

## Developing Mass Egg-Laying Medium for the Edible *Ruspolia differens* (Orthoptera: Tettigonidae)

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### Abstract

The edible katydid, *Ruspolia differens* (Serville), is an economically and nutritionally important insect species that is common and widespread in Africa. Currently, there are aims to develop mass-rearing methods suitable for this species, but a critical first step would require a suitable oviposition medium to ensure economic and easy production of eggs with high hatchability. The objective of the present study was to determine the oviposition preference and egg hatchability in different oviposition media. Based on preliminary testing of a wide range of different materials, two oviposition media, namely, artificial (folded plastic cloth) and natural medium (leaf sheath of *Pennisetum purpureum* Schumacher attached to a stem), were selected for a detailed experiment. A two-choice laboratory experiment was conducted in which *R. differens* females were able to select between the folded plastic cloth (one side covered with short fleece) and a leaf sheath of *P. purpureum* (wrapped in polyester fiber). The results show that *R. differens* laid almost three times more eggs on the plastic cloth (mean  $\pm$  SE, 169.3  $\pm$  18.0) than on the natural substrate, *P. purpureum* (69.0  $\pm$  13.9). However, the hatchability (overall mean%  $\pm$  SE, 56.5  $\pm$  4.7) of eggs in the two media did not differ. Folded plastic cloth, therefore, provided a more effective medium for egg laying by *R. differens*, and is thus a promising oviposition medium for getting natural populations to start laying eggs in future mass rearing.

**Key words:** edible bush cricket, mass rearing, nsenene, oviposition media, Uganda

The edible katydid, *Ruspolia differens* (Serville), locally known in East Africa as ‘nsenene’ (Luganda) or ‘senene’ in Kiswahili (Matojo 2017), is an economically and nutritionally notable insect in sub-Saharan Africa (Agea et al. 2008, Okia et al. 2017). It is a large, elongate, cone-headed tettigoniid that occurs throughout the Afrotropical region, including Uganda, the Democratic Republic of the Congo, Kenya, Tanzania, Mauritius, and Seychelles (Massa 2015). Individuals are predominantly active at night and remain quiescent during the day, often hidden deep down in the sheaths of grasses, and are common in areas occupied by tall *Chloris* and *Digitaria* grasses (Swaine 1964). The adults exhibit distinct swarming and non-swarming phases, and throughout development individuals express color polymorphism with six color morphs (Swaine 1964, Matojo et al. 2017). The adults have a mean fresh weight of between 0.30 and 0.86 g depending on the diet and sex (Lehtovaara et al. 2017, Malinga et al. 2018b, Rutaro et al. 2018). *Ruspolia differens* is the most highly consumed insect in Uganda (Agea et al. 2008) and is increasingly being seen as a promising solution to food and nutrition security in East Africa (Kinyuru et al. 2017). The insect

is nutritionally rich and contains on a dry weight basis 47–49% fats, 8% carbohydrates and 44–46% proteins (Kinyuru et al. 2010, Siulapwa et al. 2014), based on Kjeldahl method which overestimates the level of protein content in insects since the inaccessible proteins such as chitin are included (Jonas-Levi and Martinez 2017). It is also rich in essential polyunsaturated fatty acids, i.e., linoleic and  $\alpha$ -linolenic acids (Kinyuru et al. 2010). However, its utilization is currently based mainly on hand picking and light harvesting from wild populations (van Huis et al. 2013, Okia et al. 2017). The impact of harvesting on wild *R. differens* populations is unknown, which is important given that wild populations are also likely to be threatened by land-use changes in the region, including the conversion of grassland habitat for other uses (Maitima et al. 2009). Consequently, there is increasing interest in mass rearing this edible insect as a strategy for ensuring its sustainable utilization (Malinga et al. 2018a,b), although the technology is still in its infancy. The development of large-scale mass-rearing system would require suitable oviposition substrate for egg production, a suitable diet, suitable rearing containers for all life stages, and optimal environmental

conditions for rearing. In our earlier works, we have established that maximal survival and adult weight can be achieved for *R. differens* at temperatures of between 28 and 30°C (Lehtovaara et al. 2018) and on mixed rather than single artificial diets (Malinga et al. 2018b).

However, a major obstacle to developing an efficient and reliable mass-rearing program has been the difficulty in getting natural populations to lay large quantities of eggs required for mass production. Under field conditions, *R. differens* usually oviposits in a tight space between the leaf sheaths of various grasses in batches (Karuhize 1968), whereas in the laboratory, they lay eggs in cotton wool and between the cotton wool and the container wall (Brits and Thornton 1981, Malinga et al. 2018a,b). However, the limitation of cotton wool is that eggs often stick on the medium and, in practice, are time-consuming to remove for hatching.

In this pioneering work, our aim was to identify an easily manageable and economic medium for egg laying in wild populations of *R. differens*. The medium should allow field-collected adults to be used for starting colonies of mass rearing by letting them oviposit into the most convenient substrate. We first investigated the potential of a wide range of artificial and natural materials as an egg-laying medium (preliminary study) and then selected the two most promising media for a detailed experiment. The selected materials were folded plastic cloth (with one side covered in short fleece) and the leaf sheaths of Elephant grass, *Pennisetum purpureum* Schumach (Poales: Poaceae) attached to a stem. The advantage of plastic cloth is that eggs can hatch on the medium without having to be manually removed and it is, therefore, suitable for the mass production of nymphs. In addition, the plastic material can be reused multiple times. In order to evaluate their suitability for mass rearing, we set two specific study questions: 1) does the readiness to lay eggs differ between these two media? and 2) does hatchability differ between these two media?

## Materials and Methods

### Preliminary Selection of Oviposition Media

We conducted a preliminary study exploring the utility of a wide range of potential oviposition media. The 12 possible media studied were leaf sheaths of *P. purpureum* attached to stems wrapped in polyester fiber (Toom Tekstiil AS, [www.toomtekstiil.ee](http://www.toomtekstiil.ee)), folded moistened

tissue paper (Luxury, Global Paper Products Ltd, Mukono), plastic coated with crepe paper on both sides and wrapped in polyester fiber, moistened cotton wool (Gulf Cotton Ltd, Busega, Kampala), *Panicum maximum* Jacq. (Poales: Poaceae) stems wrapped in polyester fiber, *P. maximum* leaves wrapped in polyester fiber, polyester fiber rolled inside a polythene sheet (cut from transparent plastic bag), folded plastic cloth with one side fleece coated and wrapped in polyester fiber, stems and leaves of *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase (Poales: Poaceae) wrapped in polyester fiber, folded moistened foam mattress (Recticel Ltd., [www.recticel.fi](http://www.recticel.fi)) placed inside a jar, moistened vermiculite (Liadur Sp. z o.o., [www.liadur.pl](http://www.liadur.pl)) with stems and leaves of *P. purpureum* and moistened vermiculite alone placed inside a jar (Table 1). Each experimental unit (container: 240 mm long × 180 mm wide × 125 mm high; Thermopack, Nairobi, Kenya) contained 15 randomly selected adult females and 10 males collected from the wild. These were simultaneously offered the 12 egg-laying media (Table 1). There were three experimental units in the experiment. Moistened tissue paper placed in each container was used as a source of water. The individuals were fed ad libitum on a combination of fresh maize seed (at silking stage attached to the comb), freshly germinated millet and sesame seed cake, and the experiment was run for 2 wk. We tested for differences in the total number of eggs laid among the 12 different media used in the preliminary test with Friedman's ANOVA. Based on preliminary testing, the media differed in number of eggs laid (Friedman's test,  $\chi^2 = 23.2$ ,  $P = 0.016$ ,  $df = 11$ ,  $n = 48$ ) and the two most promising candidates for oviposition medium were folded plastic cloth and leaf sheaths of *P. purpureum* (Table 1).

### Oviposition and Hatchability Experiment

The oviposition preference was evaluated using a two-choice bioassay with 1) leaf sheaths of *P. purpureum* attached to stems wrapped in polyester fiber and placed upright in a plastic jar (53 mm wide × 71 mm high) about a quarter filled with water and sand from the bottom (Fig. 1) and 2) folded plastic cloth wrapped in polyester fiber and placed in a jar (53 mm wide × 71 mm high), half filled with water (Fig. 1).

Ten randomly selected adult females and 10 males from the wild were placed in each experimental unit (container, 240 mm long × 180 mm wide × 125 mm high), and simultaneously offered

**Table 1.** Results of preliminary testing of 12 artificial (specific name for product if available, manufacture and their contacts) and natural materials as egg-laying medium for *R. differens*

Medium	Total number of eggs laid, median (interquartile range)
Leaf sheaths of <i>Pennisetum purpureum</i> Schumach. attached to stem wrapped with polyester fiber (Toom Tekstiil AS, <a href="http://www.toomtekstiil.ee">www.toomtekstiil.ee</a> )	42 (40–54)
Folded moistened tissue paper (Luxury, Global Paper Products Ltd, Mukono)	15 (8–18)
Plastic coated with crepe paper on both sides wrapped with polyester fiber (Helmi sauna laudeliina, Delipap Oy, <a href="http://www.delipap.fi">www.delipap.fi</a> )	0 (0–5)
Moistened cotton wool (Gulf Cotton Ltd, Busega, Kampala)	22 (11–58)
<i>Panicum maximum</i> Jacq. stems wrapped with polyester fiber	18 (13–37)
<i>P. maximum</i> leaves wrapped with polyester fiber	10 (8–26)
Polyester fiber rolled inside with polythene sheet (cut from transparent plastic bag)	0 (0–9)
Folded plastic cloth with one side fleece coated (Table cloth Compact, Friedola 1888 GmbH, <a href="http://www.friedola.de">www.friedola.de</a> ) wrapped with polyester fiber	191 (155–195)
Stems and leaves of <i>Echinochloa pyramidalis</i> (Lam.) Hitchc. & Chase wrapped with polyester fiber	0 (0–10)
Folded moistened foam mattress (Recticel Ltd., <a href="http://www.recticel.fi">www.recticel.fi</a> ) placed inside jar	16 (11–21)
Moistened vermiculite (Liadur Sp. z o.o., <a href="http://www.liadur.pl">www.liadur.pl</a> ) with <i>P. purpureum</i> stems and leaves	0 (0–0)
Moistened vermiculite alone	0 (0–0)

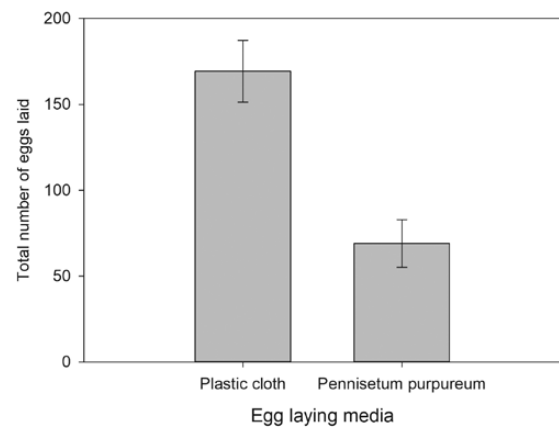
The values are the median and interquartile ranges (in parentheses) of total number of eggs laid per replicate container.

the two egg-laying media (Fig. 1). Males were added to ensure fertilization of females. Moistened tissue paper was placed in each container as a source of water. There were 14 experimental units in the experiment. The individuals were fed ad libitum on a combination of freshly germinated millet, fresh maize, and sesame seed cake. The experiment was run for 2 wk and then the number of eggs laid was counted. The eggs were left to hatch in each medium

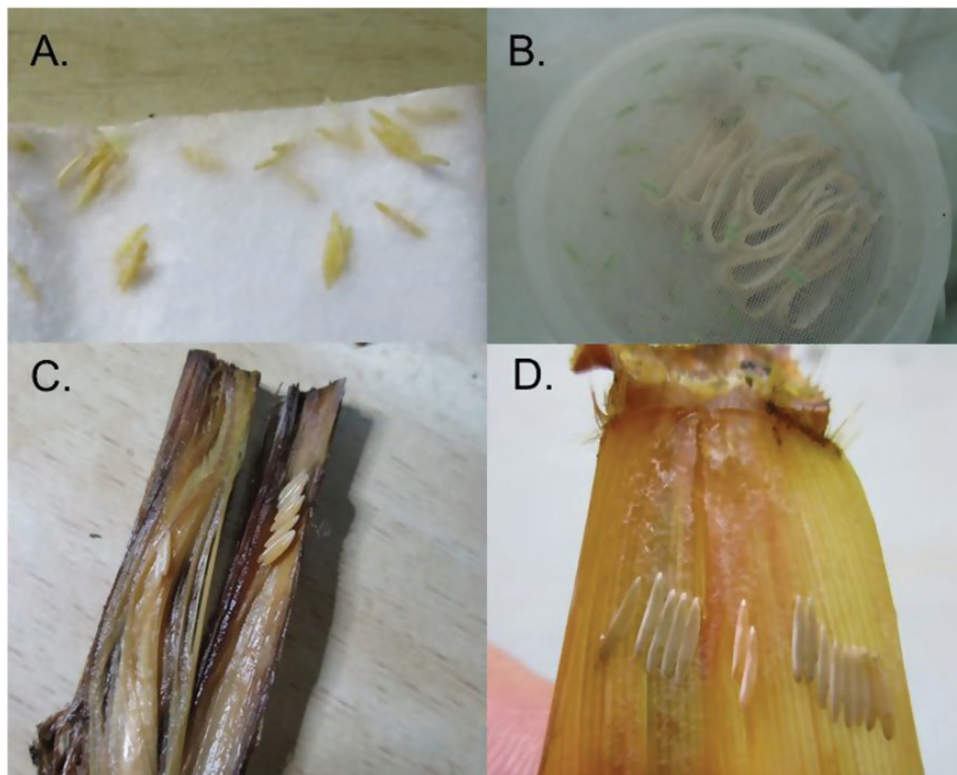


**Fig. 1.** The experimental unit in which egg-laying media were placed inside a transparent plastic jar with food for *R. differens*, (A) leaf sheaths of *P. purpureum* wrapped in polyester fiber, and (B) plastic cloth wrapped in polyester fiber (see online for color version).

for 3 wk (at a temperature of 22.7–25.6°C, relative humidity [RH] 56–71% and a 12:12 (L:D) h cycle), and sprayed daily with water to keep the eggs moist. For the hatchability experiment, we folded back the plastic cloth medium after counting the eggs and placed it in a plastic jar (53 mm wide × 71 mm high) filled with moistened cotton wool at the bottom. In the leaf sheath experiment, after opening and counting the eggs, we placed only the leaf sheath with eggs in a jar quarter filled with moistened cotton wool at the bottom. The opening on top of the jars was covered with a netting cloth to prevent the nymphs from escaping. Paired *t*-tests were conducted using SPSS, version 23 to compare the number of eggs and hatchability (percentage of neonates hatched) of the two media. Prior to analyses, the normality of the response variables was inspected.



**Fig. 2.** Total number of eggs (mean ± SE) oviposited by 10 female *R. differens* on the two studied media over 2 wk.



**Fig. 3.** *R. differens* eggs laid on (A) plastic cloth, (B) nymphs hatching from plastic cloth medium, (C and D) eggs on *P. purpureum* leaf sheath (see online for color version).

## Results

The females laid significantly more eggs on the artificial than on the natural substrate (paired *t*-test,  $t = 6.5$ ,  $P < 0.001$ ,  $n = 14$ , Figs. 2 and 3). The mean ( $\pm$ SE) number of eggs across the 14 replicates was  $169.3 \pm 18.0$  on folded plastic cloth (range 130.4–208.2), whereas it was  $69.0 \pm 13.9$  eggs on *P. purpureum* leaf sheath wrapped in polyester fiber (range 40.0–99.0; Fig. 2).

The hatchability of nymphs on the two media did not differ significantly (paired *t*-test,  $t = -0.8$ ,  $P = 0.418$ ,  $56.5 \pm 4.7$  [overall mean%  $\pm$  SE]).

## Discussion

The results of this study demonstrate that the folded plastic cloth is an effective oviposition medium for *R. differens* and can replace either cotton wool (Brits and Thornton 1981) or natural substrates such as grass leaf sheaths (Hartley 1967, Karuhize 1968). It can thus provide a promising oviposition medium for future mass rearing of this species especially when field-collected adults are used for starting colonies in mass rearing. The folding of plastic cloth to mimic the folds in a leaf sheath either stimulated the females to oviposit the eggs or helped them to insert their ovipositor and lay eggs. The advantage of plastic cloth is that eggs can hatch from medium without having to be manually removed and it is, therefore, suitable for the mass production of nymphs. In addition, the plastic material can be reused since it does not degrade easily. Overall, however, the number of eggs laid by 10 females over 2 wk in both media was small compared to the 130–160 eggs per female (green color morph) and 42–50 eggs (brown) reported in Brits and Thornton (1981). This disparity could be explained by many factors. First, when using random wild-captured females as in our experiment, their readiness to lay eggs might be low if they had only recently reached adulthood or they could include parasitized individuals with low egg-laying potential. Secondly, the seasonal food available in the field at the time of study was probably not good enough to allow females to generate many eggs. Furthermore, the low hatchability could be attributed to the development of molds (Hartley 1967) and unregulated hatching temperatures (Hartley and Yoshikazu 1988). Therefore, egg mortality could most likely be minimized in future rearing attempts through improved regulation of egg requirements.

Our laboratory results also confirm several previous field observations (e.g., Karuhize 1968) and laboratory experiments (Hartley 1967, Ssepuuya et al. 2018) that showed that adult females lay their eggs in the leaf sheaths of various grasses. The eggs laid in this situation are rather easily collected by washing (Hartley 1967). However, the disadvantage of the natural substrate in the context of mass rearing is how to keep the grasses fresh. Hartley (1967) suggested improvement by binding broad-leaved grasses with an absorbent paper and allowing them to stand in a conical flask. Improved results can be obtained by the provision of grass growing in pots but this may be impractical for large-scale mass rearing.

## Conclusion

The results of this study demonstrate a new manageable and economical method for producing large quantities of *R. differens* eggs for starting new colonies from the wild. The folded plastic cloth is a promising and effective oviposition substrate for *R. differens* that enables eggs to be easily hatched. This method can replace cotton wool or natural media such as grass leaf sheaths in the future design and development of mass-rearing methods for *R. differens*. However, the suitability of this egg producing method for long-term mass rearing still needs validation.

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