



The African citrus trioqid *Trioza erytreae* Del Guercio (Hemiptera: Triozidae): temporal dynamics and susceptibility to entomopathogenic fungi in East Africa

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Abstract

The African citrus trioqid *Trioza erytreae* Del Guercio (Hemiptera: Triozidae) is the most destructive citrus pest. Presently, biological data on *T. erytreae* are insufficient for important parameters like distribution, seasonal dynamics and entomopathogens. Therefore, we determined the temporal dynamics of *T. erytreae* along three different altitudinal gradients in Kenya. In low altitudes, females of *T. erytreae* reactivated and started laying eggs between late February and early March. The mean monthly number of immature stages of *T. erytreae* per flush shoot peaked in February, whereas adults peaked in March. For mid-altitudes, *T. erytreae* mean populations peaked in February, but adults were already present in December. In high altitudes, adults appeared in June, and females began laying eggs. *Trioza erytreae* population densities in shaded trees positively and significantly correlated with unshaded trees. There was a significant difference between adult density and maximum temperature, average temperature, solar radiation, evaporation and dew point. Furthermore, this study evaluated the effects of 11 *Metarhizium anisopliae* and 4 *Beauveria bassiana* fungal isolates against adult *T. erytreae* under laboratory conditions. *Metarhizium anisopliae* ICIPE 18 and ICIPE 69 were the most potent isolates, causing 97.5% and 82.5% mortalities within the shortest LT₅₀ time of 4.4 and 5.9 days, respectively. The presence of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) on *Zanthoxylum capense* requires further investigation. Our findings provide relevant information for early detection, monitoring and developing biopesticides against the pest.

Keywords African citrus trioqid · Biological control · Citrus · Entomopathogens · Synthetic pesticides

Introduction

Environmental, biological (including pests and diseases) and socio-economic factors are major constraints to Kenya's citrus

industry (Kilalo et al. 2009), with arthropod pests and diseases as the main challenge, of which the African citrus trioqid (ACT) *Trioza erytreae* Del Guercio (Hemiptera: Triozidae) is the most damaging pest (Ekesi 2012; Khamis et al. 2017). The sap-sucking hemipteran pest is native to Africa but has recently invaded Asia and Europe (Cocuzza et al. 2017). Yield losses up to 100% have been associated with ACT and African citrus greening disease (ACGD) (van den Berg 1990). Although several attempts have been made to prevent the spread of the pest and ACGD, for instance through intensive insecticide applications (Kilalo et al. 2009), these have met with little success. One of the reasons for the latter could be the poor knowledge on the seasonal abundance of ACT in East Africa and beyond.

Trioza erytreae prefers cool and moist environment to areas with high temperatures and frequent rainfall (Cocuzza et al. 2017). Earlier reports indicate that ACT can survive in elevations up to 1300 m above sea level (m.a.s.l.) (Cocuzza

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et al. 2017; Ekesi 2015). Another study by Shimwela et al. (2016) found more ACT in the highlands than midlands. Also, in Yemen and Saudi Arabia, ACT abundance varied across different agro-ecological zones (Cocuzza et al. 2017). The optimal temperature range for ACTs' nymphal development was around 10–12 °C (van den Berg 1990). However, depending on the temperature, the five instars may take up to 45 days to reach maturity (Moran and Blowers 1967). Higher temperatures induce high mortalities and are detrimental to eggs, nymphs and ovarian development in females (van den Berg 1990). In the field, *T. erythrae* can live for up to 50 days; however, under experimental conditions, males and females can survive for up to 73 and 82 days, respectively (van den Berg 1990).

ACTs' peak population density appears to coincide with the periods of new flush growths in citrus (van den Berg 1990). Probably, the new shoots are soft and tender to make them suitable for feeding, oviposition, and development of immatures (i.e., eggs and nymphs). Also, young flush shoots have higher nitrogen content than matured ones (Catling 1972). However, the presence of new or young shoots does not always correspond to the presence of ACT (van den Berg 1990). Moreover, Catling (1969) noted that ACT's seasonal abundance and distribution patterns are limited by factors which promote dormancy in host-plants. A wide range of natural enemies attack ACT throughout its geographical distributions (van den Berg 1990). In most cases, populations are suppressed by generalist predators like lady beetles (Coleoptera: Coccinellidae), Syrphids (Diptera: Syrphidae), Lacewings (Neuroptera: Chrysomelidae), predatory spiders and mites (Araneae) (Aubert 1987; Catling 1970) as well as by different hymenopteran parasitoids (Mc Daniel and Moran 1972). For instance, on the island of Réunion, *Tamarixia dryi* Waterston (Hymenoptera: Eulophidae) and *Psyllaephagus pulvinatus* Waterston (Hymenoptera: Encyrtidae) have been credited with up to 75% parasitism in ACT; yet in South Africa, their impacts are limited by hyperparasitoids (Mc Daniel and Moran 1972; van der Merwe 1923).

ACTs' seasonal abundance across different altitudinal gradients in East Africa is crucial to inform management decisions but poorly documented. Besides, the success of an Integrated Pest Management (IPM) strategy requires detailed knowledge of pest population dynamics, distribution across different altitudinal gradients as well as the most preferred host plants. Although such information are available for the Republic of South Africa (Cook et al. 2014), equivalent data in East Africa are lacking (Magomere 2005). One of the most important management tactics to contain the impact of a vector-borne disease is to reduce the spread of the vector, for which monitoring based on precise information on its seasonal abundance is a prerequisite.

Empirical evidence suggests that several factors, including elevation, influence ACTs' distribution and abundance

(Kilalo et al. 2009; van den Berg 1990). Therefore, the present study sought to elucidate the occurrence and population dynamics of ACT in three different altitudinal gradients in Kenya as this information could become an essential component in the development of an effective IPM program against the pest.

Currently, most citrus farmers in Kenya use pesticides to manage *T. erythrae* (Kilalo et al. 2009). Although it is effective, excessive reliance on pesticides has resulted to increased vector resistance, harmful effects on non-target species, environmental contamination, and relative unaffordability to small-scale farmers in Africa compared to other management methods (Tiwari et al. 2011). Moreover, ACT is a major pest categorized as an "A1" quarantine pest by CABI (CABI 2004).

It is important to develop and implement safe and sustainable management strategies to control *T. erythrae*. Biological control using entomopathogenic fungus has been currently identified as a sound alternative management strategy against many insect pests, not only as stand-alone control agents, but also as a component of IPM approaches (Chandler et al. 2011; Inglis et al. 2001; Mkiga et al. 2020; Onsongo et al. 2019; Opisa et al. 2018; Prince and Chandler 2020).

Whereas a number of entomopathogenic fungal species have been reported to infect the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Meyer et al. 2007; Meyer et al. 2008; Subandiyah et al. 2000; Xie et al. 1988), little is known about entomopathogenic fungus that infects *T. erythrae*. Together with the research on population dynamics, this study therefore screened entomopathogenic fungal isolates against adult *T. erythrae* for development of fungal based biopesticide against the pest.

Materials and methods

Study site and collection of population dynamics data

A survey was conducted at three sites in Kericho region of western Kenya (Table 1). Fifteen citrus orchards were identified and sampled (Fig. 1) with elevations ranging from low to high altitudes (1100–2316 m above sea level [m.a.s.l.]). The low - (site 1 - low) and mid-altitude sites (site 2 - mid) were located at 35° 08.447 E and 00° 14.072 S and 1400 m.a.s.l., and 35° 03.950 E and 00° 19.294 S and 1773 m.a.s.l., respectively, both in the Soim and Sigowet sub-county (1000–1400 m.a.s.l.). The high-altitude site (site 3 - high) was located at 35° 34.798 E and 00° 11.723 S and > 2000 m.a.s.l. in the Kiptenden orchard of the Londiani sub-county (Obiri et al. 1994).

The sites were chosen based on the availability of citrus trees and elevation. In addition, the high altitude was selected for detailed studies (i.e., weather conditions, and seasonal

Table 1. Sites used in the study including global positioning system coordinates, elevation and where *Trioza erytreae* and *Diaphorina citri* were collected.

Altitudinal gradient	County	Location	Elevation (m)	<i>Trioza erytreae</i>	<i>Diaphorina citri</i>
Low (1000–1450)	Kisumu	Kakola	1152	yes	yes*
	Kisumu	Border2	1221	yes	yes
	Kisumu	Border2	1246	yes	yes
	Kisumu	Border2	1246	yes	yes
	Kericho	Soin and Sigowet	1260	yes	yes
	Kisumu	Border2	1271	yes	yes
	Kisumu	Border2	1279	yes	yes
	Kericho	Koitaburot	1375	yes	yes
	Kericho	Koitaburot	1397	yes	yes
	Kericho	Koitaburot	1398	yes	yes
	Kericho	Soin and Sigowet	1403	yes	yes
	Kericho	Soin and Sigowet	1409	yes	yes
	Mid (1450–2000)	Kericho	Cheptuiyet	1773	yes
High (>2000)	Kericho	Londiani	2310	yes	no
	Kericho	Londiani	2316	yes	no

Note: **yes*** = apart from citrus, *Diaphorina citri* was collected on *Zanthoxylum capense* (Thunb.) Harv

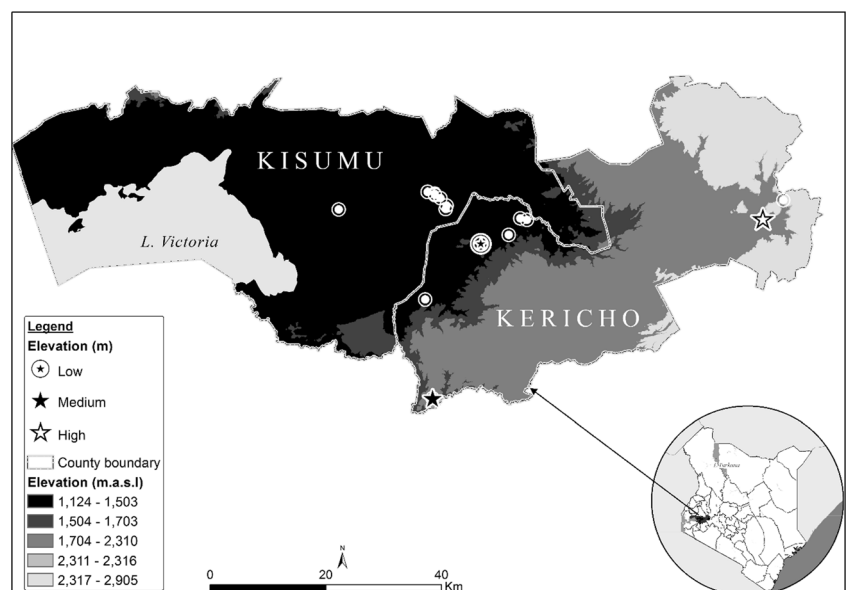
abundance in shaded and unshaded trees). Seasonal abundance across three altitudinal gradients was studied starting from December 2016 for one year. At each altitudinal gradient, one orchard was identified from which 10 trees were randomly selected for monthly sampling, with five trees in the shade (trees which received shade from other trees), and five trees in the open (trees fully exposed to the sun). The canopy of each tree was divided into four from which four flush shoots were randomly selected for eggs, nymphs and adults. Adult ACTs were counted in situ, whereas flush shoots were sent to the laboratory, observed and counted under a

Leica stereomicroscope for eggs and nymphs. Climate data were collected from the nearest meteorological station at Muhoroni, Western Kenya. In each of the study sites, no synthetic pesticides were applied during the sampling period. Geographical positioning system (GPS) data of each site were recorded using Garmin ETrex 20x.

Insect culture

Trioza erytreae adults were obtained from the Animal Rearing and Containment Unit at the International Centre of Insect

Fig. 1 Map showing sampling sites where *Trioza erytreae* and *Diaphorina citri* were found, and locations selected for seasonal abundance studies



Physiology and Ecology (*icipe*), Duduville, Nairobi Campus. The originality, mass production, and maintenance of adult *T. erythrae* colonies used in this study have been earlier reported (Aidoo et al. 2019a). Emerged adults of *T. erythrae* were exposed to seedlings in Perspex cages (75 × 65 × 75 cm) after 3 weeks post-pruning, following the appearance of new shoots. Plants were replaced monthly after adult emergence. The colony was quarterly rejuvenated, with nymphs collected from the field to reduce inbreeding. Insects were maintained under a rearing condition of 22 ± 2 °C, 65 ± 5% relative humidity and 12:12 L:D photoperiod.

Fungal cultures and preparation of conidial suspensions

All the 15 fungal isolates (11 *Metarhizium anisopliae* (Metschnikoff) Sorokin and 4 *Beauveria bassiana* (Bals.-Criv.) Vuill used in this study were obtained from the *icipe*'s Arthropod Germplasm Centre. The origin of the fungal isolates, their host source and year of isolation are summarized in Table 2. The *M. anisopliae* isolates were cultured on Sabouraud dextrose agar (SDA) while *B. bassiana* isolates were cultured on potato dextrose agar (PDA) and maintained at 25 ± 2 °C in darkness. Fungal conidia were harvested from 2 to 3-week-old sporulated cultures and suspended in 10 ml distilled water with 0.05% Triton X-100 in universal bottles containing glass beads (5–10 beads of 3 mm in diameter per bottle). The suspension was vortexed for 5 min at about 700 rpm to break the conidial clumps and ensure a homogeneous suspension. Conidial concentrations were quantified using a haemocytometer under a light microscope (Inglis

et al. 2012). A concentration of 1 × 10⁸ conidia ml⁻¹ was prepared prior to bioassays.

Viability of the isolates was determined prior to bioassays by spread-plating 100 µL of conidial suspension at a concentration of 3 × 10⁶ conidia ml⁻¹ in Sabouraud dextrose agar (SDA) plates. The plates were incubated at 25 ± 2 °C in dark conditions for 18 h after firmly sealing with parafilm. At 18 h after inoculation, lactophenol cotton blue was dropped on each plate to stop conidial growth as well as staining the conidia for successful germination assessment. The viability of fungal isolates or percentage germination was determined using a light microscope. One hundred conidia were then selected randomly, counted under a coverslip and the percentage germination calculated. The conidia were considered as viable when the germ tubes were two times the diameter of the propagule (Goettel and Inglis 1997).

Screening of entomopathogenic fungus isolates against *T. erythrae* adults

To determine the pathogenicity of fungal isolates, a standard concentration of 1 × 10⁸ conidia ml⁻¹ of all the 15 fungal isolates was sprayed on two leaves of lemon measuring approximately the same size using Burgerjons spray tower (Burgerjon 1956). The leaves were placed in a 10 ml universal bottle filled with water. Cotton wool was used to hold the leaves at the mouth of the bottle. Each universal bottle served as a replicate, and four replicates were used for each fungal isolate. The control groups were sprayed with sterilized distilled water containing 0.05% Triton X-100. After spraying, each bottle was transferred into a cage (30 × 30 × 30 cm),

Table 2 Source of origin and viability tests results of all the fungal isolates tested against *T. erythrae* adults

Fungal species	Fungal isolate	Source	Country	Year	% Germination ± SE	
<i>M. anisopliae</i>	ICIPE 91	<i>Acanthacris ruficornis citrina</i>	Senegal	2003	92.33 ± 2.33	
	ICIPE 31	<i>Locusta migratoria</i>	Madagascar	2003	96.33 ± 0.33	
	ICIPE 78	<i>Temnoschoita nigroplagiata</i>	Kenya	1990	92.67 ± 3.53	
	ICIPE 69	Soil	DRC	1990	91.00 ± 2.08	
	ICIPE 18	Soil	Kenya	1989	90.00 ± 2.31	
	ICIPE 7	<i>Amblyomma variegatum</i>	Kenya	1996	96.00 ± 1.73	
	ICIPE 20	Soil	Kenya	1996	90.33 ± 4.81	
	ICIPE 41	Soil	DRC	1990	94.33 ± 0.88	
	ICIPE 674	Soil	Kenya	2008	95.67 ± 0.88	
	ICIPE 30	<i>Busseola fusca</i>	Kenya	1989	91.67 ± 1.86	
	ICIPE 62	Soil	DRC	1990	96.00 ± 1.73	
	<i>B. bassiana</i>	ICIPE 621	Soil	Kenya	2008	96.67 ± 1.86
		ICIPE 660	Soil	Kenya	2008	93.33 ± 0.88
ICIPE 279		Coleopteran larvae	Kenya	2005	95.67 ± 1.45	
ICIPE 676		Soil	Kenya	2008	95.33 ± 2.33	

thereafter 10 *T. erythrae* adults of approximately one week old (Gandarilla-Pacheco et al. 2013) were introduced into each cage. Insect mortality was monitored for 14 days under controlled conditions of 22 ± 2 °C, 65% R.H and 12:12 D:L. The water in the universal bottle was to maintain moisture in the treated leaves. For the mycosis test, the dead insects were surface sterilized with 70% alcohol, and rinsed thrice in distilled water. They were separately kept in Petri dishes lined with sterile-moistened filter paper to record fungal outgrowth, and verify if mortality could be attributed to the respective fungal isolates.

Statistical analysis

Statistical analyses were performed on the count of different stages of ACT. The ‘glm.nb’ function in the ‘Mass’ package (Venables and Ripley 2002) was used to compute a generalized linear model with negative binomial error distribution and log-link to determine whether the different stages varied across the sampling period. For post-hoc comparisons, the least square means (‘lsmeans’) function imbedded in the lsmeans package (Lenth and Maintainer 2018) with ‘Tukey’ adjustment was employed. Spearman’s rank correlation was applied to assess the relationship between ACT population densities and climatic conditions in the high altitude. Also, ACT counts in shaded and unshaded trees were $\log(x + 1)$ transformed prior to analysis using spearman correlation to determine if abundance in shaded trees leads to a rise in ACT in unshaded trees.

Viability of the fungal isolates was analyzed using binomial regression analysis of the generalized linear model (GLM). Percentage *T. erythrae* mortality data were first corrected for natural mortality (Abbott 1925), followed by normality test (Shapiro and Wilk 1965), then arcsine transformed prior to analysis of variance (ANOVA). Lethal time (LT_{50}) for each fungal isolate was analyzed using a generalized linear model assuming binomial distribution error and probit link (probit model). The time to 50% mortality (LT_{50}) was estimated using logit analysis in SPSS version 22. All analyses were carried out in R version 3.6.1. (R Development Core Team 2019) except for LT_{50} .

Results

African citrus triozid was found in all the orchards whereas the distribution of *D. citri*, a vector of Asian strain of the citrus greening disease, was limited to elevations up to 1500 m.a.s.l. (Table 1). In addition, *Diaphorina citri*’s eggs, nymphs, and adults were found on *Zanthoxylum capense* (Thunb) Harv. (Rutaceae). The densities of ACT adults varied significantly across the different months ($\chi^2 = 940.82$, $df = 2$, $P = 0.0001$)

(Fig. 2A). In late February 2017, adult ACTs in the low-altitude resumed activities and the females started laying eggs. During the same period, density reached a maximum monthly mean of 2.5 adults/shoot. In the mid-altitude sites, adults were present throughout the entire survey period. However, the highest peak was observed in late February 2017 with a mean of 2.9 adults/shoot. The mean number of eggs was significantly higher in the mid- followed by the low-altitude and then high-altitude sites ($\chi^2 = 24.973$, $df = 2$, $P = 0.0001$) (Fig. 2B). The peak of egg counts coincided with the appearance of adults, and the maximum number of eggs varied across the different altitudinal gradients. We recorded a significantly higher mean number of nymphs in the mid-than in the low- and high-altitude sites ($\chi^2 = 24.973$, $df = 2$, $P = 0.0001$) (Fig. 2C). In the low-altitude sites, the density of nymphs peaked with a monthly mean of 40 nymphs/shoot in March. In the mid-altitude site, the density of nymphs peaked in March with a mean of 45 nymphs/shoot, whereas the maximum monthly mean of 20 nymphs/shoot was recorded in the high-altitude site in October.

Colonization of ACT in shaded and unshaded trees followed a distinctive seasonal pattern across the different altitudinal gradients (Fig. 3A–I). For instance, in the low-altitude sites, the density of adult ACT reached a maximum monthly mean of 3.2 and 0.1 adults/shoot in the shaded and unshaded trees, respectively (Fig. 3A). In March, the means were 70 and 9 nymphs/shoot for shaded and unshaded trees, respectively (Fig. 3B). In late February, the mean number of eggs were 85 and 0.7 eggs/shoot in the shaded and unshaded, respectively (Fig. 3C). ACT population densities in shaded trees positively correlated and significantly differed with unshaded trees for eggs, nymphs and adults (Fig. 4, 5, 6). Our results revealed significant differences between population density of adult ACT and maximum temperature, average temperature, solar radiation, evaporation and dew point (Table 3). However, only the latter positively influenced the seasonal abundance of ACT. Although the weather conditions affected the abundance of ACT nymphs, there was no clear evidence suggesting that any of the weather variables positively or negatively affected the seasonal abundance of ACT nymphs (Table 3). Nevertheless, only solar radiation and average temperature negatively and significantly influenced the seasonal abundance of ACT eggs (Table 3).

Conidial germination of *M. anisopliae* and *B. bassiana* isolates against adults of *T. erythrae*

All the 15 fungal isolates had viability above 90% which was not significantly different among the isolates ($\chi^2 = 30.79$, $df = 14$, $P = 0.27$). The isolates were thus considered viable for use in screening (Table 4).

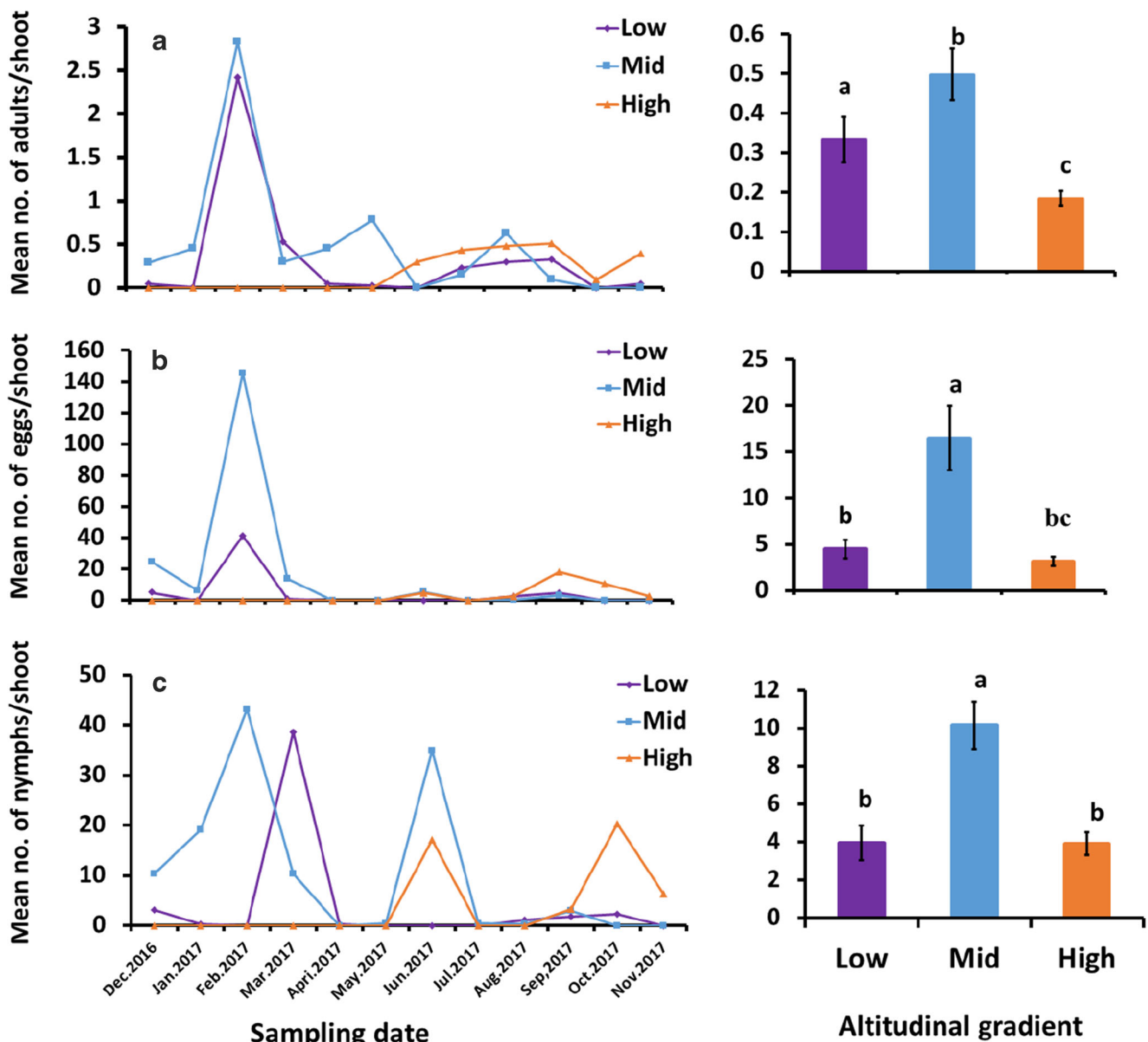


Fig. 2 Means of ACT adults, eggs and nymphs per shoot in Low (A-C), Mid (D-F) and High (G-I) altitudinal gradients of citrus groves in Western Kenya from Dec 2016 to Nov 2017: bars with different letters are significantly different at $p < 0.05$

Pathogenicity of *M. anisopliae* and *B. bassiana* isolates against adults of *T. erytrae*

There was a significant effect of fungal treatment on mortalities of *T. erytrae* adults 14 days post treatment ($F = 16.29$, $df = 14, 45$, $P < 0.0001$; Table 4). *Metarhizium anisopliae* isolate ICIPE 18 caused higher mortality than the other 14 isolates, with the shortest lethal time to 50% mortality (LT_{50}) in 4.4 days. No significant differences in pathogenicity was recorded among Isolates ICIPE 20, 69, 78; ICIPE 62, 660, 30 and ICIPE 91, 31, 621, 676, 41, 674 and 279. Although there was no significant difference in mortalities caused by ICIPE 69 and 20, a remarkable difference was observed in

their LT_{50} of 5.9 and 8.2 days, respectively. Hence, ICIPE 69 was the second most important virulent isolate (Table 4).

Discussions

ACT is a highland insect, and thus it was not a surprise that they were found in all the study sites, confirming earlier results from Green and Catling (1971) and Aidoo et al. (2019b) who reported that ACT's distribution is confined to cool and moist climates which prolong citrus flush growths, suitable for ACT's reproduction and survival. We additionally could reveal that in 2016/17, in our study sites in Kenya,

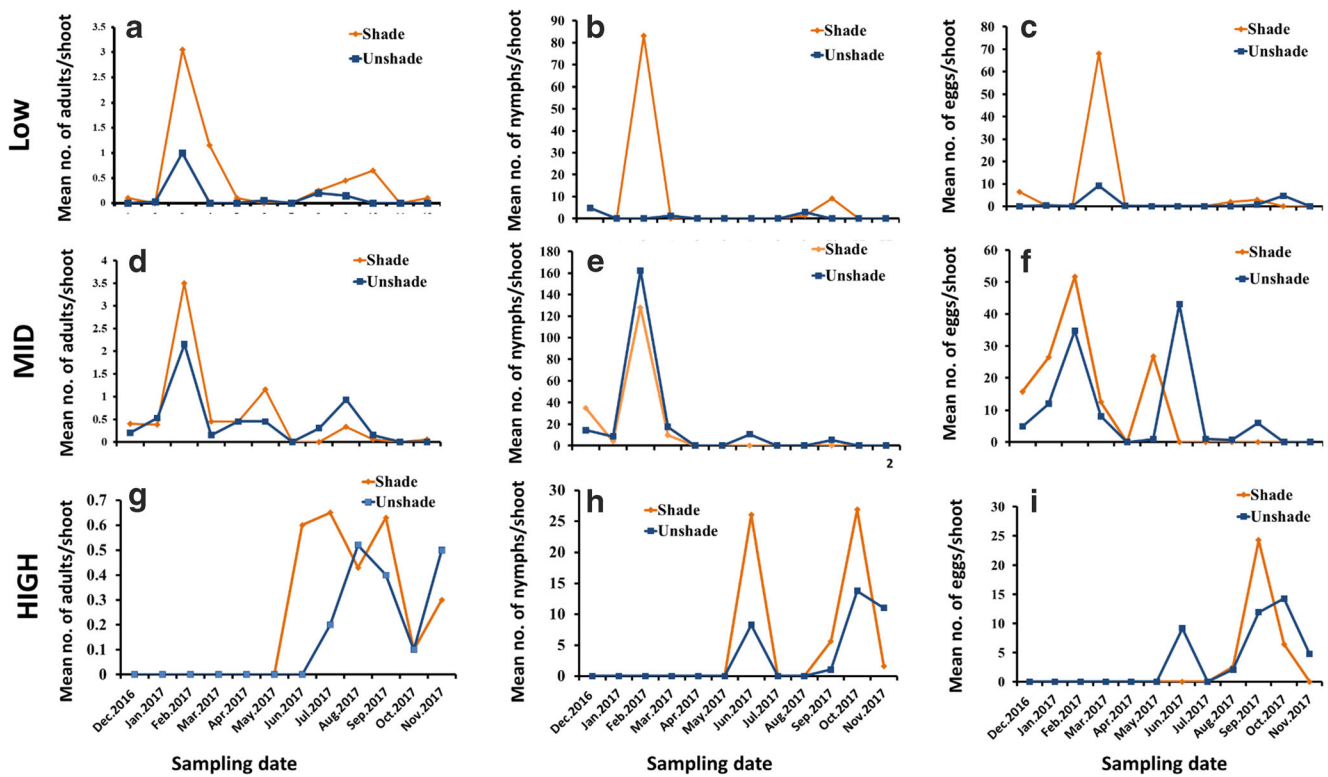


Fig. 3 Means of ACT eggs, nymphs and adults per shoot in Shaded and unshaded citrus groves in Western Kenya from Dec 2016 to Nov 2017 across low, mid and high altitudinal gradients

colonization of orchards began in the late February through early March in both low- and mid-altitudes, whereas in the high-altitude orchards adults only appeared starting in June. Across all altitudes this coincided with the appearance of new flush shoots in the host trees, corroborating earlier reports that ACT peaks overlap with periods of profuse flush growths (Cook et al. 2014; van den Berg 1990). Yet, despite the presence of new flush shoots between October and November

2017 in the mid-altitude sites, we did not find any adult *T. erytrae*, suggesting that additional factors also influence the seasonal abundance of ACT. For instance, the nutritional quality of citrus trees could have influenced the observed temporal variations in ACT abundance. However, this requires further investigation.

ACT heavily infested some of the surveyed orchards with severe damage symptoms. Consequently, the poor condition

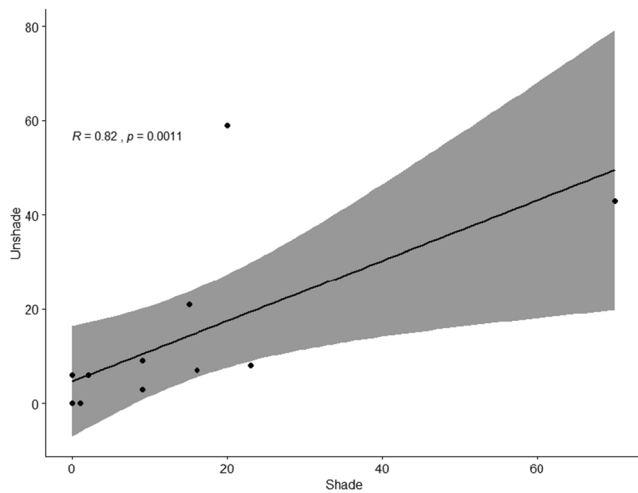


Fig. 4 Correlation analysis carried out on $\log(x + 1)$ of the count data of adults of *T. erytrae* per shoot in shaded and unshaded citrus groves in Western Kenya from Dec 2016 to Nov 2017 in the mid-altitudinal gradients

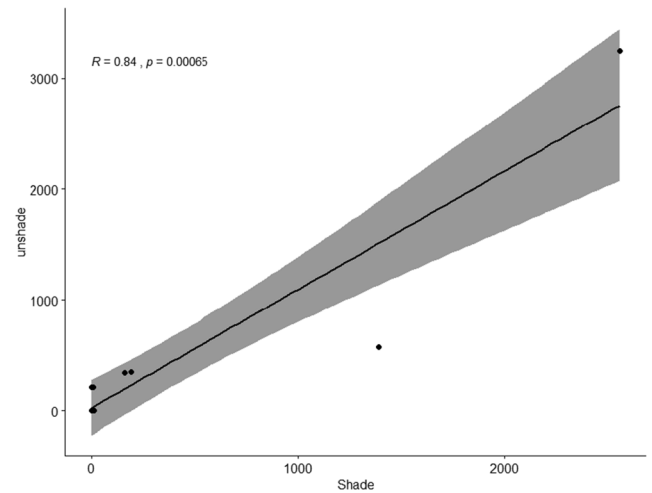


Fig. 5 Correlation analysis carried out on $\log(x + 1)$ of the count data of eggs of *T. erytrae* per shoot in shaded and unshaded citrus groves in Western Kenya from Dec 2016 to Nov 2017 in the mid-altitudinal gradients

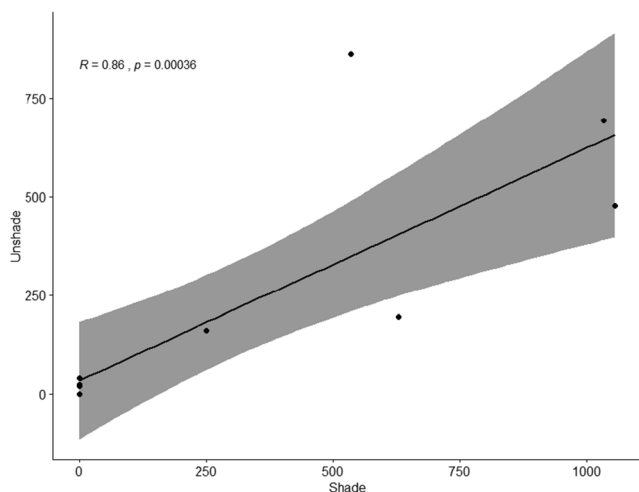


Fig. 6 Correlation analysis carried out on log (x + 1) of the count data of nymphs of *T. erytreae* per shoot in shaded and unshaded citrus groves in Western Kenya from Dec 2016 to Nov 2017 in the mid-altitudinal gradients

of the trees caused by the physical damage through ACTs’ feeding must have certainly affected the host plant quality of the trees with inherent consequences for the survival and reproduction of the insects. Moreover, poor nutritional quality of host plants can be attributed to an ACT infestation (Cook et al. 2014). We found that seasonal phenology and population dynamics of ACT adults and immatures stages differed between shaded and unshaded trees. Those in shaded trees generally dominated, probably because of the comparably lower temperatures there. Infestations usually commenced in the shaded trees then spread to the unshaded ones, once temperature conditions became more favorable for survival and reproduction (van den Berg 1990).

The climatic effects on the seasonal abundance of ACT in our survey were rather inconclusive, calling for longer and more detailed investigations as also stipulated by Samways (1987) who found weather effects on adult ACTs more pronounced over longer than shorter periods. Van den Berg et al.

(1990) observed a positive correlation between rainfall, minimum, maximum temperatures, relative humidity and all stages of ACT. However, only the minimum temperature and relative humidity significantly correlated with the number of eggs. They further observed that adults significantly correlated with minimum temperature, rainfall, maximum temperature, and relative humidity, whereas nymphs correlated with minimum temperature and relative humidity.

Results of the present study revealed that the distribution of Asian citrus psyllid, a close relative of ACT, was confined to low altitudes (elevations below 1409). Adults were found in December to the middle of February in elevations below 1409 m.a.s.l. The absence of ACP in high altitudes could be attributed to variation in temperature, air pressure, oxygen levels, and ultraviolet light (Jenkins et al. 2015). However, in Tanzania, ACPs’ distribution and abundance appear to be limited to elevations below 600 m.a.s.l. (Shimwela et al. 2016). Rwomushana et al. (2017) reported ACP in Kericho County of western Kenya. The present study revealed the presence of ACP in Kisumu County, similarly situated in western Kenya. It can thus be, speculated that ACP is expanding its ecological niche to the Kenyan highlands. Detection of ACP in elevations above 1000 m.a.s.l. in these regions is quite disturbing because of the presence of two citrus greening disease vectors. Their potentially combined effects could pose a serious threat to the Kenyan’s citrus industry, particularly in the western part of the country. Our observation of eggs, nymphs, and adults of *D. citri* on *Zanthoxylum capense* also warrants follow-up studies as this alternative host plant could influence the population dynamics of ACP by serving as a reservoir for the citrus greening disease pathogens.

Based on these results on the spread of ACTs to elevations above 1000 m.a.s.l. and given that up to now there is no cure for HLB disease therefore effective control of ACT is very important in curbing the transmission of the disease.

Table 3 Spearman’s rank correlation (r_s) between climatic variables and monthly data

<i>Triozia erytreae</i>		Minimum Temperature (°C)	Maximum Temperature (°C)	Average Temperature (°C)	Rainfall (mm)	Relative Humidity (%)	Solar Radiation (Langley)	Evaporation (mm)	Dew point (°C)	Wind run (Km/day)
Adults	S	136.840	524.460	524.230	203.810	265.720	467.470	528.840	108.490	445.05
	r_s	0.521	-0.834	-0.833	0.287	0.071	-0.634	-0.849	0.621	-0.556
	P	0.082	0.001**	0.001**	0.365	0.823	0.027*	0.0004***	0.031*	0.060
Nymphs	S	235.56	358.11	405.62	301.47	289.57	432.360	359.830	187.660	333.600
	r_s	0.179	-0.252	-0.418	-0.054	-0.012	-0.512	-0.258	0.344	-0.166
	P	0.576	0.429	0.176	0.867	0.969	0.089	0.418	0.274	0.605
Eggs	S	200.8	435.69	458.64	221.32	290.46	473.34	428.87	130.73	388.59
	r_s	0.297	-0.523	-0.604	-0.226	-0.016	-0.655	-0.499	0.543	-0.359
	P	0.347	0.081	0.038*	0.479	0.962	0.021*	0.098	0.068	0.252

Table 4 Pathogenicity of entomopathogenic fungal isolates against *Trioza erytreae* adults and their LT₅₀ values 14 days post treatment

Fungal species	Fungal isolate	% Mortality ± SE	LT ₅₀ (days) (95% CI)
<i>M. anisopliae</i>	ICIPE 91	52.5 ± 2.5d	10.7 (10.0–11.7)
	ICIPE 31	50 ± 4.1d	12.0 (10.9–13.6)
	ICIPE 78	80 ± 4.1b	7.3 (6.6–7.9)
	ICIPE 69	82.5 ± 4.8b	5.8 (5.0–6.5)
	ICIPE 18	97.5 ± 2.5a	4.3 (4.2–4.5)
	ICIPE 7	72.5 ± 6.3bc	10.5 (10.1–11)
	ICIPE 20	82.5 ± 4.8b	8.1 (7.6–8.6)
	ICIPE 41	47.5 ± 4.8d	–
	ICIPE 674	47.5 ± 2.5d	–
	ICIPE 30	57.5 ± 4.8 cd	10.3(9.6–11.1)
	ICIPE 62	62.5 ± 2.5 cd	8.8 (7.9–9.8)
	<i>B. bassiana</i>	ICIPE 621	50 ± 5.8d
ICIPE 660		60 ± 7.1 cd	10.0 (9.2–10.9)
ICIPE 279		42.5 ± 2.5d	–
ICIPE 676		50 ± 2.5d	12.1 (11.4–13.1)

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls (SNK) test ($P < 0.05$). LT₅₀ (in days); FL represents fiducially limit at 95%. LT₅₀ values for ICIPE 41, 674 and 279 were not computed as they only caused mortality <50%

Our results on pathogenicity of entomopathogenic fungi against *T. erytreae* showed variable susceptibility of the ACT to all the 15 fungal isolates tested. Such variations in psyllid mortality exposed to different entomopathogenic fungal isolates or strains have also been reported in *D. citri* (Gandarilla-Pacheco et al. 2013). This difference in susceptibility of *T. erytreae* to fungal infections has been associated with inter- and intraspecific variations in virulence among fungal species and isolates (Zélé et al. 2020).

Among the tested isolates, *M. anisopliae* ICIPE 18 and 69 caused the highest mortalities of more than 80% to *T. erytreae* adults. Both isolates induced high mortalities in the Tephritid fruit flies, *Zeugodacus cucurbitae* (Onsongo et al. 2019) and the legume pod borer, *Maruca vitrata* (Tumuhaise 2015) whereas ICIPE 69 has been effective against the false codling moth, *Thaumatotibia leucotreta* (Mkiga et al. 2020), the western flower thrips *Frankliniella occidentalis* (Niassy et al. 2012) and three species of African Tephritid fruit flies (Dimbi et al. 2003; Ekesi et al. 2002). Given that ICIPE 69 has already been commercialized, extension of labels to *T. erytreae* upon validation of its efficacy under field conditions would be useful for citrus farmers, especially the small scale farmers.

Recent study of attraction of both sexes of ACTs to lemon terpenes by Antwi-Agyakwa et al. (2019) provides a useful insight into exploring pheromones together with the potent fungal isolates in this study, and this could help to manage the pest through an autodissemination approach

using autodissemination devices, with dry conidia of the fungus and lemon terpenes that can attract both sexes of ACTs in the device, pick the fungus and infect ACTs in the wild, through contact. Autodissemination is very cost effective technology, and it is widely used to manage several pests such thrips (Mfuti et al. 2017), tsetseflies (Maniania et al. 2006) and diamondback moth (Furlong and Pell 2001), which can be exploited for *T. erytreae*.

Conclusion

Our study showed that seasonal abundance of *T. erytreae* in Kenya varied across the different altitudes. Also, ACT (all stages) seasonal dynamics varied between shaded and unshaded trees. The presence of ACP in elevations above 1000 m.a.s.l. requires further investigation as well as the observation of *Z. capense* as being a putative alternate host plant of *D. citri*. Beside, further studies should focus on the effects of climatic conditions in the low and mid-altitudinal gradients of Kenya. Our study could help inform early and effective monitoring of *T. erytreae* to limit population build-up, and reduce its spread in the future. The timing of ACTs population is crucial because residual activity of entomopathogenic fungus declines under field conditions. However, our laboratory studies on entomopathogenic fungus in managing *T. erytreae* should further be validated under field conditions due to different environmental conditions, which could affect the performance of the entomopathogens.

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Authors’ contributions OFA, SAM, CB, SE, FM, MS and CMT conceived the idea and contributed to subsequent reading.

OFA analyzed and wrote the first draft.

OFA, SO, JA, JWK, BR, and CB contributed to data collection and proofreading.

All authors contributed to proofreading.

Compliance with ethical standards

This study was not undertaken in national parks or any protected areas. No specific permission was required for these experiments or collections due to the studies not involving endangered or protected species. This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors have declared that there exists no conflict of interest.

Ethical approval Informed consent was obtained from all the participants involved in the study.

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