

Tuta absoluta (Meyrick, 1917) (Lepidoptera, Gelechiidae): a New Pest of Tomato in Serbia

Ivo Toševski¹, Jelena Jović¹, Milana Mitrović¹, Tatjana Cvrković¹,
Oliver Krstić¹ and Slobodan Krnjajić¹

¹ Institute for Plant Protection and Environment, Department of Plant Pests,
Banatska 33, 11080 Belgrade, Serbia (tosevski_ivo@yahoo.com)

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SUMMARY

Tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), a devastating pest of tomato originating from South America has been recorded in Serbia on three localities: in tomato main greenhouse and open field production area located in the vicinity of town Leskovac (South Serbia), in surroundings of the village Donji Vrtogoš (near town Vranje, South Serbia) and in a greenhouses complex in Kraljevci (60 km west of Belgrade). The presence of *T. absoluta* was confirmed by morphological and molecular study of the collected specimens.

Keywords: Leaf miner; Quarantine pest; DNA barcoding; Cytochrome oxidase subunit I

INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917), is a neotropical oligophagous moth from the family Gelechiidae (Lepidoptera), which is associated with solanaceous crops. Since the 1960s, this moth has become one of the key pests of tomato crops in South America (Garcia and Espul, 1982). In Europe, *T. absoluta* presence was initially reported in eastern Spain in late 2006 (Urbaneja et al., 2007), where after, this pest has been recorded in Morocco (EPPO, 2008) and in Tunisia (EPPO, 2009d) as well as in several European countries, France (EPPO, 2009a), Italy (EPPO, 2009b), Netherlands (EPPO, 2009c), Albania (EPPO, 2009f), Portugal (EPPO, 2009g), Bulgaria (EPPO, 2010a), Cyprus (EPPO, 2010b), Germany (EPPO, 2010c), Israel (EPPO, 2010d), Hungary (EPPO, 2010e) and Lithuania (EPPO, 2011b). The new pest on tomato crops has recently been reported from Greece (EPPO, 2011a) and from several countries in Africa and Middle East, suggesting rapid and continuous spreading of this

pest around the Mediterranean Basin (Potting, 2009; EPPO, 2011c). Intensive spreading and dissemination of tomato leaf miner should be correlated with fruit importation and commercialization (Potting, 2009). One of the possible pathways for a long distance dissemination of *T. absoluta* could be through a packaging material (boxes) coming from infested countries (EPPO, 2010e).

Since the initial detection, tomato leaf miner has become the most serious pest causing severe damage on tomato in invaded areas (Germain et al., 2009). Conspicuous economic losses and rapid spreading along the areas of traditional tomato production, promote this pest as the most serious agricultural threat to European tomato production. Cost-benefit analysis showed that existence of a new and such a severe pest in tomato crops significantly increased costs of pest management, primary related to the use of insecticides, which is causing a heavy disruption of existing Integrated Pest Management (IPM) systems (Thomas, 1999; Lietti et al., 2005).

It is still unknown how the *T. absoluta* was introduced from South America in Europe due to general prohibition to import plants for planting belonging to Solanaceae family from the third countries (Annex 3, 2000/29/EC). However, despite of intensive monitoring of the plant protection agencies throughout Europe, tomato leaf miner was subsequently reported mainly from tomato packing stations or in tomato shipments (EPPO, 2010e).

The main host plant of *T. absoluta* is tomato (*Lycopersicon esculentum*), but this moth has also been reported from several solanaceous weeds, including *Solanum nigrum* and *Datura stramonium*. Damage has been reported on some other Solanaceae crops, in the first place on eggplant (*Solanum melongena*) and pepper (*Capsicum annuum*). On potato (*Solanum tuberosum*) the larvae feed on above ground parts of the plant, but nevertheless, under appropriate climatic conditions and high population density, *T. absoluta* could become a pest on the potato crops as well (Pereyra and Sánchez, 2006).

Even tough, tomato trade is one of the main aspects for a long distance dissemination of *T. absoluta*, short and medium natural spread throughout environment is a matter of great concern. Tomato leaf miner may multiply during summer months in outdoors but it is not expected to survive winter conditions because its development stops between 6 and 9°C (Barrientos et al., 1998). It is most likely that different development stages can survive in greenhouse conditions (Potting, 2009). Alternative host plants, especially *S. nigrum*, may play very important role in rapid and continuous spreading of this pest in agro-ecosystems.

According to Directive 42/10 of the Serbian Ministry of Agriculture, Forestry and Water Management for monitoring exotic and invasive pests, during 2010 and 2011 we have surveyed the territory of Serbia for presence of tomato leaf miner *T. absoluta*. The results of this survey are presented in the paper.

MATERIAL AND METHODS

Sites selection and pheromone trapping

We assumed that, if present on territory of Serbia, *T. absoluta* should be found in main tomato production regions with appropriate temperature regime or as transient field populations on sites which are most similar to Mediterranean stands without geographic barriers. A total of 5 sites were selected for this purpose during survey in 2010 (Table 1): 1) main station for tomato packing located at Belgrade; 2) open field tomato production area in Pećinci (40 km west of Belgrade); 3) open field and greenhouse production area located around village Slance (about 10 km east of Belgrade); 4) main greenhouse and open field production area located in Navalin near town Leskovac (about 240 km south of Belgrade); 5) open field and greenhouse tomato production area around village Donji Vrtogoš (near town Vranje, about 290 km south of Belgrade) close to the border with FYR Macedonia (Figure 1). A total of 3 sticky-traps (Delta traps) were set up per locality on September 2, 2010 and armed with pheromone lure specific to attract *T. absoluta* male adults (PHERODIS, *Tuta absoluta*, Koppert). The pheromone traps were positioned inside green houses near the front door and about 1.5 m above ground. The traps were assigned to cover as much as possible wide area within green house complex or open field tomato crops on the surveyed locality. The exposed traps were collected after 20 days and transported to laboratory for further study. Trapped males were carefully removed from the sticky surface and sampled for morphological study. All alive moths captured with pheromone traps were placed in 96% ethanol for further genetic studies.

Table 1. Male moths of *T. absoluta* captured in pheromone traps at seven surveyed destinations in Serbia

Locality	District	GPS coordinate	Tomato crop description (No of inspected crops)	Number of captured specimens
Slanci*	Beogradski	N44 48.496 E20 34.379, 90 m	green houses (2) open field (1)	0
Pećinci*	Sremski	N44 53.424 E19 57.814, 84 m	open field (3)	0
Belgrade*	Beogradski	N44 49.534 E20 21.572, 96 m	main packing station	0
Navalin *	Jablanički	N43 02.281 E21 57.611, 243 m	green houses (2) open field (1)	8
Donji Vrtogoš*	Pčinjski	N42 29.192 E21 49.190, 437 m	green houses (2) open field (1)	7
Stajkovce**	Jablanički	N42 58.508 E22 03.299, 281 m	green houses (6)	>50
Kraljevci**	Sremski	N44 58.948 E19 52.351, 158 m	green houses (1)	12

* sites inspected in 2010

** sites inspected in 2011

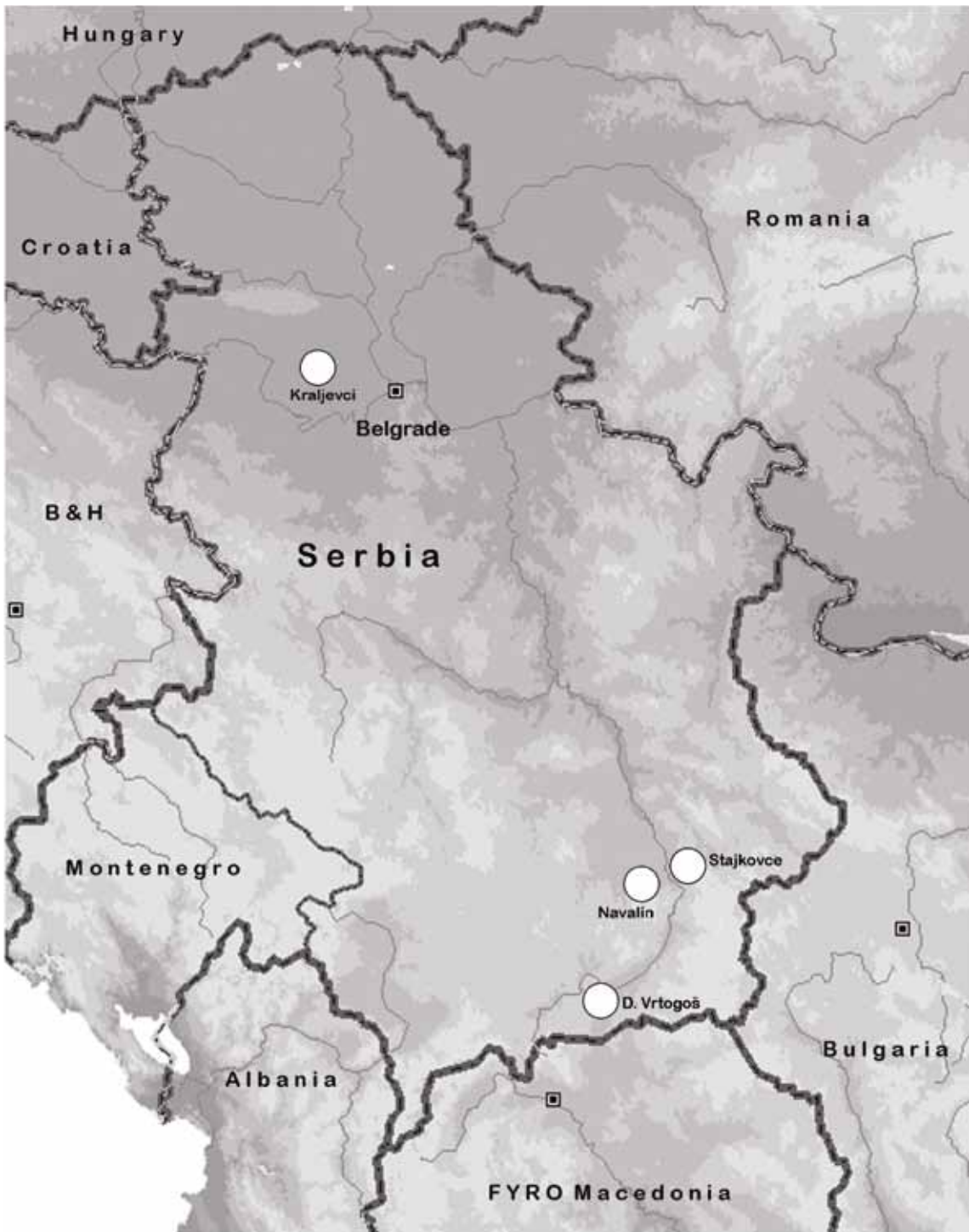


Figure 1. Localities with confirmed presence of *Tuta absoluta* on the territory of Serbia

During June and July 2011, the main greenhouse production area around village Stajkovce (near Leskovac) was surveyed for the presence of mined tomato plants reported by several tomato producers. A total of five greenhouses were visited and checked for tomato leaf miner presence. In addition, at the beginning of July 2011, a pheromone trap was set up in the village Kraljevci, some 60 km west of Belgrade after tomato producers had reported a presence of wide leaf mines in tomato crop.

Morphological study

The trapped specimens which, according to morphological characters, correspond to *T. absoluta* (Figure 2) were carefully removed from sticky surface and washed with 96% ethanol. Morphological characters of the trapped adults were examined under stereo microscope Leica MS5. For all collected specimens the abdomen was carefully removed and macerated in 10% KOH for about 15 minutes. After maceration the male genitalia were extracted and examined. The shape of valve and vinculum were studied as most appropriate characters for *T. absoluta* recognition following description provided by Cooperative Agricultural Pests Surveys (CAPS) http://caps.ceris.purdue.edu/screening/tuta_absoluta_id

DNA extraction, PCR amplification and sequencing

A single specimen previously identified according to morphological characters as tomato leaf miner, originating from the locality Navalín (Leskovac), was used for the DNA study. Total DNA from individual insect was extracted using Dneasy® Blood & Tissue Kit (QIAGEN) according to the manufacturer's instructions. The mitochondrial cytochrome oxidase subunit I gene (COI) was chosen as the appropriate gene with good genetic resolution for differentiation at species level. In order to make more precise identification and comparison with the *T. absoluta* nucleotide sequence data available in NCBI GenBank (National Center for Biotechnology Information), two regions of the COI gene were amplified. Amplification

for the barcode region of the COI gene 5'-end comprising 658 nucleotides (nt) (709 including primers) was performed using LCO1490 and HCO2198 primers (Folmer et al., 1994), while 3'-end of the same gene 814 bp in length (864 including primers) was amplified using C1-J-2195 and L2-N-3014 primer pair (Simon et al., 1994).

Polymerase chain reactions (PCR) for amplification of both portions of the COI gene contained High Yield Reaction Buffer A with Mg (1x), 2.25 mM MgCl₂, 0.6 mM of each dNTP, 0.5 μM of each primer and 1U of KAPA *Taq* DNA polymerase (Kapabiosystems) in a 20 μL final volume. PCR cycles were performed in a Mastercycler ep gradient S (Eppendorf) applying the following thermal steps with LCO1490/HCO2198 primer pair: 95°C for 5 min (initial denaturation), 35 cycles at 95°C for 1 min, 1 min at 54°C (annealing), 72°C for 2 min, and a final extension at 72°C for 10 min. For the amplification of 3' end of the COI gene with C1-J-2195/ L2-N-3014 primers cycling protocol was similar with exception of annealing temperature which was reduced to 45°C and number of cycles which were 40. PCR amplicons were purified using the QIAquick PCR-purification Kit (QIAGEN) according to the manufacturer's instructions, and sequenced on automated equipment by Macrogen (Seoul, South Korea). Comparison with sequences from GenBank was carried out using BLAST analysis (Basic Local Alignment Search Tool; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). In addition, sequence of barcode portion of the COI gene was compared with BOLD (Barcode of Life Data Systems) database.

RESULTS

Survey and pheromone trapping

In 2010 a total of 7 specimens of tomato leaf miner *T. absoluta* were collected with pheromone traps exposed in the village Donji Vrtogoš (South Serbia) and 8 specimens at village Navalín (Leskovac, South Serbia), while no adults of this moth were recorded on other surveyed localities in this year (Table 1, Figure 2).

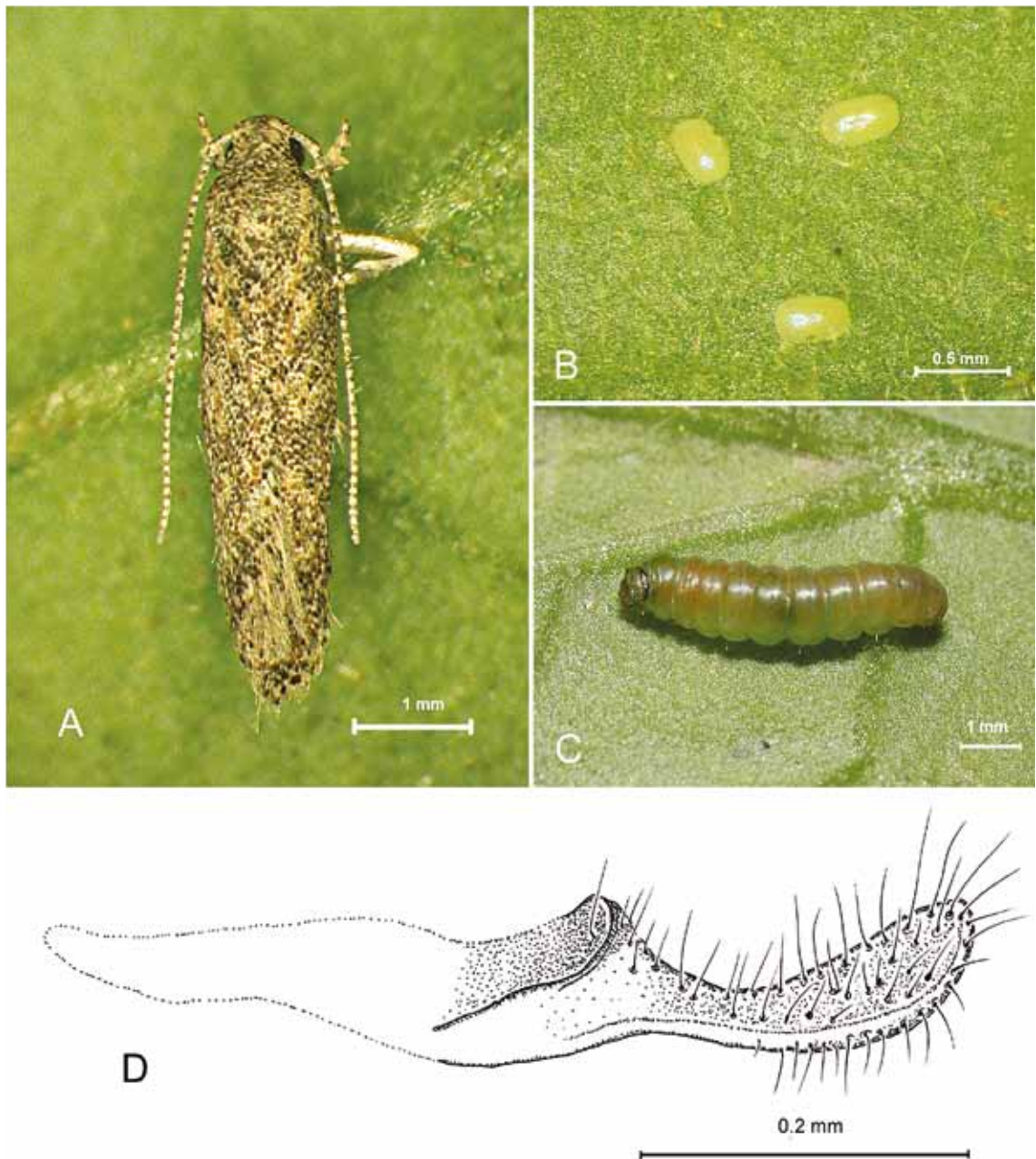


Figure 2. *Tuta absoluta* (Meyrick, 1917): A – adult; B – eggs; C – fifth instar larvae; D – detail of the male genital armature, left valve

The Donji Vrtogoš site was characteristic with scarce tomato production fields and only several small greenhouse facilities (about 30 m long and 6 m wide). For other solanaceous crops, we observed several small fields with egg plant and pepper, with total surfaces not exceeding acreage of 0.2 ha. *T. absoluta* was recorded also in Leskovac, one of the main regions for vegetable production in Serbia with intensive production of tomato, eggplant and pepper. Main characteristics of this

region are early spring vegetable production in greenhouse facilities followed by open field/greenhouse vegetable production until late autumn.

Additional survey throughout greenhouses with tomato production around village Stajkovce in late June 2011 revealed severe outbreak of *T. absoluta* in 5 inspected tomato crops locations. The attack rate was estimated as heavy (Figure 3A and 3B) according to high number of fresh and old mines (Figure 3C), damaged

fruits (Figure 3D) and uncountable number of flying moths. In addition, 12 adults were captured with pheromone sticky trap set up inside greenhouses at locality Kraljevci (North Serbia) (Table 1, Figure 2).

Morphological study

T. absoluta is a small moth (Figure 2A) with a body length of around 7 mm and wing span of 10-14 mm. The ground color of the adult moths is a greenish-brown or silvery gray, with darker patches on the fore wings. Wing apex is fringed with speckled brown scales. The antennae are long, filiform, ringed with black and brown scales; labial palpi prominent, up-curved, with the apical segment long and acute.

The eggs are oval-cylindrical, cream-white colored, 0.2x0.4 mm in size (Figure 2B). The eggs are oviposited on both sides of the leaves, but sometimes on buds or green fruits. The larvae of young instars are whitish-gray in early instars (L1-L3) with a black head. In later developmental stages (L4-L5), larvae become pinkish-green to green in color with brown head (Figure 2C). The pupae are brown, usually less than 6 mm long.

Males of *T. absoluta* can be easily distinguished from other gelechiid moths, which are occasionally trapped on pheromone trap, by examining the genital armature. The most informative character is morphology of valvae, which are digitate and setose apically, with inner margin prominent convex medially (Figure 2D).

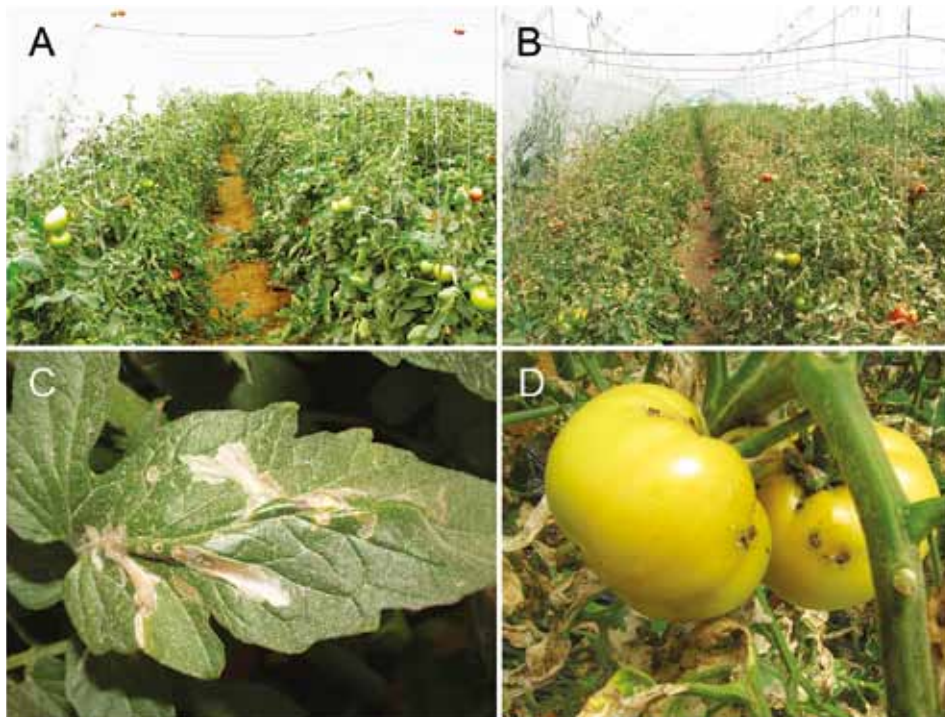


Figure 3. Severe outbreak of *Tuta absoluta* at village Stajkovce (Leskovac, South Serbia) during early summer 2011: A – attacked tomato crop at the end of the June; B – view of the same tomato crop at July 18; C – view of mined tomato leaf by *T. absoluta* larvae; D – view of tomato fruit damage caused by *T. absoluta* larvae

Molecular study

Both portions of mitochondrial COI gene were successfully amplified and sequenced and the sequences are available from GenBank under accession numbers JN417242 and JN417243. BLAST analysis of 3' portion of the COI gene revealed 100% identity of the sequenced specimen collected in Serbia with spec-

imens of *T. absoluta* from Spain (GenBank Acc. No. HQ873072-HQ873078), Chile (HQ87306), Argentina (HQ873059), Turkey (HQ873051) and Italy (HQ873048 and HQ873050) (Cifuentes et al., 2011). Comparison of COI barcode region of the sequenced specimen with sequences available in BOLD database confirmed 100% identity with the barcode sequence of *T. absoluta* from France (HQ968678; BOLD: PHLAB662-10).

DISCUSSION

T. absoluta is multivoltine and according to rapid population growth should be treated as r-selected species (Pereyra and Sánchez, 2006). The rapid population growth, potential dispersal through environment and expressed resistance to insecticides (Desneux et al., 2010), classify this pest as the most serious threat for tomato production worldwide. Even though studies of *T. absoluta* distribution and its impact in Serbia are in initial phase, it seems that three geographically distant records in Serbia have their own history.

At site Donji Vrtogoš (South Serbia) the *T. absoluta* specimens were collected at the beginning of September in low number, which strongly suggests that such late appearance of *T. absoluta*, without reported significant damage from tomato breeders, can be correlated with transition appearance because of close Mediterranean neighborhood (i.e. FYR Macedonia).

The massive outbreak recorded at Leskovac (village Stajkovce), where nearly 4000 hectares are in intensive tomato production, is of particular concern because of potential spread of this pest via trade and exporting in other regions of Serbia. Significant losses in tomato production which are observed in several tomato crops, strongly suggest that *T. absoluta* is established in this area. In addition, this is confirmed in interviews conducted with tomato breeders during June and July 2011, which reported presence of tomato leaf miner in less extent in previous years, and without significant damage. In region of the village Stajkovce the first *T. absoluta* mines in 2011 were observed at the end of April, which indicates successful overwintering of this pest in greenhouse conditions. On the contrary, presence of *T. absoluta* in Kraljevci in epidemiological sense should be correlated with infested planting material or packing boxes, introduced in greenhouses during production process.

The ongoing spread of *T. absoluta* throughout Europe and its status as a severe pest on tomato is followed by the lack of fully satisfactory effective management options (Desneux et al., 2010). Multilateral measures are proposed in control of tomato leaf miner which consider combination of biological control methods, massive trapping and chemical or microbiological treatments with authorized products in crops. Thus, it is obvious that presence of *T. absoluta* in invaded area will force changes in pest management packages, as well as in cultural practices, optimizing greenhouse structure followed by total removal of infested crop residues from greenhouse and, intensive control of weedy plants as potential alternative hosts on which *T. absoluta* can persist during winter time.

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Tuta absoluta (Meyrick, 1917) (Lepidoptera, Gelechiidae): nova štetočina paradajza u Srbiji

REZIME

Prisustvo lisnog minera paradajza *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), opasne štetočine poreklom iz Južne Amerike, utvrđeno je na tri lokaliteta u Srbiji: u plastenicima i poljima u okolini Leskovca (južna Srbija), u okolini sela Donji Vrtogoš (u blizini Vranja) i u kompleksu plastenika u selu Kraljevci (60 km zapadno od Beograda). Identifikacija *T. absoluta* je izvršena morfološkim i molekularnim analizama sakupljenih uzoraka.

Ključne reči: Lisni miner; karantinska štetočina; DNK barkoding; subjedinica 1 citohrom oksidaze