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Trapping, identification and rearing of edible palm weevils in Kenya and Uganda

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Abstract

Palm weevils, *Rhynchophorus* spp. (Coleoptera: Curculionidae) double as devastating invasive pests of palms, and delicacies in many communities. This study evaluated ethyl acetate and 3-methyl-octan-4-ol for trapping palm weevils from coconut, oil and raffia palms in Kenya and Uganda; taking into account the distance of traps on oil palm from a forest or raffia palm. Eggs from the weevils from different locations were incubated and their larvae reared on sugarcane under laboratory conditions. All the 285 weevils collected were identified by morphological and molecular techniques as *Rhynchophorus phoenicis,* and the catch was female-biased. 3-methyl-octan-4-ol attracted more weevils than ethyl acetate. More weevils were caught at ~100 m away from the forest than within 15 m outside the forest; and on raffia palm than oil palm irrespective of distance apart. Eggs took ~4 d to develop and 95-100% of pupae developed into adults, irrespective of the collection site. Larval and pupal developmental periods for weevils from Uganda were longer than those of Kenya, resulting in egg-adult development of ~6 months and ~4 months, respectively. The sex ratio of laboratory produced males and females was similar. These results can guide in designing an integrated *R. phoenicis* management system with a dual benefit of protecting palms from weevil attack and utilising the weevils to improve human nutrition.

1. Introduction

There are nine species of palm weevils, *Rhynchophorus* Herbst spp. (Coleoptera: Curculionidae) which are highly destructive invasive pests of palms and other plants worldwide (Giblin-Davis *et al.*, 2013; Wattanapongsiri, 1966). Three species of these weevils are recorded in Africa namely *Rhynchophorus phoenicis* (Fabricius), *Rhynchophorus quadrangulus* Quedenfeldt and *Rhynchophorus ferrugeneus* (Olivier) (CABI, 2019; Chebbi, 2011; Giblin-Davis, 2001; Wattanapongsiri, 1966). *R. phoenicis* is a widely distributed and most destructive pest of oil palm (*Elaeis guineensis* Jaquin) and coconut palm (*Cocos nucifera* L.) in tropical Africa. On the other hand, *R. quadrangulus* occurs in West-Central Africa; whereas *R. ferrugeneus* has been reported in five North African countries namely Algeria, Egypt, Libya, Morocco and Tunisia (CABI, 2019; Chebbi, 2011; Hill, 2008). Coconut palm is widespread in almost all tropical

coastal areas of the world (Stauffer *et al.*, 2014). It has been an important source of food, fibre, fuel, wine, water and other raw materials for communities in the East African coastline since its introduction by Asian traders in the late part of the first millennium (Boivin *et al.*, 2013). Meanwhile, oil palm is indigenous to West Africa and the Congo Basin, but it has been introduced to more than 25 African countries as a plantation crop for palm oil, sauces, soap, wine, fertiliser (ashes), roofing (leaves), building material (trunk), medicines (roots) and other raw materials (Carrere, 2013; Stauffer *et al.*, 2014). Raffia palm (*Raphia* spp.) have similar economic benefits as coconut and oil palms, and is an important host plant for the edible wild palm weevil larvae in Africa (Muafor *et al.*, 2015; Obahiagbon, 2009).

Semiochemicals – organic compounds that mediate behavioural responses of organisms to their environment e.g. locating mates, hosts, or food sources, avoiding

competition, escaping natural enemies, and overcoming natural defence systems of hosts – are widely applied in pest management programs as lures, repellents and mate disrupters (El-Shafie and Faleiro, 2017). These natural chemical signals are largely species specific and less persistent in the environment, therefore, they are harmless to non-target insects and do not pollute the environment. Some of the key drawbacks for the use of semiochemicals in pest management are their lower efficiency in eliminating pests compared to conventional insecticides, and expensive equipment needed for their extraction and characterisation. Male produced aggregation pheromones have been identified in seven species of *Rhynchophorus* weevils and applied in mass trapping and killing of the weevils in palm plantations (Gries *et al.*, 1994; Jaffé *et al.*, 1993; Oehlschlager, 2016; Weissling *et al.*, 1993). The aggregation pheromones of these weevils comprise closely related methyl-branched secondary alcohols (Oehlschlager, 2016). Besides aggregation pheromones, several palm weevils such as *R. phoenicis* (Gries *et al.*, 1994), *R. ferrugeneus* (Abdel-Azim *et al.*, 2017; Guarino *et al.*, 2011), *Rhynchophorus cruentatus* (Giblin-Davis *et al.*, 1994) and *Rhynchophorus palmurum* (Jaffé *et al.*, 1993) are attracted to fermenting host plant odours. Ethyl acetate and ethyl propionate singly or jointly stand-out as the most commonly used palm-derived lures which synergise aggregation pheromone of palm weevils (Abdel-Rahman *et al.*, 2017; Giblin-Davis *et al.*, 1994; Gries *et al.*, 1994; Guarino *et al.*, 2011; Jaffé *et al.*, 1993; Oehlschlager, 2016). Therefore, these esters are suitable for detecting occurrence of different palm weevils in an area.

Some species of palm weevils such as *R. phoenicis* and *R. ferrugeneus* are delicacies in several communities across the world, especially in Asia and Africa where the larvae are valued at US$ 7-12/kg (Anankware *et al.*, 2016; Banjo *et al.*, 2006; Cito *et al.*, 2017; Hanboonsong *et al.*, 2013; Meutchieye and Niassy, 2016). In Uganda, *R. phoenicis* is ranked the third most consumed insect after grasshoppers and termites (Okia *et al.*, 2017). Palm weevil larvae may contain up to 72% fat and 41% crude protein (Mba *et al.*, 2017; Quaye *et al.*, 2018b; Rumpold and Schlüter, 2013); 714 kcal/100 g, and good amounts of all essential amino acids and fatty acids (Mba *et al.*, 2017; Womeni *et al.*, 2009, 2012). The weevil larvae are therefore nutritionally comparable or superior to conventional animal- and plant-protein sources (Kelemu *et al.*, 2015; Raubenheimer and Rothman, 2013).

Traditional collectors of edible palm weevil larvae identify palm trees with symptoms of infestation and cut and split them up to extract the larvae (Muafor *et al.*, 2015). This practice is destructive to the affected crops and un-sustainable, hence necessitating development of a strategy of artificial rearing of the weevils to meet the demands of its consumers. Several studies have demonstrated that palm weevils can be reared on different artificial diets such as sugarcane and spoilt fruits like watermelon, pineapple and bananas (Bong *et al.*, 2008; Giblin-Davis *et al.*, 1989; Quaye *et al.*, 2018a). However, obtaining stock colonies of adult weevils for rearing is still a challenge. Whereas traps baited with aggregation pheromones and fermenting palm volatiles laced with insecticides is a global strategy of managing palm weevils (Gries *et al.*, 1994; Miguens *et al.*, 2011; Nardi *et al.*, 2011), the use of these traps to collect palm weevils for captive rearing to produce edible larvae has scarcely been explored. Palm weevil trapping, but without insecticides, could double as a pest management technique and a source of edible palm weevil larvae for improving nutrition and food security.
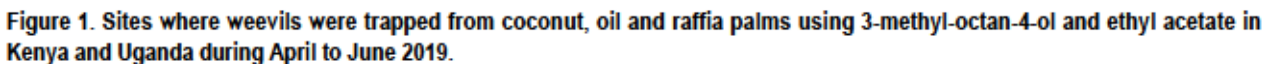
In this study, traps baited separately with ethyl acetate, a palm-derived attractant of different species of palm weevils, and 3-methyl-octan-4-ol, an aggregation pheromone of African palm weevil *R. phoenicis*, were evaluated for trapping palm weevils from different palm species in Kenya and Uganda for captive rearing. Our research hypotheses were: (1) diverse species of edible palm weevils occur at different sites on coconut and oil palms in Kenya, and raffia palm and oil palm in Uganda; (2) 3-methyl-octan-4-ol and ethyl acetate catch different quantities of palm weevils; (3) position of the baited traps on oil palms at different distances from a wild forest or raffia palm influences weevil catches; and (4) weevils collected from different sites have different developmental periods and survival rates during rearing in the laboratory.

2. Materials and methods

Study sites

The study was conducted in Kwale and Busia counties in Kenya; and Mayuge, Bugiri, Mukono, Masaka and Kalangala districts in Uganda. The geographical positioning system (GPS) data collected from the study sites were plotted on Arc Map using the Arc GIS software version 10.3 (Esri Eastern Africa Ltd, Nairobi, Kenya) (Figure 1). The sites were selected based on availability of palm weevil host plants. In Kenya, Kwale is among the major coconut palm production areas, involving largely small holder producers (Kadere *et al.*, 2009; Odenya *et al.*, 2006); whereas Busia is famous for oil palm production (Odenya *et al.*, 2006). In Uganda, Mayuge, Bugiri, Mukono and Masaka districts have research adaptive oil palm trial farms sized 1-17 ha (Baguma *et al.*, 2019); whereas Kalangala district has over 14,000 ha of oil palms (Carrere 2013; Vegetable Oil Development Project, 2016). Ages of the trees across sites were variable and not determined, as was the case with environmental conditions.

In Mukono district where the oil palm plantation was surrounded by a thick natural forest with undetermined plant species, the plantation was subdivided into two subsites, the peripheral zone within 15 m outside the forest,

Figure 1. Sites where weevils were trapped from coconut, oil and raffia palms using 3-methyl-octan-4-ol and ethyl acetate in Kenya and Uganda during April to June 2019.

and the centre of the plantation, which was approximately 100 m away from the forest. This subdivision was guided by the report by Baguma *et al.* (2019) that the symptoms of *R. phoenicis* at this site were higher on oil palms positioned 100 m away from the forest than those within 15 m outside the forest. At Kalangala district, the study site comprised an oil palm plantation which was adjacent to wild raffia palms. The site was subdivided into: (1) the raffia palms; (2) the peripheral zone of oil palms, within 15 m from the raffia palm; and (3) oil palm trees approximately 100 m away from the raffia palms.

Trap design and placement

The lids of locally purchased 19 litre white plastic buckets were fitted with yellow modified unitrap tops (ChemTica Internacional S.A., Heredia, Costa Rica) using binding wires. The unitrap top funnels were designed to prevent escape of trapped adult weevils. Each trap was fitted with a hooked wire for holding the lure pouch approximately 2 cm below the lid of the bucket. Sugarcane stems which were purchased locally at each study site were cut into pieces

of approximately 15 cm long and sliced longitudinally into two sub-equal halves. Eight sugarcane slices were placed in each bucket trap as a food bait to enhance attractiveness of the commercial lures. The traps were then tied to the trunks of the host plants (coconut, oil and raffia palms), 1.5-2 m above the ground.

Lures

Two pouched lures, P080-Lure® and P048-Lure®, were purchased from ChemTica Internacional S.A. The P080-Lure® was a kairomone containing 40 ml of ethyl acetate, with a release rate of 200-400 mg/day depending on ambient temperature. According to the manufacturer, this lure was designed to trap *R. palmarum, R. ferrugeneus, R. cruentatus, Rhynchophorus bilineatus* and *R. phoenicis* as well as *Metamasius hemipterus*. The broad-spectrum lure was therefore selected for detecting possible occurrence of these species in the study sites. On the other hand, P048-Lure® contained 700-800 mg of the aggregation pheromone of *R. phoenicis* 'phoenicol' (3-methyl-octan-4-ol) (Gries *et al.*, 1994; Perez *et al.*, 1994), released at 3-10 mg/day.

Experimental design

Preliminarily detection of sites infested with *Rhynchophorus* spp. for the one-month trapping experiment was carried out by placing 2-3 bucket traps baited separately with each lure at each site for 48-72 h. The red labels in Figure 1 show the sites where no *Rhynchophorus* spp. was caught. Based on the number of weevils caught (not shown) and logistical issues, Shimoni, Kinondo and Matuga in Kwale-Kenya; Alupe, Agriculture Training Centre and Angorom in Busia-Kenya; and Mukono and Kalangala districts in Uganda (labelled green in Figure 1) were selected for the month-long experiment. At Kwale-Kenya which was the first trapping site, two controls (unbaited trap and a trap baited with sugarcane only) were included. Unlike traps baited with the commercial lures, no *Rhynchophorus* spp. was caught in the control traps, therefore controls were excluded from subsequent trials.

At each trapping site in Kenya and subsites in Mukono and Kalangala districts in Uganda, 3-6 replicates of traps baited separately with either ethyl acetate or 3-methyl-octan-4-ol were distributed at approximately 20 m apart in a completely randomised design. The traps were inspected weekly for four weeks to record the type and number of palm weevils caught. The study was conducted from 8th April to 12th May 2019 in Kwale; 13th May to 16th June 2019 in Busia; and 27th May to 30th June 2019 in Uganda. Palm weevils recovered from the traps were sexed based on rostral setae that are only present in males (Giblin-Davis *et al.*, 2013).

Morphological identification of insects

Samples of the palm weevils collected were morphologically identified according to the dichotomous keys published by Wattanapongsiri (1966), Arnett *et al.* (2002) and EPPO (2007). The identifications were confirmed by Salvatore Anzaldo from the School of Life Sciences, Arizona State University, USA. Voucher specimens of the weevils were deposited in the Biosystematics Support Unit collection of the International Centre of Insect Physiology and Ecology (*icipe*).

Molecular identification of insects

DNA extraction and amplification

Representative adult weevil samples from Kwale, Kenya, and Bugiri, Masaka and Kalangala districts in Uganda; and a larval sample from Kalangala were stored in 95% ethanol and transported to the Arthropod Pathology Unit at *icipe*. The right hind legs of representative adult samples and whole larval sample were each surface sterilised using 3% NaOCl then rinsed thrice with distilled water. Genomic DNA was extracted from the individual legs using Isolate II Genomic DNA Kit (Bioline, London, United Kingdom), following the

manufacturer's instructions. The resultant DNA was eluted in a final 50 µl volume then quality and quantity checks done using the Nanodrop 2000/2000c Spectrophotometer (Thermo Fischer Scientific, Wilmington, DE, USA).

Polymerase chain reaction (PCR) was done to amplify the D2 region of 28S rDNA (28S) using Lep D2-Fw 5′ AGTCGTGTTGCTTGATAGTGCAG 3′ and Lep D2-Rv 5′ TTGGTCCGTGTTTCAAGACGGG 3′ (Campbell *et al.*, 1994; Goolsby *et al.*, 2006) markers. The PCR was carried out in a total reaction volume of 20 µl containing 5X My *Taq* Reaction Buffer (5 mM dNTPs, 15 mM $MgCl_2$, stabilisers and enhancers), 0.5 pmol/µl of each primer, 0.5 mM $MgCl_2$, 0.0625 U/µl My *Taq* DNA polymerase (Bioline) and 15 ng/µl of DNA template. This reaction was set up in the Nexus Mastercycler gradient (Eppendorf, Hamburg, Germany). The following cycling conditions were used: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s annealing at 58.8 °C and 1 min at 72 °C, then a final elongation step of 10 min at 72 °C. The target gene region was approximately 575 base pairs.

The amplified PCR products were resolved through a 1.2% agarose gel. DNA bands on the gel were analysed and documented using KETA GL imaging system trans-illuminator (Wealtec Corp, Meadowvale Way Sparks, NV, USA). Successively amplified products were excised and purified using Isolate II PCR and Gel Kit (Bioline) following the manufacturer's instructions. The purified samples were shipped to Macrogen Inc. Europe Laboratory (Amsterdam, the Netherlands) for bi-directional sequencing.

Sequences data analysis

The successful sequences were assembled and edited using Geneious Version 8 (http://www.geneious.com) (Kearse *et al.*, 2012). The primer sequences were identified and removed from the consensus sequences generated from both the forward and reverse reads. For conclusive identification of the species, similarity searches were conducted by querying the consensus sequences via Basic Local Alignment Search Tool (BLAST) at the GenBank database hosted by National Centre of Biotechnology Information (NCBI). BLAST algorithm finds regions of local similarity between sequences, in which consensus sequences were compared to reference sequences in the GenBank database. In addition, a query was done in Barcode of Life Database (BOLD). Sequences of the samples were then submitted to GenBank (Table 1).

Development and survival of first generation of a laboratory colony of palm weevils

Adult palm weevils collected from the different sites – two from Kenya separately (Kwale and Busia) and a combination for all samples from Uganda – were transported in ventilated

Table 1. Molecular identification of samples collected from different locations in Kenya and Uganda.

Locality	Collection point	GeneBank identity	ID %	Sample accession number
Kwale-Kenya	S04.15.965°; E039.57507°	*Rhynchophorus phoenicis*	99.0	MW301383
Kwale-Kenya	S04.15.965°; E039.57507°	*R. phoenicis*	98.9	MW301382
Bugiri-Uganda	N00° 36.354; E033° 44.353	*R. phoenicis*	97.1	MW301385
Kalangala-Uganda	S0013.857; E032.06.725	*R. phoenicis*	95.0	MW301381
Kalangala-Uganda	S0013.857; E032.06.725	*R. phoenicis*	97.1	MW301386
Masaka-Uganda	S00° 37.870; E031° 44.079	*R. phoenicis*	96.4	MW301384

plastic containers measuring 15×21 cm at the top, 12×18 cm at the bottom and 16 cm height (Kenpoly, Nairobi, Kenya) to *icipe*'s Animal Rearing and Quarantine Unit. Ventilation was attained by puncturing small holes on the lids and sides of the containers with the tips of iron nails. The weevils were reared in these containers on sugarcane as food both in transit and in the insectary. The rearing room was maintained at 27.7±0.1 °C and 61.9±0.9% RH with 12 h of light and 12 h of darkness. The choice of sugarcane as food for the weevils and the laboratory conditions were based on previous studies by Debrah (2016) and Quaye *et al.* (2018a). The sugarcane was replaced every two days during which the residues were incubated at ambient conditions of the rearing room. These residues were inspected daily to record incubation, larval and pupal durations. Only pupal survival rate was measured since eggs and larvae were concealed inside the rearing sugar cane. Adults were sexed as described above for field collected weevils.

Data analysis

Effects of type of lure and distance of traps from wild raffia palms (0, 15 and 100 m) or natural forest without raffia palms (within 15 m from the forest or 100 m away) on weevil catches at different sites; collection site on number of days of egg, larval, pupal and egg-adult development; and sex on egg-adult development were determined using generalised linear models (GLMs) with Poisson family and log link. The models were tested for goodness of fit by consideration of their dispersion parameters (i.e. ratio of residual deviance to degrees of freedom) which should be approximately one for a model to be considered fit (Breslow, 1996). Only dispersion parameters for GLMs fitted to data on developmental periods of eggs and pupae indicated proper fits, while models found to be unfit were corrected using negative binomial GLMs (Osgood, 2000), using the 'MASS' package (Ripley and Venables, 2002). There were no catches in traps baited with ethyl acetate in Busia and Mukono, therefore statistical comparisons of catches by type of lure were not carried out in these sites. The proportion of pupae that completed their respective stages were compared based on site of collection using

the prop.test in R statistical computer software (R Core Team, 2019). The percentage of male and female weevils from the field and the laboratory were also compared using the prop.test.

Where appropriate, means were separated using Tukey's multiple mean comparisons. The package 'effects' was used for generation of means (Fox and Weisberg, 2018); whereas 'multcomp' was used for mean separation (Hothorn *et al.*, 2008). All analyses were carried out in R version R i386 3.5.3 (R Core Team, 2019), at α=0.05.

3. Results

Identification of insects trapped

All collected adult palm weevil samples of undetermined ages were identified as *R. phoenicis*. The gene sequences of all samples analysed matched with those of *R. phoenicis* (GenBank accession number AY131091.1) by 95-99% (Table 1).

Catches of *R. phoenicis* in baited traps at different sites

A total of 285 weevils were caught during the survey. About 55.1% of the catch were females, which was significantly higher than males (44.9%) (χ^2=10.5, df=1, *P*=0.001). Traps baited with 3-methyl-octan-4-ol caught about 1-3 adult weevils per week in all the trial sites (Figure 2). Ethyl acetate baited traps caught the weevils in only Kwale and Kalangala, but their catches were significantly lower than those recorded for 3-methyl-octan-4-ol baited traps (χ^2=33.9; d.f.=1; *P*<0.001 and χ^2=33.5; d.f.=1; *P*<0.001, respectively).

Catches of *R. phoenicis* in traps placed on oil palms at the periphery and 100 metres away from the natural forest

The mean catches of *R. phoenicis* on oil palms which were approximately 100 m away from the natural forest (1.9±0.6 weevils per trap per week) were significantly higher than the catches in traps placed at the periphery of the natural forest (0.3±0.2) (χ^2=6.5; d.f.=1; *P*=0.011).
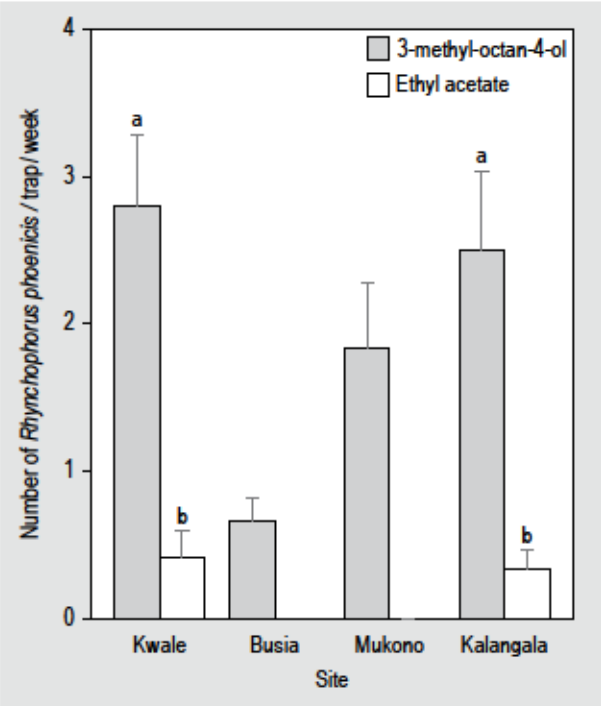
Figure 2. Mean numbers of *Rhynchophorus phoenicis* caught per week per trap baited with 3-methyl-octan-4-ol or ethyl acetate in Kenya (Kwale and Busia) and Uganda (Mukono and Kalangala) from April to June 2019. Error bars represent standard errors of means (SEM). Different letters on bars at Kwale and Kalangala indicate significant differences between the means.
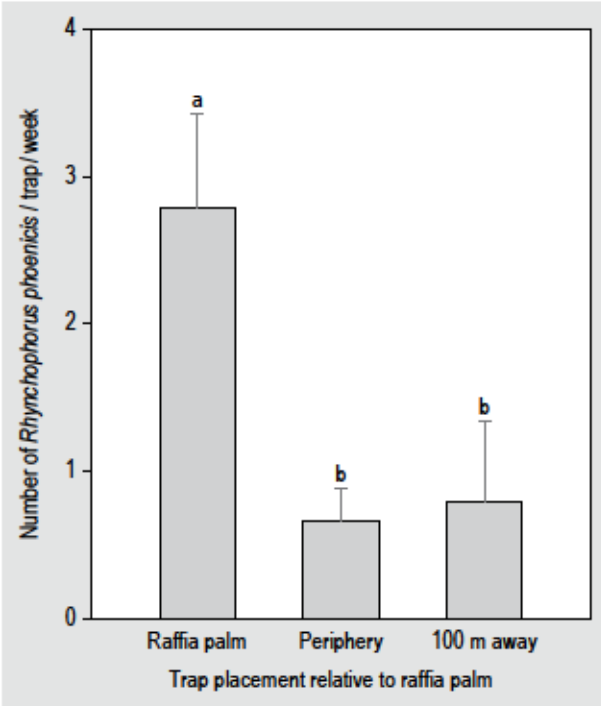

Figure 3. Mean number of *Rhynchophorus phoenicis* caught in traps placed on the wild raffia palms, and oil palms within 15 m and at 100 m away from the raffia palm from May to June 2019. Error bars represent SEM. Different letters on bars indicate significant differences between the means.

Catches of *R. phoenicis* in traps placed at different distances from the raffia palms

Catches of *R. phoenicis* on traps tied to raffia palms were significantly higher than the catches from traps placed on oil palms at both ≤15 m and 100 m away from the raffia palms (χ^2=15.3; d.f.=1; *P*<0.001; Figure 3), but the weevil catches on oil palms at different distances from raffia palms were comparable.

Development of first generation of a laboratory colony of *R. phoenicis* and survival of their pupae

Larval hatch was first detected after about four days of egg incubation, irrespective of where the eggs were collected (χ^2=1.5; d.f.=2; *P*=0.481; Table 2). However, larval, pupal and egg-adult developmental days for weevils collected from Uganda were significantly more than those of weevils collected from Kenya (χ^2=28.1; d.f.=2; *P*<0.001, χ^2=42.3; d.f.=2; *P*<0.001 and χ^2=43.0; d.f.=2; *P*<0.001, respectively). All pupae from Kenya, and 94.7% of those from Uganda successfully developed into adults, with no significant effect of collection site on pupal survival (χ^2=0.6; d.f.=2, *P*=0.741). Of the 29 adults that emerged, 58.6% were males while 41.4% were females, with no statistical difference in the percentage of the two sexes (χ^2=1.1, df=1, *P*=0.294). There

was no significant difference in egg-adult developmental days of males (116.0±6.1 d) and females (129.0±11.0 d) (χ^2=1.5; d.f.=1; *P*=0.215).

4. Discussion

All palm weevils collected from oil, coconut and raffia palms in different sites in Uganda and Kenya were identified as *R. phoenicis* using both morphological and molecular techniques. We used the 28S rDNA to characterise the insects collected from both countries to species level. This region is targeted for samples that are not successfully

Table 2. Mean (±SEM) number of developmental days of different stages of *Rhynchophorus phoenicis* produced by adult weevils collected from Kenya and Uganda.[1]

Site	Egg	Larva	Pupa	Egg-adult
Kwale-Kenya	4.3±0.4a	78.0±4.7a	35.2±1.7a	113.4±4.5a
Busia-Kenya	3.6±0.3a	80.1±5.4a	30.9±1.7a	111.9±6.6a
Uganda	4.3±0.4a	142.8±19.3b	58.7±3.3b	194.3±13.4b

[1] Different letters within a column indicate significant differences between the means.

amplified using the classical barcoding markers such as the Folmer *et al.* (1994) primers. The molecular characterisation confirms the morphological identification and corroborates previous reports that *R. phoenicis* is widespread in tropical Africa (CABI, 2019). It seems therefore that the other two palm weevil species (*R. quadrangulus* and *R. ferrugeneus*) reported in West-Central and North Africa, respectively (CABI, 2019; Chebbi, 2011; Hill, 2008), have not yet spread to Kenya and Uganda. Contrary to Baguma *et al.* (2019) who were unable to detect incidence of *R. phoenicis* in Bugiri-Uganda, we recorded the presence of this species at this site. These discrepancies can be attributed to the methods used in sampling by the two studies, thus demonstrating the superiority of semiochemical lures in detecting occurrence of *Rhynchophorus* spp. over the use of host-plant infestation symptoms. A previous report shows that palm weevils can asymptomatically live cryptically inside palm trees for a long time (CABI, 2019).

The sex ratio of *R. phoenicis* adults caught in the survey was female biased. This finding is consistent with reports on other palm weevil species such as *R. cruentatus* (Giblin-Davis *et al.*, 1989; Weissling *et al.*, 1993) and *R. ferrugeneus* (Abdel-Azim *et al.*, 2017). Higher attraction of female palm weevils to semiochemicals lures than males could be attributed to higher sensitivity of female antennae to odours than those of males (Guarino *et al.*, 2011). The higher catches of female than male *R. phoenicis* may be advantageous for obtaining more eggs for captive rearing, but it is necessary to determine the optimal sex ratio that meets the female mating needs.

Only traps baited with 3-methyl-octan-4-ol caught *R. phoenicis* in Busia and Mukono and approximately 7-8-fold more weevils were trapped by this lure than ethyl acetate in Kwale and Kalangala. This finding concurs with the report by Rochat and Avand-Faghih (2000) that *R. ferrugeneus* was less attracted to date palm odour than its aggregation pheromone, 4-methyl-5-nonanol. However, Weissling *et al.* (1994) demonstrated that cabbage palmetto attracted more *R. cruentatus* than its aggregation pheromone, 5-methyl-4-octanol. This implies that sensitivity of palm weevils to host-derived volatiles compared to their aggregation pheromones depends on the species.

We established that traps placed on oil palms approximately 100 m away from the natural forest without raffia palms caught 6-fold more *R. phoenicis* than those at the periphery of the forest. This finding corroborates that of Baguma *et al.* (2019) who documented higher incidence of *R. phoenicis* symptoms on oil palms located 100 m away from the forest than those positioned at its periphery. Low pest density near undisturbed natural forest might be attributed to rich plant diversity with suitable microclimates which could boost the build-up of natural enemies of the pests

(Altieri, 1999; Letourneau *et al.*, 2011). The rich plant diversity may also produce non-host volatiles that either repel the pests or mask host cues, thereby camouflaging the host plant (Andersson, 2007; Letourneau *et al.*, 2011). Although damage by insect pests induces release of volatiles that attract natural enemies (Ahuja *et al.*, 2011), such odours emanating from infested plants at a long distance from the forest may unfortunately not reach the natural enemies it harbours. Whereas literature on natural enemies of *R. phoenicis* is scarce (CABI, 2019), several parasitoids, predatory birds and entomopathogenic bacteria, fungi, viruses and nematodes have been reported to attack *R. ferrugeneus* in its native habitats in South East Asia (El-Shafie, 2014; Giblin-Davis *et al.*, 2013). Further investigations are required to establish occurrence of natural enemies of *R. phoenicis* in East Africa for their potential integration into palm weevil biological control programs. Investigation of interaction of *R. phoenicis* with volatiles emanating from wild plants around oil palm plantations may help in identifying repellents which can be applied to deter the pest from the host plant.

Traps placed at the wild raffia palms caught 4-fold more *R. phoenicis* than traps placed on adjacent cultivated oil palms, irrespective of distance. This suggests that *R. phoenicis* prefers wild raffia palm to oil palm, and therefore it can be a potential trap crop (Piñero and Manandhar, 2015) which can lure away the pest from the oil palm (Cook *et al.*, 2007; Eigenbrode *et al.*, 2016). This finding also opens prospects for identification of more highly attractive host odor from raffia palm for optimising trapping of *R. phoenicis*.

Prior to this study, incubation periods of *R. phoenicis* eggs have been scarcely measured (Debrah, 2016; Quaye *et al.*, 2018a; Wattanapongsiri, 1966). We found that *R. phoenicis* eggs developed in about four days, irrespective of the site of collection of the mother-weevils. This incubation period is closely similar to that of other palm weevils such as *R. ferrugeneus, R. palmarum* and *R. vulneratus* (Giblin-Davis *et al.*, 2013; Wattanapongsiri, 1966). Larvae and pupae produced by weevils collected from both Kwale and Busia in Kenya developed in approximately two and one months, respectively. However, the larval and pupal stages for samples originating from Uganda took approximately three and two months to develop, respectively. Overall, development of *R. phoenicis* samples from Kenya took about four months, while those from Uganda took more than six months. These observations contradict the reports by Wattanapongsiri (1966) that *R. phoenicis* pupal stage from unspecified location lasted for 26 days. Debrah (2016) also reported contrasting results that *R. phoenicis* larval and pupal stages developed for 20-39 d and 24-48 d, respectively, in Ghana. The differences demonstrated in the durations of development of *R. phoenicis* populations from different locations might be attributed to different factors such as genetic make of the different populations, geographical

location, environmental conditions (especially temperatures and relative humidity) and probably the type of diet used (Boivin *et al.*, 2004; Hamby *et al.*, 2016).

This study revealed that approximately 95-100% of *R. phoenicis* pupae developed into adults irrespective of collection site, which closely corroborates the findings by Quaye *et al.* (2018a) in Ghana that 85-97% of *R. phoenicis* pupae survival to adulthood. We recorded comparable proportions of *R. phoenicis* males and females emerging from pupae, which concurs with the findings by Quaye *et al.* (2018a) when the weevils were reared on different diets in Ghana. This indicates that the sex ratio of natural populations of *R. phoenicis* is approximate 1:1, which may need to be maintained under artificial mass rearing conditions. More comprehensive investigations of survival of *R. phoenicis* through lifetable analysis would be helpful.

5. Conclusion

In conclusion, *R. phoenicis* is still the only species of palm weevils attacking different palm species in Kenya and Uganda. 3-Methyl-octan-4-ol was a more potent attractant of the palm weevils than ethyl acetate. The baited traps were more efficient in attracting female *R. phoenicis* than males. Plant diversity seems to play a critical role in repelling or regulating populations of *R. phoenicis*. This weevil appears to prefer raffia palm to oil palm, but the mechanism for this preference requires further investigation. Eggs from wild-collected *R. phoenicis* from Kenya and Uganda develop for about 4 days, but larval and pupal development was markedly influenced by country of origin, resulting in egg-adult development periods of ~4 months and over 6 months, respectively. Almost all pupal samples developed into adults, irrespective of the collection site. The ratio of male and female adults that emerged from the laboratory colony was approximately 1:1. These results are useful for designing an integrated *R. phoenicis* management system through trapping the weevils from infested palms for captive mass rearing to produce edible larvae. Future studies are necessary to establish the effect of environmental conditions on weevil catches and development.

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Conflict of interest

The authors declare that they have no conflict of interest.

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