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ORIGINAL RESEARCH PAPER

Mixed artificial diets enhance the developmental and reproductive performance of the edible grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae)

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Abstract

Diet mixing is a common feeding habit among polyphagous insect herbivores and is believed to be advantageous for performance-related factors like growth, survival and oviposition. However, relatively little is known about the influence of artificial diet or their mixtures on the performance of edible insects. We examined the effects of artificial diet mixtures on the developmental and reproductive performance (survival, developmental time, fresh adult weight and female fecundity) of an edible grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae). We raised individuals from eggs and reared newly hatched nymphs to adult stage on six different dietary treatments consisting of a single diet, and mixtures of two, three, five, six and eight artificial diets. More diversified diets resulted in shorter development time and greater adult fresh weight and female fecundity compared to the single diet or less diversified diets. Even with slight diet diversification, survival to adult stage was greatly improved. Overall, these results highlight the potential of diet mixtures in achieving maximum adult weights and female fecundity and shortening development time, information which could be used when designing mass-rearing programs for this edible grasshopper.

Keywords African edible bush cricket · Captive rearing · Diet mixing · Insect farming · Mass rearing

Introduction

Diet mixing (i.e., eating different food types) is a common feeding habit among polyphagous insect herbivores and is considered beneficial for performance-related factors like survival, oviposition and growth (Singer and Bernays 2003). Diet mixing could allow individuals to balance the intake of different nutrients, feed on nutrient-deficient food resources when superior alternatives are in limited supply and avoid the ingestion of toxic doses of secondary metabolites in solitary foods (reviewed in Bernays and Bright 1993; Bernays et al. 1994). Among the polyphagous orthopterans, there are many examples of diet mixing (e.g., MacFarlane and Thorsteinson 1980; Miura and Ohsaki 2004; Singer and Bernays 2003; Unsicker et al. 2008); these studies have shown them to grow faster and exhibit higher fitness and survival rates when fed on mixed diets compared to single-resource diets. However, despite the widespread evidence of diet mixing in insect herbivores, in contrast, there is limited knowledge on the effect of artificial diets or their mixtures on the performance of edible insects (Alves et al. 2016; Ebenebe and Okpoko 2016; Miech et al. 2016; van Broekhoven et al. 2015), which hinders the development of a suitable diet or diet mixtures for sustaining mass-rearing.

Ruspolia differens (Serville) (Orthoptera: Tettigoniidae) is a multivoltine nocturnal edible grasshopper with an elongate cone-head and long filiform antenna (Matojo and Yarro 2010). It is one of the most economically valuable wild-harvested edible insect species (Agea et al. 2008; van Huis 2013), occurring in grassland and open bushes throughout tropical Africa and on some islands of the Indian Ocean

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(Massa 2015). Nymphs hatch in 11–12 days, reaching adult maturity in 2–3 months (Hartley 1967). Under laboratory conditions, *R. differens* eat flowers, seeds and flours of cultivated cereals, including maize, sorghum and rice, as well as wheat bran, germinated finger millet, ground dog biscuits, dried blood, Lucerne meal and a high protein cereal meal (Brits and Thornton 1981; Nyeko et al. 2014; Malinga et al. 2018). Currently, there is a great need to develop mass-rearing methods for this species because of concerns of over-harvesting wild populations, and the need to enhance food security in East Africa. In our previous work, we found that seed heads of rice, sorghum and finger millet, wheat bran, chicken feed egg booster and germinated seeds of finger millet were the most accepted foods by *R. differens* (Malinga et al. 2018).

In the present study, we experimentally investigated how artificial diet treatments representing a gradient of increasing food resource diversity, ranging from one food to mixtures of two, three, five, six and eight foods, affect the development and reproductive performance of *R. differens*. We tested the prediction that increased resource diversity would lead to increased nymphal survivorship, increased adult fresh weight and female fecundity, and shorter nymphal developmental times due to the improved balance of nutrients (Hägele and Rowell-Rahier 1999). Specifically, we addressed the following questions: (1) does the developmental time from the first instar to adult stage, nymphal survivorship, adult fresh weight or female fecundity in *R. differens* differ between individuals in the six diet treatments, and (2) are there directional trends in these variables along the food diversity gradient?

Materials and methods

Study insects

The laboratory colony of non-swarming *R. differens* was collected at night in June 2016 from farmlands at the Makerere University Agricultural Research Institute, Uganda. Upon collection, the grasshoppers were allowed to mate in five plastic containers (240 mm length × 180 mm width × 125 mm height; Thermopak, Nairobi, Kenya). To ensure successful mating, each container held 10 males and 10 females, and individuals were fed on fresh maize cob. Wet folded tissue paper was used as a source of water. For oviposition, we placed four small round plastic jars (53 mm width × 71 mm height) filled with moistened cotton wool, one at each corner, as a substrate. The eggs laid were collected, placed on damp cotton wool and sand (50:50) in small round plastic jars, and sprayed with water every 2 days to allow hatching in 2–4 weeks.

Diet treatments

Eight foods, hereafter “artificial diets”, were selected: rice seed heads, finger millet seed heads, wheat bran, chicken superfeed egg booster, sorghum seed heads, germinated finger millet, simsim cake and crushed dog biscuit pellets. The selection of foods was based on their acceptance in our previous work (Malinga et al. 2018). These foods were assigned to six dietary treatments which represented a gradient of increasing food resource diversity, ranging from one food (single-food treatment) to mixtures of two, three, five, six and eight foods (Table 1); an equal quantity (2 g) of each constituent food was randomly placed in each container. We used mature seed heads of finger millet, sorghum and rice which had just started to turn golden-brown, reddish-brown and yellow, respectively. Dog biscuits, wheat bran and chicken feed egg booster were acquired from local markets in Kampala, Uganda. Germinated finger millet was prepared by soaking millet

Table 1 Compositions of diets used in the performance experiments

Treatment code	Treatment name	Composition[a]
One	Single food treatment	Rice seed heads
Two	Two food mixtures	Rice seed heads
		Finger millet seed heads
Three	Three food mixtures	Rice seed heads
		Finger millet seed heads
		Wheat bran
Five	Five food mixtures	Rice seed heads
		Finger millet seed heads
		Wheat bran
		Chicken superfeed egg booster
		Sorghum seed heads
Six	Six food mixtures	Rice seed heads
		Finger millet seed heads
		Wheat bran
		Chicken superfeed egg booster
		Sorghum seed heads
		Germinated finger millet
Eight	Eight food mixtures	Rice seed heads
		Finger millet seed heads
		Wheat bran
		Chicken superfeed egg booster
		Sorghum seed heads
		Germinated finger millet
		Simsim cake
		Dog biscuit pellets

[a]The nutritional compositions of the different experimental food components are presented in Malinga et al. (2018)

grains in cotton cloth overnight, draining the water and then leaving it to sprout at room temperature for 72 h. Simsim cake was prepared by heating equal quantities of simsim seeds and sugar between 60 and 70 °C until the sugar turned into liquid, followed by cooling. To improve consumption, dog biscuit pellets, simsim cake and the seed heads of finger millet, sorghum and rice were lightly crushed with a grinding stone.

Performance experiments

Rearing experiments were carried out at the Animal Science Laboratory, Makerere University Agricultural Research Institute, at a temperature of 23–27 °C, RH, 50–60% and a natural 12:12 day:night cycle. To determine the performance of *R. differens* on the six diets, a total of 35 individual nymphs were randomly subjected to each of the six food treatments (Table 1) and reared to adults. To account for possible variations in microclimatic conditions during rearing, the containers were randomly placed on the tables in 35 groups (blocks), each of which consisted of one replicate of each dietary treatment. Altogether, the 210 newly hatched nymphs were individually placed in transparent plastic containers (80 mm high and 125 mm in diameter; TPL2035; Thermopack), with the opening on the top being covered with a netting cloth to prevent the nymphs from escaping. The food was offered ad libitum to each individual and refreshed every 3–4 days until moulting to adult, and water was supplied by the insertion of wet tissue paper. Individual insects were inspected daily from the first instar to adult moulting to observe nymphal survivorship and adult emergence. For emerged adults, we measured fresh weight within 24 h of moulting. For surviving insects, the duration of nymphal development was determined as the number of days between hatching and the emergence of the adult.

To determine the female fecundity in the laboratory, in each treatment replicate, we placed one newly emerged adult female and two male *R. differens* for mating and oviposition (two males were used to ensure the fertilization of the female). Males that died during the experiment were replaced with males from the wild. The containers (240 mm length × 180 mm width × 125 mm height; Thermopack) were arranged in a randomised complete block design comprising of six blocks, and each block had one replicate of each dietary treatment (treatment one was excluded because the few individuals that had emerged as adults died before the start of the fecundity experiment). A small round plastic jar (53 mm width × 71 mm height) filled with moistened cotton wool was provided for each female in each rearing container to serve as an oviposition substrate. The total number of eggs laid by each female in treatment was determined by weekly counts until death.

Statistical analysis

We used linear mixed models (type III sum of squares) followed by Bonferroni post hoc pairwise comparisons to analyse the effects of dietary treatment on the adult fresh weight (g), developmental time (days) (proportions of adult females to males, 49:31) and female fecundity of *R. differens*. In all models, the block was included as a random factor. To improve normality and equality of variance, we applied $\log_{10}$ transformations to the response variables. Initially, we included the longevity in days since hatching and number of days in the oviposition experiment of females as covariates in fecundity analysis, but they were excluded because the variables correlated strongly (positively) with each other and the response variable. Linear contrasts within the analysis of variance (ANOVA) were used to determine whether there was a linear trend in the insect performance variables along the food resource diversity gradient.

We fitted a logistic regression model (with generalised estimating equations procedure of SPSS) to test whether diet treatment (independent variable) predicts the nymphal survival to adulthood. For this analysis, the individuals were placed into two categories, those that emerged to adults and those that died before this stage (the response variable), and block was specified as the subject variable. The goodness-of-fit of the model was assessed using the Hosmer–Lemeshow test and type III Wald χ^2 tests were used to evaluate the significance of model parameters (Hosmer et al. 2013). All statistical analyses were performed using IBM SPSS Statistics software, v.23 (IBM, Armonk, NY, USA).

Results

Developmental time

The total nymphal development time (in days) differed significantly between the six diet treatments (linear mixed model; $F_{5,63.3} = 19.5$, $p < 0.001$; Fig. 1). Overall, there was a significant decreasing linear trend in the development time along the food resource diversity gradient (ANOVA linear contrasts, $F_{5,74} = 78.0$, $p < 0.001$), indicating that as food resource diversity increases from single to more diversified diet mixtures, development time decreases proportionally (Fig. 1).

Fresh weight of adults

The fresh weight of *R. differens* adults differed significantly between the six diet treatments (linear mixed model; $F_{5,57} = 13.4$, $p < 0.001$; Fig. 2). There was a significant increasing linear trend in the adult fresh weight along the food resource diversity gradient (ANOVA linear contrasts,

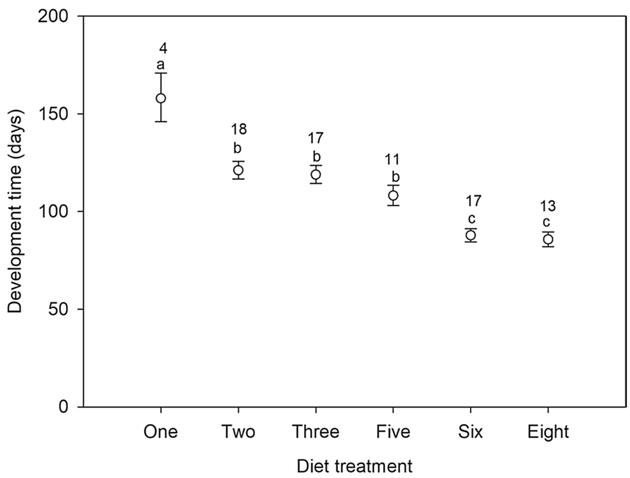

Fig. 1 Effect of dietary treatment on the developmental time (the number of days between hatching and the emergence of the adult) of *R. differens*. Values represent back-transformed estimated marginal means (± SE) from the linear mixed model. Numbers above the bars indicate the number of *R. differens* analysed. Different letters indicate treatments that differ significantly according to pairwise tests (Bonferroni test, $p < 0.05$)

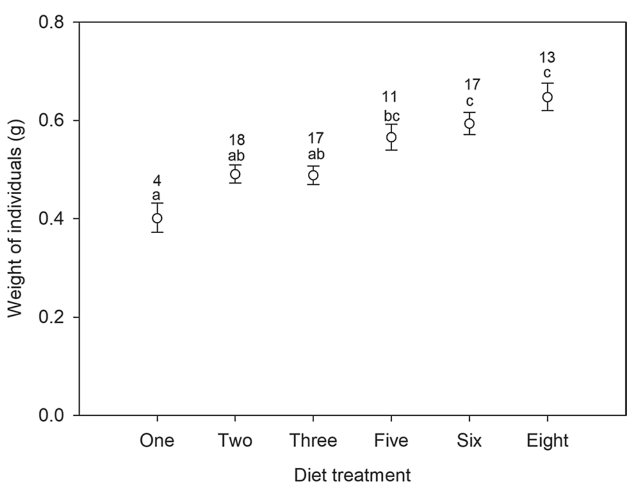

Fig. 2 Effect of dietary treatment on the fresh weight of adult *R. differens*. Values represent back-transformed estimated marginal means (± SE) from the linear mixed model. Numbers above the bars indicate the number of *R. differens* analysed. Different letters indicate treatments that differ significantly according to pairwise tests (Bonferroni test, $p < 0.05$)

$F_{5,74} = 44.2$, $p < 0.01$), suggesting that, as food resource diversity increases from single to more diversified diet mixtures, adult weight increases proportionally (Fig. 2).

Female fecundity

The female fecundity (total number of eggs laid per female) differed significantly between the five (treatment one was excluded; see "Materials and methods") dietary treatments (linear mixed model, $F_{4,25} = 7.6$, $p < 0.001$; Table 2). Longevity in treatment did not explain differences in female fecundity (one-way ANOVA, $F_{4,25} = 0.980$, $p = 0.436$). In addition, there was a significant increasing linear trend in female fecundity along the food resource diversity gradient (ANOVA linear contrast, $F_{4,25} = 19.5$, $p < 0.001$), indicating that, as food resource diversity increases from a few to the more diversified diet mixtures, the fecundity of females tends to increase.

Survival to adulthood

Overall, the nymphal survival to adulthood was 38.1%, but differed significantly between the six dietary treatments, being higher in diversified dietary treatments than in the single dietary treatment (logistic regression: $\chi^2 = 15.2$, $df = 5$, $p = 0.010$; Table 3). The odds of survival (the probability of surviving relative to dying) was 4–8 times higher for individuals fed mixtures of foods than in those fed one food only (odds ratio = exp (parameter value)).

Discussion

Our results demonstrate that the performance (developmental time and growth) of the edible grasshopper *R. differens* can be optimised by rearing on dietary mixtures rather than on less diversified or single diets. In the present study, the nymphal developmental time to adult of *R. differens* was greatly shortened and a greater adult weight was attained when individuals were reared on more diversified diets rather than on single resource diets. The improved performance could be due to the consumption of a balance of high quality (i.e., high nitrogen or carbohydrate) in diversified diets; Unsicker et al. 2008). Several studies with generalist Acrididae have shown that grasshoppers perform better on high quality (i.e., high nitrogen

Table 2 The total number of eggs laid and longevity in days since hatching of female *R. differens* on the five different dietary treatments

Diet treatment[a]	No. of eggs per female (mean ± SE)[b]	Longevity (in days since hatching) (mean ± SE)
Two	75.5 ± 19.4 ab	80.5 ± 5.9
Three	39.3 ± 13.4 a	69.7 ± 7.2
Five	127.8 ± 29.7 bc	88.0 ± 5.7
Six	257.2 ± 67.3 c	85.5 ± 9.1
Eight	189.8 ± 49.8 bc	85.3 ± 8.3

[a]For all treatments $n = 6$

[b]Different letters within a column indicate statistically significant differences according to pairwise tests (Bonferroni test, $p < 0.05$)

Table 3 The survival to adulthood of *R. differens* on the six different diet treatments

Treatment	Number of insects	Survival rate (%)[a]	Odds ratio (95% CI)[b]	*P*
One	35	11.4 (4)		Reference
Two	35	51.4 (18)	8.2 (2.4-28.4)	0.001
Three	35	48.6 (17)	7.3 (2.4-22.5)	0.001
Five	35	31.4 (11)	3.6 (1.0-12.9)	0.054
Six	35	48.6 (17)	7.3 (2.1-25.0)	0.001
Eight	35	38.1 (13)	4.6 (1.4-15.1)	0.012
Total		38.1 (80)		

[a]Percentage of insects that survived to adult

[b]*Odds ratio* exp (parameter value), the ratio of the odds of surviving to the odds of not surviving (Hosmer et al. 2013). Odds ratios and *p* values come from the logistic regression model (generalized estimating equation procedure of SPSS). Treatment one was used as a reference category

or carbohydrate) diets (Bernays and Bright 1993; Berner et al. 2005; Miura and Ohsaki 2004; van Broekhoven et al. 2015), which in turn determines their growth and developmental rate (Joern and Behmer 1997), although the mechanisms behind this are still unclear. The beneficial effects of multiple resource diets on developmental time and adult weight have been reported for the two-striped grasshopper, *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae) (MacFarlane and Thorsteinson 1980), and on generalist grasshoppers, *Parapodisma subastris* (Huang) (Miura and Ohsaki 2004) and *Chorthippus parallelus* (Zetterstedt) (Orthoptera: Acrididae) (Unsicker et al. 2008).

Furthermore, our experimental results indicated that dietary mixing greatly improved female fecundity. Thus, based on these results, it is clear that the nutritional value of mixed diets might be superior to those of single-resource diets, leading to improved oocyte development. Improved fecundity in mixed diets has also been reported for the generalist grasshoppers *P. subastris* (Miura and Ohsaki 2004) and *C. parallelus* (Orthoptera: Acrididae) (Unsicker et al. 2008).

Finally, we detected greatly improved survival rates of *R. differens* when reared on dietary mixtures rather than single resource diets. Even with a slight diet diversification, nymphal survival to adult was greatly improved. The current result is consistent with those for another generalist grasshopper, *C. parallelus* (Orthoptera: Acrididae), in which survival was highest in the food plant mixture with eight plant species and lowest in the treatments in which only one single plant species was offered as food (Unsicker et al. 2008). The survival rate of many insect herbivores has been shown to be closely linked to the quality of their diets (Joern and Behmer 1997). Thus, the relatively lower survival on single (carbohydrate-rich rice diet) than in mixed diets could be due to the low protein and high starch content in a single-resource diet, as also indicated in a previous study with mealworms (van Broekhoven et al. 2015). From the applied point of view, our results demonstrate how a cost-effective rearing program for *R. differens* could be designed with limited diet mixtures.

In conclusion, the results from this study provide evidence that *R. differens* performs better on mixed diets than on single or less diversified diets. Increasing dietary diversification resulted in higher survival, shorter development time and greater adult weight and female fecundity. These results highlight the importance of mixed diets in the optimisation of edible grasshopper, *R. differens*, production during mass-rearing programs.

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