Entomologia
Experimentalis et Applicata

DOI: 10.1111/eea.12707

The edible katydid *Ruspolia differens* is a selective feeder on the inflorescences and leaves of grass species

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Accepted: 7 May 2018

Key words: Afrotropics, East Africa, grassland, herbivory, multiple-choice, no-choice, nutritional ecology, Poaceae, swarming, Uganda, Orthoptera, Tettigoniidae, edible bush-cricket

Abstract

Ruspolia differens (Serville) (Orthoptera: Tettigoniidae, Conocephalinae) (its common names including 'African edible bush-cricket', 'edible grasshopper', and 'nsenene') is an important source of food for humans in East Africa, but its ecology and biology are poorly understood. We explored the host plants of *R. differens* with a series of no-choice and multiple-choice laboratory experiments using 18 local common grass and sedge species in Uganda. In no-choice experiments, the degree of acceptance differed significantly among the studied plant species, but in only three species were leaves rejected and in one species were inflorescences rejected. The pattern of acceptance among plant species was different in the local vs. swarming populations. Leaves were generally more accepted by the local population, whereas inflorescences were generally more accepted by the swarming population. Both leaves and inflorescences were more readily accepted by males than by females. According to the multiple-choice experiments, *R. differens* preferred inflorescences over leaves. Our results demonstrate that *R. differens* is a facultatively oligophagous grass-specialist, which has a clear preference for certain grass or sedge species (especially inflorescences), but it accepts many host plants if the preferred ones are not available. To preserve viable natural populations of *R. differens* in East Africa in the long term, our results draw special attention to the availability of grasslands where accepted and preferred host plants are available year-round.

Introduction

Ruspolia differens (Serville) (Orthoptera: Tettigoniidae, Conocephalinae), is one of the most consumed edible insects by humans and an important source of food and nutrition in eastern and southern Africa (Agea et al., 2008; Kinyuru et al., 2010; van Huis et al., 2013) (Figure S1). The common names of this species include 'African edible bush-cricket', 'edible grasshopper', and 'nsenene'. In East Africa, *R. differens* are harvested from natural populations during the two annual periods of swarming (Agea et al., 2008). The swarms are known to be capable of migrating long distances and the locations of their source populations are unknown (Bailey & McCrae, 1978). Consequently, no measures for habitat protection have been introduced so far. Recently, intensified commercial harvesting with powerful light-harvesting stations could pose a risk of overexploitation of natural populations in the long term, while the bycatch could pose a risk to populations of other light-attracted insect species. Small-scale mass-rearing methods accessible to local farmers are currently being developed with the aim of enhancing food security in East Africa. However, the major obstacle to such efforts is poor understanding of the ecology and biology of *R. differens*, including the host plant species it accepts and prefers. Such understanding is also essential for management of agricultural land and the protection of natural habitats of *R. differens* in East Africa.

Ruspolia differens is a relatively large-bodied (30–42 mm long; Matojo & Yarro, 2013) and primarily nocturnal species (Bailey & McCrae, 1978) that is found in grassland habitats (Swaine, 1964) across the Afrotropics

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and some islands in the Indian Ocean (Bailey & McCrae, 1978; Massa, 2015). The two typical color morphs of *R. differens* are green and brown, whereas morphs with superimposed purple stripes and a purple head are rarer (Bailey & McCrae, 1978). The nymphs can switch color during molting (Robinson & Hartley, 1978), possibly a plastic response allowing body coloration to match local environmental conditions (Valverde & Schielzeth, 2015), providing cryptic camouflage for individuals hiding in grass tussocks during the day (Bailey & McCrae, 1978; Matojo & Yarro, 2010). *Ruspolia differens* forms migratory swarms during and after the rainy seasons; in Uganda, this occurs around April-May and November-December (Bailey & McCrae, 1978). In the swarming populations (or 'swarming phase'; Matojo & Yarro, 2010), the behavior of individuals is different compared to the local non-swarming populations ('non-swarming phase'); they fly actively in high densities, mostly during the night and are attracted to lights (Bailey & McCrae, 1978; McCrae, 1982). Swarming of *R. differens* represents a distinct state of activity, not merely an index of population density (Bailey & McCrae, 1978). The species has strong mandibles, possibly an adaptation for cracking grass seeds (Brits & Thornton, 1981). In the field, the species has been observed on 19 grass and two sedge species (>80% of observations from *Brachiaria ruziziensis*, *Panicum maximum*, and *Chloris gayana*; R Opoke, P Nyeko, GM Malinga, K Rutaro, H Roininen, A Valtonen, unpubl.), as well as on many species of cereal (Swaine, 1964). In the laboratory, *R. differens* accepts grasses, with flowers and seeds possibly preferred over stems and leaves (Hartley, 1967), and a large variety of cereals and artificial feeds (Brits & Thornton, 1981; Malinga et al., 2018). However, detailed experiments revealing the acceptance and preference of potential host plant species are still lacking and there has been no experimental evaluation as to whether *R. differens* prefers inflorescences over leaves, or whether the host selection differs among swarming and local (i.e., non-swarming) populations, and between sexes or among color morphs.

In this study, we explored the host acceptance and preference of *R. differens* in Uganda with a series of no-choice and multiple-choice laboratory experiments using 18 Afrotropical grass and sedge species. Three study questions were posed: (1) does the acceptance of the 18 offered plant species (either inflorescences or leaves) differ in no-choice experiments, and does the pattern depend on population (local vs. swarming), sex, or color morph? (2) Does the preference of the six most accepted leaves or inflorescences differ when offered together in multiple-choice experiments, and does the pattern depend on sex or color morph? And (3) does the preference of the three most accepted leaves and inflorescences differ when offered together in multiple-choice experiments, and does the pattern depend on sex or color morph?

We hypothesize that the plant species are not equally accepted or preferred because plant species differ with respect to their chemistry, and nutrients, in particular, are important in determining the host choice among orthopterans (Behmer & Joern, 2008; Cease et al., 2012). Furthermore, we hypothesize that inflorescences are preferred over leaves because reproductive parts of plants are generally more nutritious for herbivores than leaves, due to their high levels of proteins (Bernays & Chapman, 1994). We also hypothesize that individuals representing local vs. swarming populations, and females vs. males, differ in the pattern of their acceptance or preference of the plant species on offer, due to their different physiological state or nutritional requirements (Boys, 1978; Behmer & Joern, 1994; Unsicker et al., 2008). Finally, we hypothesize that the color morphs differ in the pattern of acceptance or preference of the plant species offered due to differences in the degree to which the species potentially provide protection from predators to individuals differing in their cryptic camouflage (Karpestam & Forsman, 2011).

Materials and methods

Study site and sampling of *Ruspolia differens*

The experiments were conducted at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK) in Uganda. No-choice experiments were conducted in November and December 2015, and multiple-choice experiments in May, June, and November 2017. We studied both local and swarming *R. differens* populations. Individuals representing the local population were collected within 3 km of MUARIK from agricultural farmland, and individuals from the swarming population were collected from commercial light-harvesting stations in Kampala. Only healthy adults, as judged by visual scrutiny, were included in the experiments, but if an individual died during the experiment, or parasitoid larvae emerged from it, the data were excluded from analyses. We stored, and during experiments reared, individuals of both swarming and local populations in the exact same conditions. Prior to the experiments, insects were stored in 2-l containers (maximum of 10 individuals in each) and fed with *P. maximum* and fresh maize cobs. In almost all cases, individuals were included to experiments within 2 days after they were collected from the wild, but a small proportion of healthy individuals which were returned to test populations (see details below) were stored up to 1 week. We classified individuals based on their color morph as 'green' (including all green morphs and green morphs with purple stripes) or 'brown' (including all brown morphs and brown morphs with purple stripes).

Experimental settings

In all experiments, we reared individual *R. differens* for two consecutive nights in a circular 14-cm-diameter and 8-cm-deep plastic container with holes in the lid for air circulation, and in a natural L12:D12 photocycle. A small amount of rolled wet tissue paper was placed in each container to maintain humidity and to provide water for the insect; the relative humidity in the laboratory was ca. 67% and temperature ranged between 22 and 28 °C.

We first explored the degree of acceptance of potential hosts of *R. differens* with two no-choice experiments using (1) leaves and (2) inflorescences of 18 plant species (Table 1). The 18 plant species were selected because they represent common grass and sedge species at the study site. The undamaged plant material was collected within 5 km of MUARIK. We only used fresh, fully mature leaves and inflorescences (with visible stamens). In the no-choice experiments, we offered each solitary *R. differens* one plant species in equal quantities (estimated visually). The degree of acceptance of offered leaves was estimated visually after the second night using the following scale (% of offered leaves consumed): 0, 1, 1.1–5, 5.1–10, 10.1–20, 20.1–30, 30.1–40, 40.1–50, 50.1–60, 60.1–70, 70.1–80, 80.1–90, and 90.1–100. The degree of acceptance of inflorescences was estimated visually after the second night using the following scale (% of offered inflorescences consumed): 0, 0.1–5, 5.1–15, 15.1–50, 50.1–95, and 95.1–100. In both experiments, in total 16 individual *R. differens* from both local and swarming populations were randomized for each plant species. However, as some insects died during the experiment, the number of replicates varied between 10 and 16. The individuals were selected from test populations of >100 individuals, which were fed with *P. maximum* and fresh maize cobs. New individuals were regularly added to the test populations due to mortality. After each no-choice trial, healthy and active insects were returned to the test populations. However, due to the large size of test populations, the regular replenishment by individuals from the wild and the practice of always first randomizing the fresh individuals to experimental trials, the probability of the same individual being used twice (or more) in the no-choice trial of the same plant species was very low.

We then continued studying host preference with multiple-choice experiments using (3) the six most consumed leaves, (4) the six most consumed inflorescences, and (5) a combination of three most consumed leaves and three most consumed inflorescences based on no-choice experiments. The foods were selected by ranking the 18 species according to the average percentage consumed in no-choice experiments – we chose to use the mean value in each class, and not the median values due to many ties in data. The multiple-choice experiments were conducted separately for the local and swarming population because the six or three most consumed plant species were not

Table 1 Grass and sedge species used in the no-choice experiments. Species were identified at the Makerere University Herbarium by P. Rwaburindore, based on Clayton et al. (1974). All plant species are likely to represent the C4 photosynthetic type (Jones, 1987; Osborne et al., 2014; Ayeni et al., 2015)

Scientific name	Common name(s)	Family
Brachiaria ruziziensis R. Germ. & C.M. Evrard	Congo signal grass, Congo grass	Poaceae
Chloris gayana Kunth	Rhodes grass	Poaceae
Cyperus distans L.f.	Slender cyperus	Cyperaceae
Cyperus papyrus L.	Papyrus	Cyperaceae
Digitaria abyssinica (Hochst. ex A. Rich.) Stapf	East African couch grass	Poaceae
Echinochloa pyramidalis (Lam.) Hitchc. & Chase	Antelope grass	Poaceae
Eleusine indica (L.) Gaertn	Wild finger millet, goose grass	Poaceae
Eragrostis tenuifolia (A. Rich.) Hochst. ex Steud	Elastic grass	Poaceae
Hyparrhenia rufa (Nees) Stapf	Giant thatching grass	Poaceae
Imperata cylindrica (L.) P. Beauv.	Spear grass	Poaceae
Melinis repens (Willd.) Zizka	Rose natal grass	Poaceae
Panicum maximum Jacq.	Guinea grass	Poaceae
Pennisetum purpureum Schumach.	Elephant grass, napier grass, Uganda grass	Poaceae
Setaria homonyma (Steud.) Chiov.	'Setaria'	Poaceae
Setaria megaphylla (Steud.) T. Durand & Schinz	Ribbon bristle grass	Poaceae
Setaria sphacelata (Schumach.) Stapf & C.E. Hubb. ex Moss	Nandi grass	Poaceae
Sorghum leiocladum (Hack.) C.E. Hubb.	Wild sorghum	Poaceae
Sporobolus pyramidalis P. Beauv.	Pyramid grass	Poaceae

identical for these populations. Between 29 and 57 individual *R. differens* were randomly selected for each of the six multiple-choice experiments and each individual was used in only one experimental trial. In each multiple-choice experiment, the six foods offered were placed in the containers in a similar quantity and spread so that insects had access to each. The quantity of each food was sufficient to enable individuals to feed ad libitum. After the second feeding night, we ranked the plant material in the order in which they were preferred (1, most preferred; 2, second-most preferred; ... 6, least preferred). The mean ranks were used if two (or more) foods were preferred equally.

Statistical analysis

To answer the question whether the degree of acceptance differed among the 18 plant species in the no-choice experiments, we fitted ordinal logistic regressions (multinomial response with cumulative logit link function). As the response variables, we used the degree of acceptance (evaluated originally as percentages) transformed to ordinal scales; scores for leaves varying between 0 (0%) and 12 (90.1–100%) and for inflorescences between 0 (0%) and 5 (95.1–100%). The explanatory variables included plant species, population (local vs. swarming), sex, and color morph, as well as all two-way interactions between these factors. We first fitted a full model with all variables and their two-way interactions. For the final models, all non-significant interactions were excluded.

To determine whether the rank order of preference differed among the six foods offered in multiple-choice experiments, we fitted SPSS 'generalized estimating equations' (ordinal response with cumulative logit link function). The individual, to whom the six foods were offered simultaneously, was included as a 'subject'. The explanatory variables included the plant species, sex, and color morph, as well as two-way interactions between plant species and color morph and between plant species and sex. Interaction between sex and color morph was not included in the model, because the scores of all individuals ranged between 1 and 6. Direct effects of sex and color morph were included only because these terms appear also in interactions included in the models. We first fitted a full model with all terms, but, for the final model, all non-significant interactions were excluded.

All statistical analyses were conducted in IBM SPSS Statistics v.23 and graphs were produced with R v.3.3.2 (R Core Team, 2014). As post-hoc pairwise tests were not available in SPSS for the ordinal logistic regression (to determine which host plant or food types differed from each other in terms of preference), we inferred differences between the most preferred food(s) and the rest of the foods based on the standard SPSS output for ordinal logistic regression (which shows statistical differences between one control food and all other foods). In the case of multiple-choice experiments, where the three most consumed leaves and the three most consumed inflorescences were combined, we inferred differences between all inflorescences vs. all leaves.

Results

Acceptance of leaves in the no-choice experiment

In the no-choice experiment, *R. differens* accepted most of the offered leaves (only three plant species received a median score of 0 for both local and swarming populations; Figure 1A). The degree of acceptance of the studied plant species differed (final multinomial regression model: Wald $\chi^2 = 194.6$, d.f. = 17, $P<0.001$) and there was an interaction between plant species and population ($\chi^2 = 55.0$, d.f. = 17, $P<0.001$), indicating that the pattern of acceptance among plant species was different in the local vs. swarming populations (Figure 1A). For the local population, the most accepted leaves were *Eleusine indica*, *Setaria homonyma*, *P. maximum*, and *Cyperus distans*, whereas for the swarming population, *Setaria megaphylla*, *Echinochloa pyramidalis*, and *E. indica* were the most accepted (all with median score ≥ 8). The two populations differed in their overall degree of acceptance ($\chi^2 = 4.7$, d.f. = 1, $P = 0.030$), with individuals representing the local population having a slightly higher degree of acceptance of leaves (median score 4) than those representing the swarming population (median score 2.5; Figure 1A). Likewise, acceptance between sexes differed ($\chi^2 = 5.0$, d.f. = 1, $P = 0.026$), with males having a slightly higher degree of acceptance of leaves (median score 5) than females (median score 3). The color morphs did not differ in their acceptance of leaves ($\chi^2 = 1.7$, d.f. = 1, $P = 0.19$). Interactions between population vs. sex, population vs. color morph, plant species vs. sex, plant species vs. color morph, and sex vs. color morph were non-significant and were excluded from the final model.

Acceptance of inflorescences in the no-choice experiment

In the no-choice experiment, *R. differens* accepted almost all of the offered inflorescences (only one plant species received a median score 0 for both local and swarming population; Figure 1B). The degree of acceptance of the studied inflorescences differed (final multinomial regression model: $\chi^2 = 245.2$, d.f. = 17, $P<0.001$) and there was an interaction between plant species and population ($\chi^2 = 30.6$, d.f. = 17, $P = 0.022$), indicating that the pattern of degree of acceptance among inflorescences was different in the local vs. swarming population (Figure 1B). For the local population, the most accepted inflorescences

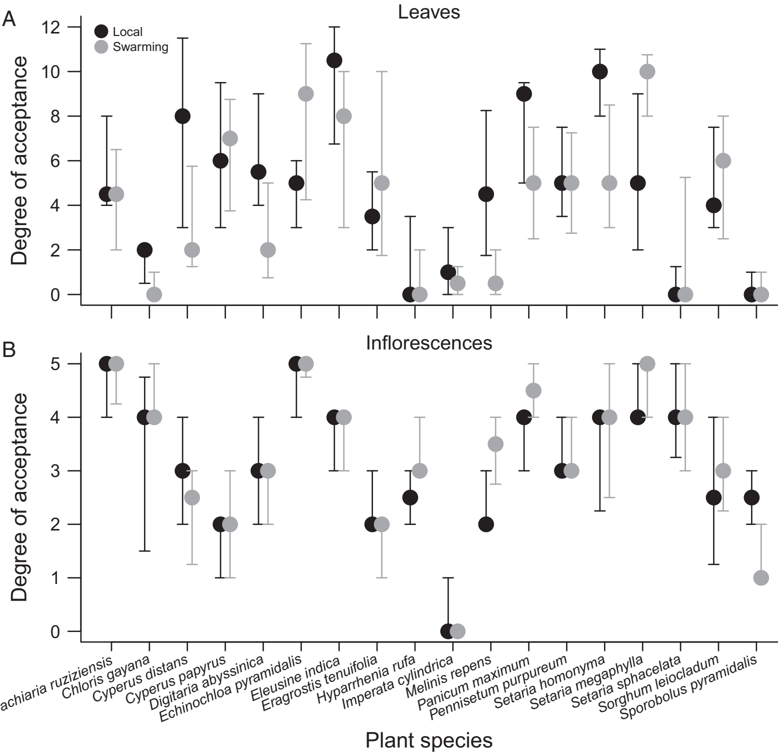

Figure 1 The degree of acceptance by *Ruspolia differens* in no-choice experiments of (A) leaves and (B) inflorescences of the 18 studied plant species. The median (± interquartile range; if not shown it equals the median) scores are indicated on ordinal scale as used in statistical analysis (0 indicating 0% acceptance). The median and interquartile range were calculated on 10–16 individual replicates.

included *B. ruziziensis* and *E. pyramidalis*, and for the swarming population, *B. ruziziensis*, *E. pyramidalis*, and *S. megaphylla* (all with median score of 5). Populations differed in their overall acceptance (χ^2 = 4.0, d.f. = 1, P = 0.047); for five plant species, the median score was higher for the swarming population and for only two species the median score was higher for the local population (although both populations received a median score of 3) (Figure 1B). Also, the degree of acceptance between sexes differed (χ^2 = 4.3, d.f. = 1, P = 0.039), males (average 3.2) having slightly higher scores than females (average 3.0), although both males and females received the same median score of 3. The color morphs did not differ in their acceptance of inflorescences (χ^2 = 0.1, d.f. = 1, P = 0.75). Interactions between population vs. sex, population vs. color morph, plant species vs. sex, plant species vs. color morph, and sex vs. color morph were non-significant and were excluded from the final model.

Preference for leaves in multiple-choice experiments

For the local population, the rank order of preference differed among the plant species (leaves) offered (final multinomial regression model: χ^2 = 45.9, d.f. = 5, P<0.001; Figure 2A) and there was also an interaction between plant species and color morph (χ^2 = 42.7, d.f. = 5, P<0.001; Figure S2), indicating that the pattern of preference for leaves differed among the color morphs. The interaction between plant species and sex was not significant and it was dropped from the final model. The most preferred leaf was *S. homonyma* and it was more preferred than any of the other five individual plant species (in all cases P<0.05).

Also for the swarming population, the rank order of preference differed among the plant species (leaves) offered (final multinomial regression model: χ^2 = 54.6, d.f. = 5, P<0.001; Figure 2B). The interactions between plant species vs. color morph and plant species vs. sex were not significant and they were dropped from the final model. Overall, the most preferred leaf was *Cyperus papyrus*, which was more preferred than *E. pyramidalis*, *S. megaphylla*, or *E. indica* (in all cases P<0.05).

Preference for inflorescences in multiple-choice experiments

For the local population, the rank order of preference differed among the plant species (inflorescences) offered (final multinomial regression model: χ^2 = 45.1, d.f. = 5, P<0.001; Figure 2C) and there were also interactions between plant species vs. sex (χ^2 = 13.6, d.f. = 5, P = 0.018; Figure S3A) and plant species vs. color morph (χ^2 = 14.4, d.f. = 5, P = 0.013; Figure S3B). This indicated that the pattern of preference for inflorescences differed between the sexes and color morphs. The two most

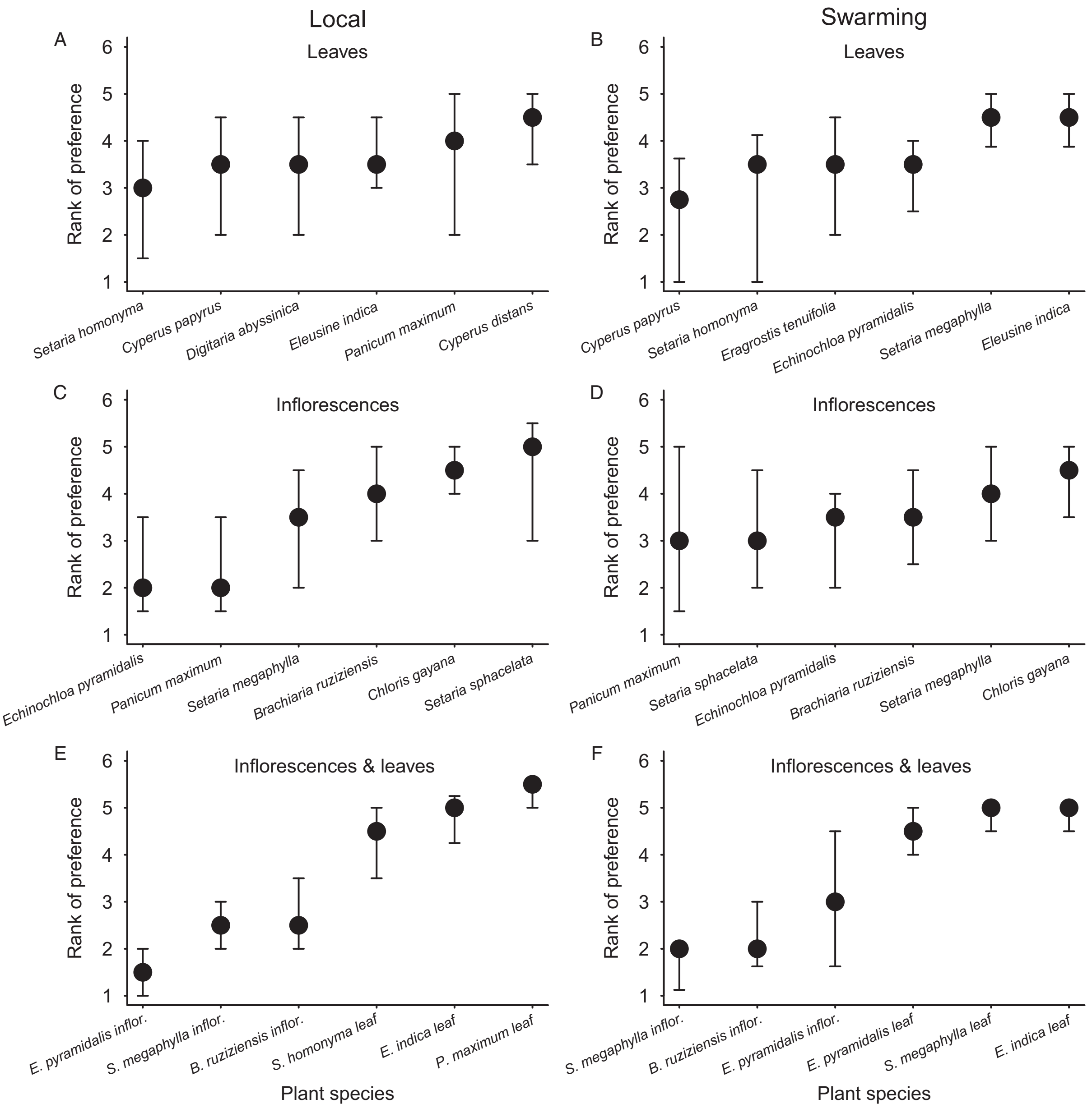

Figure 2 The rank order of preference by *Ruspolia differens* among (A) the six leaves for the local population (n = 57), (B) the six leaves for the swarming population (n = 36), (C) the six inflorescences for the local population (n = 33), (D) the six inflorescences for the swarming population (n = 29), (E) the three leaves and three inflorescences for the local population (n = 43), and (F) the three leaves and three inflorescences for the swarming population (n = 34). The rank order ranges from 1 (most preferred) to 6 (least preferred) and the dots indicate medians (± interquartile range). The foods are ordered based on the median (in case of ties based on 25% quartile).

preferred inflorescences (with the same median values) were *E. pyramidalis* and *P. maximum*. *Echinochloa pyramidalis* was more preferred than *B. ruziziensis*, *C. gayana*, or *Setaria sphacelata* (in all cases P<0.05). *Panicum maximum* was more preferred than either *C. gayana* or *S. sphacelata* (in all cases P<0.05).

Also, for the swarming population, the rank order of preference differed among the plant species (inflorescences) offered (final multinomial regression model: $\chi^2 = 19.8$, d.f. = 5, P = 0.001; Figure 2D). The interactions between plant species vs. color morph and plant species vs. sex were not significant and they were dropped from the final model. The two most preferred inflorescences (with the same median values) were *P. maximum* and *S. sphacelata* which were more preferred than *S. megaphylla* and *C. gayana* (in all cases $P<0.05$).

Preference for leaves vs. inflorescences in multiple-choice experiments

For the local population, the rank order of preference differed among the six foods offered, namely the three most consumed leaves and three most consumed inflorescences combined (final multinomial regression model: $\chi^2 = 260.4$, d.f. = 5, $P<0.001$; Figure 2E). The interactions between plant species vs. color morph and plant species vs. sex were not significant and they were dropped from the final model. The most preferred food was inflorescence of *E. pyramidalis*, which was more preferred than any other foods (in all cases $P<0.05$). This was followed by inflorescences of *S. megaphylla* and *B. ruziziensis* (with the same median values) which were more preferred than any of the offered leaves (in all cases $P<0.05$).

Also, for the swarming population, the rank order of preference differed among the six foods offered, namely the three most consumed leaves and three most consumed inflorescences combined (final multinomial regression model: $\chi^2 = 204.1$, d.f. = 5, $P<0.001$; Figure 2F). There were also interactions between plant species vs. sex ($\chi^2 = 13.3$, d.f. = 5, $P = 0.020$; Figure S4A) and plant species and color morph ($\chi^2 = 23.6$, d.f. = 5, $P<0.001$; Figure S4B), indicating that the pattern in which offered foods were preferred was slightly different among the sexes and color morphs. The most preferred foods were inflorescences of *S. megaphylla* and *B. ruziziensis* (with the same median values), followed by inflorescences of *E. pyramidalis*. All inflorescences were more preferred compared to each of the offered leaves (in all cases $P<0.05$), with the exception of the inflorescences of *E. pyramidalis* which did not differ from leaves of *E. pyramidalis* ($P = 0.30$).

Discussion

Our results indicate that *R. differens* is a facultatively oligophagous grass-feeder. It has a clear preference for certain grass or sedge species and their inflorescences, but accepts many host plants if the preferred ones are unavailable, which is consistent with previous observations (Swaine, 1964; Hartley, 1967; Brits & Thornton, 1981). In this way, *R. differens* is a typical grass-specialist, which is commonly oligophagous, feeding on species in more than one genus (Bernays & Barbehenn, 1987). It has been suggested that this trait is common among grass specialists because adaptations to cope with defenses of one grass taxon enable utilization of several taxa (Bernays & Barbehenn, 1987). For *R. differens*, the ability to utilize several host plants enables it to satisfy its nutritional and water intake needs in the highly seasonal environment where it lives. Theoretically, the capacity to use several host plants also enables a mixing of various foods in the diet, which is associated with better performance of *R. differens* (shorter developmental time and higher fresh adult weight and female fecundity; Malinga et al., 2018) and many orthopteran species due to adequate balance of nutrients or by dilution of toxins (Bernays & Minkenberg, 1997; Miura & Ohsaki, 2004; Unsicker et al., 2008).

Nevertheless, *R. differens* is selective in its host choice, as the potential grass species were not accepted or preferred to the same extent. Selective behavior is typical among orthopterans; even the polyphagous species are selective in their host use (Joern, 1979; Behmer & Joern, 1993, 2008). Interestingly, the acceptability of leaves appears not to be strongly associated with their palatability to cattle. The leaves of *P. maximum*, which are highly palatable to and nutritious for cattle (Roodt, 2015), were fairly well accepted by *R. differens*, whereas leaves of *C. gayana* and *S. spachelata*, which are also highly palatable to and nutritious for cattle, were poorly accepted by *R. differens*. Leaves of *H. rufa*, *I. cylindrica*, *M. repens*, and *S. pyramidalis*, all poorly palatable to cattle with a low nutritional content (Roodt, 2015), were also poorly accepted by *R. differens*. However, inflorescences of *P. maximum*, *C. gayana*, and *S. spachelata* were highly accepted, whereas *H. rufa*, *I. cylindrica*, *M. repens*, and *S. pyramidalis* were moderately to poorly accepted by *R. differens*.

The differences among grass species in their acceptability and preference could be explained by many factors. Physiologically, the process of host choice by phytophagous insects depends on their behavioral responses to the physical and chemical attributes of plants. When making a decision on which host to choose, an insect may use its smell, taste, and touch (leaf odor, phagostimulants, deterrents, and leaf toughness), or vision (plant shape, structure, and color) (Bernays & Chapman, 1994). The choice of the host plant could be determined by nutrient imbalance in the insect (Bernays & Bright, 1993) and nutrient status of the plant (Berner et al., 2005; Behmer & Joern, 2008; Cease et al., 2012). Furthermore, the choice of host could be determined by the water content of the plant, water imbalance in the insect (Lewis & Bernays, 1985; Bernays & Lewis, 1986), and by physical features of the host plant which make them difficult for insects to chew (Bernays & Barbehenn, 1987), including a high amount of silica (Hanifa et al., 1974; McNaughton et al., 1985; Massey et al., 2006). In addition, grasses contain secondary metabolites (Frey et al., 1997) and alkaloids produced by endophytes of grasses (Clay, 1988), which could act as feeding deterrents in host selection, although they generally lack the high diversity and high concentrations of secondary compounds typical of other plant families (Bernays & Barbehenn, 1987). Finally, choice of the host could be determined or modified by previous experiences of the insects in the wild. This could involve habituation (gradually increasing acceptance) to feeding deterrents (Jermy et al., 1982), associative learning of colors (Bernays & Wrubel, 1985) or odors (Simpson & White, 1990) of preferred plants, or aversion learning (Lee & Bernays, 1990).

Our results demonstrate that *R. differens* prefers inflorescences to leaves if they are available. Such preference is likely to be beneficial because reproductive parts of plants are generally more nutritious for herbivores than leaves, due to their high level of proteins (Bernays & Chapman, 1994). Also among Poaceae, pollen is rich in protein (Roulston et al., 2000). Our results further suggest that population of local and swarming *R. differens* differ to some degree in their host use. The generally higher acceptance rates of nutritious inflorescences than the nutritionally poorer leaves in the swarming population compared to the local population could be explained by higher energy intake demands of actively flying swarming individuals compared to non-swarming individuals for whom it might be safer to feed on leaves. Although the biology and ecology of swarming in *R. differens* is not fully understood, the behavior of swarming individuals is radically different compared to the local non-swarming individuals; they fly actively in high densities, mostly during the night and are attracted to lights (Bailey & McCrae, 1978; McCrae, 1982). During the day, swarms land on open grasslands where they are typically static (McCrae, 1982). Swarming flight is facilitated by fat reserves (Karuhize, 1972), which are likely to require intake of highly nutritious foods.

Our results also suggest that female *R. differens* are more selective than males. The overall acceptance scores of both leaves and inflorescence were lower for females compared to males in no-choice experiments. Both sexes of *R. differens* accumulate weight as adults, but females generally gain more weight than males (Lehtovaara et al., in press) and thus should have an overall higher demand for food. Previous studies have found sex-specific host preference or selection (Boys, 1978; Behmer & Joern, 1994; Franzke et al., 2010) and higher food consumption levels by females compared to male orthopterans (Delvi & Pandian, 1971; Unsicker et al., 2008).

Color polymorphism seems not to be strongly associated with host selection in *R. differens*, as there were only minor differences in plant rank order of preference among the color morphs. The physiology of color polymorphism in *R. differens* is not profoundly understood, but nymphs can change color during molting (Robinson & Hartley, 1978). It is also known that green morphs are more typical in females than in males (Bailey & McCrae, 1978), and more in isolated individuals than in those reared in groups (Robinson & Hartley, 1978).

The three grass species on which *R. differens* are overwhelmingly observed in the field (>80% of observations on *B. ruziziensis*, *P. maximum*, and *C. gayana*; R Opoke, P Nyeko, GM Malinga, K Rutaro, H Roininen, A Valtonen, unpubl.) were not accepted or preferred exceptionally well in this experimental setting, although their inflorescences were among the most accepted plant species. It has been suggested that host-finding behavior in the field has a random element to it; herbivores are not necessarily able to orientate themselves to the most preferred hosts and hosts found to be acceptable in the laboratory are often rejected in the field (Bernays & Chapman, 1994). Many ecological factors in the field may completely alter the host choice when compared to standard (laboratory) conditions. Examples of such factors include interactions with conspecifics or other species, temperature, wind, specialization on certain habitat type, plant architecture, and availability of hosts or neighboring plants (Bernays & Chapman, 1994).

Our results have several implications from a practical point of view. First, the conservation of viable natural populations of *R. differens* in the long term is only possible if grasslands with the acceptable hosts are available in East Africa. In our study region, grasslands are threatened by rapid urban expansion (Seto et al., 2012), expansion of croplands, and harmful agricultural practices, such as overgrazing, land mismanagement, and land-use conflicts (Darkoh, 2003). Second, understanding of the accepted and preferred local host plants (and their parts) is also essential when selecting the appropriate foods for the small-scale mass-rearing of *R. differens*. Food determines the growth rate, survival, and fecundity of insects (Bernays et al., 1997; Awmack & Leather, 2002; Unsicker et al., 2008), as well as their value in human nutrition (Lehtovaara et al., 2017). Development of small-scale mass-rearing methods of *R. differens* for local farmers in East Africa could help prevent overexploitation of natural populations of *R. differens* in the long term and to enhance food security in East Africa.

In conclusion, the generally higher acceptance rates by *R. differens* of nutritious inflorescences compared to the nutritionally poorer leaves in the swarming population compared to the local population could be explained by higher energy intake demands of actively flying swarming individuals. To preserve viable natural populations of

R. differens in East Africa in the long term, our results draw special attention to the availability of grasslands, where the accepted and preferred host plants are available year-round, preferably at the flowering phase.

Acknowledgements

We thank our funders Academy of Finland (Project no. 14956 to H.R.) and UniPID (FinnCEAL+ grant to A.V.). We also thank the Office of the Ugandan President and the Uganda National Council of Science and Technology for permission to conduct the study (Permit no. NS544). We thank P. Rwaburindore for identification of plant species, I. Mwesige & S. Nuwagira for assistance with data collection, the Makerere University Agricultural Research Institute, Kabanyolo, for hosting the research, and D.A. Woller and the anonymous reviewer for valuable comments on the manuscript. All datasets are available from the corresponding author upon request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. *Ruspolia differens*, brown morph, at Kabanyolo, Uganda. Photo: A. Valtonen (8 May 2016).

Figure S2. Rank order of preference (ranging from 1 = the most preferred to 6 = the least preferred) among the six plant species (leaves) offered for the local population presented separately for the two color morphs. Dots indicate the medians (± interquartile range). The offered foods are ordered based on median of green morph (in case of ties first based on 25% then on 75% quartile).

Figure S3. Rank order of preference (ranging from 1 = most preferred, to 6 = least preferred) among the six plant species (inflorescences) offered for the local population presented separately for (A) the two sexes and (B) the two color morphs. Dots indicate the medians (± interquartile range). The foods are ordered based on median of females or green morph (in case of ties based on 25% quartile).

Figure S4. Rank order of preference (ranging from 1 = most preferred, to 6 = least preferred) among the three most consumed leaves and three most consumed inflorescences combined for the swarming population presented separately for (A) the two sexes and (B) the two color morphs. Dots indicate the median (± interquartile range). The foods are ordered based on median of females or green morph (in case of ties based on 25% quartile).