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Diversity and abundance of fruit flies (family: *Tephritidae*) in the Albertine rift zone, Democratic Republic of the Congo, and preliminary prospects for biological control

Abstract

Since the invasion of the African continent by the oriental fruit fly, *Batrocera dorsalis* (Hendel) (syn. *B. invadens* [Drew, Tsuruta & White]), tephritid fruit flies have become a growing problem in the Democratic Republic of the Congo (DRC). Very few studies on these species have been conducted, and none in the South Kivu region. We carried out a preliminary survey of tephritids in the South Kivu which is part of the Albertine Rift zone. Both para-pheromone and food bait lures were used. *Bactrocera dorsalis* (70.6%) and *Ceratitis fasciventris* Bezzi (22.4%) were the most abundant species. We also recorded the species *B. cucurbitae* (Coquillett), *Perilampsis curta* Munro, *C. anonae* Graham, *C. cosyra* (Walker), *C. punctata* (Wiedemann), *C. rosa* Karsch, *Carpophthoromyia vittata* (Fabricius), *Dacus (Leptoxyda) eminus* Munro , *D. bivittatus* (Bigot), *D. punctatifrons Karsch*, *D. (Lophodacus) hamatus* Bezzi, *D. (Dacus) hargreavesi* Munro, and *D. (Leptoxyda) siliqualactis* Munro. In addition, we observed the occurrence in the study area of a weaver ant, *Oecophylla longinoda* Latreille, a predacious ant known as a biological control agent in some African orchards. We present here the first record of *B. dorsalis* in fruit orchards in South Kivu, DRC, and suggest that *Oecophylla longinoda* could be used as an important part of an IPM program for the control of fruit flies in orchards, and for reducing pesticide usage. The results also highlight future research avenues for improving fruit production in this area.

Keywords: perilampsis, orchards, dacus, bactrocera invadens, oecophyilla longinoda, bactrocera dorsalis

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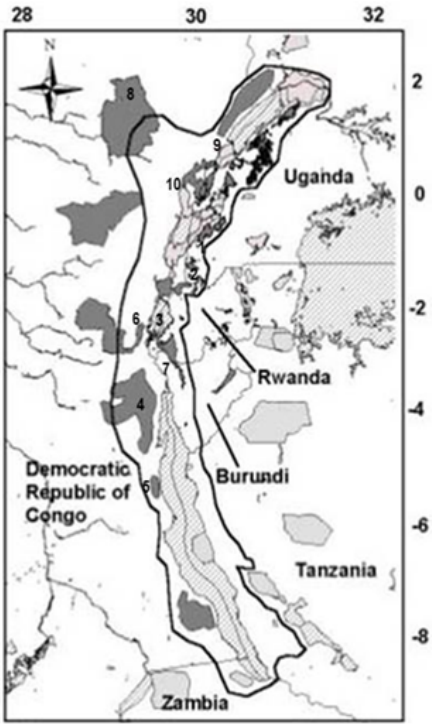
Introduction

Invasions of polyphagous fruit flies (Diptera: *Tephritidae*) cause important losses in fruit crops in tropical and subtropical areas worldwide, despite stringent quarantine controls.[1] In South-East Asia, species from the genus *Bactrocera* Macquart has been identified.[2–4] Fruit and vegetable production is one of the fastest growing agriculture sectors in Africa, providing both income and employment to growers and exporters. Since the invasion of the African continent by *Bactrocera dorsalis* (Hendel) (syn. *B. invadens* [Drew, Tsuruta & White]) fruit flies have become a growing concern, especially with respect to mango production.[5] The Family *Tephritidae* (true fruit flies) includes about 4300 described species in almost 500 genera. The larvae of most species develop in the seed-bearing organs of plants, including many commercially grown soft fruits and vegetables. This family is represented in all continents of the world except Antarctica. The major pest genera are mainly restricted to particular geographical regions, but because of increasing international movement of fresh produce and people, tephritid invasions are increasing.[6,7] found that all material examined from the East Asian (China, Thailand, Peninsular Malaysia, Taiwan, Indonesia and Philippines), African (Benin, Democratic Republic of Congo, Mozambique, Sudan and Kenya) and Indian subcontinent (Pakistan, Nepal, India and Sri Lanka) previously identified as *B. invadens* is genetically similar to *B. dorsalis* from Asia. They concluded that *B. carambolae* is a valid species and that the remaining taxa, *B. dorsalis*, *B. invadens* and *B. papayae*, represent the same species. Thus, we consider *B. dorsalis* (Hendel) as the senior synonym of *B. papayae* Drew & Hancock and *B. invadens* Drew, Tsuruta & White. Only a few studies have been published on *Tephritidae* of the Democratic Republic of the Congo (DRC)[8–11] The Albertine Rift area, where this study was conducted, is a biodiversity-rich area within the Great Lakes region in Central Africa, acknowledged for its fauna and flora Kuper et al. 2004;[12,13] The Albertine Rift is not only important for its biodiversity but also for its ecological processes and ecosystem services. The savanna parks contained some of the highest biomasses of largemammals recorded on earth in the 1960s.[14] War and poaching have led to major decreases in the numbers of large mammals in these parks but most of the species are still present and could recover to former levels with good protection.[13] The impacts of the browsing and grazing of the elephants, hippopotamuses, buffalos, and antelope species had amajor influence on the vegetation of the parks[15] and as a result of the decline innumbers of these species it is thought the parks are changing.[16] The volcanoes in the Virunga National Park are active and influence the ecology of a large portion of this park and its surroundings. The fisheries in some of the lakes are the most productive on the continent and provide a livelihood for many people.[17,18] and the rivers and streams flowing from the forests on the mountains provide clean water. In Rwanda, for example, it is estimated that more than 70% of people obtain water that comes from their national parks.[19] The spectacular land formations and rich biodiversity of the Albertine Rift mean that it has great potential for tourism. Civil wars and international conflict over the past 30 years have hampered tourism development but when peace comes to the region there is enormous potential to develop world class tourism.[13,20] The Albertine Rift has no precisely defined boundaries. The northern end is usually taken to include all of Lake Albert which ends at the

beginning of the Albert Nile (2°21' N 31°27' E). The southern end is usually taken to include most, but not all, of Lake Tanganyika, terminating at about the latitude of the Marungu Highlands (7°30'S). The Rift runs north-south with a westerly arc and provides a natural border for Democratic Republic of Congo and its western neighbors, Uganda, Rwanda, Burundi, and Tanzania. Some observers include all of Lake Tanganyika within the Albertine Rift and thus Zambia might be considered a sixth nation with Albertine Rift territory.[21]

The World Wide Fund for Nature (WWF) qualified it as an "Eco-area", and Conservation International termed the Albertine Rift "a hot point of biodiversity". It extends from the Blue Mountains in the north (in Ituri district of the Eastern Province of the DRC; 2°N, 30°30'E) down to the extreme south of Lake Tanganyika (9°S, 30°30'E) in Figure 1.[22] Considering this biodiversity it is necessary to develop methods of controling fruit flies that are compatible with the environment and safe to humans, while also providing a cost-effective control solution. Therefore, pesticides or baits are not a solution, as the former can endanger the biodiversity of the area, and both are too expensive for local smallholder farmers.[5] Alternatively, biological control using endemic species that naturally occur in fruit orchards could offer an economically and sustainable way of controling fruit flies. Two species of predatory weaver ant, *Oecophylla longinoda* (Latreille) and *Oecophylla smaragdina* (Fabricius) (Hymenoptera: Formicidae) have been used either in Africa, Asia or Australia to protect orchards from various pests including fruit flies (*e.g.* for citrus orchards:[23–25] for mango orchards:[25–29] for coconut orchards: Vanderplank 1960; for cocoa orchards: Room 1971;[30] for cashew orchards:[31–33] The efficacy of the ants is attributed to predation on larval instars, and repellency and disturbance of adult fruit flies during oviposition. To be effective the ants must be conserved in the orchard and pesticides should be avoided.[31] These weaver ants nest in the foliage of trees, shrubs and large herbs.[34–36] Our study aimed at identifying the fruit fly species present and their relative abundance in orange, tangerine and mango orchards in the South Kivu region to identify the key pest species. We also determined the natural occurrence of *O. longinoda* in these orchards to assess whether this biological control agent could potentially be used in a fruit fly IPM programme in the DRC.



Site 1: Bwindi Impenetrable National Park, site 2: Echuya Faunal Reserve and Mgahinga Gorilla National Park, site 3: Idjwi Island, site 4: Itombwe forest, site 5: Mount Kabobo, site 6: Kahuzi – Biega National Park, site 7: Kibira National Park, site 8: Okapi faunal Reserve, site 9: Ruwenzori Mountains National park, site 10: Mount Tshiabirimu.
In some instances, sampling sites in geographic close proximity and with no obvious geographic discontinuities separating them were considered a single locality; these sites are circled (Map adapted from Plumptre et al. 2003).

Figure 1 Geographic placement of the Albertine Rift (in white shading) indicating the geographic sampling sites included in the study.

Material and methods

The survey of fruit fly species was carried out in private orchards of *Mangifera indica* L.(*Anacardiaceae*), *Eriobotrya japonica* (Thunb.) Lindley (Rosaceae), *Citrus sinensis* Osbeck. (*Rutaceae*), *Averrhoa carambola* L. (*Oxalidaceae*), *Psidium guajava* L. (*Myrtaceae*) located in the Irhambi-Katana area of Kabare and for the weaver ant, *Oecophylla longinoda* was carried out in the private orchards of *Mangifera indica* L. (*Anacardiaceae*), *Citrus sinensis* Osbeck. and *Citrus reticulata* Blanco (*Rutaceae*) located in the Kiringye and Sange area of Ruzizi plain, South Kivu, in the Albertine Rift zone of eastern DRC. Irhambi-Katana is located at 2°13'30" N; 28°49'53" E, and is 1654m above sea level and Kiringye and Sange are located at 2°53'29.6"S ; 29°59'49.0" E , and 917m above sea level 3°05'28.6"S; 29°07'43.4"E, and is 916m above sea level. The fruit fly trapping method was based on the guidelines in IAEA.[37] Five types of lures in McPhail traps were used. Three were para-pheromones attractive to only male fruit flies –Methyl eugenol, for *B. dorsalis* is one of many species whose males are attracted while other males of some other *Bactrocera* species are attracted to cue lure;[38] and trimedlure, for Mediterranean fruit fly, *Ceratitis capitata* (Wied.), mango fruit fly, *C. cosyra* (Walker) and Natal fruit fly, *C. rosa* Karsch. Two attractants were food baits–liquid protein hydrolysate, Nu-Lure (Miller Chemical and Fertilizer Corporation, Hanover, USA), and a terpene, terpinyl acetate, attractive to a wide variety of insects of both sexes.[39] Food baits are not species-specific and are known to have a lower attraction

to fruit flies than male lures.[6] All the para-pheromones and one of food bait (terpene, terpinyl acetate) were used in combination with insecticide DDVP (2, 2-dichlorovinyl dimethyl phosphate) strip that were placed at the bottom of a McPhail trap to kill any attracted flies. The five types of lures were separately placed in trees of five different fruit kinds (depending on availability), and repeated two times over orchard of Kabare. Traps in the same orchards were separated by at least 50 meters. The protein bait was changed after two weeks and the four other lures after two months. All tephritid fruit flies were collected bi-weekly from 7 January until 7 August 2014 and placed in vials containing isopropyl glycol. The flies were identified at the Research Centre for Natural Sciences (CRSN, Lwiro) and when positive identification was not possible, specimens were sent for identification to the Royal Museum for Central Africa (Tervuren, Belgium). The McPhail traps remained in place for six months during the fruiting season of mango (January 2014 to August 2014). Nest observations of weaver ants were made during the flowering period of mango and citrus, when ants are searching for new food sources, new territory and migration to new areas,[40] thus, then the nests are still in the trees when the fruit fly populations are high *i.e.* during 3 months (July 2014 to September 2014). Nests of weaver ants were counted in 15 trees per orchard at Ruzizi plain. The method of observation was completely random (unrestricted random sampling).[41] The observations were made during by two persons and those persons searching for nests for 3 hours each day in the morning from 5: am for 6 days per week *i.e.* 18 hours per week and 216 hours for the 3 months.

The relative abundance and frequency of fruit fly species trapped were calculated by the following formulae**:**

$$\text{Relative abundance} = \frac{\text{Total number of fruit flies of one species trapped}}{\text{Total number of species trapped}}$$

$$\text{Frequency} = \frac{\text{Total number of fruit flies caught for one species trapped}}{\text{Total number of fruit flies of all species trapped}} x100$$

The use of the documentation and the internet: Numerous books describe attacks on fruit trees. They are used to verify the diagnosis in a systematic way.[42] A brief literature review has identified a number of publications on fruit fly over the whole of the Democratic Republic of Congo.

Statistical analyses

Statistical analyses were carried out on the five most abundant fruit fly species. A one-way ANOVA was carried out to compare the number of African weaver ant nests in the different orchards of *Mangifera indica* L. *Anacardiaceae*), Citrus sinensis Osbeck. (*Rutaceae*), Citrus reticulata Blanco (Ruzizi plain), and pairwise comparison of Oecophylla longinoda nests per tree species, mixing all orchards (Fisher LSD Method) and for the PCA figure were carried out by using the SigmaPlot software.

Results

Fruit fly species recorded

Table 1 summarizes the total number of flies collected during the six months of the experiment till January 2014 to August 2014 , a total of 6695 tephritid fruit flies with a high number of 4736 for Bactrocera species (5 Bactrocera cucurbitae, 4730 Bactrocera dorsalis and 1 Bactrocera mesomelas), after comming the Ceratitis species with 1779 (1498 Ceratitis fasciventris, 145 Ceratitis cosyra, 129 Ceratitis anonae, 3 Ceratitis capitata and 2 species respectively for Ceratitis rosa and Ceratitis punctacta), again comming Dacus species with 177 start with Dacus bivittatus (159), Dacus punctatifrons (11), Dacus eminus (4) and respectively 1 specie for Dacus hargreavesi, Dacus siliqualactis and Dacus (Lophodacus) hamatus , so that comming 2 Perilampsis curta and 1 Carpophthoromyia vittata. Concerning the relative abundance Bactrocera dorsalis are more important (0.998) and has a high frequency (70.649 %) after comming Dacus bivittatus with 0.898 of abundance, 2.374 % of frequency again comming Ceratitis fasciventris with 0.842 of abundance , 22.374% of frenquency so that coming Ceratitis cosyra with 0.081of abundance, 2.165% of frequency, Ceratitis anonae with 0.072 of abundance and 1.926 % of frenquency and at the end coming Dacus punctatifrons with 0.062 of abundance and 0.164 % of frenquency. The Figure 2 shows five most species abundante Bactrocera dorsalis , Ceratitis fasciventris, Dacus bivittatus , Ceratitis cosyra ,Ceratitis anonae. African weaver ant, *O. longinoda* The number of nests of *O. longinoda* per fruit kind for the mixing all orchards shows difference between orange, tangerine and mango orchards i.e.; orange orchard has 6 nests, tangerine 3 nests and mango 13 nests. The ANOVA of the sample is presenting at the Table 2. Again this Table 2 shows the most difference between the number of ants nests in orange, tangerine and mango species, i.e. the number of Oecophilla nest differed between those species in the orchards: In areas occupied by *O. longinoda*, well-defined paths over fallen branches and fronds were used by the workers to connect neighbouring trees that contained nests, thus the pairwise comparison. The pairwise comparison by Fisher LSD Method showed that orange and tangerine species had the similar numbers of mean nest and mango had a high number

Table 1 Relative abundance and frequency of the different species of fruit flies captured during the experiment

Species	Total number captured	Relative abundance	Frequency (%)
Ceratitis capitata	3	0.001	0.044
Ceratitis rosa	2	0.001	0.029
Ceratitis fasciventris	1498	0.842	22.374
Ceratitis cosyra	145	0.081	2.165
Ceratitis anonae	129	0.072	1.926
Ceratitis punctacta	2	0.001	0.029
Bactrocera cucurbitae	5	0.001	0.074
Bactrocera dorsalis	4730	0.998	70.649
Bactrocera mesomelas	1	0	0.0 14
Dacus bivittatus	159	0.898	2.374
Dacus punctatifrons	11	0.062	0.164
Dacus eminus	4	0.022	0.059
Dacus hargreavesi	1	0.005	0.014
Dacus siliqualactis	1	0.005	0.0 14
Dacus (Lophodacus) hamatus	1	0.005	0.0 14
Carpophthoromyia vittata	1	-	0.0 14
Perilampsis curta	2	-	0.029

Literature review of fruit fly in Democratic Republic of Congo

Munro published in 1938 and 1962 about quelques diptères trypétides du Congo Belge avec descriptions d'espèces nouvelles and, again the Mission zoologique de l'Institut de Recherche Scientifique en Afrique Centrale (I.R.S.A.C.) en Afrique orientale, Diptera *Trypetidae*. The study of Virgilio et al.,[10] was about a quantitative comparison of frugivorous tephritids (Diptera: *Tephritidae*) in tropical forests and rural areas of the Democratic Republic of Congo and Rubabura et al.,[11] studied the invasive fruit fly, Ceratitis species (Diptera: *Tephritidae*), pests in South Kivu region, eastern of Democratic Republic of Congo.

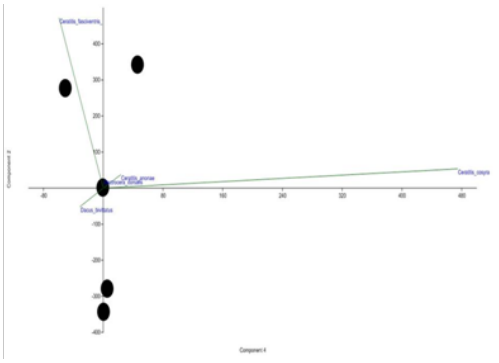


Figure 2 PCA of five most species abundante

Table 2 ANOVA of *Oecophylla longinoda* nests per tree species

Source of variation	DF	SS	MS	F	P
Between Groups	2	729.733	364.866	9.507	<0.001
Residual	42	1611.929	38.379		
Total	44	2341.661			

Comparison O. *longinoda* nests	Diff of means	LSD (alpha=0.050)	P	Diff >= LSD
Mango vs. Tangerine	9.467	4.565	<0.001	Yes
Mango vs. Orange	7.133	4.565	0.003	Yes
Orange vs. Tangerine	2.333	4.565	0.308	No

Table 3 List of fruit flies species in eastern recorded in Democratic Republic of Congo in the article of Virgilio et al.,[10] and in this study

Species	Virigilio et al.,[10]	This study
Dacus bivittatus (Bigot), 1858	*	*
Dacus punctatifrons Karsch, 1887		*
Dacus humeralis Bezzi, 1915	*	*
Dacus fumosus Collart, 1935	*	
Dacus langi Curran, 1927	*	
Dacus ciliatus Loew, 1862	*	
Dacus gypsoides Munro, 1933	*	
Dacus radmirus Hering, 1941	*	
Dacus setilatens Munro, 1984	*	
Dacus (Leptoxyda) eminus Munro,1939		*
Dacus(Dacus) hargreavesi Munro,1939		*
Dacus (Leptoxyda) siliqualactis Munro,1939		*
Daus (Lophodacus) hamatus Bezzi, 1917		*
Bactrocera dorsalis Hendel, 1912	*	* (Dominant species)
Bactrocera cucurbitae (Coquillet),1899		*
Bactrocera (Gymnodacus) mesomelas Bezzi,1908		*
Carpophthoromyia dividua Meyer, 2006	*	
Carpophthoromyia pseudotritea Bezzi, 1918	*	
Carpophthoromyia tessmanni Enderlein, 1920	*	
Carpophthoromyia vittata (Fabricius), 1794		*

Table Continued...

Species	Virigilio et al.,[10]	This study
Ceratitis capitata (Wiedemann), 1824		*
Ceratitis cosyra (Walker), 1849		*
Ceratitis rosa Karsch, 1887		*
Ceratitis fasciventris Bezzi, 1920		*
Ceratitis anonae Graham, 1908		*
Ceratitis punctacta (Wiedemann), 1824		*
Perilampsis curta Munro, 1938		*
*Present		

Discussion

We found *B. dorsalis* to be the most abundant fruit fly species in the Albertine Rift area. *B. dorsalis* has been recorded in the African continent since 2003.[43] It attacks more than 30 hosts plants species, although mango, Mangifera indica L., is clearly the preferred host;[5,44–47] The *B. dorsalis* is regarded as the most economically important and arguably one of the most important pest species in world agriculture (Clarke et al. 2005; Drew et al. 2005). Again, Vayssières et al.,[48] reported that the increase in population of *B. invadens* appeared to be directly linked to the ripening of different mango cultivars. In general, host availability and abundance are among the factors determining the population fluctuations of Bactrocera species.[49–25] as well as other fruit fly species.[53,54] Vayssières et al.,[46] also reported that the population of *B. invadens* increases with rise in temperature and rainfall. Rainfall can affect plant phenology and nutrient quality for insects[55] and is among the factors causing the rapid increase of various Bactrocera species,[56] while low population for many species has been attributed to drought through its effect on fruiting of plant species.[57] Abiotic and biotic factors affecting abundance and distribution of tephritids have been reviewed by Duyck et al.,[58] The population fluctuation and invasive behaviour of *B. invadens* suggest the species is an rstrategist (rapid population growth and colonization of new habitats, rapid decline in populations during unfavourable conditions, etc.).The species is widely acknowledged as either a serious agricultural pest where it occurs, or as a high level quarantine threat in countries where it is absent but capable of invasion and establishment. Clarke et al.,[1,6] *B. dorsalis* and *B. carambolae* have also invaded and become established in the Pacific,[59] South America[60] and Africa.[43] The other 16 species recorded in this study were less dominant. Although widely distributed in the sub-Saharan region, the Mediterranean fruit fly, *C. capitata*, appears to be a less serious pest of cultivated fruit along the equatorial belt of Africa than other Ceratitis species.[61]

In Table 3 above, we compare the species recorded by Virgilio et al.,[10] and those trapped during our study. Virgilio et al.,[10] studied fruit fly species in two different habitats (pristine and disturbed) and at four locations in the Eastern Province of the DRC (Congo, Lomami, Aruwimi, Itimbiri). And Rubabura et al.,[11] show that concerning caught per Ceratitis individual in South Kivu, Ceratitis fasciventris (1498 individuals), Ceratitis cosyra (145 individuals), Ceratitis anonae (129 individuals), Ceratitis capitata (3 individuals) and 2 individuals respectively for Ceratitis rosa and Ceratitis punctacta. Copeland et al. in 2006, in their study, showed that *C. fasciventris* had the most data for both native (n 20) and exotic (n 9) plant hosts. *C. fasciventris* also was distributed widely throughout the central Kenya highlands, collected at elevations of up to 2,220 m, but it was absent from the coast. Ceratitis fasciventris was the dominant Ceratitis species captured during the entire experiment, biweekly. *C. fasciventris* had the greatest diversity of hosts, both in terms of plant species and families.[62] Three species were common to both studies: *D. bivittatus*, *D. punctatifrons* and *B. dorsalis*, all in Mugeri-Mwanda village, near Lake Kivu. The sampling period differences between the two locations may explain the difference of fruit fly diversity. Our results highlight the necessity of repeating fruit fly surveys throughout during different seasonal periods. Observations of African weaver ants in the study orchards showed that most nests occurred in mango trees, with two to four times fewer in both orange and tangerine. The higher occurrence in mango might be explained by the fact that *O. longinoda* use leaves of mango trees for their nests, and also depend on certain plant-feeding Homoptera found on mango for much of their food.[40] Nest observations were made during the flowering period of mango, when ants are searching for new territory and migration to new areas.[40] The nesting habits and colony composition of *O. longinoda* are such that one colony may spread over a number of adjacent trees. Way[26] also showed that in Zanzibar citrus and mango were favoured host plants of O. *longinoda*. Our results, together with those of other workers, suggest that *O. longinoda* could play a role as a component of a fruit fly IPM programme in area of the DRC, although this aspect needs more investigation. Nevertheless, then presence of Homoptera species on fruit trees can have negative implications; some species are plant disease vectors (e.g. of sudden-death desease (Du Giroflier, Way, 1954b cited by Dejean 1991). The use of *O. longinoda* as predators of fruit flies should not be considered before carrying out careful experiments on the possible damage to host trees that might result from associated Homoptera on which the ants that feed.

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Conflicts of interest

The authors declared there is no conflicts of interest.

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