

Cannibalism, oviposition and egg development in the edible long-horned grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae) under laboratory conditions

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Abstract

Reliance on seasonal wild harvests of the edible long-horned grasshopper *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae) needs to be broken through developing efficient mass rearing protocols. This study aimed at boosting productivity of laboratory colonies of *R. differens* through understanding ways of minimising cannibalism, selecting suitable oviposition substrates and enhancing egg development and hatchability. Firstly, we investigated the extent of cannibalism in a colony of *R. differens* relative to total mortality, sex, diurnality, growth stage, body parts eaten and visual barriers (presence/absence of cardboard egg trays). *R. differens* cadavers recovered from cages with/without the egg trays were scored for cause of death (cannibalism/other), time of death (night/day), sex, growth stage and body part devoured. Secondly, cotton wool and leaf sheaths of *Pennisetum* sp., maize and *Panicum* sp. were evaluated for oviposition preference by *R. differens*. Eggs oviposited in each substrate were incubated to determine developmental duration and hatchability. Thirdly, we tested effect of moistening *R. differens* eggs on incubation period and hatchability in intact and opened leaf sheaths against unmoistened eggs in intact sheaths as a control. We found that cannibalism accounted for 49% of *R. differens* deaths with 83% of victims being adults. Males and females were equally susceptible to cannibalism. Over 97% of cannibalism occurred at night and presence of egg trays almost doubled incidence of cannibalism. The thorax was the most preferred body part, which was consumed in 77% of cannibalised individuals. Maize and *Panicum* sp. were preferred for oviposition over *Pennisetum* sp. and cotton wool. Opening of egg-laden leaf sheaths reduced incubation period by 0.4 days. Unmoistened eggs didn't hatch after incubation for 25 days, but 65% of them hatched within 11 days upon moistening. These findings offer guidelines for improvement of protocols for mass rearing of *R. differens* for human consumption and other uses.

Keywords: diurnality, egg-moistening, hatchability, light barrier, mortality

1. Introduction

Orthopterans have the ability to swarm in extraordinary numbers, causing massive destruction of crops during their outbreaks (Bidau, 2014). On the other hand, they are rich in protein, fat, amino acids, macro- and micro-nutrients, making them palatable for human consumption (Paul *et al.*, 2016). For example, the long-horned grasshopper, *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae), is a delicacy in several countries across Africa such as the Democratic Republic of Congo, Zambia, South Africa, Cameroon, Zimbabwe, Kenya, Uganda, Tanzania and Malawi (Kelemu *et al.*, 2015). *R. differens* contains 46-48%

fat which comprises the two essential fatty acids linoleic acid (30%) and linolenic acid (~4%); and 43-44% crude protein (Kinyuru *et al.*, 2010). The insect also contains recommended daily human requirements of retinol, α -tocopherol, niacin, riboflavin and folic acid, as well as iron, zinc, calcium and potassium per 100 g (Kinyuru *et al.*, 2010). In Uganda, *R. differens* swarms twice a year (around May and November) (Bailey and McCrae, 1978). In 2018, *R. differens* was valued at approximately US\$2.8 per kg in Uganda; which was over 20% higher than the prices of common animal products like beef and goat's meat (Agea *et al.*, 2008). However, the wild harvested *R. differens* are reportedly contaminated with carcinogenic heavy metals

such as lead, and antioxidants such as catalase (Kazosi *et al.*, 2019). Accumulation of these harmful substances in the insects are partly attributed to feeding on wild vegetation and crops which are prone to contamination from irrational use of agricultural chemicals (Kazosi *et al.*, 2019). Effective and sustainable mass rearing of *R. differens* has the potential to break the reliance on seasonal wild harvest and prevent contamination of these highly valued insects with harmful substances (Kazosi *et al.*, 2019). However, development of protocols for mass-rearing of *R. differens* has been critically constrained by lack of understanding on factors that influence cannibalistic behavior (Hartley, 1967; Lehtovaara *et al.*, 2019), scanty information on oviposition preference (Malinga *et al.*, 2019) and conditions influencing egg hatching (Ssepuuya *et al.*, 2018).

Cannibalism – preying on conspecifics – is a key factor that can cause heavy losses during artificial rearing of insects (Kuriwada *et al.*, 2009; Michaud, 2003). The vice is common among tettigoniids (Gangwere, 1967; Hartley, 1967; Lehtovaara *et al.*, 2019; Mbata, 1992; Richardson *et al.*, 2010; Ros-Santaella *et al.*, 2015; Senthilkumar, 2006; Simpson *et al.*, 2006). Cannibalism in orthopterans is mainly attributed to the deficiency in proteins and salts as demonstrated in the Mormon Cricket, *Anabrus simplex* Haldeman (Simpson *et al.*, 2006). The citations above indicate that cannibalism in tettigoniids is enhanced by high population densities, availability of weak newly moulted or injured or old post-oviposition female individuals and during swarming. Hartley (1967) reported that providing *R. differens* (then referred to as *Homorocoryphus nitidulus vicinus*) with enough water and food enables adult insects to evade cannibalism except the freshly moulted adults which were vulnerable at the wing buds. These reports on factors affecting cannibalism among tettigoniids are largely based on observations of the insects in the wild, but little has been done to investigate effect of factors such as photoperiod, sex and age on the extent of cannibalism under captive rearing. Furthermore, visual barriers such as card-board chicken egg trays are commonly used to provide refuge sites to reduce cannibalism in other related orthopterans such as gryllids (Gatheru *et al.*, 2019; Miech *et al.*, 2016). However, effectiveness of visual barriers in reducing cannibalism among reared *R. differens* has not yet been tested.

Preference of host plants for feeding by *R. differens* (Malinga *et al.*, 2018; Ssepuuya *et al.*, 2018; Valtonen *et al.*, 2018) and influence of host plant diets on nutritional composition of the insects (Rutaro *et al.*, 2018) have been investigated. However, reports on the choice of host plants as substrates for oviposition by *R. differens* are scarcely studied (Malinga *et al.*, 2019). Furthermore, environmental conditions that influence development of *R. differens* eggs needs to be critically assessed. Bailey and McCrae (1978) reported that moisture fastens the development of *R. differens* eggs in

East Africa. In the wild, eggs of *R. differens* reportedly don't develop under dry conditions, but only during the rainy season (Van Huis *et al.*, 2013). Standardisation of protocols for incubation of *R. differens* eggs under captive rearing therefore necessitates validation of the role of moistening in egg development.

This study aimed at boosting productivity of laboratory colonies of *R. differens* through understanding ways of minimising cannibalism, selecting suitable oviposition substrates and enhancing egg development and hatchability. Our research questions were: (1) how much death of *R. differens* results from cannibalism?; (2) how is the incidence of cannibalism in *R. differens* affected by sex, photoperiod, growth stage, body parts eaten and visual barriers?; (3) does *R. differens* exhibit preference for oviposition substrates?; and (4) how does moisture affect egg development and hatchability?

2. Materials and methods

Insects

A colony of *R. differens* was established at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi Kenya in November 2017 with collections from commercial traps in Kampala, Masaka, Mbarara, Hoima and Kabale districts in Uganda. The geographical positioning system (GPS) data collected from the trapping sites were plotted on Arc Map using the Arc GIS software version 10.3 (Esri Eastern Africa Ltd, Nairobi, Kenya) (Figure 1). Moderate resolution imaging spectroradiometer normalised difference vegetation index layer for January 2020 sourced from United States Geographical Surveys (<https://earthexplorer.usgs.gov/>) at 250 m spatial resolution was incorporated in the map.

Prior to the experiment, the insects were reared in ventilated Perspex cages measuring 50×50×50 cm for eight generations to allow them ample time to acclimatise to laboratory rearing conditions (Jensen *et al.*, 2017) and also to accumulate sufficient numbers for the study. These cages were stocked with 100-200 first instar nymphs (1-3 days old) depending on the size of the colony. As the nymphs grew, their numbers reduced to 30-50 adults per cage. The size of the colony at the start of the cannibalism experiment was approximately 150 adults, 700 nymphs and 2,500 eggs. The insects were mainly fed on *Panicum* sp. shoots and/or maize seedlings depending on the availability. The feed was presented as leaf bouquets packed in clean plastic bottles (diameter 6 cm by 12 cm height). The bottles were filled with water to one-third of the volume to prevent plant parts from withering. Plant materials were secured at the mouth-feed interface of the bottle with cotton wool, to prevent insects from drowning. Five bottles with leaves were placed in each cage. Feeding materials were changed and

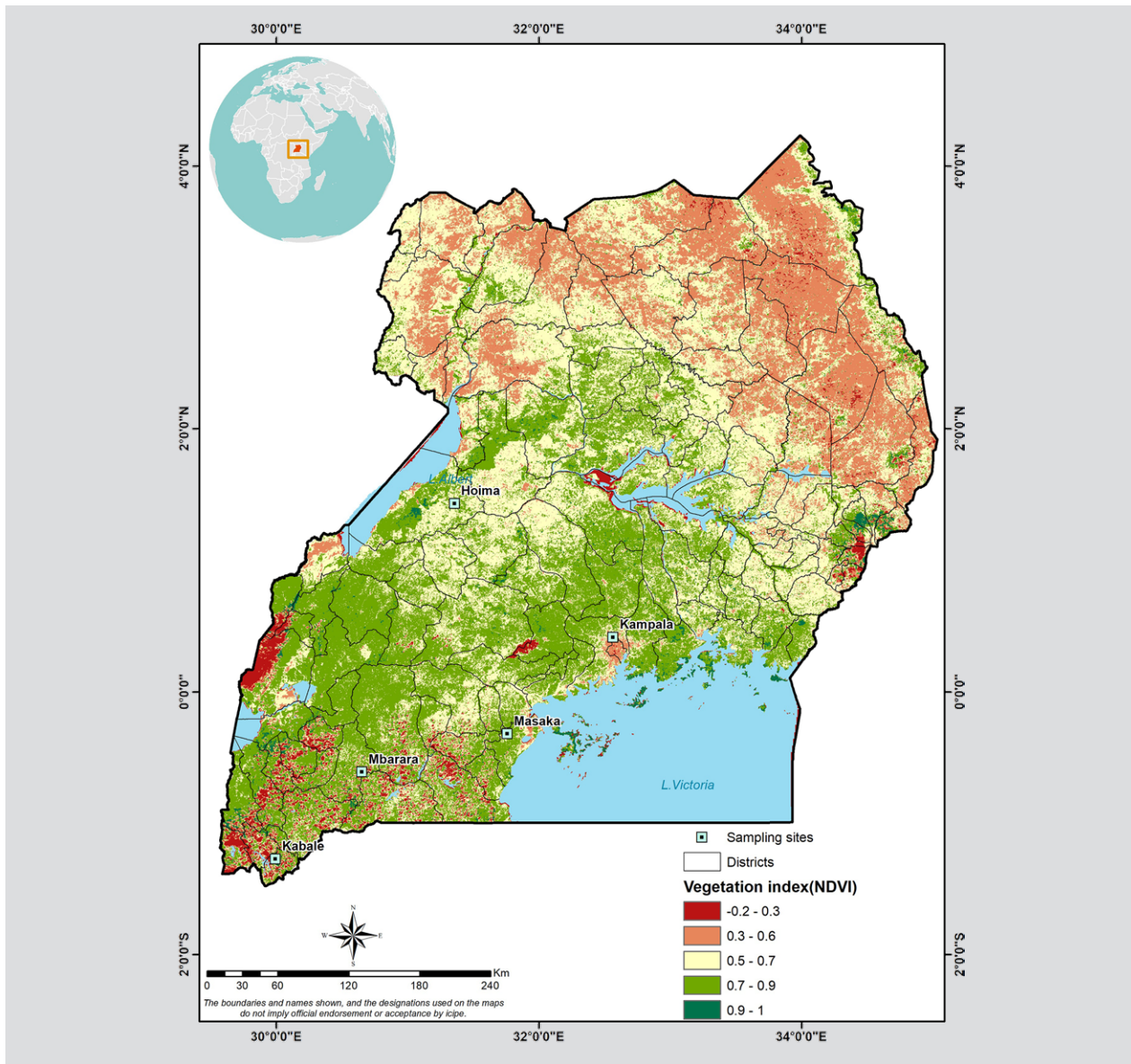


Figure 1. Location of *Ruspolia differens* collection sites in Uganda.

cages cleaned after 2-3 days depending on their condition. A dry supplement containing 53.4, 21.1, 8.5, 7.3, 5.2 and 4.5% carbohydrates, protein, moisture, ash, fat and crude fibre, respectively, formulated by Treasure Feeds Limited (Thika, Kenya) was provided to the insects. Eggs were collected from used feeding materials by opening the leaf sheaths to presumably enhance nymphal emergence, but without dislodging the eggs. The sheaths containing eggs were placed on moist cotton wool in ventilated plastic containers (21 cm × 14 cm × 8 cm) and incubated at 30 °C in an incubator (Akili Know-How® GmbH & Co., Bremen, Germany). The rearing room was maintained at 28-30 °C, approximately 50% relative humidity and a photoperiod of 12 h light: 12 h darkness. The rearing room was fitted with an air circulation system which continuously drew out air

from the room and supplied fresh air to prevent build-up of undesirable smells.

Estimation of the extent of cannibalism in *R. differens* colony relative to total mortality, sex, diurnality, growth stage, body parts eaten and visual barriers

Cohorts of the insects were stocked according to the density and age as described above. Each stage was represented by four stocked cages, two of which were fitted with five pieces of cardboard chicken-egg trays, separated by feeding bottles, to serve as visual barriers and shelter for insects under attack. The other half of the cages were maintained without the visual barriers.

The colony was monitored daily for 40 days for cases of *R. differens* death. All dead *R. differens* during the study were collected twice daily at 6:00 h local time (representing deaths that occurred at night) and 18:00 h for day-time deaths. Insect cadavers were removed from the cages and scored for cause of death (cannibalism or other), sex, growth stage and body part devoured. Following observations by Forkwa *et al.* (unpublished data) that cannibalistic *R. differens* showed great preference for live compared to dead conspecifics, all specimens that were found eaten were assumed to be victims of cannibalism.

Preference for oviposition substrate by *R. differens*

This study was conducted in the laboratory with six adult *R. differens* cages as replicates, each stocked with 22 females and 22 males. In each cage, four oviposition substrates (cotton wool, potted maize seedlings, shoots of Elephant grass (*Pennisetum* sp.) and shoots of *Panicum* sp. were randomly assigned to four corners of the cages. The cotton wool was moistened by soaking with tap water and the excess water squeezed off. The plant-based oviposition substrates were provided as leaf bouquets as described above. After three days of exposure of the various substrates to the insects for oviposition, the leaf sheaths carrying the eggs were carefully removed from plant-based oviposition substrates, the eggs counted and incubated as described above. The eggs oviposited in moist cotton wool were also carefully removed for counting and incubation. The eggs were monitored daily for nymphal hatching for 25 days. The incubation periods of each egg and total number of hatched nymphs per treatment were recorded.

Influence of moisture on incubation period and hatchability of *R. differens* eggs

Three treatments on egg-laden maize leaf sheaths namely moistened eggs in intact sheaths, moistened eggs in opened sheaths and unmoistened eggs in intact sheaths (control) were examined. Intact sheaths with eggs were gently opened to count the eggs and then closed. Each treatment was applied on approximately 50 *R. differens* eggs and replicated four to six times. The eggs were incubated as described above. The incubation boxes were arranged in a completely randomised design. Preliminary assays were carried out to establish if using 0.5% Sodium hypochlorite as a disinfectant; and distilled water instead of tap water for moistening, would improve egg development (measured by incubation period and hatchability), but these treatments had no effect on egg development. Therefore, we proceeded with moistening the eggs with tap water as a cheaper option. Since unmoistened eggs in intact sheaths did not hatch during the experiment, they were moistened and re-incubated to determine their viability.

Data analysis

Counts of *R. differens* cadavers categorised by cause of death (cannibalism or other), sex, growth stage, presence/absence of egg trays, time of death (day/night) and body part devoured were compared using the chi square goodness of fit test followed by chi square pairwise comparisons of percentages (for more than three treatment levels) using the R package 'RVAideMemoire' (Hervé and Hervé, 2019).

The counts of *R. differens* eggs oviposited per substrate and the egg incubation periods (days) across oviposition substrates and preincubation moisture treatments were subjected to generalised linear modelling (GLM) with Poisson family and logit link. Owing to overdispersion in the data (Ratios of residual deviances to degrees of freedom 855.39/16 for number of eggs oviposited, 281.82/1526 for number of developmental days across oviposition substrates and 52.415/260 for preincubation moistening of eggs), negative binomial GLMs were fitted to the data using the package 'MASS' (Ripley and Venables, 2002). For both oviposition substrates and preincubation moisture treatments, the ratios of residual deviances to degrees of freedom regarding incubation periods remained unchanged, hence Quassipoisson GLMs were fitted to the data sets. Because unmoistened eggs in intact leaf sheaths didn't hatch the first time, they were not included in statistical analysis. However, these eggs hatched after re-incubation in moist cotton wool, hence their mean development parameters were computed.

The number of hatched and unhatched eggs from different oviposition substrates and preincubation treatments were column bound (cbind) and subjected to GLM with binomial family and logit link. Owing to overdispersion of data (ratios of residual deviances to degrees of freedom of 372.47/16 and 62.259/7 for oviposition substrates and preincubation treatments, respectively), the data were subjected to Quassibinomial GLMs.

All analyses were carried out at $\alpha=0.5$ in R statistical software version 3.5.3 (R Core Team, 2019), and in cases of significant effects of treatments, means were separated using Tukey's pairwise comparisons with packages 'effects' for generation of means (Fox and Weisberg, 2018) and 'multcomp' for mean separation (Hothorn *et al.*, 2008).

3. Results

Extent of cannibalism in *R. differens* colony relative to total mortality, sex, diurnality, growth stage, body parts eaten and visual barriers

The percentage of *R. differens* cadavers which were cannibalised (49.2±4.7%) did not differ significantly from the percentage of deaths that arose from other causes

(50.8±4.7%) (Chi-square test: $\chi^2_1=0.1$, $P=0.771$). Similarly, the percentage of cannibalised *R. differens* females (56.6±4.7%) was not significantly different from that of the males (43.4±4.7%) (Chi-square test: $\chi^2_1=2.0$, $P=0.158$). On the other hand, a significantly higher incidence of cannibalism (97.2±1.4%) occurred in the night than in the day (2.8±1.4%) (Chi-square test: $\chi^2_1=142.1$; $P<0.001$). Furthermore, the percentage of *R. differens* cannibalised in the presence of chicken egg trays (64.1±4.0%) was significantly higher than those cannibalised in the absence of the trays (35.9±4.0%) (Chi-square test: $\chi^2_1=11.0$, $P<0.001$).

Cannibalism among different life stages of *R. differens* differed significantly (Chi-square test: $\chi^2_5=466.4$, $P<0.001$; Figure 2), with 83.4±3.1% of the cannibalism recorded in adults. Cannibalism among immature stages ranged from 1.4±1.0% in 4th instars to 5.5±1.9% in 1st instars. Preference for different body parts of *R. differens* varied significantly (Chi-square test: $\chi^2_8=135.2$, $P<0.001$; Figure 3). The thorax only was the most preferred body part (36.2±3.9%) while the legs only were least preferred (0.7±0.7%). The total number of victims with their thoraxes being one of the parts devoured (thorax only, thorax plus leg, thorax plus abdomen, thorax plus head and whole body) was 76.6%.

Preference for oviposition substrate by *R. differens* and the effect of the substrates on egg development

The numbers of eggs oviposited by *R. differens* in the different substrates were significantly different (Chi-square test: $\chi^2_3=39.3$, $P<0.001$; Figure 4). The highest number of

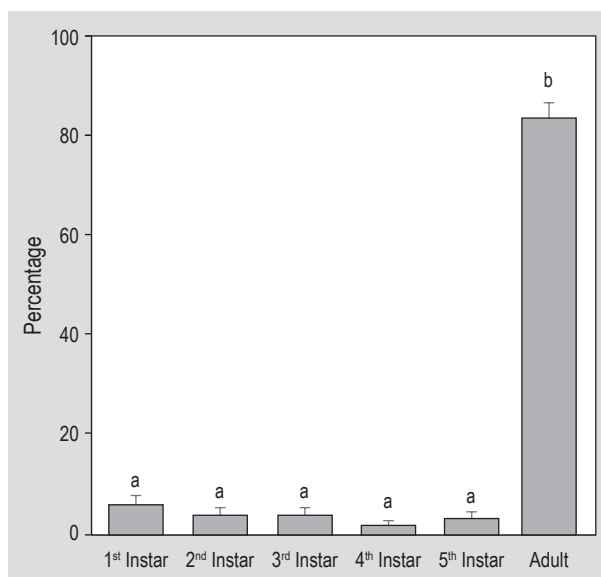


Figure 2. Percentages of cannibalised *Ruspolia differens* categorised by developmental life stages. Error bars indicate standard errors. Different letters on bars indicate significant differences in percentages.

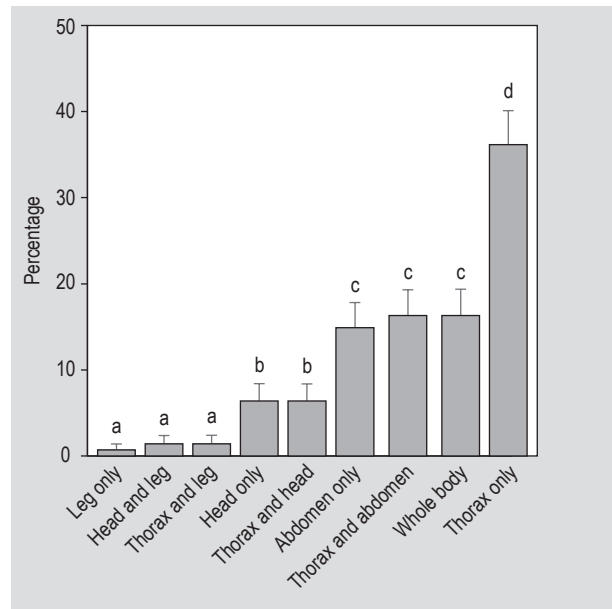


Figure 3. Percentages of cannibalised *Ruspolia differens* categorised by body part consumed. Error bars indicate standard errors. Different letters on bars indicate significant differences between percentages.

eggs were oviposited in maize (261.0±85.9) and *Panicum* sp. (241.0±27.3); whereas the lowest number of eggs was oviposited in cotton wool (14.0±5.5).

Hatchability of the eggs was not significantly affected by oviposition substrate (Chi-square test: $\chi^2_3=32.3$, $P=0.695$; Figure 5), ranging from 43.5±1.4% in maize to 73.8±6.8% in cotton wool. However, there were significant differences in egg incubation periods across the oviposition substrates (Chi-square test: $\chi^2_3=6.4$, $P<0.001$). The longest duration

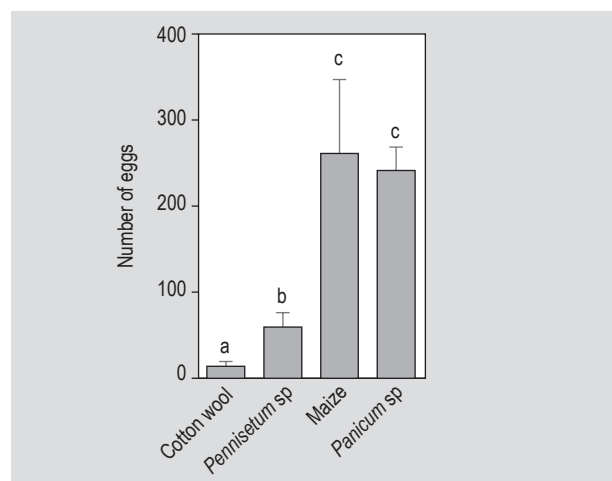


Figure 4. Mean number of eggs oviposited by *Ruspolia differens* in different substrates randomly allocated in the cage within three days. Error bars indicate standard errors. Different letters on bars indicate significant differences between means.

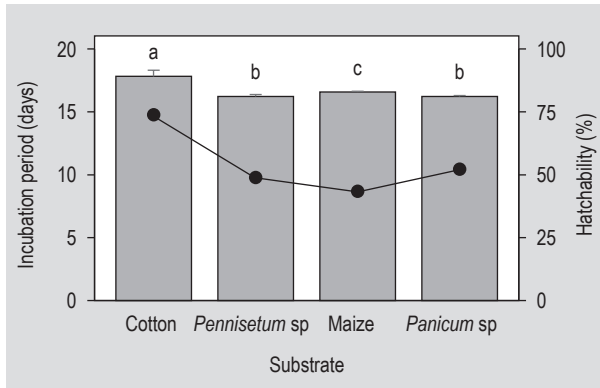


Figure 5. Incubation periods (bars) and hatchability of *Ruspolia differens* eggs oviposited in the different substrates per cage within three days. Error bars indicate standard errors. Different letters on bars indicate significant differences between means.

for the eggs to develop to first instar stage occurred on cotton wool (17.8±0.5 days); while the shortest duration was recorded in *Pennisetum* sp. and *Panicum* sp., both with 16.2±0.1 days.

Influence of moisture on incubation period and hatchability of *R. differens* eggs

The control eggs in intact leaf sheaths which were not moistened failed to hatch during 25 days of incubation. However, upon subsequent moistening and reincubation of these eggs, 65±3.5% of them hatched within 11.4±0.1 days. The incubation periods of the moistened eggs in intact leaf sheaths was statistically longer than that of eggs in opened leaf sheaths by 0.4 days (F-test: $F_{1,260}=4.2, P=0.040$; Figure 6). However, there was no significant difference in the hatchability of moistened *R. differens* eggs in intact and opened leaf sheaths (F-test: $F_{1,7}=0.5, P=0.488$).

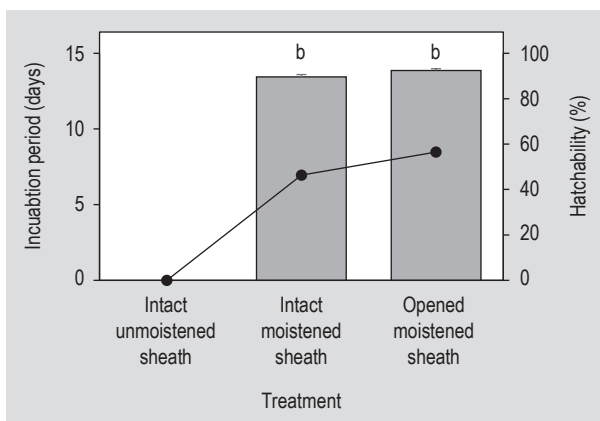


Figure 6. Mean incubation periods (bars) and hatchability of *Ruspolia differens* eggs subjected to different preincubation moisture treatments. Error bars indicate standard errors. Different letters on bars indicate significant differences between means.

4. Discussion

This study has revealed that in laboratory reared *R. differens*, cannibalism can account for almost half of the mortality. These findings corroborate reports by Hartley (1967) and Lehtovaara *et al.* (2019) that cannibalism is a major constraint in mass production of *R. differens*. The high level of cannibalism observed is an indication that the diet used for feeding the insects probably didn't provide enough protein and salts (Simpson *et al.*, 2006), which might have forced *R. differens* to practice a compensatory feeding response. The high level of cannibalism also suggests that the population density of the insects may not have been optimal (Hartley, 1967; Lehtovaara *et al.*, 2019). Determination of appropriate stocking density and diet that minimise cannibalism in *R. differens* therefore requires further research.

We found that over 83% of the cannibalism occurred in the adult stage of *R. differens*. This finding is consistent with reports by Bailey and McCrae (1978) and Richardson *et al.* (2010) that cannibalism in tettigoniids predominantly occurs at the adult stage. This finding suggests that harvesting of *R. differens* for human consumption just before or immediately after adult emergence could circumvent cannibalism, provided other factors such as nutritional contents of early harvested insects are further tested. However this study showed no detectable sexual differences in susceptibility of *R. differens* to cannibalism, indicating that the cannibalism may not be linked to mating behaviour as reported in other arthropods like praying mantis and spiders (Wilder *et al.*, 2009).

Our results provide the first evidence that most of the cannibalism at all stages of *R. differens* took place at night. This is in accordance with the report by Bailey and McCrae (1978) that *R. differens* is primarily a nocturnal insect with most of the flight and oviposition activities occurring at night. Therefore, manipulation of the lighting regime especially during the adult stage, could be another option for reducing cannibalism in *R. differens* during captive mass rearing. However, further research is warranted to establish the effect of modified light regime on mating and oviposition behaviour. Our data demonstrate that the incidence of cannibalism in *R. differens* increased drastically when egg trays were introduced as refuge sites, which is contrary to observations on gryllids in which the use of chicken egg trays prevents cannibalism (Gatheru *et al.*, 2019; Miech *et al.*, 2016). We speculate that the egg trays may have provided dark havens mimicking night conditions, which could have further increased cannibalism. The trays may have also reduced on the available space for the insects, hence increasing population density which is known to increase cannibalism (Richardson *et al.*, 2010).

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Interestingly, the thorax of *R. differens* was the most preferred part attacked by cannibalising conspecifics, with over 36% victims consumed at the thorax only; and 76.6% having their thoraxes among the parts eaten up. Insect thoraxes have extra muscles which are concerned with movement of the legs and wings (Chapman, 1998). These muscles may be richer sources of protein, which is known to be sought after by cannibalistic tettigoniids (Simpson *et al.*, 2006). This suggests that further manipulation of the composition of protein in the rearing diet of *R. differens* may be helpful in combatting cannibalism under captive rearing. However other factors such as speed of kill when the thorax is eaten as compared to other body parts and preference for live insects over dead insects for cannibalism needs to be further investigated to better understand this phenomenon.

Maize seedlings and *Panicum* sp. shoots were four- and 17-fold more preferred for oviposition by *R. differens* compared to *Pennisetum* sp. shoots and cotton wool, respectively. This partly concurs with Ssepuuya *et al.* (2018) that *Panicum maximum* had the highest survival of *R. differens* nymphs, which could be a mechanism to choose oviposition hosts that ensure survival of their progeny (Hilker and Meiners, 2011). It is also possible that the different oviposition host substrates vary in their nutritional and defensive phytochemistry which are known to influence rates of oviposition in insects (Awmack and Leather, 2002; Hilker and Meiners, 2011), though this was not tested in the current study. On a flipside, Malinga *et al.* (2019) reported that in preliminary studies, wild collected *R. differens* adults preferred ovipositing on *Pennisetum* sp. to *Panicum* sp. grass; and in subsequent studies, folded plastic cloth (with one side covered in short fleece; Table cloth Compact, Friedola 1888 GmbH, Meinhard, Germany) were preferred over leaf sheaths of *Pennisetum* sp. for oviposition by wild collected adult *R. differens*. The difference between these studies might have been due to the feeding environment provided to the insects. For example, the feeding of our insects on maize seedlings and *Panicum* sp. grass may have influenced their preference for these substrates for oviposition over *Pennisetum* sp. Further investigations to identify ideal oviposition substrates for *R. differens*, considering availability of the substrates are still required.

Although not numerically pronounced, eggs oviposited in maize seedlings took statistically longer to incubate than the eggs from *Panicum* sp. and *Pennisetum* sp. *Panicum* sp. shoots were therefore the most ideal oviposition substrates for *R. differens*, which may serve as a yardstick for further selection of alternative oviposition substrates for the insects. Besides being the least preferred substrates for oviposition by *R. differens*, the incubation period of eggs oviposited in cotton wool was longer than that of eggs laid on live plant-based substrates. This may be attributed to the positive interaction between embryonic development and plant

phytochemicals (Awmack and Leather, 2002), which are lacking in the cotton wool. The influence of host chemistry on *R. differens* egg development therefore needs more detailed investigation.

Eggs of *R. differens* which were incubated without moistening did not hatch. However, these eggs remained viable with 65% of them hatching in about 11 days upon moistening. This duration is relatively shorter than for all other treatments in this study ranging from 13-18 days (Figure 4 and 5). Our results corroborate previous reports that moistening fastens egg development in *R. differens* and other tettigoniids (Bailey and McCrae, 1978; Hartley and Warne, 1972). Bailey and McCrae (1978) also reported that development of wetted *R. differens* eggs takes 11-12 days, which coincides with the incubation period of the eggs that we moistened and reincubated upon failure to hatch without moistening. This suggests that the moisture deficient eggs undergo mild development which reduces their development period and increases hatchability once moisture is accessed. This may partly explain the seasonality of swarming of *R. differens* which coincides with peak rains (Bailey and McCrae, 1978). Further, this finding highlights the need for further investigation on desiccation on *R. differens* eggs as a means of their long-term storage in commercial production.

Our results demonstrate that opening *R. differens* egg-laden leaf sheaths modestly reduced their incubation period by 0.5 days compared to eggs which were left intact in leaf sheaths, but the opening of leaf sheaths had no detectable effect on egg hatchability. The quicker egg development may be a result of better exposure to moisture which is known to fasten *R. differens* egg development (Bailey and McCrae, 1978; Hartley and Warne, 1972). Further research on economic returns on labour for opening the leaf sheaths is required to guide *R. differens* farmers on the cost-benefit of the effort on opening the leaf sheaths.

Hatchability of *R. differens* eggs under the different preincubation moisture treatments ranged from about 46 to 65%, with no statistical effect of the treatments on hatchability. This is similar to the observation above on preference for oviposition substrates, where hatchability of *R. differens* eggs ranged from about 50 to 78%, but were not statistically variable. Ssepuuya *et al.* (2018) reported up to 89% hatchability of *R. differens* eggs in opened leaf sheaths and incubated at 30 °C in Uganda, which is similar to the current studies. The discrepancies in these findings indicate that several factors which are yet to be determined influence egg hatchability in *R. differens*. For example the tettigoniid bush cricket, *Requena verticalis* Walker males reportedly prefer to mate with younger virgin females to enhance their confidence of paternity (Simmons *et al.*, 1994), which can influence the viability of eggs based on age of the females. On the other hand, post-mating diet

of female insects influence survival of their eggs (Wedell, 1996). More research is therefore necessary to fully elucidate how such factors influence hatchability of *R. differens* eggs in laboratory colonies.

5. Conclusions

This study has shown that cannibalism is an important factor that can account for nearly half of the mortalities in laboratory reared *R. differens*, with 83% of the victims being adults. There was no marked sex differential susceptibility to cannibalism in *R. differens* but almost all the cannibalism occurred at night. The thorax was the most preferred part of cannibalised individuals. Presence of egg trays in *R. differens* rearing cages appeared to provide dark havens and increase population density, that nearly doubled the incidence of cannibalism. Maize seedlings and *Panicum* sp. shoots were preferred for oviposition by *R. differens* over *Pennisetum* sp. and moist cotton wool. Eggs oviposited in *Panicum* sp. developed faster than those oviposited on maize by about 0.4 days. Moisture was required for full *R. differens* egg development, which may explain the seasonality of swarms of the insect to coincide with peak rains. Whereas opening *R. differens* egg-laden leaf sheaths prior to incubation hastened incubation period by 0.5 days, the returns to labour for this activity should be investigated. These findings offer guidelines for improvement of protocols for mass rearing of *R. differens* for human consumption and other uses.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

- Agea, J.G., Biryomumaisho, D., Buyinza, M. and Nabanoga, G.N., 2008. Commercialization of *Ruspolia nitidula* (Nsenene grasshoppers) in central Uganda. *African Journal of Food, Agriculture, Nutrition and Development* 8: 319-332.
- Awmack, C.S. and Leather, S.R., 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47: 817-844. <https://doi.org/10.1146/annurev.ento.47.091201.145300>
- Bailey, W. and McCrae, A., 1978. The general biology and phenology of swarming in the East African tettigoniid *Ruspolia differens* (Serville) (Orthoptera). *Journal of Natural History* 12: 259-288. <https://doi.org/10.1080/00222937800770151>
- Bidau, C.J., 2014. Patterns in Orthoptera biodiversity. I. Adaptations in ecological and evolutionary contexts. *Journal of Insect Biodiversity* 2: 1-39. <https://doi.org/10.12976/jib/2014.2.20>
- Chapman, R.F. 1998. *The insects: structure and function*. Cambridge University Press, New York, NY, USA, 961 pp.
- Fox, J. and Weisberg, S., 2018. Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. *Journal of Statistical Software* 87: 1-27. <https://doi.org/10.18637/jss.v087.i09>
- Gangwere, S., 1967. The feeding behavior of *Atlanticus testaceus* (Orthoptera: Tettigoniidae). *Annals of the Entomological Society of America* 60: 74-81. <https://doi.org/10.1093/aesa/60.1.74>
- Gatheru, J., Khamis, F., Ombura, F., Nonoh, J., Tanga, C., Maina, J., Mohamed, S., Subramanian, S., Ekesi, S. and Fiaboe, K., 2019. Impact of processing methods on microbial load of reared and wild-caught edible crickets (*Scapsipedus icipe* and *Gryllus bimaculatus*) in Kenya. *Journal of Insects as Food and Feed* 5: 171-183. <https://doi.org/10.3920/JIFF2018.0042>
- Hartley, J., 1967. Laboratory culture of a Tettigoniid, *Homorocoryphus nitidulus vicinus* (Wlk.) (Orthoptera). *Bulletin of Entomological Research* 57: 203-205. <https://doi.org/10.1017/S0007485300049920>
- Hartley, J. and Warne, A., 1972. The developmental biology of the egg stage of Western European Tettigoniidae (Orthoptera). *Journal of Zoology* 168: 267-298. <https://doi.org/10.1111/j.1469-7998.1972.tb01349.x>
- Hervé, M. and Hervé, M.M., 2019. Package 'RVAideMemoire'. Available at: <https://tinyurl.com/yd4envdu>.
- Hilker, M. and Meiners, T., 2011. Plants and insect eggs: how do they affect each other? *Phytochemistry* 72: 1612-1623. <https://doi.org/10.1016/j.phytochem.2011.02.018>
- Hothorn, T., Bretz, F. and Westfall, P., 2008. Simultaneous inference in general parametric models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences* 50: 346-363. <https://doi.org/10.1002/bimj.200810425>
- Jensen, K., Kristensen, T.N., Heckmann, L.H. and Sørensen, J.G., 2017. Breeding and maintaining high-quality insects. In: Van Huis, A. and Tomberlin, J.K. (eds.) *Insects as food and feed: from production to consumption*. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 175-198.

- Kasozi, K.I., Namazi, C., Basemera, E., Atuheire, C., Odwee, A., Majalija, S. and Kateregga, J.N., 2019. Inorganic pollutants in edible grasshoppers (*Ruspolia nitidula*) of Uganda and their major public health implications. *African Health Sciences* 19: 2679-2691. <https://doi.org/10.4314%2Fahs.v19i3.44>
- Kelemu, S., Niassy, S., Torto, B., Fiaboe, K., Affognon, H., Tonnang, H., Maniania, N.K. and Ekesi, S., 2015. African edible insects for food and feed: inventory, diversity, commonalities and contribution to food security. *Journal of Insects as Food and Feed* 1: 103-119. <https://doi.org/10.3920/JIFF2014.0016>
- Kinyuru, J.N., Kenji, G., Muhoho, S.N. and Ayieko, M., 2010. Nutritional potential of longhorn grasshopper (*Ruspolia differens*) consumed in Siaya district, Kenya. *Journal of Agriculture, Science and Technology* 12: 32-46.
- Kuriwada, T., Kumano, N., Shiromoto, K. and Haraguchi, D., 2009. High population density and egg cannibalism reduces the efficiency of mass-rearing in *Euscepes postfasciatus* (Coleoptera: Curculionidae). *Florida Entomologist* 92: 221-228.
- Lehtovaara, V., Tahvanainen, J., Sorjonen, J., Valtonen, A. and Roininen, H., 2019. Space and shelter requirement of nymphs in the mass-rearing of the edible *Ruspolia differens* (Orthoptera: Tettigoniidae). *Journal of Economic Entomology* 112: 1651-1657. <https://doi.org/10.1093/jee/toz065>
- Malinga, G.M., Valtonen, A., Lehtovaara, V.J., Rutaro, K., Opoke, R., Nyeko, P. and Roininen, H., 2018. Diet acceptance and preference of the edible grasshopper *Ruspolia differens* (Orthoptera: Tettigoniidae). *Applied Entomology and Zoology* 53: 229-236.
- Malinga, G.M., Lehtovaara, V.J., Valtonen, A., Nyeko, P. and Roininen, H., 2019. Developing mass egg-laying medium for the edible *Ruspolia differens* (Orthoptera: Tettigoniidae). *Journal of Economic Entomology* 112: 2157-2160. <https://doi.org/10.1093/jee/toz124>
- Mbata, K., 1992. The biology and host plant specificity of *Acanthoplus speiseri* Brancsik (Orthoptera: Tettigoniidae: Hetrodinae), a pest of grain crops. *Journal of the Entomological Society of Southern Africa* 55: 99-106.
- Michaud, J., 2003. A comparative study of larval cannibalism in three species of ladybird. *Ecological Entomology* 28: 92-101. <https://doi.org/10.1046/j.1365-2311.2002.00481.x>
- Miech, P., Berggren, Å., Lindberg, J. E., Chhay, T., Khieu, B. and Jansson, A., 2016. Growth and survival of reared Cambodian field crickets (*C*) fed weeds, agricultural and food industry by-products. *Journal of Insects as Food and Feed* 2: 285-292. <https://doi.org/10.3920/JIFF2016.0028>
- Paul, A., Frederich, M., Uyttenbroeck, R., Hatt, S., Malik, P., Lebecque, S., Hamaidia, M., Miazek, K., Goffin, D. and Willems, L., 2016. Grasshoppers as a food source? A review. *Biotechnologie, Agronomie, Société et Environnement* 20: 337-352.
- R Core Team, 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <https://www.R-project.org/>
- Richardson, M.L., Mitchell, R.F., Reagel, P.F. and Hanks, L.M., 2010. Causes and consequences of cannibalism in noncarnivorous insects. *Annual Review of Entomology* 55: 39-53. <https://doi.org/10.1146/annurev-ento-112408-085314>
- Ripley, B.D. and Venables, W., 2002. *Modern applied statistics with S*. Springer, New York, NY, USA, 512 pp.
- Ros-Santaella, J.L., Pintus, E. and Senhadji, K., 2015. Cannibalistic behaviour of the vulnerable and endemic *Baetica ustulata* (Rambur 1838) (Orthoptera: Tettigoniidae: Ephippigerinae): first report. *Annales de la Société entomologique de France (N.S.)* 51: 89-92. <https://doi.org/10.1080/00379271.2015.1063251>
- Rutaro, K., Malinga, G.M., Lehtovaara, V.J., Opoke, R., Valtonen, A., Kwetegyeka, J., Nyeko, P. and Roininen, H., 2018. The fatty acid composition of edible grasshopper *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae) feeding on diversifying diets of host plants. *Entomological Research* 48: 490-498. <https://doi.org/10.1111/1748-5967.12322>
- Senthilkumar, N., 2006. Rearing technology of *Conocephalus maculatus* (Le Guillou) under laboratory conditions (Orthoptera: Tettigoniidae). *Journal of Entomological Research* 30: 79-81.
- Simmons, L., Llorens, T., Schinzig, M., Hosken, D. and Craig, M., 1994. Sperm competition selects for male mate choice and protandry in the bushcricket, *Requena verticalis* (Orthoptera: Tettigoniidae). *Animal Behaviour* 47: 117-122. <https://doi.org/10.1006/anbe.1994.1013>
- Simpson, S.J., Sword, G.A., Lorch, P.D. and Couzin, I.D., 2006. Cannibal crickets on a forced march for protein and salt. *Proceedings of the National Academy of Sciences* 103: 4152-4156. <https://doi.org/10.1073/pnas.0508915103>
- Ssepuyua, G., Tanga, C., Yekko, I., Sengendo, F., Ndagire, C., Fiaboe, K., Karungi, J. and Nakimbugwe, D., 2018. Suitability of egg hatching conditions and commonly available food plants for rearing the long-horned grasshopper *Ruspolia differens* Serville (Orthoptera: Tettigoniidae). *Journal of Insects as Food and Feed* 4: 253-261. <https://doi.org/10.3920/JIFF2018.0005>
- Valtonen, A., Malinga, G.M., Junes, P., Opoke, R., Lehtovaara, V.J., Nyeko, P. and Roininen, H., 2018. The edible katydid *Ruspolia differens* is a selective feeder on the inflorescences and leaves of grass species. *Entomologia Experimentalis et Applicata* 166: 592-602. <https://doi.org/10.1111/eea.12707>
- Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. and Vantomme, P., 2013. *Edible insects: future prospects for food and feed security*. Food and Agriculture Organization of the United Nations, Rome, Italy, 201 pp.
- Wedell, N., 1996. Mate quality affects reproductive effort in a paternally investing species. *The American Naturalist* 148: 1075-1088.
- Wilder, S.M., Rypstra, A.L. and Elgar, M.A., 2009. The importance of ecological and phylogenetic conditions for the occurrence and frequency of sexual cannibalism. *Annual Review of Ecology, Evolution, and Systematics* 40: 21-39. <https://doi.org/10.1146/annurev.ecolsys.110308.120238>

