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Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy

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Abstract

The tomato borer *Tuta absoluta* (Meyrick) is an invasive pest native to South America and since its arrival in Europe the tomato production has faced severe yield loss. The complex of indigenous parasitoids that colonized this new host species was monitored in Southern Italy during 2009-2011, in some of the regions where *T. absoluta* was initially detected (Campania, Sardinia and Sicily) with the aim of identifying the parasitoid complex of the tomato borer as well as finding potential biocontrol agents of this invasive pest. The survey was carried out by sampling the tomato borer on open field and protected greenhouse crops, on wild secondary hosts and by exposing sentinel infested tomato plants.

A quick shift of native parasitoids to the new invasive host was observed and the parasitoid complex associated to *T. absoluta* seems to follow the typical pattern of colonization on exotic pests. The recovered species were, in fact, mainly generalist idiobiont parasitoids causing low levels of parasitism in open field. The species found belong to 13 genera and 6 families (Ichneumonidae, Braconidae, Eulophidae, Elasmidae, Pteromalidae and Trichogrammatidae). In particular, the 10 identified species were: *Diadegma pulchripes* (Kokujev), *Bracon osculator* (Nees), *Bracon* (*Habrobracon*) *nigricans* Szepligeti, *Necremnus* sp. near *tidius* (Walker), *Necremnus* sp. near *artynes* (Walker), *Neochrysocharis formosa* (Westwood), *Pnigalio soemius* s.l. (Walker), *Pnigalio cristatus* (Ratzeburg), *Pnigalio incompletus* (Boucek) and *Halticoptera aenea* (Walker). For seven of these species, the finding on *T. absoluta* is the first host-parasitoid association report. This survey highlighted that conservation of indigenous natural enemies, also by means of habitat management techniques, should be taken seriously into account when planning integrated management strategy of the tomato borer in the Mediterranean area.

Key words: Parasitoid community, tomato borer, field sampling, spontaneous flora, sentinel plant, new host-parasitoid associations.

Introduction

The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae), is a very injurious pest affecting tomato crops during the whole cycle, both in greenhouse and open field cultivations. This neotropical species was originally widespread in South America and, starting from 2006, it rapidly invaded Southern Europe and North Africa (Urbaneja *et al*., 2007; Desneux *et al.*, 2010). In Italy it was first detected in 2008 (Tropea Garzia *et al*., 2009; Viggiani *et al*., 2009) and spread throughout the major tomato-producing regions infesting both fresh market and processing tomato. More recently, the pest was reported in most European and North African countries and in several Middle East countries as well (Desneux *et al*., 2011).

T. absoluta is a multivoltine pest that shows high reproductive potential and short life cycle (Pereyra and Sanchez, 2006). The female lays eggs, mostly singly, on leaves and stems, the young larvae bore and develop inside the plant, continuously searching for new feeding locations and pupation occurs mainly in the soil. In the Mediterranean basin, *T. absoluta* infests other Solanaceous crops (eggplant, sweet pepper, potato and tobacco) as well as spontaneous plants, such as the black nightshade, *Solanum nigrum* L.; occasional damages have been reported on green bean (Desneux *et al*., 2010).

The species is considered a key pest of tomato in its native area causing high yield losses (Silva *et al*., 2011). The primary *T. absoluta* management strategy in most South American countries is chemical control (Siqueira *et al*., 2000). However, pesticides are only partially successful because of the general endophytic behaviour of the larval instars and the rapid selection of resistant populations (Siqueira *et al*., 2000; Lietti *et al*., 2005; Silva *et al.*, 2011). Occurrence of *T. absoluta* at increasing population levels led growers to extensively use insecticides, which could cause many side-effects on natural enemies in tomato crops (Desneux *et al*., 2007; Biondi *et al.*, 2012). Several eco-sustainable control methods and integrated pest management (IPM) programs have been recently evaluated (Batalla-Carrera *et al*., 2010; Mollá *et al*., 2011; Vacas *et al*., 2011; Zappalà *et al*., 2012). In this framework a key role could be played by biological control agents (Desneux *et al.*, 2010). In Italy, like in most invaded countries, the control of *T. absoluta* is still largely based on chemical applications (Sannino and Espinosa, 2010). This approach may disrupt previous successful IPM procedures adopted during the last decades all over European countries for other pests in the field. For this reason the knowledge of the indigenous antagonists and the strategies to conserve them, have to be considered as a priority in the implementation of exotic pest management (van Lenteren and Woets, 1988). In fact, a successful establishment of non-native species is theoretically related to their higher competitiveness compared to native species as well as to the reduced control by natural enemies (Grabenweger *et al.*, 2010). In newly invaded areas there can be few natural enemy species and/or their effect on the exotic species can be weak. Another point of interest is that indigenous natural enemies need time to colonize, get adapted and effectively control the exotic species. Several examples of invading pests with poor parasitoid complexes, mainly represented by idiobiont species performing low levels of parasitism, are known. Idiobiont parasitoids do not need to adapt to their host physiology and therefore they may switch more easily to new hosts (Askew and Shaw, 1986), providing, in some cases, a substantial control of insect pests, especially of leafminers (Godfray *et al.*, 1995; Urbaneja *et al.*, 2000). Most of these examples are represented by endophytic species which are among the most heavily parasitised insects (Girardoz *et al.*, 2006).

Various indigenous species of parasitoids and predators feed on *T. absoluta* in the Mediterranean basin (Desneux *et al*., 2010; Gabarra and Arnó, 2010; Mollá *et al*., 2010; Loni *et al*., 2011). They are gradually getting adapted to the new pest and will probably play a central role as limiting factors in the near future. This study aims to identify the parasitoid complex of *T. absoluta* in Southern Italy as well as to find potential biocontrol agents of this invasive pest. These goals fall in the recent European IPM guidelines of pest control favouring the application of conservation biocontrol strategies and the use of indigenous species as biocontrol agents.

Materials and methods

Tomato borer laboratory rearing

T. absoluta colonies were started from vegetal material, mainly infested by tomato borer larvae, collected in Sicily and Campania and maintained in the laboratory on cherry-type tomato plants (cv. Shiren). The host plants were grown outdoor into 16 cm diameter pots and protected by pest infestations in screened cages (300 × 80 × 120 cm) under natural climatic and photoperiodic conditions until they had reached a height of 60-70 cm. In the laboratory the moth was reared inside cages (50 × 60 × 80 cm) covered with fine polyester mesh, at 25 ± 2 °C temperature, 50 ± 10% relative humidity and a L14:D10 photoperiod. Forty *T. absoluta* adults (1:1 sex ratio) were released on 12 tomato plants per cage and provided with a protein and sugary diet (Protonectar®, Lega Italia, Italy) in a water solution using a spongy dispenser. After 20 ± 2 days, when the majority of the larvae had reached the 4th instar, the infested material was collected and reared inside 5-liter screened plastic containers until adult emergence. Adults were collected using a mechanical aspirator to be inoculated in the rearing cages. To avoid high levels of endogamy, colonies were refreshed by introducing specimens collected from the field into the rearing system at least once every two months.

Sentinel tomato plants, 60-70 cm high and with 15 expanded leaves on average, bearing *T. absoluta* eggs or larvae were selected and exposed in the field to collect parasitoids. In order to obtain plants infested by coetaneous pre-imaginal stages, the adults were removed three days after their release, therefore getting contemporary ovipositions, and the infested plants were then moved in a separate cage. Sentinel plants infested with eggs and young larvae were obtained 2-4 days from the removal of inoculated adults, those with 3rd and 4th instar larvae after 8-10 days from the removal of inoculated adults.

Parasitoid sampling

The survey was conducted in some of the Italian regions where *T. absoluta* was first detected, namely Sicily, Sardinia and Campania (figure 1), by sampling in different growing and ecological environments (table 1). Two sampling methods were used to collect tomato borer parasitoids: a) infested sentinel tomato plants, and b) field sampling of infested cultivated plants and weeds.

a. Sentinel plants

From August 2009 to May 2011, sentinel tomato plants were placed in 7 different field sites (table 1). In order to expose all pre-imaginal *T. absoluta* instars to parasitoid attack, sentinel plants infested with eggs, young and mature larvae were used. Each plant was infested with an average of 50 pre-adult instars.

In sites 1 to 6, two sentinel plants were placed every week. One plant was infested with 50% eggs and 50% young larvae, the other one with 50% 3rd and 50% 4th instar larvae. The plants were removed from the field after one week, the infested material was observed in the laboratory under a stereomicroscope and then the parasitized instars were isolated until the adult wasp emergence. A total number of 140 sentinel plants were exposed and 6,484 *T. absoluta* instars were observed. The stages showing clear parasitization activity were isolated and reared until the emergence of adult parasitoids; besides, the apparently healthy instars were also reared in order to detect endoparasitoids.

In site 7, twice a week six sentinel plants, two bearing eggs, two young larvae and two mature larvae, were exposed to parasitoid attack. Plants were replaced every 3-4 days and the foliage of each plant stored in aerated cages to detect parasitoid emergences.

b. Field sampling

Infested material was also collected by direct inspection of cultivated solanaceous plants (open field tomato, potato and protected tomato crops) and *S. nigrum* spontaneously growing close to the cultivated sites as well as in urban areas (table 1). In sites 1-6 the samples were collected from August 2009 to February 2010, while in sites 8 and 9 sampling was performed from May to October 2010 on protected tomato crops. Infested leaves were collected weekly during the entire cultivation period; each sample consisted of 100 infested leaves. To avoid collecting empty old mines, since *T. absoluta* attack moves towards apical leaves in the plant, as the crop season advances, higher leaves bearing active infestation were progressively selected. Leaves were observed under a stereomicroscope to register the number

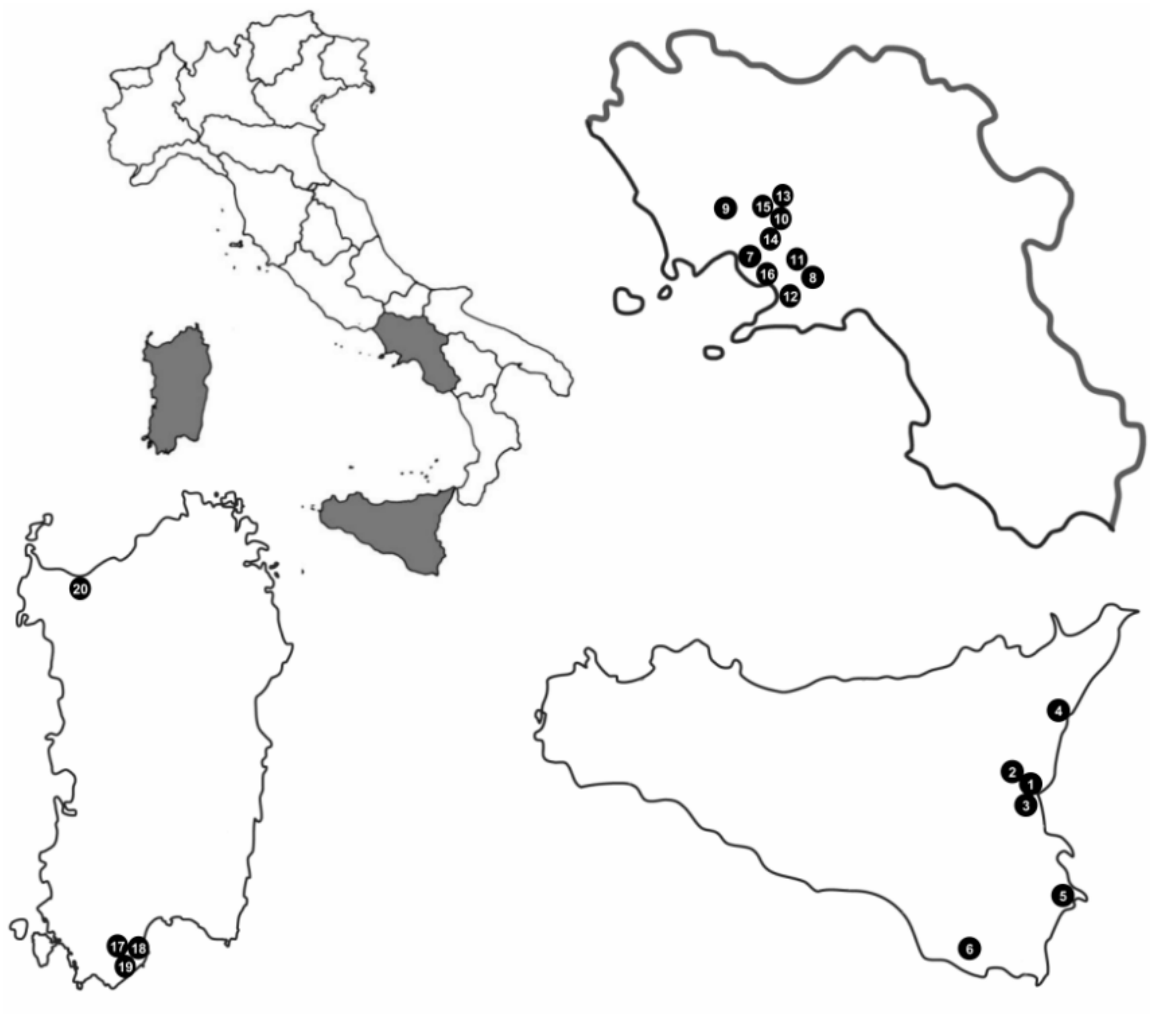

Figure 1. Geographical distribution of the survey sites (2009-11).

of *T. absoluta* and parasitoid larvae and pupae. Foliage with apparently healthy *T. absoluta* larvae was stored in aerated boxes to isolate possible emerging adult parasitoids. In addition, tomato apical shoots were collected to search for *T. absoluta* eggs and assess the egg parasitoid activity. For this purpose, 100 eggs per sampling date were examined under a stereomicroscope and then stored in aerated tubes until parasitoid emergence.

Finally, sporadic field samplings were performed from September to October 2010 in sites 10-16 where both processing and fresh market tomato crops were grown. Only one sample was collected for each site and consisted of 100 leaves infested by *T. absoluta* larvae. Collected leaves were stored in aerated boxes to isolate emerging adult parasitoids.

In site 17, samples were collected from June to July 2010 on a cherry-type tomato crop (cv. Minuetto) grown in a plastic greenhouse without insect-proof screens and transplanted in February 2010. The crop was sprayed with abamectin and spinosad at the recommended label rates on 23 April and 27 May, respectively. A total of 160, 100 and 50 infested leaves were collected on 23 June, 30 June and 7 July 2010, respectively and maintained in ventilated plexiglass cages (30 × 30 × 30 cm) in the laboratory until adult parasitoids emergence. Moreover, dead adults and larvae of *T. absoluta* were recorded in order to assess the parasitism rate, calculated as: emerged wasps / (emerged wasps + *T. absoluta* larvae + *T. absoluta* adults).

A second sampling was carried out on a tomato crop (cv. Minuetto) grown in a glasshouse with insect-proof screens and transplanted in October 2010 (table 1, site 18). Tomato plants were sprayed on 30 November with flonicamid and abamectin and on 21 December with emamectin benzoate at the recommended label rates. From November 2010 to May 2011, *T. absoluta* leaf damage, active infestation and natural parasitism were monitored weekly. The leaf damage was assessed by counting the number of mines on three randomly-chosen leaves per plant (basal, median, and apical) over 150 plants. To assess the active infestation of *T. absoluta* larvae (as the percentage of alive larvae on sampled mines) and the parasitism rate (calculated as: parasitised *T. absoluta* larvae / dead + alive + parasitised *T. absoluta* larvae), an additional sample of infested leaflets from basal, median, apical layers was collected and 70 mines from each layer (a total of 210 mines) were observed under a stereomicroscope. The number of dead and alive *T. absoluta* larvae and pre-imaginal stages of parasitoids was recorded. Parasitised larvae were iso-

Table 1. Description of *T. absoluta* parasitoid survey sites (2009-2011).

	Site Location	Ecological features	Sampling method
	Sicily		
1	Catania (CT) 37°31'8"N 15°4'18"E	Urban horticultural crops (vegetables) with scattered citrus and olive trees, abundant spontaneous flora, no pesticide treatments	Sentinel plant Field sampling from: - spontaneous flora - open field solanaceous crops
2	Catania (CT) 37°32'6"N 15°4'10"E	Uncultivated citrus orchards, scattered Mediterranean trees and shrubs (black locust tree, privet, European mettle tree, laurel), abundant spontaneous flora, no pesticide treatments	Sentinel plant Field sampling from: - spontaneous flora
3	Catania (CT) 37°28'40"N 15°3'32"E	Open field vegetables crops, scarce spontaneous flora, frequent pesticide treatments	Sentinel plant Field sampling from: - spontaneous flora - open field solanaceous crops
4	Fiumefreddo (CT) 37°46'53"N 15°12'24"E	Citrus and olive orchards, vineyards and vegetables cultivation in greenhouses, abundant spontaneous flora, organic farming	Sentinel plant Field sampling from: - spontaneous flora - greenhouse solanaceous crops
5	Siracusa (SR) 37°1'27"N 15°16'21"E	Citrus orchards and open field (potato) and protected (tomato) vegetable crops, abundant spontaneous flora, occasional pesticide application	Field sampling from: - spontaneous flora - open field solanaceous crops
6	Scicli (RG) 36°45'36"N 14°43'60"E	Protected vegetable crops, scattered olive and carob trees, abundant spontaneous flora, no pesticide applications, organic farming	Sentinel plant Field sampling from: - spontaneous flora - greenhouse solanaceous crops
	Campania		
7	Portici (NA) 40°48'54"N 14°21'05"E	Urban vegetables and ornamental crops, many species of fruit tree (citrus, olive, grapevine, apricot, etc.), holm oak (*Quercus ilex*) forest and abundant Mediterranean flora, no pesticide treatments nearby the sampling site	Continuous sampling by sentinel plants: - tomato SP infested with *T. absoluta* eggs - tomato SP infested with *T. absoluta* larvae
8	Pagani (SA) 40°46'10"N 14°36'55"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Continuous field sampling from: - greenhouse solanaceous crops open field tomato crop - spontaneous flora
9	Carinaro (CE) 41°0'06"N 14°16'36" E	Intensive greenhouse and field vegetable crops, orchards, IPM, spontaneous flora	Continuous field from: - greenhouse solanaceous crops spontaneous flora
10	Marigliano (NA) 40°56'54" N 14°27' 06"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - open field processing tomato crop
11	Poggiomarino (NA) 40°48'07"N 14°33'35"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - greenhouse solanaceous crops
12	Gragnano (NA) 40°41'46"N 14°30'33"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling (collection of infested material) from: - greenhouse solanaceous crops
13	Polvica (NA) 40°58'33"N 14°26'44"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - open field processing tomato crop
14	Somma Vesuviana (NA) 40°54'19"N 14°26'17"E	Intensive greenhouse and field vegetable crops, orchards, IPM	Occasional field sampling from: - greenhouse solanaceous crops
15	Acerra (NA) 40°58'02"N 14°24'19"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - greenhouse solanaceous crops
16	S. Maria la Bruna (NA) 40°45'49"N 14°24'18"E	Intensive greenhouse and field vegetable crops, ornamentals, frequent pesticide treatments	Occasional field sampling from: - greenhouse solanaceous crops
	Sardinia		
17	Pula (CA) 38°58'19"N 8°58'1"E	Vineyards and greenhouse (without insect-proof screens) tomato crops, abundant spontaneous flora. Occasional pesticide treatments	Field sampling from: - greenhouse solanaceous crops
18	Pula (CA) 38°58'19"N 8°57'55"E	Vineyards and glasshouse (with insect-proof screens) tomato crops, abundant spontaneous flora. Several pesticide treatments	Continuous field sampling from: - greenhouse solanaceous crops
19	Pula (CA) 38°57'25"N 8°57'08"E	Open field and greenhouse (with insect-proof screens) tomato crops, abundant spontaneous flora. Occasional pesticide treatments	Field sampling from: - greenhouse solanaceous crops
20	Sorso (SS) 40°48'31"N 8°29'47"E	Open field tomato and potato crops, abundant spontaneous flora. No pesticide treatments	Field sampling from: - open field solanaceous crops - spontaneous flora

lated in Petri dishes and kept under natural conditions of temperature, relative humidity and photoperiod until adult parasitoids emergence.

In addition, to detect pupal parasitoids, tomato fruits and leaves with *T. absoluta* pupae were collected from March to April 2011 in a plastic greenhouse with insect-proof screens (table 1, site 19). Pupae were placed in plexiglass cages, as described above, until emergence of either *T. absoluta* or adult parasitoids.

Finally, tomato borer infestations on an open field potato crop and *S. nigrum* weeds were monitored bi-weekly from November 2010 to May 2011 (table 1, site 20). Thus, 100 potato and *S. nigrum* leaves each were collected and the number of pre-imaginal stages of *T. absoluta* and parasitoids were recorded using a stereo-microscope. Since potato crops are often infested by the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera Gelechiidae), collected larvae were periodically reared until the emergence of adults and male genitalia were observed under a microscope to ensure the correct identification of the pest.

Results

Collected parasitoids

In the three surveyed regions of Southern Italy, parasitoid specimens belonging to 13 genera and 6 families (Ichneumonidae, Braconidae, Eulophidae, Elasmidae, Pteromalidae and Trichogrammatidae) were obtained and ten species were identified (table 2).

Parasitoids were collected in 14 out of 20 monitored sites, six from both Sicily and Campania and two from Sardinia. These records suggest a higher diversity of parasitoid species in sites characterized by the presence of abundant spontaneous vegetation (herbaceous, shrub and arboreal); eight species have been found in site 1, seven in site 6, four in site 7 and five in site 8 (table 2). On the other hand, parasitoids were obtained in Sardinia only from two of the three surveyed protected tomato crops (table 2). Overall, the species most abundantly recovered were *Necremnus* spp., *Bracon nigricans* Szepligeti and *Neochrysocharys formosa* (Westwood), together representing almost 70% of the specimens collected (table 2).

The essential taxonomic and biological features of the identified species are here reported.

Diadegma pulchripes (Kokujev) (= *turcator* Aubert) (Hymenoptera Ichneumonidae) is reported as endoparasitoid of mature larvae of *P. operculella* from Turkey, Cyprus, Israel, Crete and China (Pucci *et al.*, 2003; Yu and van Actherberg, 2010). In Italy, this parasitoid can constitute the major biotic mortality factor of this pest, representing up to 60% of the natural parasitism (Ortu and Floris, 1989). The wasp was introduced in India for controlling the potato tuber moth and its establishment has been proved one century later (Sankaran, 1974). In this survey, adults were obtained from *T. absoluta*-infested sentinel plants as well as from potato infested by the tomato borer in sites 1, 3 and 5 (table 2). This report is a new host-parasitoid association. The parasitoid was reared for few generations using *T. absoluta* as host, allowing laboratory observations which showed adult emergence from 4th instar larvae and pupae of the host.

Bracon osculator (Nees) (Hymenoptera Braconidae) has a Palaearctic distribution and has been recorded in almost all European countries and also in Afghanistan, Azerbaijan, Georgia, Iraq, Israel, Kazakhstan, Korea, Mongolia, Turkey and Turkmenistan. It is an idiobiont ectoparasitoid of Lepidoptera larvae belonging to the families Coleophoridae, Nepticulidae, Momphidae, Elachistidae, Choretidae, Gracillariidae, Cosmopterigidae and Tortricidae (Yu and van Actherberg, 2010). *B. osculator* has been already reported in mainland Italy on *Elachista bisulcella* (Duponchel) and *Elachista utonella* (Frey) (Lepidoptera Elachistidae) (Parenti *et al.*, 1995). This report is a new association of this species with *T. absoluta* and the first record of this braconid wasp in Sicily. During the survey, only adult females were obtained and exclusively from sentinel plants located in site 1 (table 2). Parasitoid larvae behaved as gregarious on *T. absoluta* larvae. *Bracon* species were already reported as *T. absoluta* parasitoids in the pest native areas; in Europe, an unidentified species of *Bracon* was reported from Spain (Desneux *et al.*, 2010).

Bracon (*Habrobracon*) *nigricans* Szepligeti [= *concolorans* Marshall; *concolor* Thomson; *mongolicus* (Telenga)] (Hymenoptera Braconidae) is widely distributed in the Palaearctic region and has been recorded almost all over Europe and also in Mongolia (Papp, 2009). It is a generalist larval ectoparasitoid of some Lepidoptera and one Coleoptera species. *B. nigricans* was found feeding on *P. operculella* in Sardinia (Ortu and Floris, 1989). Our report is a new association of *B. nigricans* with *T. absoluta* and the first record in Sicily. Several specimens of both sexes were collected, in the summer season, from sentinel plants located in site 1 and from open field tomato crop in site 3 (table 2). It was successfully reared for several generations with no apparent adverse effects on the parasitoid biological performances and a laboratory rearing is actually maintained to conduct studies on its main biological and behavioural traits and on its efficacy as *T. absoluta* biocontrol agent.

Necremnus sp. near *tidius* (Walker) (Hymenoptera Eulophidae) after the paper of Gibson *et al*. (2005), that reduced the limits of *N. tidius* to specimens reared on Coleoptera Curculionidae, this species is currently under revision by an integrative approach (Bernardo *et al.*, unpublished data). This parasitoid is a biparental generalist solitary ectophagous species of lepidopteran leafminers (Bernardo and Viggiani, 2003). It has been recovered on open field crops (sites 10 and 11) and in protected crops (site 17) (table 2). Another species of the same genus was detected and provisionally indicated as *Necremnus* sp. near *artynes* (Walker) (Hymenoptera Eulophidae). *N. artynes* is a biparental generalist solitary ectophagous parasitoid of lepidopteran leafminers. Its primary host is *Cosmopterix pulchrimella* Chambers (Lepidoptera Cosmopterigidae), a leafminer attacking *Parietaria diffusa* M. et K., a species very common in Italy. *N. artynes* was recently reported as a parasitoid of *T. absoluta* in Spain (Desneux *et al.*, 2010). *Necremnus* sp. near *artynes* here

Table 2. Parasitoid taxa recovered from *T. absoluta* during the survey (2009-2011). Sampling method: SP = Sentinel plant; OF = Open field collection; PC = Protected crop collection.

Parasitoid species	Sampling method	Site	Month	Host plant	N. of collected specimens (sex ratio: males/tot)	Parasitoid community composition (%)
Larval parasitoids						
Family Ichneumonidae						
Diadegma pulchripes	SP	1	Sept., Nov.	Tomato	14 (0.4)	6.1
	SP	3	September	Tomato		
	OF	5	June	Potato		
Diadegma sp.	OF	10	October	Tomato	1 (0)	0.4
Cryptinae gen. sp.	SP	2	May	Tomato	4 (0.5)	1.7
		6	April	Tomato		
Family Braconidae						
Bracon osculator	SP	1	November	Tomato	2 (0)	0.9
Bracon nigricans	SP	1	July-Sept.	Tomato	25 (0.2)	10.8
	OF	3	August	Tomato		
Family Eulophidae						
Chrysocharis sp.	SP	1	October	Tomato	5 (0)	2.2
	SP	7	May	Tomato		
	PC	8	September	Tomato		
Elachertus sp.	SP	1	October	Tomato	1 (0)	0.4
Elachertus inunctus species group	SP	7	Apr.-May	Tomato	19 (0.8)	8.2
Necremnus sp.	OF	3	August	Tomato	3 (0.7)	1.3
	OF	8	October	Tomato		
Necremnus sp. near *artynes*	SP	6	May	Tomato	89 (0.4)	38.5
	OF	3	August	Tomato		
	OF	8	September	Tomato		
	OF	11	October	Tomato		
	PC	12	October	Tomato		
	OF	10	October	Tomato		
	PC	17	June	Tomato		
	PC	18	Oct., June	Tomato		
Necremnus sp. near *tidius*	OF	10	October	Tomato	8 (0.5)	3.5
	OF	11	October	Tomato		
	PC	17	June	Tomato		
Neochrysocharis formosa	SP	1	April, Oct.	Tomato	28 (0.4)	12.1
	SP	2	Sept.-Oct.	Tomato		
	SP	4	September	Tomato		
	OF	3	August	Tomato		
	PC	6	February	Tomato		
	OF	13	February	Tomato		
Pnigalio sp. a gr. *soemius*	SP	1	May-Sept.	Tomato	6 (0.5)	2.6
		2		Tomato		
		6		Tomato		
Pnigalio sp. b gr. *soemius*	PC	8	June	Tomato	1 (0)	0.4
Pnigalio cristatus	SP	1	June-Sept.	Tomato	2 (0)	0.9
Pnigalio incompletus	PC	8	June	Tomato	1 (1)	0.4
Sympiesis sp.	SP	1	June-Sept.	Tomato	4 (0.5)	1.7
		6		Tomato		
Family Elasmidae						
Elasmus sp.	SP	2	September	Tomato	4 (0.7)	1.7
	OF	3	August	Tomato		
Family Pteromalidae						
Halticoptera aenea	SP	6	May	Tomato	1 (0)	0.4
Egg parasitoids						
Family Trichogrammatidae						
Trichogramma sp. a	SP	7	October	Tomato	1 (0)	0.4
Trichogramma sp. b	SP	7	October	Tomato	1 (0)	0.4
Trichogramma sp. a and b	SP	7	October	Tomato	6 (1)	2.6
Trichogramma sp. c	OF	6	May	Tomato	4 (0.5)	1.7
Trichogramma sp. d	PC	18	March	Tomato	1 (1)	0.4

collected is the only species, among those recovered in this survey that was found in the three regions, both on sentinel plants, open field and protected crops (table 2). This species is at moment under revision (Bernardo *et al.*, unpublished data).

Neochrysocharis formosa (Westwood) [= *Closterocerus formosus* (Westwood)] (Hymenoptera Eulophidae) is a generalist parasitoid with a cosmopolitan distribution (Burks *et al.*, 2011). It develops as primary solitary or gregarious larval endoparasitoid of a wide range of leafmining or stemboring Coleoptera, Diptera and Lepidoptera associated to various cultivated and spontaneous plants (Noyes, 2003). The species is widespread in Italy on various crop pests, such as *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) (Massa *et al.*, 2001) and *Holocacista rivillei* Stainton (Lepidoptera Heliozelidae) (Alma, 1995). It is regarded as a valuable natural enemy in South America and recently a parasitisation up to 5% of tomato borer larvae, predominantly during the late season, was reported in Argentina (Luna *et al.*, 2011). Also this morphospecies could represent a complex of at least two cryptic species, as pointed out by preliminary results (Adachi-Hagimori *et al*., 2011). This is the first report for this species on *T. absoluta* in the Mediterranean basin. During the survey, various specimens of both sexes of *N. formosa* were found, all year round, on tomato borer both collected in field sampling (open field and protected crops) and sentinel plants from Campania and Sicily (sites 1, 2, 3, 4, 6 and 13) (table 2).

Pnigalio soemius s.l. (Walker) (Hymenoptera Eulophidae) is a very common Palaearctic species parasitising over 130 leafminers and gall makers belonging to Coleoptera, Diptera, Lepidoptera and Hymenoptera, many of which considered of economic interest (Noyes, 2003; Bernardo *et al.*, 2008). In consideration of the high phenotypic intra-specific variation and the coexistence of cryptic species, the whole *P. soemius* "taxonomic concept" is currently under revision also by molecular and behavioural approaches (Bernardo *et al.*, 2008; Gebiola *et al.*, 2012). Preliminary data suggest that *P. soemius* s.l. is a complex of generalist and stenophagous species, with an intense predatory behaviour both as larva and adult (host feeding and host killing). This species complex includes resilient and potentially effective natural enemies in different agricultural ecosystems in Mediterranean environment (Bernardo *et al.*, 2006; Gebiola *et al.*, 2012). Also in this case, the report is a new host-parasitoid association. Specimens collected belong at least to two different cryptic species; a few couples of one species were obtained from sentinel plants exposed in sites 1, 2 and 6, while a second species emerged from infested material collected on protected tomato crop (site 8) (table 2).

Pnigalio cristatus (= *Ratzeburgiola cristata*) (Ratzeburg) (Hymenoptera Eulophidae) has been recently synonymised on the basis of molecular and comparative morphological analysis with related species (Gebiola *et al.*, 2010). It is a European species reported on various Coleoptera, Diptera and Lepidoptera leafminers feeding on spontaneous and cultivated plants, such as *P. citrella* (Massa *et al.*, 2001; Noyes, 2003; Vercher *et al.*, 2005). Based on currently available literature, this species is reported as associated to *T. absoluta* for the first time. A few specimens of both sexes were found on sentinel plants in site 1 (table 2).

Pnigalio incompletus (= *Ratzeburgiola incompleta*) (Boucek) (Hymenoptera Eulophidae) has been recently synonymized on the basis of molecular and comparative morphological analysis with related species (Gebiola *et al.*, 2010). Also in this case, preliminary results suggest that this is a complex of monophagous species. It is an ectophagous solitary (rarely gregarious) parasitoid of several leafminers (Diptera, Lepidoptera and Coleoptera) feeding on spontaneous and cultivated plants; it is often reared in association with *P. cristatus*, with which it shares many hosts (Gebiola *et al*., 2010). Also in this case on the basis of currently available literature, this species is for the first time reported on *T. absoluta* and it was recovered from protected tomato crop in the site 7 (table 2).

Halticoptera aenea (Walker) (Hymenoptera Pteromalidae) is a cosmopolitan polyphagous species associated mainly with Diptera leafminers (Agromyzidae) infesting various plants also of economic interest, trees and shrubs; it is an endoparasitoid emerging from pupae of the host (Noyes, 2003). Only a female of this species was obtained from one *T. absoluta* larva on a sentinel plant in site 6 (table 2); the report is a new host-parasitoid association.

Parasitism rates by *Necremnus* spp. in greenhouse

Percentages of parasitism by *Necremnus* spp. in a tomato crop under greenhouse without insect-proof screen (site 17) were in general low throughout the cropping season, ranging from 5 to 14% (table 3). *T. absoluta* density (estimated as emerged parasitoids + *T. absoluta* larvae + *T. absoluta* adults) ranged from 1.6 to 6.77 individuals/leaf (table 3).

In the other monitored glasshouse, insect-proof screened (site 18), *Necremnus* sp. near *artynes* was the only larval parasitoid emerged from samples collected in 2010-2011 (table 4). However, parasitism rates never exceeded 8% across the cropping cycle (figure 2). The infestation levels (measured as percentage of mines with feeding larvae) were > 40% most of the season, although showing at least three peaks on 21 December (75%), 2 March (65%), and 15 April (66%). After two insecticide applications, flonicamid plus abamectin and emamectin benzoate respectively, at the beginning of the season, the larval infestation was partially reduced. Afterwards, the active infestation increased progressively (figure 2). The infestation ranged from 2 mines/leaf (14 December) to a minimum of 0.5 mines/leaf (2 March), increasing afterwards to 2.8 mines/leaf on 4 May. The seasonal pattern of *T. absoluta* infestation on leaves was affected by the frequent pruning of basal leaves. *T. absoluta* dead larvae by host feeding were also recorded, although this type of mortality never exceeded 6%. Beside the larval ectoparasitoid, an egg parasitoid, *Trichogramma* sp., emerged from leaf samples collected in this site (table 2).

Table 3. Density of *T. absoluta* per leaf and parasitism rate [wasps / (emerged wasps + *T. absoluta* larvae + *T. absoluta* adults)] of *Necremnus* sp. near *artynes* and *Necremnus* sp. near *tidius* in a greenhouse without insect-proof screens (Sardinia, 2010, site 17).

	T. absoluta instars/leaf (n.)	*Necremnus* sp. near *artynes* parasitism (%)	*Necremnus* sp. near *tidius* parasitism (%)
June 23, 2010	6.77	11.3	2.9
June 30, 2010	1.64	4	1
July 7, 2010	1.60	7.3	1.9

Table 4. Number of *T. absoluta* larvae (alive and parasitised) and *Necremnus* sp. near *artynes* adults emerged from parasitised larvae collected in a glasshouse with insect-proof screens (Sardinia, 2010-2011, site 18).

Date	*T. absoluta* larvae	*Necremnus* sp. near *artynes* emerged adults	
		Total number	Sex ratio (males/total)
November 23, 2010	78	1	1
December 1, 2010	100	2	0.5
December 7, 2010	121	2	1
February 23, 2011	108	5	0.6
March 9, 2011	115	6	0.5
March 17, 2011	80	1	1
March 23, 2011	63	5	0.8
March 30, 2011	122	8	0.6
April 7, 2011	176	2	0
April 15, 2011	231	10	0.3
April 21, 2011	178	2	0
May 4, 2011	191	7	0.6

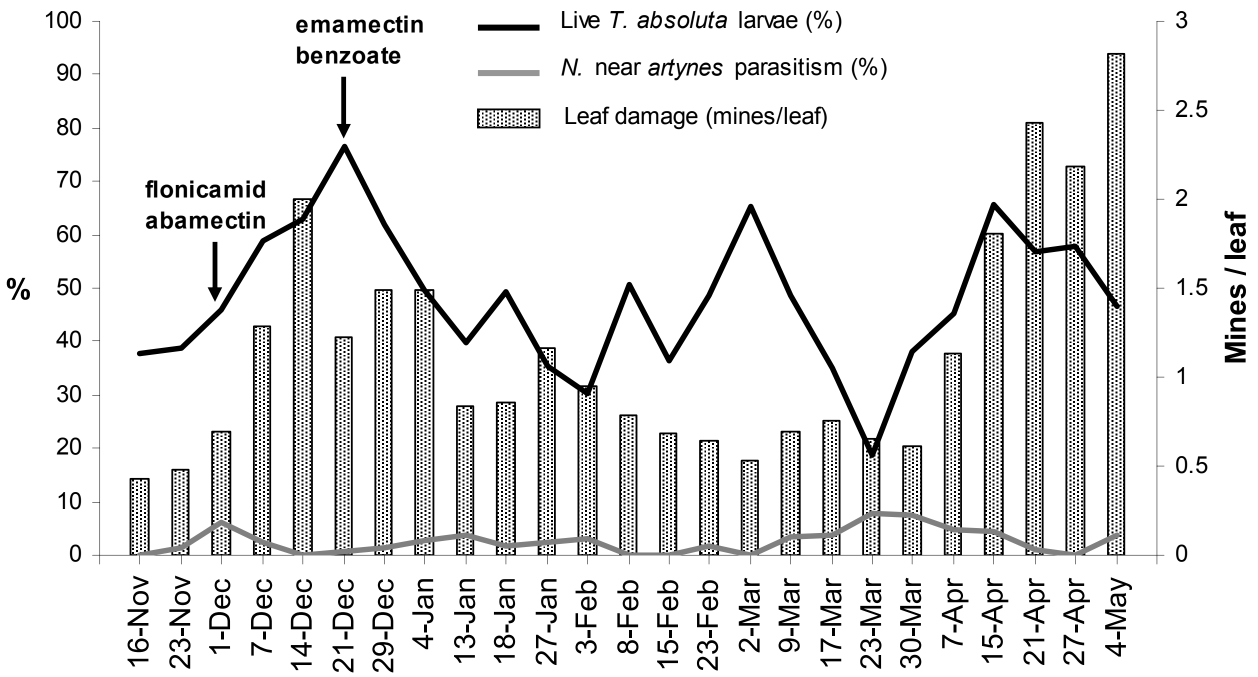

Figure 2. Seasonal pattern of active infestation of *T. absoluta* larvae, natural parasitism rate by *Necremnus* sp. near *artynes* and leaf damage in a glasshouse with insect-proof screens (Sardinia 2010-2011, site 18). Arrows indicate dates of insecticide applications.

Discussion and conclusions

The results obtained from this survey of indigenous parasitoids of *T. absoluta* conducted in a variety of different habitats (open field and protected tomato crops, other solanaceous crops and spontaneous vegetation) in Southern Italy, provided novel and relevant information contributing to the knowledge of this exotic pest in the Mediterranean basin.

Particularly, some major points are worth being mentioned. First, a prompt adaptation of native parasitoids to the new invasive host was observed, as highlighted by the natural parasitism recorded few years after the first detection of the moth. Overall six families (Ichneumonidae, Braconidae, Eulophidae, Elasmidae, Pteromalidae and Trichogrammatidae) with 13 genera and 10 identified species were recorded and in particular, the family Eulophidae was the most abundant in terms of number of species recovered. Besides, comparing *T. absoluta* parasitoid composition in Italy with that of South America, similarities arise in terms of guilds (egg, early larval, mature larval, ecto/endo, pupal, gregarious/solitary parasitoids) and families, although the number of species recorded is lower (Luna *et al.*, 2007; Desneux *et al.*, 2010). This lower species richness is typical of parasitisation pattern on exotic invasive herbivorous species, performed mainly by generalist idiobionts with relatively low levels of parasitisation in open field (Cornell and Hawkins, 1993). However, the detection of seven new associations between *T. absoluta* and the species *D. pulchripes*, *B. osculator*, *B. nigricans*, *P. soemius*, *P. cristatus*, *P. incompletus*, and *H. aenea* is noteworthy. Furthermore, *N. formosa* is the only species currently recovered on *T. absoluta* both in Europe and in South America, where it was mentioned as a potential biocontrol agent based on its previous use in other crops (Luna *et al.*, 2011). Overall, the low parasitism rate found in this survey may not support the role of the indigenous parasitoid community in effectively controlling *T. absoluta*. However, previous biological control programs of several exotic pests demonstrated the importance of indigenous natural control agents in the regulation of pest populations (Viggiani, 2000).

Species with concealed habit are among the most attractive hosts for parasitoids and one of the most recent examples of invasive leafminer species in the Mediterranean countries is *P. citrella*. Despite *T. absoluta* and *P. citrella* occupy different ecological niches, there are some interesting analogies between them. Four out of the 12 eulophids collected on *T. absoluta* were also recorded on *P. citrella* in the Mediterranean basin (Massa *et al.*, 2001; Vercher *et al.*, 2005). These parasitoid species may also develop on alternative hosts living on spontaneous flora which is very common also in the sites here surveyed. The data collected indicated that the abundance of parasitoid species was generally connected with the presence of spontaneous flora and evergreen crops as critical component of functional biodiversity. Although additional assessment of potential role of biodiversity abundance and habitat management techniques should be conducted to confirm this hypothesis, the pest populations can be reduced by enhancing the efficacy and local abundance of the existing natural enemies' community by landscape management (Gardiner *et al.*, 2009). Thus, we encourage further investigations to enhance the native parasitoid community activity through a rational habitat management within the crop as well as within the farm (Landis *et al.*, 2000). For example, evidences of negative effects of leaf pruning found in this study, which causes the removal from greenhouses of *T. absoluta* larvae suitable for parasitisation, were highlighted and a possible solution could be keeping pruned material inside greenhouses into selective mesh cages, allowing only parasitoids to emerge and move onto the crop. The increase in the abundance and diversity of the natural enemy community could be also obtained by the use of '*banker plants*', a tri-trophic system which typically consists of a non-crop plant that is deliberately infested with a non-pest herbivore (Frank, 2010). This technique was already successfully tested on tomato to control the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Rhynchota Aleyrodidae) and Diptera leafminers, *Liriomyza* spp. (van der Linden, 1992; Lambert *et al.*, 2005). However, negative interactions, such as intraguild predation, among natural enemy species may result in a long-term reduction of their ability to suppress pest populations, especially when increasing the species richness in an agricultural context (Straub *et al.*, 2008).

In this framework, improving the knowledge of endemic parasitoid host communities, still largely unknown, and of the performances of some selected key parasitoid species, both endemic and exotic, would be essential to address future studies. Besides, considering the closeness between the two moths, it could be interesting to better investigate the parasitoid complex of the potato tuber moth, *P. operculella*, for its potential role as source of parasitoids for the tomato borer. This is the case of *Copidosoma koehleri* Blanchard (Hymenoptera Encyrtidae), *B. nigricans* and *D. pulchripes*. The first species is an egg-larval parasitoid of Nearctic origin, reported in Chile on *T. absoluta* (Desneux *et al.*, 2010) and which has been recently reintroduced in Italy to control the potato tuber moth (Guerrieri and Noyes, 2005), while the two Ichneumonoidea species that were found in this survey on *T. absoluta* had already been reported as *P. operculella* natural enemies.

Necremnus sp. near *artynes*, was recovered in all the monitored regions, from May to October, and its activity could be related to the presence, in the sampled sites, of its primary hosts such as *C. pulchrimella* on *P. diffusa* (Bernardo and Viggiani, 2003). Even if this species was the most abundant and widespread, among the recovered parasitoids, its low parasitism rate does not suggest to consider this parasitoid as a key species in *T. absoluta* biological control in the Mediterranean basin. By contrast, *Necremnus* sp. near *artynes* was the only species, among those recovered, able to build up the population in treated protected tomato crops. This finding should be taken into account when applying tomato IPM programmes that should emphasize the role of natural mortality factors by selective pesticides application, by regular monitoring (both pest and its natural enemies) and by applying economic thresholds (van

Lenteren and Woets, 1988). In conclusion, information collected in this paper, supported by the theory on recruitment and accumulation of native parasitoid species on introduced herbivores (Cornell and Hawkins, 1993), may open to interesting perspective on *T. absoluta* conservation biocontrol in the Mediterranean basin.

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