Impact of Reproductive Activities on the Tissues of Zonocerus variegatus Grasshopper Adults (Orthoptera: Pygomorphidae)

Authors: Olutoyin Ademolu, Kehinde, Adewunmi Idowu, Babatimde, and Oke, Olubode A.

Source: Florida Entomologist, 94(4) : 993-997

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.094.0437

IMPACT OF REPRODUCTIVE ACTIVITIES ON THE TISSUES OF *ZONOCERUS VARIEGATUS* GRASSHOPPER ADULTS (ORTHOPTERA: PYGOMORPHIDAE)

KEHINDE OLUTOYIN ADEMOLU, BABATIMDE ADEWUNMI IDOWU AND OLUBODE A. OKE
Biological Sciences Department, University of Agriculture, P.M.B 2240, Abeokuta, Nigeria
E-mail: kennyademolu@yahoo.com

ABSTRACT

The adult phase of insects' life is primarily for reproduction of young ones that makes continuity of life possible. The influence of reproductive activities like mating and oviposition were investigated in adult males and females variegated grasshopper, *Zonocerus variegatus.* The adult stage was divided into four phases according to activities performed following days of emergence, namely: early somatic phase, late somatic phase, copulation and oviposition. During each phase, the insects were dissected and the somatic tissues (haemolymph, fat body and femoral muscles) were removed analyzed for both organic and inorganic substances. The mean concentration of organic substances (protein, glucose and lipids) and inorganic substances (Na^+, K^+,ca^{2+}, and Cl^-) in both sexes' tissues increased significantly ($p < 0.05$) from early somatic to late somatic phase. However, there was a significant decrease in concentration of the metabolites in the three tissues during copulation in both sexes which further decreased during oviposition in female adult. In contrast to the female, there was increase in the concentration of the metabolites after copulation in the male adult. Copulation and oviposition are activities that exhaust tissues nutrients in adult *Zonocerus variegatus*.

Key Word: copulation, oviposition, tissues, *Zonocerus variegatus,* nutrients

RESUMEN

La fase adulta de vida de los insectos es mayormente para la reproducción de progenies que hace posible la continuidad de la vida. Se investigó la influencia de las actividades reproductivas, como el apareamiento y la oviposición en los machos y hembras adultos del saltamonte variegado, *Zonocerus variegatus*. Se dividió el estadio adulto en las siguientes cuatro fases de acuerdo a las actividades realizadas después de días de emergencia: la fase somática temprano, la fase somática tardio, la cópula y oviposición. Durante cada fase, los insectos fueron disecados y los tejidos somáticos (hemolinfa, la grasa corporal y los músculos femorales) fueron obtenidos y analizados para sustancias orgánicas e inorgánicas. La concentración media de las sustancias orgánicas (proteínas, glucosa y lípidos) y sustancias inorgánicas (Na^+, K^+,Ca^{2+} y Cl^-) en los tejidos de ambos sexos se incrementó significativamente ($p < 0.05$) a partir de la fase somática temprano hasta la fase somática tardio. Sin embargo, hubo una disminución significativa en la concentración de los metabolitos en los tres tejidos durante la cópula en ambos sexos, que disminuyó aún más durante la oviposición en hembras adultas. En contraste con las hembras, hubo un aumento en la concentración de los metabolitos después de la cópula en el macho adulto. La cópula y oviposición son actividades que agota los nutrientes de los tejidos de los adultos de *Zonocerus variegatus*.

The African grasshopper, *Zonocerus variegatus* (L.) is a tropical insect that belongs to Order Orthoptera and Family Pyrgomorphidae. In Nigeria, it usually occurs on uncultivated land with the nymphs and adult stage sharing the same habitat which extends from rain forest zone to the Guinea Savannah in the north (Youdeowei 1974).

The life cycle of *Z. variegatus* is built around 6 nymphal stages (Chapman et al. 1977). Nymphs' development is usually completed in 3-5 mo while the adult life takes 15 wk in the laboratory (Muse 2003) which is enough time for egg maturation, oviposition and remating. Nevertheless, the complexity and size of *Z. variegatus* increases during post embryonic development.

The description and morphometrics of the nymphs of *Z.variegatus* was reported by Chapman et al. (1977). The antennae increased in length with each instar as well as the number of annuli. Ademolu et al. (2009) observed that the weight, length and width of the hind femur increased as the insect increased in age with the adult life recording a significantly higher size and weight compared to lower instars.

Chemical composition of the tissues of *Z. variegatus* was examined by Ademolu et al. (2007), who discovered that the concentration of the organic and inorganic substances in the tissues increased during post embryonic development. Previously Modder (1977) had observed that varia-

tions existed in the concentrations of metabolites of the somatic tissues of the penultimate instar of *Z. variegatus*.

Z. variegatus is a polyphagous insect that causes serious damage to both food and cash crops in West Africa (Toye 1982). However, not all food plants contribute to its growth and development (Tamu 1990). For instance, while *Manihot esculenta* Crantz (cassava leaves) supports the growth, *Chromolaena odorata* (L.) R. M. King & H. E. Robins and *Aspilia africana* C. D. Adams do not. Likewise, in a study by Idowu & Sonde (2004) it was found that the survivability and longevity of nymphs and the reproductive performance of adult *Zonocerus* was enhanced in insects fed on cassava leaves compared to those fed on *Acalypha wilkesiana* Müll. Arg. and *Carica papaya* L.

The alimentary canal system of *Z. variegatus* is divided into 3 parts, i.e., foregut, midgut and hindgut, but major digestion and absorption of nutrients take place at the midgut (Modder 1984). Similarly, the activities of glycosidases, proteinase and lipase increased with age during post-embryonic development (Ademolu & Idowu 2011). In a recent study (Idowu et al. 2009) it was observed that the micro flora present in the gut regions of *Z.variegatus* were able to produce digestive enzymes that assist the insect in neutralizing the toxic effect of cyanogenic glycosides present in its major food plant.

Mating and oviposition are two energy sapping necessary processes that influence the longevity and development of adult insects including *Z. variegatus* (Gibbs et al. 2009; Muse 2003). There has not been any report on the effect of reproductive activities on tissue physiology of *Z. variegatus*. The few existing reports have focused on the effect of reproductive activities on weight and longevity. Thus the thrust of this present study is to examine the influence of reproductive activity on the tissues of both sexes during the adult life.

Materials And Methods

Insect Collection

The penultimate (6th) instar stage of *Z. variegatus* was collected from uncultivated farmland at the University of Agriculture, Abeokuta (UNAAB), Nigeria. They were reared in wire cages (30 × 30 × 45cm) placed at the insectary of the Department of Biological Sciences of UNAAB. They were maintained on fresh leaves of cassava (*M. esculenta)* until they reached adult stage.

Experimental Procedure

As soon as the insects molted into the adult stage, they were separated into different cages. 20 adult insects were placed into each cage (10 males and 10 females). There were 12 cages and a total of 240 insects (120 males and 120 females). The cages were divided into 4 groups with 3 replicates according to the phases of life during the adult stage or days after emergence as follows: early somatic phase (3-5 d); late somatic phase (10-15 d); copulating phase (23-35 d) and oviposition phase (45-58 d) as described by Idowu & Modder (1998). Each cage has 4 special holes containing sand for oviposition by the females.

At each phase, 10 insects (5 males and 5 females) were removed and dissected for tissue collection. Insect samples for early and late somatic phases were retrieved from their cages at d 3 and d 10, respectively, after emergence. Samples for copulation were collected at d 30 after emergence (they were taken after mating for 6 h), while at d 50 (few hours after oviposition) insect samples for oviposition phase were taken.

Tissue Collection and Preparation

Fat body

The insects were dissected as described by Youdeowei (1974) and the fat body was collected following the method of Modder (1984). The fat body from the body wall was removed with forceps and 5 g of the sub-sample was weighed into a test tube using a sensitive weighing balance (Mettler-PM-11-K) and homogenized in 5 mL of distilled water and the homogenate was kept in the freezer for further analysis.

Hemolymph

The hemolymph was collected by the method described by Ademolu et al (2007). A microneedle was inserted into the mid-ventral axis of the thorax and the haemolymph oozing out was collected into a calibrated syringe, and 100 µL of each haemolymph sample was centrifuged at 1300 rpm. for 15 min to spin down the hemocytes and debris. The supernatant was used for further analysis.

Femoral Muscle

The femoral muscles were collected following method described by Ademolu et.al. (2009). The hind femora was opened with a sharp razor and all the femoral muscles were removed by forceps into the Petri dishes, dried to constant weight at 50 °C in an oven for 12 h. 0.5 g of the sub-samples was vortexed in 0.05 M KCl. The homogenate was centrifuged at 500 rpm (5°C) for 30 min. The supernatant obtained was kept in the freezer for further use.

Chemical Analysis

Organic Substances

The protein content of the tissues was determined by the method of Henry et al. (1997), while

the Baunmniger (1994) method was adopted for glucose determination. Lipid assay was done by the method of Grant et al. (1997).

Inorganic Substances

For these analyses, samples were digested with a mixture of per chloric acid and nitric acid (1:2 v/v) and cooled to room temperature. Na^+ and K^+ were determined by flame photometer while Ca^{2+} was determined using an atomic absorption spectrophotometer (Model AA.403). The Henry et. al. (1997) method was used to assess the $PO_4$2- and Cl^- contents of the tissues.

The analyses were done in triplicates and the data were subjected to analysis of variance; and when significance differences occurred, mean separation was done by the Student-Newman-Keul test (SNK).

Results

Hemolymph

The mean concentration of the organic and inorganic substances in the haemolymph of adult females increased significantly ($P < 0.05$) from early somatic phase to the late somatic phase of the insect. However, there was a decrease in concentration during the copulation and oviposition phases (Table 1). In the *Zonocerus* adult males similar observations were recorded, i.e., increased concentration during somatic phases and a significance decrease during the mating phase. However, there was increase in concentration of the metabolites during the post copulatory phase in adult males.

Fat Body

The mean concentration of the metabolites in the fat body of adult females increased from the early somatic phase to late somatic phase, but dropped during copulation and oviposition (Table 2). In adult males, no significant difference occurred in the concentration of the fat body metabolites between the somatic phases on one hand and the copulatoory and post copulatory phases on the other hand.

Femoral Muscle

The changes in concentrations of metabolites in the femoral muscles of adult males and adult females followed same patterns as described above for fat body and haemolymph (Table 3). Comparison of means, however, showed that significantly higher lipid concentrations were recorded in the femoral muscles of both adult female and male *Zonocerus* than in their fat body and haemolymph.

Discussion

Reproductive activities have a significance influence on the physiology of *Z. variegatus* (Muse 2003); and the present study agrees with this finding. During somatic phases of adult *Z. variegatus* concentrations of both organic and inorganic substances increased from early to late somatic phases. Idowu & Modder (1998), likewise, had observed increases in the concentration of haemolymph protein during somatic phase. This increase in metabolite concentrations might be due to the buildup of substances necessary for the sexual activity lying ahead of the insect, because the adult stage is mainly devoted to reproductive processes.

The concentrations of metabolites in tissues in both sexes dropped during mating and also during oviposition in the adult female. During mating, the male releases a spermatophore, which contains nutrients, and this contributes to the male's exhaustion. Mating has been described as an energy sapping exercise that affects the weight and fat-body nutrients of adult *Melontha melontha* (L.) (Leopold 1976). Ofuya et al. (2008), reported that mated males of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) had significantly shorter life spans than unmated males, and, probably, this is due to the energy used by mated male for reproductive activities.

Mating females are assumed to have built up concentrations of metabolite substances preparatory to reproduction, but their non-eating habit and the stress of carrying their partner for hours during copulation might have exhausted or consumed the nutrients gained from the male. Himuro & Fujisak (2010) had earlier reported that mating is costly for both sexes of the seed bug. *Togo hemipterus* (Scott) (Heteroptera; Lygacidae).

Oviposition similarly reduced the concentration of tissue metabolites of *Z. variegatus* adult females. During oviposition weight is reduced and less food is eaten (Mccaffery et al. 1978). Muse (2003) observed that *Zonocerus* females without access to an oviposition substrate (thus could not lay eggs) lived longer than females that oviposite. Mortality was very high after the first oviposition owing to the stress of oviposition activities, which involve the stretching of the abdominal segments (Chapman & Page 1979). The oviposition process, which includes digging the soil is common in *Z. variegatus*. This digging activity is likely be responsible for the decreases in concentrations of tissue metabolites.

In contrast to the female, the concentrations of tissue metabolites increased during the post-copulatory phase in *Z. variegatus* adult males. After mating, while females were busy with egg production and preparation of oviposition sites, the adult males resumed normal feeding and thus replenished nutrients lossed during mating.

TABLE 1. THE CONCENTRATIONS OF METABOLITES IN THE HAEMOLYMPH OF MALE AND FEMALE ADULT *Z. VARIEGATUS* (MG/DL).

	Glucose		Protein		Lipids		Ca^{2}		K^{+}		Po^{4}		Na^{+}		Cl^{-}	
Phases	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Early somatic phase	15.0[b]	13.0[b]	10.5	11.9[ab]	20.2[a]	20.2[a]	7.8	7.4[a]	2.6	2.1	1.7	1.2[b]	17.0[a]	18.0[ab]	11.1	10.3
Late somatic phase	17.0[a]	16.7[a]	11.2	15.3[a]	22.4[a]	22.4[a]	7.9	7.5[a]	2.5	2.5	2.0	3.5[a]	22.3[a]	22.0[a]	12.0	12.0
Copulating phase	13.20[b]	11.3[b]	10.0	13.4[a]	19.3[a]	19.3[a]	5.1	7.5[a]	2.7	2.3	1.6	2.1	13.0[a]	19.0[a]	8.3	11.0
Oviposition/post mating phase	15.20[b]	9.2[b]	11.5	10.5[b]	15.1[b]	15.1[b]	7.5	4.3[b]	2.8	2.2	1.9	2.0[b]	15.0[a]	15.0[b]	8.2	10.0

Mean values in the same column having the same superscript are not significantly different ($P > 0.05$).
M - males; F - females.

TABLE 2. THE CONCENTRATIONS OF METABOLITES IN THE FAT BODY OF MALE AND FEMALE ADULT *Z. VARIEGATUS* (MG/DL).

	Glucose		Protein		Lipids		Ca^{2+}		K^{+}		Po^{4}		Na^{+}		Cl^{-}	
Phases	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Early somatic phase	22.0[ab]	22.1	14.8	16.3[b]	27.0[a]	25.1[a]	6.20	6.9[b]	2.1	1.5	2.0	3.0	30.0	19.0	10.9	10.3
Late somatic phase	24.1[a]	24.7	15.2	20.2[a]	29.0[a]	25.4[a]	6.10	7.6[a]	2.3	1.9	2.0	3.2	32.0	19.0	11.0	12.0
Copulating phase	19.2[b]	20.9	12.3	16.6[b]	22.0[b]	22.7[b]	6.00	6.9[b]	1.7	1.8	1.8	2.9	27.0	16.0	8.2	11.0
Oviposition/post mating phase	20.0[b]	20.0	12.5	14.4c	24.7[b]	20.3[b]	6.00	4.2[c]	2.4	1.8	2.1	2.1	28.0	17.0	10.0	10.2

Mean values in the same column having the same superscript are not significantly different ($P > 0.05$).
M- Males; F- females.

TABLE 3. THE CONCENTRATIONS OF METABOLITES IN THE FEMORAL MUSCLES OF MALE AND FEMALE ADULT *Z. VARIEGATES* (MG/DL).

	Glucose		Protein		Lipids		Ca^{2+}		K^{+}		Po^{4}		Na^{+}		Cl^{-}	
Phases	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Early somatic phase	15.0[b]	14.2[b]	22.7	23.4	35.2[a]	36.6[a]	7.0	9.0[b]	8.3	10.0	11.50[b]	12.7	27.0	27.0	17.0	17.0
Late somatic phase	18.0[b]	18.5[a]	23.1	26.1	35.8[a]	38.2	7.5	14.2[a]	8.3	12.0	13.0[a]	12.6	27.0	27.0	17.0	18.0
Copulating phase	13.0[c]	12.2[c]	20.2	23.2	30.3[b]	36.5	4.2	11.3[b]	6.4	12.0	10.0[b]	11.0	25.0	25.0	15.0	15.0
Oviposition/post mating phase	16.20[b]	12.2[c]	21.0	20.1	33.3[ab]	34.2	7.3	7.6[c]	7.20	10.0	12.0[a]	10.1	22.0	22.0	17.0	14.0

Mean values in the same column having the same superscript are not significantly different ($P > 0.05$).
M- Males; F- females.

In conclusion, copulation and oviposition have remarkable impacts not only on body weight but also on the somatic tissues of *Z. variegatus*.

References Cited

ADEMOLU, K. O., AND IDOWU, A. B. 2011. Activity of digestive enzymes in *Zonocerus variegatus* (Orthoptera: Pygomorphidae) gut homogenates during post-embryonic development. Int. J. Tropical Insect Sci. (in press).

ADEMOLU, K. O., IDOWU, A. B., AND OLATUNDE, G. O. 2009. Morphometrics and enzymes activities in the femoral muscles of variegated grasshopper, *Zonocerus variegatus* (Orthoptera: Pygomorphidae) during post embryonic development. Int. J. Tropical Insect Sci. 29: 53-56.

ADEMOLU, K. O., IDOWU, A. B., AND AMUSAN, A. A. S. 2007. Chemical analysis of tissues of *Zonocerus variegatus* (l) (Orthoptera: Pyrgomorphidae) duringpost embryonic development in Abeokuta, southwestern Nigeria. Nigerian J. Entomol. 24: 24-34.

BAUMNIGER, R. M. 1974. Analytical Biochemistry. Oxford Press, London. pp. 83-85.

CHAPMAN, R. F; COOK, A. G., MITCHELL, G. A., AND PAGE, W. W. 1977. Description and morphometrics of nymphs of *Zonocerus variegatus* (L) (Orthoptera:Acridoidea). Bull. Ent. Res. 67: 427-437.

CHAPMAN, R. F., AND PAGE, W. W. 1979. Factors affecting the mortality of grasshopper, *Zonocerus variegatus* in Southern Nigeria. J. Animal Ecol. 48: 247-270

GIBBS, M., BREUKER, C. J., AND DYCK, H. V. 2009. Flight during oviposition reduces maternal egg provisioning and influences offspring development in *Pararge aegeria* (L). Physiol. Entomol. 35: 29-39

GRANT, G. H. 1987. Fundamentals of Clinical Chemistry WB Saunders Company, USA. pp. 81-83.

HIMURO, C., AND FUJISAKI, K. 2010. Mating experience weakens starvation tolerance in the seed bug *Togo hemipterus* (Hemiptera: Lygaedae). Physiol. Entomol. 35(2): 128-133.

HENRY, R. J., CANON, D. C., AND WINKELMAN, J. W. 1997. Clinical Chemistry. Principles and Techniques. 2nd ed. Harper and Row Publishers, New York. pp. 54-56.

IDOWU, A. B., AND MODDER, W. W. D. 1998. Preliminary chemical analyses of the repellent secretion of the African variegated grasshopper, *Zonocerus variegatus*. Insect Sci. Appl. 18(2): 129-137.

IDOWU, A. B., AND SONDE, O. A. 2004. The contribution of food plants to the growth, development and fecundity of *Zonocerus variegatus* (L). Nigerian J. Entomol. 21: 24-28.

IDOWU, A. B., EDEMA, M. O., AND OYEDEPO, M. T. 2009. Extracellular enzyme production by microflora from the gut region of the variegatus grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorhidae). Int. J. Tropical Insect Sci. 29: 229-235.

LEOPOLD, R. A. 1976. The role of male accessory glands in insect reproduction. Ann. Rev. Entomol. 21: 199-222.

MCCAFFERY, A. R., COOK, A. G., PAGE, W. W., AND PERFECT, T. J. 1978. Utilization of food by *Zonocerus variegatus* (L) (Orthoptera: Pygomorphidae). Bull.Ent.Res. 68:589-606.

MODDER, W. W. D. 1984. Diurnal variation in the feeding and growth activity in nymphs of the African pest, grasshopper *Zonocerus variegates* (L.) Insect Sci. Appl. 5: 527-531.

MODDER, W. W. D. 1977. Changes in somatic tissues in the penultimate instars of the African grasshopper, *Zonocerus variegatus* (L.) (Acridiodea: Pygomorphidae). Acrida 6: 19-32

MUSE, W. A. 2003. Effect of sex, age, starvation and feeding, isolation and crowding and oviposition on longevity of *Zonocerus variegatus* (L.) (Orthoptera: Pygomorphidae). J. Biol. Res. Biotechnol. 1(2): 11-20.

OFUYA, T. L., IDOKO, J. E., AND OLAWALE, B. A. 2008. Influence of male adult size on fecundity and progeny size in *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae). Nigerian J. Entomol.25: 94-97

TAMU, G. 1990. Feeding behavior of the variegated grasshopper *Zonocerus variegatus*. Ph.D. Thesis, University of Ibadan.

TOYE, S. A. 1982. Studies on the biology of the grasshopper pest *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae) in Nigeria. Insect Sci. Appl. 3: 1-7.

YOUDEOWEI, A. 1974. Dissection of the variegated grasshopper, *Zonocerus variegatus* (L.). Nigeria Oxford University Press, Ibadan. pp. 69-73.