Mutamiswa *et al. Agric & Food Secur (2017) 6:49*
DOI 10.1186/s40066-017-0128-2

Agriculture & Food Security

RESEARCH

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First report of tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in Botswana

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Abstract

Background: The tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) is an invasive insect pest of tomato and other solanaceous plants which is rapidly expanding its geographic range. It has a highly damaging effect on tomatoes and potential of threatening food production and consequently national food security. Here, we report on the first detection of *T. absoluta* in Botswana, its consequences on agriculture and food security, and recommend on management strategies. The pest was observed feeding on *Solanum lycopersicum* L. plants at Genesis farm, Matshelagabedi village in northern Botswana. Following detection, we incubated infected tomato fruits until adult eclosion. External morphology was conducted and confirmed at Botswana International University of Science and Technology (Botswana). Molecular identification and morphological male genitalia were confirmed at Stellenbosch University (South Africa). In addition, we set up some sex-specific pheromone (Tuta optima PH-937-OPTI) at the core detection site and surrounding areas.

Results: Morphological genitalia features of pheromone-baited trap catches confirmed the insect pest was indeed *T. absoluta*. Molecular analysis also confirmed the morphological identification and thus confirming this first report of *T. absoluta* in Botswana.

Conclusion: This first record of *T. absoluta* in Botswana is worth reporting to promote coordinated efforts amongst stakeholders, research specialists and extension officers in Botswana and across the southern African region in monitoring and managing the pest.

Keywords: Insect pest invasion, Food security, Molecular identification, Solanaceous plants

Background

Insect pest invasions have been rapidly increasing worldwide, and with increased movement of people and goods from one country to another, there are high chances for increased numbers of invasive species conquering many regions [1]. Furthermore, climate is changing [2], and in consequence, impact on the rate of biological invasions, insect distribution, abundance and impacts of such invasions on a global scale [3]. In Africa, the risk and rate of invasion has dramatically increased [1], with destructive insect pests like *Chilo partellus* Swinhoe [4], *Prostephanus truncatus* (Horn) [5], *Phenacoccus manihoti* Matile-Ferrero [6] and *Bactrocera dorsalis* (Hendel) [7] invading the continent over the past decades. These invasions pose a significant risk in a continent where ~70–80% of the population depends on agriculture for household food security and sustenance [8]. Exports of vegetables from developing countries have immensely increased in the past decades, and horticultural exports have been reported to contribute to improvement in food security and livelihoods in these developing countries [9]. Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae) is highly ranked as a food and cash crop [10, 11] in Africa and indeed Botswana [12]. However, biotic factors such as insect pests hamper production [11], which may compromise household and national food security.

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The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) also known as South American tomato pinworm, is one of the key insect pests of tomato [13]. This may be because of the extensive damage it elicits, lack of ecologically relevant methods for management [14] and pesticide resistance [15]. It is very destructive to tomato plants and fruits causing between 80 and 100% yield losses if left uncontrolled [16]. Furthermore, it has a wide host range and has been reported on cultivated solanaceous plants such as eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), tobacco (*Nicotiana tabacum* L.), wild solanaceous weeds like black nightshade *Solanum nigrum*, *Solanum elaeagnifolium*, *Solanum puberulum*, *Datura stramonium*, *Datura ferox*, *Nicotiana glauca*, and garden bean (*Phaseolus vulgaris* L.) [17]. It is of South American origin, Andes region, Peru, and was first detected in eastern Spain in 2006 [13, 16]. Thereafter, it invaded the Mediterranean Basin, Europe, Middle East, South Asia (India), and north, east and west Africa [13, 16, 18]. In north Africa, *T. absoluta* was detected north of the Sahel, Tunisia and Morocco in 2008 [16, 19, 20], west Africa; in Niger and Nigeria in 2010, and in Senegal in 2012 [21] east Africa; in Sudan and Ethiopia in 2011 [21, 22], in Kenya in 2013 [11], in Tanzania in 2014 [23] and in Uganda in 2015 [11], southern Africa; in Zambia [24] and in South Africa in 2016 [25, 26] (see distribution map, Fig. 1).

Tuta absoluta is nocturnal, and adults spend the day hiding between leaves [16]. It is multivoltine, producing between 10 and 12 generations per year [27, 28]. It is highly tolerant to high temperature, with a developmental optimum of 30 °C and a wide developmental thermal window [29, 30]. This implies that during its geographic range expansion, *T. absoluta* has a high probability of thriving in novel variable thermal environments and even with global climate change [2]. The biological cycle takes an average of 24–38 days at 27 °C, and females can lay 250–300 cylindrical creamy yellow eggs, mostly singly on aerial parts and young fruits of the host plant [16, 27, 28]. After 4–6 days, the eggs develop into 0.5-mm yellow or green larvae [28, 29]. The larval stage takes 12–15 days and goes through four developmental instars [28, 32]. The first 2 instars have been reported to mine between the epidermal layers of the leaf leading to a reduction in the photosynthetic area and premature senescence [21]. Thereafter, larvae leave the mines as 3rd and 4th instars, boring into stalks, apical buds and fruits [17]. Larval damage also promotes the entry of secondary pathogens causing fruit rot [28] and forms the most destructive developmental stage [16, 31, 32]. Pupation takes place in the soil, within the mines, on the leaves or in packaging materials [21, 31] and can last 9–11 days at benign climatic conditions [28]. With the rapid north to south movement and high invasion potential of *T. absoluta* over wide geographic areas in Africa (Fig. 1), it still poses a biosecurity threat to countries where it has not yet been detected. Here, we make the first report of *T. absoluta* in the north-eastern part of Botswana and recommend management strategies following detection. Detection and description of new species upon introduction in novel environments is of paramount importance in crafting management plans for invasive species [33]. We then explore preventative and control measures to be undertaken by various stakeholders to reduce its spread into pest-free areas or further invasions into neighbouring countries.

Methods

The first economic damage of *T. absoluta* was reported at Genesis farm (S21.14776; E27.64744), Matshelagabedi village in North-East District of Botswana. A report was made to the Department of Plant Protection in the Botswana Ministry of Agriculture. The second detection point was Noka Farm (S21.12860; E27.48830), Francistown, in the same district. Following detection, a physical assessment of crop damage symptoms for the presence of the pest was conducted and infested tomato fruit samples were collected and incubated in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) at 24 ± 1 °C, 70 ± 5% RH [34] in insect cages (35 cm^3) until adult eclosion. Emerged adult moths were collected and examined for confirmation.

Morphological identification

Morphological characters were confirmed at Botswana International University of Science and Technology (BIUST), Botswana, and Stellenbosch University, Cape Town, South Africa. Adult moths were collected and knocked down in killing jars containing chloroform absorbed in cotton wool. Using a stereomicroscope, Bestscope (model BS3060BT), connected to a computer, morphological features were examined and photographed using a microscope-mounted 5.0-MP digital camera (DCM-510) (Hangzhou Scopetek® Opto Electric Co, Hangzhou, China). *T. absoluta* can be reliably identified morphologically using male genital features, by examining the valvae and gnathos [35]. Males have broad, horseshoe-shaped gnathos and a digitate valva, with a medial hump and constriction [36].

Plant damage symptoms

Damaged tomato plants, plant parts and fruits from tunnels, open fields and net shades were collected and examined visually using illuminated bench magnascopes (RBM 101 model, Radical Instruments, India). Tunnelling

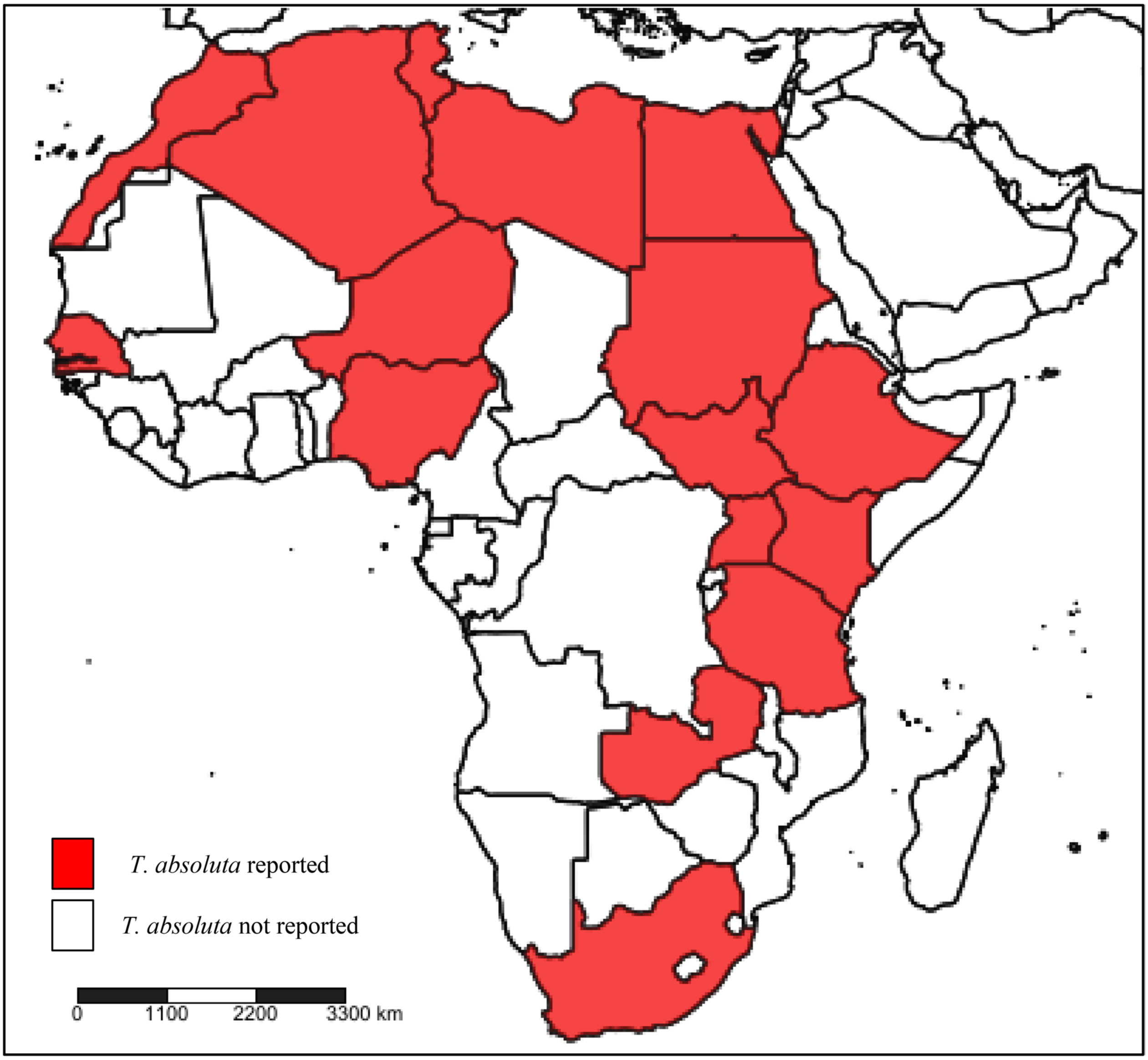

Fig. 1 Current distribution of *Tuta absoluta* in Africa. Redrawn from [52]

and feeding behaviour in fruits, stems and leaves was observed.

Sex pheromone trapping

Yellow delta traps (Chempac, Progressive Agricare®) equipped with sticky pads were placed in tomato tunnels, open fields on and around the core detection point (Fig. 2). A synthetic sex pheromone, *T. absoluta* optima PH-937-OPTI (Russel IPM), was used as the lure (see Fig. 3).

The traps were set following a survey protocol developed by [37] with necessary modifications (Table 1).

Molecular analysis

We extracted total genomic DNA from moths and larvae from the field-collected and incubated samples. DNA extractions were performed using the QIAmp®DNA Micro Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. Extracted DNA concentrations were measured using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Inc.). One nanogram of DNA was used in subsequent PCR amplifications. PCR products of ~685 bp in length were amplified for the COI gene using the primer set LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′)

Fig. 2 *Yellow delta* pheromone trap placed at **a** Genesis farm (S21.14776; E27.64744; 987 m.a.s.l) (tunnel agroecosystem) and **b** open forest (S21.16684; E27.57120; 1013 m.a.s.l) (wild habitat) with predominant *Colophospermum mopane*, North-East District of Botswana. Photos by H. Machekano and R. Mutamiswa

Fig. 3 *Tuta absoluta* PH-937-OPTI female sex pheromone (Russell IPM®) used in the delta traps

and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′), which amplifies a 710-bp fragment of the COI gene in a wide range of invertebrate taxa [38, 39]. The TopTaq Master Mix kit (Qiagen) was used in all reactions. Thermocycling conditions consisted of denaturation at 95 °C for 1 min, followed by 35 cycles of 95 °C for 45 s, 51 °C for 45 s and 72 °C for 1 min, with a final extension at 72 °C for 3 min. The PCR product was visualized on a 1.2% agarose gel and purified using the Wizard® Genomic DNA Purification Kit (Promega Corporation). Purified products were sequenced both ways using BigDye® Terminator v3.1 chemistry (Applied Biosystems) with the same primer pair used for the PCRs. Sequences were edited and aligned using the CLC main Workbench 6.9.

Table 1 Delimited detection zones for *Tuta absoluta* detection in Matshelagabedi village, northern district of Botswana

Location	Distance from the core detection site (m)	Number of traps/zone
Core detection point (genesis farm)	0	6
First (open forest)	500	6
Second (open forest)	8000	2
Third (open forest)	16,000	2
Fourth (tomato fields)	24,000	2

Data were collected 7 days (03 December–10 December 2016), and trapped moths were counted using dyed pointers and tally counters

Data analysis

Insect morphological features were identified under microscope (1:10 zoom ratio, 0.8 × ~0.8 obj. mag, 50–75-mm binocular head) (BestScope®, China). Qualitative data on crop damage symptoms were collected by observation and sample incubation. Moth trap count data were collected from the sticky pads and counted. Detected adult moth counts were presented as graphs. The COI sequence data from molecular analysis was compared to the standards for confirmation. Sequence data was compared with existing sequences in the GeneBank (KX443111, KX443108, KP793741, KP814057 and JQ749676) and (KJ657881, KJ657680, KC852871, KT452897, KP793742, KC852872, KP324753 and an outgroup KX862248) in MEGA6 [38], the latter of which were used to draw the phylogenetic tree.

Results

Morphological features

Morphological features on the collected moths show head vertex covered with appressed scales that appear flattened against the head. The labial palps were also scally and had the same colour as the head with a distinguishingly forward projecting, up-curved shape with a relatively long pointed apical segment. Other general features like body size and colour also concurred with those of *T. absoluta*. Observed male genitalia conformed

Fig. 4 *Tuta absoluta*: male genitalia. Photograph by R. Ramukhesa

to that of *T. absoluta* as described by [35] and also in agreement with [26] (Fig. 4). The aedeagus/phallus was characterized by a broader, basal prominent caecum. The uncus was hood-shaped and quite broad at the apex. The uncus was attached to a tegument basally broadened with an ovate gnathos. The valvae were digitate within inner margin convex shape, and each was covered with lightly dense setae. The vinculum was broad and well developed, with an elongated and broad saccus.

Crop damage symptoms

Survey of the tomato fields and surrounding habitats showed extensive damage inflicted by *T. absoluta*. The damage was characterized by extensive wilting of whole plants associated with severe leaf and stem. The shoots had distorted shoots with signs of die back, dead hearts and wilting. The leaves showed lesions of different sizes, large necrotic areas, wilting and chlorosis (Fig. 5a). Frass was largely visible on all damaged parts of the plants. *T. absoluta* damage was also observed on wild hosts, e.g. *Solanum lichtensteinii* (Willd), a wild solanaceous plant native to southern Africa (Fig. 5b). The damage was also characterized by lesions and necrotic damage. However, the damage on *S. lichtensteinii* was less severe than that on tomato plants (see Fig. 5a, b).

Tomato fruit damage symptoms were characterized by internal feeding with distinct exit holes (Fig. 5c, d) and substantial frass. Fruits attacked in their early developmental stages had distorted shapes and relatively smaller size (Fig. 5c). Most damaged mature fruits showed signs

Fig. 5 Typical *Tuta absoluta* damage symptoms on **a** tomato plants, **b** wild host *Solanum lichtensteinii*, **c** distorted and damaged fruit following larval fruit infestation and **d** advanced damage signs with exit holes, secondary infection, hanging skins with consumed internal contents and accelerated senescence at Genesis farm (S21.14776; E27.64744; 987 m.a.s.l). Photos by H. Machekano and R. Mutamiswa

of secondary infection, subsequent decomposition and loss of internal fruit contents (Fig. 5d).

Pheromone trapping

Sex pheromone traps caught varying numbers of *T. absoluta* male moths (Fig. 6) depending on location and whether the traps were in the open tomato fields, in tunnels or open natural/forest habitat. We recorded *T. absoluta* male moths on all pheromone-baited traps and sites in the north-eastern part of Botswana (see Fig. 7 for detection sites). The core detection site, however, showed generally higher mean number of moths per trap than the rest of the sites. More moths were caught inside the tunnels than outside (see Fig. 8). The capture of male moths using a species-synthetic equivalent of female-emitted species-specific sex pheromone [14] confirms the moths reported here were indeed *T. absoluta*.

Molecular analysis

The consensus sequence was used in a BLASTN (basic local alignment search tool) search [40] to find matching sequences [41]. The COI sequence and phylogenetic tree matched the *T. absoluta* (100% identity) sequences KJ657881, KJ657680, KC852871, KT452897, KP793742, KC852872 (see Fig. 9), as well as KX443111 and KX443108 [42], KP793741 (first report from India, Asokan et al. 2015, unpublished data), KP814057 [43] and JQ749676 [44]. Phylogenetic analysis showed genetic distances between 0.00 and 0.59 between the Botswana *T. absoluta* sample and other samples deposited in the GeneBank, and between 12.67 and 12.84 between *T. absoluta* samples and the outgroup *Ephysteris promptella*. The molecular data confirmed the present specimens as *T. absoluta*.

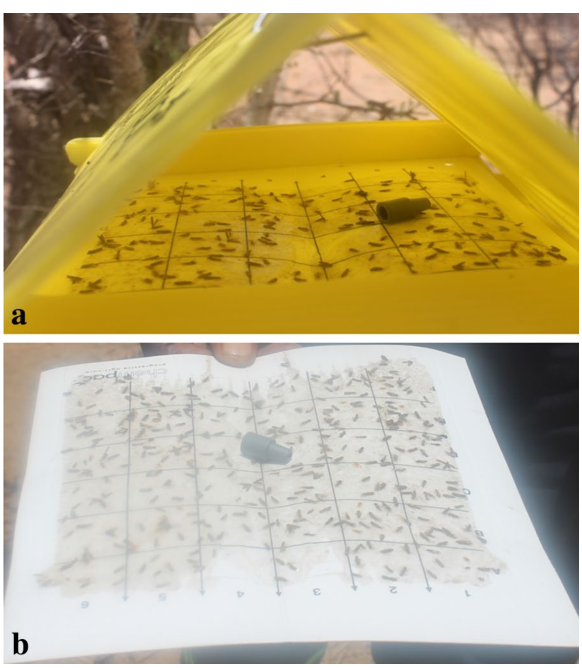

Fig. 6 Sex pheromone trap catches for *T. absoluta* male moths **a** set up inside the yellow delta trap and **b** sticky pad retrieved from the yellow delta trap following 1-week trapping period at Genesis Farm (S21.14776; E27.64744; 987 m.a.s.l). Photos by H. Machekano and R. Mutamiswa

Discussion

The recent invasion and rapid geographic spread of *T. absoluta* poses a major threat to both natural and agro-ecosystems [16] in the African region. While *T. absoluta* has been reported in east, north and west Africa [16, 19, 20], to our knowledge, this is the first scientific report, detailing the presence of this pest in Botswana and generally southern Africa.

Tuta absoluta was caught at all the sites where the traps were set in the two districts of Botswana suggesting that there are high chances of widespread distribution of the insect pest in these and other districts. This is also supported by the detection of moths at distant places from the core detection site (Genesis farm) and in natural habitats. Indeed, this finding agrees with previous study by [16] who detected adult *T. absoluta* at ~10 km from the tomato fields. Presence of *T. absoluta* in the wild may explain its host polyphagy and also means it may be capable of migrating distances beyond 10 km. Indeed, the adult moths have been reported to fly distances of up to 100 km [45], and given their small size, they are easily blown by wind currents. This characteristic further improves spread of propagules upon introduction in new habitats and thus invasion success.

The mitochondrial cytochrome c oxidase subunit I (COI) sequencing through DNA Barcoding linked the Botswana trap catches to *T. absoluta* samples from Tunisia (Accession number JQ749676) and India (Accession number KP793741) (also see Fig. 9), the characteristic host damage symptoms and sex-specific pheromone trapping consequently supporting morphological identification that was done in Botswana and South Africa. This confirmed moth identity as *T. absoluta*, suggesting that this alien pest is highly invasive and is expanding its geographic distribution. The insect pest has been reported being on a downward incursion from north Africa towards the south, and this may have been promoted by its high reproductive capacity [46], wide host range [31], wide developmental thermal windows [29, 30], continuous vegetable production across political borders, absence of effective surveillance and monitoring systems, lack of effective sanitary and phytosanitary

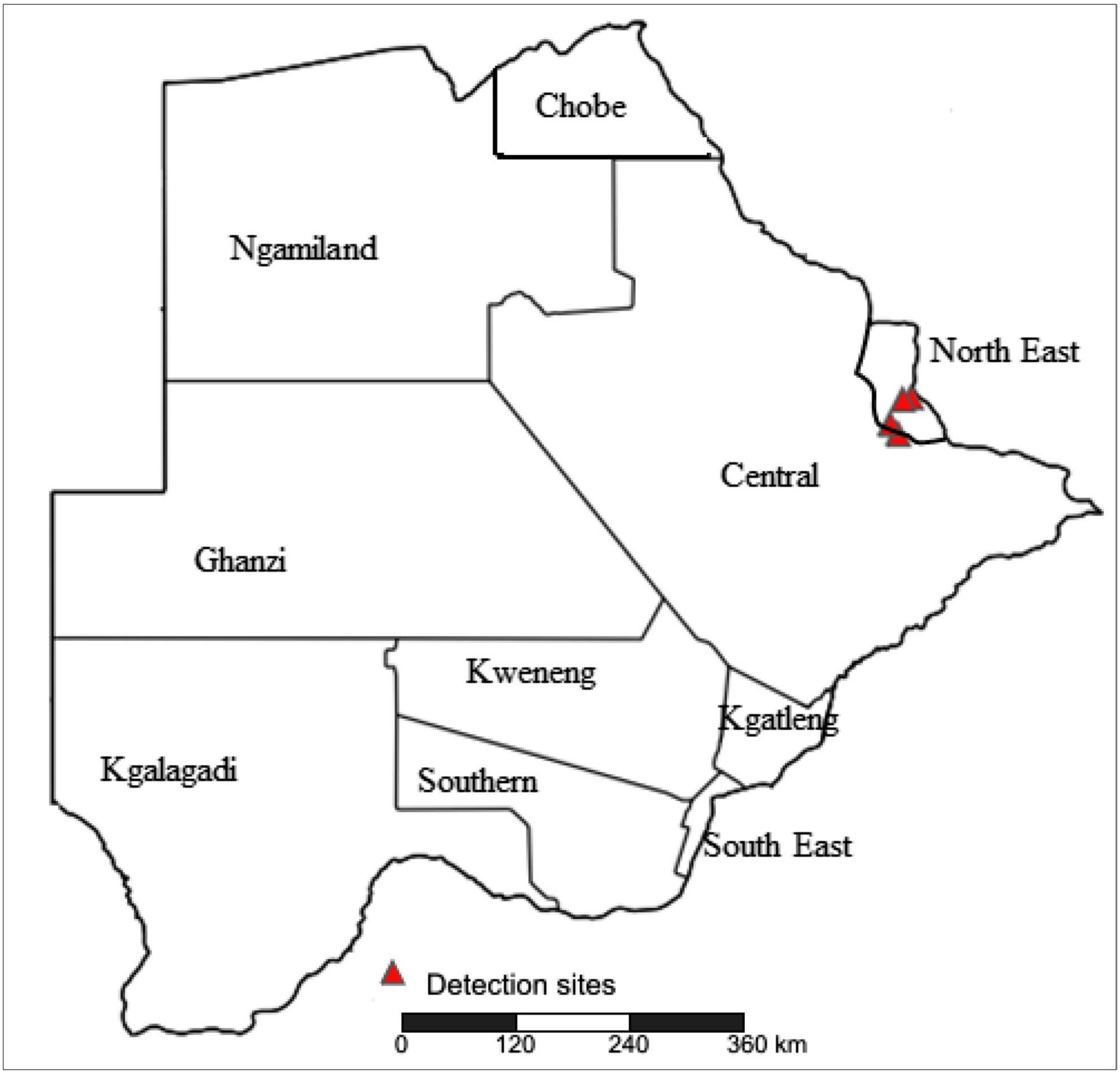

Fig. 7 A *map* of Botswana showing detection sites for *Tuta absoluta*

measures and increase in intra-continental trade [47]. Moreover, resistance to conventional and new chemicals [48, 49] has been reported as a contributing factor towards its invasion success [15, 50]. In addition, African ecological and climatic conditions are similar to those of South American countries [16], suggesting that *T. absoluta* may establish and invade other countries in the southern African region.

Most of the farmers in Botswana who are into tomato production rely on importing seedlings from neighbouring countries. In addition, retailers are known to import tomato fruits from Zambia, Zimbabwe and South Africa for supplying domestic market (personal observation). Zambia reported its first detection of *T. absoluta* in May 2016 [24], while South Africa reported hers in September 2016 [25]. Therefore, there are high chances that *T. absoluta* may have found its way into Botswana through tomato fruits, seedlings from its trade partners through, e.g. packing materials, boxes, crates, pallets and to some extent outdoor markets selling the fruits from infested areas. Furthermore, the insect may have migrated across borders as flying adult moths. Given its high invasion

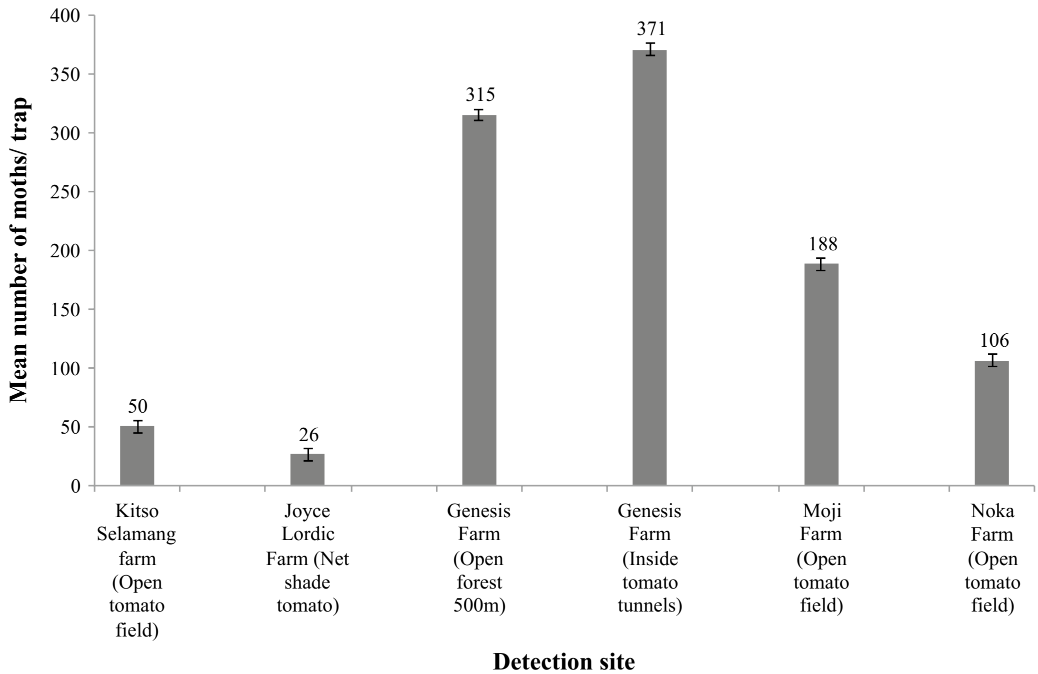

Fig. 8 Mean number of *Tuta absoluta* male moths/trap over a period of 7 days from different sites distant from core detection point in north-east Botswana. Traps were baited using *Tuta absoluta* PH-937-OPTI (Russel IPM)

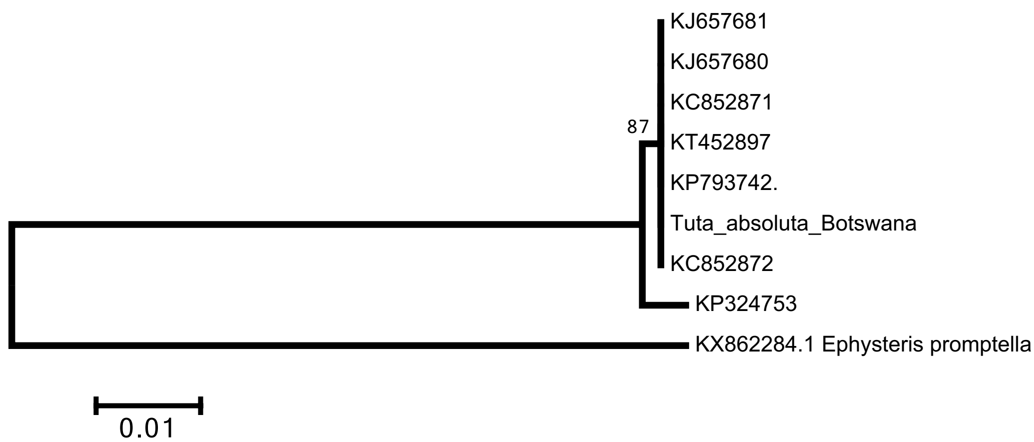

Fig. 9 A comparison of the sequences of Botswana specimens with existing sequences on GenBank; KJ657881, KJ657680, KC852871, KT452897, KP793742, KC852872, KP324753 and an outgroup KX862248. The evolutionary history was inferred using the neighbour-joining method [53]. The optimal tree with the sum of branch length = 0.13050185 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches [54]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method [55] and are in the units of the number of base substitutions per site. The analysis involved nine nucleotide sequences. Codon positions included were first + second + third + noncoding. All positions containing gaps and missing data were eliminated. There were a total of 510 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [38]

potential, the moth has been reported to drift with wind currents [16], fly up to 100 km and move between non-screened greenhouses and outdoor crops [45], suggesting that they can move long distances in a country or across borders colonizing novel environments. The recent invasions by *T. absoluta* in Africa were reported in Tanzania

[23], Uganda [11], Zambia and South Africa [24, 25]. Botswana, therefore, records the latest detection of the invasive species in the southern African region. This implies that all the countries bordering Botswana, e.g. Namibia and Zimbabwe, and others, e.g. Mozambique, Malawi, Angola, may be at significant risk. It may also imply that, the pest may have already invaded these countries but not yet reported and hence the need for strict surveillance and quarantine regulations in these countries. Although tomato is the preferred host by *T. absoluta*, this invasion poses a threat to other solanaceaous crops in the country and region such as *S. tuberosum*, *C. annuum*, *N. tabacum* and leguminous *P. vulgaris*. Given the socioeconomic values of these commodities to the African communities, the current *T. absoluta* invasion may have negative consequences on the agricultural export market, household and national food security and thus livelihoods.

The detection of *T. absoluta* in Botswana specifically on tomatoes poses a threat to tomato production in the country and region, thereby affecting food and nutritional security. This is because it prefers tomatoes to other solanaceous crops [11, 51]. Given the rate at which this pest is spreading across the continent [1, 11, 21] and its potential damage on tomato plants, there is need to come up with effective management options to avoid further invasions. In this regard, we recommend effective monitoring of its spread, conducting pest risk assessments for the country and region, developing effective sanitary and phytosanitary measures, awareness campaigns, farmer trainings, countrywide surveys to determine pest-free zones and introduction of biological control agents. In addition, coordinated efforts amongst stakeholders, research specialists and extension officers in Botswana and across the southern African region should be employed to implement effective monitoring systems and area-wide pest management.

Conclusion

We conclude that morphology of the male genitalia, and confirmation through molecular data, positive sex-specific pheromone lure trapping and host plant damage symptoms all confirmed association with *T. absoluta*. Therefore, based on these findings we confirm the first record of *T. absoluta* in Botswana.

Authors' contributions

CN was involved conceptualization; RM and HM helped in data curation; RM, HM and CN were involved in formal analysis; CN contributed to funding acquisition; RM, HM and CN helped in investigation; RM, HM and CN were involved in methodology; CN contributed to project administration; CN was involved in resources; CN helped in supervision; RM, HM and CN helped in validation; RM and HM contributed to visualization; RM and HM helped in writing—original draft; RM, HM and CN were involved in writing, review and editing. All authors read and approved the final manuscript.

Acknowledgements

We acknowledge the support of Botswana International University of Science and Technology, Pia Addison (Stellenbosch University), Russel IPM for *T. absoluta* lures, Ministry of Agriculture, Plant Protection division for the samples and Welma Pieterse for morphological and molecular analyses for confirmation. We also acknowledge Yoseph Assefa and Monamodi Kesamang for assistance with phylogenetic analysis.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Collected and analysed data during the current study are available upon request from the corresponding author.

Consent for publication

The authors consent to manuscript publication.

Ethics approval and consent to participate

Not applicable since the study involved tomato plants attacked by the insect pest.

Funding

The project was funded through Botswana International University of Science and Technology (BIUST) Research Office Grant.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 10 April 2017 Accepted: 21 June 2017
Published online: 01 November 2017

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