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Distribution and molecular identification of *Tuta absoluta* (MEYRICK, 1917) (Lepidoptera, Gelechiidae) populations in Bosnia and Herzegovina and Montenegro

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ABSTRACT. Since 2010, the South American tomato moth – *Tuta absoluta* (MEYRICK, 1917) (Lepidoptera, Gelechiidae) – has been recorded in Bosnia and Herzegovina (B&H) and Montenegro. In 2012, pheromone traps were placed among tomato crops growing in greenhouses and open fields in 14 localities in B&H and Montenegro; the pest was caught at all the sites. Adults were caught in both greenhouses and open fields in Trebinje (B&H) on 4th June 2012 and in Ulcinj (Montenegro) in mid-May. Species identification was confirmed by means of morphological characters of adults and male genitalia. In addition, molecular identification of specimens from B&H and Montenegro was performed using mitochondrial (mtDNA) markers.

KEY WORDS: South American tomato moth, pheromone trapping, molecular identification, COI.

INTRODUCTION

The South American tomato moth – *Tuta absoluta* (MEYRICK, 1917) (Lepidoptera, Gelechiidae) – is one of the most devastating pests of tomatoes, both in greenhouse and open field locations in South America, Europe and North Africa (in the Sahel savannah) (DESNEUX et al. 2010). It has also been reported to attack potatoes (*Solanum tuberosum* L.), eggplants (*Solanum melongena* L.), peppers (*Capsicum annum* L.), weeds (*Datura*

stramonium L., *Nicotiana glauca* G.) (KORYCINSKA & MORAN 2009) and green beans (*Phaseolus vulgaris* L.) (EPPO 2009).

T. absoluta is originally from South America and was first described in Peru in 1917 as *Phthorimaea absoluta* (MEYRICK, 1917) (DESNEUX et al. 2010). This has other synonyms: *Scrobipalpuloides absoluta* (POVOLNY, 1987), *Scrobipalpula absoluta* (POVOLNY, 1964) and *Gnorimoschema absoluta* (CLARKE, 1962) (EPPO 2005).

In Europe, the first record was in Spain in 2006 (EPPO 2008a), followed by other Mediterranean countries: Tunisia (EPPO 2009d), Algeria (GUENAOUI 2008, cited in OSTRAUSKAS & IVINSKIS 2010), Morocco (EPPO 2008b), France (EPPO 2009a), Italy (EPPO 2009b), Portugal (EPPO 2009g), Malta (EPPO 2009h) and Albania (EPPO 2009f). Its prevalence and distribution has continued in Europe and the presence of *T. absoluta* was also recorded in the countries of the former Yugoslavia: in Slovenia (KNAPIČ & MAROLT 2009, cited in OSTRAUSKAS & IVINSKIS 2010); Croatia (GOTLIN ČULJAK et al. 2010), where the pest was found in coastal areas and sporadically in inland parts of the country (ŠIMALA pers. comm., cited in ŠKALJAC et al. 2012); then in Kosovo (EPPO 2010d), Serbia (TOŠEVSKI et al. 2011) and Montenegro (HRNČIĆ & RADONJIĆ 2011). In Bosnia and Herzegovina, its presence was confirmed for the first time in 2010, at Čapljina (West Herzegovina, Bosnia and Herzegovina) (OSTOJIĆ 2010) and in Banja Luka (Republic of Srpska, Bosnia and Herzegovina) (ĐURIĆ & HRNČIĆ 2010).

T. absoluta spreads mostly from one tomato plant to another while these are being planted, and from one region to another as a result of the trans-border trade (import/export) in tomatoes (TOŠEVSKI et al. 2011, VAN DEVENTER 2009, cited in BETTAIBI et al. 2012). It has also been observed that adults can cover a few kilometres by flying (VAN DEVENTER 2009, cited in BETTAIBI et al. 2012) and in a year can often be carried more than 1000 km on the wind (LEVY 2010).

T. absoluta is a serious threat to tomato production, as it can decrease fruit quality and result in 50 to 100% yield losses (EPPO 2005, VIGGNIANI et al 2009, DESNEUX et al. 2010). The larvae destroy tomato plants by mining the leaves, stems and buds, and by burrowing tunnels in the fruits, thereby rendering fresh tomatoes unmarketable (VIGGNIANI et al. 2009).

This pest is of great economic importance and has spread to other regions of Europe. The distribution, as well as the morphological and molecular identification of *T. absoluta* populations in B&H and Montenegro, has been studied.

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MATERIALS AND METHODS

Survey and pheromone trapping

The survey was conducted from May to October 2012, in the main tomato production areas of Republic of Srpska (B&H) and Montenegro. A total of 14 localities (Table 1, Fig. 1) – 7 each in B&H and Montenegro – were selected for the survey.

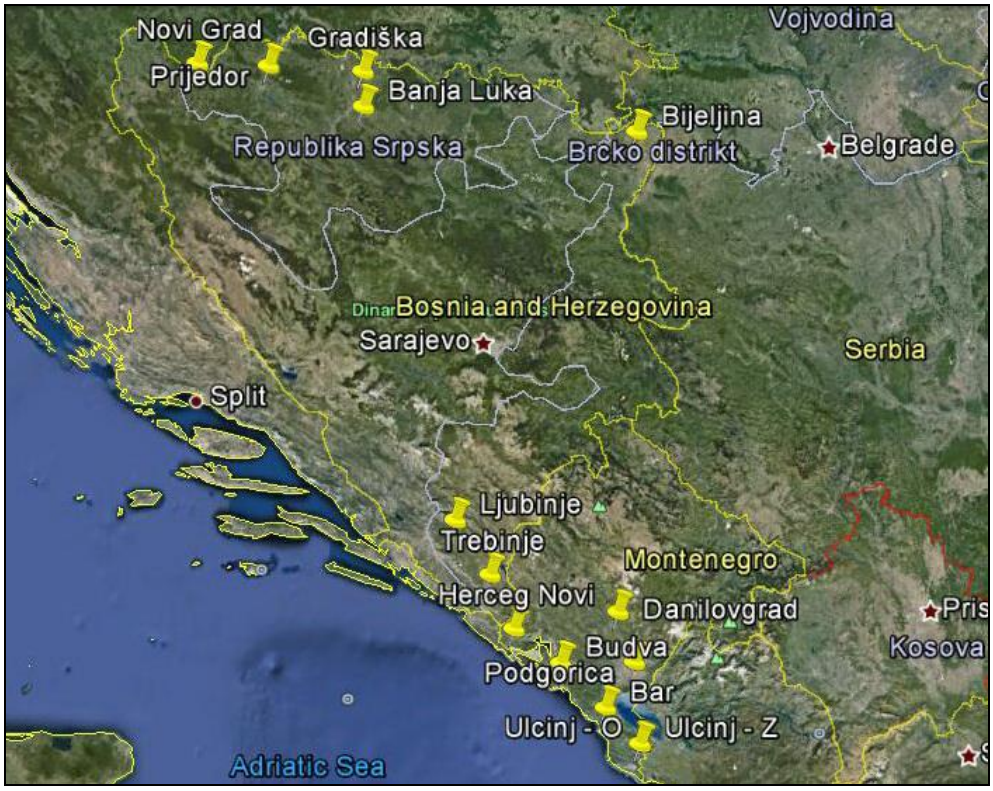

Fig. 1. Map showing the localities surveyed in Bosnia and Herzegovina and Montenegro.

Csalomon® pheromone traps were used as an early detection tool for *T. absoluta*. One trap per site was placed inside greenhouses or in open fields, depending on where the tomato crops were growing. They were hung 20 cm above the plant, checked and moved up once every 2 weeks, while pheromone plugs were replaced every 6 weeks. Sticky trap bottoms were changed as needed. Identification of the species was confirmed using the morphological characters of adults and the male genitalia. For this purpose, adults were

obtained from infested tomato leaves collected from surveyed sites. Additionally, live specimens, collected directly from tomato plants using a pooter, were preserved in 96% ethanol and submitted for further molecular identification.

Table 1. Overview of the localities surveyed.

Locality	Country	GPS coordinate	Tomato crop production
Banja Luka	Bosnia and Herzegovina	44°50'01.8" N, 17°22'00.2" E	greenhouse
Prijedor	Bosnia and Herzegovina	44°59'48.1" N, 16°43'48.2" E	greenhouse
Novi Grad	Bosnia and Herzegovina	44°59'13.4" N, 16°15'42.8" E	open field
Gradiška	Bosnia and Herzegovina	44°59'53.0" N, 17°20'59.8" E	greenhouse
Bijeljina	Bosnia and Herzegovina	44°46'44.1" N, 19°09'35.5" E	greenhouse
Trebinje	Bosnia and Herzegovina	42°42'07.1" N, 18°19'56.3" E	open field
Ljubinje	Bosnia and Herzegovina	42°56'44.2" N, 18°05'49.5" E	greenhouse
Podgorica	Montenegro	42°19'25.0" N, 19°15'48.0" E	open field
Danilovgrad	Montenegro	42°33'12.0" N, 19°08'53.0" E	greenhouse
Herceg Novi	Montenegro	42°27'25.0" N, 18°29'55.0" E	greenhouse
Budva	Montenegro	42°18'24.0" N, 18°48'09.0" E	open field
Bar	Montenegro	42°06'22.0" N, 19°05'37.0" E	greenhouse
Ulcinj	Montenegro	41°56'44.0" N, 19°19'44.0" E	open field
Ulcinj	Montenegro	41°56'41.0" N, 19°19'03.0" E	greenhouse

Morphological identification

Adults obtained from infested tomato leaves collected from the survey sites were analysed at the entomology laboratory under a stereomicroscope. Also, a certain number of adults were preserved in 96% ethanol for further study.

Selected specimens were macerated, after which the male genitalia were extracted and examined. All adults caught were identified to species level, according to the keys and descriptions presented by different authors (EPPO 2005, KORYCINSKA & MORAN 2009, USDA 2011). Identification of *T. absoluta* was based on morphological characters of adults, as well as on the shape of the uncus, digitate, setose valvae and the well-developed vinculum and phallus of the male genitalia.

DNA extraction

Genomic DNA was extracted from two adult abdomens (two individuals from each location monitored; Table 1) using DNeasy Blood and Tissue Kit (QIAGEN), following the manufacturer's instructions. In total, 14 samples were submitted for DNA extraction procedure and PCR analyses.

PCR analyses

The mitochondrial cytochrome oxidase subunit I (COI) gene proved suitable for PCR because of its good genetic resolution, which is ideal for differentiation at the species level (KAMBHAMPATI & SMITH 1995). Therefore, primers amplifying mtCOI fragments were employed for the PCR analyses.

Amplification of the COI gene 5'-end was performed with LCO1490 and HCO2198 primers (FOLMER et al. 1994), while the COI gene 3'-end was amplified using C1-J-2195 and L2-N-3014 primer pairs (SIMON et al. 1994) (Table 2). PCR was set up in 20 μL final volume and the master mix contained: High Yield Reaction Buffer A with Mg (1x), 2.25 mM $MgCl_2$, 0.6 mM of each dNTP, 0.5 μM of each primer and 0.1U/μ of KAPA*Taq* DNA polymerase (Kapabiosystems). Amplification was performed in a Thermal Cycler 2720 (Applied Biosystems) and the temperature profile for the amplification in the reaction with LCO1490/HCO2198 primers was: 5 min. at 95 °C (initial denaturation) followed by 1 min. at 95 °C, 1 min. at 54 °C, 1 min. and 30 sec. at 72 °C for 35 cycles, with a final extension for 7 min. at 72 °C. The amplification protocol with C1-J-2195/L2-N-3014 primer pair was distinguished by the annealing temperature (45 °C) and the number of cycles (40).

The PCR products were visualised after running 1% agarose gel (100 V) for 1 hour. The gel was stained with a 0.05% solution of ethidium bromide.

Table 2. Sequences of oligonucleotide primers used in PCR analyses.

Primer sequence (5'-3')	Reference
COI gene 5'-end (709 bp) LCO1490**:** GGTCAACAAATCATAAAGATATTGG HCO2198: TAAACTTCAGGCTGACCAAAAAATCA	FOLMER et al. (1994)
COI gene 3-end (864 bp) C1-J-2195: TTGATTTTTTGGTCATCCAGAAGT L2-N-3014: TCCAATGCACTAATCTGCCATATTA	SIMON et al. (1994)

Sequencing and sequence analyses

PCR products of DNA samples from Bijeljina (B&H) and Ulcinj (Montenegro) were subjected to sequencing and sequence analyses. The Bijeljina (B&H) and Ulcinj (Montenegro) populations of *T. absoluta* are geographically and climatically distinct (Fig. 1), so they were selected for further analysis.

Selected PCR products were purified using the QIAquick PCR purification Kit (QIAGEN) following the manufacturer's instructions and sent to Macrogen (the Netherlands) for sequencing. BLAST analysis (Basic Local Alignment Search Tool; http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare the sequences obtained with those from the GenBank.

RESULTS AND DISCUSSION

Distribution and pheromone trapping

The presence of *T. absoluta* was confirmed for the first time in 2010 in Bosnia and Herzegovina (ĐURIĆ & HRNČIĆ 2010, OSTOJIĆ 2010) and in Montenegro (HRNČIĆ & RADONJIĆ 2011).

In order to determine the distribution of the pest, pheromone traps were placed at 14 localities in B&H and Montenegro in May 2012 (Table 1, Fig. 1). At all the localities surveyed, *T. absoluta* was caught from May to October 2012. Records show that the first adults in B&H were caught in Trebinje on 4th June, Ljubinje on 14th June, Bijeljina on 27th June, Novi Grad on 18th August, Banja Luka on 11th September, Prijedor on 12th September and Gradiška on 7th October. In comparison with the study in B&H during 2010 and 2011 (ĐURIĆ et al. 2012), this survey confirmed the presence of *T. absoluta* at the Novi Grad, Prijedor and Gradiška localities for the first time.

The first adults in Montenegro were caught in mid-May in the Ulcinj locality, both in greenhouses and open fields, in Herceg Novi at the end of May, and in the other localities from 1st to 5th June.

The results of this survey show that in Montenegro and in the southern parts of B&H, where a Mediterranean climate prevails, the first adults were trapped earlier than in northern B&H, which has a continental climate (Fig. 1). This can be explained by the fact that *T. absoluta* prefers areas with a warmer climate, because it is a native of South America and also because its spread through Europe started in the Mediterranean region (DESNEUX et al. 2010). On the other hand, this survey confirmed that the pest is also present in greenhouses and open fields in the continental climate of B&H, such as in Bijeljina, Novi Grad, Banja Luka, Prijedor and Gradiška. In accordance with many authors (VAN DEVENTER 2009, cited in BETTAIBI et al. 2012, TOŠEVSKI et al. 2011, ĐURIĆ et al. 2012), this is partly caused by the intensive tomato production in some areas, as well as the cross-border tomato trade in the country.

Molecular identification

The main aim of the present work was to identify the presence, and estimate adult emergence and distribution of *T. absoluta* specimens in different localities in the two countries. In addition to morphological identification, molecular tools such as PCR, sequencing and sequence analyses were also employed for *T. absoluta* identification, and mitochondrial markers were used for the analyses.

Both COI gene fragments were successfully amplified in all 14 DNA samples. Amplified PCR products from populations from two climatically and geographically distinct locations (Bijeljina/B&H and Ulcinj/Montenegro) (Fig. 1) were sequenced.

Sequences were obtained from GenBank under accession numbers: KC852871 (B&H), KC852872 (Montenegro) (5'COI) and KC852869 (B&H), KC852870 (Montenegro) (3'COI). No polymorphism was observed when PCR products from mixed DNA samples were sequenced.

No difference was observed in the BLAST analyses of 5'COI sequences from B&H and Montenegro, as they were 100% identical. The BLAST analyses also revealed that they were 100% identical with those from Serbia (GenBank Acc.No. JN417242) (TOŠEVSKI et al. 2011) and Tunisia (GenBank Acc.No. JQ749676).

Comparing the sequenced 3' COI fragments, we discovered that the B&H and Montenegro sequences were 99% identical. The B&H sequence revealed a 100% identity with *T. absoluta* specimens collected from Murcia-Spain (HQ873080), Crete-Greece (HQ873053) and Turin-Italy (HQ873049) (CIFUENTES et al. 2009). In contrast to this, the Montenegro 3' COI fragment sequence was confirmed to have 100% similarity with *T. absoluta* sequences from Chile (HQ873062), Sicily-Italy (HQ873048), Turin-Italy (HQ873050), Andalusia-Spain (HQ873076), Izmir-Turkey (HQ873051) and Argentina (HQ873057) (CIFUENTES et al. 2009).

Although it focused primarily on the identification of *T. absoluta*, this work was also designed to analyse the variability of *T. absoluta* populations in different regions, including different markers, by employing different molecular methods. Mitochondrial markers have frequently been used to study the genetic variability of many invasive insect species (KAMBHAMPTI & SMITH 1995). However, CIFUENTES et al. (2009) studied the genetic variability of *T. absoluta* from the Mediterranean and South American populations. They used mitochondrial and ribosomal markers, and the results of the phylogenetic analysis show that no genetic variability was found between the Mediterranean and South American populations. Therefore, other molecular markers and methods should be used for studying variability in *T. absoluta* populations. For example, RAPD-PCR was used to examine the genetic variability of the Tunisian population (BETTAIBI et al. 2012): the results revealed a high genetic variability among the 7 populations tested. Using AFLP, the Brazilian population of *T. absoluta* was separated into two groups according to resistance to insecticides (SUINAGA et al. 2004). In view of this, future projects should make use of other markers and molecular methods, which will enable a comprehensive study of the variability of the *T. absoluta* populations in B&H and Montenegro.

Conclusions

T. absoluta is a major threat to tomato production. It was detected in B&H and Montenegro in 2010. Since then, it has spread within both countries and in all 14 localities surveyed during this study. Pheromone traps have been shown to be a very useful tool for the early detection of this pest.

This morphological and molecular study confirms that the pest spread rapidly through both countries and adapted to continental climate conditions.

However, other markers and molecular methods could be used in future studies. These would provide a clearer insight into the dispersal mode, phylogenetic structure and insecticide resistance mechanisms of *T. absoluta*, which is crucial for developing an effective pest management strategy.

REFERENCES

BETTAIBI A., MEZGHANI-KHEMAKHEM M., BOUKTILA D., MAKNI H., MAKNI M. 2012. Genetic variability of the Tomato Leaf Miner (*Tuta absoluta* MEYRICK; Lepidoptera: Gelechiidae) in Tunisia, inferred from RAPD-PCR. Chilean Journal of Agricultural Research **72**(2): 212–216.

CIFUENTES D., CHYNOWETH R., BIELZA P. 2011. Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (POVOLNY, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. Pest Management Science **67**(9): 1155–1162.

DESNEUX N., WAJNBERG E., WYCKHUYS K.A.G., BURGIO G., ARPAIA S., NARVAEZ-VASQUEZ C.A., GONZALEZ-CABRERA J., CATALAN RUESCAS D., TABONE E., FRANDON J., PIZZOL J., PONCET C., CABELLO T., URBANEJA A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. Journal of Pest Science **83**(3): 197–215.

ĐURIĆ Z., HRNČIĆ S. 2010. Tomato leaf miner – *Tuta absoluta* MEYRICK in Republic of Srpska. Agroznanje Journal **11**(3): 17–23. (in Serbian)

ĐURIĆ Z., HRNČIĆ S., VUJANOVIĆ M., ĐURIĆ B., MITRIĆ S. 2012. *Tuta absoluta* (MEYRICK) (Lepidoptera, Gelechiidae) in the Republic of Srpska (Bosnia and Herzegovina). EPPO Bulletin **42**(2): 337–340.

EPPO 2005. *Tuta absoluta*. Data sheets on quarantine pests. EPPO Bulletin **35**(3): 434–435.

EPPO 2008a. First record of *Tuta absoluta* in Spain. EPPO Reporting Service **1**(001): 2.

EPPO 2008b. First record of *Tuta absoluta* in Morocco. EPPO Reporting Service 9(174): 2.

EPPO 2009. *Tuta absoluta* found on *Phaseolus vulgaris* in Sicilia (IT), EPPO Reporting Service 8(154): 3.

EPPO 2009a. First record of *Tuta absoluta* in France. EPPO Reporting Service **1**(003): 2–3.

EPPO 2009b. First record of *Tuta absoluta* in Italy. EPPO Reporting Service **2**(023): 6.

EPPO 2009d. First record of *Tuta absoluta* in Tunisia. EPPO Reporting Service **3**(042): 2.

EPPO 2009f. First report of *Tuta absoluta* in Albania. EPPO Reporting Service **9**(170): 2.

EPPO 2009g. First report of *Tuta absoluta* in Portugal. EPPO Reporting Service **9**(171): 3.

EPPO 2009h. First record of *Tuta absoluta* in Malta. EPPO Reporting Service **10**(188): 2.

EPPO 2010d. First record of *Tuta absoluta* in Kosovo. EPPO Reporting Service **11**(114): 2.

FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology **3**(5): 294–299.

GOTLIN ČULJAK T., RAŽOV J., GOMBOC S., GRUBIŠIĆ D., JURAN I., ŽANIĆ K. 2010. The first record of tomato leaf miner *Tuta absoluta* POVOLNY, 1994 (Lepidoptera: Gelechiidae) in Croatia. Glasilo biljne zaštite **10**(4): 273–281. (in Croatian)

HARIZANOVA V., STOEVA A., MOHAMEDOVA V. 2009. Tomato leaf miner, *Tuta absoluta* (POVOLNY) (Lepidoptera: Gelechiidae) – first record in Bulgaria. Agricultural Science and Technology **1**(3): 95–98.

HRNČIĆ S., RADONJIĆ S. 2011. *Tuta absoluta* MEYRICK (Lepidoptera, Gelechiidae), a new pest in Montenegro. IOBC-WPRS Bulletin **68**: 71–74.

KAMBHAMPATI S., SMITH P.T. 1995. PCR primers for amplification of four insect mitochondrial gene fragments. Insect Molecular Biology **4**(4): 233–236.

KORYCINSKA A., MORAN H. 2009. South American tomato moth *Tuta absoluta*: plant pest factsheet. Sand Hutton, York, UK.

LEVY D. 2010. Meet the *Tuta absoluta*. Tomato News **6**: 7–10.

OSTRAUSKAS H., IVINSKIS P. 2010. Records of the tomato pinworm (*Tuta absoluta* (MEYRICK, 1917)) – Lepidoptera: Gelechiidae – in Lithuania. Acta Zoologica Lituanica **20**(2): 151–155.

OSTOJIĆ I. 2010. Tomato leaf miner (*Tuta absoluta*) – new pest in Bosnia and Herzegovina. Green Garden **69**: 16–17. (in Bosnian)

SIMON C., FRATI F., BECKENBACH A., CRESPI B., LIU H., FLOOK P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America **87**(6): 651–701.

SUINAGA F.A., DIAS CASALI V.W., PICANCO M., FOSTER J. 2004. Genetic divergence among tomato leafminer population based on AFLP analysis. Pesquisa Agropecuária Brasileira **39**(7): 645–651.

ŠKALJAC M., KOSTANJŠEK R., ŽANIĆ K. 2012. The presence of *Wolbachia* in *Tuta absoluta* (Lepidoptera: Gelechiidae) populations from coastal Croatia and Montenegro. African Entomology **20**(1): 191–194.

TOŠEVSKI I., JOVIĆ J., MITROVIĆ M., CVRKOVIĆ T., KRSTIĆ O., KRNJAJIĆ S. 2011. *Tuta absoluta* (MEYRICK, 1917) (Lepidoptera, Gelechiidae): a New Pest of Tomato in Serbia. Pesticides and Phytomedicinea **26**(3): 197–204.

USDA 2011. New Pest Response Guidelines, Tomato Leafminer (*Tuta absoluta*), Emergency and Domestic Programs. United States Department of Agriculture. Internet: http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/Tuta-absoluta.pdf

VIGGIANI G., FILELLA F., DELRIO G., RAMASSINI W., FOXI C. 2009. *Tuta absoluta*, a new moth recorded also in Italy. L'Informatore Agrario **2**: 66–68. (in Italian)

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