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Rapid Communication

First report of the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and its damage in the Democratic Republic of Congo

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Abstract

Tuta absoluta (Meyrick) (Lepidoptera, Gelechiidae), a highly devastating and invasive pest attacking tomatoes either in greenhouses or in open fields, is currently spreading throughout many countries in Africa. Six major vegetable production areas in Kinshasa, Democratic Republic of Congo (DRC), were surveyed between October and November 2016 to report any possible occurrence of the invasive *Tuta absoluta*. Morphological identification of the sampled adult insect individuals was based on phenotypic characters using a stereomicroscope while molecular identification was carried out using polymerization chain reaction-based approaches targeting the mitochondrial cytochrome oxidase subunit I (COI) gene. Results showed that *T. absoluta* was found in a commercial tomato farm in Nsele area, while it was absent in the five other sampled sites. Phylogenetic analysis revealed that *T. absoluta* populations found in Nsele were genetically close to those from Tunisia and Kenya. This is the first report of *T. absoluta* occurrence and damage in tomatoes grown in DRC where effective and sustainable pest management programs against this invasive insect should be developed and implemented to avoid major crop yield and economic losses.

Key words: tomato crop damage, Integrated Pest management, Kinshasa Hinterland, DRC, sub-Saharan Africa

Introduction

The development of urban horticulture around the main cities of the Democratic Republic of Congo (DRC) is a major agricultural event over the last decade (Huart 2014). The DRC has experienced many years of war that has led to rural exodus, resulting in a higher rate of unemployment in the main cities and towns. To support their food needs, displaced people are engaged in the vegetable crop production around the cities (Deschytener 2005), for income, livelihood, social stabilization and to supply the large consumers based in Kinshasa (Kasongo 2009). There are many vegetable-growing areas around Kinshasa, such as Ndjili-Brasserie, Ndjili-Secomaf,

Maluku, Matadi-Mayo, Matadi-Kibala, Kimwenza, Funa, Nsele, and Mbudi-Lutendele. In each location, several vegetable species are grown in a mixed or pure cropping system (Huart 2014; Kasongo 2009).

Tomato (*Solanum lycopersicum* L.) is one of the important high value vegetable crops, consumed by a large number of people and it is one of the major sources of income for farmers, especially during the rainy season (Cishesa 2016). However, tomato cultivation has been facing several abiotic and biotic constraints including low soil fertility, price fluctuations, seed quality, pests and diseases. The major biotic constraints of tomato production in DRC are viral diseases such as Tomato mosaic virus (ToMV) characterized by the presence of mosaic, the Tomato yellow leaf curl virus (TYLCV); bacterial diseases caused by *Ralstonia solanacearum, Pseudomonas syringae, Erwinia carotovora*; and fungal diseases caused by *Fusarium* spp. Similarly, insect pests such as aphids, thrips and fruit borer (*Helicoverpa armigera* Hübner) represent a major challenge for tomato production in DRC. The South American tomato pinworm, *Tuta absoluta* (Meyrick) has been considered for several years a key, devastating insect pest of tomato and other solanaceous plants worldwide (Desneux et al. 2010; EPPO 2012; Campos et al. 2017; Biondi et al. 2018; Mansour et al. 2018; Han et al. 2019). This lepidopteran leafminer is a multivoltine insect originated in South America, then it spread as invasive insect to Europe where it was first reported in Spain in 2006 (Desneux et al. 2010, 2011; Guedes and Picanço 2012; Biondi et al. 2018). In Africa, *T. absoluta* was first reported in Morocco, Algeria and Tunisia in 2008, then it spread rapidly to several other countries in either Northern, Eastern, Western or Southern Africa (Mansour et al. 2018). Based on a report mentioning serious infestations of tomatoes by an insect in the Hinterland of Kinshasa during October 2016, the Plant Clinic-International in Kinshasa led an investigation in six different tomato production areas in order to identify the insect and the levels of its damage. In this context, the aim of the present study was to report with evidences, for the first time in DRC, any possible occurrence and spread of *T. absoluta* in tomatoes grown in the Hinterland of Kinshasa and to describe its induced crop damage.

Materials and methods

Study sites

The city of Kinshasa is made up of urban and rural areas where most of agricultural activities are carried out. For this study, surveys were conducted from October to December 2016 in the following vegetable production areas: Nsele, Nswenge, Ndjili-brasserie, Ndjili-Secomaf, Funa and Kimbondo-Ceprosem. The sampling period coincided with the rainy season in western part of DRC. The criteria used for the selection of the study sites were the (i) accessibility of the site, (ii) Report of damage from an unknown pest on

Table 1. Agro-ecological characteristics of the sampled sites.

Sites	Cropping system	Seed source	Fertilizer	Pesticide	Symptoms and Damage	Growth stage
Funa	Mono-cropping and mixed intercropping system	Seed from Local Market and from Ceprosem,	Organic fertilizer (leaves, pig's faeces and Mineral fertilizer	Mancozeb, Twigathoate, Purimifoce	Necrosis, chlorosis and perforation of green and red fruits	Nursery-bed, flowering, fruiting
Nswenge	Mono-cropping and mixed intercropping system	-Ceprosem, Technisem (France) -Local markets in DRC	Organo-mineral: chicken dropping, cow dung	Ioda, Ivori, Cypermetrine, doudoutrine, Macozebe	Holes on fruits and stem; galleries on the leaves;	Flowering and fruiting
Kimbondo	Mono-cropping system for seed production and in some sites, tomato are grown for consumption	Local seed from previously cultures, from local market	Organo-mineral as NPK, uree and pigs stool, chicken dropping	Imidachlopride, Zalangue, Purimiforce	Insect damages on leaves, stems, fruits and wilting of roots	Nursery-bed, flowering,
Nsele	Tomato in green house and Intercropping system (tomato- pepper out of the green house	Imported seed from south Africa, Israel; Seed from Ceprosem, Technisem	NPK, Uree DAP, pigs feaces, organic fertilizer	Dimethoate, Cypermetrine	Wilting of whole plant, mining of leaves, stems; rotting of ripe fruits; Apical necrosis of the fruit	Flowering,
Ndjili-brasserie	Tomato growing with other Solanaceae as Solanum *melongenae*	Local Market at Secomaf et marché locale	Organo-mineral	Ash, concoction of plant biopesticide, Thiodan, cypermetrine	Wilting of plant, fruit and collar rot	Before flowering
Ndjili-Secomaf	Location here tomato is growing in mono-cropping system.	Local Market at Secomaf	Only Organic fertilization	DDT, Thiodan, Manèbe, Doudoutrin	Dieback and plant wilt	Flowering, fruit ripening

tomato in the site, (iii) potential for vegetable production, and (iv) more specifically the abundance of tomato and other solanaceous crops, including tomato and eggplant, in the site. In each location, three fields were randomly chosen for sample (insect) collection, but in Nsele, one of the fields surveyed was the farm characterized previously with the occurrence of pest damage. The characteristics of each sampling site are presented in Table 1.

Sample collection

Insects sampling was performed using yellow traps containing 0.55 L of formaldehyde (40%) solution for a good preservation of the samples. In each site, three plots were surveyed and, in each plot, three yellow traps were placed on the ground and next to the collar of tomato plants for a period of two days. Traps were replaced after two days, during all the study period. Samples from each plot were pooled in order to have one composite sample per site. Afterwards, insects were stored in a fridge at 4 °C.

Morphological identification

Morphological identification of insects was carried out based on the phenotypic characters of the adults, using a stereomicroscope and the

taxonomical identification was achieved using dichotomous key implemented by (Mignon 2016). The taxonomic identification was limited to the family of each collected insect. Following stereomicroscopy observation, for each surveyed site, three adult insects with characteristics close to *T. absoluta* were selected, and a total of 18 insect samples were used for DNA extraction and molecular analyses.

Molecular analyses

Genomic DNA extraction

Genomic DNA was extracted from a single insect selected after stereomicroscopy observation, following the protocol adopted by (Gawel 1991). This protocol consisted of the preparation of 50 mL of an extraction buffer (1001 mM of Tris-HCl (1 M), pH 8.0, 1.4% NaCl (5M), 20 mM EDTA (500 mM), 2% MATAB, 1% PEG 600, 0.5% Sodium Sulfite). Samples were ground in 100 µl of the extraction buffer and the solution was vortexed for 20 seconds for homogenization. The obtained solution was incubated at 74 °C for 20 minutes, cooled at room temperature, and then 500 µl of CIAA (24/l) was added. After centrifugation at 13.000 rpm for 30 minutes, the supernatant was precipitated using one volume of Iso-Propanol. The mixture was shaken until the formation of pellet and centrifuged for 10 minutes at 13.000 rpm. The supernatant was discarded, and the pellet was washed with 500 µl of 70% ethanol, air dried and eluted in 50 µl with sterile water.

Mitochondrial Cytochrome Oxidase Subunit I (COI)-gene analysis

Polymerization chain reaction (PCR) was carried out using two different set of primers (LCO1490_Forward_GGTCAACAAATCATAAAGATATTGG/ HCO2198_Reverse_TAAACTTCAGGCTGACCAAAAAATCA) and (C1-J-2195_Forward_TTGATTTTTTGGTCATCCAGAAGT/L2-N-3014_Reverse_ TCCAATGCACTAATCTGCCATATTA) targeting respectively the partial part (709 bp and 864 bp) of the mitochondrial cytochrome oxidase subunit I (COI) gene, which is recognized to have a good genetic resolution and is ideal for the differentiation of species (Folmer 1994). This gene is recognized to provide a suitable genetic information allowing the differentiation of insects at the species level (Kambhampati 1995).

PCR was carried out with 2.5 µl of DNA template in 25 µl of final volume of master mix (2.5 µl of $MgCl_2$, 0.75 µl of dNTP (10 µM), 5 µl of Gotaq buffer, 13.2 µl of water, 0.5 µl of LCO 1490. Forward primers (0.5 µM), 0.5 µl of HCO2198 revers primers (0.5 µM) and 0.1250.5 µl of Gotaq polymerase). In order to certify the quality of the analysis, the solution of sterile water and Gotaq buffer was used as a negative control. PCR conditions were the initial denaturation of 5 min at 95 °C, followed by 35 cycles of 1min at 95 °C, 1 min at 54 °C, 30 seconds at 72 °C, 7 min at 72 °C. The PCR products were visualized using the Bioradtrans luminator machine

(BioRad, USA), after running 1.5% agarose gel (100 V) for 45 minutes. The gel was stained with a 0.05% solution of ethidium bromide.

Sequencing and Phylogenetic analysis

PCR products from positive samples were purified using the Stratec DNA purification Kit, supplied by Biomed-Germany, following the manufacturer protocol. Pure DNA was sequenced using the LCO1490/HCO2198 primers to Macrogen manufacturer (Macrogen, Netherland). Molecular phylogenetic analysis was conducted in MEGA 7. The phylogenetic relationship was inferred using the evolutionary-based Maximum Likelihood method based on the Kimura 2-parameter model (1) and the trees with the highest log likelihood is shown (Kumar 2016). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites. Corresponding sequence of *Gnorimoschema gallaesolidaginis* (*Lepidoptera*, genus *Gnorimoschema*; GenBank Accession number: DQ233565.1) was used as the outgroup. Phylogenetic relationships analyses were conducted in MEGA7 (Kumar 2016). Accession numbers of *Tuta absoluta* isolated from Democratic Republic of Congo: Tuta-Nsele1-DRC (MG693224), Tuta-Nsele-7-DRC (693223), Tuta-Nsele-2-DRC (MG693222), Tuta-Nsele3-DRC (MG3221), Tuta-Nsele8-DRC(MG693220), Tuta-Nsele4-DRC (MG693219), Tuta-Nsele5-DRC (MG693218), Tuta-Nsele6-DRC (MG693217), Tuta-Nsele9 (MG693216).

Finally the pairwise identity between the *T. absoluta* sequences from the samples and with those from the GenBank database was done with the sequence demarcation tool (SDTv1.2) (Muhire, 2014).

Results

Tuta absoluta *damage*

Damage associated with *T. absoluta* larvae and adults was observed both on the tomato leaves and fruits in Nsele, the site from where the first damage was reported. Different types of damage were observed. This included perforation of variable sizes on the leaves, and on the green fruits, associated with the presence of larvae near the damaged holes. Adult insects were found on the leaves (Figure 1).

Morphological identification

The morphological examination of the insects, which was based on the types of antennae, buccal organs, paws and wings (type, color), is summarized in Table 2 that shows a great diversity of insects infesting tomatoes. Insects

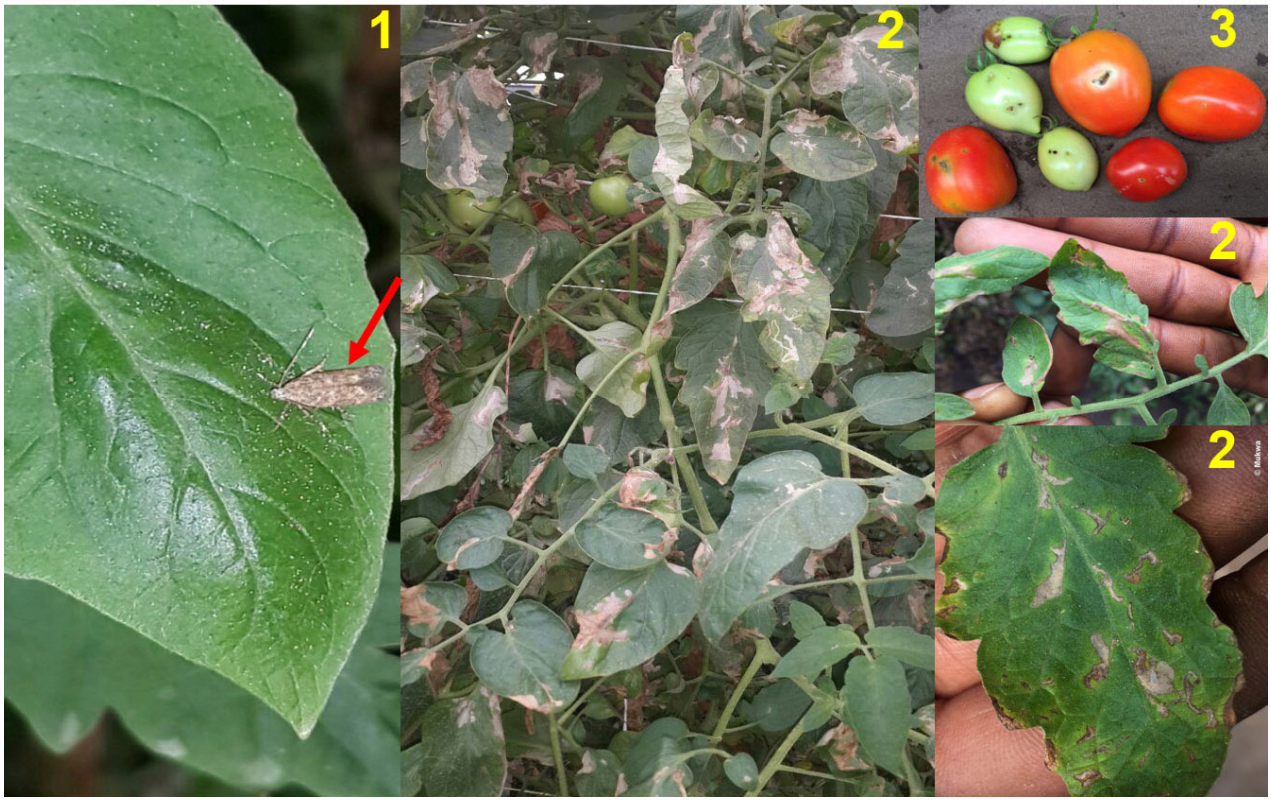

Figure 1. (1) *Tuta absoluta* (Meyrick) adult on tomato leaves, (2) damage caused by *T. absoluta* larvae on leaves, (3) damage caused by *T. absoluta* larvae on tomato fruit observed in Nsele in Kinshasa, Democratic Republic of Congo. Photo by Lyna F.T. Mukwa

collected from Funa belonged to six orders and eight families (Table 2). The family Potamanthidae was the most represented with three species. Other insects belonging to the order Hemiptera (Asilidae, Cantharidae, Cercopidae, Siricidae and Acridoidea families) and Hymenoptera, which are probably predators, were also recorded in this site.

Morphological analysis of samples collected from Matadi-Kibala showed the presence of five orders and 18 families of insects; the Orthoptera order with many polyphagous (multivoltine) species with the Synphidae family having the predatory species (Table 2). However, most of the insects (14) belonged to Bombyliidae and Syrphidae families. In Kimbondo-Ceprosem, many insect families were also recorded in the tomato crops. In terms of number of insects, the most commonly found groups were the Coleoptera-Carabidae represented by 19 insect individuals (Table 2). In Ndjili-secomaf and Ndjili-brasserie, insects from the order Homoptera were the most commonly found, followed by those belonging to the families Forficulididae, Cicindalidae from Forficulididae, Cicindelidae, Acrididae, Trichoceridae and Telligonidae. In Nsele, where tomato crops were cultivated in fields and in greenhouses, several insect families were recorded.

Molecular identification

Insects morphologically similar to *T. absoluta* were selected and used for the molecular analysis. In PCR assays, the mitochondrial cytochrome oxidase

Table 2. Families of insects observed in tomato crops in the six locations

Location name	Orders	Sub-orders	Super families	Family	Number of insects observed
Funa	Ephemeroptera			*Potamanthidae*	*3*
	Diptera	*Brachycera*		*Asilidae*	*1*
				Empididae	*2*
	Coleoptera			*Cantharidae*	*1*
	Hemiptera			*Cercopidae*	*1*
			Coccoidea	*Coccoidea*	*2*
	Hymenoptera	*Symphytes*		*Siricidae*	*1*
Matadi-Kibala	Coleoptera			*Silpidae*	*1*
				Curculionidae	*2*
				Cicindelidae	*1*
				Carabidae	*1*
	Diptera	*Brachycera*		*Asilidae*	*2*
				Tabanidae	*1*
				Bombyliidae	*14*
		Nematocères		*Trichoceridae*	*2*
				Cecidomyiidae	*1*
		Cyclorrhaphes		*Syrphidae*	*4*
	Hymenoptera	*Apocrites*		*Formicidae*	*2*
				Cholcididae	*2*
				Proctotrupidae	*3*
				Vespidae	*4*
				Apidae	*2*
	Heteroptera			*Gerridae*	*1*
	Orthoptera	*Ensifera*	*Grylloidea*	*Gryllidae*	*2*
		Caelifera	*Acridoidea*	*Acrididea*	*1*
Ceprocem	Coleoptera			*Carabidae*	*19*
				Cicindelidae	*10*
				Dytiscidae	*7*
	Dermaptera			*Forficulididae*	*9*
	Diptera	*Brachycera*		*Asilidae*	*13*
				Bombyliidae	*1*
		Nematocera		*Cecidomyiidae*	*9*
				Trichoceridae	*4*
	Heteroptera			*Anthocoridae*	*1*
	Hymenoptera	*Apocrites*		*Chrisididae*	*1*
	Orthoptera	*Caelifera*	*Acridoidea*	*Acrididae*	*6*
Ndjili-Cecomaf	Homoptera	*Ensifera*	*Tettigonioidea*	*Tettigonidae*	*3*
				Jassidae	*19*
				Forficulididae	*5*
				Cicindelidae	*5*
				Acrididae	*7*
				Trichoceridae	*2*
				Telligonidae	*4*
				Anthocaridae	*1*
				Noctuidae	*> 20*
Ndjili-Brasserie				*Cecidomyiidae*	*5*
				Cantharidae	*11*
				Curculionidae	*25*
				Trichoceridae	*4*
				Carabidae	*9*
Nsele	Lepidoptera			*Sphingidae*	*7*
				Noctuidae	*10*
				Gelechiidae	*> 50*
				Aphididae	*> 50*
				Cecidomyiidae	*11*

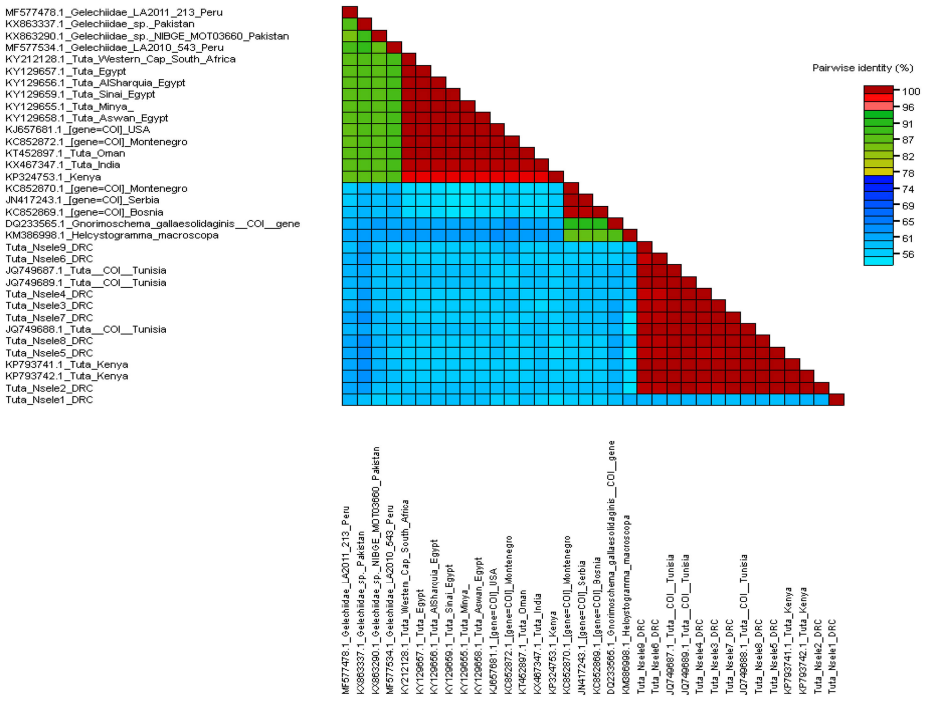

Figure 2. Graphical representation of percent pairwise nucleotide sequence identity of partial mitochondrial cytochrome oxidase subunit I (COI) gene of nine samples of *Tuta absoluta* collected in Democratic Republic of Congo and 23 *T. absoluta* from other countries obtained from the GenBank database.

subunit I (COI) gene (mtCOI-gene) primers LCO1490 forward and HCO2198 revers designed to amplify the fragment of 709 bp were used. These amplicons were sequenced from nine *T. absoluta* positive samples. Pairwise comparison of *T. absoluta* individuals from the different samples showed that 8/9 samples were 100% similar, but one isolate, Tuta-Nsele1_DRC showed a difference with 84% of identity. Comparison of the *T. absoluta* isolate from the samples with those from the GenBank database, from Tunisia and Kenya, shared 99–100% identities at the nucleotide and amino acid levels. The pairwise identity between *T. absoluta* isolates from DRC compared to those from the GenBank database showed that the DRC isolates had two clusters. In the first cluster, isolates were very close to those from Tunisia and Kenya, but there was 71% pairwise identity with the isolates from Egypt, South Africa, Montenegro, Oman, USA, India, Pakistan and Peru (Figures 2 and 3). Only one isolate, Tuta Nsele1 DRC gather the second cluster. This isolate seems to be close to other DRC, Kenyan and Tunisian isolates, with 84% pairwise identity but far from USA, Pakistan, and all other isolates analysed here.

The nucleotide sequences reported here have been deposited in the GenBank database under accession numbers: MG693216 to MG693224.

Discussion

The South American tomato pinworm *T. absoluta* is a devastating invasive pest for which the introduction and dispersion can be favored by international

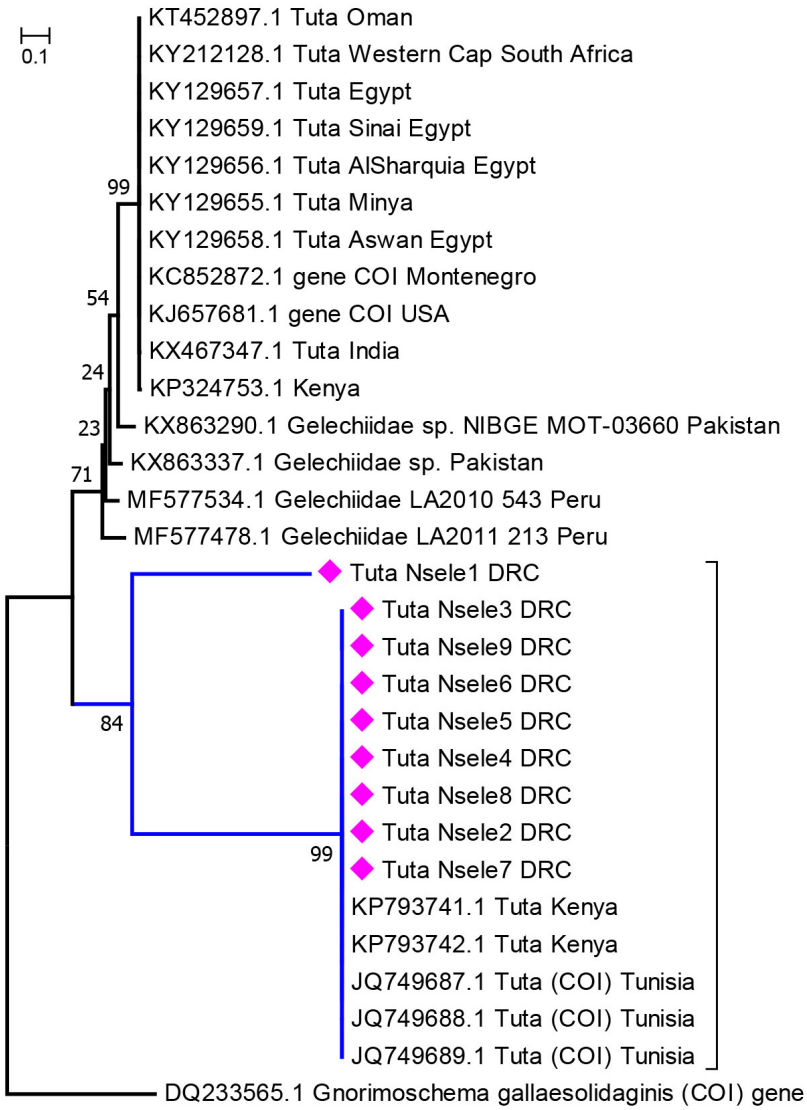

Figure 3. Locations of new records of *Callinectes sapidus*. Previous records in the Iberian captures in this study are shown with white circles. For details see Supplementary material.

trade and commerce, especially for African countries where quarantine measures are inefficient, and in some cases unavailable. *Tuta absoluta*, native to South America, is now present in many countries of the Mediterranean Basin of Africa and Europe (Abbes 2012; Brévault 2014; Chidege 2016; Mutamiswa 2017; Pfeiffer 2013; Son 2017; Visser 2017). This study confirmed, for the first time, the occurrence of *T. absoluta* in the Hinterland of Kinshasa, DRC. The results presented here represent, to our best knowledge, the first record of *T. absoluta* in tomato crops grown throughout the DRC. Based on the phylogenetic analysis, *T. absoluta* isolates from The DRC seemed to be genetically close to those from Tunisia and Kenya. Given the early introduction of *T. absoluta* in Kenya in 2013, and with exchanges of agricultural inputs (seeds, plantlets, etc.) between the DRC and other African countries (Zambia, Tanzania and Kenya), the emergency of *T. absoluta* in the Western part of DRC, with isolates genetically close to those of Kenya is not surprising.

Analysis of the historic situation of one of the largest farms in Nsele site and from discussions with farmers showed that, some months ago, seeds, pesticides and fertilizers were imported from Israel to one of the farms, where the damage was first reported both in greenhouses and open-fields. It is known that *T. absoluta* can spread through infected fruits or plants after harvest, through transportation systems, by wind over 10 km around the host plant (Brévault 2014; EPPO 2012; Tabone 2014), by chrysalids infested-soil, other crops from the infected fields, and the residues of previously cultivated crops (EPPO 2012; Tabone 2014). According to Tonnang et al. (2015), *T. absoluta* is a serious and a very invasive pest, which adapts in different agro-climatic conditions. The Climex model developed by Tonnang et al. (2015) for global prediction of current and future climate inducted change in distribution shift, suggests that temperature and moisture characteristics stimulate the growth of *T. absoluta* population. The life cycle of *T. absoluta* at 15 °C is 89 days, which includes 10 days as eggs stage, 36 days as larvae stage, 20 days as pupae, and 23 days as an adult (Brévault 2014; Son 2017; Tonnang 2015). It has been demonstrated that increases in the temperatures shorten the duration of these stages. For example, at 30 °C, the life cycle of *T. absoluta* is about 29 days, while tomatoes take 90 days to develop (Leite 2001). In the last ten years, the daily temperature in Kinshasa has been increasing and turn around 35 °C (Malanda-Nsumbu 2005; Plan 2005). This means that, in DRC, *T. absoluta* life cycle can be shortened with three generations within the tomato crop cycle, and then result in 14 generations in a year.

Apart from the temperature effects on the proliferation of *T. absoluta* in the Congolese agriculture, the insect resists to a range of pesticide active substances. In some cases, various mutations were observed when the insect was exposed to various pesticides (Abd El-Ghany 2016; Haddi 2012; Luna 2012; Polaszek 2012). In DRC, farmers use different pesticides for pests and disease management, and most of the time the recommended and registered doses are not respected (Mukwa et al. 2014). This makes *T. absoluta* management difficult within the Congolese agricultural context, which may facilitate further spread of this pest in the country. Moreover, *T. absoluta* can exist in infested soils for many years (EPPO 2012; Tabone 2014), and the sandy soils of the western part of DRC might be favourable for the development and spread of this insect pest.

Considering all the above listed factors, the risk of dispersion of *T. absoluta* in DRC remain very high. Pest management strategies should be implemented by considering the quarantining of the uninfected areas and the implementation of control using the most suitable Integrated Pest Management (IPM) approaches in the infested areas. This can be done, for example, by removal of infested plant materials on the ground or near greenhouses in order to get rid of the pest. In greenhouses, disinfestations will be needed to destroy the pupae, especially in mono-cultivation.

Sustainable pheromone-based monitoring and mass trapping systems, and biorational, eco-friendly control using either biopesticides as *Bacillus thuringiensis* or augmentative releases of natural enemies (Desneux et al. 2010; Biondi et al. 2018; Mansour et al. 2018, 2019) should be tested and developed in the near future as alternatives to the possible repetitive applications of broad-spectrum chemical pesticides.

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