BIOTIC MORTALITY FACTORS IN FIELD POPULATIONS OF *TUTA ABSOLUTA* MEYRICK (LEPIDOPTERA: GELECHIIDAE) IN SOUTH BULGARIA

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Abstract

The tomato leafminer *Tuta absoluta* Meyrick is an invasive species for Europe, North Africa and the Middle East, attacking cultivated and wild *Solanaceae* plants. After it was recorded in Bulgaria in 2009 it spread in all tomato growing regions of the country. The aim of the study was to establish the biotic mortality factors, influencing the field population of the leaf miner in the region of Plovdiv (South Bulgaria). The mortality, caused by predators, parasitoids and pathogens varried from 12.59% in August 2011 to 45.65% in September 2013.

During the years the mortality increased from 19.38% in 2011 to 31.57% in 2013. Three insect species (*Macrolophus pygmaeus, Nabis* sp. and *Neochysocharis formosa*), and one mite species (*Trombidium* sp.) were found to feed on the eggs and larvae of the moth. A fungal pathogen (*B. bassiana)* was isolated from the larvae. For the larval parasitoid *N. formosa* the tomato leafminer is a new host in Bulgaria. In laboratory tests aimed at evaluating the consumption abilities of the most numerous predator *M. Pygmaeus*, the nymph killed an average of 40.75 ± 1.61 eggs or 2.25 ± 0.45 larvae of the leaf miner per day, and the adult – respectively 75.88 ± 1.55 and 4.88 ± 0.61.

Key words: tomato leafminer, parasitoids, predators, entomopathogenic fungi, mortality rate.

INTRODUCTION

The tomato leafminer *Tuta absoluta* (Meyrick, 1917) is originating from South America and is an invasive pest on cultivated and wild Solanaceae plants in Europe, North Africa and the Middle East. It develops many generations per year and attacks plants throughout the entire growing season. The moth damages all aerial parts of the tomato plants (leaves, stems, flowers and fruits) which makes it especially dangerous pest capable of causing to 100% loss of yield (Vargas, 1970; Estay, 2000).

The biotic mortality factors were studied in both the center of origin and the new area of distribution. A variety of predators and parasitoids attacking eggs, larvae or pupae of the tomato leafminer was reported In South America (Uchôa-Fernandes and Campos, 1993; Colomo et al., 2002; Marchiori et al., 2004; Colomo and Berta, 2006). After the established of the pest in the Mediterranean basin, there are reports for a range of local natural enemies, the suitability of which as biological control agents is currently being evaluated. Many parasitoids belonging to families Eulophidae, Braconidae, Ichneumonidae, Pteromalidae, Chalcididae and Trichogrammatidae (Hymenoptera) have been established, along with predatory species from families Miridae, Anthocoridae, Nabidae (Heteroptera), Chrysopidae (Neuroptera), Sphesidae, Formicidae (Hymenoptera) and Phytoseiidae (Acari: Mesostigmata) (Zappalà et al., 2013). Some pathogenic microorganisms (*Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisoplia)* were established in the places of distribution to causes diseases on *T. absoluta* (Giustolin et al., 2001; Rodriguez et al., 2006a; Inanli et al., 2012).

Predatory bugs as *Macrolophus pygmaeus* Rambur and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) are the most common predators on the tomato plants. Under laboratory conditions both predators feed on the eggs and the larvae of the moth. The adults consume an average of 100 eggs/day, and the nymph of *M. pygmaeus* eats fewer eggs than the nymph of *N. tenuis* (Arnó et al., 2009). On the basis of 281 different observations on tomato plants grown in greenhouses and in open field Arnó et al. (2009) estimated that the presence of 4.5 predatory bugs on plant reduced the level of damage to below 4%. According to studies of Mollá et al. (2009), when both predators were established well in a crop they were able to reduce the attack on the leaves by 75-97%, and on the fruits - by 56-100%.

One of the most widespread larval parasitoids is *Neochrysocharis formosa* Westwood (Hymenoptera: Eulophidae) which was reported to parasitize the larvae of *T. absoluta*, both in Europe and in South America (Zappalà et al., 2013; Luna et al., 2011). It has a cosmopolitan distribution and is not found only in Australia. More than 100 species from Coleoptera, Hemiptera, Diptera, Lepidoptera and Hymenoptera (National History Museum, 2013) have been reported as hosts. It is an idiobiont-endoparasitoid and lays its eggs inside the host body where the larvae develop. Mature larvae leave the dead body of the host and pupate in the mines. The adults emerge after eight days (Osmankhil et al., 2010).

In Bulgaria *Neochrysocharis formosa* was reported by Pelov et al. (1993), Pelov and Tomov (1998), Radeva et al. (1999), Kutinkova and Andreev (2001), Tomov (2001) – such as *Achrysocharella formosa* Westwood and by Boyadzhiev (2003, 2004) - such as *Nechrysocharis formosus* Westwood. Without of the Boyadzhiev's reports, other authors have published it as a part of the beneficial entomofauna in Bulgaria and as a bio-agent against the leaf miners (Lepidoptera: *Leucoptera malifoliella* Costa (Lyonetiidae), *Phyllonorycter blancardella* Fabricius, *Phyllonorycter corylifoliella* Hübner (Gracillariidae) and *Stigmella malella* Stt. (Nepticulidae)) on the fruit trees.

Despite the importance of entomopathogenic fungi as *Beauveria bassiana* Vuillemin and *Metarhizium anisopliae* Sorokin (Sordariomycetes: Clavicipitaceae) (Giustolin et al., 2001; Rodriguez et al., 2006a, 2006b) their impact on *T. absoluta* is relatively poorly documented in South America. Some results demonstrate high pathogenicity of *B. bassiana* on larvae (L1–L4) - 95.0%, 73.3%, 80.8% and 38.7% respectively (Giustolin et al., 2001). Rodriguez et al. (2006a) studied the pathogenicity of the two entomopathogens on the eggs of the tomato leafminer and established that *B. bassiana* (80.00%) has a greater ability of infecting than *M. anisopliae* (60.00%). Recently, the studies in Europe showed that *M. anisopliae* has an effect both on the eggs and the larvae, first instar, while *B. bassiana* infects mainly the eggs (Inanli et al., 2012).

It is obvious that biological control could successfully complement other strategies for control of the pest, including sex pheromone based-plant resistance, and chemical control. Being an alien pest for Europe, studies are needed about the local predators, parasitoids and pathogens which could influence its population density in the different countries.

In this connection the aim of the present study was to establish the biotic mortality factors, influencing the field population of the tomato leafminerr in the region of Plovdiv.

MATERIALS AND METHODS

The studies were conducted in 2011-2013 in the region of Plovdiv, South Bulgaria in different field tomato crops. For establishing the predatory and parasitoid species and pathogens, attacking different stages of *T. absoluta*, surveys were conducted weekly in not treated with pesticides tomato plots and on Solanaceae weeds. Predatory insect and mite species were collected using visual observation and method of beating. Damaged leaves with mines and larvae of *Tuta absoluta* were examined at the laboratory and the total number of mines, mines with larvae and number of sick or dead larvae was recorded. Each mined leaf with larva inside the mine was placed separately in a Petri dish and the larva was reared under lab conditions (25±2ºC, RH 50–60% and 16:8 h L:D) till emergence of adult moth or parasitoid, or death. Emerged adults of parasitoids were counted and stored in 70% ethanol. Parasitoids were identified at the Department of Zoology, University of Plovdiv. The rates of parasitism was calculated as percent of all larvae and pupae. The larvae with symptoms of disease were placed individually in Petri dishes and observed daily until death. The larvae with symptoms of mycosis after the death were stored at 6°C and subsequently sent for identification of the pathogen to the Institute of Soil Science, Agricultural Technology and Plant Protection "N. Pushkarov", Sofia.

In 2013 the predatory potential of nymphs and adults of the predatory bug *M. pygmaeus* (Heteroptera: Miridae) when fed on eggs and larvae of *T. abso*luta was tested under laboratory conditions. Couples of *T. absoluta* were released to mate and lay eggs on laboratory grown tomato plants, placed separately in plastic cages (20x20x30 cm), covered with organza. After two days the adults were removed from the cages, and the plants were inspected for eggs. The consumption abilities of the predator's nymphs were tested on 150 eggs/plant, and of the adults – on 200 eggs/plant. To determine the predatory potential when fed on young larvae of the moth, the laid eggs on plants were observed daily to record the beginning of hatching. On the next day after hatching of the moth's larvae, a nymph or an adult of the predator wes released singly in each cage. After one day of feeding the plants were inspected again and the dead or alive eggs and larvae of the moth were counted and recorded. The experiment was set in four variants with eight replications for variant. The variants were: feeding (1) adult of the predator with eggs of the moth; (2) adult of the predator with larvae of the moth; (3) nymph of the predator with eggs of the moth; (4)nymph of the predator with larvae of the moth. *M. pygmaeus* nymphs and adults were collected from the field.

The results were analyzed using a package for statistical analysis SPSS 13.0 for Windows.

RESULTS AND DISCUSSION

In the region of Plovdiv in field tomato crops in 2011-2013 in which chemical pesticides were not applied, a total of 6 species, feeding on *Tuta absoluta* were found: 2 species of predatory bugs (Miridae and Nabidae), 1 parasitoid species from family Eulophidae, 1 predatory mite species from family Trombidiidae, an entomopathogenic bacteria (Bacillaceae) and an entomopathogenic fungus (Clavicipitaceae) (Tabl. 1). The predatory bug *Campylomma verbasci* (Miridae) was quite common on tomato plants, infested by *T. absoluta*, but at laboratory experiments it did not attack or eat eggs or larave of the moth and on the base of that it was excluded from the list of the predators of *T. absoluta.*

An increasing percent mortality of *Tuta absoluta* in the region of Plovdiv due to biotic mortality factors was observed during the years of the experiment (2011-2013). The rate of mortality was greates in September in all three years of the experiment, when the population density of the moth in the field was highest.

In 2011 from the sampled 700 mined leaves, a total of 640 larvae of the moth were found, of them 124 dead or sucked by predatory bugs. The highest percent mortality was recorded in September (23,84%), which coinsides with the high population density of the pest and the favorable climatic conditions (average monthly temperature 21,2°C; RH – 63%) for the development of the beneficial insects. The percent mortality was lowest in August (12,59%) (average monthly temperature 23,7ºC and RH – 64%). For the three-month period of the observations the percent mortality was 19,38% (Fig. 1).

Table 1. Species composition of predator, parasitoid and entomopathogens, attacking stages of *T. absoluta* at field conditions in the region of Plovdiv in 2011-2013

Class	Order	Family	Species
Insecta	Heteroptera	Miridae	*Macrolophus pygmaeus* Rambur
		Nabidae	*Nabis* sp.
	Hymenoptera	Eulophidae	*Neochysocharis formosa* Westwood
Arachnida	Prostigmata	Trombidiidae	*Trombidium sp*.
Bacilli	Bacillales	Bacillaceae	*Bacillus thuringiensis* Berliner
Fungi	Ascomycota	Clavicipitaceae	*Beauveria bassiana* Vuill

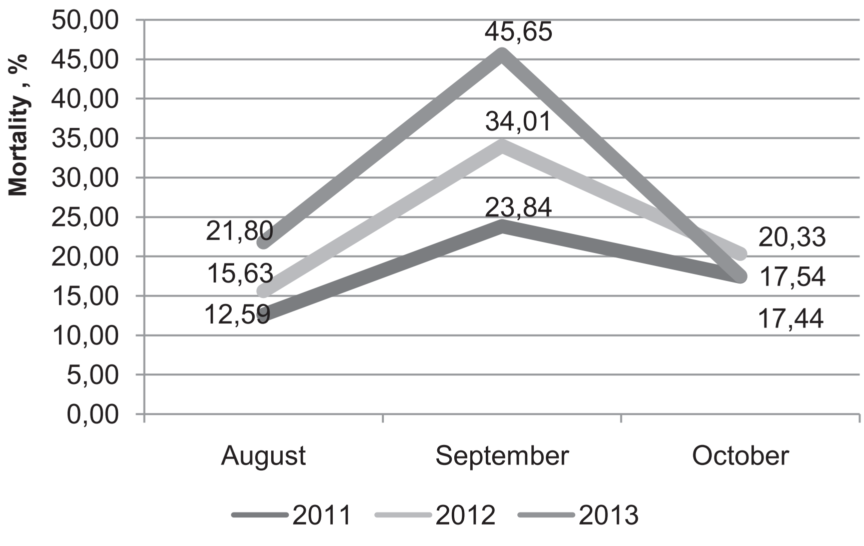

Fig. 1. *Rate of mortality of T. absoluta due to biotic factors at field condition in the region of Plovdiv in 2011-2013*

In 2012 from the sampled 900 mined leaves, 766 larvae were found, of them 185 dead. The percent mortality was higher compared to the previous year – 24,15% Fig. 1). In September the mortality was 30,01% (at average monthly temperature 20,6ºC and RH 61%), and much lower in August (15,63%) (average monthly temperature 25,1ºC and RH – 51% (Fig. 1).

The tendency of increase in percent mortality in the field population of *T. absoluta* continued in 2013, reaching the highest level for the three – year period of observations - 31,57% (Fig. 1). Out of 700 mined leaves sampled, 567 were with larvae inside, of them 179 dead. The results, recorded for each month showed highest mortality again in September – 45,63% (average monthly temperature 19,7ºC and RH – 55%), but was lowest in October – 17,54%. By the end of the vegetation period the mortality dropped down to the levels in 2011 which was attributed to the sudden decrease of the daily temperatures in October 2013.

All adult parasitoids emerged at the lab, were identified as *Neochrysocharis formosa* Westwood (Hymenoptera: Eulophidae) (Fig. 2-4). The species has already been reported as parasitizing the larvae of the tomato leafminer in Europe (Zappalà et al., 2013), and in South America (Luna et al., 2011). In Bulgaria so far this species has been izolated only from larvae of leafmining moths on fruit trees.

Fig. 2. *Larva of Neochrysocharis formosa*

Fig. 3. *Pupa of Neochrysocharis formosa*

Fig. 4. *Adult of Neochrysocharis formosa*

Fig. 5. *Beauveria bassiana* on larva of *T. absoluta*

It was estabished that the parasitoid attacks L2 larvae, and its larva is developing inside the hosts body. At completion of larval stage, it leaves the dead body of the host and pupates next to it or inside the mine.

The mean rate of parasitism was quite low – from 0,99 ± 0,50 in August 2011 to 5,22 ± 0,83 in September 2013 (Table 2). A tendency of increase was observed during the years – 1,14 ± 0,30 in 2011, 2,79 ± 0,55 in 2012 to 3,64 ± 0,86 in 2013.

The fungal pathogen causing death to some of the larvae in the field population of the moth in the region of Plovdiv was identified as *Beauveria bassiana* (Fig. 5). The infected larvae had the typical symptoms for the disease – retarded locomotion, supressed feeding, leaving the mines, and after the death, the corpse hardened and was covered by the fastgrowing white mycellium of the fungus.

Preliminary observations showed that the predatory bug *M. pygmaeus* feeds on eggs (Fig. 6) and young larvae (Fig. 7) of the moth, including those inside the mines, but did not attack older larvae (L3-L4) and eventually dies of starvation. The same was reported by Urbaneja et al. (2009), which was the reason to include in the experiment for consumption abilities only eggs and young larvae L1-L2 of *T. absoluta.*

Table 2. Rate of parasitism on the larvae ot *T. absoluta* at field conditions in the region of Plovdiv in 2011-2013

Year/Month		N	Min, %	Max, %	Mean, %	Std. Deviation
2011	August	3	0	1,74	0,99 ± 0,50	0,87
	September	3	0,73	1,96	1,38 ± 0,36	0,62
	Total	6	0	1,96	1,14 ± 0,30 b*	0,72
2012	August	3	1,09	2,06	1,67 ± 0,29	0,51
	September	3	3,22	4,63	3,92 ± 0,41	0,71
	Total	6	1,09	4,63	2,79 ± 0,55 ab	1,35
2013	August	3	1,35	2,41	1,92 ± 0,31	0,53
	September	3	3,7	6,36	5,22 ± 0,83	1,44
	Total	6	1,35	6,36	3,64 ± 0,86 a	2,12

**a, b – Duncan's multiple test, P = 0,05*

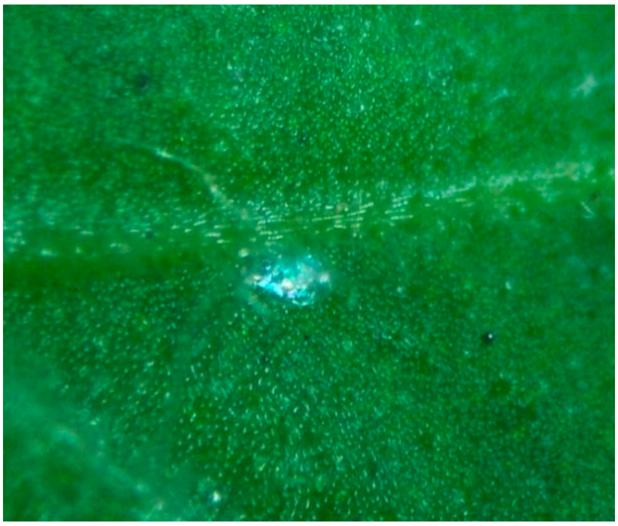

Fig. 6. *Egg of T. abosluta sucked by M. pygmaeus*

Fig. 7. *Larva of T. abosluta sucked by M. pygmaeus*

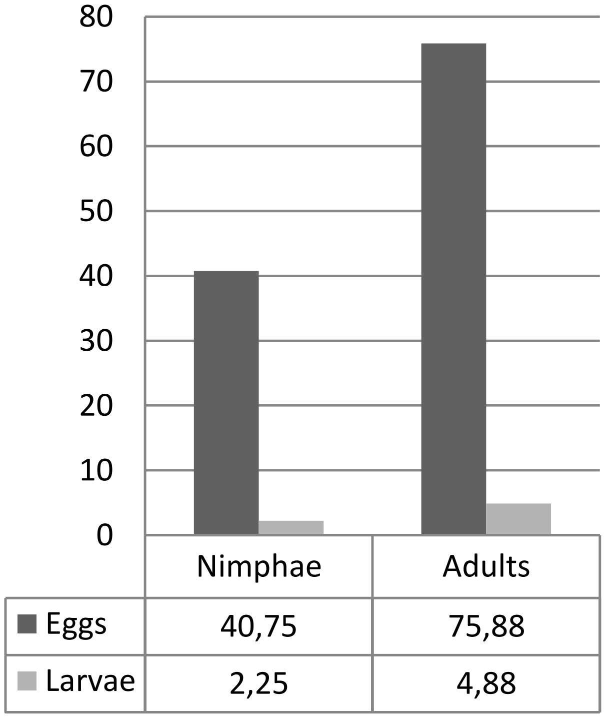

Fig. 8. *Average daily consumption ability of nymphs and adults of Macrolophus pygmaeus fed on eggs and larvae (L1-L2) of T. abosluta*

At lab conditions the average daily consumption abilities of *Macrolophus pygmaeus* were: of the nymph - 40.75 ± 1.61 eggs or 2.25 ± 0.45 larvae of the pray per day; of the adult - 75.88 ± 1.55 eggs and 4.88 ± 0.61 larvae of the pray per day (Fig. 8).

CONCLUSIONS

1. The natural mortality of *T. absoluta* at field conditions caused by biotic factors varies from 12,59% in August 2011 to 45,65% in September 2013. During the years the natural mortality increased from 19,38% in 2011 to 31,57% in 2013.

2. A limited number of natural enemies were found – 6 predators, parasitoids and pathogens. Among them the parasitoid *N. formosa* and the fungal pathogen *B. bassiana* are reported for the first time in Bulgaria on larvae of *Tuta absoluta*.

3. The rate of parasitism by *N. formosa* at field conditions was comparatively low – from 1,14 ± 0,30% in 2011 to 3,64 ± 0,86 in 2013.

4. The average daily consumption abilities of the predatory bug *Macrolophus pygmaeus* at lab conditions was greater for the adults – reaching 40,75 ± 1,61 eggs or 2,25 ± 0,45 larvae.

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