



Diet acceptance and preference of the edible grasshopper *Ruspolia differens* (Orthoptera: Tettigoniidae)

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Abstract

The edible grasshopper *Ruspolia differens* (Orthoptera: Tettigoniidae) gathered seasonally from the wild is a highly valued and an economically important edible insect, particularly in East Africa. To reduce the pressure on wild populations, a sustainable mass production technique needs to be developed. Unfortunately, however, basic biological know-how on feeding habits of *R. differens* is poorly understood, which poses a constraint on the development of mass-rearing technology. Here, we evaluated the acceptance and feeding preference of *R. differens* for 16 cultivated or processed foods using no-choice and multiple-choice laboratory bioassays. The results indicated that adult *R. differens* can eat a wide variety of foods but does not necessarily accept all the foods equally. Furthermore, our experiments showed that *R. differens* has high ability to select diet among those available. The order of decreasing preference was wheat bran > germinated finger millet > rice seed head > finger millet seed head > chicken feed egg booster > sorghum seed head. Finally, our study indicated that sex and color morph are not associated with the order of acceptance and preference of diets in *R. differens*. These results show potential foods that could be utilized for developing future mass-rearing methods for *R. differens*.

Keywords African edible bush cricket · Artificial food · Feeding test · Insect rearing · Uganda

Introduction

The edible grasshopper *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae) is a highly valued insect with a widespread distribution throughout several countries in the Afrotropical region, e.g., Angola, Ghana, Ivory Coast, Central African Republic, Democratic Republic of Congo, Rwanda, Tanzania, Kenya, Uganda, Zimbabwe, Mauritius, Madagascar, and in some islands of the Indian Ocean, e.g., Seychelles, Zanzibar (Bailey and McCrae 1978; Massa 2015). It is a slender grasshopper (30–42 mm long) with a filiform antenna, a slanted face, and a distinctly longer

than wide cone, which narrows from base to apex (Matojo and Yarro 2010). *Ruspolia* species are primarily nocturnal feeders (Bailey and McCrae 1978), and are common in savannas and *Hyparrhenia* grasslands at altitudes between 800 to 1700 m asl (Hemp 2005). The body length varies among sex (males, mean $\pm$ SE, 35.3 ± 0.8 mm, females 38.0 ± 0.6 ; Matojo and Yarro 2013). They may be solitary or gregarious forming high-density seasonal flying swarms in East Africa in May and November–December (Bailey and McCrae 1978).

In Eastern Africa, *R. differens* (locally called “nsenene” by its Luganda name) is among the most consumed species of insects (Kinyuru et al. 2010; Okia et al. 2017; van Huis 2003) with potential to contribute to both rural and urban livelihood and employment opportunities (Agea et al. 2008). Unfortunately, basic biological know-how on feeding habits of *R. differens* either in the laboratory or in the field is scant, which poses a constraint on the development of sustainable mass-rearing technology as an alternative to the seasonal harvest of natural populations. Laboratory studies conducted in temperate regions showed that *Ruspolia* species can be reared on grass as well as

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some artificial diets consisting of ground dog biscuits, dried blood and lucerne meal, and a high protein cereal (Brits and Thornton 1981; Hartley 1967) under controlled temperature and relative humidity. Preliminary observation conducted in Makerere University, Uganda, showed that in the laboratory, *R. differens* can eat wild grass leaves or their flowers, and artificial plant mixtures like millet and maize flours (Nyeko et al. 2014). However, there is apparently still very little information on potential locally available diets which *R. differens* can accept for food and their preference of accepted diets.

The aim of the present study is to determine the feeding acceptance and preference of adult *R. differens* on 16 cultivated or processed foods in the laboratory using no-choice and multiple-choice experiments, to screen suitable foods for mass-rearing of *R. differens*. Furthermore, we intend to examine whether the acceptance and preference of food are correlated between males and females or between green and brown color morphs. Specifically, we answer the following questions: (1) Does the feeding acceptance vary among the 16 foods? (2) Are there any variations in the preferences among the most readily accepted foods? (3) Does the feeding acceptance and preference correlate between sexes or color forms?

Materials and methods

Study insects

To evaluate the feeding acceptance and preference of *R. differens*, we used populations of swarming adult grasshoppers that were captured from artificial lights in Natete suburb of Kampala, Uganda. Upon collection, the grasshoppers were kept for one night in transparent ventilated plastic jars (height 140 mm, width 110 mm) in groups of 10 insects per container. To limit the possibility of the *R. differens* feeding on non-preferred foods during the feeding experiments because of hunger (Smith and Capinera 2005), we allowed individuals to feed ad libitum on the inflorescence of its natural food plant, *Panicum maximum* Jacq. (Poaceae) prior to the feeding tests. There are eight polymorphic forms of *R. differens*, namely green, brown, brown stripped purple, light green, pale green, purple light green, green stripped purple, and light brown (Bailey and McCrae 1978; Nyeko et al. 2014). We classified the studied grasshoppers into either green or brown. The other six color morphs are rare, so light brown and brown stripped purple were included in the brown color morph group, whereas the light green, pale green, purple light green, and green stripped purple were included in the green color morph group.

Test foods

In total 16 different foods that differ in their nutritional quality were used (Table 1). The unprocessed foods such as maize, millet, sorghum, simsim, groundnut, pumpkin, and banana were selected because they can be locally cultivated in a wide range of soils and climates, and are commonly available in most communities in Uganda. Processed foods, e.g., dog biscuit, soya millet, and wheat bran, were included because they are common and available in local markets year round. Finger millet was included because it is one of the most nutritious of all cereals being rich in essential amino acids (e.g., methionine, valine, and lysine), iron, and calcium (Barbeau and Hilu 1993). For this test, undamaged mature seed heads of finger millet, sorghum, and rice that had just started to turn golden brown, red/reddish brown, and yellow, respectively, were used. The groundnut paste was prepared by grinding the raw shelled seeds and adding a little water to make a paste. To prepare simsim cake, equal parts of simsim seeds and sugar were heated over a low temperature (60–70 °C) until the sugar liquefies and allowed to cool. The coarse rice and maize flours were prepared by grinding dried rice or maize seeds into coarse texture on a traditional grinding stone. Maize bran was prepared from husks of maize grains. Germinated millet was prepared by soaking millet grains in tap water overnight. The water was drained and the grains were wrapped in a moist netted fabric and left to sprout at room temperature for 72 h until the rootlets and shoots just begin to grow. The dog biscuit pellet consisted of high protein cereals, essential vitamins, minerals, and antioxidants. The chicken feed egg booster is a high protein diet, consisting of essential amino acids such as lysine, methionine, threonine, and tryptophan, and high levels of calcium, phosphorus, sodium, as well as antioxidants.

Experimental setup

The feeding tests were performed using no-choice (acceptance) and multiple-choice (preference) experiments, under laboratory conditions (ambient temperature, 22–32 °C, 60–72% RH, and a photoperiod of 12:12 h, L:D). In both feeding experiments, adult *R. differens* were placed individually in transparent jars. To account for possible microclimatic variation during the feeding tests, the jars were placed on tables in groups (blocks; see details below). To maintain the humidity and prevent dehydration of the insect during the experiment, each rearing jar was provided with a moistened tissue paper. The same populations of *R. differens* were used in both experiments, except that fewer individuals were used for the multiple-choice experiment.

Table 1 Foods used in the acceptance and preference test of adult *R. differens*

Common or trade name	Scientific name	Family	Manufacturer	Energy kcal ^a	Protein (g/100 g) ^a	Fat (g/100 g) ^a	Fiber (g/100 g) ^a	Carbo-hydrate (g/100 g) ^a	Ash (g/100 g) ^a	Water (g/100 g) ^a	Ca (mg) ^b	Mg (mg) ^b	Fe (mg) ^b	Na (mg) ^b
Finger millet seed head ^d	<i>Eleusine coracana</i> Gaertn.	Poaceae		336	7.7	1.5	3.6	72.6	2.6	10.9	350	114	3.9	5
Sorghum seed head ^e	<i>Sorghum bicolor</i> Moench (cv. Seredo)	Poaceae		354	9.3	3.9	9.9	65.5	2	9.4	24	311	3.9	14
Rice seed head ^e	<i>Oryza sativa</i>	Poaceae		349	6.9	0.6	1.4	78.3	0.7	12.1	12	35	1.4	5
Wheat bran ^e	<i>Triticum aestivum</i> L.	Poaceae	The East African Basic Foods Ltd, Kampala, Uganda	282	15.9	4.8	4.5	23.2	5.6	9	74	546	14.8	15
Superfeed chicken egg booster ^c			Super Feeds Farm Products Ltd, Kampala, Uganda	12.5	3.4		3.8				2			6.1
Fresh maize cob ^e	<i>Zea mays</i> L.	Poaceae		349	9.2	4.1	9.7	63.9	1.8	11.4	19	82	3.1	11
Germinated finger millet ^{fg}	<i>Eleusine coracana</i> Gaertn.	Poaceae		303.2	8.6	0.6	0.9	80.9	6.3	9.6	3		16.3	
Crushed dog biscuit pellet ^c			Martin and Martin (Pty) Ltd, Namibia		22	9	4.5		10	9				
Soya millet flour ^c			The East African Basic Foods Ltd, Kampala, Uganda	420.4	19.9	9.9	11.5							

Table 1 (continued)

Common or trade name	Scientific name	Family	Manufacturer	Energy kcal ^a	Protein (g/100 g) ^a	Fat (g/100 g) ^a	Fiber (g/100 g) ^a	Carbo-hydrate (g/100 g) ^a	Ash (g/100 g) ^a	Water (g/100 g) ^a	Ca (mg) ^b	Mg (mg) ^b	Fe (mg) ^b	Na (mg) ^b
Maize bran ^d			Super Feeds Farm Products Ltd, Kampala, Uganda	224	8.4	0.9	79	85.6	0.4	4.7	42	64	2.8	7
Boiled pumpkin ^e	<i>Cucurbita maxima</i> Duchesne ex. Lam.	Poaceae		31	1.1	0.1	0.8	6.1	0.8	91.2	6	29	0.3	2
Wet groundnut paste ^e	<i>Arachis hypogaea</i>	Fabaceae		594	25	47.2	1.5	16.5	2.6	7.2	61	191	4	17
Simsim cake ^{hi}	<i>Sesamum indicum</i> L.	Pedaliaceae		2753	44.4	13.1	8.8	35.4	14.2	8.3	1.9	0.7	304.9	25.1
Coarse rice flour ^d	<i>Oryza sativa</i>	Poaceae		366	5.9	1.4	2.4	80.1	0.6	11.9	10	35	0.4	0
Yellow dessert banana ^e	<i>Musa sp.</i>	Musaceae		106	1.4	0.2	2	23.6	0.8	72	6	29	0.3	2
Coarse maize flour ^e	<i>Zea mays</i> L.	Poaceae		351	9.7	4	9	64.5	1.5	11.5	18	93	3.8	11

^aProximate nutrient composition^bMicronutrients^cNutritional facts provided by the manufacturer^dUSDA National Nutrient Database for Standard Reference, Release 28 (2016)^eFAO (2016)^fMuyanja et al. (2003)^gCheme and Chinma (2008)^hBabiker (2012)ⁱBukya and Vijayakumar (2013)^jKumar et al. (2016)

No-choice experiment

In the no-choice laboratory bioassay, one individual in each treatment was randomly assigned to the 16 food treatments with each receiving approximately the same amount of food (about 2 g), i.e., each jar contained a single grasshopper (used only once in this experiment) and a single food (Table 1). The jars were randomly assigned to 10 blocks, each of which consisted of two replicates of the no-choice test. Overall, 320 individual *R. differens* (86 males, 234 females; 212 green and 108 brown color morph groups) were placed in an experimental transparent plastic jar (1000 mL, TPL 2035, Thermopack Limited, Nairobi), and the opening on top was tightly closed with a lid containing eight equally sized holes to allow air circulation. Each jar was inspected after 48 h to screen what food is accepted and how much is consumed by individual *Ruspolia*. The proportion of each food consumed was visually estimated using a scale of 0–5 (modified from Capinera 1993), with 0 representing no feeding, 1 representing less than 10% consumed, 2 representing 10–30% (some obvious feeding), 3 representing 31–50% (readily eaten), 4 representing 51–80%, and 5 representing 81–100% consumed.

Multiple-choice experiment

In the multiple-choice tests, each individual was simultaneously offered (once) approximately equivalent weights (about 2 g) of each of the six most accepted foods (rice seed head, finger millet seed head, wheat bran, chicken feed egg booster, sorghum seed head, and germinated finger millet), selected on the basis of their acceptance scores in the no-choice test. A total of 50 individual *R. differens* (4 males, 46 females; 43 green and 7 brown color morph groups) were randomly placed in experimental jars (one insect per jar), and the jars were placed on tables in 25 groups (blocks), each of which consisted of two replicates of the multiple-choice test. Assays were checked after 48 h and the proportion of each food consumed scored as described earlier.

Statistical analysis

To examine whether the food acceptance differed among the 16 foods in the no-choice bioassay, a non-parametric Kruskal–Wallis one-way analysis of variance (ANOVA) was used, followed by post hoc pairwise comparisons (default method in SPSS for Kruskal–Wallis). We tested for differences in the ranks of food preference among the six most readily accepted foods in the multiple-choice bioassay using Friedman's ANOVA, which is a non-parametric alternative to unreplicated block design ANOVA. When significant differences were detected, two-tailed Wilcoxon matched pairs signed-ranks tests (Zar 1996) corrected for multiple testing

(Bonferroni correction; Quinn and Keough 2002) were used to ascertain which diets were preferred more than the other. Spearman's rank order correlation analyses were conducted to test if the median feeding acceptance and preference scores of males and females or color morphs are associated with each other. All statistical analyses were performed with SPSS software (version 23, IBM Corporation, Armonk, NY, USA).

Results

In the no-choice bioassay, all of the 16 foods were accepted by adult *R. differens*. However, the median amount of foods eaten varied significantly (Kruskal–Wallis test, $df = 15$, $H = 105.4$, $p < 0.001$). The six most eaten foods were rice seed head, finger millet seed head, wheat bran, chicken feed egg booster, sorghum seed head, and germinated finger millet (Fig. 1). The boiled pumpkin, groundnut paste, and coarse rice flour were the least eaten foods. The feeding acceptance of the 16 foods correlated positively between males and females (Spearman's correlation, $\rho = 0.53$, $p = 0.035$, $n = 16$), and between brown and green color morphs (Spearman's correlation, $\rho = 0.53$, $p = 0.035$, $n = 16$).

In the multiple-choice experiments, preference of feeding differed significantly among the six foods offered (Friedman's test, $\chi^2 = 90.7$, $p < 0.001$, $n = 50$). The order of decreasing preference was wheat bran > germinated finger millet > rice seed head > finger millet seed head > chicken feed egg booster > sorghum seed head (Fig. 2). Feeding preference scores for wheat bran and germinated finger millet were significantly higher than that of finger millet seed head, rice seed head, chicken feed, and sorghum seed head (Wilcoxon test: $p < 0.001$; Fig. 2). The feeding preference

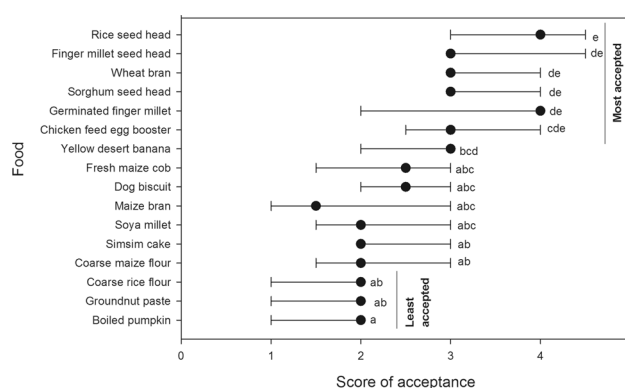


Fig. 1 Variation in acceptance scores [expressed as median (closed circles) and interquartile ranges] for the 16 cultivated and/or processed foods in the no-choice feeding tests. Bars with the same lower-case letters on the right side are not significantly different (Kruskal–Wallis one-way ANOVA followed by default post hoc pairwise comparisons)

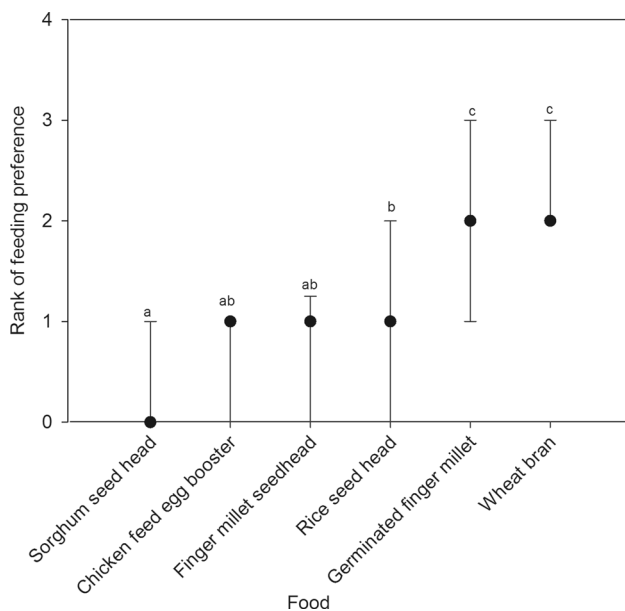


Fig. 2 Variation in the rank of feeding preference [expressed as median (closed circles) and interquartile ranges] for the six foods in multiple-choice preference tests. Bars with the same lowercase letters do not differ significantly from each other (Friedman's test followed by Wilcoxon test with Bonferroni corrected levels of observed significance)

score of males was significantly positively correlated with that of females (Spearman's correlation, $\rho = 0.93$, $p = 0.005$, $n = 6$). Also, the preference for the six foods was strongly correlated between color forms (Spearman's correlation, $\rho = 0.89$, $p = 0.018$, $n = 6$).

Discussion

In the present study, we showed that adult *R. differens* can eat a wide variety of foods but do not necessarily accept all the 16 foods equally. The high ability to utilize different foods by *R. differens* adults has shown them to be highly polyphagous like most other orthopterans (Abushama and Elhag 1971; Capinera 2014; Miura and Ohsaki 2004), allowing them to consume secondary foods if preferred foods are not available. Seed heads of rice, finger millet, wheat bran, chicken feed egg booster, seed head of sorghum, and germinated seed of finger millet were the six most accepted foods. These results confirm several previous sporadic observations that *R. differens* can eat various types of grains (Bailey and McCrae 1978; Matojo and Yarro 2010; Matojo and Hosea 2013; Nyeko et al. 2014; Swaine 1964). The mandibles of *Ruspolia* are robust and are most likely specialized for grain feeding (Brits and Thornton 1981).

Additionally, our results show that sex or color morph is not associated with the order of acceptance and preference

of diets in *R. differens*, suggesting that it is possible to feed all *Ruspolia* individuals with similar diet mixtures. These results contradict those of Franzke et al. (2010) on the meadow grasshopper, *Chorthippus parallelus* (Zetterstedt) (Orthoptera: Acrididae) in which diet selection depended on sex. Also in contrast to our results, Karpestam and Forsman (2013) showed that among the pygmy grasshoppers *Tetrix subulata* (Linnaeus) (Orthoptera: Tetrigidae), utilization of food types differed among color morphs within a population. However, sufficient nutrition should be considered for growth, egg production, and survival during future rearing. Recently, we have found out that the performance (as described by adult fresh weight, survival to adult, adult fecundity) was better on mixtures of the foods than on single or less diversified diets (Malinga et al. 2018).

Our results emphasize the high ability of *R. differens* to select diet among the available ones but also its flexibility in host use if limited range of diet is available. The high preference of *R. differens* for wheat bran and germinated finger millet is unclear, but it is possible that these foods are nutritionally superior to the others offered. It is theoretically also possible that the selection could be determined by the water content of food (Lewis and Barnays 1985) but in the present study no such effect was evident (Table 1), and therefore the nutrition content more likely explains what we found. Typically, nitrogen content (as protein) in a diet has been shown to be the most limiting nutrient, needed for the growth, reproduction, and survival of any herbivore (Mattson 1980). Thus, differences in the quantity of protein might explain the differences in diet preference. For example, of the six foods offered in the multiple-choice experiments, wheat bran had the highest total protein content. Furthermore, germination has been shown to improve the nutritional quality of cereals by, e.g., increasing the bioavailability of essential nutrients and decreasing the levels of anti-nutrient factors like phytate and tannins (Mbithi-Mwikya et al. 2000; Sripriya et al. 1997) which might explain the preference of *R. differens* for germinated finger millet. However, considering that wheat is not cultivated in this area and its price for local farmers is high, germinated finger millet produced in the area would be a more recommended food which local farmers can utilize for captive rearing of *R. differens* compared to wheat bran.

Finally, our result shows that willingness to accept diet does not necessarily determine preference order, i.e., rankings of different diets by *R. differens* differed between the no-choice and multiple-choice experiments. For example, in the no-choice test, the most accepted food was rice seed head (Fig. 1), whereas, in the multiple-choice experiment, the most preferred food was wheat bran (Fig. 2). This apparent discrepancy could be due to the behavioral habits of polyphagous insects which allow them to consume greater amounts of a lower grade food as compensation for its low

nutritional value (Roa 1992). However, if high-quality foods are available (multiple-choice experiments) they consume low quantities of these low-grade foods. Alternatively, it could simply be that different foods are processed in the gut at different speeds, partly explaining the differences in meal sizes in no-choice tests (Bernays and Barbehenn 1987). Our results emphasize the need to conduct both no-choice (acceptance) and choice (preference) testing when considering potential diets of edible insects in a laboratory setting.

In conclusion, we provide evidence that *R. differens* can eat a wide variety of foods but do not necessarily accept all the foods equally. Furthermore, our results show that *R. differens* have high preference for the most readily accepted foods with wheat bran and germinated finger millet being the most preferred foods. Additionally, it is evident that sex and color morph are not associated with the acceptance and preference of diets in *R. differens*. Thus it is possible to rear all of the *Ruspolia* individuals irrespective of sex and color morph with similar diet mixtures. These results show that many foods could be used for mass-rearing of *R. differens*. It also shows that acceptance does not necessarily determine preference order, highlighting the need to involve both acceptance and preference testing when developing ideal foods for rearing this edible insect species.

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