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Rapid Communication

First report of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the Republic of Benin

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Abstract

Tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a serious pest of tomato *Solanum lycopersicum* L. (Solanaceae) originating from South America. Due to previous outbreaks in neighbouring West African countries, surveillance was ongoing since 2014 in Benin. Tomato plants were regularly inspected and pheromone traps placed in tomato-producing localities in northern, southern and central Benin, including fields close to the border with Niger and Nigeria. In late 2017 and beginning of 2018 Gelechiidae moths were obtained from both the traps and the reared plant material. Morphological examination and molecular analysis of the gained specimens allowed to confirm the presence of the pest in the northern and central regions of Benin. We hence confirm the presence of *T. absoluta* in yet another West African country.

Key words: Cytochrome Oxidase I, invasive pest species, morphology, Solanaceae, tomato leafminer, West Africa

Introduction

Invasive species constitute a threat to native wildlife, human health and food production (Masters and Norgrove 2010). Biological invasions can cause strong negative economic effects worldwide and invasive insect pest species constitute currently a serious threat to agricultural production in tropical Africa (Pysek and Richardson 2010; Goergen et al. 2016). Among representative examples for such threats figures the tomato leafminer *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) that started to invade the African continent since 2007 (Desneux et al. 2011; Sylla et al. 2017). The larvae of this moth are devastating for tomato production, causing 30% damage on tomato in Tunisian greenhouses (Chermiti et al. 2009) and nearly 100% losses in open-field tomato in Tanzania (Chidege et al. 2016).

Originating from South America, *T. absoluta* reached Europe by 2006 (Urbaneja et al. 2007) and spread quickly (Desneux et al. 2010; Tropea Garzia et al. 2012; Biondi et al. 2018). It expanded to Africa in 2007 (Mohamed et al. 2012; Abbes et al. 2014; Adamou et al. 2017; Son et al. 2017), and soon after to Asia (Campos et al. 2017; Hossain et al. 2016; Saidov et al. 2018). In West Africa, it was reported from costal Senegal in 2012 (Pfeiffer et al. 2013) and one and three years later it occurred in Niger and Nigeria respectively (Adamou et al. 2017; Borisade et al. 2017), a close expansion towards Benin. *Tuta absoluta* was present in 7 of the 8 administrative regions of Niger three years after its introduction (Adamou et al. 2017). This development was predicted in *T. absoluta*-spread simulations based on vegetation and climatic conditions in Africa (Guimapi et al. 2016). Furthermore, given the vast tomato trade ongoing between West African countries, the risk of spreading to Benin was evident (Adamou et al. 2017; Sylla et al. 2017). Following the invasion of Senegal, several West African countries deployed surveillance systems to ensure early detection of the moth. Therefore, pheromone traps were placed in a north-south transect in Benin e.g. in Tomboutou (Malanville), Kargui (Karimama) and Dédoumè (Kpomasè), by the Benin National Institute of Agricultural research (INRAB) from 2014 to 2016, and at Malanville, Savè and Grand Popo by the International Institute of Tropical Agriculture (IITA) from 2014 to 2015, yet no *T. absoluta* individuals were caught during that period of time. However, following sustained field collections by the authors and first inquiries from vegetable farmers by the end of 2017, some Gelechiidae specimens that were likely to be the tomato leafminer were found. Here, we report for the first time the presence of *T. absoluta* in Benin, a prerequisite information to initiate appropriate management options against this newly introduced invasive species.

Methods

Following first complaints by farmers of devastating attacks by unknown larvae on tomatoes, INRAB and IITA scientists organized a joint field visit to central and northern Benin, in Savè, Parakou, Kandi, and Malanville communes (Collines, Borgou, and Alibori departments), where tomatoes were commercially grown. Tomato plots in each of these sites were inspected in a cross-sectional pattern and upon the detection of damage symptoms, tomato plants were sampled by uprooting and subsequently taken to the lab for emergence monitoring. White delta traps, baited with pheromone (Tutrack, Kenya Biologics, Nairobi) containing 3E,8Z,11Z-tetradecatrien-1-yl-acetate and 3E,8Z-tetradecadien-1-yl acetate, were placed in the tomato fields visited. The traps were checked for catches after three days. A sub-sample of insects so collected was isolated into tubes individually and stored in absolute ethanol (96%).

Molecular identification was made using specimens caught in the pheromone traps and those reared from the tomato plants. DNA was extracted following the LIVAK protocol (1984). Extracted DNA from a total of 25 specimens from two sites and 4 sampling events was sent for subsequent PCR amplifications and identification by INQABA Biotech (Pretoria, South Africa). A 685 pb target region of the Cytochrome Oxidase I (COI) gene was amplified using the barcoding primers LCO-1490 (5'-GGTCAACAAAT CATAAAGATATTGG-3') and HCO-2198 (5'-TAA ACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). The PCR products were gel extracted, purified and sequenced in the forward and reverse direction, using the PCR primers, on the ABI PRISM™ 3500xl Genetic Analyse (INQABA Biotech).

Sequences were edited in BioEdit (vers. 7.2 (Hall 1999)), manually inspected for nucleotide scoring, and translated into protein sequences to help detect sequencing errors, since mtCOI is an expressed sequence. The forward sequences were then aligned to join with the reverse complements of the reverse sequences to assess fidelity of the sequences. They were then compared with the nucleotide sequence collection in NCBI using nucleotide and protein BLAST to identify similarities with previously reported sequences. Nucleic acid sequences from sequenced species were deposited in GenBank and voucher specimens were deposited in the reference insect collection at IITA-Benin.

Male genitalia were dissected by dipping the abdomen in 96% ethanol briefly before it was left overnight in 10% potassium hydroxide (KOH). Subsequently, genitalia were stained with Eusine dye for 4 hours and then washed in 20% and 70 % ethanol baths respectively. For photographs, genitalia were mounted on a slide in pure glycerine. The genitalia were studied under an inverted reflected light stereomicroscope (Olympus XZS10) to differentiate Gelechiidae species by species-specific morphological features (Hayden et al. 2013; Huemer and Karsholt 2010).

Results

We obtained a total of 185 Gelechiidae specimens reared from the field-collected tomato plants and 136 Gelechiidae specimens from pheromone traps in Savè and Malanville communes (Figure 1) (Savè, 8°08'24′50.9″N; 2°38′04.6″E, 14.iii.2018, coll. M.F. Karlsson, R. Sikirou; Malanville, 11°51′55.4″N; 3°18′10.7″E, 16.iii.2018, coll. M.F. Karlsson, R. Sikirou).

All the extracted sequences were identified as *T. absoluta.* The 658 bp fragments had 100% homology (e = 0^E00; Gaps = 0/658) to sequences previously deposited in GenBank from insects collected in e.g. South Africa and Oman (Visser et al. 2017; Patankar and Al-Wahaibi 2015). Our samples were archived in GenBank (Table 1). Translation yielded no stop codon and nucleotide BLAST yielded cytochrome oxidase subunit I, partial (mitochondrion) [*Tuta absoluta*] (e = 4e-140).

The internal (30 males) and external (males and females) morphology of adult moth samples were examined and found to conform to the characters used to diagnose adults of *T. absoluta* (Hayden et al. 2013; Huemer and Karsholt 2010). Aedeagus had short,

Table 1. Sampling sites and accession number for specimen identified molecularly.

Site	Longitude	Latitude	Altitude (m)	GenBank accessions no.	Collection date	Collection method
Malanville	11,865389	−3,302972	161	MH882431	14.03.2018	pheromone trap
				MH882451	14.03.2018	tomato plant rearing
Savè	8,414139	−2,634611	191	MH882438	16.03.2018	pheromone trap
				MH882444	16.03.2018	tomato plant rearing

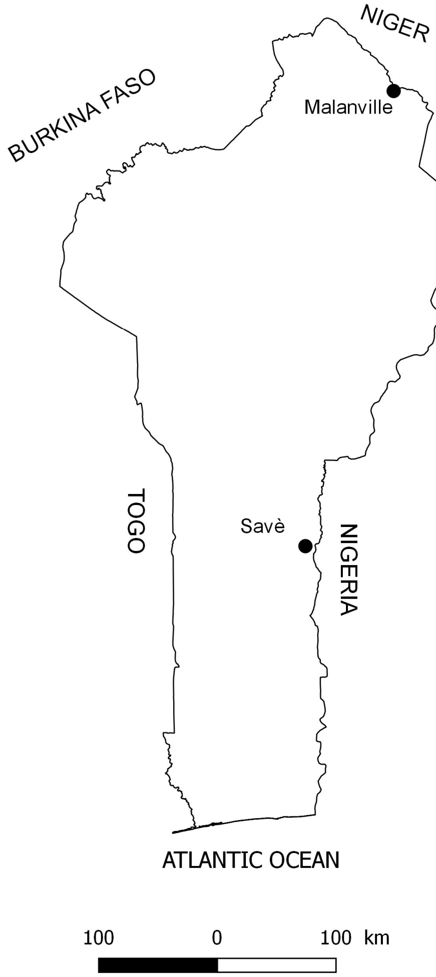

Figure 1. Site references for records of *Tuta absoluta* in the republic of Benin.

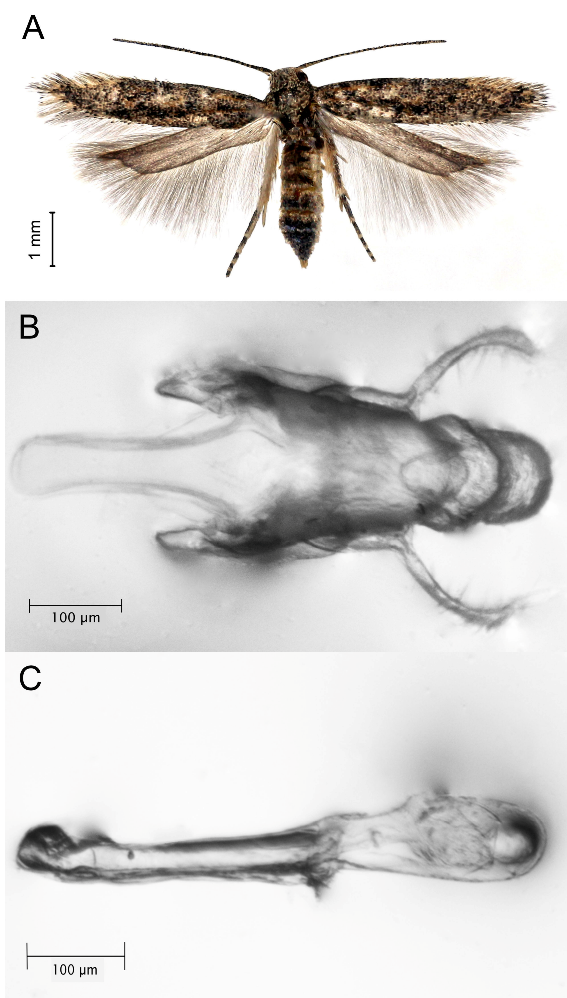

Figure 2. Morphological features of *Tuta absoluta* A) adult (G. Goergen) and B) male genitala with C) aedeagus (J.A. Zannou).

broad, and inflated coecum, uncus were broadly sub-quadrate apically nearly flattened, valva weakly curved, covered with long setae with apex extending almost to the tip of the uncus. Saccus were long and subrectangular about length of valva and vinculum, slightly smaller than uncus and the gnathos and vinculum broad while tegumen were comparatively short (Figure 2).

Discussion

We here report the presence of *Tuta absoluta* in Benin, for the first time. Morphological and molecular evidence of its present expansion follow previous spreading trends in the neighbouring countries that placed Benin at potential risk. Thus, Tomboutou in Malanville commune is situated only 4 km from the

Niger border, and Savè 30 km from Nigeria border. Reports from Niger confirm infestations of *T. absoluta* close to the Benin border (Adamou et al. 2017). Similarly, data from Nigeria indicates its occurrence in Oyo state (Borisade et al. 2017), a region bordering the Collines Department of Benin, where Savè is situated. The entry point of the moth is nevertheless unknown, alike its mode of introduction. Country-wide surveillance is now ongoing to elucidate the full geographical coverage of *T. absoluta* in Benin. Given that pheromone traps were already placed from February 2014 without result, the presence of *T. absoluta* in Benin seems to be recent. The absence of damage due to the pest in other important tomato growing areas support this view.

Whereas tomato is grown throughout the republic of Benin, the main production period varies, being the rainy season in the southern and central regions and the dry season in the extreme north. According to climatic index analysis, a high risk of *T. absoluta* establishment was predicted for northern Benin while in the southern and central parts, models projected a very high likelihood of long-term survival (Tonnang et al. 2015). Whilst the dispersal and colonization of new areas by *T. absoluta* populations are hampered by high humidity (Guimapi et al. 2016), the moth is well adapted to hot harsh environments as underpinned by population increases towards the end of dry seasons (Sylla et al. 2017). Hence, a long-term establishment of the moth throughout Benin with decreasing impact towards coastal regions is a likely development, yet the availability of tomato and alternative host plants will additionally define the population build-up (Guimapi et al. 2016; Sylla et al. 2017). The risk of spreading to the neighbouring Togo in the near future is very high, particularly because of transboundary trade, the dispersal capacity of the moth, its multivoltine life cycle, and fast-reproductive cycle (Desneux et al. 2010).

Like many Gelechiidae species, the larvae of this micromoth feed internally on various parts of their host plants and are therefore particularly difficult to manage. *Tuta absoluta* larvae consume the mesophyll layer of the leaves, stems and fruits, which reduces yield and makes tomato fruits unsuitable for trade (Desneux et al. 2011). Beside tomato, it also attacks other species of Solanaceae such as eggplant *Solanum melongena* L., black nightshade *Solanum nigrum* L., potato *Solanum tuberosum* L., tobacco *Nicotiana tabacum* L., tree tobacco *Nicotiana glauca* Graham, and datura *Datura stramonium* L., where economic damage is generally minimal (Desneux et al. 2010; Tropea Garzia et al. 2012; Ingegno et al. 2017). Tomato is an important source of income for Beninese vegetable farmers (Agossou et al. 2001; James et al. 2010) and annual production of tomato has more than doubled within a decade, increasing from 149,000 t in 2006 to 335,000 t in 2016 (FAO 2018). A decrease in the tomato production due to *T. absoluta* would be devastating for the entire West African region. Nigeria, by far the largest tomato producer in West and Central Africa, has already suffered severe production losses. Though these are compensated by importations from Cameroun and Benin, they led to a tremendous increase of the tomato price in Nigeria (Borisade et al. 2017). Several diseases and pests are already constraining the yield potential of tomato (Sikirou et al. 2001, 2017) and thus the newly introduced tomato leafminer creates a further serious threat to the tomato production (Chidege et al. 2016; Visser et al. 2017). Moreover, the higher pest pressure constitutes an elevated risk for insecticide overuse, with potential negative impact on human health, the environment, and build-up of resistance (Biondi et al. 2018). Tomato growers' immediate response to the recent invasion of *T. absoluta* into Africa and Europe has been an extensive use of insecticides and weak application of Integrated Pest Management (IPM) practices (Lietti et al. 2005; Biondi et al. 2018). However, due to the caterpillar endophytic feeding behaviour and the development of resistant strains, the efficacy of insecticides has remained relatively poor (Silva et al. 2015; Lietti et al. 2005). Additional problems in open-field African tomato production is that pesticides are often linked to inappropriate use, overdosing, manual weeding and harvesting in recently treated fields, and high levels of intoxications (Son et al. 2018; Perrin et al. 2014).

Further research is needed to assess the poorly studied native fauna of natural enemies associated with *T. absoluta* in tropical Africa. Additionally, to increase the accuracy of trapping data, we propose taxonomic studies on related species, also attracted to the pheromone traps. Our own observations show that apart from *T. absoluta,* other Gelechiidae species are regularly caught by *T. absoluta* pheromone baits. As pests can easily be confused with other closely related Gelechiidae species that might co-exist (Hayden et al. 2013), it is important to clarify the identity of these by-catches that may play an important role as potential alternative hosts of natural enemies.

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