Sexual differences in destructive capability and midgut enzyme activities in adult variegated grasshoppers *Zonocerus variegatus* (LINNAEUS, 1758) (Orthoptera: Pyrgomorphidae)

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ABSTRACT. The variegated grasshopper *Zonocerus variegatus* is a polyphagous insect, feeding on numerous food and cash crops. The present study aimed to investigate the sexual variations in the destructive capability of the adult insects and the composition of leaves damaged by them, as well as in the levels of midgut microbial flora and digestive enzymes (cellulase, amylase and α -glucosidase). The results showed that females consumed and caused more damage to cassava leaves than their male congeners. The leaves damaged by males contained more nutrients than those damaged by females. The gut microbial flora and enzyme assay showed that females had significantly larger colony forming units and a non-significant difference in enzyme activities. It can thus be concluded that adult females are more destructive than males.

KEY WORDS: dimorphism, microbial flora, digestive enzymes, leaf composition.

INTRODUCTION

Zonocerus variegatus (LINNAEUS, 1758) is a tropical African grasshopper, inflicting great losses on both cash and food crops. Both nymphs and adults are commonly found in the same habitat, which is mainly uncultivated land. TOYE (1982) observed that the life cycle of *Z. variegatus* occupies a full year with two populations: a dry season population

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(November – March) and a wet season population (April – October). Biologically, *Z. variegatus* metamorphoses through a nymphal stage (six nymphal instars) and an adult stage. The nymphal periods are 7.0, 7.6, 10.0, 19.1, 20.5 and 46.9 days for the 1st – 6th instars, respectively. The time spent in the lower nymphal instars is thus shorter than that spent in the higher instars. The adult stage lasts 147.9 days (ADEMOLU et al. 2013). Earlier, MODDER (1986) reported that the characteristics of the early instars (1st – 3rd) are different from those of the later instars (5th – 6th) and the adult stage. While the early instars live in dense aggregations, the later ones are more dispersed. Also, while the early instars survive on weeds such as *Chromolaena odorata* (L.) KING & H.E. ROBINS. and *Aspillia africana* (PERS.) C.D. ADAMS, the later instars prefer *Manihot esculenta* CRANTZ (CHAPMAN et al. 1986).

The variegated grasshopper harbours microorganisms in its alimentary canal that aid digestion (MEAD et al. 1988) and also contribute to the host's nutrition. The gut is a continuous tube running from the mouth to the anus and is divided into three regions: foregut (or stomodaeum), midgut (or mesenteron) and hindgut. Some studies have revealed that numbers of microbes and enzyme activities in the gut increased with the grasshopper's age, causing the adult stage to be the most destructive one (IDOWU & EDEMA 2003, ADEMOLU & IDOWU 2011).

Sexual dimorphism is a common phenomenon in animals, including insects. These differences reflect the outcome of natural selection in each sex. MUSE (2003) reported that in *Zonocerus variegatus*, females lived longer than males both in isolation and communally. ADEMOLU et al. (2013) likewise reported that adult females spent more days (127.9 days) feeding than their male counterparts (101.2 days). Furthermore, enzyme activities in the femoral muscles were significantly higher in adult females than adult males. In contrast, lower ionic concentrations in the antennae were recorded in adult females than in males during mating (ADEMOLU 2013). However, as both adult males and females behave and perform different functions at the adult stage, we hypothesise that their consumption patterns will not be the same. Therefore, the aim of the present study was to investigate sexual variations in the destructive capability and damaged leaf composition, as well as in the levels of midgut microbial flora and digestive enzymes in the adult stage of *Z. variegatus*.

MATERIAL AND METHODS

Sample collection

Sixth instar nymphs (identified by their coloured wing pads) of *Z. variegatus* were collected using a sweep net from uncultivated farmland on the campus of the Federal

University of Agriculture, Abeokuta (FUNAAB), Nigeria. The collections were made between 06:00 and 08:00 hrs in the morning and between 17:00 and 19:00 hrs in the evening. They were taken to the Insectary of the Department of Pure and Applied Zoology, where they were reared until the adult stage. Fresh leaves of cassava (*Manihot esculenta*) were given to the insects every morning (08:00 hrs) throughout the experiment.

Experimental Procedure

Soon after the insects had metamorphosed into the adult stage (2 days after emergence), they were divided into males and females. One hundred and fifty males and females were picked from the stock and separated into different trial cages.

Assessment of Leaf Damage

Twenty adult males and females were placed in two separate cages (30x30x45cm) and fresh leaves of *M. esculentus* were provided every day. Leaf area damage was measured by the graphical method described by OSIPITAN et al. (2012). This was done for eight weeks and the experiment was replicated three times. The amount of leaves consumed by the insects was calculated as follows: Feeding intake = Feed given – feed leftover.

Microbial flora in the midgut

The microbial flora in the midgut of adults was assessed by the method described by Idowu and Edema (2003). Ten males and females were dissected and their midgut contents emptied into Petri dishes. Then these were homogenised in 1 ml of sterile distilled water. The homogenate was decanted into labelled bottles containing 9 ml of sterilised water. Potato Dextrose Agar (PDA) was used for fungi enumeration and Nutrient Agar (NA) was used for bacteria enumeration. After incubation for 2 days and 5 days for bacteria and fungi, respectively, colony forming units were determined by visual counting.

Enzyme Assay in the Midgut

The insects were first asphyxiated in a deep freezer for 30 minutes, after which their guts were carefully removed. The midgut was cut away and homogenised in 0.05M KCl and centrifuged at 500 rpm for 30 minutes. The supernatant (enzyme extract) was stored in the freezer for further analysis. The method described by ADEDIRE & BALOGUN (1995) was adopted for determining carbohydrase (α -glucosidase, amylase and cellulase) activities in the midguts of the adult males and females. The assays were done in triplicate.

Proximate composition of the damage leaves and experimental insect

The proximate composition of the damaged leaves and the adult males and females was assessed according to HORWITZ (1980) methods. Thirty separate adult males and females

were used for this analysis. Crude fibre, ash, protein and fat were determined, while carbohydrate was determined by the difference (carbohydrate = 100 – moisture content + ash + fat + protein + fibre). All the above experiments were also carried out on adult females of *Zonocerus variegatus*. Each parameter was replicated three times.

Statistical analysis

All the data from the experiments were analysed using the *t*-test, and significant means were separated using Duncan's Multiple Range Test.

RESULTS

Data on the level of leaf damage of *Monihot esculenta* and food intake by adults male and females of *Zonocerus variegatus* are presented in Table 1. There was a significant difference in the amount of leaves consumed and damaged by the two sexes, since females damaged the leaves more significantly than males. Also, females exhibited a significantly higher feed intake than the males. Data relating to colony forming units (cfu) (bacteria and fungi) in the midguts of adults are set out in Table 2. The adult females had more cfu of bacteria and fungi than the males. Table 3 lists data on carbohydrase activities in the adult midgut. Statistical analysis revealed that there were no significant differences in the activities of the three enzymes. It is worth noting that α -glucosidase demonstrated the highest level of activity and amylase the lowest. The proximate composition of the damaged cassava leaves by adult *Z. variegatus* is shown in Table 4. The leaves damaged by female *Z. variegatus* had significantly lower crude fat, ash, protein and carbohydrate contents than those damaged by males. Table 5 contains data on the proximate composition of the experimental insects after eight weeks of consuming cassava leaves. The adult females had statistically higher levels of crude fibre, protein and ash than their male counterparts.

Table 1. Food intake and leaf area damage in *Monihot esculenta* by male and female *Zonocerus variegatus*.

Parameter	Males	Females
Leaf area damage (cm ²)	356.7 $\pm$ 0.1 ^b	410.13 $\pm$ 0.02 ^a
Feed intake (g)	42.9 $\pm$ 0.01 ^b	47.2 $\pm$ 0.1 ^a

Mean values across the columns with different superscripts are significantly different ($p < 0.05$).

Table 2. Microbial load (cfu x 10³) of the midgut of adult *Z. variegatus*.

Parameter	Potato Dextrose Agar (fungi)	Nutrient Agar (Bacteria)
Males	10 ±0.01 ^b	3.0 ±0.12 ^b
Females	30 ±0.03 ^a	90 ±0.35 ^a

Mean values across the columns with different superscripts are significantly different ($p < 0.05$).

Table 3. Carbohydrase activities (mg/g) in the midgut of adult *Z. variegatus*.

Parameter	Cellulase	Amylase	α – Glucosidase
Males	0.69 ±0.01	0.33 ±0.05	0.89 ±0.20
Females	0.68 ±0.12	0.31 ±0.13	0.88 ±0.42

Table 4. Proximate composition of leaf damaged by adult *Z. variegatus*.(g/100g).

Parameter	Moisture content	Fat content	Ash content	Fibre content	Protein content	Carbohydrate content
Leaves damaged by male	45.86 ±0.1 ^b	1.63 ±0.2 ^a	1.16 ±0.0	1.33 ±0.3	2.89 ±0.1 ^a	47.13 ±0.0 ^a
Leaves damaged by female	55.23 ±0.1 ^a	1.28 ±0.1 ^b	1.02 ±0.1	1.45 ±0.2	2.12 ±0.1 ^b	38.90 ±0.1 ^b

Mean values across the columns with different superscripts are significantly different ($p < 0.05$).

Table 5. The proximate composition of adult *Z. variegatus* fed cassava leaves (g/10g).

Parameter	Moisture content	Fat content	Ash content	fibre content	protein content	Carbohydrate content
Males	67.74 ±0.1 ^a	5.47 ±0.1	2.05 ±0.3	10.36 ±0.1 ^a	12.42 ±0.2 ^a	1.96 ±0.0 ^b
Females	63.11 ±0.3 ^b	5.82 ±0.0	2.45 ±0.1	11.19 ±0.0 ^b	14.45 ±0.1 ^b	2.98 ±0.1 ^a

Mean values across the columns with different superscripts are significantly different ($p < 0.05$).

DISCUSSION

The damage done to leaves of the cassava plant by *Z. variegatus* depends to a significant extent on the sex of the insect. Adult females inflicted significantly more damage and consumed more cassava leaves than their male congeners. The bigger size of the adult female *Z. variegatus*, as earlier observed by ADEMOLU et al. (2013), requires a higher food intake than the male in order to satisfy the demand for nutrients by the higher number of cells. Also, in preparation for egg production and oviposition, which are energy sapping processes (ADEMOLU et al. 2011), more nutrients are needed by adult females.

Microorganisms are acquired by *Z. variegatus* longitudinally, that is, through the food plants they eat (IDOWU et al. 2009). The occurrence of a higher microbial load in the female midgut than in that of the male was not unexpected, as adult females consumed more leaves than males, thereby ingesting more microbes along with the leaves. The present results also showed that more bacteria species were isolated from the midgut than fungi. This reflects the characteristics of the organisms: while bacteria are cosmopolitan and ubiquitous, fungi are more selective in their choice of host (MARTIN & KUKOR 1984).

Z. variegatus is endowed with the enzymes required for the digestion of its multifarious diets (ADEMOLU & IDOWU 2011, MODDER 1984). The presence of carbohydrases (amylase, cellulase and α -glucosidase) in the midgut of adult males and females, ascertained in the present study, confirmed this finding. However, no significant difference was recorded in the activities of the three enzymes between the sexes. This might be a reflection of the non-significant difference in the sizes of the adult male and female midguts. ADEMOLU et al. (2011) also reported that during the somatic phase of the adult *Zonocerus*, there was no significant difference in the concentration of metabolites in the tissues of either males or females.

As the present investigation has clearly shown, the protein, fat and carbohydrate contents of leaves damaged by adult female *Z. variegatus* were also significantly lower than those eaten by males. The damaged leaves has a smaller surface area or size available for photosynthesis, thus limiting the amount of nutrients that they could produce. CHAPMAN et al. (1986) noted that the root yield of cassava plants with leaf area reduced as a result of *Z. variegatus* attack was very much smaller compared to those plants with intact leaves. The results of this study show that adult females of *Z. variegatus* had higher carbohydrate, protein and fibre contents than adult males. Their higher levels of food consumption and shorter movements (because of their greater weight) could have been responsible for this observation. Adult females of *Z. variegatus* travel shorter distances than the male, thereby conserving and storing nutrients in their tissues for use at a later time (ADEMOLU et al. 2011). Similarly, the higher microbes in the female midgut might be living symbiotically in their guts, supplying them with nutrients, vitamins and alcohol (CHAPMAN 1990).

CONCLUSION

Adult females of *Z. variegatus* have a greater destructive capability than their male counterpart.

REFERENCES

- ADEDIRE C.O., BALOGUN R.A. 1995. Digestive enzymes and regional localization of proteolytic endopeptidase in alimentary canal of Kola nut weevil, *Sophrorhinus insperatus* FAUST (Curculionidae). *Entomon* **20**(3): 183–189.
- ADEMOLU K.O. 2013. Sexual dimorphism in somatic tissues' response to copulation in adult *Zonocerus variegatus* (L). *Nigerian Journal of Entomology* **30**(1): 164–169.
- ADEMOLU K.O., IDOWU A.B. 2011. Activity of digestive enzymes in *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) gut homogenates during post embryonic development. *International Journal of Tropical Insect Science* **31**(1-2): 29–33.
- ADEMOLU K.O., IDOWU A.B., OKE O.A. 2011. Impact of Reproduction Activities on the Tissues of *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). *Florida Entomologist* **94**(4): 993–997.
- ADEMOLU K.O., IDOWU A.B., OKE O.A. 2013. Life cycle and morphometric studies of variegated grasshopper *Zonocerus variegatus* (LINNAEUS, 1758). *Munis Entomology and Zoology* **8**(1): 375–381.
- HORWITZ W. (ed.) 1980. Official methods of analysis of the Association of Official Analytical Chemists. 13th edition. Association of Official Analytical Chemists, Washington, D.C.
- CHAPMAN R.F. 1990. The Insects Structure and Function. The English Language Book Society and Hodder and Stoughton, London.
- CHAPMAN R.F., PAGE W.W., MC GIFFERY A.R. 1986. Bionomics of the Variegated Grasshopper (*Zonocerus variegatus*) in West and Central Africa. *Annual Review of Entomology* **31**(1): 479–505.
- IDOWU A.B., EDEMA M.O. 2003. The Microbial flora of the different gut regions of *Zonocerus variegatus* (L) (Orthoptera: Pyrgomorphoidea). *Nigerian Journal of Plant Protection* **20**(1): 19–30.
- IDOWU A.B., EDEMA M.O., OYEDEPO M.T. 2009. Extracellular enzyme production by microflora from the gut region of the variegatus grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). *International Journal of Tropical Insect Science* **29**(4): 229–235.
- MARTIN M.M., KUKOR J.J. 1984. Role of mycophagy and bacteriophagy in invertebrate nutrition. [in:] J.M. ANDERSON, D.M. RAYNER, D WALTON (eds.). *Microbial Ecology*. Cambridge University Press, Cambridge, 257–263.
- MEAD L.J., KHACHATOURIANA G.G., JONES G.A. 1988. Microbial Ecology of the Gut in Laboratory Stocks of Migratory Grasshopper, *Melanoplus sanguinipes* (FAB) (Orthoptera: Acrididae). *Applied and Environmental Microbiology* **54**(5): 1174–1180.
- MODDER W.W.D. 1984. Diurnal variation in the feeding and growth activity in nymphs of the African pest, grasshopper *Zonocerus variegatus* (L.). *Insect Science and Application* **5**(6): 527–531.
- MUSE W.A. 2003. Effect of sex, Age Starvation and Feeding, Isolation and Crowding and Oviposition on Longevity of *Zonocerus variegatus* (Orthoptera; Pyrgomorphidae). *Journal of Biological Research and Biotechnology* **1**(2): 11–20.
- OSIPITAN A.A., OLAMIPOSI B., GARBA I.G. 2012. Evaluation of the role of spacing on yield and in management of flea beetle, *Podagrica* spp infestation in Okra (*Abelmoschus esculentus* (L.) MOENCH). *African Entomology* **20**(1): 52–59.

TOYE S.A. 1982. Studies on the biology of the grasshopper pest *Zonocerus variegatus* (L) (Orthoptera: Pyrgomorphidae) in Nigeria. *Insect Science and Application* **3**(1): 1–7.

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