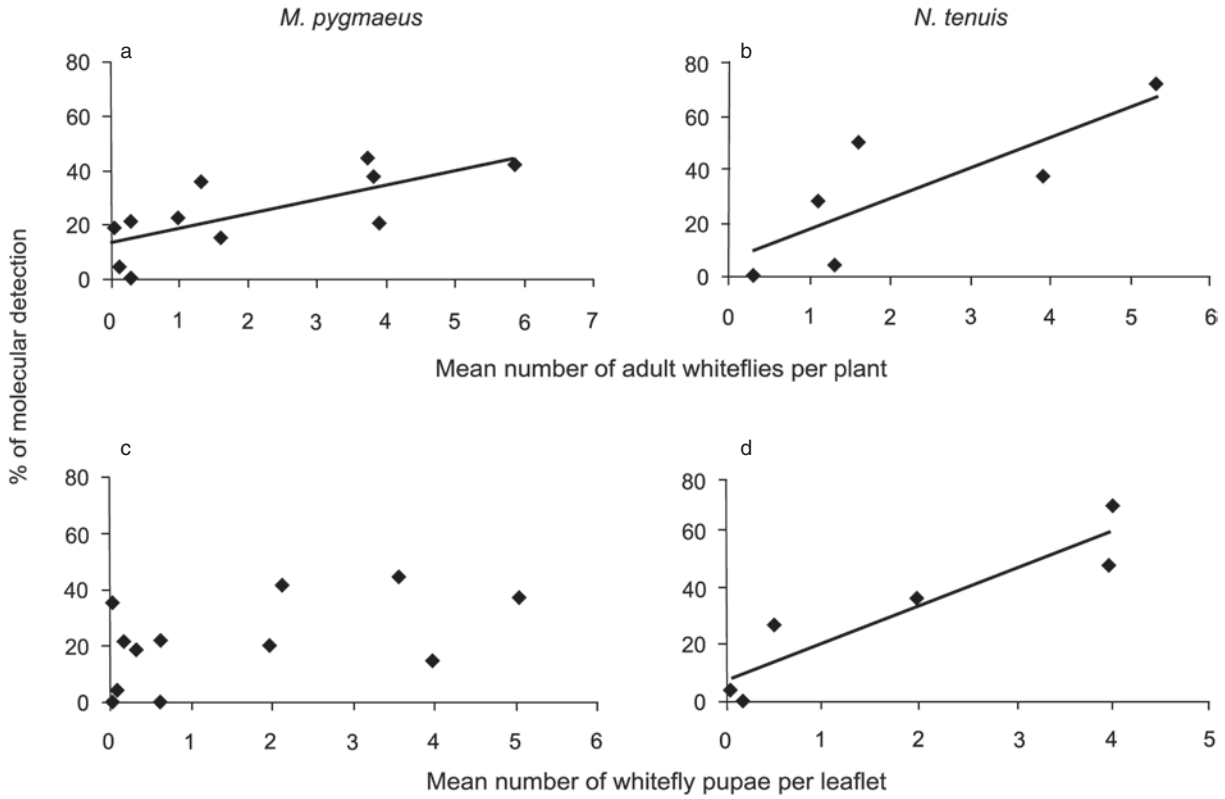

Fig. 2. (a) and (b) Relationship between percentages of whitefly detected in both predator species and whitefly adult presence; and (c) and (d) whitefly pupae presence.

Table 5. Molecular detection percentages of all prey combinations within *M. pygmaeus* and *N. tenuis*.

Prey		*M. pygmaeus* (%) ($n=56$)	*N. tenuis* (%) ($n=28$)
Pest	Parasitoid		
Bt	–	21.4	25.0
Tv	–	48.2	10.7
Bt + Tv	–	7.1	25.0
–	Em	5.4	10.7
Bt	Em	0.0	14.3
Bt + Tv	Em	0.0	3.6
–	Ep	1.8	0.0
Tv	Ep	14.3	7.1
Bt + Tv	Ep	1.8	3.6

Bt, *B. tabaci*; Tv, *T. vaporariorum*; Em, *E. mundus*; Ep, *E. pergandiella*.

species, 46% for two and 7% for three, showing possibly greater polyphagy by this predator species.

Forty-five per cent of the predators positive for *E. mundus* were also positive for *B. tabaci*, while 92% of the predators positive for *E. pergandiella* were also positive for *T. vaporariorum*.

Prey molecular detection depending on predator species

Considering the particular case of greenhouse H, where both whitefly species and both predators where present ($n=20$ *M. pygmaeus* and $n=16$ *N. tenuis*), whitefly detection was higher within *N. tenuis* than within *M. pygmaeus*, and significantly higher (two-tailed Fisher's exact test, $P=0.034$) in the case of *T. vaporariorum* (fig. 3).

Discussion

In the present study, it was demonstrated that both predators were feeding on both whitefly species, as well as on both parasitoid species under greenhouse conditions. Prey molecular detection was possible in greenhouses where prey abundance was very low or even not observed under a microscope. This suggests that predators are also feeding on small life stages of parasitoids (eggs and early larvae), which are very difficult or impossible to detect inside the whitefly by direct observation or dissection. This molecular technique provides improved detection of prey consumption in greenhouse crops, as well as the possibility to identify which prey species were fed by each predator species present in the same greenhouse, which was impossible in previous studies using traditional methods (Castañé *et al.*, 2004; Arnó *et al.*, 2005).

Whitefly molecular detection within both predators was significantly related to the adult whitefly abundance found in the greenhouses, as well as to whitefly pupae abundance in the case of *N. tenuis*. Montserrat *et al.* (2000b) observed a higher efficiency of *M. pygmaeus* preying on second-instar larvae of *Frankliniella occidentalis* (small and mobile) than on fourth-instar nymphs of *T. vaporariorum* (bigger and sessile). This suggests that *M. pygmaeus* could be attracted to mobile prey, which could explain the significant relationship between whitefly molecular detection and the abundance of adult

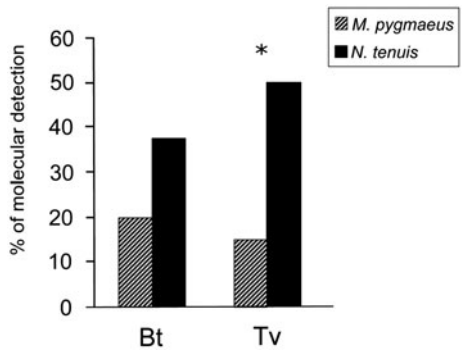

Fig. 3. Prey molecular detection percentages within *M. pygmaeus* and *N. tenuis* from greenhouse H. *, significant difference between *M. pygmaeus* and *N. tenuis*. Bt, *B. tabaci*; Tv, *T. vaporariorum*.

whitefly within the same predator. Another explanation would be related to the distribution on the plant. Adult whiteflies, mainly *T. vaporariorum*, tend to congregate in the upper leaves of the tomato plant (Arnó *et al.*, 2006), an area which also supports larger predator populations (Arnó *et al.*, 2010).

As expected, no relationship was found between whitefly DNA detected in predators and the observed predation on whiteflies. The number of predated whitefly pupal husks observed on the leaves is the result of predation over an extended period of time. It is not possible to know when these pupae were consumed or which predator species had fed on them. Also, no relationship was observed between parasitoid molecular detection and parasitoid abundance, as estimated by visual inspection of leaves. This suggests an underestimation of parasitism under the microscope because of the difficulty of observing parasitoid egg and larval stages. Even if these predators have been reported previously to prey on *E. mundus* pupae, immature stages and adults in laboratory studies (Malo, 2009), molecular methods are probably optimal for future work looking at the predators' impact on parasitoids, as these techniques provide more sensitive species-specific detection.

The molecular analysis of field collected predators allowed the identification of up to three different prey species in some of the analysed predators. Intraguild predation may be advantageous when pest species are scarce (van Baalen *et al.*, 2001) or disadvantageous when predators reduce the effectiveness of parasitoids by feeding on them (Rosenheim *et al.*, 1995). Therefore, analysis of predation rates on parasitized prey can be important when determining the effectiveness of polyphagous predators simultaneously with parasitoids (Hoelmer *et al.*, 1994). Although the number of specimens analyzed in the present study was fairly low, we show that both predators fed mainly on whiteflies (76.7% for *M. pygmaeus* and 60.7% for *N. tenuis*) but also on parasitoids (23.3% and 39.3%, respectively). As mentioned before, previous studies have already shown intraguild predation on parasitoids under field conditions using molecular tools. Chacón *et al.* (2008) showed predation on the parasitoid *Aphidius colemani* by *Harmonia axyridis* and *Chrysoperla carnea*. Similarly, Traugott *et al.* (2011) showed predation on several species of aphid parasitoids by generalist predators. Other studies indicate that joint presence of these predators and these parasitoids could be complementary (Castañé *et al.*, 2004; Gabarra *et al.*, 2006). The present study shows that predation on *E. mundus* and *E. pergandiella* by *M. pygmaeus* and *N. tenuis* under greenhouse conditions is common and could have a negative effect on biological control. However, further experiments or larger scale collections are necessary to confirm the existence and impact of such interactions on the success of biological control of whiteflies.

Although *E. pergandiella* has been described as a *B. tabaci* parasitoid (Liu & Stansly, 1996), the combination of *B. tabaci* and *E. pergandiella* DNA was not detected in predators. This suggests preference by this parasitoid for *T. vaporariorum* when both whitefly species are available. Similar results were found when natural parasitism by both parasitoids on whiteflies in tomato and cucumber crops were studied (Arnó *et al.*, 2005).

Molecular prey detection was higher in *N. tenuis* than in *M. pygmaeus*. Although it can be the result of a lower digestion rate in *N. tenuis*, a higher voracity of this predator species could also explain this difference. This would agree with previously published studies, like Arnó *et al.* (2009), who compared the predatory capacity of both predators on *T. absoluta* eggs and observed that *M. pygmaeus* nymphs preyed significantly less than *N. tenuis* nymphs. Barnadas *et al.* (1998) also observed that *M. caliginosus* consumed fewer *B. tabaci* and *T. vaporariorum* pupae than the mirid *D. tamaninii*. Finally, Montserrat *et al.* (2000a) found lower prey searching activity in *M. caliginosus* than in *D. tamaninii*.

This study has demonstrated the effectiveness of molecular markers to study predation in agroecosystems, including greenhouse tomato crops. Here, trophic interactions were detected between the polyphagous predators *M. pygmaeus* and *N. tenuis* in the presence of the whiteflies *B. tabaci* and *T. vaporariorum* and two parasitoids under field conditions. This predation on the parasitoids indicates the existence of intraguild predation, which could interfere with the biological control of those whitefly pests. The extent of this impact on a biological control program needs to be investigated further. Nonetheless, the molecular markers described here provide valuable information that would be difficult or impossible to obtain by other methods.

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