DR. GEOFFREY MAXWELL MALINGA (Orcid ID : 0000-0002-6551-2897)
DR. ANU KATRIINA VALTONEN (Orcid ID : 0000-0003-1532-1563)

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Performance of the African edible bush-cricket *Ruspolia differens* on single and mixed diets containing inflorescences of their host plant species

Geoffrey M. Malinga[1,2]*, Anu Valtonen[1], Minna Hiltunen[1], Vilma J. Lehtovaara[1], Philip Nyeko[3] & Heikki Roininen[1]

[1]Department of Environmental and Biological Sciences, University of Eastern Finland, PO Box 111, 80101 Joensuu, Finland, [2]Department of Biology, Gulu University, PO Box 166, Gulu, Uganda, and [3]Department of Forestry, Biodiversity and Tourism, Makerere University, P.O. Box 7062, Kampala, Uganda

Running title: *Performance of* Ruspolia differens *on host plant inflorescences*

***Correspondence:** Geoffrey M. Malinga, Department of Environmental and Biological Sciences, University of Eastern Finland, PO Box 111, 80101 Joensuu, Finland. E-mail: malingageoffrey@yahoo.com

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Abstract

The African edible bush-cricket, *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae), is an economically valuable insect in sub-Saharan Africa. For its mass-rearing, suitable diets that can sustain its performance need to be identified. This study aimed at finding inflorescence diets of 10 host plant species and their mixtures that result in the highest *R. differens* production. We reared newly hatched nymphs to adults on inflorescences of single host species, and also on a gradient of five diversifying dietary mixtures consisting of one and mixtures of two, three, five, and seven host plant species. For the single host-plant diets, the developmental time was shortest on *Pennisetum purpureum*, *Chloris gayana*, and *Brachiaria ruziziensis* (approximately 63 days), whereas the fresh weight was highest on *B. ruziziensis* and *P. purpureum* (approximately 0.383 g). Survival was highest (>65%) on *C. gayana*, *P. purpureum*, *B. ruziziensis*, *Setaria sphacelata*, and *Sporobolus pyramidalis*, and lowest on *Panicum maximum*, *Hyparrhenia rufa*, *Cynodon dactylon*, and *Paspalum scrobiculatum* (<40%). With respect to diet mixtures, the developmental time was significantly shorter (on average 16 days shorter), and survival was higher, in the most diversified diets compared to the least diversified diets. The contents of polyunsaturated fatty acids (PUFA) and fatty acid composition did not differ significantly among the diets. However, the content of monosaturated fatty acids (MUFA) differed significantly among the diets and there was a significant diet*sex interaction. From the applied point of view, based on our results, for maximal weight and survival plus fast development, we recommend rearing on *P. purpureum*, *C. gayana*, *B. rizuziensis*, and *S. sphacelata*, and on dietary mixtures of inflorescences. These findings are important in designing large-scale rearing programs for this species.

Abbreviated abstract (2-3 sentences, max. 80 words)

The African edible bush-cricket, *Ruspolia differens* (Orthoptera: Tettigoniidae), is an economically valuable insect in sub-Saharan Africa. Its type of food (natural host plants) significantly modifies the development time, weight, survival, and ω6:ω3 ratio of edible *R. differens* but not the composition and contents of monosaturated and polyunsaturated fatty acids (MUFA and PUFA). *Pennisetum purpureum*, *Chloris gayana*, *Brachiaria rizuziensis*, and *Setaria sphacelata* are the best natural plant foods for rearing *R. differens*. These findings may help design large-scale rearing programs. [79 words]

Graphic for Table of Contents

Ruspolia differens for TOC.jpg

Introduction

The economically valuable African edible bush cricket, *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae, subfamily Conocephalinae), is native to sub-Saharan Africa (van Huis et al., 2013; Kelemu et al., 2015; Massa, 2015), where its consumption contributes to food and nutritional security (Agea et al., 2008; Nyeko et al., 2014; Okia et al., 2017; Odongo et al., 2018). It is a multivoltine species which has distinct non-swarming and swarming phases, with swarms occurring in East Africa during rainy seasons in November-December and April-May (Bailey & McCrae, 1978). The species is a facultatively oligophagous grass specialist, accepting and feeding on a wide range of grasses and some sedge species (Swaine, 1964; Bailey & McCrae, 1978; Valtonen et al., 2018; Opoke et al., 2019). In the field, *R. differens* was observed most frequently on the most common grass of each study site, with *Panicum maximum* Jacq. (Poaceae) being the preferred host of the youngest nymphs (Opoke et al., 2019). At present, communities collect these insects through wild harvesting which is seasonal and very unpredictable (Okia et al., 2017). Consequently, there is a great need to develop mass-rearing methods for this species to improve food and nutrition security (Ssepuuya et al., 2018), as well as to prevent overexploitation of its population due to wild harvesting. However, the development of a successful and sustainable mass rearing strategy for local communities requires knowledge of potentially suitable host plants that can support the insect's growth, development, and survival. This is because not all host plants accepted are necessarily adequate for the insect's performance, which may depend on the nutritional quality of the food (Joern & Behmer, 1997; Ganguly et al., 2010; Das et al., 2012).

The development of optimal diets for mass rearing also requires knowledge of host plant mixtures which best support the insect's growth, development, and survival. This is because several orthopteran species have been shown to enhance their performance by diet mixing and exhibit higher fitness and survival rates, and faster growth on mixtures of food plants rather than on single plant species (Singer & Bernays, 2003; Miura & Ohsaki, 2004; Unsicker et al., 2008). Yet, mixed diets might be important only when low-quality foods are available (Miura & Oshaki, 2004). In our previous studies using artificial diets, we found that *R. differens* performs better on mixed diets than on single or less diversified diets (Malinga et al., 2018a, b). Ssepuuya et al. (2018) recorded the highest wet nymphal weight and growth rate of adult *R. differens* after 8 weeks on wild finger millet, *Eleusine africana* Kenn.-O'Byrne, followed by a mixture of three food plants [*Cynodon dactylon* (L.) Pers. (Poaceae), *E. africana*, and *P. maximum*], and the highest survival on *C. dactylon* and *P. maximum*. However, information on the performance of *R.*

differens on different host plants, their mixtures, and how the host plants modify the nutritional value of the insect for humans, is still very limited.

In the present study, we investigated the performance of *R. differens* fed on single host plants (inflorescences), and dietary mixtures of host plants (inflorescences) occurring in their natural habitats, with the purpose of finding a diet that results in the highest *R. differens* production for mass rearing of this edible insect. We reared *R. differens* from the first instar to adult, recording their developmental time, weight, and survival. Furthermore, we evaluated the fatty acid content and composition of the host plants and of *R. differens* individuals fed with them. We conducted two experiments. In experiment 1, diet treatments represented 10 single host plants, and in experiment 2, diet treatments represented a gradient of five diversifying mixtures of host plants. We hypothesized that increased food resource diversity would lead to increased nymphal survivorship, increased adult fresh weight, and shorter nymphal developmental times due to the improved balance of nutrients (Hägele & Rowell-Rahier, 1999). We also predicted that the nymphal survivorship and adult fresh weight would differ among the single diet treatments due to variation in the nutritional quality of host plants (Joern & Behmer, 1998). The questions asked in this study were: do the (1) developmental time from the first instar to adult, (2) fresh weight when reaching adult stage; or (3) survival rate to adult differ among diet treatments? For single-host diets (experiment 1), we further asked, do the content of saturated fatty acids (SFAs), monosaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and the ratio of omega-6 to omega-3 (ω6:ω3), or fatty acid composition (i.e., the proportions of fatty acids) differ among *R. differens* individuals fed on the various diets?

Materials and methods

Study site and study species

The experiments were done at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK), Uganda. *Ruspolia differens* is exclusively native in sub-Saharan Africa including Uganda, Kenya, Congo, Burundi, Rwanda, Tanzania, and some islands of the Indian Ocean (Massa, 2015), where it is widely consumed (Matojo, 2017). It is a nocturnal species which exhibits unique colour polymorphism with six morphs including brown, green, purple-stripped green, purple-striped brown, purple-suffused green, and purple-suffused brown (Bailey & McCrae, 1978; Matojo & Njau, 2010). It has an elongate cone-head and long filiform antenna (Matojo, 2017); male adults have about 1.5× longer antenna than females and have a characteristic pair of tongue-like metathoracic flaps located at the dorsal side of the proximal base of the wing along the radius vein (Matojo & Yarro, 2013). The insect lays eggs in the leaf sheaths of grasses and hatching occurs after 17-18 days (Hartley, 1967).

Food plants

The 10 food plants selected for the experiments were *Brachiaria ruziziensis* R Germ. & CM Evrard, *P. maximum*, *Hyparrhenia rufa* (Nees) Stapf, *Chloris gayana* Kunth, *C. dactylon*, *Sporobolus pyramidalis* P Beauv., *Pennisetum purpureum* Schumach., *Paspalum scrobiculatum* L., *Setaria sphacelata* (Schumach.) Stapf & CE Hubb. ex Moss, and *Digitaria abyssinica* (Hochst. ex A Rich.) Stapf (all Poaceae). All these species are known host plants of *R. differens* at our study location in Uganda (Opoke et al., 2019). In experiment 1, the diet treatments represented these 10 host plant species. In experiment 2, diet treatments represented a gradient of five diversifying dietary mixtures of host plant species ranging from one to mixtures of two, three, five, and seven species (Table 1). The plant selection in experiment 2 was based on our earlier survey of potential food plants of *R. differens* used in the field in Uganda (Opoke et al., 2019). The gradient represents the hierarchy of use, whereby *B. ruziziensis* was the most used and *B. ruziziensis* and *P. maximum* were the two most used host plants in the field, respectively. Throughout rearing, we used only freshly opened inflorescences, and in mixtures, the florets were randomly placed relatively close (<20 cm) to each other.

Experimental settings

We used newly hatched *R. differens* nymphs, which were reared from eggs in the laboratory, using a parent population which originated in agricultural farmland around MUARIK. However, parental identity of the egg masses was not recorded. In both experiments, newly-hatched (1 day old) nymphs were placed individually in rearing containers (21 cm high, 19 cm diameter), which represented the replicates. The opening on top of the containers was covered by a netting cloth. To minimize the effects of possible microclimatic variations in the laboratory, the replicates were arranged as blocks, each containing one replicate of each diet treatment. Food was offered ad libitum and water was supplied by inserting moistened tissue paper. All nymphs were checked daily from the first instar to adult moulting, to observe nymphal survival. The adults were weighed within 24 h after emergence and their sex was determined. For surviving individuals, the nymphal development time was determined as the days between hatching and adult emergence. For experiment 1, the laboratory experiments were conducted at ambient 25.8–30.1 °C, 56–72% r.h., and L12:D12 photoperiod. Experiment 2 was performed in the laboratory at 22.7–25.1 °C, 61–78% r.h.. and L12:D12 photoperiod. To minimize differences in the quantity of food due to differences in sizes of florets of different species, the florets were cut to relatively uniform sizes.

Experiment 1: Ruspolia differens *on single host plant species*

To determine the performance of *R. differens* on single host plants, in total 430 newly hatched nymphs (replicates) were randomly subjected to the inflorescence of 10 single host plant species (the 10 diet treatments, each with 43 replicates) and reared to adults. The rearing containers were placed in 43 blocks, each containing one replicate of each host plant with one *R. differens* individual per container. In each container, we randomly placed roughly equal quantities of host inflorescences, and food was replenished after every 2nd or 3rd day.

Experiment 2: Ruspolia differens *on a gradient of diet mixtures*

To determine the performance of *R. differens* on the diet treatments representing a gradient of diversifying plant species mixtures (Table 1), a total of 200 newly hatched nymphs (replicates) were randomly subjected to each of the five diet treatments (each with 40 replicates) and reared to adults. The rearing containers were placed in 40 blocks, each containing one replicate of each host plant with one *R. differens* individual per container. In each container, we randomly placed roughly equal quantities (two florets) of host plant species inflorescence, i.e., the nymphs on the single food diet received two florets, nymphs on the two-feed diet received one floret of the two species, nymphs on the three food mixtures received two-thirds of each floret, and so forth.

Sample preparation and fatty acid analysis of diets and *Ruspolia differens*

To investigate the nutritional quality of the plant species, we separately analysed the fatty acids of the inflorescences of the 10 host plants and of randomly selected *R. differens* adults reared on the seven hosts that supported growth and development. Briefly, after harvesting, the freshly emerged adults were killed by freezing. Overall, 25 individual *R. differens* (four individuals per diet treatment, except *P. maximum* for which only one individual was available) were randomly selected for fatty acid analysis. The host plant inflorescences and insect samples were dried first in hot air at 35-40 °C for 48 h at MUARIK, and then at the laboratory of the University of Eastern

Finland where *R. differens* and the seven host plant inflorescences were freeze-dried and ground prior to the analyses. A subsample of ca. 4 mg for host plant inflorescences and 20-120 mg for *R. differens* (excluding the wings, ovipositor, and hind legs) was weighed and placed in glass tubes that had been heated at 440°C for 4 h and solvent-rinsed to remove any organic residuals. Lipids were extracted with 2:1 chloroform: methanol (vol:vol) (Folch et al., 1957), and 0.88% aqueous KCl was added to remove non-lipid components. The fatty acids were derivatized to fatty acid methyl esters (FAMEs) with 1% H_2SO_4 in methanol. The FAMEs were dissolved in n-hexane and run with a gas chromatograph-mass spectrometer (Agilent 6890N and 5973N; Agilent, Santa Clara, CA, USA) using the DB-23 column (60 m × 0.25 mm × 0.15 µm). The average velocity of the carrier gas (helium) was 28 cm s^{-2}. For plant samples, the FAMEs were injected splitless at 250 °C, and due to higher lipid content, the *R. differens* samples were injected with 20:1 split ratio. We used the same temperature program for both sample types: initial oven temperature of 50 °C was held for 1 min, raised 15 °C per min to 150 °C, then 1.5 °C per min to 210 °C, and finally 3 °C per min to 230 °C where it was held for 5 min. Peak areas were converted to concentrations using a series of known concentrations of a standard FAME mixture (GLC-538; Nu Chek Prep, Elysian, MN, USA), and nonadecanoic acid (19:0) was used as the internal standard. Retention times and mass spectra were used for identification of FAMEs. The amounts of each fatty acid were expressed as a percentage of the total fatty acids and as content (mg per g dry weight) of *R. differens*. Fatty acids in the three remaining host plant inflorescences were analysed at the Bio-competence Centre for Health Dairy Products, Tartu, Estonia, using a direct transmethylation method (Sukhija & Palmquist, 1988) with minor modifications (Lehtovaara et al., 2017).

Statistical analysis

Linear mixed models (type III sum of squares) were fitted to analyse the effects of dietary treatment on developmental time (days) from the first instar to adult and fresh weight (g) when reaching the adult stage of *R. differens* in both experiments. In all models, we also included sex, and diet treatment*sex interaction, as fixed factors, and block as a random factor. The fit of the model was checked by inspecting the distribution of the residuals. For analyses of experiment 1 (except survival analyses), we had to exclude two diet treatments – host plants *C. dactylon* and *P. scrobiculatum* – with either no or fewer than five emerged adults. If differences among diet treatments were found, we conducted Bonferroni post hoc pairwise comparisons (except for development time in experiment 2, where we had to use the less conservative least significant difference test, as Bonferroni could not find pairwise differences even when the mixed model indicated significant differences among the diet treatments).

We fitted generalized linear mixed-effects models with a binomial error term (also known as random effect logistic regression) to determine whether diet treatment (independent variable) predicts the nymphal survival to adulthood (whether an individual survives or dies, as the response variable). In these models, the block was included as a random effect (it was not possible to include sex in the model because this was recorded only for those individuals that survived to adulthood). Model goodness-of-fit was assessed with the Hosmer-Lemeshow test and type III Wald χ^2 tests were used to evaluate the significance of model parameters (Hosmer et al., 2013). If differences among diet treatments were found, we conducted Bonferroni post hoc pairwise comparisons.

We tested for differences in the contents of SFA, MUFA, PUFA, and ω6:ω3 ratio among *R. differens* reared on the six hosts (all treatments with four replicates), between sexes, and for their interaction using univariate PERMANOVA (using Euclidian distance as a similarity measure, type III sum of squares, 999 permutations, untransformed data), with treatment and sex (and their interactions) as fixed factors. Whenever significant differences were found, we performed pairwise PERMANOVA tests to determine differences between levels of each diet treatment.

We applied permutational multivariate ANOVA (PERMANOVA, type III sum of squares, 999 permutations, untransformed data) to test whether the composition of fatty acids of *R. differens* (i.e., proportions of fatty acids) differed significantly among the dietary treatments (all treatments with four replicates), sexes and whether there was an interaction between diet and sex. Among the 24 fatty acids detected in *R. differens*, we included only those with levels of 0.05% and above in a sample (i.e., 21 fatty acids) and used Bray-Curtis as a measure of similarity.

A non-metric multidimensional scaling (NMDS; 50 restarts, Bray-Curtis as a measure of similarity) was used to illustrate the similarity of fatty acid compositions among the 10 host plant species (inflorescences) and *R. differens* fed with the single host diets. For clarity, we used the average percentages of each fatty acid across *R. differens* individuals fed on each host. In the analysis of *R. differens*, 24 fatty acids were detected, whereas for the seven grasses (*P. maximum*, *C. gayana*, *B. ruziziensis*, *S. pyramidalis*, *P. purpureum*, *D. abysinnica*, and *S. spacelata*) analysed in Finland, 16 fatty acids were detected, and for the three grasses (*H. rufa*, *C. dactylon*, and *P. scrobiculatum*) analysed in Estonia, 34 fatty acids were detected. To make the two datasets comparable, among the detected fatty acids, we included only those with levels of 0.5% and above (in a sample) in the NMDS. Furthermore, we tested how closely related the two resemblance matrices are (grasses and *R. differens* fed on those grasses) using the routine RELATE (Clarke & Gorley, 2006). The multivariate analyses were run with PRIMER v.6 software (Clarke & Gorley, 2006) and the PERMANOVA+add-on package (Anderson et al., 2008).

Results

Ruspolia differens performance on single host plant species (experiment 1)

The total nymphal developmental time differed among the eight diets included in the analyses (excluding host plant species *C. dactylon* and *P. scrobiculatum*, for which either no or fewer than five emerged adults were found) (linear mixed model: $F_{7,159.1} = 9.8$, $P<0.001$; Figure 1A) and there was a diet treatment*sex interaction ($F_{7,172.7} = 2.5$, $P = 0.019$). However, there was no significant difference in developmental time between the sexes ($F_{1,173.7} = 3.5$, $P = 0.063$). When the pairwise tests were presented separately for males and females, the nymphal developmental time in males was significantly shorter when individuals were reared on *P. purpureum* or *B. ruziziensis* (approximately 65 days) than on *S. pyramidalis* (approximately 89 days) or *H. rufa* (104 days; Figure 1A). Also, for the females, the development time was significantly shorter in individuals reared on *P. purpureum* (approximately 57 days) and *C. guyana* (61 days) compared to *H. rufa* (101 days) or *D. abyssinica* (74 days; Figure 1A).

The survival from neonate nymph to adult ranged from 0 to 88.4% with mean nymphal survival across all diet treatments of 46.3% (Figure 1B). Survival to adult differed across the 10 diets ($F_{9,420} = 9.5$, $P<0.001$; Figure 1B). Survival was highest on *C. gayana*, *P. purpureum*, *B. ruziziensis*, *S. sphacelata*, and *S. pyramidalis* (>65% survived) and lowest on *P. maximum*, *H. rufa*, *C. dactylon*, and *P. scrobiculatum* (0% survived; Figure 1B).

The fresh weight of *R. differens* when reaching adult stage differed among the eight diets (linear mixed model: $F_{7,142,7} = 6.8$, $P<0.001$; Figure 2A). However, there were no differences between sexes ($F_{1,148.4} = 0.7$, $P = 0.42$), and there was no diet treatment*sex interaction ($F_{7,148.5} = 1.7$, $P = 0.11$). According to the pairwise tests, the fresh weight was significantly higher when individuals were fed on *B. ruziziensis* and *P. purpureum* (ca. 0.383 g) than when fed on *P. maximum* or *S. pyramidalis* (ca. 0.294 g; Figure 2A).

Ruspolia differens performance on diet mixtures (experiment 2)

The nymphal development time differed among the five diets (linear mixed model: $F_{4,22.3} = 3.4$, P

= 0.025) and between the sexes ($F_{1,23.8}$ = 7.2, P = 0.013; Figure 3A) but there was no diet*sex interaction ($F_{4,26.0}$ = 2.2, P = 0.093). Females developed, on average, more slowly than males (marginal mean for females vs. males was 88.9 vs. 77.4 days). According to the pairwise tests, the nymphal developmental time was significantly shorter when individuals were reared on more diverse diets (>90 days for diets with 1-2 plant species; <80 days for diets with 5-7 plants; Figure 3A).

The survival to adult stage differed among the five diet treatments ($F_{4,195}$ = 8.6, P<0.001), being higher in the most diversified diet mixture than in the least diversified diets (Figure 3B). Overall, survival to adults ranged from 12.5-15% in the three least diversified diet treatments to 40-65% in the two most diversified diets with an overall average nymphal survival of 30% (Figure 3B).

The fresh weight of *R. differens*, when reaching adult stage, did not differ among the five diet treatments ($F_{4,40.7}$ = 1.85, P = 0.14; Figure 2B) and there was no diet treatment*sex interaction ($F_{4,46.2}$ = 0.4, P = 0.79). However, the sexes differed in adult fresh weight ($F_{1,42.1}$ = 7.8, P = 0.008), females being heavier than males (marginal mean ± SE = 0.290 ± 0.15 vs. 0.233 ± 0.015 g).

Fatty acid content and composition of the diets (experiment 1)

The most abundant fatty acids in the host plant inflorescences were palmitic acid, linoleic acid, α-linolenic acid, stearic acid, oleic acid, and arachidic acid, together comprising 90% of total fatty acids in both datasets (Table 2). According to the NMDS ordination, the 10 host plants represent a gradient with respect to the similarity of their fatty acid compositions (symbols under G in Figure 4). The host plants *S. pyramidalis* and *S. sphacelata* had a very low percentage of α-linolenic acid (2 and 6%, respectively) compared to 16-32% in the other species. This resulted in very high ω6:ω3 ratios in *S. pyramidalis* and *S. sphacelata* (13 and 6, respectively) compared to other grass species (ca. 1-2). The total fatty acid (TFA) contents of the host plant diets ranged from 12-21 mg g^{-1} dry weight depending on the species. The highest TFA (21 mg g^{-1} dry weight) was recorded on *C. dactylon*, followed by *B. ruziziensis* and *S. sphacelata* (20), *C. gayana* and *D. abyssinica* (17), *P. scrobiculatum* (15), *P. maximum* (14), *P. purpureum* (13), and *S. pyramidalis* and *H. rufa* (12).

Fatty acid content and composition of *Ruspolia differens* on single host plant species (experiment 1)

The monounsaturated fatty acid (MUFA) content differed among the diet treatments

(PERMANOVA: pseudo-$F_{6,12}$ = 2.99, P = 0.048) but not between the sexes (pseudo-$F_{1,12}$ = 1.415, P = 0.27), and there was an interaction between sex and diet (pseudo-$F_{5,12}$ = 5.817, P = 0.007; Figure 5). The saturated fatty acids (SFA) content did not differ among the diets (pseudo-$F_{6,12}$ = 0.814, P = 0.61), nor between the sexes (pseudo-$F_{1,12}$ = 2.248, P = 0.14), and there was no interaction between diet and sex (pseudo-$F_{5,12}$ = 2.066, P = 0.12).

Furthermore, the polyunsaturated fatty acids (PUFA) content did not differ among the diets (pseudo-$F_{6,12}$ = 1.827, P = 0.16) nor between the sexes (pseudo-$F_{1,12}$ = 2.241, P = 0.16). There was also no interaction between diet and sex (pseudo-$F_{5,12}$ = 1.189, P = 0.39). Finally, the ω6:ω3 ratio did not differ among the six host plant diets (pseudo-$F_{6,12}$ = 1.898, P = 0.20) nor between the sexes (pseudo-$F_{1,12}$ = 0.182, P = 0.70), and there was also no interaction between diet and sex (pseudo-$F_{5,12}$ = 1.280, P = 0.36).

The total PUFAs in *R. differens* ranged from 32% in the *S. pyramidalis* treatment to 47% in *C. gayana* treatment (Table 3). The most predominant PUFAs in *R. differens* fed with the six host plant diets were linoleic acid (C18:2ω6) and α-linolenic acid (C18:3ω3) (Table 3). The proportions of linoleic acid ranged from 28% in *S. pyramidalis* treatment to 38% in *B. ruziziensis* treatment, whereas α-linolenic acid ranged from 4% in *S. pyramidalis* and *S. sphacelata* treatments to 17% in *C. gayana* treatment. The proportion of SFAs ranged from 25% in *R. differens* fed *C. gayana* to 30% in *R. differens* fed *S. pyramidalis*.

The fatty acid composition (i.e., proportions of fatty acids) of *R. differens* did not differ among the six host plant diets which had sufficient data (PERMANOVA; pseudo-$F_{5,12}$ = 1.162, P = 0.34), nor between the sexes (pseudo-$F_{1,12}$ = 0.344, P = 0.68), and there was no interaction between diet and sex (pseudo-$F_{5,12}$ = 2.224, P = 0.10). However, when average percentages of each fatty acid across *R. differens* individuals fed on each host were used for NMDS ordination, they represented a gradient with respect to the similarity of their fatty acid compositions (symbols under R in Figure 4; the proportions of each fatty acid shown in Figure S1). Furthermore, the gradient corresponds with gradient in host plants (G); according to the routine RELATE, the resemblance of fatty acid compositions among grasses correlate with resemblance of fatty acid compositions of *R. differens* which fed on these grasses (ρ = 0.601, P = 0.002).

Discussion

Our results demonstrated that the host plant can significantly modify the performance (developmental time, weight, and survival) of edible *R. differens*. Based on the developmental time, fresh adult weight, and nymphal survival, inflorescences of *P. purpureum*, *C. gayana*, *B. ruziziensis*, and *S. sphacelata* seem to be the most suitable food for mass-rearing of *R. differens*. However, *P. maximum* that was used more frequently by the youngest nymphs than expected by its abundance in the field (Opoke et al., 2019) recorded a much lower survival compared to plants such as *B. ruziziensis*, *H. rufa*, and *P. purpureum* that were less frequently used. This suggests that the most preferred host plants in the field are not necessarily the most suitable for completion of the entire life cycle of *R. differens* in the laboratory rearing. Host selection in the field might also be influenced by other factors such as predator avoidance (Bernays & Chapman, 1994), more than the host nutritional quality.

Furthermore, our results show that a dietary mixture of grass species inflorescences is beneficial for the survival and for shortening the development time of the edible *R. differens* but not for achieving a higher adult emergence weight. As in our study, Ssepuuya et al. (2018) also observed greatly improved survival of *R. differens* on mixtures (in their case, consisting of green stems with both mature and unfolded leaves of *C. dactylon*, *P. maximum*, and *E. africana*). This result is also in agreement with previous studies on generalist grasshoppers, *Parapodisma subastris* (Huang) (Miura & Ohsaki 2004) and *Chorthippus parallelus* (Zetterstedt) (Unsicker et al., 2008) in which survival was highest in the food plant mixtures with six and eight grass species, respectively, compared to treatments in which only a single plant species was offered as food. The improved survival rate and shorter developmental time on diet mixtures could be explained by the fact that in mixtures of host plants, a full complement of nutrients is better gained than in single host plants (Unsicker et al., 2008).

Although we did not find statistically significant differences in the fatty acid content (SFA, PUFA) and composition of *R. differens* among the diets, the gradient in fatty acid composition corresponded with that of the grass inflorescences on which they were fed. Notably, the proportion of α-linolenic acid (C18:3ω3) seems to follow that of the hosts, suggesting that certain fatty acids can be modified by their hosts, agreeing with results of our previous study using artificial diets (Lehtovaara et al., 2017). Nonetheless, the host plant species used as diet sources in the current study had low fat content (11-21 mg g^{-1} dry weight), which likely explains the lack of differences in fatty acid composition of *R. differens*. The lack of differences in the SFA and PUFA contents and in the composition of *R. differens* when reared on the various host plant species could also indicate a physiological regulation of certain SFAs and PUFAs as observed in other insect species (Stanley-Samuelson et al., 1988). This finding is consistent with results of our earlier study

(Rutaro et al., 2018) in which diversifying natural plant diet (inflorescences of grasses) did not alter the content and composition of the most common fatty acids in *R. differens* when reared throughout the entire life-cycle, although the composition of rare fatty acids was altered.

In conclusion, our work indicates that the type of food (natural host plants) significantly modifies the developmental time, weight, and survival of edible *R. differens* but not the composition and contents of SFA and PUFA. From the applied point of view, based on our results, for maximal weight, maximal survival, and shorter development time, we recommend rearing on *P. purpureum*, *C. gayana*, *B. ruziziensis*, or *S. sphacelata*, and on dietary mixtures of grass inflorescences. Furthermore, the association of fatty acid compositions of *R. differens* with that of the grasses on which they are fed suggest that diets can modify the fatty acids of *R. differens* to make them more suitable for humans. These findings create an important basis for upscaling the rearing program of *R. differens* in the future.

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References

Agea JG, Biryomumaisho D, Buyinza M & Nabanoga GN (2008) Commercialisation of *Ruspolia nitidula* (Nsenene grasshoppers) in central Uganda. African Journal of Food Agriculture, Nutrition and Development 8: 319-332.

Anderson M, Gorley RN & Clarke RK (2008) Permanova+ for Primer: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.

Bailey WJ & McCrae AWR (1978) The general biology and phenology of swarming in the East African tettigoniid *Ruspolia differens* (Serville) (Orthoptera). Journal of Natural History 12: 259-288.

Bernays EA & Chapman RF (1994) Host-Plant Selection by Phytophagous Insects. Chapman & Hall, New York, NY, USA.

Clarke KR & Gorley RN (2006) PRIMER v6: User Manual/Tutorial. Primer-E, Plymouth, UK.

Das M, Ganguly A & Halder P (2012) Determination of optimum temperature and photoperiod for mass production of *Oxya hyla hyla* (Serville). Turkish Journal of Zoology 36: 329-339.

Folch J, Lees M & Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

Hägele BF & Rowell-Rahier M (1999) Genetic and environmental-based variability in secondary metabolite leaf content of *Adenostyles alliariae* and *A. alpina* (Asteraceae). A test of the resource availability hypothesis. Oikos 85: 234-246.

Hartley JC (1967) Laboratory culture of a Tettigoniid, *Homorocoryphus nitidulus vicinus* (Wlk.) (Orthoptera). Bulletin of Entomological Research 57: 203-205.

Hosmer JDW, Lemeshow S & Sturdivant RX (2013) Applied Logistic Regression, 3rd edn. John Wiley and Sons, Hoboken, NJ, USA.

van Huis A (2013) Potential of insects as food and feed in assuring food security. Annual Review of Entomology 58: 563-583.

Joern A & Behmer ST (1997) Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia 112: 201-208.

Joern A & Behmer ST (1998) Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. Ecological Entomology 23: 174-184.

Kelemu S, Niassy S, Torto B, Fiaboe K, Affognon H et al. (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.

Lehtovaara VJ, Valtonen A, Sorjonen J, Hiltunen M, Rutaro K et al. (2017) The fatty acid contents of the edible grasshopper *Ruspolia differens* can be manipulated using artificial diets. Journal of Insects as Food and Feed 3: 253-262.

Malinga GM, Valtonen A, Lehtovaara VJ, Rutaro K, Opoke R et al. (2018a) Diet acceptance and preference of the edible grasshopper *Ruspolia differens* (Orthoptera: Tettigoniidae). Applied Entomology and Zoology 53: 229-236.

Malinga GM, Valtonen A, Lehtovaara VJ, Rutaro K, Opoke R et al. (2018b) Mixed artificial diets enhance the developmental and reproductive performance of the edible grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae). Applied Entomology and Zoology 53: 237-242.

Massa B (2015) Taxonomy and distribution of some katydids (Orthoptera Tettigoniidae) from tropical Africa. ZooKeys 524: 17-44.

Matojo ND (2017) A review work on how to differentiate the longhorn grasshoppers *Ruspolia differens* and *Ruspolia nitidula* (Orthoptera: Tettigoniidae). Journal of Applied Life Sciences International 2: 1-4.

Matojo ND & Hosea KM (2013) Phylogenetic relationship of the longhorn grasshopper *Ruspolia differens* Serville (Orthoptera: Tettigoniidae) from Northwest Tanzania based on 18S ribosomal nuclear sequences. Journal of Insects 2013: 504285.

Matojo ND & Yarro JG (2010) Variability in polymorphism and sex ratio of the conehead *Ruspolia differens* Serville (Orthoptera: Conocephalidae) in north-west Tanzania. International Journal of Integrative Biology 9: 131-136.

Miura K & Ohsaki N (2004) Diet mixing and its effect on polyphagous grasshopper nymphs. Ecological Research 19: 269-274.

Nyeko P, Nzabamwita PH, Nalika N, Okia CA, Odongo W & Ndimubandi J (2014) Unlocking the Potential of Edible Insects for Improved Food Security, Nutrition and Adaptation to Climate Change in the Lake Victoria Basin (Project Report no. NR-05-10). The Lake Victoria Research Initiative, Inter-University Council of East Africa (IUCEA), Kampala, Uganda.

Odongo W, Okia CA, Nalika N, Nzabamwita PH, Ndimubandi J & Nyeko P (2018) Marketing of edible insects in Lake Victoria basin: the case of Uganda and Burundi. Journal of Insects as Food and Feed 4: 285-293.

Okia CA, Odongo W, Nzabamwita P, Ndimubandi J, Nalika N & Nyeko P (2017) Local knowledge and practices on use and management of edible insects in Lake Victoria basin, East Africa. Journal of Insects as Food and Feed 3: 83-93.

Opoke R, Nyeko P, Malinga GM, Rutaro K, Roininen H & Valtonen A (2019) Host plants of the non-swarming edible bush cricket *Ruspolia differens*. Ecology and Evolution 9: 3899-3908.

Rutaro K, Malinga GM, Lehtovaara VJ, Opoke R, Nyeko P et al. (2018) Fatty acid content and composition in edible *Ruspolia differens* feeding on mixtures of natural food plants. BMC Research Notes 11: 687.

Sukhija PS & Palmquist DL (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry 36: 1202-

1206.

Ssepuuya G, Tanga CM, Yekko I, Sengendo F, Ndagire CT et al. (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.

Stanley-Samuelson DW, Jurenka RA, Cripps C, Blomquist GJ & de Renobales M (1988) Fatty acids in insects: composition, metabolism, and biological significance. Archives of Insect Biochemistry and Physiology 5: 1-33.

Singer MS & Bernays EA (2003) Understanding omnivory through food-mixing behaviour. Ecology 84: 2532-2537.

Swaine G (1964) The bush-cricket, *Homorocoryphus nitidulus* Walker (Tettigoniidae). East African Agricultural and Forestry Journal 29: 340-342.

Unsicker SB, Oswald A, Köhler G & Weisser WW (2008) Complementary effects through dietary mixing enhance the performance of a generalist insect herbivore. Oecologia 156: 313-324.

Valtonen A, Malinga GM, Junes P, Opoke R, Lehtovaara VJ et al. (2018) The edible *Ruspolia differens* is a selective feeder on the inflorescences and leaves of grass species. Entomologia Experimentalis et Applicata 166: 592-602.

Figure captions

Figure 1 Performance of *Ruspolia differens* on single host plant species: (A) marginal mean (± SE) development time (days) from newly-hatched nymph to adult stage, and (B) survival (%) to adult stage. Numbers within the columns in B indicate the numbers of surviving *R. differens* in the experiment. No individuals survived to adulthood on *Paspalum scrobiculatum*, whereas on *Cynodon dactylon* only a few individuals survived to adulthood; therefore, these diets were excluded from developmental time analysis.

Figure 2 Box-plot of weight (g) of emerging adults of *Ruspolia differens* reared on (A) single host plant species, and (B) dietary mixtures (see Table 1 for explanation of the treatment codes). Dashed lines represent either the maximum value or 1.5 times the interquartile range of the data, whichever is the smaller, the outer limits of the boxes indicate the interquartile range, and the thick line within each box represents the median. Treatments capped with different letters indicate significant differences among diets (Bonferroni pairwise tests: P<0.05).

Figure 3 Performance of *Ruspolia differens* on dietary mixtures: (A) marginal mean (± SE) development time from newly hatched nymphs to adult stage, and (B) survival (%) to adult stage. Numbers within the columns in B indicate the numbers of surviving *R. differens* in the experiment. Means with different letters are significantly different (LSD tests: P<0.05).

Figure 4 Non-metric multidimensional scaling (NMDS) ordination (using Bray-Curtis similarity) showing patterns in fatty acid composition of the inflorescences of the 10 host grasses (G) and *Ruspolia differens* (R) which fed solely on these grasses. Lines connect the host and *R. differens* which fed on it. For clarity, we used the average percentages of each fatty acid across *R. differens* individuals fed on each host.

Figure 5 Mean (± SE) content of monosaturated fatty acids (MUFA) (mg g^{-1} dry weight) of *Ruspolia differens* females and males on the six single host plant diets.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1 Percentage of the most common fatty acids among (A) grass inflorescences, and (B) *Ruspolia differens* which fed on them. The hosts are ordered in the same order as they appear on the NMDS ordination (Figure 4). Ten fatty acids with the highest levels in grasses and insects are shown, other fatty acids are compiled as ‘Other’. Fatty acids 24:0 and 26:0 were found in grasses but not in insects.

Table 1 Host plant combinations used in mixtures of experiment 2

Treatment code	Treatment name	Composition
One	One host plant	*Brachiaria ruziziensis*
Two	Two host plants mixture	*B. ruziziensis*, *Panicum maximum*
Three	Three host plants mixture	*B. ruziziensis*, *P. maximum*, *Hyparrhenia rufa*
Five	Five host plants mixture	*B. ruziziensis*, *P. maximum*, *H. rufa*, *Chloris gayana*, *Cynodon dactylon*
Seven	Seven host plants mixture	*B. ruziziensis*, *P. maximum*, *H. rufa*, *Ch. gayana*, *Cy. dactylon*, *Sporobolus pyramidalis*, *Pennisetum purpureum*

Table 2 Composition (%) of fatty acids and total fatty acid content (TFA, mg g^{-1} dry weight) of the 10 single host grass species on which *Ruspolia differens* was reared. Fatty acids with levels of ≥0.5% are shown. Fatty acids with lower levels as well as trans- and iso/anteiso fatty acids are combined as ‘unresolved’

Fatty acid	*Panicum maximum*	*Chloris gayana*	*Brachiaria ruziziensis*	*Sporobolus pyramidalis*	*Pennisetum purpureum*	*Digitaria abysinnica*	*Setaria sphacelata*	*Hyparrhenia rufa*[1]	*Cynodon dactylon*[1]	*Paspalum scrobiculatum*[1]
Lauric acid (C12:0)	0.10	0.00	0.12	0.07	0.16	0.14	0.05	0.72	0.28	0.33
Myristic (C14:0)	0.31	0.28	0.45	0.19	0.43	0.39	0.25	1.99	0.92	1.08
Pentadecylic (C15:0)	0.15	0.11	0.12	0.06	0.15	0.09	0.10	0.83	0.41	0.37
Palmitic (C16:0)	32.55	31.96	30.57	19.44	31.82	27.58	20.63	24.83	27.38	23.53
Palmitoleic (C16:1ω7c)	0.00	0.00	0.13	0.00	0.00	0.08	0.03	0.66	0.26	0.27
Stearic (C18:0)	5.15	2.43	6.34	5.98	3.59	5.21	5.41	6.94	6.36	5.74
Vaccenic (C18:1ω7c)	0.00	0.00	0.20	1.05	0.32	0.11	0.26	1.01	0.94	0.92
Oleic (C18:1ω9c)	3.40	3.99	13.14	32.49	2.90	3.32	20.26	12.38	17.43	15.02
Linoleic (C18:2ω6)	28.28	26.63	24.52	28.93	26.15	28.65	37.55	20.18	21.29	28.15
α-Linolenic (C18:3ω3)	25.38	31.55	18.48	2.25	28.32	30.29	6.05	17.49	16.17	15.62
Nonadecanoic (C19:1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.57	0.29	0.39
Arachidic (C20:0)	1.95	1.55	2.21	3.41	3.85	2.50	3.30	2.40	1.94	1.03
Dihomo-γ-linolenic acid (C20:3ω6)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.03	0.31	0.42
Behenic (C22:0)	1.23	0.61	1.13	2.56	0.79	0.87	2.46	1.91	1.20	1.42
Tricosylic acid (C23:0)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.37	0.35
Lignoceric acid (C24:0)	0.52	0.62	1.43	1.53	0.44	0.38	1.88	2.63	2.17	1.55
Nervonic acid (C24:1ω9)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.88
Cerotic acid (C26:0)	0.55	0.00	0.86	1.84	0.47	0.00	1.41	0.00	0.00	0.00
Unresolved	0.42	0.26	0.32	0.19	0.61	0.41	0.36	3.79	2.03	2.93

Sum SFA	42.95	37.82	43.54	35.28	41.98	37.55	35.85	44.07	41.76	36.54
Sum MUFA	3.40	3.99	13.47	33.53	3.55	3.50	20.55	16.16	19.55	18.52
Sum PUFA	53.65	58.19	42.99	31.19	54.47	58.94	43.60	39.77	38.68	44.94
ω6:ω3	1.11	0.84	1.33	12.84	0.92	0.95	6.20	1.22	1.36	1.85
TFA	13.47	16.78	20.08	11.45	12.65	16.80	20.42	12.16	20.61	14.62

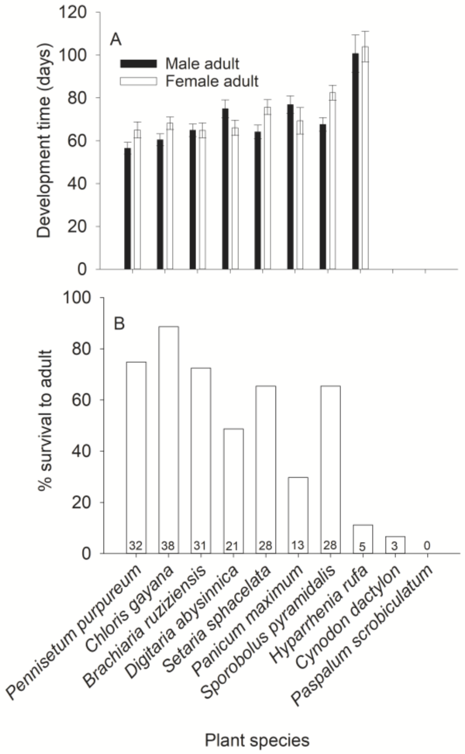
[1] Excluded from subsequent analysis because of insufficient data.

Table 3 Mean (± SE; n = 4) composition (%) of fatty acids, total saturated fatty acids (SFA), mono- and polyunsaturated fatty acids (MUFA, PUFA), and ratio of omega–6 to omega–3 fatty acids (ω6:ω3) in *Ruspolia differens* feeding on the six single grass species for which data were sufficient

Fatty acid	*Brachiaria ruziziensis*	*Chloris gayana*	*Digitaria abysinnica*	*Pennisetum purpureum*	*Setaria sphacelata*	*Sporobolus pyramidalis*
Myristic (C14:0)	0.41 ± 0.12	0.25 ± 0.08	0.32 ± 0.15	0.37 ± 0.05	0.49 ± 0.16	0.39 ± 0.11
Pentadecylic (C15:0)	0.08 ± 0.05	0.08 ± 0.03	0.06 ± 0.05	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.04
Palmitic (C16:0)	16.99 ± 3.61	15.22 ± 2.78	17.16 ± 3.96	17.76 ± 1.28	20.13 ± 2.87	20.0 ± 3.39
Hexadecenoic (C16:1ω9c)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.03
Palmitoleic (C16:1ω7c)	0.65 ± 0.27	0.29 ± 0.05	0.86 ± 0.42	0.71 ± 0.12	0.76 ± 0.32	1.09 ± 0.45
Margaric acid (C17:0)	0.50 ± 0.18	0.41 ± 0.13	0.32 ± 0.18	0.36 ± 0.06	0.26 ± 0.03	0.30 ± 0.19
Heptadecenoic (C17:1)	0.05 ± 0.04	0.05 ± 0.03	0.02 ± 0.02	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.02
Heptadecenoic (C17:1)	0.05 ± 0.04	0.02 ± 0.01	0.08 ± 0.07	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.03
Stearic (C18:0)	8.98 ± 1.88	7.38 ± 1.01	6.37 ± 0.60	7.48 ± 0.46	6.24 ± 0.46	7.84 ± 0.73
Oleic (C18:1ω9c)	24.57 ± 2.80	27.46 ± 2.84	30.40 ± 3.68	30.06 ± 1.53	32.39 ± 0.98	36.75 ± 6.61
Vaccenic (C18:1ω7c)	0.19 ± 0.05	0.02 ± 0.02	0.10 ± 0.06	0.16 ± 0.03	0.26 ± 0.04	0.18 ± 0.07
Linoleic (C18:2ω6)	37.63 ± 5.10	30.61 ± 5.45	34.91 ± 8.63	32.96 ± 2.48	34.83 ± 4.07	28.08 ± 8.36
α-Linolenic (C18:3ω3)	6.98 ± 1.97	16.78 ± 1.37	8.14 ± 2.85	8.41 ± 1.20	3.79 ± 0.92	3.84 ± 2.48
Nonadecanoic (C19:1)	0.03 ± 0.02	0.00 ± 0.00	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Nonadecanoic (C19:1)	0.12 ± 0.10	0.14 ± 0.09	0.05 ± 0.05	0.09 ± 0.03	0.03 ± 0.02	0.09 ± 0.09
Arachidic (C20:0)	2.13 ± 1.32	0.93 ± 0.11	0.85 ± 0.10	1.09 ± 0.24	0.49 ± 0.07	0.95 ± 0.45
Heneicosanoic (C21:0)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Behenic (C22:0)	0.57 ± 0.39	0.29 ± 0.06	0.21 ± 0.04	0.29 ± 0.07	0.11 ± 0.02	0.23 ± 0.13
Sum SFA	29.67 ± 2.03	24.57 ± 3.90	25.32 ± 4.67	27.54 ± 4.29	27.83 ± 2.61	29.79 ± 2.20
Sum MUFA	25.68 ± 3.06	28.00 ± 2.83	31.56 ± 4.18	29.80 ± 2.15	33.55 ± 1.15	38.25 ± 7.07
Sum PUFA	44.61 ± 3.84	47.40 ± 4.98	43.05 ± 8.69	42.64 ± 5.96	38.61 ± 3.27	31.92 ± 9.10
ω6:ω3	8.84 ± 4.63	1.89 ± 0.42	6.67 ± 2.26	3.38 ± 0.92	11.93 ± 3.98	15.19 ± 5.26

Unresolved[1]	0.03 ± 0.02	0.01 ± 0.01	0.08 ± 0.03	0.02 ± 0.02	0.00 ± 0.00	0.04 ± 0.04

[1] Fatty acid whose identity could not be established.



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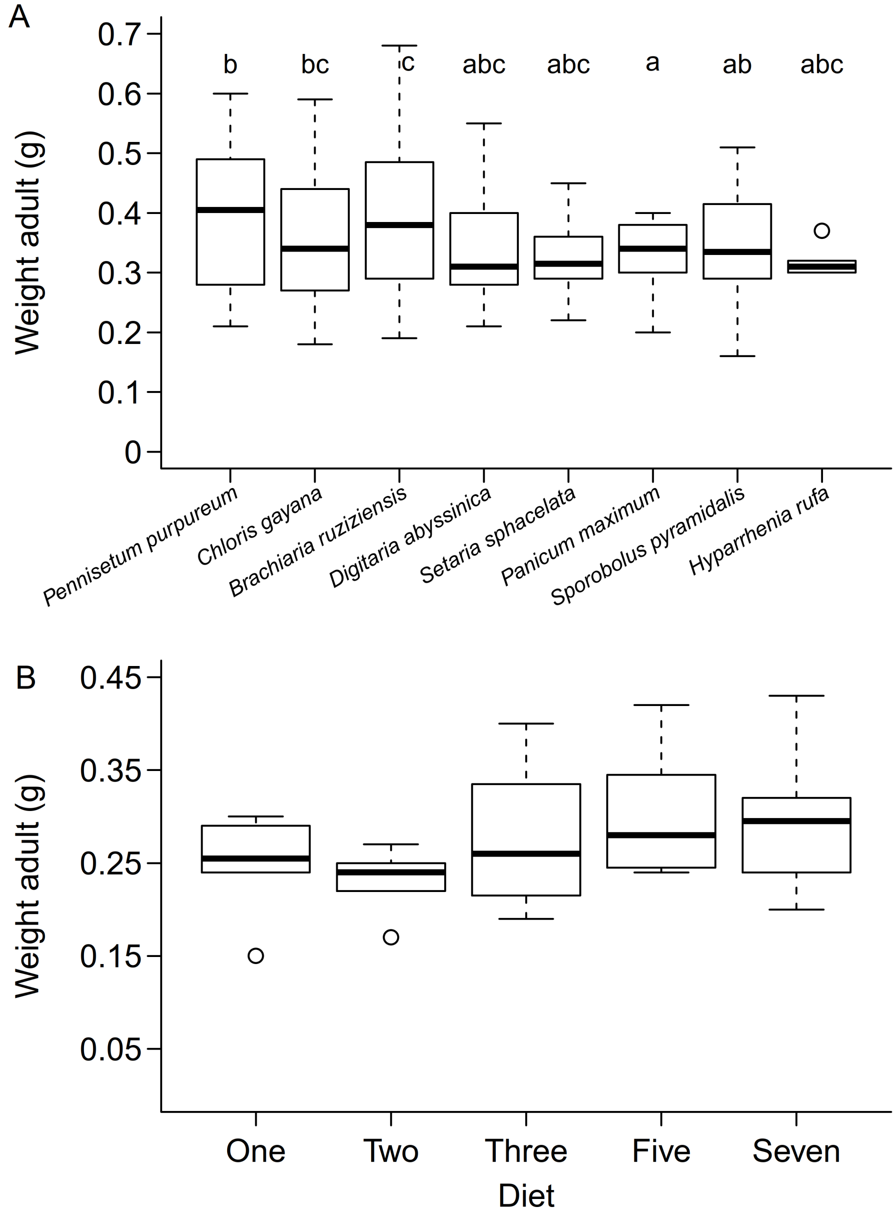

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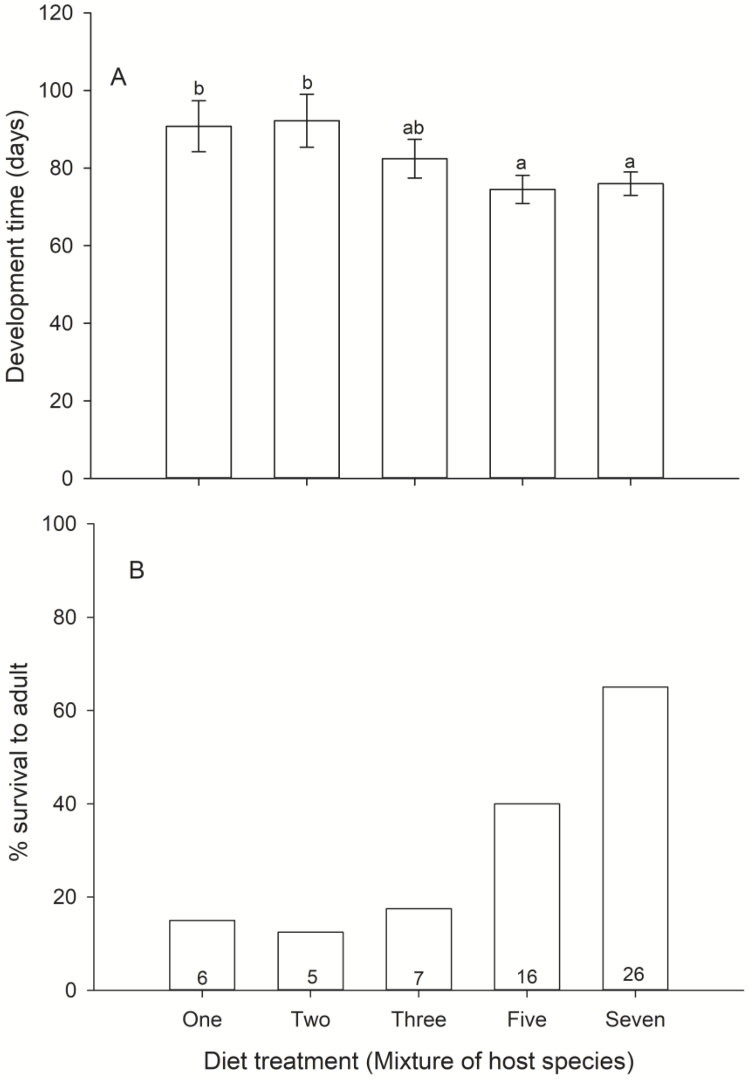

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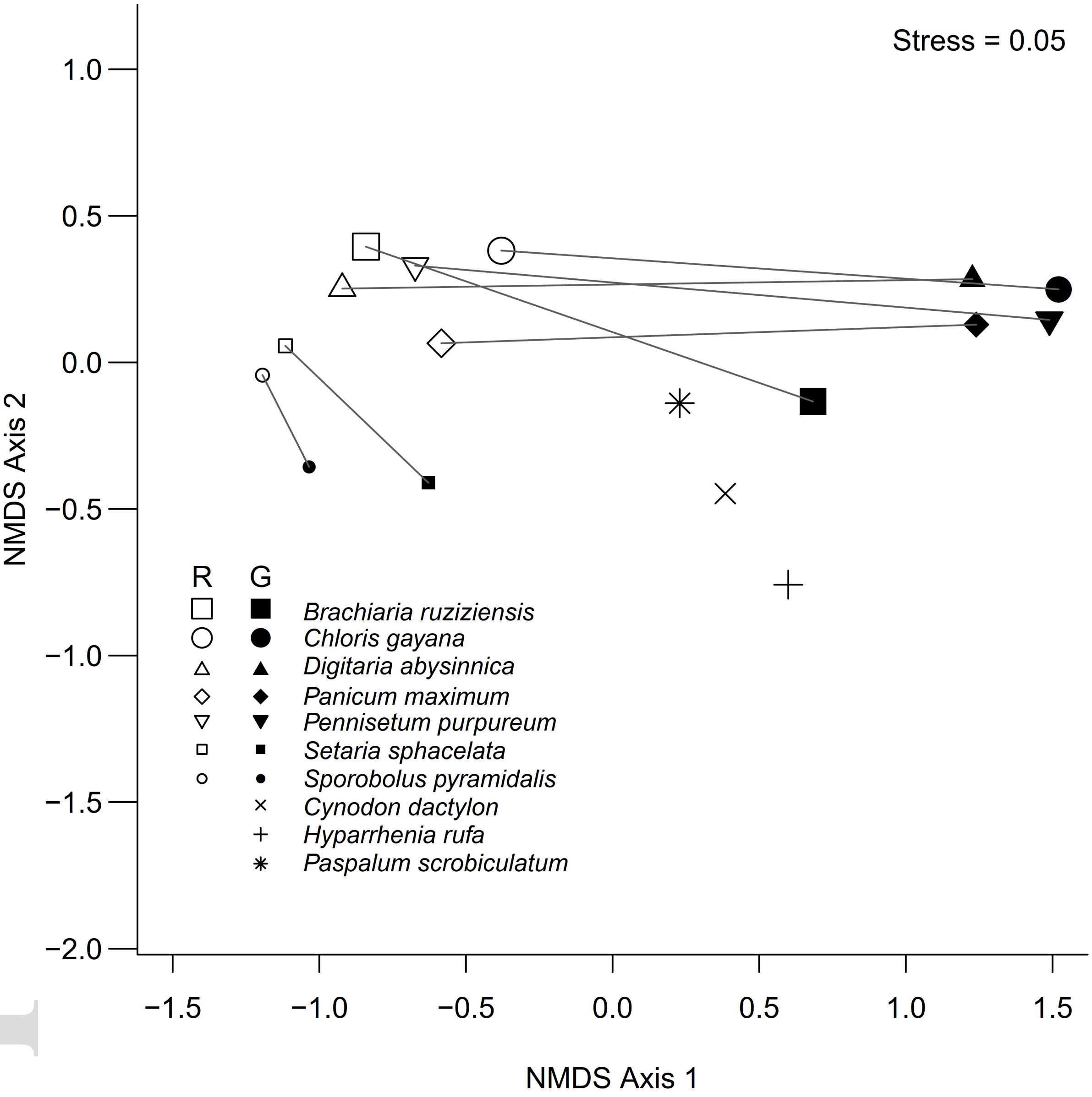

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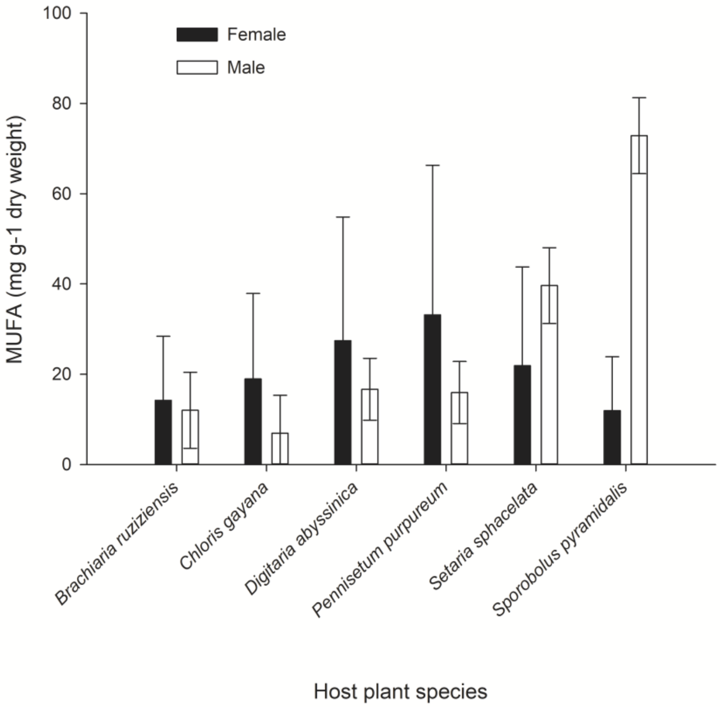

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