Population dynamics and insecticide resistance in *Tuta absoluta* (Lepidoptera: Gelechiidae), an invasive pest on tomato in Kenya

Maureen Adhiambo Ong'onge [a,b], Inusa Jacob Ajene [a], Steven Runo [b], Bonoukpoé Mawuko Sokame [a], Fathiya Mbarak Khamis [a,*]

[a] International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya
[b] Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya

ARTICLE INFO

ABSTRACT

Tuta absoluta feeds on solanaceous plants with preference on tomato. Management of the pest is mostly with chemical insecticides. This study identified insecticide resistant populations and predicted resistance to insecticides. Insecticide resistance development was modelled using system thinking, and system dynamics approaches. The model showed the pest resistance development is alarming with an exponential increase of the resistance strength mostly in recent years. Furthermore, we used seven insecticide-resistance gene markers to resolve the population structure and genetic differentiation of insecticide-resistant populations in Kenya. The genes for resistance (knockdown resistance (*kdr*) mutations, acetylcholinesterase (AChE) and voltage gated sodium channel (para)) were detected in all populations. Population structure analyses separated *T. absoluta* populations into three genetic clusters with resistant genes that are interconnected. A better insight on the population dynamics and the genetic structure *T. absoluta* resistant genes in Kenya will help estimate resistance strength and determine the most effective pest control strategies.

1. Introduction

Dreadful pests such as leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) (Meyrick) are among the most difficult pest to control in tomato production, the pest is highly invasive and widely distributed in many parts of the world [1]. The spread of *T. absoluta* has been reported in Afro-Eurasia and continues to spread posing a threat to tomato production worldwide [2,3]. Furthermore, *T. absoluta* has currently been reported in a majority of African nations. This pest has also become a threat to tomato farming in Africa, Europe and the Middle East [4]. However, only in the reports for 22 countries was the date of the first record of *T. absoluta* indicated [5]. Around the world, the most widely adopted technique to manage different insect populations is the application of chemical compounds [6]. Recently this pest's insecticide resistance has been reported and demonstrated for both open field and greenhouse populations [3,7]. The enormous population of the pest is countered by farmers, recurrently increasing the application of high dosages of the insecticide, and as a result, there is no significant change in the pest population [1]. Chemical insecticides mismanagement may possibly result in the rapid development of insecticide resistant strains, as reported in different populations worldwide [8]. Insecticide resistance is increasingly becoming a major concern as more than 500 insect and mite species are now resistant to at least some insecticides used for

---

their control [9]. Recurrent resistance of *T. absoluta* to insecticides of different insecticidal groups (organophosphates (Ops), pyrethroids, abamectin, cartap, spinosad, and diamide) was early reported, according to Zibaee et al. [10]. However, the escalated use of insecticides for the management of the pest has resulted in the spread of resistance and target site insensitivity to different classes of insecticides such as organophosphates, carbamates, synthetic pyrethroids, benzoylureas, avermectins insecticides of tomato *T. absoluta* populations [11] and recently reported resistance to the diamide chlorantraniliprole, the oxadiazine indoxacarb and spinosyn spinosad insecticides to the pest population [12].

However, the resistance of many insect pest species, including *T. absoluta,* has escalated over the years due to the extensive and intensive use of insecticides in the last few years [13]. As a result of their continued use, they pose a significant and escalated threat conferred by an SNP missense mutation triggering a leucine to phenylalanine substitution in the domain II S6 transmembrane segment a leucine to phenylalanine substitution in the domain II S6 transmembrane segment, which is responsible for the *kdr* phenotype [14]. Knockdown resistance (*kdr*) has been elucidated to be a common mechanism of resistance to pyrethroid-based insecticides, which reduces the sensitivity of the insect's nervous system to these compounds by altering the proteins target sites (point mutations) in the para-type voltage gated sodium channel. A single point mutation (SNP) in the S6 segment of domain II of the *vgsc* gene is a result of a leucine to phenylalanine (L1014F) substitution, and it is the most prevalent mutation seen in a variety of arthropod pests and is sometimes abbreviated as "*kdr.*" [11]. In house flies, a report of secondary mutations of methionine to threonine substitution (M918T) has also been identified and described as super-*kdr* mutations within the sodium channel domain II, which is attributed to higher levels of pyrethroid resistance [15]. Acetylcholinesterase (AChE) stability can be decreased and altered by any small accumulation of mutations. These variations should be the source of the fitness costs associated with resistance, as well as the cause of allelic frequency in Drosophila populations because the least altered alleles are also the most common. One is expressed in the central nervous system and is responsible for acetylcholine hydrolysis in the synapse; its inhibition causes the insect to die, and mutation provides resistance [16]. Acetylcholinesterase, encoded by the *ace* gene, which causes the neurotransmitter acetylcholine to quickly hydrolyze, which stops synaptic transmission at cholinergic synapses in the central nervous systems of insects. Research has focused on two major classes of insecticides of AChE because it targets organophosphate (OP) and carbamate (CB) insecticides for insect control, thus critical for pest management in horticultural and vector-borne pests [16,17]. Unfortunately, farmers have extensively used insecticides for control *T. absoluta* populations, but the pest has developed resistance to different classes such as carbamates, benzoylureas, organophosphates, indoxacarb, synthetic pyrethroids, avermectins, spinosyns and recently diamide insecticides [11,12,18,19]. Over the years, the extensive application of these insecticides has resulted in pressure selection of resistant strains [20]. The novel most class of insecticides are the diamides, although they have a shallow activity spectrum to Hemipteran, Lepidopteran, and Coleopteran insects and the first insecticidal compounds to be availed in the market from this class are the flubendiamide and chlorantraniliprole [4]. The most recent cases of resistance to chlorantraniliprole in populations of *T. absoluta* have been reported in Greece, Spain, Italy and Brazil, where *T. absoluta* gene sequencing for the ryanodine receptor (RyR)*,* uncovered a 1353 bp region of the RyR gene which harbours mutations that confer diamide resistance [12]. Furthermore, resistance to flubendiamides has been reported in *T. absoluta* populations in Brazil and Pakistan [4,21].

Despite the fact that insecticides have demonstrated efficiency in many contexts, the monetary expense of their application can be restrictively high [22]. Furthermore, their widespread application in farms is logistically difficult due to the evolution of resistance in their target insects and its relatively unstable [23]. The emergence of insecticide resistance has received a lot of attention empirically and theoretically, but no specific genetic models that describe when and how resistance to these chemicals evolves have been published. There are also no detailed genetic models describing adaptation to resistant crop cultivars [24]. Simulation models can be used to analyze the evolution of the impacts and help determine tactics and strategies to control the pests [25]. System dynamics (SD) was developed to understand and describe different interactions using simulation models; it was formerly developed for engineering and administration, but several studies have shown that its progressively been applied to other applications and have spread to a number of disciplines in scientific studies [26,27]. This method takes into account a set of components that interact in real time as a component with structure that changes over time, but clearly placing a focus on strategic issues and policy innovation. System dynamics models solve hitches by updating all variables with positive and negative feedback, as well as adding time delays to structure interactions and control in very short time intervals [28,29].

This study aimed to screen genes for resistance to chemical insecticides in *T. absoluta* populations, identify the population structure of insecticide-resistant *T. absoluta* populations from major tomato growing regions in Kenya (historical and current) samples and model the resistance strength of the pest. We present a model that simulates the pest's response to insecticide-treated fields and later, when high densities of insect pests invade and its sensitivity to the insecticides.

2. Results

2.1. Variation in insecticide-resistant populations

These outcomes are based on seven (7) insecticide-resistance genes genotyped in 300 *T. absoluta* individuals across ten insect populations. The designed markers successfully amplified the expected gene regions (acetylcholinesterase (*ace1*), voltage-gated sodium channel (para) gene (*vgsc*) and sodium channel (*kdr*)) in the tested *T.* absoluta samples (Supplementary Fig. S1). After scoring, the marker frequency showed that all markers were represented in all samples at different frequencies, with Tuta-TA1-F2 having the least frequency (0.094) in the current Naivasha population (Supplementary Fig. S2). The analysis of molecular variance (AMOVA) across all groups indicated high variance within the populations (87.4 %) and low variance among populations (12.6 %). The fixation Indices FST: 0.12582 was highly significant ($p < 0.01$) (Table 1).

Table 1
Analysis of molecular variance (AMOVA) of *Tuta absoluta* populations from different tomato-growing regions in Kenya.

Source of variation	Sum of squares	Variance components	Percentage variation (%)
Among populations	35.238	0.06398	12.58218
Within populations	246.338	0.44453	87.41782
Total	281.576	0.50851	

Table 2
Average coefficient of ancestry obtained from STRUCTURE with K = 3 for the 300 individuals of *Tuta absoluta* from five major tomato growing regions in Kenya. Co-ancestry higher than 10 % of each population in a cluster is shown in bold.

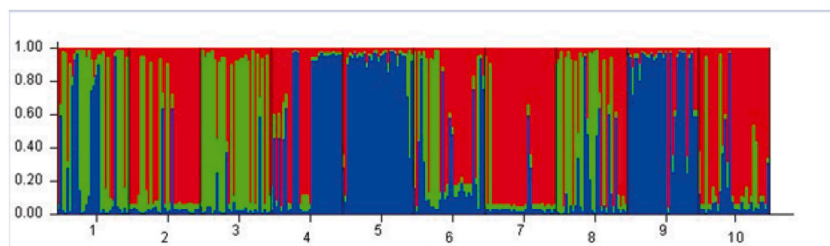| Given Population	Inferred Clusters			Number of Individuals
	1	2	3	
1	0.216	**0.501**	0.283	30
2	**0.742**	0.191	0.067	30
3	0.296	**0.640**	0.064	30
4	0.365	0.049	**0.586**	30
5	0.081	0.054	**0.866**	30
6	0.446	0.284	0.270	30
7	**0.854**	0.090	0.056	30
8	0.369	**0.464**	0.168	30
9	0.140	0.030	**0.830**	30
10	**0.743**	0.135	0.122	30

Fig. 1. Bayesian cluster analysis using STRUCTURE of *Tuta absoluta* resistant population: Graphical representation of the data set for the most likely k (k = 3), three hypothetical clusters (K1–K2-k3) based on seven insecticide-resistant genotypes where each color corresponds to a suggested cluster and each individual is represented by a vertical bar. The numbers in the X-axis correspond to a specific sample: 1-Naivasha current, 2-Loitoktok historical, 3-Loitoktok current, 4-Mwea historical, 5-Mwea current, 6-Naivasha historical, 7-Meru current, 8-Meru historical, 9-Taita Taveta current and 10-Taita Taveta historical. The Y-axis represents the probability of the assignment of an individual to each cluster. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.2. Population structure

The populations with the resistant genes fell into three genetic clusters. STRUCTURE analyses revealed that K = 3 yielded the highest value for the estimated likelihood of K (Table 2). The average ancestry probability (Q) values for each population in the three clusters revealed that the resistant genes of *T. absoluta* from Meru (current) had the highest ancestry in cluster one (Q = 0.854), resistant genes of *T. absoluta* from Naivasha (Historical samples) had the highest ancestry in cluster two (Q = 0.501), and resistant genes of *T. absoluta* from Mwea (Historical samples) had the highest ancestry in the cluster three (Q = 0.866). Visualization of cluster membership coefficients showed that among populations, the resistant genes of *T. absoluta* from Mwea (Current) and Taita Taveta (Current) grouped in one cluster (Blue), while those from Naivasha (Current), Loitoktok (Current) and Meru (Historical) were in the second cluster (green) and samples from Loitoktok (Historical), Meru (Current) and Taita Taveta (Historical) formed the third cluster (red) (Fig. 1).

Results from the Discriminate Analysis of Principal Components (DAPC) indicated three major clusters. All historical samples had a large proportion of their genotypes clustered in one group. The current samples from Taita Taveta, Mwea and Naivasha had a higher proportion of unique genotypes and clustered separately into two of the major clusters while the third major cluster had all other remaining populations with high proportions of shared genotypes (Fig. 2).

2.3. Dynamics of Tuta absoluta resistance development to chemical insecticides in tomato agrosystems

The model's results revealed that the use of chemical insecticides was efficient in controlling *T. absoluta* at the beginning, as the susceptibility of the pest population to the application of the chemical insecticides in the tomato agrosystems increased while no
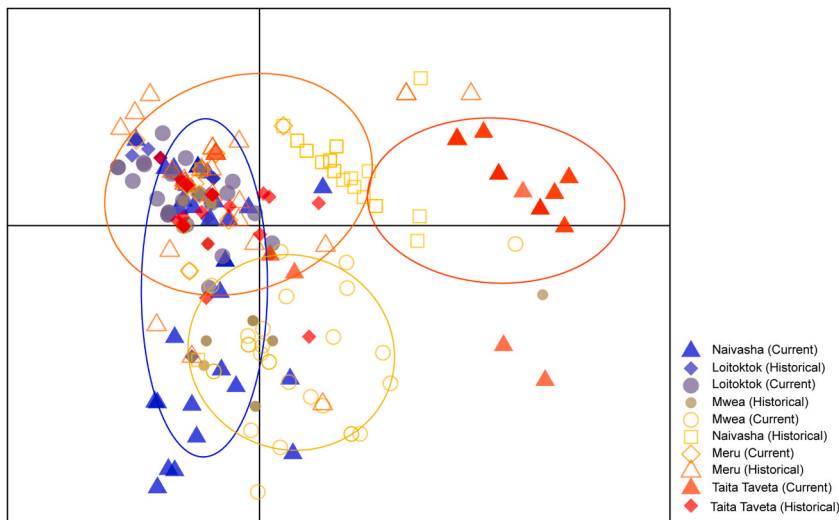
Fig. 2. Multivariate analyses of population structure of 300 *Tuta absoluta* samples from five major tomato growing regions in Kenya using Discriminant Analysis of Principal Components (DAPC).
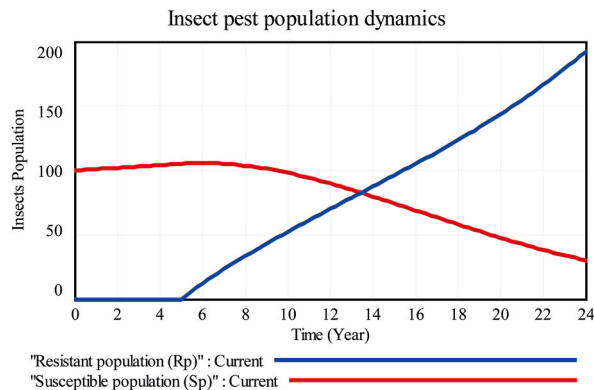

Fig. 3. Predicted susceptible and resistant *Tuta absoluta* population dynamics under synthetic pesticide management strategies.
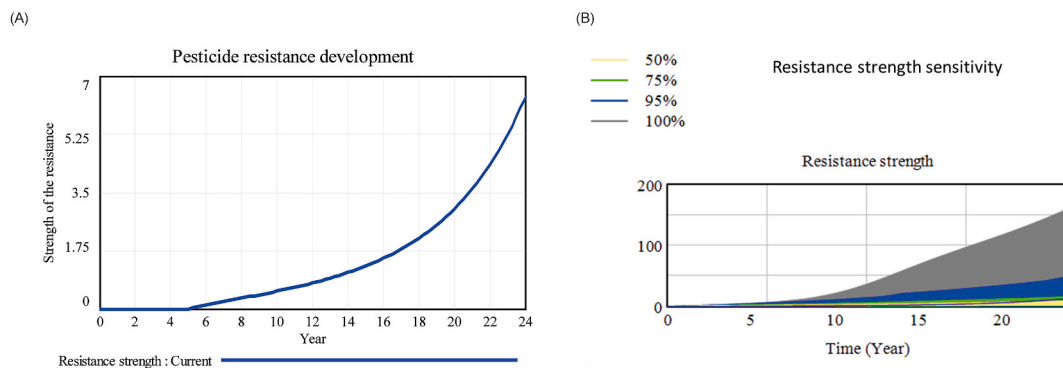

Fig. 4. Insecticide resistance strength and evolution of the strength of insecticide resistance by Tuta absoluta.

resistance to these insecticides was reported. The pest was very susceptible to the insecticides used, but after a period estimated from the model of about five years, the susceptibility to the insecticides started to drop (Fig. 3), and the resistant population marginally increased without reaching the equilibrium after 24 years of simulation (Fig. 3). Therefore, after about five years of chemical insecticide control, the pests started to develop resistance to any form of chemical insecticide used for its management where the

resistant population is still exponential after 24 years of simulation, which may lead to 100 % loss of tomato production as a result of the pest, showing the failure of insecticide efficacy in controlling the pest with time.

From the time the pest started developing resistance, the model's output showed that the problem of pest resistance is very critical as the insecticide resistance development strength exponentially increased, especially in recent years (Fig. 4a). From our simulation, the resistant strength is directly proportional to both the susceptible and resistant populations. The sensitivity analysis provided for investigating through different parameter standards in operation revealed that the number of years followed a constant distribution with a minimum value of zero (0) years. The strength of the resistance is highly influenced by the continuous use and misuse of chemical insecticides in the agrosystem. The evolution of the strength of insecticide resistance developed by the pest is very sensitive and the sensitivity strength is still exponential and predicted to be 100 % even after 20 years (Fig. 4b).

3. Discussion

Tuta absoluta's recent invasion and rapid spread in Africa and the confirmed strains of resistant populations to insecticides have raised concerns on tomato farming globally. We modelled the dynamics and interactions of *T. absoluta* populations in this study in response to chemical insecticide application as the major control method with even calendar-based applications and evaluated *T. absoluta* genetic structure of resistant populations (current and historical) of major tomato growing regions in Kenya. The strategy of chemical overuse for pest control has already been shown to increase the risk of developing insecticide resistance to numerous insecticides of different modes of action [30]. Our study showed that the resistance level of *T. absoluta* to chemical insecticides has been increasing exponentially over years. The strength of resistance after ten years from our study has started developing, and this has been seen in *Musca domestica*, *Anopheles gambiae*, and *Pediculus corporis* [31–33], where insecticides rapidly broke down due to resistance development over time. When the pest is detected, local farmers use chemical insecticides, but this management strategy has been proven to be ineffective against the control of *T. absoluta*. It is likely that as a result of globalization, with an increase in existing international trade and human trade levels, similar climatic patterns have brought the risk of invasive species introduction to unprecedented heights, would result in resistance being further widespread than previously thought and its management is still largely unknown [34,35]. Therefore, understanding the infestation dynamics and shaping the suitability would be imperative towards coming up with effective, sustainable strategies to manage the alarming increase in the insecticide resistance in this pest. Furthermore, although our model uses simulations, timing of resistance strength of *T. absoluta* was still predicted. The model sounds the alarm of insecticide use impact on pest resistance development and gives researchers and stakeholders advice on integrating other IPM strategies that will reduce chemical insecticide and pest damages to economically manageable levels [5,36].

All populations tested were positive for the common *kdr* mutation at different frequencies. Most populations were positive for all the resistance genes tested, indicating that the insect population has undergone a strong mutation to the different classes of insecticides used and indicating the presence of resistance mechanisms on some of the strains. It further indicated that the mutations are widespread across the regions studied; in fact, no insect sample was discovered that lacked at least two or more of the three mutations. Knockdown resistance mutation seemed stable across all populations studied. Our results are congruent with a study done by Ref. [11]. Our findings indicated a strong first migration gene flow occurring freely across the trade zone. This pattern could be correlated to geographical proximity of these regions since all the population are from the same country. This pattern of migration has also been observed in other invasive species, for instance *Bactrocera invadens* [37] and *Diaphorina citri* [38], these invasive pests can also be dispersed by wind. In this study, the Bayesian clustering analysis of insecticide-resistant genotypes illustrates that, there was a high level of estimated resistant gene flow across all samples, both the current and historical samples, there were no conserved resistant genes in one population. We investigated the underlying principles of the shift in the resistant genetic population dynamics and discovered a significant influence by (i) the populations are not natural, (ii) it is human-mediated transportation through trade, (iii) No restricted transboundary migration and (iv) the pest is considered to be a new population and no significant physical barriers to prevent the natural movement of this pest in Kenya [38,39]. Environmental climate events might have triggered population alterations, amplifying genetic drift with low genetic diversity, such as that found in a study on tsetse fly [40].

The tomato leaf miner has been reported to have developed resistance in several countries against a wide range of insecticides [4, 18,41]. The discovery of mutation in *T. absoluta* populations previously linked to organophosphate and carbamates resistance strengthens the theory that insecticide resistance may have aided the prompt spread the invasive pest. *Tuta absoluta* originated in Chile, according to recent genetic studies, because of strong selective pressure from human activities, a genetically homogenous population was able to expand through South America before reaching the Mediterranean basin [42,43]. This proposition was corroborated by Haddi et al. [44], who confirmed the presence of three *kdr* alterations at very high incidences in *T. absoluta* populations.

There are two kinds of *ace* genes in insects, both of which have esterase-specific active centres, implying that the two have similar basic roles linked to resistance in carbamate and organophosphate. Investigation revealed that the insecticides insensitivity caused by *ace1* mutation was the molecular basis of resistance in insects. Our study also found the incidence of *ace* genes in *T. absoluta* from field-collected current and historical samples. Previous research has shown that in *D. melanogaster* and *M. domestica*, mutations at the *ace-2* locus has led to resistance [45]. Many other insects' *ace-1* genes, particularly those of closely related Lepidopterans, have previously been linked to insecticide-insensitive forms of AchE A[44]. Furthermore, our study identified all three genes for resistance in which insects were positive from all the sampling sites. The mutations were not only conserved to one population but were distributed in all populations at different rates as early as 2015 to 2016 when the pest was first reported in Kenya. Knockdown resistance (*kdr*), of pyrethroid has been demonstrated to result from changes (point mutations) in the voltage-gated sodium channel (*vgsc*) and the insect nervous system's sensitivity to these compounds. Samples from the field of *T. absoluta* from Europe and South America had a high frequency of these mutations [10,44]. In the past, three dominant mutations against pyrethroids in several populations of *T. absoluta*

Table 3
Genotyping markers used in PCR amplification and genotyping of the resistance genes in *Tuta absoluta* in this study. Ta – Annealing temperature.

Locus	Prime code	5' - 3' sequence	Size	Ta (°C)	Target	Tags
Tuta-Ace-F1	TA1	CACACTCGTGCGTCCAAATC	175	56.3	Acetylcholinesterase (*ace1*) gene	HEX
Tuta-Ace-R1		GAGTAAAACCCTCCGCCGAA				
Tuta-Ace-F2	TA2	ACACTCGTGCGTCCAAATCA	264	56.3	Acetylcholinesterase (*ace1*) gene	FAM
Tuta-Ace-R2		GAGGCAACTCGGTACTGCAT				
Tuta-TA1-F1	TA3	AGTGTAAGTGCAGCATGGTCT	254	58	Voltage gated sodium channel (para) gene	HEX
Tuta-TA1-R1		GGTCTCCATCGGGAAAACGA				
Tuta-TA1-F2	TA4	CCGATGGAGACCTACCACGA	157	58	Voltage gated sodium channel (para) gene	FAM
Tuta-TA1-R2		ATGACGACGGTGGCTAAGAA				
Tuta-kdr-tnf 1	TA5	TGGCCGACGTTTAATTTACTCA	300	54.1	Sodium channel (*kdr*)	FAM
Tuta-kdr-tnr1		ACACTTGGAAAAGTTGGTGCT				
Tuta-kdr-2-F1	TA6	GTAGGACGATGGGTGCCTTG	160	58	Sodium channel (*kdr*)	HEX
Tuta-kdr-2-R1		CGTCCACCAACACGTCGATA				
Tuta-kdr-2-F2	TA7	AGTGTAAGTGCAGCATGGTCT	254	58	Sodium channel (*kdr*)	FAM
Tuta-kdr-2-R2		GGTCTCCATCGGGAAAACGA				

have been found [11,46]. Due to single or multiple substitutions in the sodium channel gene, the insecticide's affinity for its binding site on the sodium channel changes, resulting in *kdr* mutations in *Anopheles* and *Aedes* [47]. Recently, it was discovered that the sodium channel mutations are linked to pyrethroid resistance in *T. absoluta* [48]. The *kdr* mutations have been found in *An. Arabiensis* by independent groups in several widely dispersed locations [15]. *Tuta absoluta* populations in Iran have also been reported to have developed insecticide resistance at a faster rate, as a result of emergence of resistant populations because of selective pressure from insecticides used by farmers [10,11]. A study done by Fang et al. [49], found no *kdr* mutations in any of the specimens gathered. This tends to suggest that the insecticide resistance-related *kdr* mutations are likely recessive. The findings by Fang et al. [49] contrast with our study, where all our samples had *kdr* mutations.

The origins of the possible alteration in expression levels of these genes are mysterious, according to a study by Sandeu et al. [50], on the malaria causing vector, although it is possible that it is related to the type of selection that led to the resistance. The natural populations' genetic flexibility and capacity to respond to different selection pressures are highlighted by this change in gene expression. No significant relationship between genetic diversity and terrestrial distance was found by the genetic test of isolation by distance, suggesting that this difference may be caused by ecological or geographic reasons. Due to the prevalence of resistant genes, their dominance, and selection pressure, insect pest populations become tolerant of the damaging effects of insecticides, which results in the development of insecticide resistance [10]. Therefore, with extensive use of insecticides for the management of this pest, the insecticide resistance has escalated.

4. Conclusion

One of the most well-documented and instantaneous examples of evolutionary adaptation to environmental change is insecticide resistance. Our simulations have shown that fast and early decisions for alternative control methods to chemical insecticide use are required to protect farmers against likely invasions and establishment of *T. absoluta* and prioritize the management strategies needed where necessary. This study demonstrates that target site resistance is common in *T. absoluta* populations, and we found a high frequency of *kdr* and *ace-1* mutations in the Kenyan populations. Thus, the application of the insecticides currently used in the management this pest in these areas may adversely affect the resistance problem Therefore, sustainable integrated management options for *T. absoluta* need to be explored and up scaled to control the pest.

Data availability statement

All other relevant data are within the paper and supplementary materials.

Materials and methods

Tuta absoluta sample collection and DNA extraction

Tuta absoluta samples were collected from five major tomato-growing regions in Kenya in two batches (historical and current). The sampling sites were Mwea, Meru, Naivasha, Loitoktok and Taita Taveta. Historical samples were collected between 2015 and 2016, while the current samples were collected between March 2020 and November 2020. The samples were then stored in 96 % ethanol. Following the manufacturer's instructions, extraction of DNA from individual *T. absoluta* samples using the Isolate II genomic DNA Kit (Bioline, United Kingdom). Thirty insects per site from both current and historical samples were extracted using the protocol stated above and the DNA extracted was then stored at −20 °C for further downstream analysis. Using a Nanodrop 2000/2000c Spectrophotometer, the DNA extracts' amount and quality were evaluated (Thermo Scientific, United States). Three hundred samples were extracted from five current and five historical sampling sites. Seven primers were used for screening the samples for the presence of

genes for resistance to chemical insecticides. Specific markers targeting *T. absoluta* acetylcholinesterase (ace1) gene region, *T. absoluta* strain TA1 voltage-gated sodium channel (para) gene, and *T. absoluta* voltage-gated sodium channel (para) gene were designed from sequences retrieved from GenBank (KU985167.1, JQ701800.1 and KY767010.1) The *T. absoluta* sodium channel (kdr) markers were adopted from Zibaee et al. [10]. The markers were designed using Primer-BLAST [51]. The markers were optimised and then tagged at the 5′ end with fluorophores (FAM and HEX). Thirty samples from each of the sampling sites were genotyped using seven insecticide-resistance gene markers (Table 3). Amplifications were performed with fluorescently labeled (FAM and HEX) forward primer of each of the seven primer sets using a Mastercycler Nexus gradient thermal cycler (Eppendorf, Hamburg, Germany). 20 ml of 5X MyTaq Reaction Buffer (5 mM dNTPs, 15 mM MgCl2, stabilisers, and enhancers), 0.5 pmol of each primer, 0.5 mM MgCl2, 0.0625 U of MyTaq DNA polymerase, and 15 ng of DNA template were used to set up the reaction. The following cycling parameters were used: an initial denaturation at 95 °C for 2 min, 40 cycles at 95 °C for 30 s, annealing temperature (Ta) for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. On a Mastercycler gradient, PCR amplifications were conducted. On an ABI 3730xL Automated Sequencer, PCR products were multiplexed in batches of two or three primers and genotyped at the DNA Analysis Facility on Science Hill at Yale University (http://dna-analysis.yale.edu/). Specific PCR primers were designed in Geneious software version 10. using the default Primer 3 program.

Marker scoring and population structure

After genotyping, the software GENEMARKER v2.4.0 (Soft Genetics, State College, PA, USA), was used in scoring, and the automatically scored peaks were manually edited. The MS Tools Excel add-in was used to prepare genotyping data for analysis. Standard genetic distances were used to estimate genetic differentiation. GenAlEx [52] was used to calculate marker frequencies by population.

AMOVA was done to understand the partitioning of the different population groupings of insecticide-resistant genes. Population clustering was done using two methods for ten populations: Bayesian clustering was implemented in STRUCTURE V.2.2 [53], and we performed replicate runs for each K = 1 ± 10 with an admixture model. Using the online resource STRUCTURE SELECTOR, the most likely number of K was selected [54]. We performed Discriminant Analysis of Principal Components (DAPC) in addition to STRUCTURE. It was done with the "adegenet" [55] and "poppr" [56] packages in R version 3.5.1 with R-Studio [57]. Conversions of various data sets for the different software programs were performed in CONVERT version 1.31 [58].

System structure and model simulation assumptions

In tomato agrosystems, the populations of *Tuta absoluta* treated with insecticides were all susceptible. Following the misusing and continuous spraying of insecticides, some individuals in the population start developing resistance at a rate (alpha) which will increase and constitute the resistant population of the pest within the community. The two types of population (susceptible: S_p and resistant: R_p) each increase with a given growth rate and naturally die with a given death rate and as well as inter-specific competition coefficient for resource exploitation and availability. As the susceptible population continue developing resistance, a fraction (gamma) of the resistant population will lose the resistance character and become susceptible with a probability to develop resistance again as the spraying of insecticide continues. The evolution of the proportion of resistant population in the whole community of the *T. absoluta* population in tomato agrosystem defines the resistance strength over time.

To develop the model, the following assumptions were made.

(1) The natural flight ability of *T. absoluta* drives the time scale of the invasion process.
(2) Tomato agrosystem considered was opened with a possibility of pest to flight from out into the system and vice versa.
(3) The pest population growth is limited by tomato plants during the cropping season but 10 % will survive on alternative host plants during non-cropping season giving a raising to a new population in the next cropping season.
(4) When the cropping season starts, the pest will fly from alternative hosts to tomato crop plants.
(5) Simulations were run under the assumption that each system was in equilibrium.

Models' formulation

The aim here was to develop models that represent the evolution of susceptibility and resistance of *T. absoluta* to chemical insecticides, ordinary differential equations were developed (Equations (1)–(4)) to reflect the phenomenon of the system and the generic formulations and other equations are displayed below:

$$\frac{dP_T}{dt} = \frac{dS_p}{dt} + \frac{dR_p}{dt} \tag{1}$$

$$\frac{dS_p}{dt} = \left(\alpha_{S_p} \times S_p + \varepsilon \times R_p\right) - \left(\delta_{S_p} \times S_p + a_{12} \times S_p \times R_p\right) \tag{2}$$

$$\frac{dR_p}{dt} = \left(\alpha_{R_p} \times R_p + \gamma \times S_p\right) - \left(\delta_{R_p} \times R_p + a_{21} \times R_p \times S_p\right) \tag{3}$$
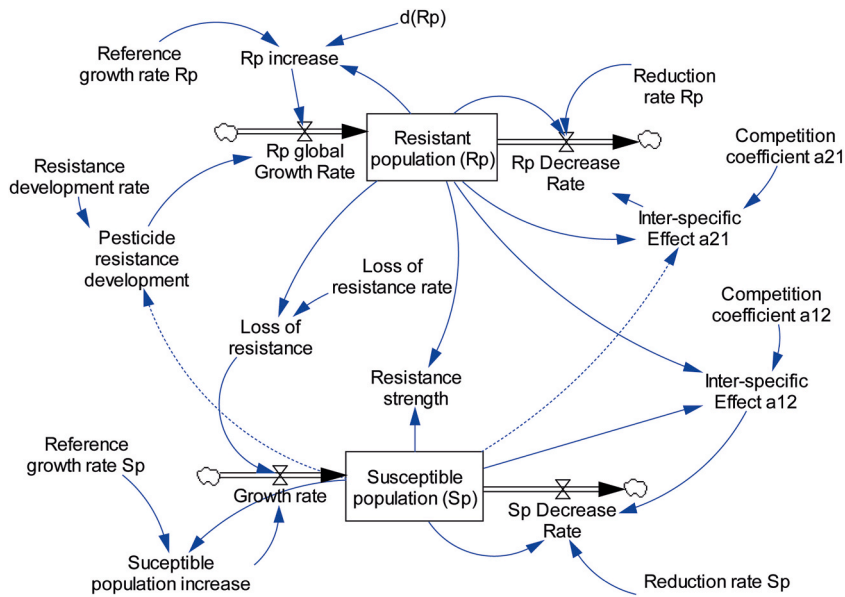
Fig. 5. Stock and flow diagram of susceptible and resistant insect pest populations in farmer's vegetable garden under the use of synthetic insecticides as a control method.

Table 4
Differential equation parameters.

Parameters	Designation	Units/sources
$\frac{dP_T}{dt}$	Total population of *Tuta absoluta* in the agrosystem at time t	insect
$\frac{dS_p}{dt}$	Susceptible population of *Tuta absoluta* in the tomato agrosystem at time t	insect
$\frac{dR_p}{dt}$	Resistant population of *Tuta absoluta* in the tomato agrosystem at time t	insect
$\mu(t)$	Resistance strength	dmnl
Rpi	Normal Growth rate of resistant population	1/year
dRp	Growth rate of resistant population	1/year
dDRp	Decrease of resistant population	1/year
Spi	Normal Growth rate of susceptible population	1/year
dSp	Growth rate of susceptible population	1/year
dDSp	Decrease of susceptible population	1/year
PRD	Pesticide resistance development	insect
a12	Inter-specific effect a12	dmnl
a21	Inter-specific effect a21	dmnl
d(Rp)	Resistance delay	year
α_{S_p}	Natural growth rate of susceptible population in the tomato agrosystems	0.13 day^{-1} (from dataset)
α_{R_p}	Natural growth rate of resistant population in the tomato agrosystems	0.11 day^{-1} (from dataset)
ε	Rate of loss of resistance character	0.06 day^{-1} (Shalaby et al.) [59]
γ	Rate of resistance development	0.5 dmnl (from dataset)
δ_{S_p}	Natural mortality rate of susceptible population	0.02 day^{-1}
δ_{R_p}	Natural mortality rate of resistant population	0.025 day^{-1} (from dataset)
a_{12}	Effect that susceptible population can cause to resistant population	4.15e-4 dmnl (from dataset)
a_{21}	Effect that resistant population can cause to susceptible population	8e- [05] dmnl (from dataset)

$$\mu(t) = \frac{\frac{dR_p}{dt}}{\frac{dP_T}{dt}} \tag{4}$$

Other equations: Rpi = αRp × Rp; dRp = PRD × Rpi; dDRp = a21 × rRP × Rp; Spi = αSp × Sp; dSp = ε × Spi; dDSp = a12 × rSp × Sp; PRD = ϒ × Sp.

Model implementation, simulation and data sources

System thinking and system dynamics approaches (a computer-aided approach for analyzing and resolving complex problems

based on a feedback mechanism of the systems) [26] was used to implement the model where the total population was initially susceptible and with time generate resistant sub-population. To analyze the population dynamics of these sub-populations (susceptible and resistant) Vensim PLE 8.0.9 software (Ventana Systems, Harvard, USA), was used to develop Stock and flow diagram (SFD) and carry out simulations of *T. absoluta* resistance and susceptibility. Vensim PLE 8.0.9 software includes a graphical environment in which users can draw CLD, stocks, and flows diagrams and run simulations [27]. The SFD is typically built with these variables: stocks, flows, connectors, and auxiliary. Stocks variables represent here susceptible and resistant sub-population state in the system. Flow variables represent resistance population global growth rate, susceptible population global growth rate and their respective death rate (Fig. 5). The mathematical equations that represent the relationships between the variables were formulated as presented in model formulation section. The parameters within the equations that control the behaviour of the system such as natural growth rate of susceptible and resistant populations, rates of resistance development and losses, mortality rate of each category, competition coefficients, etc. were identified. Experimental data sets from a field survey on prevalence, knowledge, perceptions and management practices of the pest and effectiveness of insecticides obtained in this study were used to estimate model parameter variables for model simulations. Furthermore, publicly available datasets used in this study included: *T. absoluta* occurrence data, relative humidity, the yield of tomato production, reports on high-density pest's resistance and report on data from experiments, as well as an estimation of all the parameters associated with various development rate functions. Therefore, the resistance development rate was calculated from bioassay with field recommended concentration as follow:

$$\gamma = \frac{LC_{50} \text{ of field population}}{\text{Field pesticide recommanded concentration}}$$

A multiple regression procedure was conducted using the R software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) with the experimental datasets of species interaction to estimate the competition coefficients of categories pests combination and specific values outputs and other constants from the datasets used in model simulation are presented in Table 4.

CRediT authorship contribution statement

Maureen Adhiambo Ong'onge: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Inusa Jacob Ajene:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Steven Runo:** Writing – review & editing, Supervision. **Bonoukpoè Mawuko Sokame:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis. **Fathiya Mbarak Khamis:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21465.

References

[1] V. Tumuhaise, F.M. Khamis, A. Agona, G. Sseruwu, S.A. Mohamed, First record of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Uganda, Int. J. Trop. Insect Sci. 36 (3) (2016) 135–139, https://doi.org/10.1017/S1742758416000035.

[2] N. Desneux, E. Wajnberg, K.A.G. Wyckhuys, et al., Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control, J. Pest. Sci. 83 (2010) 197–215, https://doi.org/10.1007/s10340-010-0321-6.

[3] N. Desneux, M.G. Luna, T. Guillemaud, A. Urbaneja, The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production, J. Pest. Sci. 84 (4) (2011) 403–408, https://doi.org/10.1007/s10340-011-0398-6.

[4] J.E. Silva, C.P.O. Assis, L.M.S. Ribeiro, H.A.A. Siqueira, Field-evolved resistance and cross-resistance of Brazilian *Tuta absoluta* (Lepidoptera: Gelechiidae) populations to diamide insecticides, J. Econ. Entomol. 109 (5) (2016) 2190–2195, https://doi.org/10.1093/jee/tow161.

[5] R. Mansour, T. Brévault, A. Chailleux, A. Cherif, K. Grissa-Lebdi, K. Haddi, S.A. Mohamed, R.S. Nofemela, A. Oke, S. Sylla, H.E.Z. Tonnang, L. Zappalà, M. Kenis, N. Desneux, A. Biondi, Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa, Entomol. Gen. 38 (2) (2018) 83–112, https://doi.org/10.1127/entomologia/2018/0749.

[6] A.K. Misra, R. Patel, N. Jha, Modeling the effects of insecticides and external efforts on crop production, Nonlinear Anal. Model Control 26 (6) (2021) 1012–1030, https://doi.org/10.15388/NAMC.2021.26.24442.

[7] A. Biondi, R.N.C. Guedes, F.-H. Wan, N. Desneux, Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta* : past, present, and future, Annu. Rev. Entomol. 63 (1) (2018) 239–258, https://doi.org/10.1146/annurev-ento-031616-034933.

[8] G. Kinyanjui, F.M. Khamis, F.L.O. Ombura, E.U. Kenya, S. Ekesi, S.A. Mohamed, Distribution, abundance and natural enemies of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) in Kenya, Bull. Entomol. Res. (2021), https://doi.org/10.1017/S0007485321000304.

[9] T. Van Leeuwen, W. Dermauw, K. Mavridis, J. Vontas, Significance and interpretation of molecular diagnostics for insecticide resistance management of agricultural pests, Current Opinion in Insect Science 39 (2020) 69–76, https://doi.org/10.1016/j.cois.2020.03.006.

[10] I. Zibaee, K. Mahmood, M. Esmaeily, A.R. Bandani, M. Kristensen, Organophosphate and pyrethroid resistances in the tomato leaf miner Tuta absoluta (Lepidoptera: Gelechiidae) from Iran, J. Appl. Entomol. 142 (1–2) (2018) 181–191, https://doi.org/10.1111/jen.12425.

[11] B.M. Haddi, P. Bielza, D. Cifuentes, L.M. Field, K. Gorman, C. Rapisarda, M.S. Williamson, C. Bass, Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (Tuta absoluta), Insect Biochem. Mol. Biol. 42 (7) (2012) 506–513, https://doi.org/10.1016/j.ibmb.2012.03.008.

[12] C. Grant, R. Jacobson, A. Ilias, M. Berger, E. Vasakis, P. Bielza, C.T. Zimmer, M.S. Williamson, R.H. ffrench-Constant, J. Vontas, et al., The evolution of multiple-insecticide resistance in UK populations of tomato leafminer, Tuta absoluta, Pest Manag. Sci. 75 (8) (2019) 2079–2085, https://doi.org/10.1002/ps.5381.

[13] S. Williamson, A. Ball, J. Pretty, Trends in pesticide use and drivers for safer pest management in four African countries, Crop Protect. 27 (10) (2008) 1327–1334, https://doi.org/10.1016/j.cropro.2008.04.006.

[14] R.A. Carvalho, C. Omoto, L.M. Field, M.S. Williamson, C. Bass, Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm spodoptera frugiperda, PLoS One 8 (4) (2013), https://doi.org/10.1371/journal.pone.0062268.

[15] M.J. Donnelly, V. Corbel, D. Weetman, C.S. Wilding, M.S. Williamson, W.C. Black IV, Does kdr genotype predict insecticide-resistance phenotype in mosquitoes? Trends Parasitol. 25 (5) (2009) 213–219, https://doi.org/10.1016/j.pt.2009.02.007.

[16] D. Fournier, Mutations of acetylcholinesterase which confer insecticide resistance in insect populations 158 (2005) 257–261, https://doi.org/10.1016/j.cbi.2005.10.040.

[17] B. Li, Y.H. Wang, H.T. Liu, Y.X. Xu, Z.G. Wei, Y.H. Chen, W.D. Shen, Genotyping of acetylcholinesterase in insects 98 (2010) 19–25, https://doi.org/10.1016/j.pestbp.2010.04.004.

[18] M.M.M. Lietti, E. Botto, R.A. Alzogaray, Insecticide resistance in Argentine populations of *Tuta absoluta* (meyrick) (Lepidoptera: Gelechiidae), Neotrop. Entomol. 34 (1) (2005) 113–119, https://doi.org/10.1590/s1519-566x2005000100016.

[19] E. Roditakis, K. Mavridis, M. Riga, E. Vasakis, E. Morou, J.L. Rison, J. Vontas, Identification and detection of indoxacarb resistance mutations in the para sodium channel of the tomato leafminer, *Tuta absoluta*, Pest Manag. Sci. 73 (8) (2017) 1679–1688, https://doi.org/10.1002/ps.4513.

[20] R. Nauen, D. Steinbach, Resistance to diamide insecticides in lepidopteran pests, in: Advances in Insect Control and Resistance Management, Springer International Publishing, 2016, pp. 219–240, https://doi.org/10.1007/978-3-319-31800-4_12.

[21] L.S. Zang, Z.R. Akhtar, A. Ali, K. Tariq, M.R. Campos, Flubendiamide resistance and its mode of inheritance in tomato pinworm Tuta absoluta (meyrick) (Lepidoptera: Gelechiidae), Insects 13 (2022) 1023, https://doi.org/10.3390/insects13111023.

[22] F. Chepchirchir, B.W. Muriithi, J. Langat, S.A. Mohamed, S. Ndlela, F.M. Khamis, Knowledge, attitude, and practices on tomato leaf miner, *Tuta absoluta* on tomato and potential demand for integrated pest management among smallholder farmers in Kenya and Uganda, Agriculture (Switzerland) 11 (12) (2021), https://doi.org/10.3390/agriculture11121242.

[23] E.A. McGraw, S.L. O'Neill, Beyond insecticides: new thinking on an ancient problem, Nat. Rev. Microbiol. 11 (3) (2013) 181–193, https://doi.org/10.1038/nrmicro2968.

[24] F. Gould, Role of behavior in the evolution of insect adaptation to insecticides and resistant host plants, Bull. Entomol. Soc. Am. 30 (4) (1984) 34–41, https://doi.org/10.1093/besa/30.4.34.

[25] Fao, Transboundary threats to food and nutrition security in southern Africa, Issue 1 (1) (2017). http://www.fao.org/3/a-i7691e.pdf.

[26] J.E. Hansen, P. Bie, And sodium model - I 3 (2) (1987) 116–135.

[27] K. Jagan Mohan Reddy, A. Neelakanteswara Rao, K. Lanka, P.R.C. Gopal, System dynamics modelling of fixed and dynamic Kanban controlled production systems: a supply chain perspective, J. Model. Manag. (2021), https://doi.org/10.1108/JM2-06-2020-0168.

[28] K. Jagan Mohan Reddy, A. Neelakanteswara Rao, L. Krishnanand, Manufacturer performance modelling using system dynamics approach, Mater. Today: Proc. (2020) 142–146, https://doi.org/10.1016/j.matpr.2020.03.563.

[29] B.M. Sokame, H.E.Z. Tonnang, S. Subramanian, A.Y. Bruce, T. Dubois, S. Ekesi, P.A. Calatayud, A system dynamics model for pests and natural enemies interactions, Sci. Rep. 11 (1) (2021) 1–14, https://doi.org/10.1038/s41598-020-79553-y.

[30] E. Roditakis, D. Steinbach, G. Moritz, E. Vasakis, M. Stavrakaki, A. Ilias, L. García-Vidal, M. Martínez-Aguirre, R. del, P. Bielza, et al., Ryanodine receptor point mutations confer diamide insecticide resistance in tomato leafminer, Tuta absoluta (Lepidoptera: Gelechiidae), Insect Biochem. Mol. Biol. 80 (2017) 11–20, https://doi.org/10.1016/j.ibmb.2016.11.003.

[31] A.W. Lindquist, H.G. Wilson, Development of a strain of houseflies resistant to DDT, Science 107 (1948) 276.

[32] R.L. Metcalf, Implications and prognosis of resistance to insecticides, in: G.P. Georghiou, T. Saito (Eds.), Pest Resistance to Pesticides, Springer US, New York, NY, 1983, pp. 703–733.

[33] H.G. Wilson, J.B. Gahan, Susceptibility of DDT-resistant houseflies to other insecticidal sprays, Science 107 (1948) 276–277.

[34] I.J. Ajene, F.M. Khamis, B. van Asch, G. Pietersen, N. Seid, I. Rwomushana, F.L.O. Ombura, G. Momanyi, P. Finyange, B.A. Rasowo, et al., Distribution of candidatus liberibacter species in eastern Africa, and the first report of candidatus liberibacter asiaticus in Kenya, Sci. Rep. 10 (1) (2020) 1–10, https://doi.org/10.1038/s41598-020-60712-0.

[35] M.R. Campos, A. Biondi, A. Adiga, R.N.C. Guedes, N. Desneux, From the western palaearctic region to beyond: *Tuta absoluta* 10 years after invading Europe, J. Pest. Sci. 90 (3) (2017) 787–796, https://doi.org/10.1007/s10340-017-0867-7.

[36] R.Y.A. Guimapi, S.A. Mohamed, G.O. Okeyo, F.T. Ndjomatchoua, S. Ekesi, H.E.Z. Tonnang, Modeling the risk of invasion and spread of *Tuta absoluta* in Africa, Ecol. Complex. 28 (2016) 77–93, https://doi.org/10.1016/j.ecocom.2016.08.001.

[37] F.M. Khamis, N. Karam, S. Ekesi, M. De meyer, A. Bonomi, L.M Gomulski, C.R. Guglielmino, Uncovering the tracks of a recent and rapid invasion: the case of the fruit fly pest *Bactrocera invadens* (Diptera: Tephritidae) in Africa, Mol. Ecol. 18 (23) (2009) 4798–4810.

[38] I.J. Ajene, F.M. Khamis, B. van Asch, G. Pietersen, N. Seid, A.W. Wairimu, F.L. Ombura, K.S. Akutse, M. Sétamou, S. Subramanian, et al., Genetic diversity of Diaphorina citri (Hemiptera: liviidae) unravels phylogeographic structure and invasion history of eastern African populations, Ecol. Evol. 12 (7) (2022) 1–15, https://doi.org/10.1002/ece3.9090.

[39] Y. Zhang, C. Zhang, L. Wu, C. Luo, X. Guo, R. Yang, Y. Zhang, Population genetic structure and evolutionary genetics of Anopheles sinensis based on knockdown resistance (kdr) mutations and mtDNA-COII gene in China–Laos, Thailand–Laos, and Cambodia–Laos borders, Parasites Vectors 15 (1) (2022) 1–13, https://doi.org/10.1186/s13071-022-05366-9.

[40] R. Opiro, N.P. Saarman, R. Echodu, E.A. Opiyo, K. Dion, A. Halyard, A.W. Dunn, S. Aksoy, A. Caccone, Genetic diversity and population structure of the tsetse fly Glossina fuscipes fuscipes (Diptera: Glossinidae) in Northern Uganda: implications for vector control, PLoS Neglected Trop. Dis. 11 (4) (2017) 1–29, https://doi.org/10.1371/journal.pntd.0005485.

[41] E. Roditakis, K. Mavridis, M. Riga, E. Vasakis, E. Morou, J.L. Rison, J. Vontas, Identification and detection of indoxacarb resistance mutations in the para sodium channel of the tomato leafminer, *Tuta absoluta*, Pest Manag. Sci. 73 (8) (2017) 1679–1688, https://doi.org/10.1002/ps.4513.

[42] D. Cifuentes, R. Chynoweth, P. Bielza, Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers, Pest Manag. Sci. 67 (9) (2011) 1155–1162, https://doi.org/10.1002/ps.2166.

[43] T. Guillemaud, A. Blin, I. Le Goff, N. Desneux, M. Reyes, E. Tabone, A. Tsagkarakou, L. Niño, E. Lombaert, The tomato borer, Tuta absoluta, invading the Mediterranean Basin, originates from a single introduction from Central Chile, Sci. Rep. 5 (2015) 1–5, https://doi.org/10.1038/srep0837.

[44] K. Haddi, M. Berger, P. Bielza, C. Rapisarda, M.S. Williamson, G. Moores, C. Bass, Mutation in the ace-1 gene of the tomato leaf miner (Tuta absoluta) associated with organophosphates resistance, J. Appl. Entomol. 141 (8) (2017) 612–619, https://doi.org/10.1111/jen.1238.

[45] P. Fort, A. Berthomieu, M.P. Dubois, N. Pasteur, M. Raymond, A Novel Acetylcholinesterase Gene in Mosquitoes Codes for the Insecticide Target and Is Non-homologous to the Ace Gene in *Drosophila*, 2016, https://doi.org/10.1098/rspb.2002.212.

[46] T.B.M. Silva, W.M. Silva, M.R. Campos, J.E. Silva, L.M.S. Ribeiro, H.A.A. Siqueira, Susceptibility levels of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to minor classes of insecticides in Brazil, Crop Protect. 79 (2016) 80–86, https://doi.org/10.1016/j.cropro.2015.10.01.

[47] J. Hemingway, N.J. Hawkes, L. McCarroll, H. Ranson, The molecular basis of insecticide resistance in mosquitoes, Insect Biochem. Mol. Biol. 34 (7) (2004) 653–665, https://doi.org/10.1016/j.ibmb.2004.03.01.

[48] W.M. Silva, M. Berger, C. Bass, V.Q. Balbino, M.H.P. Amaral, M.R. Campos, H.A.A. Siqueira, Status of pyrethroid resistance and mechanisms in Brazilian populations of Tuta absoluta. Pesticide Biochemistry and Physiology 122 (2015) 8–14, https://doi.org/10.1016/j.pestbp.2015.01.011.

[49] Y. Fang, W.Q. Shi, J.T. Wu, Y.Y. Li, J.B. Xue, Y. Zhang, Resistance to pyrethroid and organophosphate insecticides, and the geographical distribution and polymorphisms of target-site mutations in voltage-gated sodium channel and acetylcholinesterase 1 genes in Anopheles sinensis populations in Shanghai, China, Parasites Vectors 12 (1) (2019) 1–13, https://doi.org/10.1186/s13071-019-3657-7.

[50] M.M. Sandeu, C. Mulamba, G.D. Weedall, C.S. Wondji, A differential expression of pyrethroid resistance genes in the malaria vector Anopheles funestus across Uganda is associated with patterns of gene flow, PLoS One 15 (11 November) (2020) 1–21, https://doi.org/10.1371/journal.pone.0240743.

[51] J. Ye, G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen, T.L. Madden, Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction, BMC Bioinf. 13 (2012) 1–11.

[52] R. Peakall, P.E. Smouse, GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research- - an update, Bioinformatics 28 (19) (2012) 2537–2539.

[53] J.K. Pritchard, M. Stephens, P. Donnelly, Inference of population structure using multilocus genotypic data, Genetics 155 (2000) 945–959.

[54] Y.-L. Li, J.-X. Liu, STRUCTURESELECTOR: a web- based soft- ware to select and visualize the optimal number of clusters using multiple methods, Molecular Ecology Resources 18 (2018) 176–177.

[55] T. Jombart, Adegenet: a R package for the multivariate analysis of genetic markers, Bioinformatics 24 (2008) 1403–1405.

[56] Z.N. Kamvar, J.F. Tabima, N.J. Grünwald, Poppr: an R pack- age for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction, PeerJ 2 (2014) e281.

[57] R Development Core Team, R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, 2008, 3– 900051– 07- 0, http://www.R-project.org.

[58] J.C. Glaubitz, CONVERT: a user- friendly program to reformat diploid genotypic data for commonly used population genetic soft- ware packages, Mol. Ecol. Notes 4 (2004) 309–310.

[59] S.E.M. Shalaby, M. Mahmmod, M. Soliman Mmm, A.E.M. Abd El-Mageed, Evaluation of some insecticides against tomato leaf minor (Tuta absoluta) and determination of their residues in tomato fruits, Appl. Biol. Res. 14 (2) (2012) 113–119.