Zentrum für Entwicklungsforschung

Bio-ecology of the African citrus triozid *Trioza erytreae* Del Guercio (Hemiptera: Triozidae) on citrus in Kenya

implication for its management

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ABSTRACT

Citrus production is a major industry in Kenya. Apart from providing income and jobs, it contributes to food and nutritional security. For the last decade, citrus production has experienced a continuous decline, particularly in the Kenyan highlands. Consequently, production is unable to meet local demands, which has led to increased imports of citrus. Low production has been attributed to pest and diseases, of which the African citrus triozid (ACT) *Trioza erytreae* Del Guercio (Hem.: Triozidae) is the economically most important pest species due to its ability to vector "*Candidatus* Liberibacter africanus" (CLaf), the causal agent of the African citrus greening disease. Currently, the disease has no cure, hence the need to manage the pest in order to prevent the further spread of it. Through field surveys and laboratory studies, this thesis sought to determine the bio-ecology of ACT on citrus in Kenya and implications for its management. The results showed that ACT was widely distributed, causing more damage in shaded than unshaded citrus trees, particularly in the southeastern canopy quadrants. A newly developed predictive model revealed hotspots out of the distributional range under current and future climate scenarios. *Trioza erytreae* reproduction was confined to plants belonging to the family Rutaceae. Adult *T. erytreae* survived and reproduced on *Zanthoxylum usamberensis* (Engl.), *Murraya koenigii* (L.), *Calodendrum capense*(L.f.) Thunb., *Teclea nobilis* (Del.), *Clausena anisata* (Willd.) Hook. f. ex Benth. and *Vepris bilocularis* (Wright & Arn.) Engl. Also, plants which appeared to have superior host plant quality, as reflected by several of ACTs' tested biological parameters, also produced adults with larger wing size and greater shape. Two host plants, i.e. *V. bilocularis* and *T. nobilis*, were reported here for the first time as alternative host plants for ACT. A spatial survey was conducted to assess seasonal variations of ACT occurrence across different altitudinal gradients with ACT populations significantly varying across different altitudinal gradients, resulting in higher pest densities in mid elevations (1,500-2,000 meters above sea level [m.a.s.l.]). The closely related Asian citrus psyllid *Diaphorina citri* Kuwayama (Hem.: Liviidae) was found and sampled in elevations up to 1,409 m.a.s.l. Yellow sticky card traps proved to be most efficient in trapping adult ACT. The result of a study to compare the effects of host plant quality on wing shape and size of ACT clearly showed that both geometric and traditional morphometry were useful in separation different populations of *T. erytreae*. The findings of this thesis thus provide relevant information to improve monitoring of ACT and to design ecologically sound integrated pest management strategies for control of this pest.

KURZFASSUNG

Die Zitrusproduktion ist ein wichtiger Wirtschaftszweig in Kenia. Neben der Bereitstellung von Einkommen und Arbeitsplätzen trägt sie zur Lebensmittel- und Ernährungssicherung bei. Im letzten Jahrzehnt hat die Zitrusproduktion insbesondere im kenianischen Hochland kontinuierlich abgenommen. Folglich kann die Produktion der lokalen Nachfrage nicht gerecht werden, was zu einem zunehmenden Import von Zitrusfrüchten geführt hat. Der geringe Ertrag wird Schädlingen und Krankheiten zugeschrieben. Der wichtigste Schädling ist der Afrikanische Zitrustriozid (ACT) *Trioza erytreae* Del Guercio (Hem.: Triozidae) aufgrund seiner Fähigkeit "*Candidatus* Liberibacter africanus" (CLaf), den Erreger der Aftikanischen Zitrusgrünung, zu übertragen. Derzeit hat die Krankheit keine Heilung, daher muss der Schädling bekämpft werden, um eine weitere Ausbreitung der Krankheit zu verhindern. Durch Feld- und Laborstudien wurde versucht, die Bioökologie von ACT auf Zitrusfrüchten in Kenia und die Implikationen für die Bekämpfung des Schädlings zu bestimmen. Die Ergebnisse zeigten, dass ACT in beschatteten Zitrusbäumen stärker verbreitet war als in nicht beschatteten, insbesondere in den südöstlichen Baumkronenquadranten. Ein neu entwickeltes Vorhersagemodell beschrieb ‚Hotspots' außerhalb des derzeitigen Verbreitungsgebietes unter aktuellen und zukünftigen Klimaszenarien. Die Reproduktion von *T. erytreae* war auf Pflanzen der Familie Rutaceae beschränkt. Adulte ACT überlebten und reproduzierten auf *Zanthoxylum usamberensis* (Engl.), *Murraya koenigii* (L.), *Calodendrum capense* (L.f.) Thunb., *Teclea nobilis* (Del.), Clausena *anisata* (Willd.) Hook. f. ex Benth. und *Vepris bilocularis* (Wright & Arn.) Engl. Auf Pflanzen mit hoher Wirtspflanzenqualität entwickelten sich ACT mit größerer Flügelgröße und -form. Zwei Wirtspflanzen, *V. bilocularis* und *T. nobilis*, wurden zum ersten Mal als alternative Wirtspflanzen für ACT beschrieben. Eine räumliche Untersuchung wurde durchgeführt, um saisonale Variationen des ACT-Vorkommens über verschiedene Höhengradienten hinweg zu erfassen, wobei ACT-Populationen zwischen verschiedenen Höhengradienten mit höheren Schädlingsdichten in mittleren Höhenlagen (1.500-2.000 Meter über dem Meeresspiegel [M.ü.d.M.]) signifikant variierten. Die nahe verwandte asiatische Zitrusfrucht-Psyllide *Diaphorina citri* Kuwayama (Hem.: Liviidae) wurde in Höhen bis zu 1.409 M.ü.d.M. gefunden. Gelbe Klebe-Kartenfallen erwiesen sich als am effizientesten zum Fang von erwachsener ACT. Das Ergebnis einer Studie zum Vergleich der Effekte der Wirtspflanzenqualität auf die Flügelform und die Größe von ACT zeigte, dass sowohl die geometrische als auch die traditionelle Morphometrie zur Trennung verschiedener Populationen von *T. erytreae* geeignet ist. Die Ergebnisse dieser Arbeit liefern daher relevante Informationen zur Verbesserung des Monitorings und ACT zur Entwicklung von ökologisch verträglichen integrierten Pflanzenschutzstrategien gegen den Schädling.

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LIST OF ACRONYMS AND ABBREVIATIONS

icipe	International Centre of Insect Physiology and Ecology
USAID	United States Agency for International Development
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
IITA	International Institute of Tropical Agriculture
IPM	Integrated pest management
HLB	Huanglongbing
EPPO	European Plant Protection Organization
OIRSA	Office of Institutional Research and Student Assessment
CLaf	"*Candidatus"* Liberibacter africanus
Clam	"*Candidatus"* Liberibacter americanus
CLas	"*Candidatus"* Liberibacter asiaticus
RSA	Republic of South Africa
LafCl	"*Candidatus"* Liberibacter africanus Subsp. Clausenae
LafZ	"*Candidatus"* Liberibacter Subsp. Zynthoxyli'
LafV	*Candidatus* Liberibacter africanus Subsp. Vepridis
DNA	Deoxyribonucleic acid
EPF	Entomopathogenic fungi
STG	Shoot Tip Grafting
AfCP	African citrus psyllid

ACGD	African citrus greening Disease
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
SNK	Student Newman Keuls
MaxEnt	Maximum Entropy
GCMs	Global Climate Models
RCPs-AR5	Representative Concentration Pathways Scenarios, Fifth Assessment Report
HadGem2	Hadley Centre Global Environment Model version 2
m. a. s. l.	Meters Above Sea Level
NE	North East
SW	South West
AUC	Area Under the Curve
ACT	African Citrus Triozid
DAP	Di-ammonium phosphate
ARCU	Animal Rearing and Containment Unit
UPGMA	unweighted paired group method with arithmetic mean
PCA	principal component analysis
16S rRNA	16Svedberg Ribosomal Deoxyribonucleic acid

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Citrus

1.1.1 History of citrus

Citrus (*Citrus* spp.) belong to the family Rutaceae. Historically, citrus fruits and plants are collectively called agrumes (literally "sour fruits") by the Romans (Liu et al. 2012). Although the origin of citrus is marked with several contradictory reports, some researchers have attributed its origin to the tropical and subtropical regions of Asia including parts of SE Asia, mainly China, India, and the Malay Archipelago (Bartholomew and Sinclair 1952; Gmitter and Hu 1990; Liu et al. 2012; Ramana et al. 1981; Scora 1975; Sinclair 1961). Ancient Chinese documents revealed the earliest reference to citrus during the reign of Ta Yu (around 2,205 to 2,197 BC) when citrus was highly cherished (Nagy and Attaway 1980; Webber 1967). However, a recent study postulates that citrus originated from the southeastern foothills of the Himalayas (Wu et al. 2018). Another Study suggested that commercial citrus species including mandarins, oranges, and lemons originated from SE Asia, whereas the true origin of citrus fruits was attributed to Australia, the island of New Caledonia and New Guinea (Anitei 2007).

It is believed that the introduction of citrus to northern and southern Africa, as well as to other regions of the world, was slow (Liu et al. 2012). In Africa, citrus was first described on St Helena in 1502 by the Portuguese explorer Juan de Nova Castella on his way from India(http://www.turtlesa.com/oranges.html). It is possible that the crop had been previously introduced into St. Helena by other sailors as it was commonly used to prevent and cure scurvy (http://www.turtlesa.com/oranges.html).

1.1.2 Citrus production, importance and constraints

The commercially most important citrus fruits are oranges, followed by lemons, limes, grapefruit, and tangerines. Although citrus fruits are grown all over the world in over 140 countries, most of the crops grow on either side of a belt around the equator covering the tropical and subtropical areas of the world between latitudes 35°N and 35°S (Liu et al. 2012). For the past few decades, annual global citrus fruit production has witnessed a strong, rapid and continuous growth, from an estimated 30 million metric tons (MT) in the late 1960s (FAO 1967) to an estimated 146 million MT in 2016, with oranges contributing more than half of the world's total citrus production (FAOSTAT 2018). According to FAOSTAT (2009), China, Brazil, U.S.A., India, Mexico, and Spain are the world's leading citrus fruit-producing countries, representing close to two-thirds of global production (FAOSTAT 2018). Although many citrus fruits, such as oranges, tangerines, and grapefruits, can be eaten fresh, about a third of all citrus fruits are utilized after processing, with orange juice production accounting for nearly 85% of total processed citrus fruits for consumption (USDA 2006).

Table 1.1 Sweat orange production in leading countries in the world, 2010 -2016 (1,000 short tons), production (tons) and yield (hg/ha)

Country	2012		2013		2014		2015		2016	
	Production	Yield	Production	Yield	Production	Yield	Production	Yield	Production	Yield
Brazil	18,012	246	17,549	249	16,928	248	16,939	249	17,251	261
China	7,258	155	7,466	152	7,964	157	8,191	162	8,550	169
USA	8,268	336	7,500	305	6,139	256	5,786	248	5,160	231
Mexico	3,666	113	4,409	137	4,533	140	4,515	141	4,603	146
India	4,360	88	6,426	101	7,317	110	7,693	134	7,503	129
Spain	2,942	193	3,394	225	3,494	240	2,935	199	3,127	207
Kenya	**61**	**102**	**61**	**132**	**135**	**149**	**107**	**151**	**85**	**117**
World	70,352	178	73,112	177	72,357	178	72,794	183	73,187	184

Source: Yearbook/FAOSTAT-2018/October)

Citrus is one of the world's most economically important fruit crops, recognized for its popularity in contributing to food and nutritional security. The health benefits of sweet orange and other citrus fruits are well documented, especially in the provision of vitamin C, carotenoids, and polyphenols (Liu et al. 2012). The majority of the fruits produced in Kenya are consumed locally as a fresh product, and a smaller quantity is processed into juices, jam, and marmalades (MMA 2008). With the exception of Banana and pineapple, citrus is the most extensively cultivated fruit crop in Kenya (Kilalo et al. 2009). Citrus production and processing provide income to farmers, jobs (cultivation, harvesting, packaging, transportation, research etc.) and food for human consumption. However, citrus production appears to be experiencing a continued decline, particularly in the Kenyan mid - and highlands.

The main constraints associated with this decline are pests and diseases, shortage of planting materials, inadequate capital, and poor orchard management practices in descending order of importance (Kilalo et al. 2009). Pests and diseases are recognized as the most important production constraints (Kilalo et al. 2009). Pests that cause severe damage to citrus in Kenya are the African citrus triozid (ACT) *Trioza erytreae* Del Guercio (Hem.: Triozidae), False codling moth *Thaumatotibia leucotreta* Meyrick (Lep.: Tortricidae), Red scale *Aonidiella aurantii* Maskell (Hem.: Diaspididae), *Toxoptera citricida* Kirklady (Hem.: Aphididae), *Toxoptera aurantii* Boyer de Fonscolombe (Hem.: Aphididae), the exotic fruit fly *Bactrocera invadens* Drew, Tsura & White (Dipt.: Tephritidae) and Spiralling whitefly *Aleurodicus dispersus* Russell (Hem.: Aleyrodidae) (Kilalo 2004; Nyambo 2009). However, *T. erytreae* is the economically most important pest of citrus, particularly in the Kenyan highlands, due to its ability to vector "*Candidatus* Liberibacter africanus" (CLaf), the causal agent of African citrus greening disease (ACGD). Currently, the disease has no cure and has been associated with the collapse of thousands of orchards in the Kenyan highlands.

1.1.3. Management of citrus pests

Scales and aphid infestation on citrus can be problematic. However, natural enemies often provide effective control. In addition, synthetic insecticides have been efficient in managing citrus pest, especially when selective and environmentally less harmful products are used within the framework of a citrus Integrated Pest Management (IPM) program. However, new reports indicate that ACT has developed resistance to some synthetic insecticides (Tiwari et al. 2011).

1.2 African citrus triozid, *Trioza erytreae* Del Guercio (Hem.: Triozidae)

1.2.1 Description, biology and lifecycle

Trioza erytreae eggs are oval, smooth when freshly deposited and pear-shaped (Figure 1.1). Eggs are usually laid on the leaf margin, using a tapered short stalk at the posterior end of the female abdomen which is inserted into the plant tissue (van den Berg 1990). The mean length of the egg is 0.5 mm (Annecke and Cilliers 1963; van der Merwe 1923) and 0.28 mm (0.22-0.31 mm long) (Catling 1967; Moran and Blowers 1967). The eggs turn dark yellowish as the embryo within it develops (van den Berg 1990). Prior to exclusion two red nymphal eye spots become well pronounced towards the anterior end of the egg (Moran and Blowers 1967). Many eggs may be found on shoots and sometimes on the tender young thorns of the host plant, but occasionally eggs are laid on flower buds and on immature fruits (van den Berg 1990). Depending on the temperature, it may take up to three weeks for the eggs to hatch (van den Berg 1990).

Figure 1.1. Citrus shoot with adult *Trioza erytreae* and eggs

The nymphs undergo five nymphal stages. The general color of the nymph is pale yellow with red eyespots (Figure 1.2A). It has a white waxy fringe of filaments which enclose the nymphal body. The length of the filaments varies depending on the nymphal stage which usually increases in length during each intermoult phase. According to van den Berg (1990) the number of filaments increases from 50, 100, 200, 300 and 450 in the 1st, 2nd, 3rd, 4thand 5th nymphal instars, respectively. Also, the two pale brown eyespots appear on the abdomen of the 4thinstar after molting and become dark in the 5thinstar (Moran and Blowers 1967). The mean length of the various stages of ACT nymphs are as follows; first instar nymph 0.345 mm (range 0.25-0.41 mm), second instar nymph 0.50 mm (0.44-0.56 mm), third instar nymph 0.72 mm (0.63-0.75 mm), fourth instar nymph 1.025 mm (0.94-1.125 mm) and fifth instar nymph 1.52 mm (1.38-1.66 mm) (Moran and Blowers 1967). The early instars move only when they are disturbed or overcrowded whereas older nymphs and adults are highly mobile. Nymphs are usually attached to the underside of leaves and induce pit-like galls (Fig. 1.2B). Mean nymphal development from egg to adult ranges from 17 days and 43 days at 21.8°C and 14°C, respectively(van den Berg 1990).

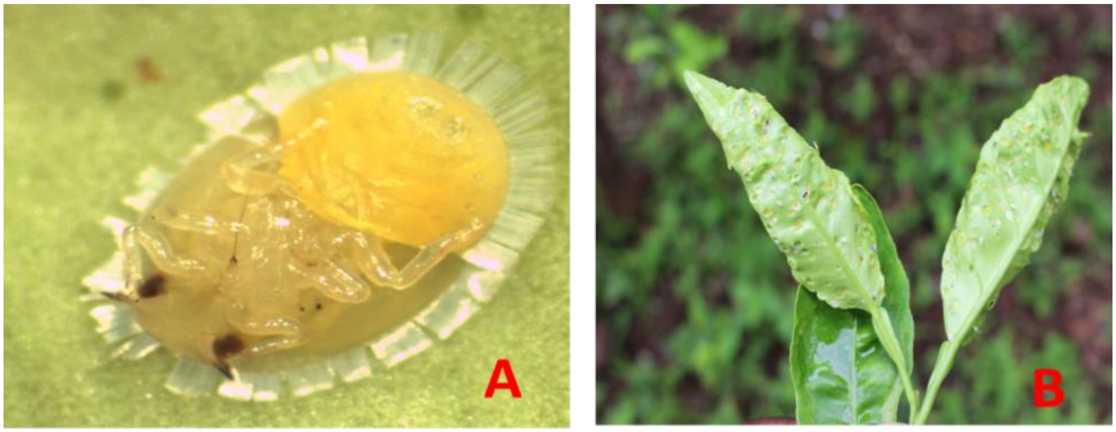

Figure 1.2.Citrus shoots with *Trioza erytreae* nymphs (with filaments) (A) and adults (B)

Adult ACT is pale green with black eyes. As the adults mature, the body color turns dark brown while the wings remain clear. The male abdominal segment is blunt whiles the female abdominal tip is sharp and pointed. The body length of males measures 2.17 mm (range 1.88-2.50 mm) compared to 2.24 mm (range 1.88-2.50) in females (Moran and Blowers 1967). The body size may be used to distinguish between the two sexes. According to (EPPO 2014), newly emerged adults reach reproductive maturity within 2-3 days after the onset of adulthood. *Trioza erytreae* can live for up to 50 days in the field, and under experimental conditions, males and females can survive for up to 73 and 82 days, respectively.

1.2.2 Geographical distribution

The threat pose by *T. erytreae* to citriculture remains unresolved. Also, the socio-economic impacts and global concern of ACT damage to citrus make it stands out as one of the most important pests of citrus requiring urgent worldwide attention. Although ACT is native to Africa, it has spread to over 20 countries in three continents (Figure 1.3), underlining that the pest is highly invasive within and outside its native range (CABI 2015).

Figure 1.3. Map showing countries where *Trioza erytreae* has been reported (Source: CABI 2015)

In the late 1890s, ACT was initially considered as a minor pest of nursery trees and frequently observed on new flush shoots in South Africa. However, its status was raised from a minor to that of a major pest of citrus in 1965, when it was found to vector CLaf, the causal agent of ACGD (McClean and Oberholzer 1965). *Trioza erytreae* was first reported in Kenya in 1922 (Waterston 1922) with subsequent detection of the ACGD in 1984 (Seif and Whittle 1984).

1.2.3 Flight activity

Dispersion patterns indicate that ACT tends to aggregate in the row adjacent to the source of infestation and along the edges of orchards bordered by windbreaks (van den Berg et al. 1991). The Author further noted that ACTs dispersal is influenced by the prevailing wind direction and that adult act can disperse for more than 1.5 km, with the females being better flyers than males. On the contrary, Catling (1973) claimed that ACT has a weak dispersal power and cannot sustain extended flights. Data on dispersal and infestation patterns for ACT suggest random tree-to-tree distribution, yet neighboring groves are the main source of infestation (Samways and Manicom 1983).

van den Berg (1989) demonstrated that the height of flights by the insect is inversely proportional to the number of catches which indicates that ACT is a low flier. He further noted that ACT flight activity is higher during the day. ACT adults remain in feeding position for several hours on suitable host plants (Figure 1.4). Aerial dispersal usually occurs shortly before sunset (1987; van den Berg 1989). *Trioza erytreae* feeds exclusively on young shoots, and nymphs and adults can move to another shoot when the earlier leaves become hard. This feeding behavior can further increase within and between orchard infestations.

Figure 1.4 Adult *Trioza erytreae* feeding on citrus shoots

1.2.4 Factors affecting population dynamics of citrus flush

Trioza erytreae prefers soft tender shoots. ACTs population fluctuations coincide with the flushing period of the citrus host (Cook et al. 2014). Females exclusively lay their eggs on newly emerged shoots which should be able to support the development of eggs through the nymphal stages until the emergence of the adults. Alternative host plants serve as breeding sites for ACT when citrus is out of season. According to van den Berg (1990), in the absence of a preferred host plant, ACT turn to alternative hosts within the vicinity of the orchard for feeding and reproduction. During the onset of citrus flush periods, they then return and attack the citrus trees. Moran (1968) reported that alternate host plants surrounding citrus orchards influence the population dynamics of ACT, serving as a source of re-infestation even when synthetic insecticides are sprayed. In South Africa, van den Berg (1990) documented four rutaceous host plants of ACT, namely *Clausena anisata* (Willd.) Hook.f. ex Benth. (Figure 1.5) *Vepris lanceolata* (Lam.) G. Don*, Zanthoxylum capen*se (Thunb.) Harv. and *Oricia* sp., and recommended similar investigations in other pest affected areas. In comparison, for the closely related Asian citrus psyllid *Diaphorina citri* Kuwayama (Hem.: Liviidae) over 50 host plants have so far been detected (Halbert and Manjunath 2004).

Several studies indicate that ACT mortalities (at all stages) are highest at extreme temperatures and low humidity (Catling 1969; Moran and Blowers 1967; van der Merwe 1923). Relative humidity close to saturation deficit leads to the development of fungal epizootics in the 2nd to 5th instar nymphs. Relative humidity in excess of 87-90% is associated with nymphal mortality of 60-70% (Aubert 1987).

Figure 1.5 Trioza erytreae nymphs (yellowish) and adults feeding on leaves of *Clausena anisata leaves* (A) and *Vepris bilocularis* (B).

Higher altitudes where conditions are cool and moist (low saturation deficit) are more favorable to ACT than low altitudes marked by hot and dry weather conditions (Aubert 1990). According to Samways (1990), population densities of ACT increase with altitude. Also, a recent study by Shimwela et al. (2016) confirmed that abundance of triozids and citrus greening are affected by altitudes, with higher densities of ACT and greater incidence of citrus greening in the Tanzanian high- than lowlands.

1.2.5 Detection and monitoring

Monitoring and surveillance of ACT using visual observations and direct counting of colonies have been effective in better timing of control measures, and risk and population assessments (Cook et al. 2014; van der Kooij et al. 1986). Eggs and nymphs of *T. erytreae* usually congregate on shoots/leaves whereas adults are randomly distributed within the canopy of a host plant or within the orchard. Elsewhere, detection and population dynamics of adult ACT can be determined using yellow sticky-trap, stem-tap samples, vacuum samples per unit time, sweep netting, suction pumps and visual searches per unit time, as well as collecting samples of flush shoots or pairs of mature leaves (Cook et al. 2014; Gilbert 1984).

1.2.6 Vector pathogen-interactions

Globally, ACGD is now recognized as one of the worst diseases of citrus (Bové 2006; Halbert and Manjunath 2004; Saponari et al. 2010). The citrus greening pathogens are highly fastidious phloem-inhabiting bacteria in the genus "*Candidatus* Liberibacter". The dynamics, epidemiology and molecular characteristics of the complex are poorly understood (Gottwald 2010; Halbert and

Manjunath 2004) but generally three strains are recognized namely the African CLaf strain, the Asian strain "*Candidatus* Liberibacter asiaticus" (CLas) and the Latin American isolate "*Candidatus* Liberibacter americanus" (CLam) (Bassanezi et al. 2005; Garnier et al. 2000). A subspecies of CLaf named "*Candidatus* Liberibacter africanus subsp. capensis" (CLafc) has been described in the Western Cape region of the Republic of South Africa (RSA) from the native rutaceous *Calodendrum capense* (L.f.) Thunb.tree. Moreover, in a recent study three novel CLaf subspecies were detected, i.e. "*Candidatus* Liberibacter africanus subsp. clausenae", "*Candidatus* Liberibacter africanus subsp. vepridis" and "*Candidatus* Lineribacter subsp. zynthoxyli". In an attempt to compile global infection statistics on citrus greening, Toorawa (1998) estimated that 53 million trees are infected in South and SE Asia and 10 million trees in Africa.

The epidemiology of citrus greening disease is difficult to assess due to the fastidious nature of the causal agent, its persistent and or propagative mode of transmission by the triozid vectors, the long latent period needed for symptom development, as well as the variability of citrus greening-associated symptoms due to climate, horticultural practices and varietal differences (Gottwald 2010; Gottwald et al. 2007). This has often led to disparate observations in estimates of disease incidence. However, the recent use of polymerase chain reaction (PCR) to accurately diagnose citrus greening infections in trees and vectors has vastly helped in resolving these problems (Saponari et al. 2010; Teixeira et al. 2008).

ACGD infection quickly disperses in a citrus orchard and eventually kills the citrus trees, particularly the younger ones (Gottwald 2010; Hall et al. 2013; Xu et al. 1988). All citrus varieties are susceptible to ACGD regardless of rootstock (Massenti et al. 2016).

In Kenya, ACGD is believed to be more prevalent in the highlands, since CLaf is heat sensitive. The disease can cause yield losses up to 100% (Kilalo 2004; Nyambo 2009; Swai et al. 1992). According to Massenti et al. (2016), citrus greening symptomatic fruits are smaller, yield less juice, have higher acidity and reduced sugar and peel color than uninfected fruits. They further noted that greening-infected fruits accumulate high amounts of flavonoids in the peel and pulp. The yield of affected trees is reduced through fruit drop, dieback, and tree stunting, as well as poor quality fruits (Cocuzza et al. 2017; van den Berg 1990). Also, the symptomatic leaves show yellowish venation (Figure 1.6).

Although a single ACT individual can vector the greening pathogen in sufficient quantities for the citrus plant to develop the symptoms (Catling and Atkinson 1974; van den Berg 1990), high populations are required before a considerable disease transmission can occur (Catling 1969; van den Berg 1990). Infected triozids are comparatively fewer than the non-carriers (Cook et al. 2014). ACT nymphs sometimes acquire the pathogen by feeding on infected citrus plants, but adults have the highest disease acquisition and transmission potential (Moll and Vuur 1977).

Trioza erytreae transmits citrus greening within 7 days after initial feeding on citrus greening-infected plants (Graca 1991).

Figure 1.6. African citrus greening symptomatic leaves

1.2.7 Morphometry of *Trioza erytreae*

Morphometrics is the analysis and measurement of size and shape. It can be used to assess the potential of host plant quality of a given (host) plant through measuring certain morphological traits. Morphometric analysis can sometimes detect subtle variations in organisms which are undetectable by genetic data. Moreover, phenotypic expression can be influenced by phenotypic plasticity (Vasconcellos et al. 2008). For instance, a study on genetic and morphometric differences between the yellowtail snapper *Ocyurus chrysurus* (Bloch) (Lutjanidae) populations of the tropical West Atlantic found differences in the morphometric analysis which were undetected in the genetic data (Vasconcellos et al. 2008).

Traditional morphometrics, involving measurements of size and ratios of size, have been utilized as a classical approach for quantifying morphometric variation in living organisms and to determine the various life stages of and compare genetic, climatic and phenotypic variations (Daly 1985). In addition, a variation of size of an organism has a smaller variation than that of shape; hence shape has received a greater attention in systematics and evolutionary studies. Recently, a new approach known as geometric analysis provides a mechanism for evaluating shape independent of size (Dryden and Mardia 1998). It has been used to evaluate morphometric variation patterns associated with wild species and laboratory-reared colonies (Ludoški et al. 2014), between wild species (Santos et al. 2015), for discriminating between species (Laurito et al. 2015) or to detect synonymy within species (Schutze et al. 2015). Geometric morphometric analysis has been crucial in determining morphometric variation in relation to factors such as competition of sympatric species (Adams and Rohlf 2000), climatic variables (Benítez et al. 2014), and temporal (Drake and Klingenberg 2008) as well as geometric variation (Lashkari et al. 2013). Host plant genotypes are known to affect the morphometrics of the Asian citrus psyllid *D. citri*, a close relative of ACT (García-Pérez et al. 2013), yet the morphometric variability, by means of traditional and/or geometric morphometry, of ACT from different host plants remains to be elucidated.

ACTs utilize a wide variety of host genotypes, principally with the family Rutaceae. Several studies have reported that ACT prefers certain species to others (Aubert 1987; van den Berg 1990). However, the consequences of these feeding preferences of ACT remain unclear. Also, ACTs performance on different host plants has been measured only through reproductive parameters like longevity, fecundity, oviposition and among others. However, no data exist on the effects of host plant on phenotypic expression of ACT.

Damage caused by *T. erytreae*

Feeding by ACT on citrus causes curling or notching and even death of newly emerged flush shoots. Heavy oviposition or larval activity sometimes kills developing terminals or cause abscission of leaves or entire terminals (Michaud 2004). Direct plant damage by ACT can also occur as a result of honeydew excretion (Figure 1.7A) and leaf distortion on infested plants at high pest population densities (Aubert 1987) (Figure 1.7B). In the Maranjau region of Kenya, ACT ranked highest among the foliar pests, causing 65% and 50% infestation of leaf clusters in young and old orchards, respectively (Ekesi 2012). ACT is also a major quarantine pest. Despite the importance of direct damage, ACT is most importantly known for its transmission of the CLaf pathogen, the causal agent of the African citrus greening disease.

Figure 1.7 Citrus leaves with *Trioza erytreae* damage symptoms, i.e. sooty mold (A) and galls/pits (B).

1.3.1 Management of *Trioza erytreae* and ACGD

1.3.2 Chemical control

Synthetic insecticides have been used to control pests on fruit trees after the discovery of DDT in the mid-1940s until the 1960s. Earlier sprays made with tobacco extract and paraffin emulsion were used to control ACT (Lounsbury 1897; van den Berg 1990). In South Africa, several foliar insecticides like dimethoate, endosulfan, and the nematicide aldicarb have been registered and are used to control ACT (Le Roux et al. 2006).

Yet even intensive insecticide programs against ACT are generally considered ineffective in preventing the spread of CGD, especially in new citrus plantings (Tiwari et al. 2011). However, insecticides applied on the stems and during the periods of profuse flush growths and offseason can greatly reduce ACT populations (Hall et al. 2012), and are recommended for ACT management.

1.3.3 Biological control

ACT populations are often suppressed by generalist predators(Aubert 1987; EPPO 2014). About 50 predators on citrus and other Rutaceae in RSA have been documented(van den Berg et al. 1987), though such information are generally lacking for East Africa and specifically for Kenya. In Réunion, two parasitoids have been credited with up to 75% parasitism in ACT but in RSA, their impact is limited by hyperparasitoids (McDaniel and Moran 1972; van der Merwe 1923). According to Catling (1970), predators such as lacewings, syrphids, coccinellids, and predatory mites can suppress ACT populations.

Tamarizia dryi it is a solitary ectoparasitoid that attacks ACT and is indigenous to Kenya (van den Berg and Greenland 2000). Female *T. dryi* attack mainly the 4th and 5th instar of ACT and rarely younger stages. The developing parasitoid larvae feed externally on the body content of the triozid host, eventually transforming it into a mummy sealed to plant tissue (McDaniel and Moran 1972). According to McDaniel and Moran (1972), the proportion of male ACT emerging from 3rd, 4th and 5th instars are 100%, 73% and 28%, respectively.

Globally, several species of entomopathogens have been reported to attack ACT, with some of them used as biopesticides. Psyllids are known to be highly susceptible to a number of entomopathogenic fungi (Avery et al. 2009; Meyer et al. 2007;2008), and among the species known to attack ACT are *Isaria fumosorosea* Wize, *Isaria javanica* Friedrichs & Bally*,* and *Hirsutella citriformis* Pat. (Avery et al. 2009; Étienne et al. 2001).

1.3.4 Cultural control

The meaningful manipulation of the ecosystem to reduce pest infestation and damage is the single most important strategy adopted by early pest managers (Racette et al. 1992). It is considered the core of IPM because it can substantially reduce the need to use synthetic pesticides that may pose a threat to the environment. For ACT management, the most commonly used cultural management option is the removal and disposal of either greening-infected citrus leaves/shoots and or the complete removal of an infected tree. In the early 1980s, almost 100% of the Tanzanian citrus orchards were ACGD infected (Swai 1988). Subsequently a country-wide Citrus Improvement Program (CIP) was initiated which aimed at the selection of clean planting material of both local and foreign origin, indexing of the parent trees for citrus greening, obtaining plants by Shoot Tip Grafting (STG), maintenance of citrus greening disease-free plants and release of citrus greening disease-free budwood to citrus nurseries. ACT management also involves the removal of alternate host plants within the vicinity of citrus groves (CABI 2015). However, despite such efforts, the citrus greening disease still poses the greatest threat to citriculture in Africa and beyond (Cocuzza et al. 2017).

1.3.5 Justification of the study

Compared with its Asian relative *D. citri*, ACT has received less attention in terms of understanding its biology and ecology to guide management decision and reduce ACGD transmission (van den Berg 1990). In Kenya, there are no detailed studies on the distribution, abundance, dynamics and host-pathogen interaction of ACT. ACGD has been implicated in the collapse of the citrus industry in the Kenyan highlands (Kavoi and Tschirley 2004; Kilalo et al.

2009), despite the widespread use of synthetic insecticides. Such ill-guided and poorly implemented insecticide applications often result in high levels of toxic residues on the produce that can negatively affect human health, and reduce lucrative export opportunities. Moreover, indiscriminate use of insecticide often leads to resistance development in key pests such as ACT, disruption of potentially useful natural enemies and higher production costs (Weinberger and Lumpkin 2005).

In South Africa, an integrated pest management (IPM) strategy based on pest monitoring, cultural control, biological control, and use of target-specific insecticides has been developed for the major citrus regions (Cocuzza et al. 2017). However, such an ecologically friendly program has not been introduced in Kenya. Due to the lack of information on effective, suitable and sustainable alternative control and pest and disease management approaches, which build upon a sound understanding of the pest's biology and ecology, citrus farmers in Kenya are presently disinclined to adopt IPM (Cocuzza et al. 2017). Hence, an improved knowledge of the bio-ecology of the pest will aid in the development of an ecologically sound IPM program in Kenya.

1.4.1 Aim and objectives

The aim of this thesis is to determine the bio-ecology of ACT on citrus and the implications for its management. The dissertation has the following specific objectives:

a) Determine the distribution, abundance and degree of damage and areas at risk of spread;
b) Determine the host suitability and feeding preference of selected native rutaceous and non-rutaceous host plants (with trioza-like galls) for ACT;
c) Determine the seasonal abundance of ACT along three altitudinal gradients;

d) Compare different sticky card traps for ACT monitoring and surveillance; and

e) Evaluate the effect of host plants on wing size and shape of ACT, using traditional and geometric morphometry.

1.4.2 Structure of the thesis

The thesis is arranged in four results sections, preceded by a general introduction (chapter 1) and concluded by a synthesis of the results, focusing on the key findings (chapter 7) an outlook for future research and application of the findings (chapter 8). Section 1 (chapters 2 and 3) focuses on the distribution, the degree of damage and areas at risk of spread as well as the host suitability and feeding preference of ACT. The seasonal phenology of ACT was investigated in section 2 (chapter 4). The use of traditional and geometric morphometry for separating different populations of ACT was investigated in section 3 (Chapter 5). The determination of the effectiveness of sticky card traps for *T. erytreae* monitoring and surveillance were investigated in section 4 (chapter 6).

2. DISTRIBUTION, INFESTATION LEVELS AND RISK OF SPREAD OF *TRIOZA ERYTREAE* (HEMIPTERA: TRIOZIDAE) IN KENYA

2.1 Abstract

The African citrus psyllid (ACT) *Trioza erytreae* Del Guercio (Hem.: Triozidae), is a very destructive pest particularly on citrus, and vectors "*Candidatus"* Liberibacter africanus (Laf), the causal agent of the African citrus greening disease (ACGD). Our study seeks to establish ACTs distribution and host-plant relationship across citrus production areas in Kenya. We also modeled the risk of spread using the Maximum Entropy Modeling (MaxEnt) algorithm with known occurrence data. Our results infer that ACT is widely distributed and causes severe damage to five host plants belonging to the family Rutaceae. The developmental stages, galls, and the percentage of infested leaves were significantly higher in the shaded than unshaded trees. The average area under the curve (AUC) of the model predictions was 0.97, indicating an optimal model performance. The environmental variables that most influenced the prediction were the precipitation of wettest quarter, precipitation of wettest month, mean diurnal range, temperature seasonality and mean temperature of the coldest quarter. The current prediction of ACT exceeded its existing range, especially in the Western, Central and Eastern regions of Kenya. The model predicted a contraction of suitable habitats for a potential spread in 2040 with an inland shift to higher altitudes in the cooler Counties (Meru and Nyeri). The potential for further expansion to climatically suitable areas was much pronounced for the 2080 forecast. These findings provide relevant information to improve monitoring/surveillance and designing IPM strategies to limit its spread and damage.

2.2 Introduction

Citrus, *Citrus* spp. (Sapindales, Rutaceae), includes some of the world's major fruit crops recognized for its global availability and contribution to food and nutritional security (Liu et al. 2012). It ranks first internationally in trade value among all fruits (UNCTAD 2013). The most commonly known species of commercial importance are sweet orange (*C. sinensis* (L.) Osbeck), lemon (*C. limon* (L.) Osbeck), lime (*C. aurantifolia* (Christm.) Swingle), grapefruit (*C. paradisi* (Macfad.), and tangerine (*C. reticulate* (Blanco)) (FAOSTAT 2018). Sweet orange is the main species, cultivated globally and representing more than 65% of the total citrus output. Annual global production of citrus was at 146,429,018 tonnes in 2016, with sweet orange varieties contributing to more than half of the world's total with approximately 85.233 tonnes from 7.268 ha of land in Kenya (FAOSTAT 2016). Although the majority of these fruits produced are consumed locally as fresh products, a smaller quantity of the Kenyan citrus is processed into juices, jam, and marmalades (MMA 2008). Yet, local market demand is so high that the production is currently supplemented by imports from South Africa and Egypt, the leading citrus producers in Africa (Chadwick 2017; MTI 2017), which represent 5-21% of the domestic production (MMA 2008; Tschirley et al. 2004).

Citrus greening disease is associated with three nonculturable gram-negative bacterial strains belonging to "*Candidatus"* Liberibacter, identified through 16S rDNA sequence analysis (Bové 2006). The main bacteria species are "*Candidatus"* Liberibacter africanus (Laf), from Africa, and "*Candidatus"* Liberibacter asiaticus (Las), from Asia and the

Americas, and "*Candidatus"* Liberibacter americanus (Lam), from Brazil that are associated with the Asian form of citrus greening or Huanglongbing (HLB). These phloem-limited greening pathogens are transmitted from infected to uninfected plants through grafting or by citrus psyllids (Gottwald 2010). Laf is transmitted mainly by the African citrus psyllid (ACT) *T. erytreae* Del Guercio (Hemiptera: Pysliidae) prevalent in Africa (Aubert 1987) and more recently in Europe (Cocuzza et al. 2017; Hernández 2003), while Las and Lam are transmitted by the Asian citrus psyllid *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae) prevalent in Asia and America (Ammar et al. 2016; Chiyaka et al. 2012). The main symptoms of HLB -infected trees include yellow shoots, leaves with blotchy mottle, small lopsided fruits with a curved central core and bitter taste, whereas infected branches show twig die-back and affected trees progressively decline and eventually die (Lee et al. 2015). Currently, there is no known cure for both forms of the disease (Gottwald 2010). This may be responsible for the near collapse of citrus industries, particularly in Kenya highlands (Ekesi 2012).

ACT, native to Africa, was detected for the first time in Kenya in 1922 (Waterston 1922). It is the only species of triozids (Hemiptera: Triozidae) that attacks citrus and induce pit-like galls on leaves (Cocuzza et al. 2017; Hodkinson 1984). Although direct damage from ACT feeding can be significant, the economic importance of the pest stemmed from its ability to vector HLB pathogens (Aubert 1987). Currently, ACT is a major pest categorized as an ‘A1’ quarantine pest by CABI (CABI 2015).

The seasonal population fluctuations of ACT follow the flushing rhythm of the citrus host (Cook et al. 2014). However, flush abundance is an inconsistent indicator of the mean density of *T. erytreae* per flush shoot as stated for *D. citri*, a close relative of ACT (Hall et al. 2008). Large infestations of adult *D. citri* in citrus orchards can occur at any time of the year, depending on environmental factors and flush availability (Catling 1972; Cook et al. 2014). The females lay their eggs exclusively on newly emerged flush shoots on which immature complete their development. The adults can live up to three months, and even under drought conditions can still survive on their host plants (van den Berg 1990). During the citrus offseason, alternative hosts are essential breeding sites for ACT. In South Africa, van den Berg (1990) reported four such Rutaceae alternative hosts, namely *Clausena anisata* (Willd.) Hook. f. ex Benth., *Veprislanceolata* (Lam.) G. Don, *Zanthoxylum capense* (Thunb.) Harv. And *Oricia* sp., and recommended similar investigations in other pest affected areas. In addition, ACT population fluctuation is driven by various climatic factors including temperature, rainfall, and light (Cook et al. 2014).

Better understanding of the biotic and abiotic factors influencing the biology and population dynamics of ACT could facilitate the development of appropriate models to predict the spread of the pest and develop appropriate control strategies. Despite ACT status as a major pest and disease vector of citrus in Africa, there is scant information on its distribution, abundance, infestations in different citrus growing areas of East Africa, and prediction of potential areas at risk of possible invasion. Hence, modeling the current distribution and forecasting areas at risk of ACT invasion are central to the development of its management strategies and spread prevention plans. Various species distribution models (SDS) have been used to describe and estimate the ecological pattern and areas at risk of pest invasion across time and space (Elith and Leathwick 2009). Maximum entropy (MaxEnt) (Phillips et al. 2006) is one such model widely used to project current and future distribution of pests at different time periods(Elith and Leathwick 2009) because of its better predictive performance compared to other modeling algorithms (Elith et al. 2006) even with reduced numbers of samples (Pearson et al. 2007). In addition, MaxEnt models perform better when presence or absence-only data are available (Phillips et al. 2006).

Knowledge of the geographical distribution, abundance, pest damage, the number of individuals present, when, and why they are found at a given location is essential for the development of appropriate and effective management strategies. Understanding the distribution of insects is crucial because it fills the knowledge gap of the biology and ecology of the species, and also enhances the development of reliable sampling plans (Binns and Nyrop 1992) that are often prerequisite for the implementation of effective IPM strategies. Hence, our goal was to evaluate ACT's bio-ecological parameters including its distribution, population densities, and damage levels in different citrus growing regions of Kenya to inform on the development of appropriate IPM strategies. We also attempted a prediction of suitable climatic areas for ACT invasion.

2.3 Materials and methods

2.3.1 Data collection

To determine the geographical distribution, population densities, leaf damage levels (galls) and climatic areas suitable for ACT, field surveys were conducted from December 2015 to April 2018 in the different citrus growing regions of Kenya (Figure 2.1). During these surveys, data were collected on numbers of ACT adults and developmental stages, and percentage of leaves showing galls symptoms. Sampling was carried out in home gardens, commercial and small-scale orchards in the high, mid and lowlands to cover a wide range of altitudinal breadth (Figure.1). In total 16 counties spreads across 28 sub-counties were visited. The counties and sites were selected based on the availability of citrus orchards. In each county, at least one orchard from different sub-counties was selected. However, the sampling technique used was the same for each orchard. In each orchard, ten trees were randomly selected within the orchard i. e. five trees under shade and five unshaded trees/ in the open. A tree was considered “shaded” when the canopy receives full shade from the canopy of another tree (taller one), and “unshaded” when a tree is fully exposed fully in the open/was directly exposed to the sun. The survey was conducted when there were profuse flush shoots based on the description by Cifuentes-Arenas et al. (2018) namely; phase 1 (emergence) consists of one stage (V1), phase 2 (development) consists of two stages (V1 and V2), Phase 3 (maturation) consists of two stages (V4 and V5) and Phase 6 (Dormancy) consists of only one stage (V6). Each groove was visited once. We followed the general procedure outlined by Sétamou et al. (2008)with slight modifications. The canopy of each tree was divided into four cardinal points (Northeast (NE), Northwest (NW), Southeast (SE) and Southwest (SW) using a hand-held analog plastic compass (Suunto–A10 NH measuring 56 x 104 x 10mm with 2.5˚ and 2 ˚ accuracy and resolution, respectively). In each quadrant, five flush shoots as described by Catling (1969) were randomly selected, i.e. NE, NW, SE, and SW for a total of 20 flush shoots per tree. The number of ACT galls per shoot, number of leaves per shoot, number of leaves with ACT galls (triozid pits), number of all the developmental stages, host plant type, geographical locations, host plant species, and the environment (shade/unshade) of the tree were recorded for each location. Sites with any occurrence of developmental stages or galls of ACT were considered infested based on the judgment that galls per shoot have been previously used to study ACT presence (Aubert 1987).

2.3.2 Data analysis

The data on percentage flush shoots with triozid galls/pits and the number of ACT adults and developmental stages on the different citrus varieties namely; Citrumelo (*Citroncirus* spp.), Lemon (*Citrus limon*), Tangerine (*C. tangerina*), "Washington navel" and "Valencia" (both *C. sinensis*) were analyzed using mixed model which takes into account all the random factors associated hierarchical sampling. This was followed by an analysis of deviance using the chi-square distribution. Akaike Information Criteria (AIC) was used to confirm the best model (Cifuentes-Arenas et al. 2018). In case they were statistically significant, means were separated using *post-hoc* multiple comparison procedures with an "Adjusted Tukey". For the alternate host plants, count numbers of ACT developmental stages and galls per shoot on different alternative host sampled were log ($x' = Log (x+1)$) transformed and subjected to analysis of variance (ANOVA) followed by Student-Newman Keuls (SNK) test. Shaded and unshaded trees were considered as treatments for environment comparison. Environment (shade and unshaded trees) and variety (host species) were considered as treatments. Analyses were conducted in R software (R Development Core Team 2009).

2.3.3 Model development

To predict the full ecological range of ACT, we fit its geographic expansion models using MaxEnt based on presently available occurrence data and climate data for Kenya downloaded from the Bioclim dataset (Fick and Hijmans 2017). To minimize multicollinearity among predictor variables (Merow et al. 2013) and also reduce over and underfitting of the suitability map of the species (Kuhn 2008; USGS 2004), we performed a Pearson correlation test between all the predictors (Bioclim variables) and elevation data from the United States Geological Survey 30m SRTM dataset using the find correlation function in caret package in R Statistical Software (Version 3.4.4). From the Pearson correlation analysis, at a threshold of r > 0.7 (Dormann et al. 2013), the number of bioclimatic variables reduced from 20 to five namely; Bio2 (Mean Diurnal Range), Bio4 (Temperature Seasonality), Bio11 (Mean Temperature of Coldest Quarter), Bio13 (Precipitation of the Wettest Month) and Bio 16 (Precipitation of the Wettest Quarter), which were least correlated were selected for running the final MaxEnt model.

For simulation of the ACT distribution, current climatic conditions at one-kilometer grid resolution from the Bioclim data set were used (Fick and Hijmans 2017). This dataset contains grids of temperature, rainfall and derived bioclimatic summary variables, which represent annual trends, seasonality and limiting environmental factors. For running the MaxEnt model, the presence data and the six predictor variables were used with, 30% of the presence samples used for testing and the remaining 70% used for validation. For prediction of distribution under future climatic condition simulations, downscaled global climate models (GCMs) HadGEM2-ES data, based on the Representative Concentration Pathways Scenarios, Fifth Assessment Report (RCPs-AR5) (IPCC 2013) future year 2055 (mean over 2041–2070) were considered. We used future climate simulations from Bioclim to project future scenarios and due to the absence of vegetation projection, we assumed vegetation and topography to be unchanged over the projection period. The same six variables were used to run the MaxEnt models for future scenarios to generate the suitability maps.

2.4 Results

2.4.1 Distribution, abundance and damage levels

ACT was found in all citrus growing areas sampled except for Makueni and Kilifi counties (Figure 2.1). Our results show that ACT was widely distributed and causes severe damage to four alternative host plants belonging to Rutaceae families.

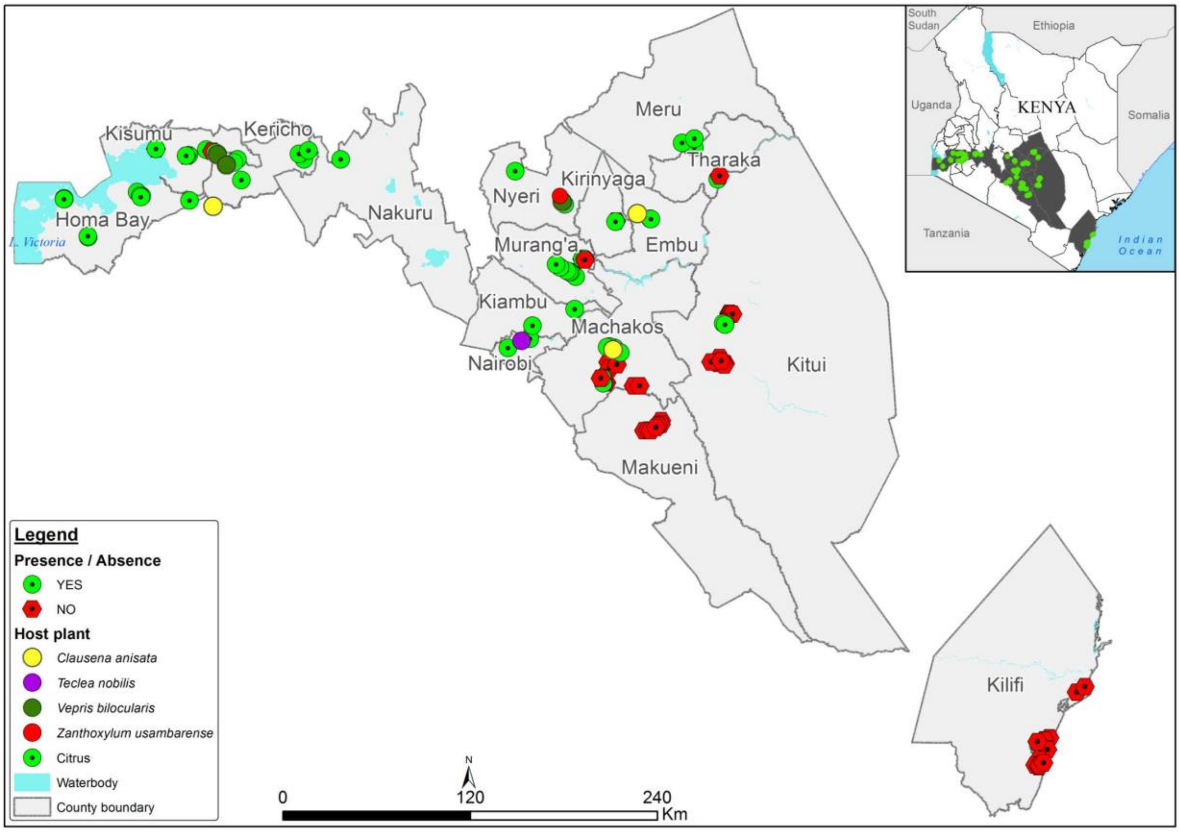

Figure 2.1 Map showing sites where *Trioza erytreae* was sampled and alternative host plants

The sites surveyed in Makueni and Kilifi had elevations ranging from 1,000-1,100 and 20-300 meters above sea level (m. a. s. l.), respectively. The other citrus growing sites visited had elevations ranging from 1,200 to 2,315 m. a. s. l. There was a significant difference between the citrus cultivars and the mean number of eggs, nymphs and adults (eggs: $\chi^2 = 57.018$, df = 4, $P =$

0.0001, nymphs: $\chi^2 = 15.671$, df = 4, $P = 0.004$), except for galls, the mean density of adult ACT and the percentage of infested leaves differed significantly across the different citrus cultivars (adults: $\chi^2 = 17.21$, df = 4, $P = 0.018$, galls: $\chi^2 = 4.218$, df = 3, $P = 0.377$, % infested leaves: $\chi^2 = 16.738$, df = 4, $P = 0.002$) (Table 2.1).

Table 2.1. Number of *Trioza erytreae* and infestation on shoots of five citrus cultivars selected from different compass quadrants

Cultivar	Eggs	Nymphs	Adults	Galls	% infested leaves
Lemon	15.55 ± 3.67b	35.02 ± 6.33b	0.87 ± 0.21b	155.06 ±12.53b	20.28 ± 1.1b
Tangerine	0.00 ± 0.00c	0.61 ± 0.10d	0.00 ± 0.00d	223.59 ± 44.2a	22.83 ± 2.46b
Valencia	12.85 ± 5.87b	36.82 ± 7.2ab	1.29 ± 0.39a	143.27 ±17.56b	18.1 ± 2.46c
Washington Navel	32.54 ± 4.96a	18.67 ± 2.82c	0.80 ± 0.20b	124.99 ±8.73b	18.51 ± 1.45c
Citrumelo	45.7 ± 14.04a	128.2 ± 25.84a	0.30 ± 0.20c	258.93 ± 39.62a	36.19 ± 2.90a

Across each host plant species (Column), means followed by different letters are significantly different (*post-hoc* test with adjusted Tukey).

However, the developmental stages, galls and infested leaves significantly varied between shaded than unshaded trees ($\chi^2 = 12.282$, df = 1, $P = 0.0005$, nymphs: $\chi^2 = 9.252$, df = 1, $P = 0.002$, galls: $\chi^2 = 41.506$, df = 1, $P = 0.0001$ and percentage leaves infested: $\chi^2 = 16.74$, df = 1, $P = 0.0001$). In addition, the mean number of adults differed significantly among the citrus cultivars ($\chi^2 = 4.552$, df = 1, $P = 0.033$) (Figure 2.2). Nested analysis of variance showed that ACT (all stages) differed significantly within and among host plants (Table 2.2).

Table 2.2 Nested analysis of variance of ACT infestation levels and densities as affected by citrus host plant, site, tree, and canopy quadrant in Kenya

Source of Variation	No. of eggs	No. of nymphs	No. of adults	No. of galls	% leaves infested
	% Variance component (Random effects)				
Random effects					
Site	18.69	39.39	16.18	23.84	25.7
Quadrant	0.35	0	0.52	1.22	0.87
Tree	0.26	3.32	0.92	1.23	1.52
Residual	80.71	57.29	82.38	73.7	7.91
Fixed effects					
Host plant	χ^2_4 =57.02***	χ^2_4 =15.67**	χ^2_4 =17.21**	Fixed effect	Fixed effect
Environment	χ^2_1 =2.28***	χ^2_1 =9.25***	χ^2_1 =4.55*		

Variance components are unbiased estimates obtained using linear mixed model In R software (*lme4* package)

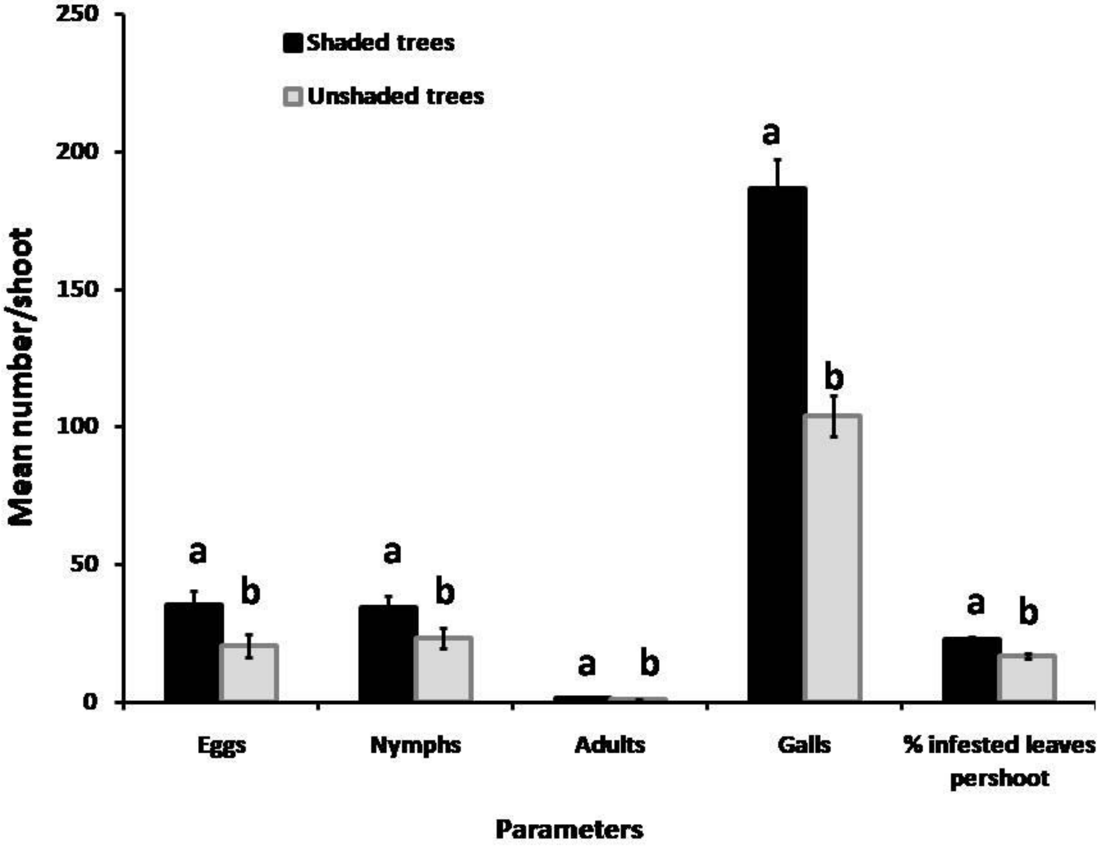

Figure 2.2 Mean number of *Trioza erytreae* and infestation levels on shaded and unshaded citrus tree

The mean number of eggs, nymphs and galls did not differ significantly among the different ACT alternate host plants (eggs: $\chi^2 = 3.054$, df = 3, $P = 0.3838$, nymphs: $\chi^2 = 4.490$, df = 3, $P = 0.213$ and galls: $\chi^2 = 3.022$, df = 3, $P = 0.388$) (Table 2.4).

Table 2.4. Number of *Trioza erytreae* and level of damage on alternate host plants

Parameters	Host plants			
	Clausena anisata	*Teclea nobilis*	*Vepris bilocularis*	*Zanthoxylum usambarense*
Eggs	146.29 ± 18.11	106.05 14.04	81.70 ± 13.04	90.50 ± 16.24
Nymphs	72.36 ± 15.11	63.20 ± 14.84	40.02 ± 9.09	78.90 ± 17.21
Adults	2.12 ± 0.42a	1.75 ± 0.45ab	1.20 ± 0.30ab	0.30 ± 0.15b
Galls	123.40 ± 21.18	75.70 ± 19.21	72.48 ± 14.48	66.70 ± 9.79

Within each row, means followed by different letters are significantly different (*pos-hoc* test with adjusted Tukey- test).

2.4.2 Current and future scenarios

Model predictions indicate that rainfall conditions were more important than other variables in creating the map. The possible future distribution map based on the current climate and occurrence data of ACT is shown in figure 2.3.

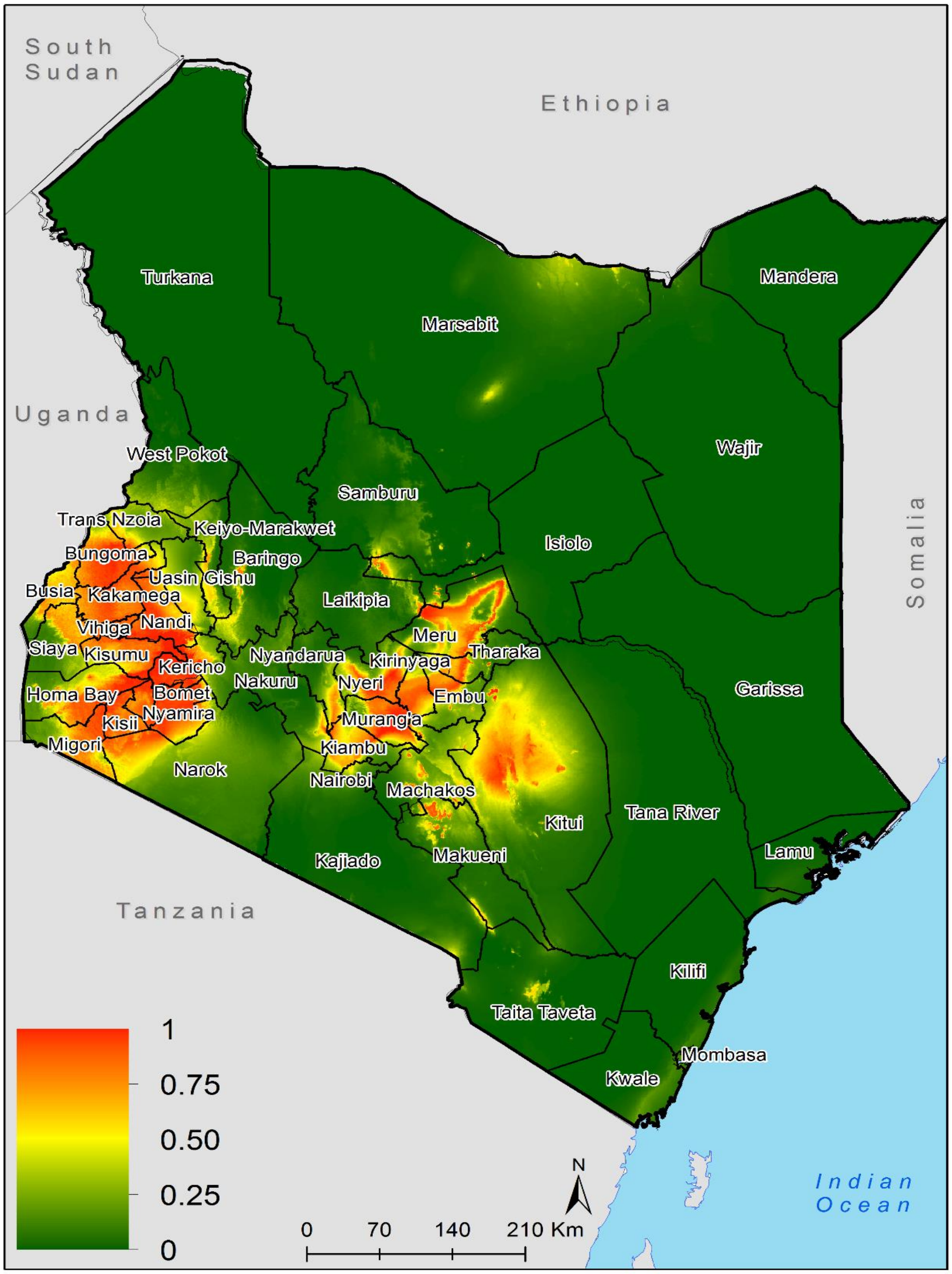

Figure 2.3 Map showing the current suitable (warm) and unsuitable (green) climate areas at risk of *Trioza erytreae* incursion (green regions represent unsuitable areas for *Trioza erytreae* invasion)

The model predicts most areas in Meru, Kakamega, Muranga, Embu, Kiambu; Nyeri, Bungoma, Kakamega, Nandi, Kisumu, Bomet, Nyamira, and Kisii counties as hotspots areas while Mandera, Turkana, Garissa, and Wajir counties appear to be unsuitable habitats for ACT. In addition, some few areas in the Mombasa, Kilifi, Kwale, Lamu, Marsabit, Kajiado, Taita, Taveta, and Isiolo regions were areas predicted with low possibility of an ACT spread. The two future predictions showed variable results (Figure 2.4). The 2040 climate change model (Figure 2.4A) predict a contraction in suitable climate areas while the 2080 prediction (Figure 2.4B) an expansion of the pests' ecological niche. For the 2040 scenario, there was a substantial reduction in the suitable habitat areas for ACT throughout the counties with a noticeable shift, particularly in the Nyanza and Coastal regions (i.e. some areas in Homabay, Migori, Busia, Siaya, Narok, Kajiado, Taita Taveta, Kwale, Mombasa, Kilifi, and Kwale). A similar observation was made in most of the counties in central Kenya. Moreover, for the 2040 scenario, there was an inland shift of the potential distribution range of ACT in the coastal regions. In some parts of Meru, Embu, Kirinyaga, Nyeri, and Muranga counties there was a decline in areas suitable for ACT. There was a considerable expansion of potentially suitable climate areas under the 2080 scenario, particularly in Turkana, Marsabit and Narok counties.

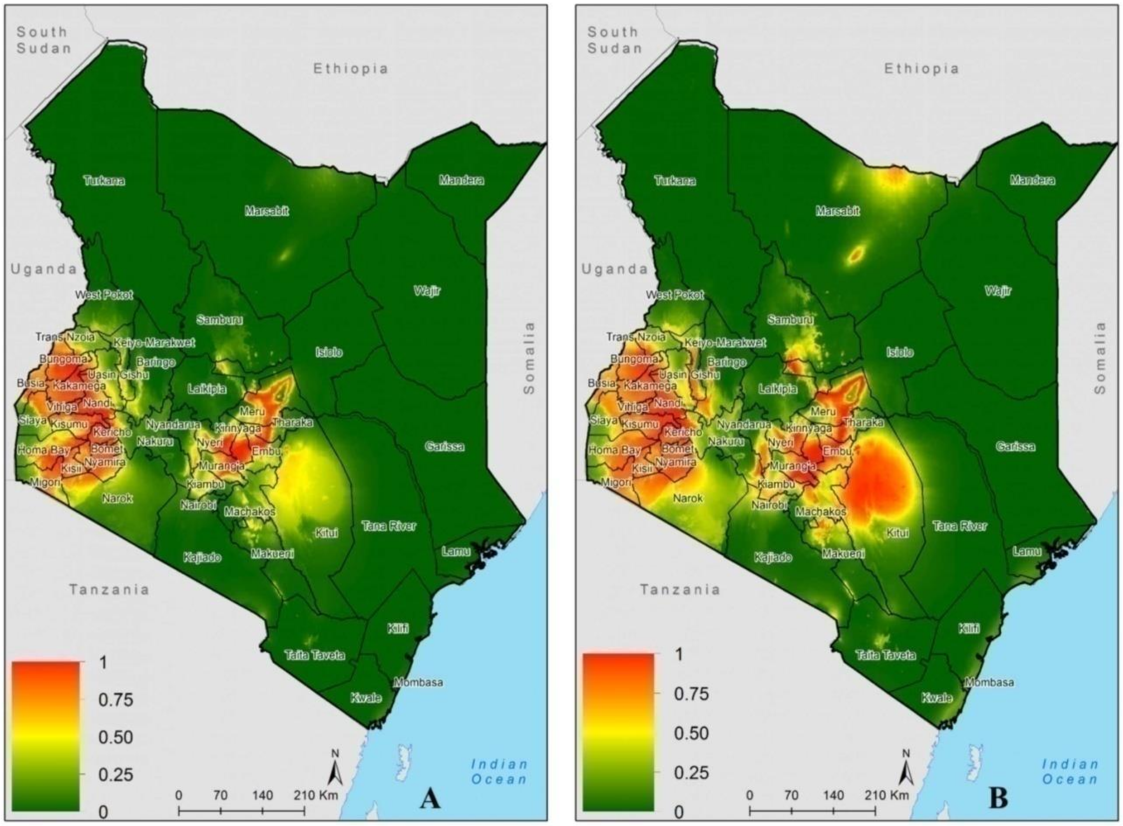

Figure 2.4 Map showing the potential areas at risk of *Trioza erytreae* incursion in A (2040) and B (2080) (green regions represent unsuitable areas for *Trioza erytreae* invasion)

The model performance for ACT was better than random, with a training area under the curve (AUC) and test values of 0.946 and 0.926, respectively, for the current scenario. The 2040 prediction gave 0.939 and 0.929 for training and test data, respectively, while 0.936 and 0.956 were recorded for training and test data for the 2080 scenario, hence the model performed well in predicting the potential habitats of the pest. A maximum training sensitivity plots specificity threshold value of 1.720 from the 10th percentile training presence occurrence of the triozids was recorded. Among the climatic variables, the percentage contribution varied from each other (Figure 2.5). Bio16 contributed to 77% of the current, 53% to 2040 and 50% to 2080

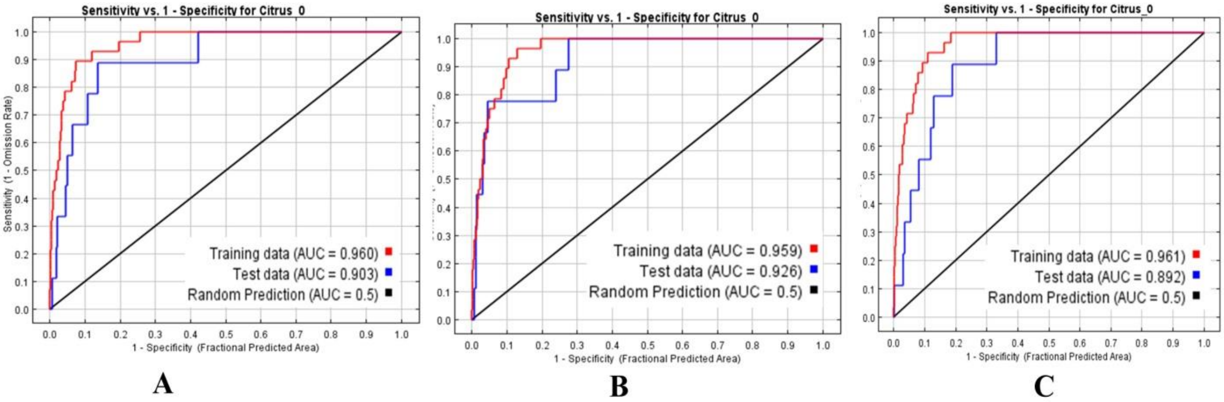

Figure 2.5 ROC curve and AUC value under the current period for A (Current), B (2040) and C (2080)

scenarios, while Bio13 contributed 8%, 30% and 38% for current, 2040 and 2080 distribution of AfCP, respectively. These two factors explain about 86%, 83% and 88% for the current, 2040 and 2080 models, respectively. Elevation contributed to 6%, 5% and 6% for current, 2040 and 2080 scenarios, respectively. Bio11 contributed the least with 0%, 0.2% and 0% for current, 2040 and 2080 predictions, respectively (Figure 2.6, Table 2.5).

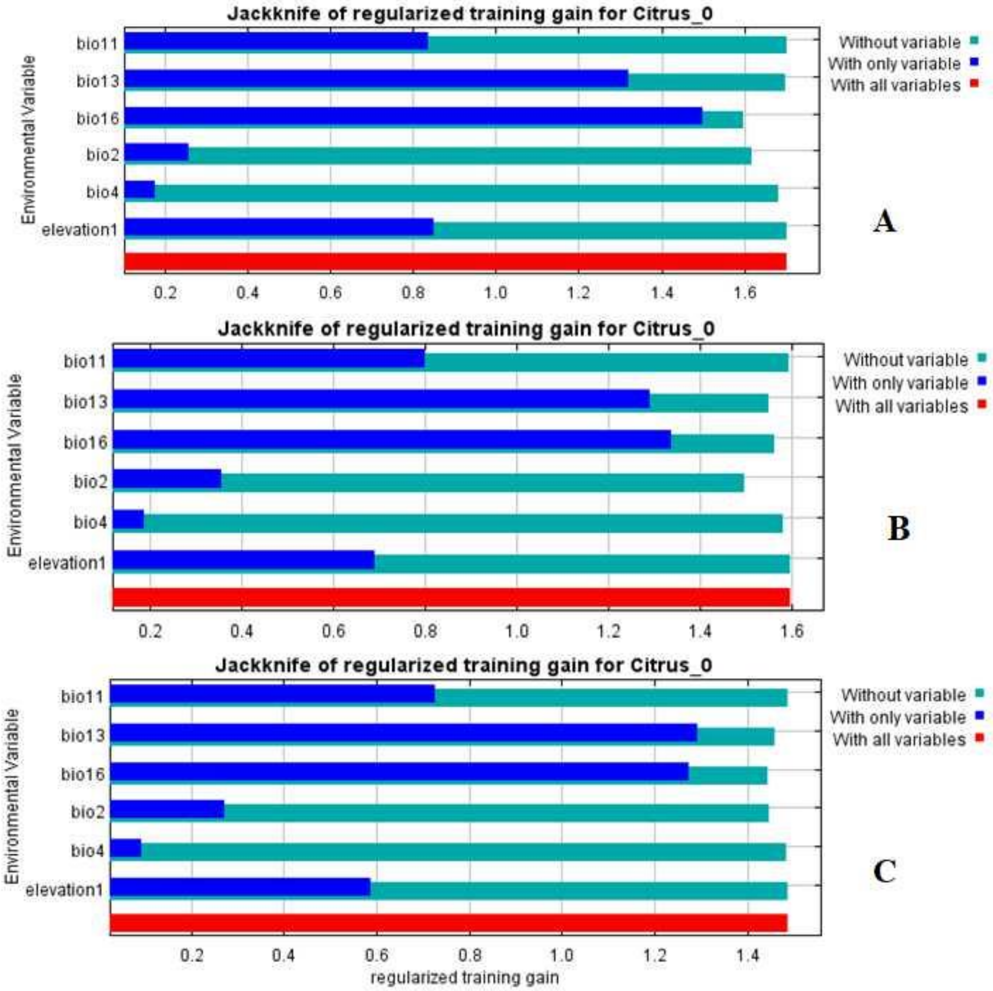

Figure 2.6 Jackknife test for the variable importance of *Trioza erytreae* habitat suitability distribution over 10 replicate runs A (Current), B (2040) and C (2080)

Table 2.5. Estimates of contribution and permutation importance of environmental variables in MaxEnt modeling of *Trioza erytreae.*

Environmental variables	Percent contribution			Permutation importance		
	Current	2040	2080	Current	2040	2080
Precipitation of Wettest Quarter (mm)	77.8	53.1	50	88.7	27.5	56.8
Precipitation of Wettest Month (mm)	8.6	30.5	38.2	0.5	48.6	23.7
Elevation (m)	6.5	5.3	6	0.7	0	0.6
Mean Diurnal Range (Mean of monthly (max temp - min temp)) (˚C)	6.1	9.8	5.5	9	19.1	16.1
Temperature Seasonality (standard deviation *100) (˚C)	0.9	1	0.2	1.1	2	2.7
Mean Temperature of Coldest Quarter (˚C)	0	0.2	0	0	2.7	0.2

2.5 Discussion

2.5.1 ACT distribution, abundance and damage levels

Adults, developmental stages and damage symptoms of ACT appear to be widely distributed in Kenya and the pest seems to be more prevalent in cooler habitats. In the hot and humid Makueni and Kilifi counties all the orchards visited had no ACT developmental stages or symptomatic leaves. A combination of factors including suitable habitats, host plant, natural enemies, diseases and heritable traits has been reported to influence the distribution and establishment of a species in a new area (Wallner 1987). This can further be explained by the fact that ACT eggs are highly vulnerable to desiccation, as such might not have been able to hatch, develop and establish in these dry habitats where temperatures were high with low relative humidity (Green and Catling 1971). This suggests that in addition to elevation other factors can influence ACT's distribution and abundance. Citrus farms in Kilifi County were not infested by ACT, possibly because of the prevailing high temperatures as reported above. However, these areas are perfectly suitable for *D. citri*, ACT's Asian counterpart which is more adapted to warm climates as shown in recent invasions in Kenya and Tanzania (Rwomushana et al. 2017; Shimwela et al. 2016).

The densities of different ACT developmental stages and damage levels (galls per shoot and infested leaves) were significantly higher in shaded than unshaded trees when compared across the citrus cultivars. This is probably attributed to greater exposure to sunlight and consequently higher temperatures in unshaded trees. Both ACGD and ACT favor cooler and moist climates, which for the latter are more suitable for oviposition and the development of the developmental stages (Catling 1973; Schwarz and Green 1970). Also, it was observed that more adults occupied the southeastern quadrant of the tree canopy. However, this observation requires further investigation.

An earlier study reported that ACT prefer leaves of lemon over other host plants for reproduction (Moran 1968; Moran and Buchan 1975). However, we found higher mean numbers of ACT developmental stages, galls and infested leaves on citrumelo than on the other varieties, which might be partially attributed to volatiles produced by citrumelo. This is consistent with results from Fancelli et al. (2018) who demonstrated a positive relationship between host plant volatiles and attraction of *D. citri* to its preferred host plants. Additional studies to address ACT-citrumelo volatiles and their attraction are crucial to understanding the underlying mechanisms involved in their preference for feeding and oviposition on this host and for the development of attractants for ACT. Although, volatiles have been implicated in the attraction of psyllids for feeding and reproduction, other factors such as the nutritional status of the host plant cannot be overlooked. For instance, Sétamou et al. (2016) observed that *D. citri*, when raised on different host plants, showed considerable variation in terms of feeding and reproductive preferences. Apart from these, several other factors are well documented to strongly influence the abundance of ACT, key among them are availability of young soft shoots and the quality of the host plant (Cocuzza et al. 2017). Similarly, studies on ACT population dynamics revealed that peak population densities coincided with the flushing of the citrus tree (Cook et al. 2014). In addition, ACT can disperse and identify new flush shoots over more than 500 meters (van den Berg and Deacon 1988). It is worth noting that in the absence of suitable foliage ACT can only survive for a few hours (Catling 1973) before dying because of desiccation (van den Berg and Deacon 1988).

2.5.2 Current and future scenarios

Species distribution models have been extensively used to describe a range of applied and theoretical ecological issues (Peterson et al. 2011). Although there are many available techniques, MaxEnt is widely used (Elith et al. 2006). We used ACT presence-only data to model the current and future potential habitats under different climate change scenarios. Although rainfall appears to be the key factor limiting ACT's distribution, high temperatures prevent its establishment, particularly in the Kenyan lowlands. Temperature and precipitation are known to influence species distributions at broad scales (Wiens 2011). In addition, the distribution of ACT and the pathogen it transmits are restricted to cool habitats that are below 30˚C (Bové 2006; Cocuzza et al. 2017). High temperature coupled with low humidity has been shown to increase ACT egg and nymphal mortality (Samways 1987b).

Our results indicate a possible change in the current and future distributions of ACT. As the climate changes, some areas that are presently suitable for the pest and/ or where ACT occurs may become unsuitable in the future. The model accurately predicted many suitable habitats at risk of possible spread. However, the ability of a species to establish outside its distributional range depends on a wide variety of other factors: natural enemies (predators, parasitoids, and fungi), vegetation of the area, and presence of its host plant, as well as anthropogenic activities. All these factors need to be further investigated to better understand the ecology and potential risk of spread of this devastating pest across different citrus agroecosystems.

2.6 Conclusion

Our findings clearly demonstrate that citrus cultivars significantly affected the distribution of ACT within the tree canopy with more shaded citrus trees more prone to ACT attack than unshaded ones, which is possibly due to temperature effects. We successfully modeled the current and future risk of spread and potential hotspots of ACT in Kenya to guide the development of effective IPM strategies.

3. HOST SUITABILITY AND FEEDING PREFERENCE OF THE AFRICAN CITRUS TRIOZID TRIOZA ERYTREAE DEL GUERCIO (HEMIPTERA: TRIOZIDAE), VECTOR OF CITRUS GREENING DISEASE PATHOGEN

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3.1 Abstract

African citrus greening disease (ACGD) is worldwide one of the most damaging diseases of citrus. Currently, the disease has no cure and has been attributed to the collapse of the citrus orchards in several countries. In Africa, the causative agent "*Candidatus* Liberibacter africanus" is vectored by African citrus triozid (ACT) *Trioza erytreae* Del Guercio (Hemiptera: Triozidae). ACT is native to Africa but has been recently reported in Asia and Europe. Apart from citrus, *Murraya koenigii* (L.) and *Clausena anisata* (Willd) Hook. F. ex Benth. are preferred host plants. At present, there is scanty information on host plant suitability and preference of *T. erytreae*. Also, there are contradictory reports on its reproduction and survival on rutaceous and non-rutaceous host plants. In the present study, we tested the suitability and preference of rutaceous and non-rutaceous trees and shrubs as potential ACT host plants in choice and no-choice bioassays. The development from egg to the adult stage was longest on *Calodendrum capense* (Wright & Arn.) Engl. Plants which appeared to have superior host plant quality as reflected by several of ACT's biological parameters also produced *T. erytreae* with superior morphometric features. Our findings on the host status of the five rutaceous plants imply that these plants can greatly influence the population dynamics of ACT as well as the epidemiology of ACGD, and these can be a useful guide in the area-wide management of the pest in Kenya.

3.2 Introduction

The African citrus triozid (ACT) *Triozae erytreae* Del Guercio (Hemiptera: Triozidae) is the most economically important vector of the African citrus greening (ACGD) disease (Khamis et al. 2017; Kilalo 2004) caused by the phloem-limited bacteria *"Candidatus* Liberibacter africanus" (Bové 2006). The disease affects citrus production by attacking the vascular system of plants and its effect can easily be seen on an infected tree showing twig dieback, leaf yellowing, green, misshapen fruit, blotchy mottle on leaves (a random yellowing pattern), yellow leaf veins, salty, bitter tasting fruit that has no economic value, and off-season blooming (Bové 2006; van den Berg 1990). The disease is associated with a decline of citrus trees and is probably widespread in all the citrus growing countries where it occurs. It possesses a great potential to affect citrus trees of all cultivars and cause a negative impact on the citrus industry by shortening the productive lifespan of trees. It is suspected that greening disease has caused colossal losses in some parts of Asia and Africa (Batool et al. 2007).

Currently, ACGD has no cure and generally citrus greening disease (CGD) is the most destructive disease of citrus worldwide (Bové 2006; Grafton-Cardwell et al. 2013; Pimpinato et al. 2017). CLaf transmitted by ACT adults and nymphs (van den Berg 1990) could have led to the near collapse of the citrus industry in Kenya, particularly in the lower midlands and high altitudinal gradients (Magomere et al. 2009). Currently, local production has to be supplemented by imports from South Africa and Egypt which ranges between 5-21% of the domestic production (Tschirley et al. 2004). There have been several contradictory reports on the host range of ACT. Aubert (1987) reported that *T. erytreae* can exploit an extensive range of host plant species, but that reproduction is confined to rutaceous plants. Contrarily, non-rutaceous plants have been reported as host plants of ACT (Abate 1988; Khamis et al. 2017). According to Moran (1968), all citrus species are generally susceptible to attack by ACT. Thus, filling the knowledge gap of alternate hosts of ACT is crucial for pest management as they can serve as a source of re-infestation of citrus trees. This is in accordance with the report by van den Berg (1990)in South Africa, who observed that indigenous alternate host plants significantly influence ACT's population dynamics by serving as a source of re-infestation. Important host plants reported as host of ACT by van den Berg (1990) included: *Clausena anisata* (Willd.) Hook. f. ex Benth., *Vepris lanceolata* (Lam.) G.Don.*, Zanthoxylum capense* (Thunb.) Harv. and *Oricia*sp. (all Rutaceae). However, in Kenya, no information exists on the suitability and preference of different host plants to infestation by ACT. Moreover, over 50 host plants have been reported for the closely related Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), the principal vector of "*Candidatus* Liberibacter asiaticus", the causal agent of CGD or HLB disease, the most important citrus disease outside Africa (Halbert and Manjunath 2004).

This study sought to establish the effects of indigenous host plants on the development, survival, reproduction, and morphometric parameters of ACT as a prerequisite to the development of rational and effective integrated pest and vector management strategies of the pest in citrus.

3.3 Materials and methods

3.3.1 Host plants

Field surveys were conducted throughout the citrus growing regions of Kenya and nurseries across different agro-ecological zones to identify rutaceous plants with *Trioza*-like gall forming. Host plants recorded during the survey were *Ficus thonningii* (Blume), *F. sycomorus* L. (both

Moraceae), *Stephania abyssinica* (Quart, Dill & A.Rich) Walp. (Menispermaceae), *Murraya koenigii* (L.), *C.anisata, Teclea nobilis* (Del.)*, Calodendrum capense* (L.f.)Thunb., and *Vepris bilocularis* (Wright & Arn.) Engl. (all Rutaceae). The most commonly known and documented hosts of ACT observed during the survey included *M. koenigii*, commonly grown in residential areas as a spice plant and *C.anisata* which was widely considered by the communities to have medicinal properties (Williams et al. 2016). Potted plants with 25–33cm height were purchased from a certified commercial nursery in Embu, Kenya (Table 1).

The plants were later transplanted into 3l white plastic pots containing steam-sterilized, humus-rich soil and 1g of di-ammonium phosphate (DAP) per plant. The potted plants were maintained under ambient conditions in a greenhouse at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya [S 01° 13' 14.6''; E 036° 53' 44.5'', 1,612 meters above sea level (m. a. s. l.)]. The experimental plants were watered regularly as needed and fertilized twice a month with a foliar fertilizer (Gleeco balance^{TM} N: P: K 17:17:17). Plants with profuse flush

shoots were used for the experiments. Prior to the experiment, the plastic pots containing seedlings of the different host plants were covered with aluminum foil to prevent any natural infestation. One seedling of each host plant was selected and placed in a portable rearing cage measuring 40 x 40 x 60 cm (Model No.: BYC-003, Fujian, PR China) (Aidoo et al. 2018). No fertilizer was applied to the plants during the experimental period. The bottom of each cage was initially lined with a purpose towel for easy identification of any dead insect in the cage.

3.3.2 *Trioza erytreae* culture

Laboratory culture ACT originally collected from *T. nobilis* during a country-wide survey was used to initiate a colony at the *icipe'*s Animal Rearing and Containment Unit (ARCU). Infested leaves of the plants from the field samples containing immature life stages of ACT were carefully placed on new flush shoots of rough lemon *C. jambhiri* (Lush.) and reared for five generations as a stock colony prior to the beginning of the experiments. We used rough lemons in the rearing based on the availability of seedlings and their suitability as ACT host. Insects were kept in portable collapsible rearing cages made up of polyester mesh and steel wire (40 x 40 x 60 cm) with one side clear view (vinyl) and another side with a zipper opening (Model No.: BYC-003, Fujian, PR China) under controlled conditions of 12:12D photoperiod, mean daily temperature 22 ± 2°C and relative humidity (r.h.) of 70 ± 5%.

3.3.3 No-choice and choice-experiments

Before the commencement of the experiment, all selected host plant species were scouted for the presence of *T. erytreae* and ensured they showed no blotchy mottle on the leaves. Furthermore, genomic DNA was extracted from the petioles and midribs of each plant using methods described by Doyle (1990). Primers and probe described by Li et al. (2006) were used to ensure experimental plants were not infected with "*Candidatus* Liberibacter africanus". The selected host plants were trimmed to stimulate the development of new flush shoots.

To establish the suitability and performance of ACT in a no-choice bioassay, host plant species were placed individually in portable collapsible rearing cages as described above. Thereafter, 20 triozids (10 males and 10 females) of newly emerged adults (3-5 days-old) were introduced into each cage and allowed to settle. The leaves of each plant were examined at the 5th day for the presence of ACT eggs. The eggs deposited on each host plant by the ACT adults were counted and recorded with the aid of a Leica LAS EZAD stereomicroscope (Leica Ltd., Switzerland) at ×25). Plants with eggs on the leaves were carefully removed and transferred into new cages and labeled accordingly. Egg hatchability, nymphal development, survival rate and adult emergence were monitored and recorded for each host plant species. The experiments were conducted in the laboratory under controlled conditions at 12L: 12D photoperiod, mean daily temperature range of 22 - 24°C and relative humidity (rh) of 70 ± 5%. Each experiment was repeated four times.

For the choice test, the same experimental set-up as described above was used. Only five plant species that were observed to demonstrate suitability for the development of the ACT were used for the choice experiments. The same procedures were carefully followed as described above for the no-choice test except that the five host plant species were introduced together in the same cage. A total of 16 pairs of newly mated (3-5 days old) adults (16 males: 16 females) were exposed to the plants. Adult ACTs were allowed to settle and later examined for the similar parameters as described above. The positions of each host plant were rotated after every 24 hrs until and changed at 5th day throughout the experiment for each set up to avoid bias, given all psyllids in each cage equal access to the five plants species. The experiment was repeated five times while rotating the position of plants for each treatment.

3.3.4. Morphometric characteristics of ACT of five different host plants

Adult ACTs from the five tested host plants was collected and their body structures measured. The length of the hind tibia was quantified (Figure 3.1A), while the wing size was measured (Figure1B).

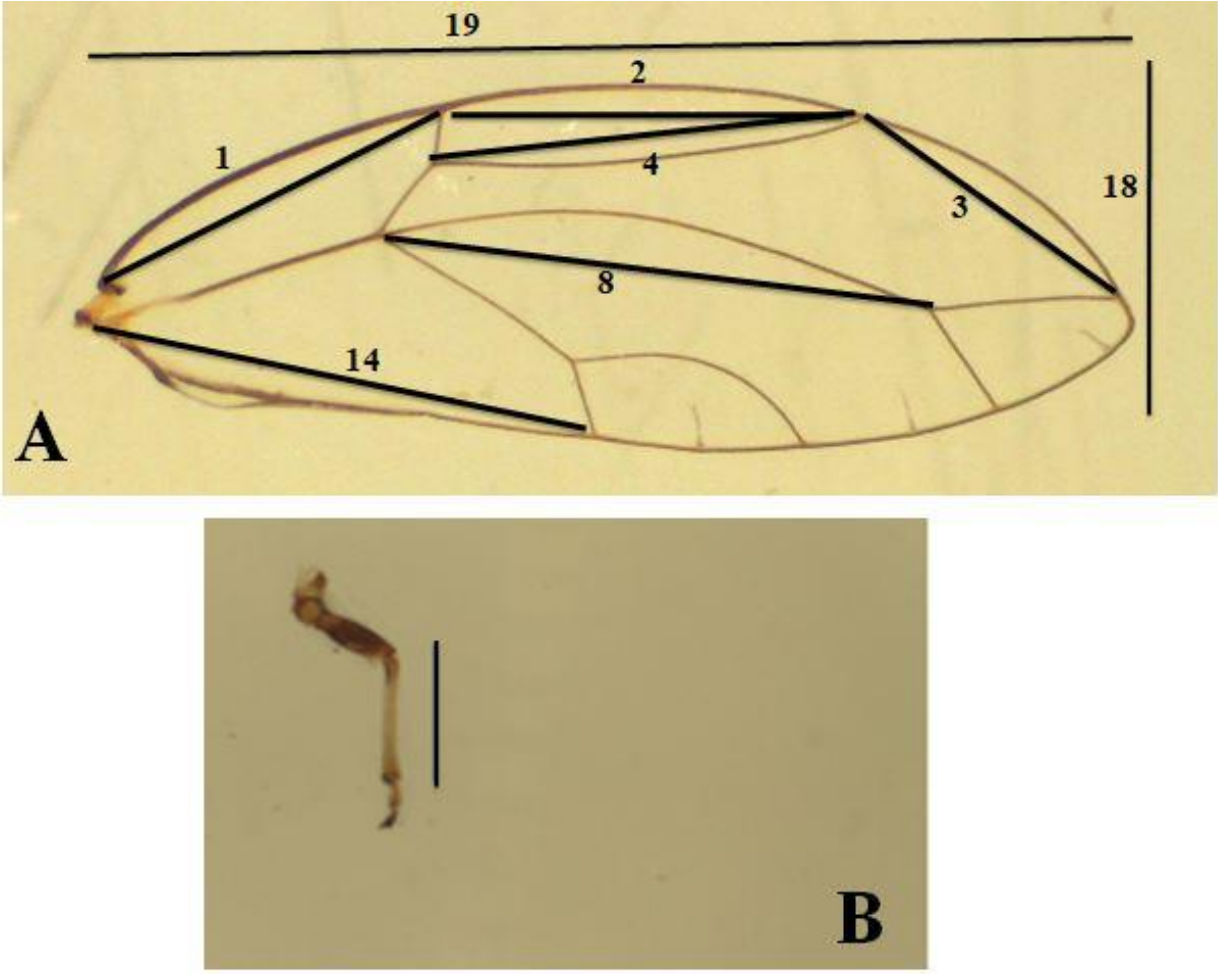

Figure 3.1 Wing measurements (A) and hind tibia length (B),

Wing loading was computed as wing length divided by wing width. Wing aspect ratio was computed as the ratio between the wing length and the wing width. To ensure consistency in the measurements, only the right wings were removed and measured. Only females were used in the morphometric analysis. Emerging ACT progeny were aspirated, preserved in 75% ethanol in a labeled microcentrifuge tube. Adult ACT was prepared for digital images at *icipe*'s Biosystematics Unit, where reference samples have been kept. The body structures were mounted on clean microscopic glass-slides [ground edges of 25.4 x 76.2 mm (1ˈ x 3ˈˈ), 1mm - 1.2mm thick (Kamfit Surgical, Cat.No.7105)] using euparol as the mounting medium. Each specimen was placed in clove oil to avoid brittleness and to enhance the observation of the body structures. The traits of interest were mounted on a clear glass-slide (dimensions 25.4 x 76.2mm and 1-1.2m thick). Images from the slide-mounted specimens were captured and measured using a Leica LAS EZAD stereomicroscope (Leica Ltd., Switzerland) at ×25. In total, 76 females were measured: *C. anisata* = 18, *M. koenigii* = 18, *T. nobilis* = 15, *C. capense* = 10 and *V. bilocularis* = 15. To ensure consistency in the measurements, only the right wings, and the right hind tibia were removed and used for the measurements.

3.3.5 Data analysis

The survival function of ACT lifetime data was estimated using Kaplan-Meier analysis whereas the curves were compared by the log-rank test. *Trioza erytreae* settling and oviposition preferences in the choice test were analyzed using logistic regression models of analysis of deviance followed by mean separation using the Tukey's. Data for the mean number of eggs, nymphs, adults, and egg and developmental time of nymphs were analyzed using analysis of variance (ANOVA). When treatment means were significant ($P < 0.05$), means were separated using Student Newman Keul's test. Prior to analysis, the data were log (x +1) transformed. Data for the morphometric traits were also subjected to analysis of variance (ANOVA) while mean separation was conducted using Student Newman Keuls (SNK) test ($P \leq 0.05$) in R Software (version 3). Hierarchical clustering/dendrograms using squared Euclidean distances of size data between groups were plotted using the unweighted paired group method with arithmetic mean (UPGMA). To study which trait contributed to most of the variation observed in the principal component analysis (PCA), we used PAST software (Hammer et al. 2001).

3.4 Results

3.4.1 Survivorship, development and reproduction in Choice and no-choice and tests

In the no-choice bioassay*,* Adult longevity up to 14th day was observed on only rutaceous host plants (Figure 3.2).

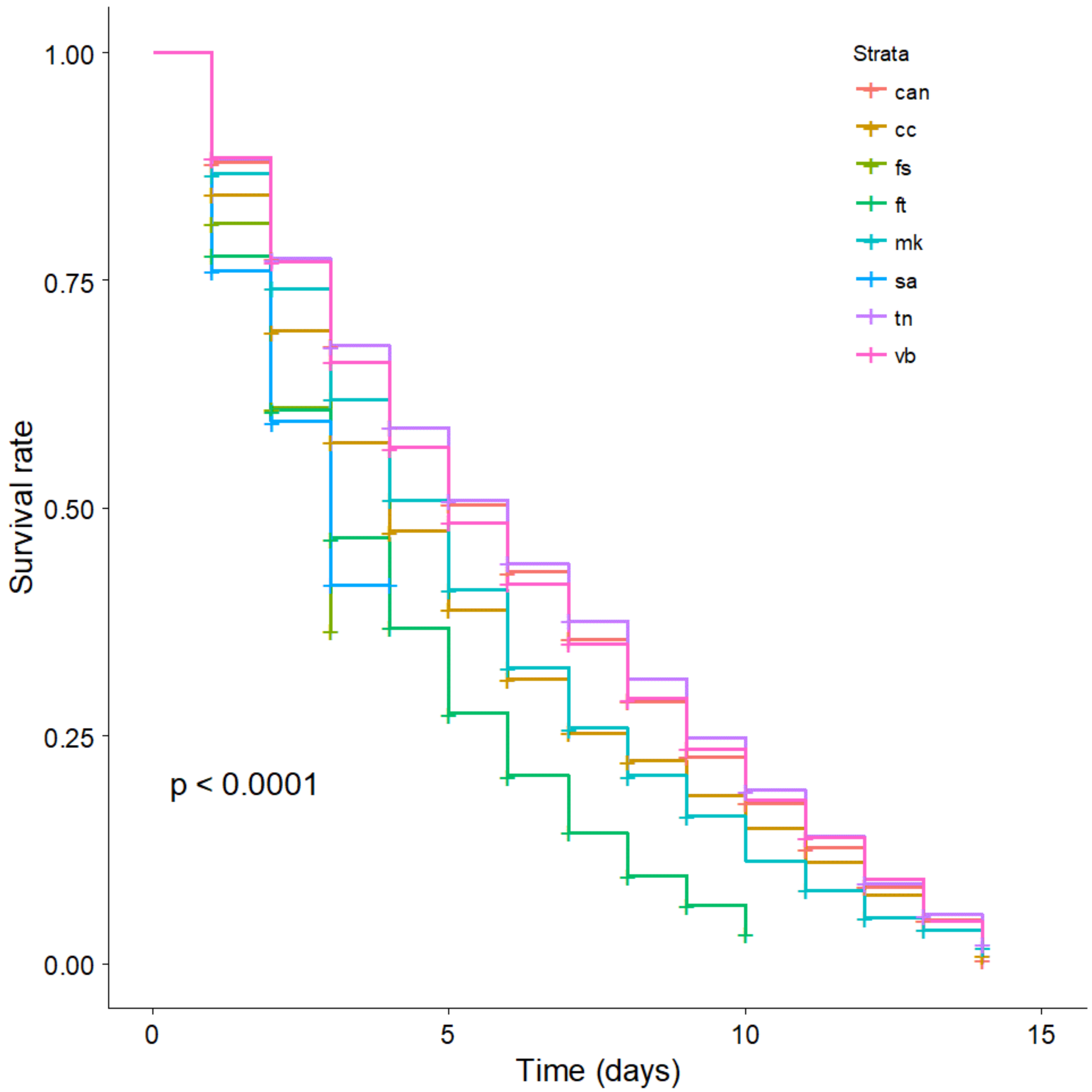

Fig. 3.2 Kaplan-Meier curve showing the survival of adult *Trioza erytreae* on different host plants for two weeks in rearing cages; *Clausena anisata* = can, *Calodendrum capense* = cc, *Ficus sycomorus* = fs, *Ficus thonningii* = ft, *Murraya keonigii* = mk, *Stephania abyssinica* = sa, *Teclea nobilis* = tn, *Vepris bilocularis* = vb.

ACT females significantly laid the highest number of eggs on *C. anisata* followed by *V. bilocularis*, *M. koenigii*, *T. nobilis* and *C. capense* (Table 3.1)

Table 3.1: Means (± SE) for biological parameters of *Trioza erytreae* reared on different host plants in a no-choice experiment

Host plant	No. of eggs laid per flush	Egg development period (days)	Nymph development time (days)	Total no. of adults emerged	Growth Index (GI)
Clausena anisata	273.3 ± 30.9a	7.3 ± 0.3a	16.0 ± 0.0a	48.3 ± 0.6b	2.1ab
Vepris bilocularis	208.0 ± 49.5b	9.3 ± 0.3b	19.0 ± 0.0c	31.7 ± 1.1c	1.1b
Murraya koenigii	206.7 ± 82.2b	7.0 ± 0.0a	16.7 ± 0.3b	97.0 ± 2.4a	4.0a
Calodendrum capense	76.7 ± 55.9d	9.5 ± 0.4b	20.0 ± 0.5d	2.5 ± 0.4d	0.08b
T. nobilis	193.3 ± 47.2c	7.0 ± 0.0a	16.3 ± 0.3ab	44.7 ± 1.7b	1.92b

Means followed by the same letter within a column are not significantly different (SNK test, P ≤ 0.05).

(F = 3.787, d.f. = 4, P= 0.0254). There were significant differences (F = 42.95, d.f. = 4, P= 0.0001) in the egg development period for the tested host plants with fastest egg development on *T. nobilis* and *C. anisata* whereas those oviposited on *C. capense* and *V. bilocularis* needed the longest time to hatch. Significantly, a higher number of nymphs emerged on *M. koenigii* than any of the other host plants (F = 3.705, d.f. = 4, P= 0.0001). Similarly, nymphal development time was significantly slowest in *C. capense* compared to the other host plants (F = 32.2, d.f. = 4, P= 0.0273). The numbers of adults recorded per flush shoot were significantly affected by the host plants (F = 15.43, d.f. = 4, P = 0.0001). Growth index which is the ratio of the total number of emerged adults and the mean development time from egg to adult (Sétamou et al. 1999) was significantly dependent on the host plants (F = 6.719, d.f. = 4, P = 0.0036).

In the choice bioassay, the significantly highest and lowest mean number of adults settling per flush shoots were recorded on *M. koenigii* and *V. bilocularis*, respectively, with a similar pattern found in terms of percentage of adults settling per host plant (Table 3.2). The mean number of eggs laid per flush shoot was not significantly affected by the host plant species. Host plants clearly affected the survival of ACT in the no-choice.

Table 3.2. *Trioza erytreae* settling and oviposition preferences in a choice test (n=25 for each host plant)

Plant species	No. of adults settling per flush shoot	% Adult settling per host plant	% Flush shoots *with* *T. erytreae* eggs
C. capense	3.6 ± 1.3ab	11.3 ± 4.1ab	24.0 ± 4.0
C. anisata	7.2 ± 2.1ab	22.5 ± 6.4ab	52.0 ± 4.9
T. nobilis	6.4 ± 1.5ab	20.0 ± 4.7ab	24.0 ± 7.5
M. koenigii	10.0 ± 2.0a	31.25± 6.3a	44. 0 ± 11.7
V. bilocularis	2.8 ± 1.3b	9.4 ± 4.1b	28.0 ± 10.2

Mean values expressed as means ± SEM of all 25 flush shoots per host plant. Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ using the *post – hoc* test with adjusted tukey

3.4.2. Effect of host plants on morphometry of ACT

Host plants significantly affected the studied morphometric traits of ACTs. Adults emerging from *C. capense* were smaller than those from the other tested host plant species. All traits explanations of the morphometric parameters see captions of table 3.3. Adults that emerged from *M. koenigii* were larger than those from *C. capense* but similar to the ones from the other host plants, particularly from *C. anisata*. Adult ACT emerging from *C. anisata* were slightly larger than those from *T. nobilis* except for R_2, M, wing width, wing length, hind tibia length, and wing aspect ratio. No significant difference was found among the different host plant species for the wing aspect ratio (wing length/wing width) though wing loading differed significantly among the tested host plant species (tibia length/wing length) with the lowest wing loading found for *C. capense*.

Table 3.3. Size of morphological traits (means ± SEM) measured from female *Trioza erytreae* raised on different host plants

Fig.1B Code	Trait	*Vepris bilocularis*	*Clausena anisata*	*Murraya koenigii*	*Teclea nobilis*	*Calodendrum capense*	*F*	*df*	*P-value*
1F.1	C+Sc	0.981 ± 0.03b	1.047 ± 0.02ab	1.090 ± 0.01a	1.017 ± 0.02b	0.978 ± 0.09b	5.089	4	0.0011
1F.2	R_1	1.007 ± 0.02b	1.036 ± 0.08ab	1.097 ± 0.01a	1.032 ± 0.02ab	0.985 ± 0.10b	3.017	4	0.0176
1F.3	R_2	0.837 ± 0.02bc	0.852 ± 0.02ab	0.193 ± 0.02a	0.892 ± 0.02ab	0.799 ± 0.10c	5.099	4	0.0011
1F.4	R_s	1.040 ± 0.02bc	1.083 ± 0.02ab	1.136 ± 0.01a	1.079 ± 0.02ab	0.981 ± 0.13c	4.819	4	0.0017
1.F 8	M	1.347 ± 0.02b	1.395 ± 0.02ab	1.492 ± 0.02a	1.413 ± 0.03ab	1.306 ± 0.13b	4.894	4	0.0015
1.F14	A to A/Cu_{1b}	1.245 ± 0.02b	1.305 ± 0.02ab	1.378 ± 0.02a	1.289 ± 0.02b	1.225 ± 0.04b	5.270	4	0.0001
1.F18	Wing Width	0.887 ± 0.02ab	0.922 ± 0.02a	0.961 ± 0.01a	0.939 ± 0.02a	0.881 ± 0.00b	3.474	4	0.0120
1.F19	Wing Length	2.603 ± 0.03bc	2.676 ± 0.02bc	2.847 ± 0.02a	2.717 ± 0.03ab	2.532 ± 0.08c	5.621	4	0.0005
F1.A	Hind tibia length	0.581 ± 0.02b	0.609 ± 0.01ab	0.639 ± 0.01a	0.617 ± 0.02ab	0.514 ± 0.08c	10.72	4	0.0001
	Wing loading	0.224 ± 0.003a	0.228 ± 0.003a	0.225 ± 0.005a	0.226 ± 2.91a	0.205 ± 0.07b	4.032	4	0.001
	Wing aspect ratio	2.94 ± 0.03	2.904 ± 0.02	2.962 ± 0.01	2.91 ± 0.03	2.853 ± 0.90	1.439	4	0.23

The scatter plot resulting from the principal component analysis (PCA) did not show a complete separation (Figure 3.3). However, Euclidean distances elucidated that ACT emerging from *M. koenigii* could be separated from the offspring of the other host plants in a single branch, with the latter clustering in a single multilevel branch (Figure 3.3 and 3.4).

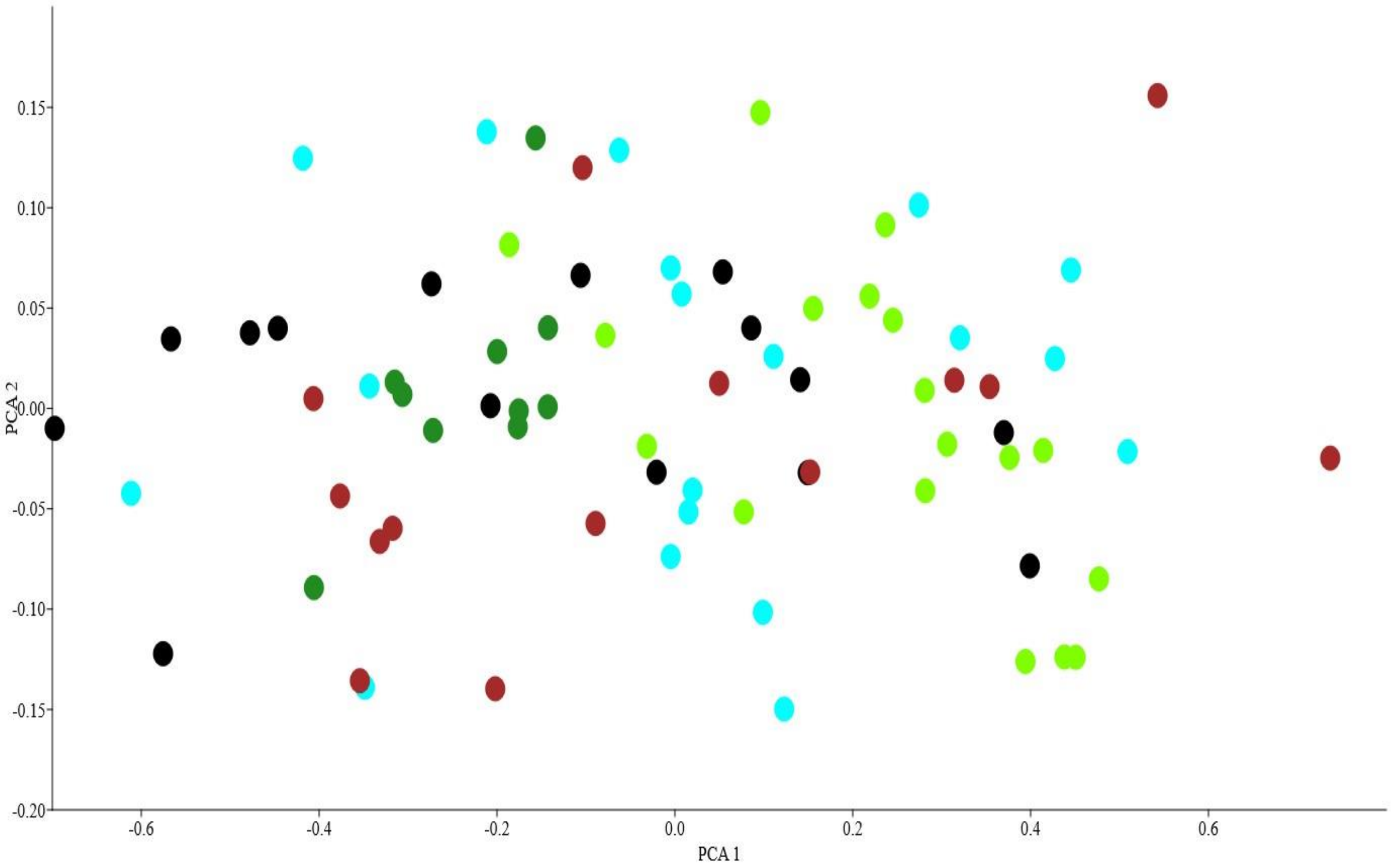

Fig. 3.3 Scatter plot showing the first two principal components emerged from the different host plant species of ACT *Vepris bilocularis* = Aqua, *Teclea nobilis* =Black, *Calodendrum capense* = Green, *Murrya koenigii* = Chartreuse, *Clausena anisata* = Red)

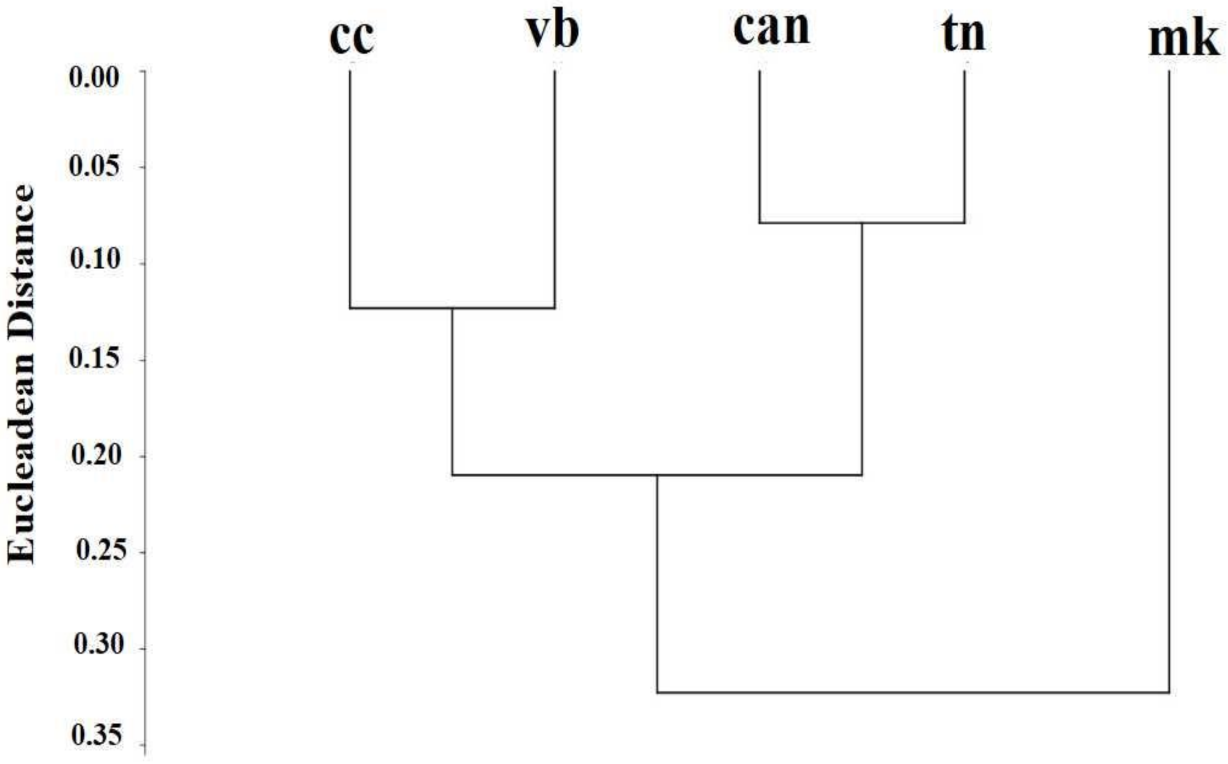

Fig. 3.4. Dendogram formed by means of UPGMA procedure using squared Euclidean distances of ACT emerged from different plant species (cc = *Calodendrum capense*, vb = *Vepris bilocularis*, can = *Clausena anisata*, tn = *Teclea nibilis* and mk = *Murraya koenigii*).

The multivariate analysis of the first principal components yielded an accumulated explained variance of 86.5% with an eigenvalue of 0.102 using the first principal components, whereas the second principal component explained 4.6% of the variation with an eigenvalue of 0.005. Wing length was the trait that contributed to most of the variation observed in PCA1 followed by M and A to A/Cu_{1b}, whereas Rs and R_2 were the main factors in PCA2. The negative loading illustrates the negative correlation between PCA2 and R_2 vein (Figure 3.5). Overall, PCA1 and PCA2 contributed to an accumulated explained variation of 93.9% of the morphometric variation found in ACTs reared on the five tested host plants.

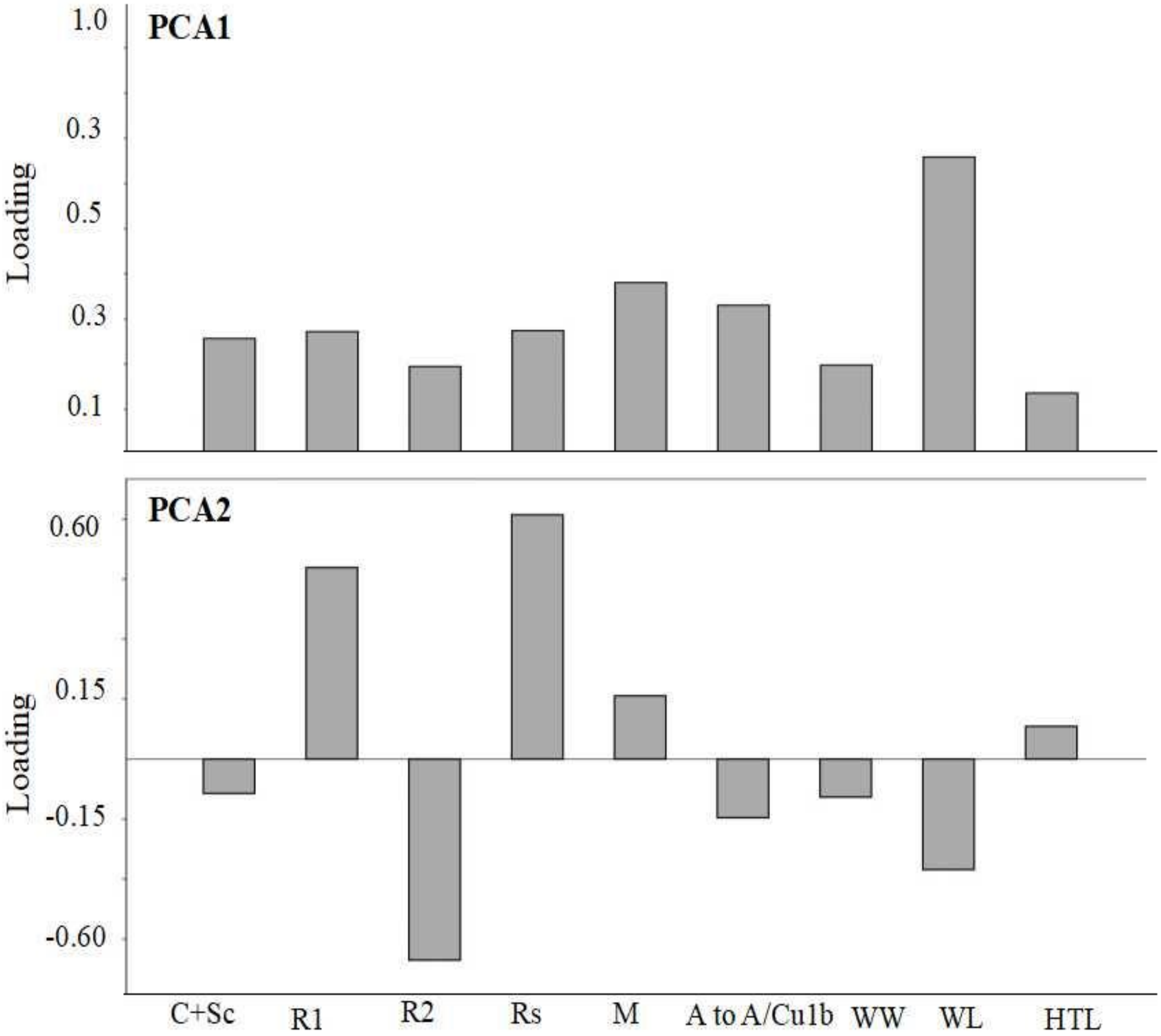

Fig. 5. The coefficient of the first two principal components (PCA1 and PCA2) of the PCA of *Trioza erytreae* reared on five different host plants showing contributions of traits to the variation observed in PCA1 and PCA2.

3.5 Discussion

Variations among individuals within populations of herbivores insects in terms of host acceptance have been attributed to behavioral plasticity, probably as a result of variability in learning, physiological conditions and search time for an oviposition site (Jaenike 1990). Moreover, co-evolution of herbivores insects and higher plants is associated with the chemical composition of the latter (Ehrlich and Raven 1964). Insect-plant interactions can lead to the development of insect host resistance, often via detoxification of secondary plant metabolites, tolerance, and/ or avoidance (Jaenike 1990). In our study, all tested putative host plants of ACT were native to Africa except for *M. koenigii* which originates from India. Because of the frequent use of *M. koenigii* leaves for medicinal purposes in different countries(Gahlawat et al. 2014), the seeds were presumably introduced into Kenya from India. We found all tested rutaceous plants suitable for ACT reproduction, with *M. koenigii* appearing to be the most preferred host plant.

During our field survey, we found ACT repeatedly on *S. abyssinica*, corroborating recent observationsKhamis et al. (2017). However, in several laboratory trials, we could only demonstrate adult survival for up to four days with no signs of oviposition. As *S. abyssinica* is a menispermaceous plant, this adds further support to an earlier report that ACT reproduction is confined to rutaceous plants(Aubert 1987). In addition, a survey conducted in Ethiopia found ACT on *S. abyssinica*, *Prunus africana* (Hook.f.) Kalkman (Rosaceae) and *Ficus sycomorus*(Abate 1988), probably a reflection of the nutritional composition of *S. abyssinica* and the other plants (Sétamou and Bartels 2015). Thus, the observed high mortalities and unsuccessful reproduction of ACT on *S. abyssinica* in the laboratory merit further investigation. As in *S. abyssinica* we also could not detect any ACT oviposition on *F. thonningii* though adults survived for up to 11d on these plants. Possibly the tough leaves of *F. thonningii* did not instill egg laying by *T. erytreae* females. Development of ACT on *C. capense* was very low as only a few adults emerged in the no-choice test, and adults survived for a maximum of on 14d which was the lowest among the five rutaceous plants that proved to be suitable for ACT development. Females laid a considerable number of eggs on *C. capense* but most of the emerging nymphs subsequently died, probably because of the hardness of the leaves and possibly also because of the low nutritional quality of the shoots. We reported here for the first time *T. nobilis* and *V. bilocularis* as indigenous host plants of ACT in Kenya. The potential of these hosts serving as a reservoir for CLaf and their impact on the epidemiology of ACGD and the population dynamics of the vector warrants further research.

The growth index, that is, the effect of nutritional quality of host plants on the survival rate and development times of insects (Sétamou and Bartels 2015; Sétamou et al. 1999), was highest in *M. koenigii* followed by *C. anisata* and *T. nobilis*. Similarly, number of nymphs emerged and the number of emerging adults were highest in *M. koenigii* followed by *C. anisata* and then *T. nobilis*. The same pattern was observed in the choice test in terms of the number of adults settling on flush shoots. Besides, *M. koenigii*, *C. anisata,* and *T. nobilis*we found the highest percentage of adults settling per host plant, and in *C. anisata*and*M. koenigii* the highest percentage of flush shoots carrying eggs. Yet, ACT performed very poorly on *C. capense* in terms of growth index, survival, oviposition, and development times of the immatures. Feeding and oviposition of *D. citri*, a close relative of ACT, varied significantly between *Helietta apiculata* Benth. and *M. exotica* L. (both Rutaceae) (Pimpinato et al. 2017). In general, food consumption influences the growth, development, and reproductive potential of insects (Slansky Jr and Scriber 1985).

Our results clearly indicated that ACT morphometric traits vary among different host plants. Plant quality affects the morphological and physiological variability in *T. erytreae*(Moran 1968), and the nutritional quality of the different host plants could be responsible for the observed variation in the size of the emerged adults. Lower wing loading in female Monarch butterflies *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) was associated with efficient flight and migratory success (Davis and Holden 2015). Tibia length was significantly longest in adult ACTs reared on *M. koenigii*, underlining the good host plant quality of this species as also noted in most of the studied biological parameters. Tibia length often positively correlates with overall body size (Kjærsgaard et al. 2015) and fertility (Reeve et al. 2000). Our results clearly showed that adults used for the experiments, originally collected from *T. nobilis* were not favored as suggested by Hopkins host selection principle(Hopkins 1917). For instance, more adults with bigger sizes emerged from *M. koenigii* than any of the other tested host plants.

3.6 Conclusion

From the findings of the present study, it could be concluded that the survival, reproduction, and morphometry of *T. erytreae* in Kenya were supported by five main rutaceous host plants namely, *C. anisate, V. bilocularis, M. koenigii, T. nobilis and C. capense. Murraya koenigii* proved to be the most suitable host plant species in terms of ACT reproduction but also in several morphometric traits. Our results suggest that the five studied rutaceous host plants can possibly influence the population dynamics of ACT as well as the epidemiology of ACGD by acting as reservoirs of the CLaf pathogen.

4. SEASONAL OCCURRENCE OF *TRIOZA ERYTREAE* DEL GUERCIO (HEMIPTERA: TRIOZIDAE) IN THREE DIFFERENT ALTITUDINAL GRADIENTS OF KENYA

4.1 Abstract

The African citrus triozid (ACT) *Trioza erytreae* Del Guercio (Hem.: Triozidae) is one of the most destructive citrus pests in Africa. Apart from inflicting physical damage to citrus, it vectors the unculturable "*Candidatus*" Liberibacter africanus, the causal agent of the African citrus greening disease (ACGD). Notwithstanding, biological data on ACT are insufficient for important parameters like distribution and seasonal dynamics across different agro-ecological zones. Therefore, this study sought to determine the temporal dynamics of ACT from December 2016 to November 2017 by assessing the occurrence of adults and immatures (i.e., eggs and nymphs) and the degree of damage along three different altitudinal gradients in Kenya. Ten trees were selected per orchard from which four flush shoots were randomly sampled. Adults and immatures, as well as galls and parasitized nymphs per shoot were counted. Adults and immatures peaked differently across the different altitudinal gradients. Densities of ACT (all stages) and damage were consistently higher in the mid than in the other altitudes. In low altitudes, females started laying eggs in late February. For mid-altitudes ACT populations peaked in February, but adults were already present in December. In high altitudes, adults appeared in June and females started laying eggs. The percentage of infested shoots was highest in lemon trees (58%). The closely related Asian citrus psyllid *Diaphorina citri* Kuwayama (Hem.: Liviidae) was found on *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae), an observation that requires

further investigations. *Tamarixia dryi* Waterston, *Tetrastichus* sp. (both Hym.: Eulophidae), *Psyllaephagus pulvinatus* Waterston, *Aphidencyrtus cassatus* Annecke (both Hym.: Encyrtidae), and coccinellids were the most common parasitoids and predators, respectively, found in the orchards. This information could help inform an early and effective monitoring of ACT to limit the population build-up and reduce its spread in the future.

4.2 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 triozid (ACT) *Trioza erytreae* Del Guercio (Hem.: 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). Apart from inflicting physical damage through direct feeding on citrus shoots, it vectors the phloem-limited bacteria "*Candidatus*" Liberibacter africanus, the causal agent of the African citrus greening disease (ACGD) (Aubert 1987). Yield losses up to 100 % have been associated with ACT and 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 1,300 meters above sea level (m.a.s.l.) (Cocuzza 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 is around 10-12^{o}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. erytreae* 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).

ACT peak population density appears to coincide with the periods of new flush growths in citrus (Cook et al. 2014; 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 (Col.: Coccinellidae), Syrphids (Dip.: Surphidae), Lacewings (Neur.: Chrysomelidae), predatory spiders and mites (Araneae) (Aubert 1987; Catling 1970) as well as by different hymenopteran parasitoids (McDaniel and Moran 1972). For instance, on the island of Réunion, *Tamarixia dryi* Waterston (Hym.: Eulophidae) and *Psyllaephagus pulvinatus* Waterston (Hym. : Encrytidae) have been credited with up to 75% parasitism in ACT; yet in South Africa, their impacts are limited by hyperparasitoids (McDaniel 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.

4.3 MATERIALS AND METHODS

4.3.1 Study sites

A survey was conducted at three sites in the Kericho region of western Kenya. We identified and sampled 15 citrus orchards (Figure 3.1) with elevations ranging from low to high altitudes (1,100-2,316 meters 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 1,400 m.a.s.l., and 35° 03.950 E and 00° 19.294 S and 1,773 m.a.s.l., respectively, both in the Soin/Sigowet sub-county (1,000-1,400 m.a.s.l.). The high-altitude site (site 3 - high) was located at 35° 34.798 E and 00° 11.723 S and >2,000 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.

4.3.2 Data collection

Seasonal abundance across different 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 tree, and five trees in the open trees fully exposed to the sun). The canopy of each tree was divided into four flush shoots from which four flush shoots were randomly selected for eggs, nymphs, adults, and galls. During the survey, predators and parasitoids found were collected and identified using an insect dichotomous key. Temperature, rainfall, humidity data were obtained from the nearest meteorological station in Muhoroni town. Percentage parasitism (P %) was determined using the following formula $o/ (o + w) \times 100$, where o = number of emerged parasitoids and w = number of adult ACT emerged; a similar formula is also used for determining parasitism in fruit flies (Steck 1986; Badii et al. 2016). In each of the study sites, no synthetic pesticides were applied during the survey period. Geographical positioning system (GPS) data of each site was recorded using Garmin ETrex 20x).

4.3.3 Statistical analysis

Statistical analyses were performed on the count of the different stages of ACT using analysis of variance (ANOVA) and generalized linear models (GLM) with an Adjusted Tukey. Shaded and unshaded trees were considered as treatments to evaluate seasonal fluctuations. Pearson's product correlation was applied to assess the relationship between ACT population densities and climatic conditions. ACT counts per month in shaded trees and was correlated with unshaded ones to determine if abundance in shaded trees leads to a rise in ACT in unshaded trees. Analyses were carried out in R version 3.5.0.

4.4 Results

Sites, where ACT was recorded as well additional information about the orchards where the specimens were collected, are listed in Table 4.1 and illustrated in Figure 4.1. ACT was found in all the orchards whereas the distribution of the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Hem.: Liviidae), a vector of the Asian strain of the citrus greening disease, was limited to elevations up to 1,500 m.a.s.l.

Table 4.1: Sites used in the study including the global positioning system coordinates and elevation

Altitudinal gradient*	**County**	**Location**	**Latitude**	**Longitude**	**Elevation (m)**	***Trioza erytreae***	***Diaphorina citri***
Low (1000-1450)	Kisumu	Kakola	E034 55.207	S00 10.853	1152	yes	**yes**^{zbc}
	Kisumu	Border2	E035 03.506	S00 09.215	1221	yes	yes
	Kisumu	Border2	E035 05.187	S00 10.821	1246	yes	yes
	Kisumu	Border2	E035 04.206	S00 09.511	1246	yes	yes
	Kericho	Soin	E035 04.163	S00 09.389	1260	yes	yes
	Kisumu	Border2	E035 05.271	S00 10.497	1271	yes	yes
	Kisumu	Border2	E035 04.700	S00 09.804	1279	yes	yes
	Kericho	Koitaburot	E035 12.769	S00 11.838	1375	yes	yes
	Kericho	Koitaburot	E035 12.155	S00 11.697	1397	yes	yes
	Kericho	Koitaburot	E035 11.075	S00 13.247	1398	yes	yes
	Kericho	Soin	E035 08.447	S00 14.072	1403	yes	yes
	Kericho	Soin	E035 03.280	S00 19.294	1409	yes	yes
Mid (1450-2000)	Kericho	Cheptuiyet	035 03.950	00 28.522	1773	yes	no
High (>2000)	Kericho	Londiani	035 34.798	00 11.723	2310	yes	no
	Kericho	Londiani	035 36.699	00 09.982	2316	yes	no

Notes**: * in** meters above sea level **yes**^{zbc} **=** apart from citrus, *Diaphorina citri* was collected on *Zanthozylum capense*

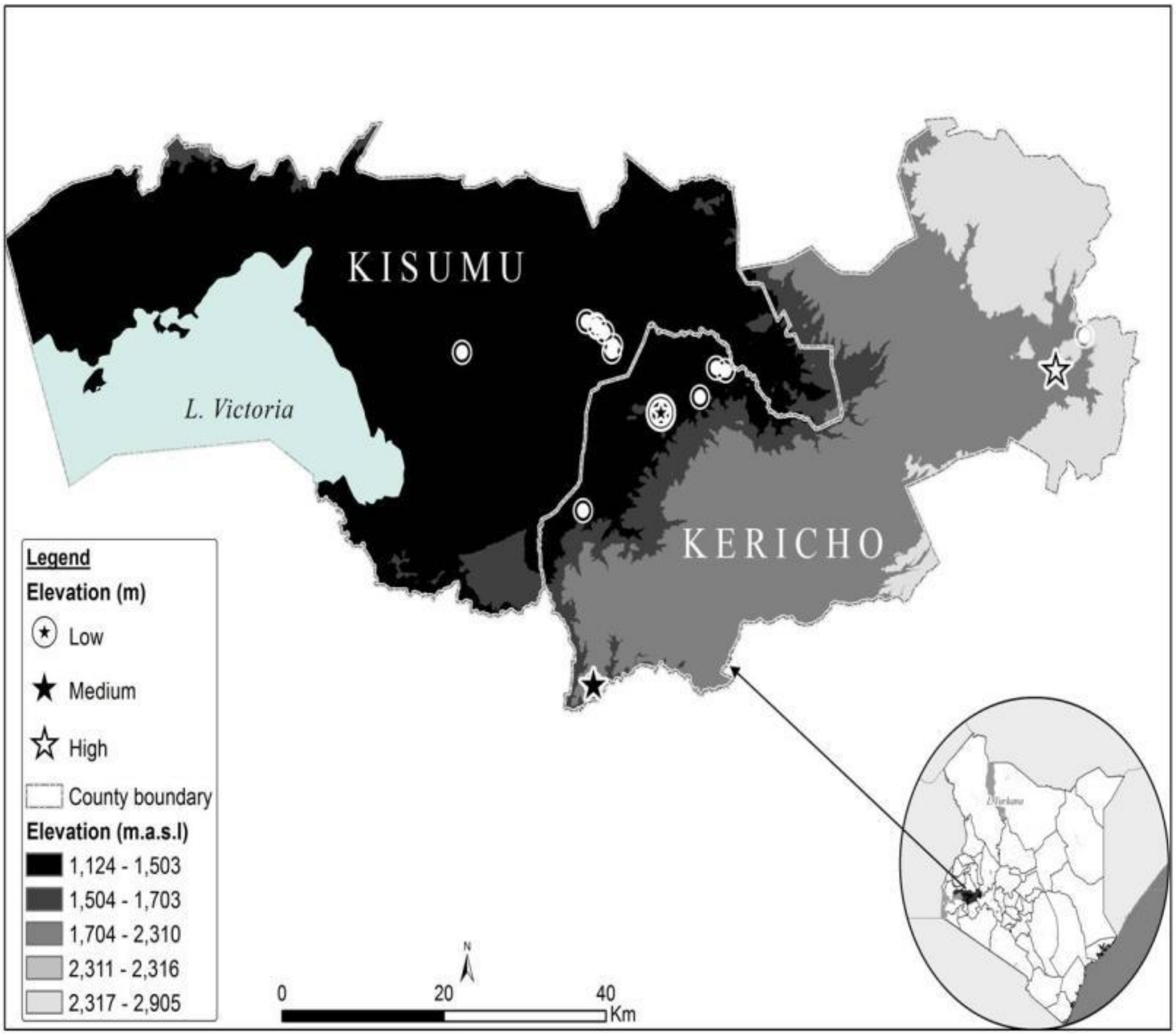

Figure 4.1: Map showing sites where *Trioza erytreae* was sampled.

There were no significant differences between climatic conditions, i.e., relative humidity, average, minimum and maximum temperature, and rainfall, and the density of ACT adults (Figure 4.2A). Similarly, the number of eggs and nymphs did not significantly differ with the said climatic parameters. However, percentage parasitism varied significantly with relative humidity (relative humidity: $r = -0.675$, df = 10, $P = 0.015$).

The densities of ACT (all stages) varied significantly across the different months ($\chi^2 = 940.82$, df = 2, $P = 0.0001$) (Figure 4.3A-C). 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 numbers of eggs were significantly higher in the mid- followed by the low- and then high-altitude sites ($\chi^2 = 24.973$, df = 2, $P = 0.0001$). 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, $P = 0.0001$). 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. There were no significant differences between percentage parasitism in ACT, with the highest means of 55 and 58% parasitism were found in the low- and mid-altitude site, respectively (Figure 4.3D).

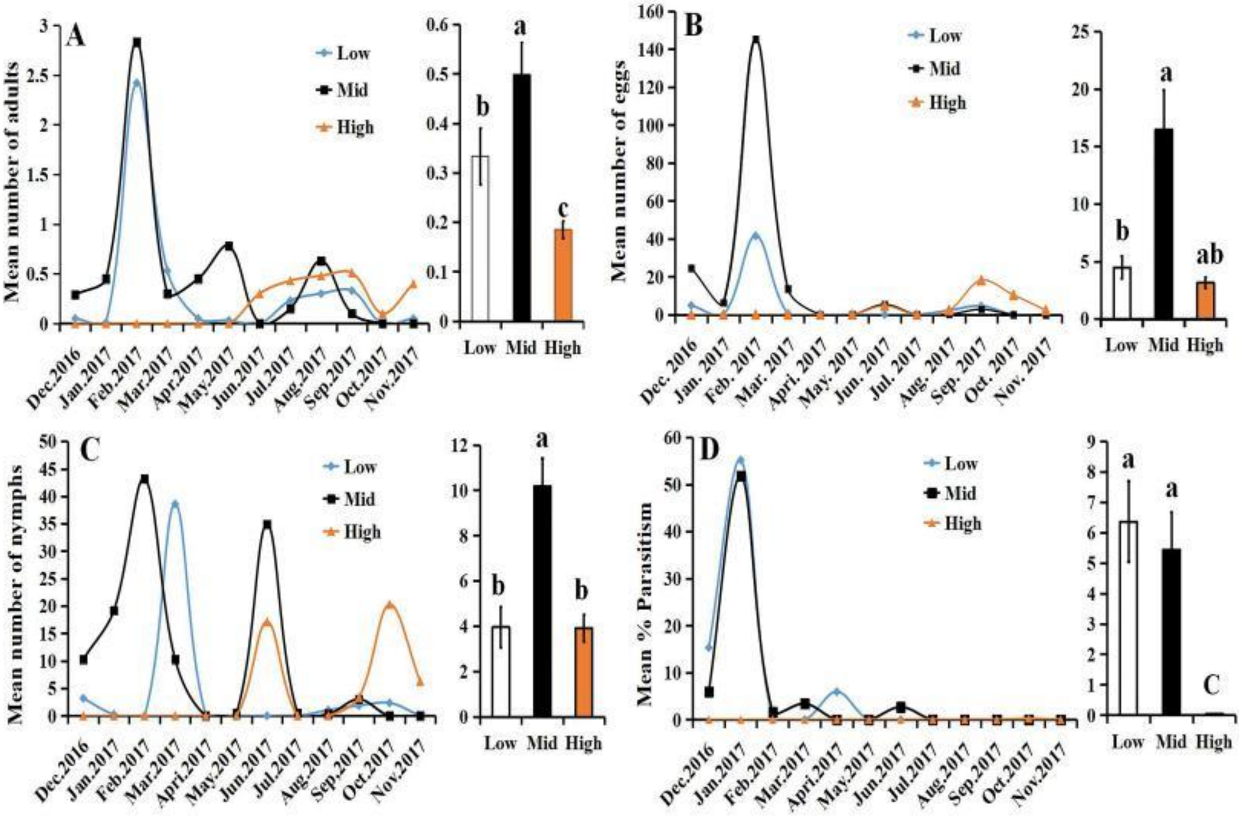

Figure 4.3 Means of *Trioza erytreae* individual per shoot in low, mid and high altitudes for adults (A), EGGS (B), nymphs (C) and percentage parasitism (D).

Colonization of ACT in shaded and unshaded trees followed a distinctive seasonal pattern across the different altitudinal gradients (Figure 4.4A-I). For instance, in the low-altitude sites, the density of ACT reached a maximum monthly mean of 3.2 and 0.1 adults/shoot in the shaded and unshaded trees, respectively. In late February, the mean numbers of eggs were 85 and 0.7 eggs/shoot in the shaded and unshaded, respectively. In March, the means were 70 and 9 nymphs/shoot for shaded and unshaded trees, respectively (Figure 4.4A-I). ACT population densities in shaded trees positively correlated and significantly differed with unshaded trees for eggs, nymphs and adults (Egg; r = 0.810, df = 34, P = 0.00001; nymphs r = 0.642, df = 34, P = 0.00001; Adults r = 0.827, df = 34, P = 0.00001) (Figure 4.5).

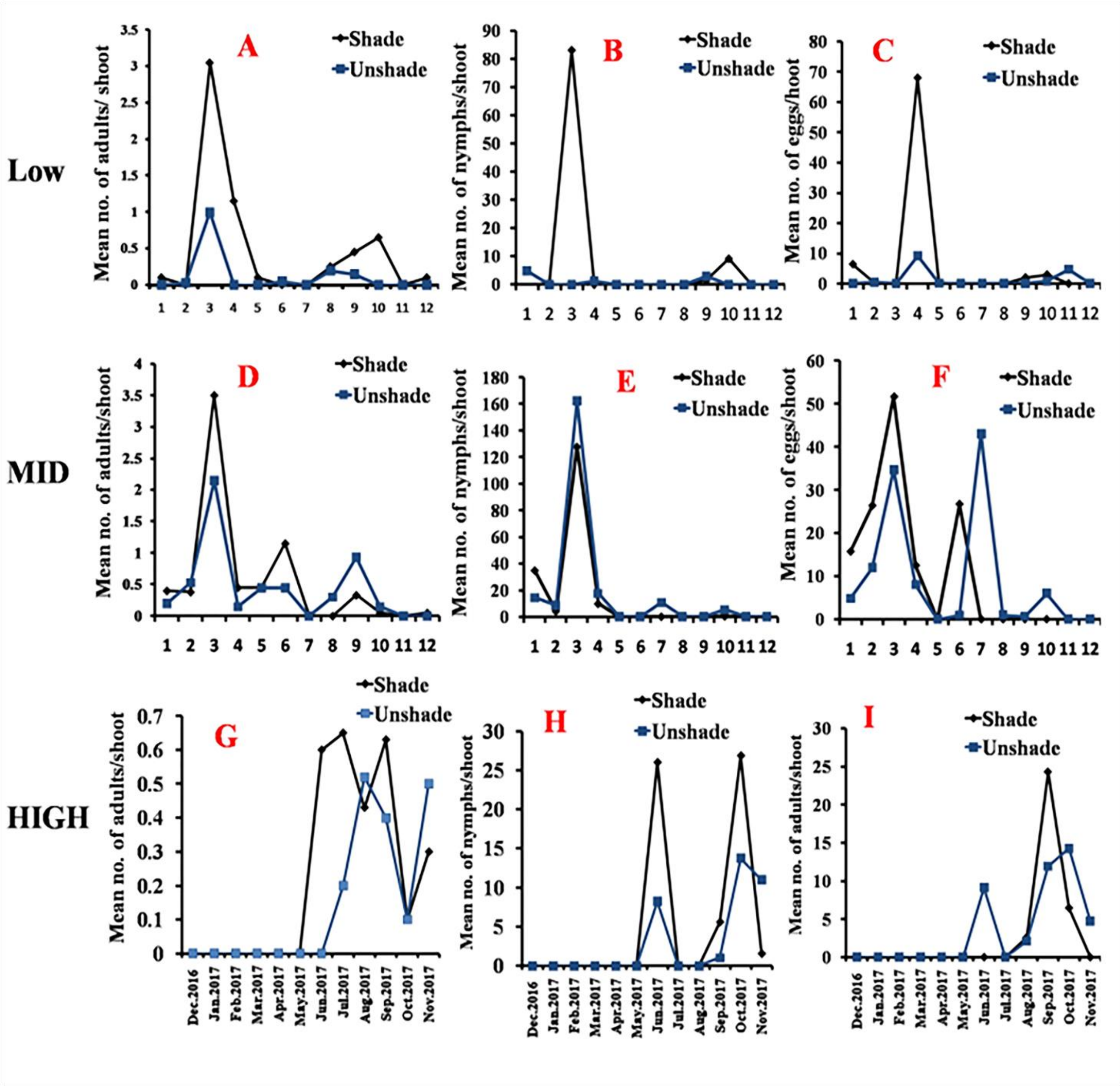

Figure 4.4 *Trioza erytreae* infested leaves per shoot in shaded and unshaded trees.

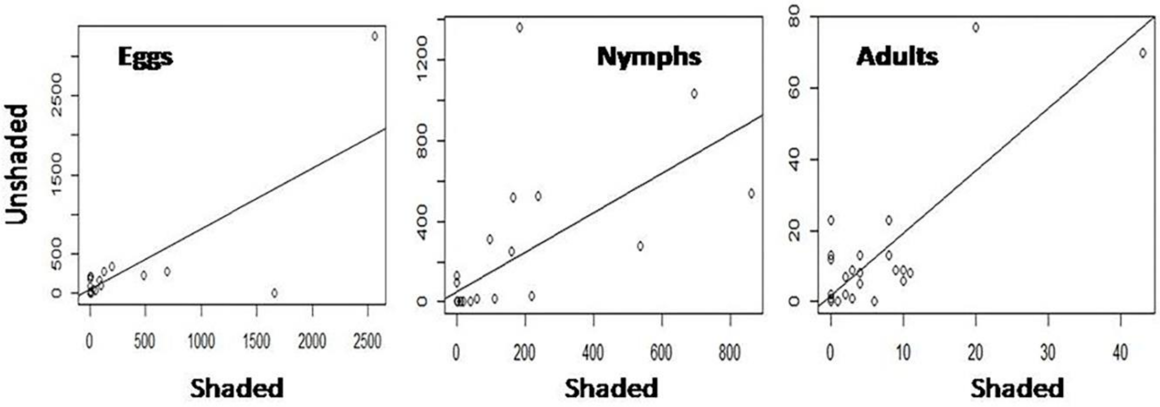

Figure 4.5 Correlation between *Trioza erytreae* (all stages) in shaded and unshaded trees

The percentage of citrus species infested was greater in lemon trees, followed by tangerine and then “Valencia” orange trees (Figure 4.6). Although the density of eggs sampled from the different citrus species/ varieties did not differ significantly (*P* = 0.3822), more eggs were collected from lemon trees than other host plants. Significantly higher mean numbers of ACT nymphs were found in lemon trees than on the other host plants (χ^2 = 10.309, df = 3, *P* = 0.016). Moreover, significantly more galls/shoot were found on lemon trees compared to the other citrus species/ varieties (χ^2 = 11.395, df = 3, *P* = 0.009). There was no significant difference between host plants and percentage parasitized nymphs per flush shoot. The percentage of shoots infested by ACT was higher in lemon than any other citrus species/ varieties (Figure 4.6). Four parasitoid species and six coccinellid predators were found to be associated with ACT and are presented in Figure 4.7. In addition, mycosed adult ACT, presumably infected by *Metarhizium* and *Beauveria* strains, were occasionally observed.

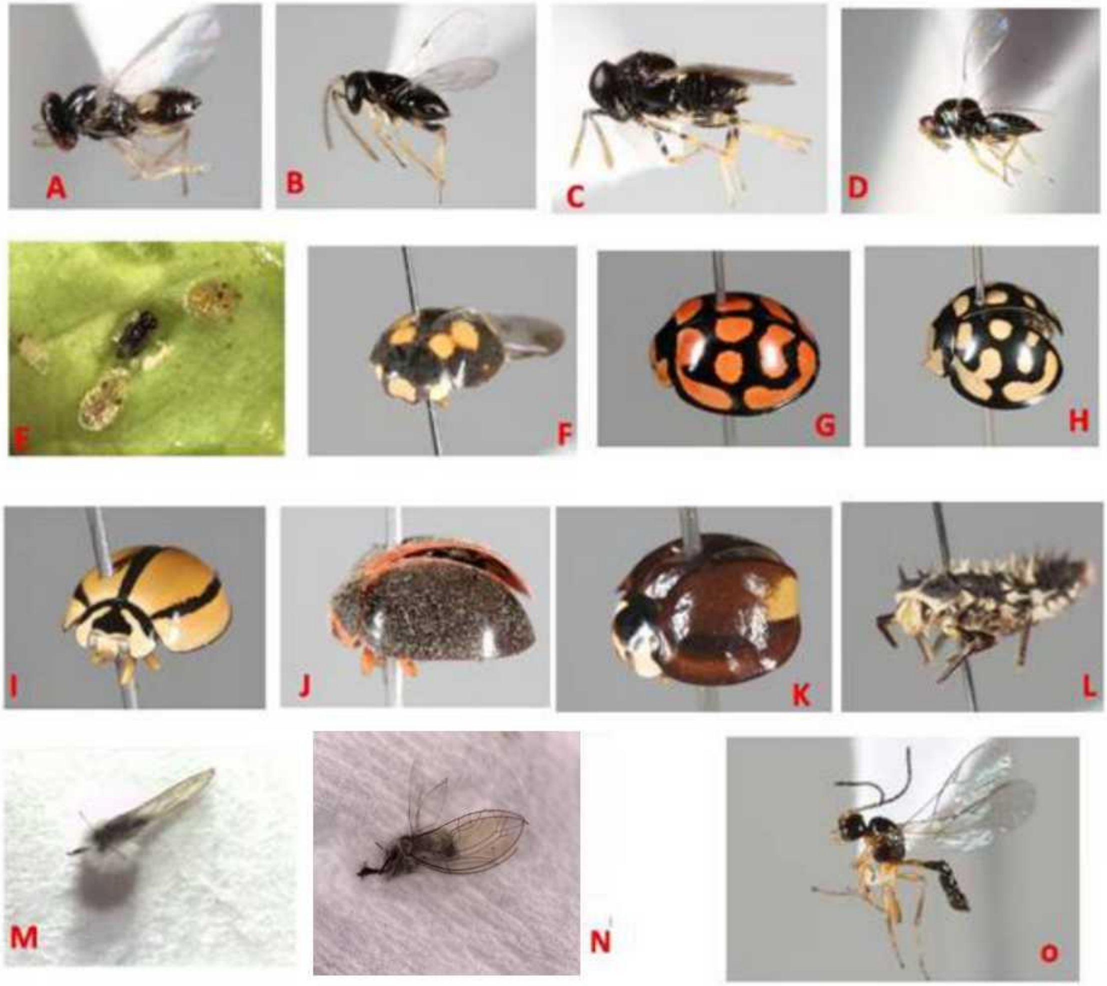

Figure 4.7 Natural enemies collected during the survey. *Tamarixia dryi* Waterston (Hym.: Eulophidae) (A), *Psyllaephagus pulvinatus* Waterston (Hym.: Encrytidae) (B),*Aphidencyrtus cassatus* Annecke (Hym.: Encyrtidae)(C), *Tetrastichus* sp. (Hym.: Eulophidae) (D), mummified nymphs with unidentified parasitoid (E), unidentified coccinellid adults (F-K), unidentified coccinellid larva (L), adult ACT with mycosis from a *Beauveria* strain infection (M), adult ACT with mycosis from a *Metarhizium* strain infection (N), *Braconid* sp. (O).

4.5 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) 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, 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 (Catling 1969; 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. erytreae*, suggesting that additional factors also influence the seasonal abundance of ACT. For instance, the nutritional quality of citrus trees could have impacted the observed temporal variations in ACT abundance. Some of the surveyed orchards were heavily infested by ACT with severe damage symptoms. Consequently, the poor condition 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 ACGD infection (Cook et al. 2014).

We found that seasonal phenology and population dynamics of ACT adults and immatures (i.e., eggs and nymphs) 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 and ACTs started to spread to the unshaded ones once temperature conditions became more favorable for survival and reproduction (van den Berg 1990).

The climatic effects on the population dynamics of *T. erytreae* in our survey were rather inconclusive, calling for longer and more detailed investigations as also stipulated by Samways (1987b) 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. We observed the highest ACT infestation levels in lemon trees, confirming earlier results of (Aubert 1987) who found lime and lemon the most preferred host plants of *T. erytreae*. Probably, lemon trees produce more regular flush shoots compared to the other citrus species and cultivars. However, lemon trees appear to be more resistant to citrus greening disease, especially compared to sweet oranges (Graca 1991; Obergolzer et al. 1965).

Results of the present study revealed that the distribution of ACP*,* a close relative of ACT, was confined to low altitudes (elevations below 1409). Adults were found in December throughout the middle of February in elevations below 1,409 m.a.s.l. The absence of ACP in high altitudes could be attributed to, among others, 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 1,000 m.a.s.l. in these regions is quite disturbing because of the then presence of two citrus greening disease vector species. 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* (Thunb) Harv. (Rutaceae) 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.

In this study, four parasitoids were identified as indigenous parasitoids attacking ACT in Kenya. The inventory of parasitoids and predators in Kenya provides crucial baseline data for future conservation and/ or introduction of parasitoids and predators for biological control efforts in Kenya. In the light of this, *T. dryi* was introduced from South Africa and successfully reduced ACT populations on Réunion Island. Also, ACT was successfully used in classical biological control in South Africa and Mauritius (McDaniel and Moran 1972; van den Berg and Greenland 2000) reported that *A. cassatus* was most dominant among 13 different parasitoids collected in Zimbabwe. Several predators contribute to ACT population control, however, not to economically acceptable levels (van den Berg et al. 1987).

4.6 Conclusion

This study showed that the seasonal abundance of *T. erytreae* in parts of Kenya varied across different altitudes. Also, the results clearly showed that ACT (all stages) seasonal dynamics varied between shaded and unshaded trees. The presence of ACP in elevations above 1,000 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*. Identified parasitoids namely, *T. dryi, P. pulvinatus, A. cassatus*, *Tetrastichus* sp., would be useful in developing a biological control method for the pest. The natural enemies provided in this study would be useful in developing a biological control strategy for the pest. Further studies to identify these predators to species level are required.

5 SURVEILLANCE AND MONITORING OF “*CANDIDATUS*” LIBERIBACTER SPP. VECTORS: AFRICAN CITRUS TRIOZID *TRIOZA ERYTREAE* DEL GUERCIO (HEMIPTERA: TRIOZIDAE) AND ASIAN CITRUS PSYLLIDS *DIAPHORINA CITRI* KUWAYAMA (HEMIPTERA: LIVIIDAE)

5.1 Abstract

The African citrus triozid (ACT) *Trioza erytreae* (Del Guercio) (Hem.: Triozidae) and the Asian citrus psyllid (ACP) *Diaphorina citri* (Kuwayama) (Hem.: Liviidae) are primary vectors of the “*Candidatus”* Liberibacter spp. pathogens. ACT vectors “*Candidatus”* Liberibacter africanus (CLaf) which is associated with the African citrus greening disease (ACGD). ACP also vectors “*Candidatus”* Liberibacter asiaticus (CLas) and “*Candidatus”* Liberibacter americanus (CLam) which are the causal agents for greening disease in Asia and the Americas, respectively. Reliable and efficient detection and sampling procedures for both vectors are critical in identifying geographical areas being invaded by the vectors, and for the development of economically sound Integrated Pest Management (IPM) strategies. An earlier study in Kenyan citrus groves had revealed that yellow sticky traps (Asacp) were not effective in detecting and monitoring ACT and ACP. Therefore, this study sought to compare eight different double-sided sticky traps to evaluate which color is most effective for detecting ACT and ACP, particularly at low densities. Sticky card traps varying in color, sticky material, wavelength, and reflectance were assessed for ACT and ACP in citrus orchards in western Kenya. The traps were coded as Red, Blue, Asgreen1, GLMgreen, Asacp, Asyellow, White, and Black. Data were collected on a weekly basis for eight weeks. The results showed that Asyellow and GLMgreen captured more ACT and ACP, respectively. Asyellow

significantly captured more ACTs than any other trap type. Asyellow proved also to be more effective in detecting both ACP and ACT than the other tested trap types. The results clearly showed that the Asyellow trap was most suitable for trapping ACT. Since there was no clear evidence suggesting that GLMgreen would be more useful in trapping ACP, the Asyellow trap type would be economically useful when both ACP and ACT are present. This information are relevant in developing ecologically friendly IPM strategies for citrus greening vectors.

5.2 Introduction

Citrus is one of the economically most important fruit crops in Kenya with an annual production and about 85,000 tons (FAOSTAT 2016). Most of these citrus fruits are produced in the western, central and coastal regions of the country. The Kenyan citrus industry provides jobs, income, food and other benefits from citrus related activities. However, for the past few decades, production has experienced a continuous decline (Kilalo et al. 2009), and attempts made to reverse the trend and increase production have met with little success. Currently, production is far below the global averages (FAOSTAT 2016) and is unable to meet the local demands, leading to supplemental citrus imports from other countries like Egypt and South Africa (Chadwick 2017; MTI 2017).

Several abiotic and biotic constraints are associated with this decline. For the latter, the main ones are arthropod pests and diseases, of which the African citrus triozid (ACT) *Trioza erytreae* Del Guercio (Hem.: Triozidae) and *Diaphonira citri* (ACP) Kuwayama (Hem.: Lividae) are the most important. *Trioza erytreae* is a very destructive pest in citrus production, transmitting "*Candidatus*" Liberibacter africanus (CLaf), the causal agent of the African citrus greening disease (ACGD). Also, its physical damage to citrus causes gall leaves, irregular blossom, stunted tree growth, and malformed fruits. Yield losses up to 100% have been reported for ACT (Kilalo et al. 2009). Although the pest is native to Africa it has spread to Asia and Europe (Coccuza et al. 2016). On the other hand, *D. citri*, a close relative of ACT that vectors "*Candidatus"* Liberibacter asiaticus (CLas) in Asia and America and "*Candidatus"* Liberibacter americanus (CLam) in Brazil, native to Asia but recently introduced to Africa (Saponari et al. 2010), has equally been associated with serious losses to the Kenyan citrus industry (Bové 2006; Khamis et al. 2017).

The dual presence of ACT (Waterston 1922) and ACP (Rwomushana et al. 2017) in Kenya implies additional risks associated with the potential introductions of the CLas and possibly also CLam pathogens. ACGD and the Asian citrus greening disease often referred to as Huanglongbing (HLB) disease, have no cure and present in Africa (Saponari et al. 2010). Presently citrus greening disease has no cure and symptoms include yellowing of shoots, malformed fruits, fruit drop, and tree dieback; whereas infected trees may live for several years, during this period there is, however, a significant reduction in productivity and production of unmarketable fruits (Bové 2006).

Simple and efficient detection sampling procedures for both ACT and ACP are critical in identifying geographical areas being invaded by the pests and for the development of an economically sound Integrated Pest Management (IPM) strategy. Methods employed to monitor ACT and ACP populations include removal of flush shoots and counting of insects (all stages) (Hall and Albrigo 2007, Sétamou et al. 2008), a stem-tap sampling procedure to count adults, as well as the use of yellow sticky card traps (Hall and Hentz 2010, Hall et al. 2010). Especially yellow sticky card traps are commonly used for the adult ACT and ACP monitoring (Aubert and Quilici 1988; Aubert and Hua 1990; Flores et al. 2009; Cook 2014), and proved to be superior for ACP to blue sticky card traps (Hall et al. 2010). However, so far only a few available sticky trap types have been thoroughly evaluated, and never simultaneously for both ACT and ACP. Therefore, the purpose of this study was to evaluate the potential of eight sticky card traps, differing in color and adhesive materials, for monitoring of adult ACT and ACP, with a particular emphasis of detecting the pests at low densities.

5.3 Materials and methods

5.3.1 Sticky card traps

Four citrus orchards were selected for detecting adult ACT and ACP using eight different double-sided sticky traps with where categorized as yellow (2 shades), green (2 shades) red (1), white (1), blue (1) and black (1). Traps were deployed and removed on a weekly basis for eight weeks in total (Table 1). Adult ACT and ACP monitoring were carried out in non-commercial farms. In each study site, three of each trap were placed every week for eight weeks.

Table 5.1. Sticky card traps evaluated for monitoring adult *Trioza erytreae* and *Diaphorina citri*

Trap code	Source	Adhesive material	Trap dimensions (cm)	Sticky sides	Price per trap ($)	Price per trap (Ksh)
Black	Locally made	Soveurode®aerosol glue	13.8 x 19.8	Two	0.2	20
Asacp	AlphaScents (Pantone 396)	No Mess Sticky Card™	13.8 x 19.8	Two	1.2	120
Asyellow	AlphaScents	No Mess Sticky Card™	13.8 x 19.8	Two	1.2	120
White	Locally made	Soveurode®aerosol glue	13.8 x 19.8	Two	0.2	20
Asgreen1	AlphaScents (Pantone 7487)	No Mess Sticky Card™	13.8 x 19.8	Two	1.4	140
						100

GLMgreen	Great Lakes IPM	Traditional	13.8 x 19.8	One(both sides)	1	
Blue	Locally made	Soveurode®aerosol glue	13.8 x 19.8	Two	0.2	20
Red	Locally made	Soveurode®aerosol glue	13.8 x 19.8	Two	0.2	20

A 512-channel ASD handheld spectroradiometer with a wavelength ranging from 325–1,075 nanometers (nm) was used to determine the percentage reflectance of each trap. The spectroradiometer was initially calibrated with a white filter paper in order to minimize the effect of a change in sun illumination. Each trap was suspended under the spectroradiometer to record the wavelength within a 6 m radius. To minimize error, five readings were taken and averaged for each trap.

5.3.2 Study sites

Figure 5.1 Spectroradiometer for recording wavelength of a trap

In the study site 1, the orchard was located in Nyando, a sub-county in Kisumu County with an elevation of 1,246 meters above sea level (m.a.s.l.). In the study site 2, the orchard was located in Soin/Sigowet, sub-county in Kericho County with an elevation of 1,398 m.a.s.l. In the site 3, the orchard was located in Soin/Sigowet, sub-county in Kericho County with an elevation of 1,397 m.a.s.l. In the study site 4, the orchard was located in Soin/Sigowet sub-county in Kericho county and consisted of *Citrus limon*. It was a moderate-sized home orchard. The elevation of the site was 1,375 m.a.s.l. In all the sites, no systemic or foliar synthetic insecticides were applied at least six months prior to the start or during the course of the experiment.

5.3.3 Data collection

The traps were hung out above the eye level. Prior to deployment, a hole was created at the center of the upper edge of each trap for easy hanging out to the branches. Each trap was tied to the outside of the tree canopy using a twist tie. To ensure consistency, traps were placed on only unshaded trees (trees exposed directly to the sun). Traps were randomly placed. Thereafter, the position of each trap was changed weekly to remove bias. Adult ACTs and ACPs trapped were sent to the laboratory and counted under a stereomicroscope (×25).

5.3.4 Data analysis

Data analysis followed Hall et al. (2010). For each experiment, the numbers of adult ACTs and ACPs were log (x+1) transformed prior to analysis. The transformed data were subjected to an analysis of variance (ANOVA), and in case of a significant *p*-value, means were separated using the Student Newmans' Keul (SNK) test ($P \leq 0.05$). The data for each site was then pooled to determine which trap was most efficient in detecting ACT and ACP. The numbers of adult ACPs and ACTs at each location were compared using two-sample t-test. Data analyses were performed in R software (version 3.4.2).

5.4 Results

The spectral reflectance of each trap and wavelengths are presented in Figure 5.1. The white trap had the highest relative reflectance intensity in the blue wavelength region (0.6 – 0.8 %). Red had the lowest reflectance (0.25 nm) whereas GLMgreen, Asyellow and white traps had the highest reflectance (0.8-1.75 %) in the yellow wavelength regions (560-590 nm). Relative reflectance for red was highest (0.3 %) with the lowest reflectance being black in the orange wavelength region (590-635 nm). In the blue region, the blue trap recorded the highest reflectance (0.35 %) followed by GLMgreen and Asgreen1 with reflectance between 0.2 – 0.3 % while Asacp and Asyellow had less than 0.2 % reflectances

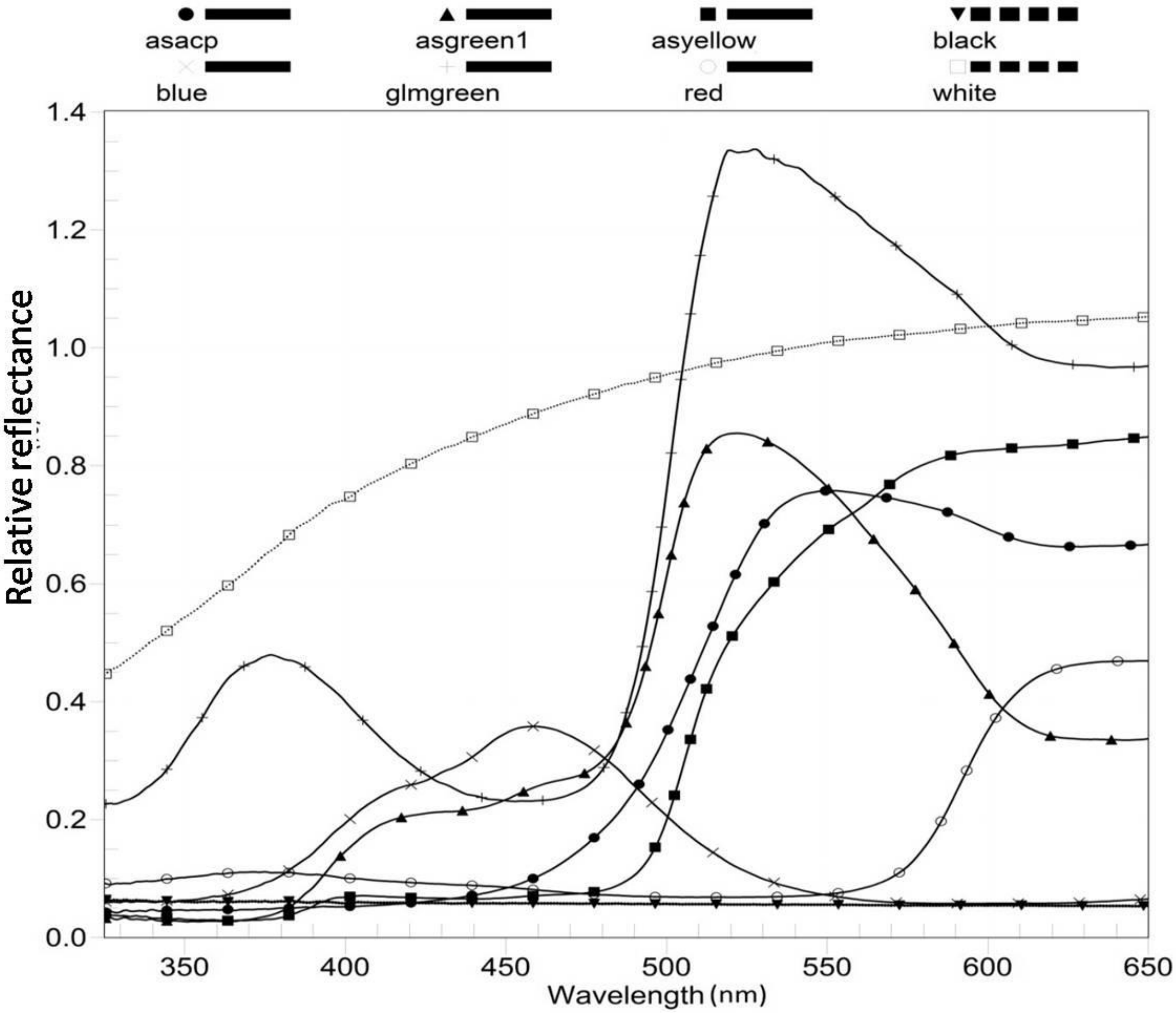

Figure 5.1. Spectral reflectance of eight different double-sided sticky card traps for monitoring of ACT and ACP.

The results showed a significantly higher number of ACT than ACP was collected on Asacp. Similarly, a significantly higher mean number of adult ACT than ACP were collected on the Asyellow trap. Trap catches for both ACP and ACT varied significantly on White, GLmgreen, Red, Asyellow and Asacp (Table 5.2 and Figure 5.2).

Table 5.2 Mean (SEM) number of adult *T. erytreae* and *D. citri* captured per week on different sticky card traps deployed in citrus. All traps were of the same size measuring 13.9 × 19.8 cm and were sticky on both sides

Location	Species	Asacp	Asgreen1	Asyellow	Black	Blue	GLMgreen	Red	White
Study site 1	*Trioza erytrea*	2.33 ± 1.2abA	1.67 ± 0.8abA	4.0 ± 0.57aA	0.0 ± 0.0Bb	0.0 ± 0.0b	6.33 ± 0.8aA	0.00 ± 0.0b	2.67 ± 0.4aA
	Diaphorina citri	0.67 ± 0.4aB	1.33 ± 0.3aA	0.33 ± 0.3aB	0.67 ± 0.4aA	0.0 ± 0.0bB	0.33 ± 0.3aB	0.00 ± 0.0b	0.00 ± 0.0aB
Study site 2	*Trioza erytrea*	24.33 ± 13.3abA	12.67± 8.0bcA	53.67 ± 2.2abA	0.67 ± 0.4cA	0.0 ± 0.0bB	123.0 ± 55.6aA	0.00 ± 0.0b	1.3 ± 0.4cA
	Diaphorina citri	0.0 ± 0.0cB	0.0 ± 0.0cB	0.67 ± 0.3bB	0.0 ± 0.0cB	0.0 ± 0.0bB	1.67 ± 0.3aB	0.00 ± 0.0b	0.0 ± 0.0cB
Study site 3	*Trioza erytrea*	6.67 ± 5.6abA	0.3 ± 0.3bB	103 ± 73.3aA	1.0 ± 0.5bA	0.0 ± 0.0bB	9.33 ± 1.3abA	0.00 ± 0.0bB	3.3 ± 0.5abA
	Diaphorina citri	0.67 ± 0.3aA	3.0 ± 0.5aA	0.7 ± 0.3aB	1.67 ± 0.8aA	1.67 ± 0.3aA	2.7 ± 0.5aB	2.00 ± 0.4aA	0.0 ± 0.0aB
Study site 4	*Trioza erytrea*	9.67 ± 6.5abA	5.67 ± 0.8abA	8.67 ± 3.3abA	0.33 ± 0.3abB	0.67 ± 0.3abB	11.3 ± 4.0aA	0.0 ± 0.0bB	5.00 ± 0.6abA
	Diaphorina citri	3.67 ± 1.3abA	0.0 ± 0.0cB	5.0 ± 1.7aA	1.67 ± 0.5abcA	1.3 ± 0.5bcA	3.33 ± 0.3abB	1.33 ± 0.88abcA	4.3 ± 0.7abA

For each study, means in the same row followed by the same letter are not significantly different (P ≤ 0.05), ANOVA followed by SNK test. Analysis on log-transformed data, untransformed means presented.

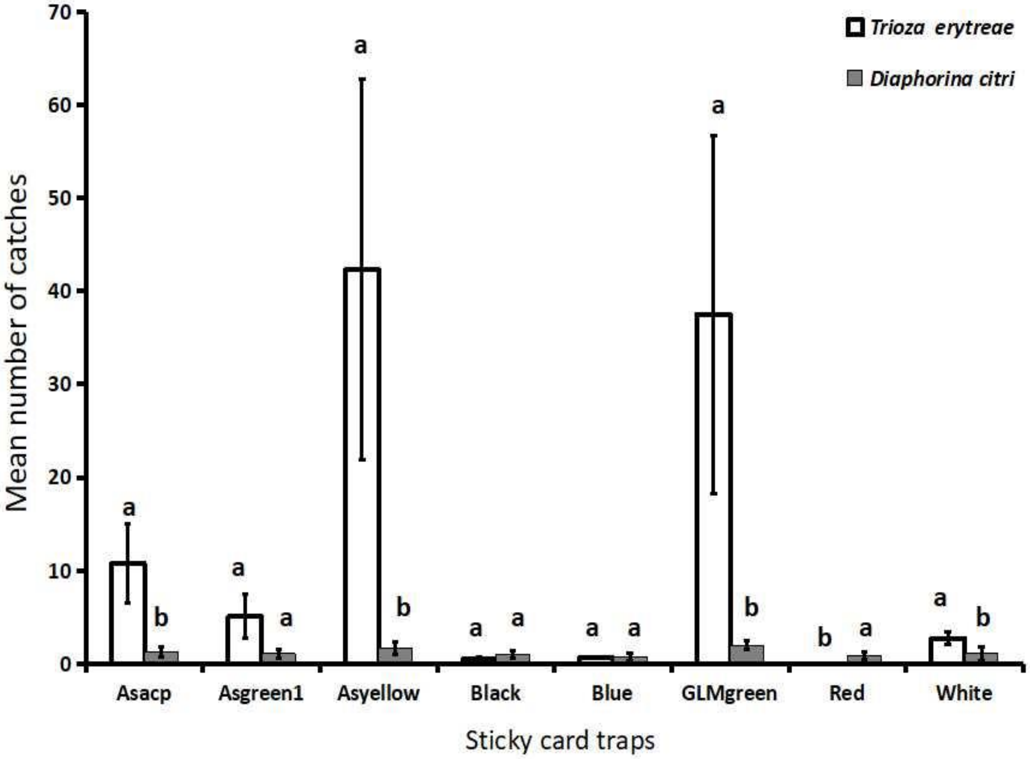

Figure 5.2 Overall mean number of adult *Trioza erytreae* and *Diaphorina citri* captured on eight different sticky trap types.

The mean number of the adult ACT were consistently higher in Asyellow and GLMgreen traps for throughout the sampling period (Figure 5.3). However, the maximum number of Asyellow trap catches was highest in week three, concurrently with the peak of the GLMgreen trap catches.

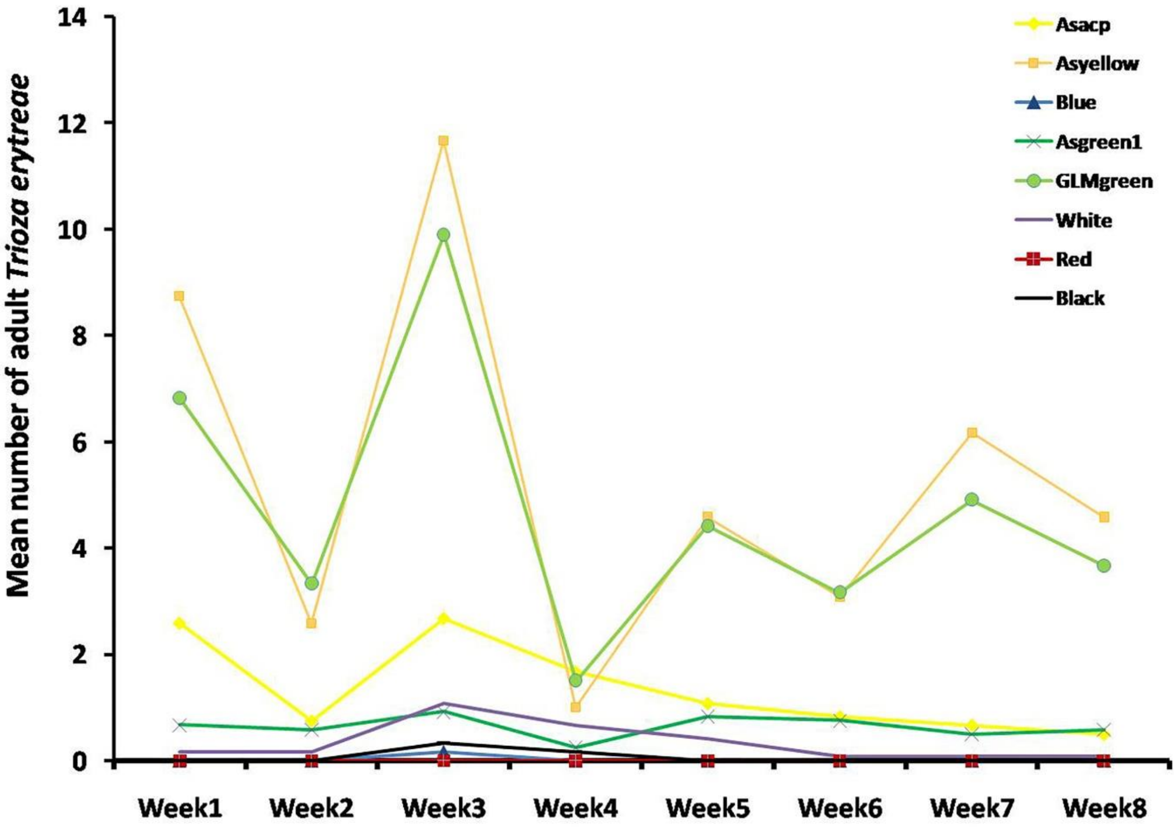

Figure 5.2 Weekly mean number of adult *Trioza erytreae* captured on eight different sticky traps.

The mean numbers of ACP adults on GLMgreen were highest in week seven whereas in Asyellow traps in week three (Figure 5.4).

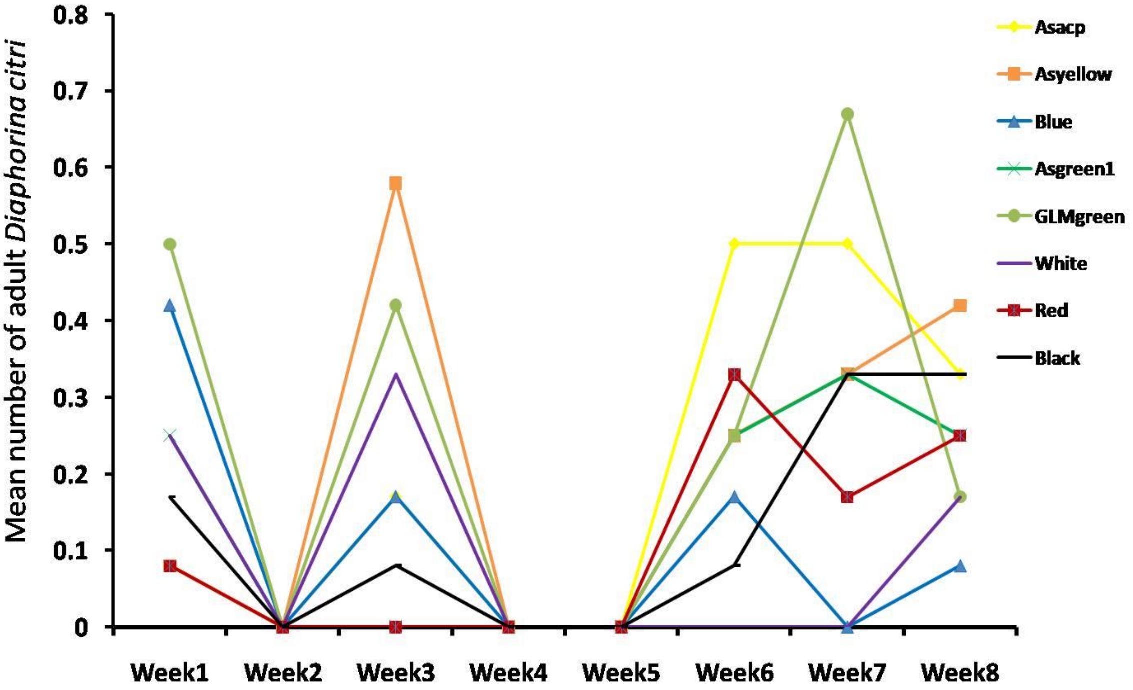

Figure 5.3 Weekly mean number of adult *Diaphorina citri* captured on eight different sticky traps.

5.5 Discussion

Higher numbers of adult ACTs were captured using Asyellow traps, followed by the GLMgreen and Asacp ones. These traps had a relatively high wavelength in the yellow region with low reflectance in the blue region. Wavelength and reflectance of objects influence ACTs' visual attraction to them (Samways 1987; van den Berg 1990). Asyellow, GLMgreen, and white traps recorded the highest reflectance in the yellow wavelength region and Asyellow had the lowest reflectance in the blue region. According to Urban (1976), ACT is highly responsive to light wavelengths of 550 nm (yellow-green). He further noted that traps with high reflectance in the yellow region and simultaneously low reflection in the blue region are suitable for detecting ACT. Consequently, yellow sticky traps have been widely used to study the seasonal phenology of ACT (Aubert and Quilici 1988; van den Berg 1989; Aubert and Hua 1990; Cook et al. 2014).

Greater numbers of ACP were captured on GLMgreen traps than with any other trap type. GLMgreen also had the overall highest reflectance in the yellow region and higher reflectance in the blue region than Asyellow traps. According to Hall et al. (2007), yellow traps are more effective than blue traps in detecting ACP at low densities. Yet, we did not observe significantly different ACP catches between GLMgreen and Asyellow traps, suggesting that both types of traps could be useful in detecting ACPs at low densities.

Asian citrus psyllids feed on both new and old shoots (Husain and Nath 1927; Hall et al. 2010). However, immatures (eggs and nymphs) are found on new flush shoots which could be due to higher nutritional contents in the new soft leaves than old shoots. Also, the biology of ACP has been strongly associated with young flush shoots because new flush shoots are required for feeding and reproduction (Husain and Nath 1927; Hall et al. 2010). Patt and Sétamou (2010) found that adult ACP was attracted to volatiles emitted from young flush shoots and recommended further studies into a color spectrum of the flush shoot if there is a visual component of the ACP finding flush. Similarly, the biology of ACT is closely linked with young flush shoots of its host plants because young shoots are required for oviposition and development of young ones (van den Berg 1990). Therefore, a similar study for ACT will help develop an appropriate attractant for both pests.

All our study sides were located in elevations > 1000 m.a.s.l. *Trioza erytreae* is a highland insect and prefers a cool and moist climate to hot and dry ones (Van den Berg 1990). However, the reverse is true for ACP, with decreasing numbers with increasing elevation (Jenkins et al. 2015).

According to Hall et al. (2010), the attractiveness of ACP to traps can vary depending on the time of year due to factors such as the direction and intensity of the sun and their interaction with the traps. They further indicated that seasonal variation could have a significant impact on the background color of the trees in which traps are placed making it difficult or easier for ACP to detect the traps (Hall et al. 2010).

Greater numbers of an adult ACT than ACP were captured during the eight-week sampling period, suggesting that climatic conditions were more favorable for the former than the latter vector species, respectively. Adult ACTs prefer certain citrus species/ cultivars to others as host plants.

5.6 Conclusion

The results clearly showed that the Asyellow trap type was most suitable for trapping adult ACTs. Since there was no clear evidence suggesting that GLMgreen would be more useful than any of the other traps in trapping ACP. It is thus suggested that Asyellow would be economical in detecting both ACP and ACT are present. This information is relevant in developing ecologically friendly IPM strategies for citrus greening vectors. A further study to compare GLMgreen and Asyellow for adult ACP trapping is required. Also, the presence of a few adults of ACP in the study sites could have affected our results hence a further study is required for both traps.

6 SIZE AND SHAPE ANALYSIS OF TRIOZA ERYTREAE DEL GUERCIO (HEM. TRIOZIDAE), VECTOR OF CITRUS HUANGLONGBING DISEASE

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6.1 Abstract

The African citrus triozid (ACT) *Trioza erytreae* Del Guercio (Hemiptera: Triozidae), is one of the most devastating pest of citrus with a well-known role as a vector of the phloem-limited bacteria [*Candidatus* Liberibacter africanus] associated with huanglongbing (HLB; citrus greening disease), currently considered the world's most serious disease of citrus. Although the pest can successfully develop and reproduce on non-citrus host plants, there is no documented information on geometric morphometry of ACT. We determine the effect of host plants on ACT geometric morphometry under controlled laboratory conditions. ACT was reared on six different known host plant species for five successive generations; *Citrus limon*, *Citrus sinensis* "Valencia", *Murraya koenigii*, *Citroncirus* spp., *Clausena anaisata,* and *Citrus tangelo.* Adults were examined for morphometric variation using traditional and geometric analysis. ACT reared on *C. limon* and *Citroncirus* spp. was significantly larger than those reared on the other host plant species. ACT reared on *C. anisata* and *C. tangelo* was consistently smaller than those from *M. koenigii* and *C. sinensis.* Based on warped outline drawings, ACT reared on *Citroncirus* spp. and *Murraya koenigii* had narrower wings than those reared on *C. anisata*, *C. limon* and *C. sinensis* with slightly broader wing patterns. This study clearly demonstrates that host plant species affect morphometric

variation in ACT, which might have a direct impact on fitness parameters of the pest as well as its potential for dispersion. Wing shape and size appear to be useful in separating populations of ACT into different groups.

6.2 Introduction

The African citrus triozid (ACT) *Trioza erytreae* Del Guercio (Hemiptera: Triozidae) was first reported in Kenya in 1922 (Waterston 1922) as a serious pest of citrus. Its presence in the region led to the near collapse of the citrus industries in the Kenyan highlands due to its ability to transmit the phloem-limited bacteria (*Candidatus* Liberibacter spp.), which is strongly implicated in huanglongbing (HLB) or citrus greening disease, the world's most devastating disease of citrus (Bové 2006; McClean and Schwarz 1970). ACT are mostly found in the mid and high altitudes, where it threatens citrus production (Shimwela et al. 2016). After, the initial discovery of ACT in Africa (native home), it has spread and invaded over 30 countries with subsequent detection in Europe and Asia (van den Berg 1990). The spread of ACT from its aboriginal home in Africa to Europe and Asia illustrates the pest's potential to adapt and establish in new citrus-producing regions of the world with different environmental conditions.

The symptoms of this highly invasive hemipteran sap-sucking polyphagous pest reviewed by Khamis et al. (2017) include distorted leaves, stunted and twisted flush shoots, sooty molds and honeydew, and premature dropping of leaves and shoots whiles HLB causes mottling and patchy discoloration of citrus fruits and trees, fruit drop, stunted tree growth and bitter fruits among others. These symptoms are usually followed by severe dieback and subsequent death of the infected plant. Besides citrus, *T. erytreae* can complete its development and reproduction on most citrus and several citrus-related host species in Rutaceae (van den Berg 1990). In South Africa, four indigenous alternate host plants of *T. erytreae* belonging to the family Rutaceae have been documented, namely *Clausena anisata* (Willd.) Hook. f. ex Benth., *Vepris lanceolata* (Lam.) G. Don.*, Zanthoxylum capense* (Thunb.) Harv. and *Oricia* sp. (van den Berg 1990). These alternate host plant species may probably serve as a reservoir for a breeding population of *T. erytreae* when citrus is out of season.

ACT populations have been observed to peak when alternate host plants such as *C. anisata* were found growing in the proximity of citrus orchards (van den Berg 1990). Therefore, rearing of ACT on optimal host plant species would be crucial for understanding the dynamics and the stability of ecological systems given that insects show morphometric variations associated with the environment, which may be the result of phenotypic response or genetic inheritance, or both. For example, rearing of the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama on the curry tree *Murraya koenigii* (L.),(Martini et al. 2014; Simmons et al. 2013) *Citrus medica* L.,(Hall and Hentz 2016) *Ci. macrophylla* Wester (Hall and Richardson 2013) and orange jasmine *M. paniculata* (L.) Jack (all Rutaceae) (Hall et al. 2007; Paris et al. 2015) revealed differences in development times and times to peak emergence under greenhouse conditions(Hall and Hentz 2016). Also, adult ACP collected from different host plants in the field in Mexico were reported to exhibit a morphometric variation (García-Pérez et al. 2013), although the role of biotic and abiotic factors in this variation was unclear. Knowledge of these factors influencing the biology and dynamics of the ACP and its close relatives would facilitate the development of appropriate models to predict the spread and the development of appropriate management strategies. Although several studies have been conducted on host preference of ACT on some citrus and non-citrus host plants, little is known about effects of different host plants on the biology of ACT, especially with respect to body size, wing size, and shape.

The use of morphometric analyses as tools to evaluate phenotypic variations that result from both biotic and abiotic factors as well as differences associated with the development and genetic factors have been demonstrated by several authors (Daly 1985; Paris et al. 2016). These differences may be related to and can provide insight into the effects of host plants on factors affecting attributes such as survival and fecundity (Alves et al. 2014; Hall and Hentz 2016; Nava et al. 2007). According to Daly (1985) and Paris et al. (2016), traditional morphometrics analysis of size and size ratios is the classical approach for quantifying variation in biological specimens and it is used to determine instar, and to compare genetic, environmental and phenotypic variations (Daly 1985). Other reliable approaches used include geometric morphometrics analysis that focuses on mechanisms that provide an in-depth evaluation of shape independent of size through the use of landmarks on two-and-three-dimensional surfaces (Bookstein 1991; Bookstein 1996; Dryden and Mardia 1998; Rohlf and Marcus 1993; Rohlf and Slice 1990). It can be used to remove confounding effects of size, rotation, and orientation and is sensitive enough to detect subtle differences characteristic of subpopulations within a single species (Lashkari et al. 2013; Paris et al. 2016). For example, it has been used to elucidate patterns of morphological variation associated with wild populations and laboratory colonies of the common housefly *Musca domestica* L. (Diptera: Muscidae) (Ludoški et al. 2014), wild sandfly populations(Santos et al. 2015), the discrimination of four species of *Culex* mosquitoes (Diptera: Culicidae)(Laurito et al. 2015) and synonyms of two *Bactrocera* species (Diptera: Tephritidae) (Schutze et al. 2015).

Traditional and geometric morphometrics have also been used for differentiation of psyllids collected from different geographic locations (Lashkari et al. 2015; Lashkari et al. 2013), or experiencing different chemical control programs or environmental rearing conditions (Mostafavi et al. 2017; Paris et al. 2017). The effect of host plant species on the morphometric measurements of tomato/potato psyllid *Bactericera cockerelli* (Sulc) and ACP have been well documented with considerable variation (Killiny 2016;2017; Vargas-Madríz et al. 2013), which could be attributed to the nutritional quality of the different host plant species (Hail et al. 2012; Killiny 2017; Sétamou et al. 2017), such as sugars and amino acids that support survival and reproduction (Vargas-Madríz et al. 2013). However, it was also found that amino acids not obtained from phloem-feeding, may be produced by the symbiotic bacteria found in the psyllid (Cocuzza et al. 2017; Subandiyah et al. 2000; Thao et al. 2000), thus potentially ameliorating differences in nutrition levels provided from plants.

Although, it has been widely documented that ACT feeds on a range of rutaceous host plant species (Moran 1968), with significant impact on their development and survival (Doyle 1990) information on morphometric characteristics remain largely unknown. Thus, the purpose of this study was to assess the effect of host plant species on morphometric variation in ACT under controlled laboratory conditions. The choice of optimal and suboptimal host plant species for ACT in key areas of citrus groves and geographical range of the disease has been proposed as a possible component of an ACT management strategy. Therefore, the use of both approaches of morphometric analysis would provide results that could be compared to other studies, such as that of ACP, whereby both traditional (Killiny 2016; Lashkari et al. 2015), and geometric (Lashkari et al. 2013) morphometric analyses were used. In addition, both approaches would be helpful in guiding an extended literature review to allow the interpretation of results, particularly regarding dispersal and other biological fitness parameters like developmental, survival, size and fecundity.

6.3 Materials and methods

6.3.1 Host plants and insect culture

Based on extensive field survey conducted throughout the major citrus growing regions across different agro-ecological zones in Kenya, six most important and readily available host plant species known to support all the life stages of ACT were selected: *Citrus limon* (L.) (lemon), *M. koenigii,Citrus sinensis* L. Osbeck (Valencia), *C.anisata*, *Citrus tangelo* (Minneola)and *Citroncirus* spp. (Citrumelo). The seedlings of the selected plant species were sourced from a commercial nursery located in Nairobi, Kenya. The plants were later transplanted into 3-litre white plastic pots containing steam-sterilized, humus-rich soil and 1 g of di-ammonium phosphate (DAP) per plant. The potted plants were maintained under ambient conditions in a greenhouse at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya [S 01° 13' 14.6''; E 036° 53' 44.5'', 1,612 m above sea level]. The experimental plants were watered regularly as needed and fertilized twice a month with a foliar fertilizer (Gleecobalance^{TM} N: P: K 17:17:17+TE). Prior to purchasing the seedlings, the nursery was carefully examined for ACT and ACP presence and symptomatic leaves. There were no immature, adults and/ HLB or citrus greening symptomatic leaves in the nursery where the seedlings were purchased. In addition, all seedlings were obtained from the same location and none of the seedlings showed HLB or citrus greening symptoms during and after experiments.

Wild-caught populations (nymphs and adults) of ACT were originally collected from the field during the country-wide survey on lemon (*C. limon*). A laboratory culture was initiated at the Animal Rearing and Containment Unit (ARCU) of *icipe*. Infested plant leaves from the field samples containing immature life stages of ACT were carefully placed on new shoots of *M. koenigii* in a screenhouse and reared for three generations as stock colony prior to the beginning of the experiments. The host plant (*M. koenigii*) was selected for rearing based on the availability of seedlings. Thereafter, ACT colonies were established on the six selected host plant species after trimming to stimulate the development of new flushes. Five plants from each host species were placed individually in portable collapsible rearing cages made up of polyester mesh and steel wire (40 x 40 x 60 cm) with one side clear view (vinyl) and another side with a zipper opening (Model No.: BYC-003, Fujian, PR China). One hundred (100) ACT adults were introduced into each cage and allowed to oviposit for three days and then removed. After hatching, the resulting immature ACTs were allowed to develop to the adult stage for five generations under controlled conditions of 12L:12D photoperiod, mean daily temperature range of 22 - 24°C and relative humidity (rh) of 65 ± 5%.

6.3.2 Specimen preparation and measurement

After five generations on the respective host plant species, ACT adults were collected seven days upon emergence to allow for sexual maturity and development of full body colorations. Adult ACTs were collected using an aspirator and killed by placing them in a freezer (- 20°C). The dead ACTs were later preserved in 70% ethanol in well-labeled microcentrifuge tubes and held in a freezer until processed. Adult ACT was prepared for digital images at *icipe*'s Biosystematics Unit where a reference collection is kept. The body structures were mounted on clean microscopic glass-slides [ground edges of 25.4 x 76.2 mm (1ˈ x 3ˈˈ), 1mm - 1.2mm thick (Kamfit Surgical, Cat.No.7105-Stig Jiangsu Garments IMP. & FX P Corp. Ltd.)], using euparol as the mounting medium. Prior to mounting, each specimen was placed in clove oil to avoid brittleness and to enhance the observation of the body structures. The digital images of the body structures from the slide-mounted specimens were captured using a stereomicroscope [Leica MZ 125 Microscope (Leica Microsystems, Switzerland)], fitted with a Toshiba 3CCD camera using the Auto-Montage software (Syncroscopy, Synoptics Group, Cambridge, UK) at magnification of X25 (Li et al. 2006). The measurements were taken using the program Image-Pro® Plus version 4.1 for Windows™ (Khamis et al. 2012; Thévenaz 2013).

6.3.3 Traditional morphometrics

The traditional morphometric analysis was based on linear measurements of specific structures. For the female psyllids, body length was measured from the outermost tip of the genal combs to the outermost tip of the proctiger (Fig. 1A).

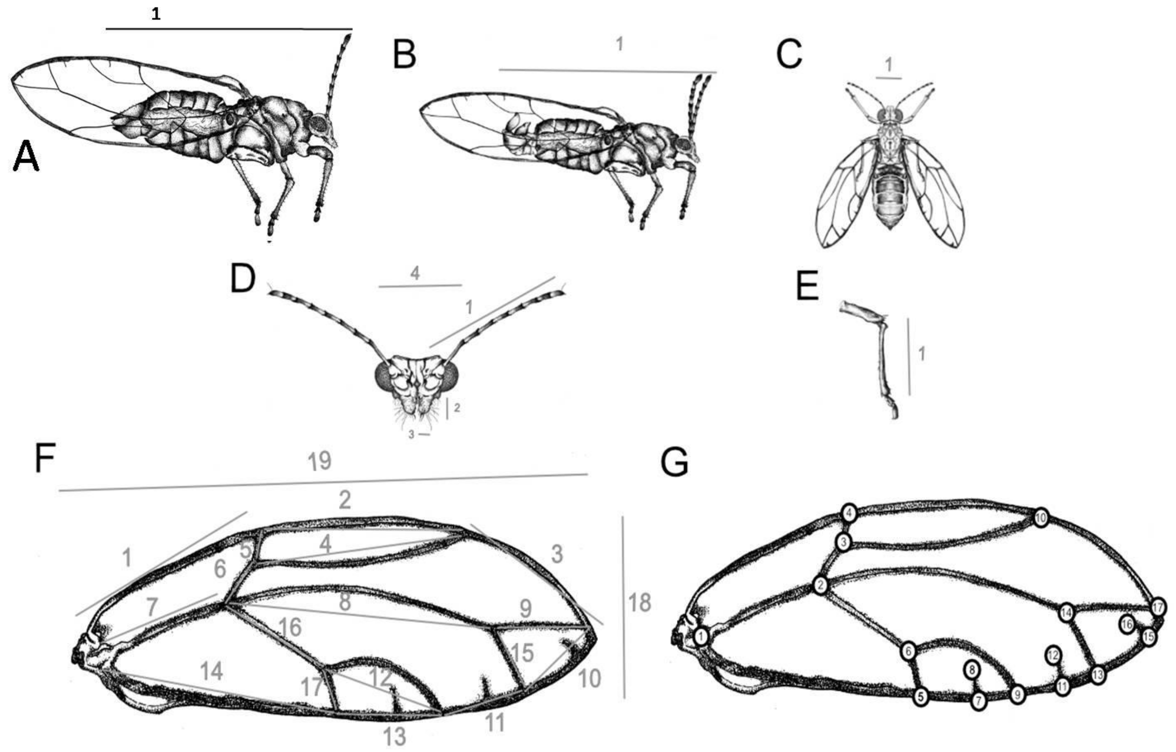

Figure 6.1. Features measured for morphometric analysis. A. Lateral view of female psyllid and the body length (1) B. Lateral view of male ACT and body length measurement (1) C. Female psyllid dorsal view. D. Dorsal view of the head with antennal (1) and genal comb measurements (2, 3) E. Tibia illustration and measurement (1) F. Wing view and wing vein measurements (1:20) G. Wing view and morphometric landmarks (1:17).

Body length of the male was measured from the outermost tip of the genal combs to the outermost tip of the sub-genital plate (Fig. 1B). The width of the thorax was obtained from a dorsal measurement (Fig. 1C). Measurements from the head included antenna length, genal process length, and width and head capsule width (Fig. 1D). Tibial length measurements were also obtained (Fig. 1E). Traditional morphometrics were based on 17 linear measurements based on wing veins (Fig. 1-F, 1-17), including the length from the base of the wing C+Sc from the intersection with $R+M+Cu_1$ to the intersection with the pterostigma (Fig. 1F,1), C+Sc from the intersection of the pterostigma to the intersection with R (Fig. 1F, 2), R from the intersection with

C+Sc to the intersection with Rs (Fig. 1F, 3), and R during the overlap with the pterostigma (Fig. 1F, 4). Additionally, measurements were made of the wing width from the costal margin to the anal margin (Fig. 1F, 18) and the wing length from the base of the wing to its apex (Fig. 1F, 19). Each body part and wing vein was measured three times and averaged to reduce measurement error. Wing ratio was calculated as the wing length/wing width.

6.3.4 Geometric morphometrics

Wing shape was based on a set of 17 homologous landmarks (*x* and *y* coordinates in Cartesian space) (Fig. 1G). The landmarks were made on the image of the psyllid wing using ImageJ (Cartesian space) (Fig.1G) and the plugin point picker (Racine 2012). (A landmark is identified as an intersection between the wing vein (Fig. 1G, 1-3, 6, 14), the base and apex of radular spinules (Fig. 1G, 7-8, 11-12, 15-16), or between the wing vein and wing margin (Fig. 1G, 4-5, 9-10, 13, 17). Two sets of landmarks were made for each specimen to reduce measurement error.

6.3.5 Data Analysis

Data analyses for traditional and geometric morphometrics were conducted similarly to previous morphometric studies on ACP (Paris et al. 2016; Paris et al. 2015). Traditional linear morphometric measurements of male and female psyllids were compared using the Welch's t-test or the Student t-test. The type of t-test used depended on the equality (Student t-test) or inequality (Welch's t-test) of the variance of data as determined by Bartlett's test. Comparisons were made between measurements of male and female psyllids to test for sex-related differences in linear measurements. Similarly, linear measurements of female psyllids reared on different host plants were analyzed using one-way ANOVA and in case of significance followed by multiple means' separation using Tukey HSD test (*P* ≤ 0.05). The linear measurements conformed to a normal distribution; thus, data were not transformed. ANOVA and t-tests were conducted in R studio (R Development Core Team 2009), and R(Hope 2013), using Rmisc (Wickham et al. 2017a), dplyr (Fox and Weisberg 2011), car (Wickham et al. 2017b), readr (De Mendiburu 2016) and agricolae (Hammer et al. 2001). Permutational multivariate analysis of variance (PERMANOVA) was used to compare group means of different linear measurement. Ordination of data was conducted using principal component analysis (PCA) and canonical variate analysis (CVA) using Paleontological Statistics Software (PAST) version 3.04 software (Klingenberg 2011). A *posteriori* procedures, cross-validation, and jackknife analysis were conducted to confirm the validity of the assignment of individual psyllids to groups.

For geometric morphometric analysis, the coordinates of the 17 different landmarks were derived using Procrustes superimposition to produce shape data without the influence of size, position, or orientation (Dryden and Mardia 1998; Rohlf and Slice 1990). Additionally, shape analysis was undertaken using residuals of log-transformed centroids (measures of wing size) to remove allometric effects (Paris et al. 2017). Ordination techniques used to discern patterns between treatments in the shape data included both PCA and CVA. Individuals assigned to different groups based on size data were determined to be significantly different by Mahalanobis distances derived from permutations (10,000 rounds) and validated using a cross-validation confusion matrix. Statistical analysis of shape data and visualization of shape data differences between psyllids reared on different host plants and the average shape of *T. erytreae* was using the MorphoJ software (Klingenberg 1996). Overall, 166 specimens were used for both traditional and geometric analysis; 15 females each from *C. anisata*, *M. koenigii*, *C. limon*, *C. sinensis*, *Citroncirus* spp., and *C. tangelo*, there were 15 males each from all the varieties except *Citroncirus* spp. which had 16 specimens.

6.5 Results

6.5.1 Traditional morphometrics

Female psyllids were significantly larger than males in every measurement analyzed (Table 6.1). For example, female body length was on average 2.20 ± 0.02 mm and significantly larger than those of males 1.82 ± 0.02 mm (t = 13.03, df = 164, $P < 0.001$).

Table 2. Size of morphological traits (mm ± SE) measured from female and male African citrus psyllids reared from different host plants. *P-values* are provided for Student or Welch's *t*-test depending on the variance of the data for comparison.

Fig. 1 Code	Trait	Female	Male	T	df	*P- value*
1F.1	C+Sc	1.10 ± 0.01	1.02 ± 0.01	8.09	161.63	< 0.001
1F.2	R_1	1.12 ± 0.01	0.98 ± 0.01	9.43	151.74	< 0.001
1F.3	R_2	0.94 ± 0.01	0.81 ± 0.01	8.26	139.54	< 0.001
1F.4	R_s	1.15 ± 0.01	1.02 ± 0.01	10.05	164.0	< 0.001
1F.5	R/R_s to R/R_1	0.16 ± 0.02	0.14 ± 0.00	8.63	158.75	< 0.001
1.F6	R/R_s to R/M	0.24 ± 0.00	0.20 ± 0.00	11.31	161.70	< 0.001
1.F 7	$R+M+Cu_1$	0.82 ± 0.01	0.78 ±0.01	4.83	163.10	< 0.001
1.F 8	M	1.52 ± 0.01	1.34 ± 0.01	10.98	164.0	< 0.001
1.F 9	M_{1+2}	0.56 ± 0.01	0.49 ± 0.00	9.13	164.0	< 0.001

1.F10	A/M$_{1+2}$ to A/M$_{3+4}$	0.47± 0.00	0.40 ± 0.00	10.36	164.0	< 0.001
1.F11	A/M$_{3+4}$ to A/Cu$_{1a}$	0.54 ± 0.00	0.49 ± 0.01	7.80	164.0	< 0.001
1.F12	Cu$_{1a}$/A to Cu$_{1b}$/Cu$_{1}$	0.67 ± 0.01	0.59 ± 0.01	8.77	157.78	< 0.001
1.F13	Cu$_{1b}$/A to Cu$_{1a}$/A	0.58 ± 0.00	0.52 ± 0.00	8.72	161.85	< 0.001
1.F14	A to A/Cu$_{1b}$	1.41 ± 0.01	1.28 ± 0.01	8.03	160.94	< 0.001
1.F15	M$_{3+4}$	0.36 ± 0.00	0.32 ± 0.00	7.34	163.84	< 0.001
1.F16	Cu$_{1}$	0.64 ± 0.01	0.56 ± 0.00	11.33	158.93	< 0.001
1.F17	Cu$_{1b}$	0.22 ± 0.00	0.20 ± 0.00	5.36	147.47	< 0.001
1.F18	Wing Width	0.97 ± 0.01	0.86 ± 0.01	12.14	162.31	< 0.001
1.F19	Wing Length	2.89 ± 0.02	2.58 ± 0.02	10.62	164.0	< 0.001
1.A1,1. B2	Body Length	2.20 ± 0.02	1.82 ± 0.02	13.03	164.0	< 0.001
1.C1	Thorax Width	0.54 ± 0.01	0.47 ± 0.00	9.76	155.48	< 0.001
1.D1	Antenna Length	0.95 ± 0.01	0.90 ± 0.02	2.10	89.57	0.04
1.E1	Tibia Length	0.66 ± 0.00	0.63 ± 0.00	4.73	162.73	<0.001
1.D2	Genal Comb Length	0.12 ± 0.00	0.10 ± 0.00	7.50	150.52	< 0.001
1.D3	Genal Comb Width	0.06 ± 0.00	0.06 ± 0.00	3.39	162.51	< 0.001
1.F19/1.F18	Wing Ratio	2.96 ± 0.01	3.01 ± 0.01	-2.82	148.54	<0.01

Subsequent comparisons were made based on female psyllid measurements. Host plant species impacted female morphometric measurements as shown by the significant differences between host plants for each linear measurement (Table 6.2). Additionally, there were clearly significant differences between the impacts of host plants on measurements of psyllids reared on the different host plants (Table 6.2). In general, psyllids reared on *C. limon* and *Citroncirus* spp. were significantly larger than those reared on other host plants. Psyllids reared on *C. anisata* and *C. tangelo* were consistently the smallest with psyllids from *M. koenigii* and *C. sinensis* intermediary in size (Table 6.2). There were no significant differences between the wing ratio scores of female psyllids reared on different plants (Table 2, $F = 0.96_{5,\,84}$, $P = 0.45$).

Table 6.3. Size of morphological traits (mm ± SE) measured from female African citrus psyllids reared from different host plants. *P-values* are provided for ANOVA analysis for comparison of each trait between host plant cultivars (BK = *Bergera koenigii*, CA = *Clausena anisata*, CL = *Citrus limon*, CS = *Citrus sinensis* ‘Valencia’, CC = *Citroncirus* spp., CT = *Citrus tangelo*). Values marked with different letters within a row indicate significant differences between host plants.

Trait	BK	CA	CL	CS	CC	CT	*P-value*
C+Sc	1.09 ± 0.01b	1.06 ± 0.02bc	1.20 ± 0.01a	1.08 ± 0.01b	1.15 ± 0.01a	1.02 ± 0.01c	< 0.001
R_1	1.08 ± 0.01bc	1.06 ± 0.02bc	1.20 ± 0.01a	1.15 ± 0.05ab	1.21 ± 0.02a	1.02 ± 0.01c	< 0.001
R_2	0.91 ± 0.01b	0.88 ± 0.02b	0.98 ± 0.01ab	0.94 ± 0.02ab	1.06 ± 0.06a	0.87 ± 0.01b	< 0.001
R_s	1.12 ± 0.02bc	1.11 ± 0.08bc	1.24 ± 0.01a	1.13 ± 0.02b	1.25 ± 0.02a	1.06 ± 0.01c	< 0.001
$R/R_s$to R/R_1	0.15 ± 0.00bc	0.16 ± 0.00bc	0.17 ± 0.00a	0.16 ± 0.00bc	0.16 ± 0.00ab	0.14 ± 0.00c	< 0.001
$R/R_s$to R/M	0.24 ± 0.02bc	0.24 ± 0.01bc	0.25 ± 0.01b	0.23 ± 0.01c	0.28 ± 0.00a	0.22 ± 0.01c	< 0.001
$R+M+Cu_1$	0.83 ± 0.01b	0.78 ± 0.01c	0.89 ± 0.01a	0.81 ± 0.01bc	0.84 ± 0.01b	0.77 ± 0.01c	< 0.001
M	1.51 ± 0.02b	1.45 ± 0.03bc	1.65 ± 0.01a	1.51 ± 0.03b	1.60 ± 0.03a	1.40 ± 0.02c	< 0.001
M_{1+2}	0.54 ± 0.01c	0.53 ± 0.01c	0.50 ± 0.01b	0.55 ± 0.01bc	0.64 ± 0.01a	0.52 ± 0.01c	< 0.001
A/M_{1+2} to A/M_{3+4}	0.45 ± 0.00cd	0.44 ± 0.01cd	0.49 ± 0.01ab	0.47 ± 0.01bc	0.51 ± 0.01a	0.43 ± 0.01d	< 0.001
A/M_{3+4} to A/Cu_{1a}	0.54 ± 0.01ab	0.52 ± 0.01b	0.58 ± 0.01a	0.55 ± 0.01ab	0.55 ± 0.01ab	0.52 ± 0.01b	< 0.001
Cu_{1a}/A to Cu_{1b}/Cu_1	0.63 ± 0.01b	0.63 ± 0.02b	0.73 ± 0.01a	0.65 ± 0.02b	0.74 ± 0.01a	0.63 ± 0.01b	< 0.001
Cu_{1b}/A to Cu_{1a}/A	0.54 ± 0.01b	0.55 ± 0.01b	0.622 ± 0.01a	0.58 ± 0.01b	0.61 ± 0.01a	0.55 ± 0.01b	< 0.001
A to A/Cu_{1b}	1.40 ± 0.02b	1.35 ± 0.03bc	1.55 ± 0.01a	1.36 ± 0.02b	1.49 ± 0.01a	1.28 ± 0.02c	< 0.001
M_{3+4}	0.33 ± 0.00c	0.35 ± 0.01bc	0.36 ± 0.00b	0.35 ± 0.01bc	0.42 ± 0.01a	0.34 ± 0.01bc	< 0.001
Cu_1	0.65 ± 0.01bc	0.62 ± 0.01c	0.70 ± 0.01a	0.62 ± 0.01c	0.68 ± 0.01ab	0.58 ± 0.01d	< 0.001

Cu_{1b}	0.21 ± 0.01b	0.21 ± 0.01b	0.24 ± 0.01a	0.20 ± 0.01b	0.25 ± 0.01a	0.19 ± 0.00b	< 0.001
Wing Width	0.97 ± 0.01b	0.94 ± 0.02bc	1.05 ± 0.01a	0.96 ± 0.02b	1.03 ± 0.02a	0.90 ± 0.01c	< 0.001
Wing Length	2.84 ± 0.02b	2.77 ± 0.05bc	3.11 ± 0.02a	2.87 ± 0.05b	3.07 ± 0.03a	2.69 ± 0.02c	< 0.001
Body Length	2.18 ± 0.03b	2.18 ± 0.04b	2.38 ± 0.02a	2.16 ± 0.06bc	2.31 ± 0.03ab	2.01 ± 0.04c	< 0.001
Thorax Width	0.55 ± 0.01b	0.52 ± 0.01bc	0.59 ± 0.01a	0.53 ± 0.01b	0.56 ± 0.01ab	0.48 ± 0.02c	< 0.001
Antenna Length	0.88 ± 0.01d	0.93 ± 0.01bcd	0.99 ± 0.02ab	0.98 ± 0.01abc	0.99 ± 0.01a	0.93 ± 0.01cd	< 0.001
Tibia Length	0.66 ± 0.00b	0.61 ± 0.01c	0.71 ± 0.00a	0.66 ± 0.01b	0.68 ± 0.00ab	0.62 ± 0.01c	< 0.001
Genal Comb Width	0.06 ± 0.00abc	0.06 ± 0.00c	0.06 ± 0.00ab	0.06 ± 0.00bc	0.06 ± 0.00a	0.06 ± 0.00abc	< 0.001
Genal Comb Length	0.12 ± 0.00b	0.11 ± 0.00b	0.13 ± 0.00a	0.11 ± 0.00b	0.12 ± 0.00b	0.11 ± 0.00b	< 0.001
Head Capsule Width	0.29 ± 0.00b	0.28 ± 0.00b	0.31 ± 0.00a	0.29 ± 0.01b	0.32 ± 0.01b	0.27 ± 0.00b	< 0.001
Wing Ratio	2.94 ± 0.02a	2.95 ± 0.02a	2.97 ± 0.02a	2.99 ± 0.02a	3.00 ± 0.02a	2.98 ± 0.02a	< 0.45

PCA provided a good fit for the observed data. The first two coefficients accounted for 78.8% of the variation (PC1 = 71.0%, PC2 = 7.8%). In the first principal component, the wing length (0.54) and body length (0.43) measurements contributed most to the variation of the data with the contribution of measurements from the M vein (0.31), and A to A/Cu_{1b} vein (0.30) (Table 6.3). In the second principal component, body length (-0.77), the R_1 (0.34) and R_2 (0.41) veins contributed most to data variation (Table 6.3).

Table 6.4. Coefficients of the first two principal components (PC1 and PC2) of the principal component analysis of traditional morphometrics of African citrus psyllids reared on different host plant cultivars.

Trait	PC1	PC2
C+Sc	0.20	0.00
R_1	0.20	0.34
R_2	0.20	0.41
R_s	0.24	0.14
R/R_s to R/R_1	0.03	-0.01
R/R_s to R/M	0.05	0.02
$R+M+Cu_1$	0.13	-0.01
M	0.31	0.11
M_{1+2}	0.09	0.09
A/M_{1+2} to A/M_{3+4}	0.09	0.04
A/M_{3+4} to A/Cu_{1a}	0.08	0.01
Cu_{1a}/A to Cu_{1b}/Cu_1	0.15	0.15
Cu_{1b}/A to Cu_{1a}/A	0.09	0.12
A to A/Cu_{1b}	0.30	-0.05
M_{3+4}	0.06	0.07
Cu_1	0.14	-0.01
Cu_{1b}	0.06	0.03
Wing Width	0.18	0.04
Wing Length	0.54	0.14
Body Length	0.43	-0.77
Thorax Width	0.10	-0.05
Antenna Length	0.10	0.06
Tibia Length	0.11	-0.04

Genal Comb Length	0.00	0.01
Genal Comb Width	0.01	0.00
Head Capsule Width	0.05	-0.01
Eigenvalues	0.13	0.01
Proportions	71.02	7.82

Negative loading for body length indicated a negative correlation between body length and the second component. PERMANOVA revealed significant differences between psyllids reared on different host plant cultivars (N_{perm}= 10,000, $F_{5,89}$ = 16.02, $P < 0.001$) based on linear measurements. According to the CVA results, the first two canonical variates explained 70.86% of the variance (CV1=39.9%, CV2=31%). The most important sources for variation for the first canonical variate were wing (0.07) and body (0.05) lengths, with contributions from A to A/Cu_{1b} (0.04) and M vein (0.04) vein lengths (Table 6.4).

Table 6.4. Coefficients for the first two canonical variates (CV1 and CV2) for analysis of traditional morphometrics of African citrus psyllids reared on different host plant cultivars.

Trait	CV1	CV2
C+Sc	0.03	-0.02
R_1	0.03	-0.01
R_2	0.03	0.00
R_s	0.03	-0.01
R/R_s to R/R_1	0.00	0.00
R/R_s to R/M	0.01	0.00
R+M+Cu_1	0.01	-0.01

M	0.04	-0.02
M_{1+2}	0.02	0.01
A/M_{1+2} to A/M_{3+4}	0.01	0.00
A/M_{3+4} to A/Cu_{1a}	0.01	-0.01
Cu_{1a}/A to Cu_{1b}/Cu_1	0.02	0.00
Cu_{1b}/A to Cu_{1a}/A	0.01	0.00
A to A/Cu_{1b}	0.04	-0.03
M_{3+4}	0.01	0.01
Cu_1	0.02	-0.01
Cu_{1b}	0.01	0.00
Wing Width	0.02	-0.01
Wing Length	0.07	-0.03
Body Length	0.05	-0.03
Thorax Width	0.01	-0.01
Antenna Length	0.01	0.01
Tibia Length	0.01	-0.01
Genal Comb Length	0.00	0.00
Genal Comb Width	0.00	0.00
Head Capsule Width	0.01	0.00
Eigenvalues	4.28	3.32
Proportions	39.89	30.97

In the second canonical variate the A to A/Cu_{1b} (-0.03), wing length (-0.03) and body length (-0.03) were the most important loadings (Table 6.4). CVA yielded noticeable separation between ACT groups reared on the different host plants (Figure 2).

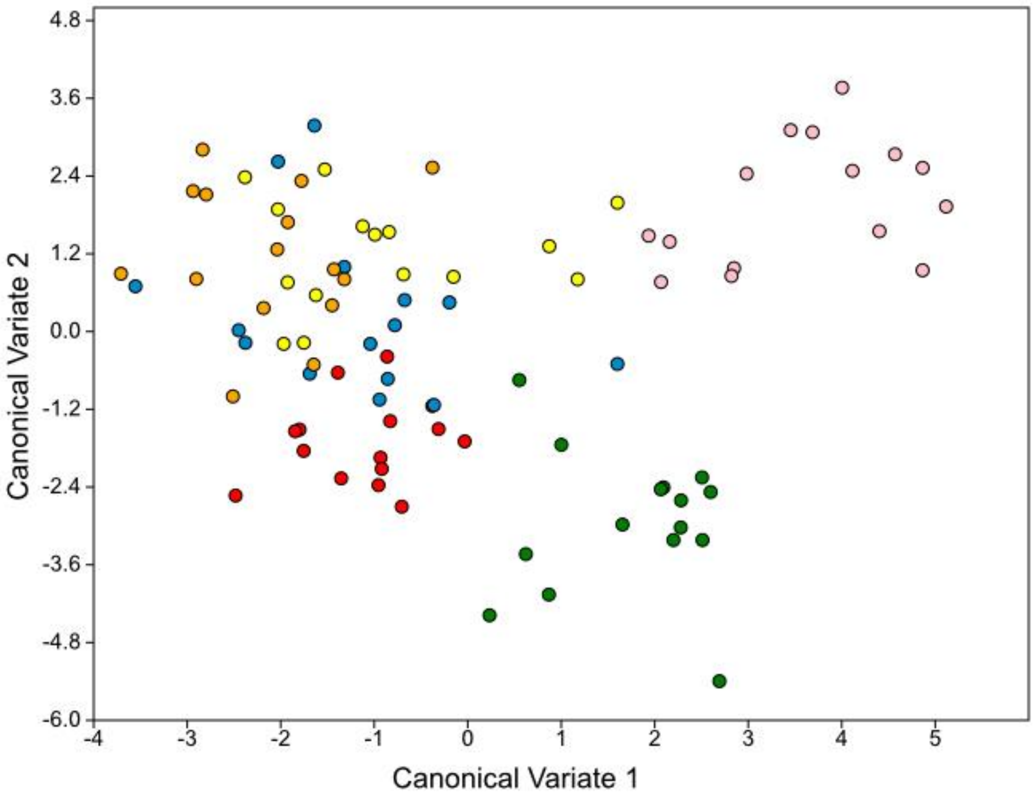

Figure 6.2. Scatterplot depicting the first two canonical variates of a canonical variate analysis of distance variables of female African citrus psyllids reared on different host plant species (*Murraya koenigii*=red, *Clausena anisata*=blue, *Citrus limon*=green, *Citrus sinensis* 'Valencia'=yellow, *Citron cirus* spp.=pink, *Citrus tangelo*=orange).

Trioza erytreae reared on *Citroncirus* spp. were most noticeably separated from the remainder of the psyllids (Figure 6.2), primarily due to body length and wing length differences in the first canonical variate (Table 6.4). ACT reared on *C. sinensis*'Valencia', *C. limon, C. tangelo*, *C. anisata* and *M. koenigii* were loosely separated (Fig. 6.2), with contributions of this separation based on body length, wing length, and the A to A/Cu_{1b} vein lengths as indicated by the loadings of the first canonical variate. There was no significant difference between wing aspect ratios of the psyllids reared on the different host plants (Table 6.2). Mahalanobis distances were significantly higher for ACT reared on *Citroncitrus* spp. compared to those reared on other host plant species (Table 6.5).

Table 6.5. Mahalanobis distances based on traditional morphometrics among female African citrus psyllids reared on different host plants (BK = *Murraya koenigii*, CA = *Clausena anisata*, CL = *Citrus limon*, CS = *Citrus sinensis* 'Valencia', CC = *Citroncirus* spp., CT = *Citrus tangelo*).

Cultivar	BK	CA	CL	CS	CC	CT
BK						
CA	15.40					
CL	17.51	28.14				
CS	19.22	17.39	27.06			
CC	37.21	32.56	31.24	28.18		
CT	14.17	14.02	34.25	8.47	35.52	

A posteriori classification indicated that 93.3% of the psyllids were correctly classified to the proper host plant on which they were reared, however, jackknife cross-validation indicated that only 66.7% of them were correctly classified (Table 6.6).

Table 6.6 *A posteriori* classification summary for discriminant analysis of traditional morphometric measurements for African citrus psyllids reared on different host plants (BK = *Murraya koenigii*, CA = *Clausena anisata*, CL = *Citrus limon*, CS = *Citrus sinensis* 'Valencia', CC = *Citroncirus* spp., CT = *Citrus tangelo*).

Cultivar	Total N	Classified Correctly		Misclassified		Number of specimens misclassified					
		N	%	N	%	BK	CA	CL	CS	CC	CT
BK	15	14	93.33	1	6.67	-	1	0	0	0	0
CA	15	14	93.33	1	6.67	0	-	0	1	0	0
CL	15	14	93.33	1	6.67	0	0	-	1	0	0
CS	15	13	86.67	2	13.33	0	0	0	-	0	2
CC	15	15	100	0	0	0	0	0	0	-	0
CT	15	14	93.33	1	6.67	0	1	0	0	0	-
Total	90	84	93.33	6	6.67	0	2	0	2	0	2
Jackknife cross-validation											
BK	15	10	66.67	5	33.33	-	2	1	0	0	2
CA	15	7	46.67	8	53.33	3	-	1	3	0	1
CL	15	10	66.67	5	33.33	3	1	-	1	0	0
CS	15	8	53.33	7	46.67	0	0	1	-	3	3
CC	15	15	100	0	0	0	0	0	0	-	0
CT	15	10	66.67	5	33.33	1	2	0	2	0	-
Total	90	60	66.67	31	34.44	7	5	3	6	3	6

6.5.2 Geometric morphometrics

Individual variation was 1,198 times greater for size and 37 times greater for shape than measurement error due to landmark placement, indicating that the measurement error was small compared to the biological variation (Table 6.7).

Table6. 7. Procrustes ANOVA for the determination of error of the centroid and shape of female African citrus psyllids with respect to landmark placement for geometric morphometric analysis.

Effect	SS	MS	df	F	*P*
Centroid					
Individual	2221061.60	26130.14	85	1198.26	< 0.0001
Residual	1875.39	21.81	86		
Shape					
Individual	0.1445	0.0001	2550	37.29	< 0.0001
Residual	0.0039	0.0000	2580		

The ordination method of CVA revealed significant differences between the confidence ellipses of psyllids reared on the different host plants (Figure 6.3). Confidence ellipses of psyllids reared on different host plants were clearly separated between each host plant except for *C. sinensis*'Valencia' and *C. anisata* which displayed partial overlap. Psyllids reared on *Citroncirus* spp. were the most separated along the first canonical variate (Fig. 6.3). The remaining psyllids reared on *M. koenigii*, *C. anisata*, *C. limon*, and *C. sinensis* 'Valencia' were separated along the second canonical variate (Figure 6.3).

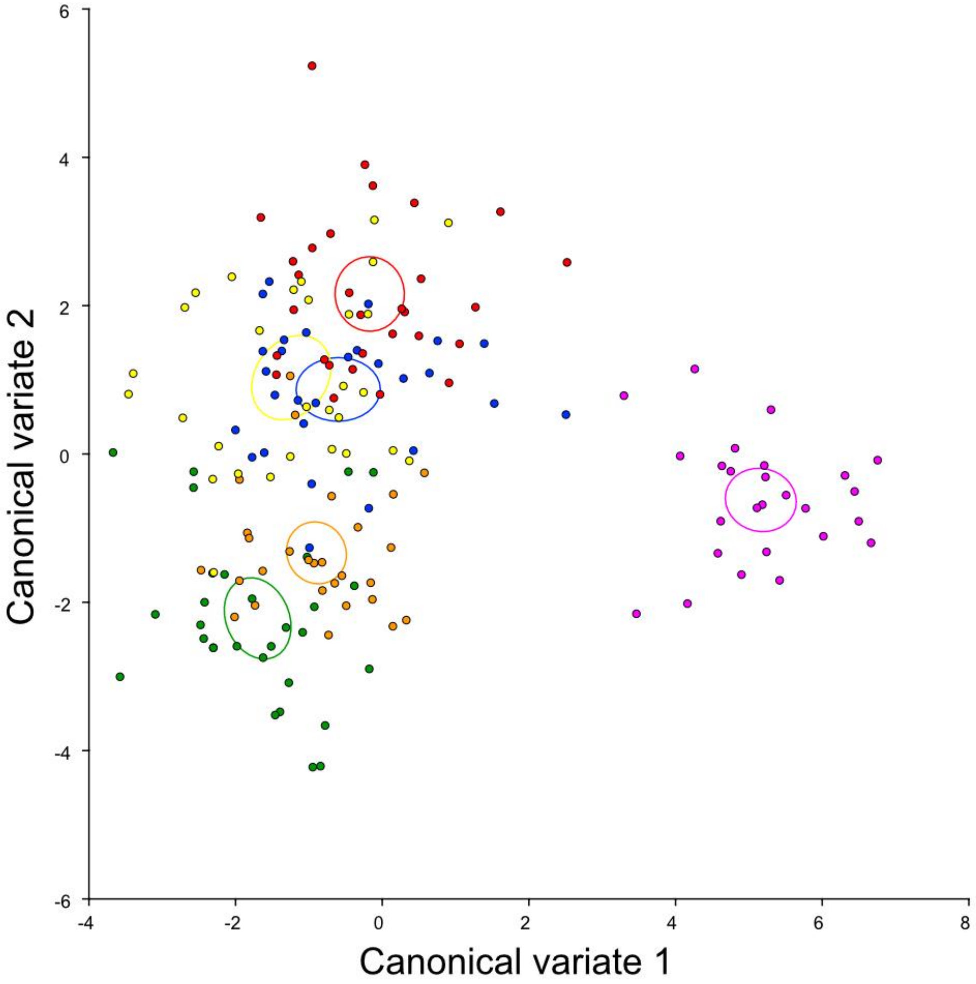

Figure 6.3. Scatterplot depicting the first two canonical variates of a canonical variate analysis of geometric morphometric data for wing shape variation of female African citrus psyllids reared on different host plant species (*Murraya koenigii*=red, *Clausena anisata*=blue, *Citrus limon*=green, *Citrus sinensis* 'Valencia'=yellow, *Citron cirus* spp.=pink, *Citrus tangelo*=orange). Confidence ellipses (95%) represent means of wing shape.

Shape differences between psyllids reared on the different host plants were also noted, with significant comparisons based on Mahalanobis distances and Hotelling's T^2 tests (Table 6.8).

Table 6.8. Mahalanobis distances between populations of female African citrus psyllids reared on different host plant cultivar using geometric morphometric measurements. *P*-values for Hotelling's T^2 tests with 10,000 permutations are in parentheses (BK = *Bergera koenigii*, CA =

Clausena anisata, CL = *Citrus limon*, CS = *Citrus sinensis* ‘Valencia’, CC = *Citroncirus* spp., CT = *Citrus tangelo*).

Cultivar	BK	CA	CL	CS	CC	CT
BK						
CA	3.14 (< 0.001)					
CL	4.95 (< 0.001)	3.90 (0.001)				
CS	3.87 (0.04)	3.04 (< 0.001)	4.08 (< 0.001)			
CC	6.31 (< 0.001)	6.18 (< 0.001)	7.12 (< 0.001)	6.85 (< 0.001)		
CT	4.07 (< 0.001)	3.52 (0.09)	3.05 (< 0.001)	4.07 (< 0.001)	6.45 (< 0.001)	

The average shape of psyllid wings reared on different host plants was produced as warped outline drawings using the thin-plate spline in MorphoJ. To visualize these changes, the warped outline drawings of individual plants were superimposed over the average warped outline of all host plants (Figure 6.4). Wings of *Citroncirus* spp. and *M*. *koenigii* were narrower than the average wing shape of psyllid wings (Figure 6.4). Wings of psyllids reared on *C. anisata*, *C. limon*and *C. sinensis*‘Valencia’ were slightly broader than the average shape of psyllid wings (Figure 6.4).

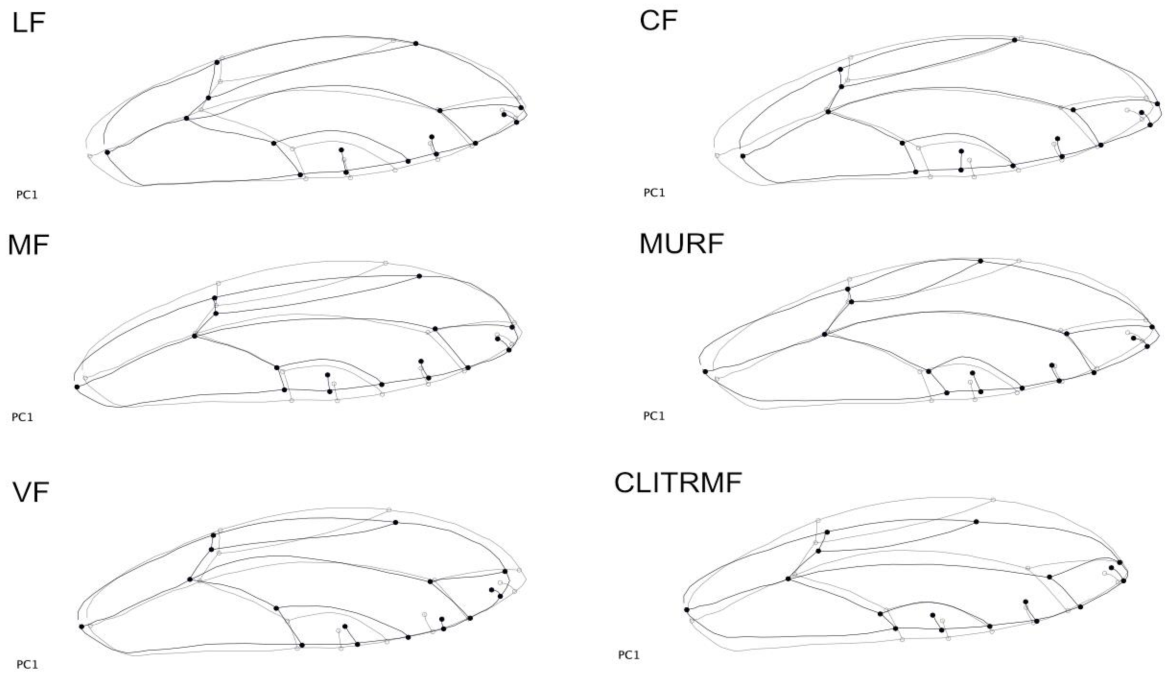

Figure 4. Wireframe visualizations of the average wing shape variation of the first principal component of female African citrus psyllids reared on different host plants. The black lines of the wings occurring on each host plant show the shape changes from the average shape (gray line) of all African citrus psyllids measured. (A) LF=*Citrus limon*, (B) CF=*Clausena anisata*, (C) MF=*Murraya koenigii*, (D) MURF=*Citrus tangelo*, (E) VF=*Citrus sinensis* 'Valencia', (F) CLITRMF=*Citroncirus* spp.

6.6 Discussions

Traditional morphometrics has been useful in studying growth and evolution as well the genetic basis of variations in phenotype (Klingenberg 2002; Reeve et al. 2000), geometric morphometrics utilizes configuration of landmarks and possesses a strong statistical power and is more robust due to its ability to detect a small morphological change which could have gone undetected using a less powerful approach (Klingenberg 2002). In our present study, we showed that both geometric and traditional morphometrics could be useful in separating ACT species. For instance, ACT adults undergoing different pesticide application regimes, sampled from different agro-ecological zones. These significant morphometric differences potentially could play a significant role in fecundity, dispersal, and fitness (Hassanalian et al. 2017). Both methods have been previously used to group ACP, the Asian counterpart from field populations (Lashkari et al. 2015)and populations raised on different host plants under laboratory conditions (Paris et al. 2016).

This finding is consistent with some demographic analyses where fitness was assessed for the ACP (Paris et al. 2016). The potential differences in the nutritional value of ACT host plant species found in the field compared to those offered in our laboratory study could help to explain the varying size between parents and offspring fed on similar host plants. The importance of size and shape with regard to psyllid dispersal are still unresolved. An evaluation of flight duration of ACP on a flight mill did not find a correlation between wing length and flight duration (Arambourou et al. 2017). A study of seven insects examined wing shape to determine aerodynamic performance, found that bumble bee and honey bee were the best at providing forward thrust (Bayles et al. 2017). How the psyllid wing shape compares to insect wings that have superior aerodynamic qualities remains largely unknown. Only individuals having the same wingspan and surface area wings were used in the said study (Bayles et al. 2017). As a result, the impact of natural variation on performance was not observed and no intraspecific differences could be tested for fitness or dispersal capabilities. In another study, not only did rising temperature decrease wing size, development times and flight duration of the blue-tailed damselfly, *Ischnura elegans* (Vander Linden) (Odonata: Coenagrionidae) also played a significant role (Gottwald et al.

2007). Future studies are needed to evaluate the natural variation of wing size and shape on ACT dispersal ability.

Dispersal of ACP occurs between managed groves through habitats that do not contain cultivated citrus and to islands as far away as 470 Km (Lewis-Rosenblum et al. 2015; McCulloch et al. 2017; Paris et al. 2016; Rundle et al. 2007). Their dispersal throughout Florida can be pieced together by incidence data on the presence of "*Candidatus* Liberibacter asiaticus" in citrus groves, which indicates that ACP individuals traveled at least 88 to 144 km (Moran and Blowers 1967). Furthermore, ACP dispersion in southern California, also deciphered through disease incidence reports, indicates that dispersal occurred anisotropically and in a nonrandom manner(Gottwald et al. 2007). The widespread distribution of ACP compared to the more geographically restricted ACT may be indicative of certain morphometric variations that are suitable to dispersal and may prove useful in identifying potential management solutions for the two psyllid species. Using biogeographical data, mayfly, stonefly, and damselfly distribution of the genus *Enallagma* was correlated with wing size and area (Catling 1973; van den Berg and Deacon 1988; Whitman and Vincent 2008). Similarly, future studies should explore possible correlations between wing shape of psyllid wings and dispersal capabilities.

Our study showed that in every measurement analyzed all female traits were significantly larger than those of males. The body size, wing length, and width measurements were consistent with previous studies (Dial et al. 2008; Moran 1968). However, Cocuzza et al. (2017), reported slightly higher means for male antennal lengths than we found in our study. The influence of different host plants, coupled with biotic and abiotic factors, may play a key role in the morphometric variability of ACT. Yet, the small sample size in our study could have contributed to the lower mean antennal lengths. In the absence of ACT's host plants, adults can disperse with the aid of the prevailing winds to a distance beyond 1.5 Km (Awmack and Leather 2002; van den Berg 1990), with female ACT dispersing significantly further than males(Whitman and Vincent 2008). The authors further indicated that this could enhance their dispersal to establish new localities and infect new and uninfected host plants. However, the role of ACT's wing shape in its dispersal to a longer distance remains unclear. Although, the population of ACT fluctuates in the open field, however, females always dominate (Derr et al. 1981; van den Berg 1990). The latter can probably be attributed to the larger size of females than males. Smaller orthopteran and early instar stages are attacked by invertebrate predators while larger species and advanced instar stages by vertebrate predators; thus, body size also influences both predators and prey tactics (Boggs 1986; Johansson et al. 2009).

ACT reared on *C. limon* and *Citroncirus* spp. were larger than those kept on any of the other host plants/ cultivars tested. In an earlier study, *T. erytreae* preferred *C. limon* for feeding and oviposition over *Vepris undulala* (Th.) Verdoorn et Sm., *C. anisata*, *Fagara capensis* Thunb., and *Calodendrum capense* (L. f.) Thunb (Doyle 1990). Host plant quality, in general, affects the fecundity of herbivorous insects at both individual and population level (Outomuro et al. 2013). Similarly, the quality of citrus shoots affects ACTs phenotype as nymphs developed quicker and bigger on nourished than unnourished leaves (van den Berg 1990). Larger females mate more times than smaller ones (Breuker et al. 2007). In the laboratory, the size of a female insect is usually a good predictor of potential fecundity (Kaufmann et al. 2013; Kjærsgaard et al. 2015; Musundire et al. 2012). However, further studies would be required to evaluate the relationship between body size, fecundity, and survivorship of both the ACT and ACP.

The wing shape of ACT developed on *C. anisata, C. limon, and C. sinensis* “Valencia” wasslightly broader than ACT’s average wing shape. Dispersal and migration abilities of dragonfly (Kaufmann et al. 2013; Kjærsgaard et al. 2015), and butterflies (Berwaerts et al. 2002), were observed to be influenced by wing shape. A positive correlation between tibia length and total body size (Dudley and Srygley 1994; Nava et al. 2007), and the overall number of eggs laid (Hassanalian et al. 2017; Paris et al. 2016) have been earlier reported. The tibia of ACT developed on *C. limon* was longer compared to those kept on other host plants. Higher preference of *C. limon* by ACT compared to other indigenous host plants has been previously reported (van den Berg 1990). It can be speculated that large-bodied adult ACT would be able to fly longer distances than their smaller bodied counterparts as is the case of ACP (Paris et al. 2016), *Scathophaga stercoraria,* and *Aedis aegyepti* (Mozaffarian et al. 2007*)*. In our study, the tibia of ACT developed on *C. anisata* was shorter compared to those kept on the other host plants tested. There was no significant difference in the wing aspect ratios which may indicate that the plants are not affecting the psyllids in terms of flight performance or are affecting the flight performance of the psyllids equally whether that be improving or reducing flight performance. However, wing aspect ratio was significantly lower in females than in males. There have been contradictory reports on the relationship between wing aspect ratio and flight performance (Paris et al. 2016). For instance, acceleration capacity in the male butterfly *Pararge aegeria* was found to be positively correlated with wing aspect ratio aspect ratio (Denno et al. 2000) whereas a different study found a negative correlation between wing aspect ratio and flight speed (Denno et al. 1986).

Several reproductive and population dynamics parameters have been associated with food quality (Nevo and Coll 2001; Paris et al. 2016). The nutritional quality of the host plant affects wing size and shape as shown for instance in the Carob moth *Ectomyelosis ceratoniae* (Zeller), a pest of pomegranate in Iran (Morais et al. 2010). The wing shape and size of potato psyllids varied on different host plants (Wilke et al. 2016). In planthoppers, body length increased in specimens raised on nutritionally superior host plants, and the nutritional effect on body size was greater in females than males (Denno et al. 2000). The wing shape and size and other morphometric characters in our study can potentially be attributed to the varying nutritional quality of the different tested host plant species/ cultivars, though this requires further studies. Nutritional quality of host plants is known to influence the body size(Denno et al. 1986), the performance of herbivores and their parasitoids (Musundire et al. 2012). Moreover, varying nitrogen contents in cotton plants cause variation in body size and colour of *Aphis gossypii* Glover, reared under different nitrogen treatments (Nevo and Coll 2001).

Body length, wing length, M and A to A/Cu_{1b} veins contributed to most of the variations observed in both PCA and CVA while R_1 and R_2 also contributed to some of the variations observed in PCA in the six host plants. A study on ACP found body length, wing length and wing width as the main traits that contributed to the variation of populations of ACP (Lashkari et al. 2015). This could provide baseline information for grouping populations of for further morphometric studies on ACT. Wing shape analysis has been successfully used to distinguish morphologically similar species of mosquitoes (Morais et al. 2010; Wilke et al. 2016) and also to study the origin and population structure of *D. citri* (Lashkari et al. 2015).

6.7 Conclusion

Our study clearly demonstrates host plant species on which ACT nymphal stages feed and develop have a direct effect on their morphometric characteristics, particularly on wing shape and size. The wing shape of ACT reared on *Citroncirus* spp. and *M. koenigii* were narrower compared to those kept on other host plants, while the ones that developed on *C. anisata*, *C. limon* and *C. Sinensis* were slightly broader than the average shape of ACT wings. Thus, ACT reared on *Citroncirus* spp. and *M. koenigii* would likely produce offspring that are less likely to disperse compared to those reared on *C. anisata*, *C. limon* and *C. sinensis* that have an ideal capacity associated with long distance flight. The observed phenotypic plasticity enhances our understanding of morphometric variability associated with host plants. Both the traditional and geometric analyses were effective for the detection of differences in size and shape among ACT reared on different host plant species. Future studies should explore possible correlations between wing shape of ACT and dispersal capabilities as evidenced by known geographic distribution of the insects in biogeography studies. In addition, further studies on ACT are required to evaluate the relationship between body size, fecundity, dispersal, and survivorship. Finally, our study was largely confined to host plants of interest for establishing laboratory colonies and as such, they should be expanded to incorporate citrus species and cultivars commonly used in commercial production.

7 Discussion

7. 1 Past and present management of ACT.

The growing concern of over 60 million trees infected with the citrus greening disease has drawn global attention given that the substantial decline was recorded in Asia followed by Africa and other parts of the world where the disease has been reported. Unfortunately, in Kenya, a thorough study on ACT and the recently reported ACP has received a little attention (Kilalo et al. 2009). For example, during the 1920s – 1970s, ACT was considered as a minor pest in Kenya until 1980s when the citrus greening disease was first discovered and started causing huge losses to the local citrus industry (Garnier and Bové 1996; Rwomushana et al. 2017). Control measures to eradicate the disease and/ or reduce population build–ups focused on the removal of infested/infected leaves, shoots, branches, trees and, pesticide application through trunk injection with tetracycline hydrochloride and direct application of insecticides and fungicides (Kilalo et al. 2009). Despite these management strategies, ACT still remains a major threat to the Kenyan citrus industry.

7.2 Distribution and the sympatric relationship between ACT and ACP

The ACT distribution survey showed that it was confined to highlands as reflected in absence data in the Kenyan lowlands indicating that ACT prefers cool and moist climates (van den Berg 1990). For instance, Kilifi County, characterized by low elevations coupled with prolong drought period and warm climates, is not suitable for ACT's reproduction and survival (Coccuzza et al. 2017). In contrast, a recent report of ACP in Kenya showed a high preference for low altitudes despite its presence also in the mid-altitudes (Rwomushana et al. 2017), which was confirmed in the present study. Although ACT and ACP are both present in Kenya, so far only ACGD has been detected, suggesting that proper management techniques could prevent the introduction of the Asian greening pathogen in Kenya. According to Cocuzza et al. (2017), the presence of ACP in many regions is associated with the movement of infected and infested host plants from the neighboring countries.

7.3 Comparison of sticky card traps for monitoring and surveillance of ACT and ACP

Different sticky card traps were available in the market but had not been compared for effectiveness in monitoring and surveillance of ACT and ACP. The data gathered from the trap analysis confirmed that yellow sticky card traps were more effective than any of the other traps for detecting adults of ACT and ACP. Preference of yellow to green traps has been earlier reported for ACT (Samways 1987b) and ACP (Hall et al. 2010). ACP appeared to be more attracted to the GLMgreen than Asyellow trap type; however, there was no evidence to show that any of the tested traps were more effective in monitoring ACP at low adult densities. Although sticky traps are useful in reducing population build-ups, traps alone are not effective in maintaining citrus greening vector densities below economic threshold levels. Hence, integrating biological and chemical control methods would be economically efficient in managing both greening vector species (Samways 1987b).

7.4 Climate change impacts on ACTs distribution

A combination of factors including suitable habitats, host plant, natural enemies, diseases and heritable traits has been reported to influence the distribution and establishment of a species in a new area (Wallner 1987). However, climate change and elevation are additional crucial factors that influence the distribution and establishment of an organism in a new area. Our species distribution modeling showed that precipitation of the wettest quarter, precipitation of wettest month, mean diurnal range, temperature seasonality and mean temperature of the coldest quarter were the most important factors that influenced ACTs' distribution in Kenya. Also, the possible expansion of ACTs suitable climate areas may favor the ACP. Hence, strict control measures to prevent the spread of both citrus greening vectors to non-infested regions of Kenya are pivotal.

8 Conclusion and Outlook

8. 1 Conclusion

In the past 35 years, the African citrus triozid has become one of the most difficult pests to control. In Kenya, several attempts to manage the pest have met with a little success. Recently, ACTs' resistance to insecticides has been reported in many citrus growing regions. As a prelude to their management and in order to further improve our knowledge, information on the bio-ecology of ACT are required. Also, knowledge about biotic and abiotic factors influencing their populations should facilitate the development of an ecologically friendly IPM strategy for the pest. There is, hence, a real need for extensive investigations on the pests' dynamics, distribution and seasonal abundance on citrus. Although ACT has been observed attacking various citrus species/ varieties and has been associated with ACGD in Kenya, there is no information on its indigenous alternative host plants and indigenous parasitoids.

This study fills the knowledge gap of the biology, ecology, and management of ACT which is crucial for the development of appropriate IPM strategies for the pest. The following relevant conclusions can be drawn:

- Distribution - the degree of damage and areas at risk of invasion study indicates that ACTs' distribution is confined to higher altitudes, suggesting a shift of citrus production to lower altitudes would be one of the best options. However, the recent detection of ACP in Kenya puts this suggestion into question.

- Understanding pest population dynamics is fundamental in developing an appropriate IPM strategy for the pest. ACTs activities varied across different altitudinal gradients, suggesting that management strategies should consider altitudinal gradients.
- A study on host range revealed *Clausena anisata*, *Calodendrum capense*, *Murraya koenigii*, *Zanthoxylum usamberense*, *Vepris bilocularis* and *Teclea nobilis* as alternative host plants of ACT, with *V. bilocularis* and *T. nobilis* here being reported for the first time as host plants of ACT. These plants can support the development of the vector in citrus growing areas where citrus trees or other preferred alternative host plants are absent, an important factor in the dispersal and epidemiology of ACGD.
- Comparison of sticky card traps for monitoring and detection of ACT and ACP demonstrate a potential utility of ‘’Asyellow’’ sticky card traps for especially ACT detection in citrus orchards in Kenya. However, there was no evidence to suggest that any of the traps were particularly suitable for detecting ACP.
- Findings from the shape and size study provide evidence of wing shape and size variations of ACT based on host plant species which might affect the fitness parameters like fecundity, dispersal, and survival of the greening disease vector.

8.2 Outlook

In 2015, a preliminary survey to identify a transect to study ACTs’ population dynamics was conducted in citrus growing regions of Kenya. Surprisingly, orchards present during the preliminary study were not available when this research commenced. The orchards had collapsed due to either heavy infestation by ACT or cut down to cultivate a different crop due to unmarketable fruits. The findings from this research indicate that ACT was widely distributed with greater infestations in the highlands where conditions were more conducive for its reproduction and survival. However, further studies to monitor an expansion of its ecological niche to the low altitudes are warranted.

The use of alternative host plants for food, medicine, and the house fence would make ACTs management very difficult. However, removing these plants within and near citrus orchards would facilitate the development of appropriate management strategies for the pest. Data on ACTs preference of shaded to unshaded trees is very crucial. This information is particularly important for sampling and monitoring purposes. Also, the presence of ACT in shaded than unshaded trees even at low densities suggests that pruning of unwanted new stem shoots would be very important in developing effective IPM strategies for the pest. Also, trees which provide shade for ACT found within the vicinity of an orchard must be cleared

Asyellow and GLMgreen traps should be reviewed and included a scented bait or lure. Investigation into the potential of lure sticky card to predict certain damage parameters like an injury to citrus fruits would be very useful. This information would be relevant in detecting and monitoring ACPs and ACTs invasion. Although GLMgreen trap captured more ACP, there was no clear evidence suggesting that GLMgreen trap was more efficient any of the other traps in detecting ACP. However, adding attractants and killing lures to the trap would improve its effectiveness in detecting ACT.

Data on ACTs seasonal dynamics in different altitudinal gradients are very relevant as this would create awareness of when to expect colonization of orchards by ACT. The population dynamics of ACT varied across the different altitudinal gradients indicating the need to develop different monitoring and management schedules according to altitude. In addition, another study on the seasonal fluctuation of alternative host plants in relation to citrus would add to the body of knowledge of ACT.

A follow-up study on insect morphometry to determine the role of host plants on fitness parameters like fecundity, survival, and dispersal would help develop an appropriate IPM strategy for the pest. Also, a future study to evaluate the chemical composition of each of the tested host plant would help develop an attractant for the pest.

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