Nigerian J. of Ent. (1991), 12, 51 - 60 DOI:10.36108/NJE/1991/21.0160

Activity of Invertase in the Alimentry Canal of the Kola Weevil, *Sophrorhinus* insperatus Faust

C.O. ADEDIRE* AND R.A. BALOGUN

Department of Zoology, Obafemi Awolowo Universty, Ile-Ife, Nigeria. *(Accepted 22 June, 1992)*

ABSTRACT

The nature of alpha-glucosidase in the alimentary canal of the kola weevil, *Sophrorhinus insperatus* Faust has been studied with respect to sucrose. In both larval and adult stages of the weevil, the optimum conditions for invertase activity involve a reaction mixture of pH 6.0 and 1% sucrose at 37°C.Enzymatic activity was consistently higher in the larva than in the adult, indicating that the larva is the more destructive stage of the weevil.

Extracts of the nuts of *Cola nitida* and *C. acuminata* inhibited invertase activity to varying degrees *in vitro*, thus suggesting the presence of naturally occurring inhibitors in the two kola varieties. The possible roles of these inhibitors in plant resistance mechanisms are discussed.

INTRODUCTION

The kola nut weevils, notably *Balanogastris kolae Desbr.* and members of the genus *Sophrorhinus* are the major insects responsible for heavy post-harvest losses and quality deterioration of kola nuts in the kola groves of West and Central Africa. *Sophrorhinus insperatus* Faust, is a monophagous principal storage pest of *Cola nitida* (Vent) Schott and Endl. and *C. acuminata* (Pal de Beauv) Schott and Endl. in the tropics. The feeding and developmental activities of these weevils coupled with fouling of the nuts by their excrements often render the nuts unift for human consumption and of little industrial value (Gerard, 1967; Daramola, 1947a). Their nutritional value is also presumed to be lowered.

Attempts have been made to control these weevils both in the field and at storage but very little success has been achieved. The growth habit of the kola trees, such as their great height and dense foliage coupled with the irregular flowering and fruiting patterns, are some of the factors which have hindered field control of the weevils (Daramola, 1974a; Ivbijaro, 1978). Thus,

*Present Address: Department of Biology, Federal University of Technology, P.M.B. 704, Akure, Nigeria

measures aimed at the control of the pests have been restricted to the stores. Alibert and Mallamaire (1955) reported a successful control of the weevils with a fumigant, methylbromide, under reduced pressure. Fumigation of infested nuts with 3g of aluminium phosphide (Phostoxin R) for about 15 hours has been reported to have successfully controlled all stages of *B. kolae* and *S. insperatus* without affecting the germination potential of the fumigated nuts (Gerard, 1967). Irradiation of infested kola nuts with 8 - 15 Krads of Cobalt 60 gave an effective control of the kola weevils, *B. kolae* and *S. insperatus* without any detectable physical damage to the nuts (Daramola, 1974b). Ojo (1969) and Ivbijaro (1977) reported that 0.03% and 0.06% of gamma - BHC (Gammalin 20) gave effective control of the weevils. Daramola (1983) also recommended the use of 0.03% Unden 20, a carbamate of low mammalian toxicity, for the control of the adult and immature stages of the kola weevils. However, the possible physiological effects of ionising radiation and bio-accumulated insecticide residues in treated nuts on the consumers are not yet fully understood.

It is against this background that the present study was designed to determine the optimal conditions for invertase activity in the alimentary canal of *S. insperatus* and to investigate the possible roles of naturally occurring invertase inhibitors in plant resistance.

MATERIALS AND METHODS

Insect rearing: The adult kola weevils for the establishment of the insect colonies used in this study were initially obtained from field infested nuts of *C. nitida* which were kept until emergence in hand-woven baskets lined with banana leaves. The raising and maintainance of insect colonies and the preparation of enzyme extract were done as described in detail elsewhere by Adedire (1990). Biochemical assays were carried out on both larvae and adult insects as follows.

Determination of invertase activity

The activity of invertase (sucrase)in the enzyme extract was measured with the alkaline 3, 5 - dinitrosalicyclic acid (DNSA) procedure of Noelting and Bernfeld (1948) as described by Ishaaya and Plaut (1974) Except, where otherwise stated, each reaction mixture consisted of 0.4ml of 1% sucrose, 0.2ml of 100mM phosphate buffer, pH 6.0 and 0.2ml of the enzyme extract. The mixture was incubated for 60 minutes at 37^{o}C and the reaction was terminated by the addition of 1.6ml of the alkaline 3, 5-dinitrosalicylic acid reagent. The colour was developed by heating the reaction mixtures at 100^{o}C for 5 minutes. Samples were diluted with 1.6ml of distilled water and the absorbance read at 550mm in a CE 343 single sample spectrophotometer.

Determination of optimal conditions for invertase activity

The optimum conditions for invertase activity such as reaction velocity (incubation

period), pH, enzyme and substrate concentrations, were estimated in a series of preliminary investigations in which individual factors were varied while others were kept constant.

The pH - dependent activity curves for invertase were determined for a pH-range of 3.0 to 8.0 using 100mM of the following buffers: potassium hydrogen - phthalate, pH 3.0; acetate, pH 4.0 - 5.5; and phosphate, pH 6.0 - 8.0

The velocity curves for invertase in the gut extract of the larva and adult weevil were determined in reaction mixtures that contain 0.4ml 1% sucrose, 0.2ml phosphate buffer (pH 6.0) and 0.2ml of enzyme extract. Duplicate aliquots from two groups of enzyme preparations incubated at 37°C were removed at intervals of fifteen minutes and assayed for the products of hydrolysis as described earlier.

The temperature - activity curves of the enzyme in the gut extracts of both larva and adult weevil were determined over a range of 20°C to 55°C at 5°C increments. The effect of substrate concentration on the reaction velocity was tested in serially diluted solutions ranging from 2.5 to 20mg/ml sucrose.

Effect of kola nut extracts on invertase activity

Kola nut extract was prepared by homogenizing 1.0g of either *C. nitida* or *C. acuminata* in 100ml of phosphate buffer, pH 6.0. The extract was heated for 10 minutes at 70°C as described by Baker (1983). Each reaction tube contained 0.2ml of the buffered extract and 0.2ml of enzyme solution. The mixture was pre-incubated for 30 minutes at 37°C prior to the addition of 0.4ml of 1% sucrose solution. Then, the reaction mixture was later re-incubated at 37°C for one hour and the glucose produced was determined with DNSA reagent. The buffer for the control did not contain any kola nut extract. The spectrophotometer was adjusted to zero by a reaction mixture containing denatured enzyme extract.

RESULTS

Optimal conditions for invertase activity:

(a) *Effects of incubation period and enzyme concentration on invertase activity*

The relationship obtained between incubation period and invertase activity was linear over a period of 120 minutes (Figure 1) for both larval and adult gut homogenates. A reaction time of 60 minutes which fell on the linear section of the activity curve was chosen for subsequent experiments. A correlation coefficient (r) of 0.99 was obtained for both larval and adult invertase. The curves for the rate of sucrose digestion as a function of enzyme concentration are shown in Figure 2. There is a linear relationship between concentration of the enzymes and the reaction velocity up to 12 guts/ml of buffer in both larval and adult weevils.

(b) *Effects of pH and substrate concentration on invertase activity*

The pH-profiles for the invertase from the larva and adult kola weevil are presented in Figure 3. Invertase activity was

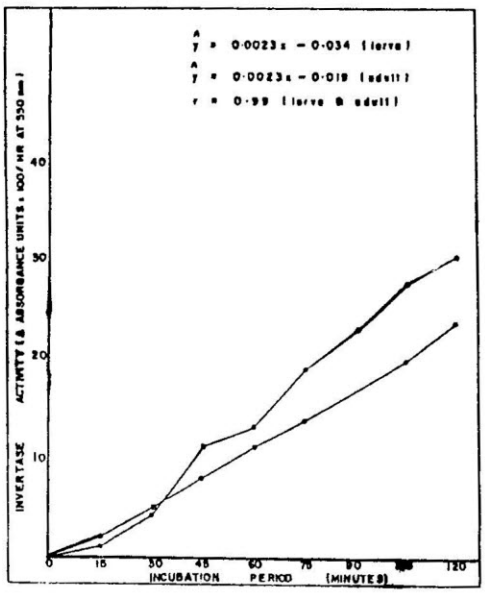

Fig. 1: Effect of incubation period on invertase activity of the gut homogenate of Sophrorhinus insperatus. (●—●) larva; (o—o) adult

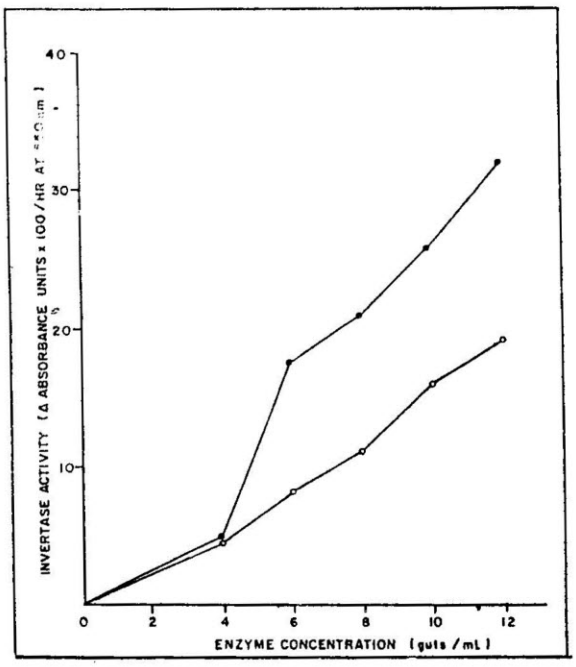

Fig. 2: Effect of enzyme concentration on invertase activity of Sophrorhinus insperatus (●—●) larva; (o—o) adult Each point is a mean of duplicate analysis of two separate whole gut preparations.

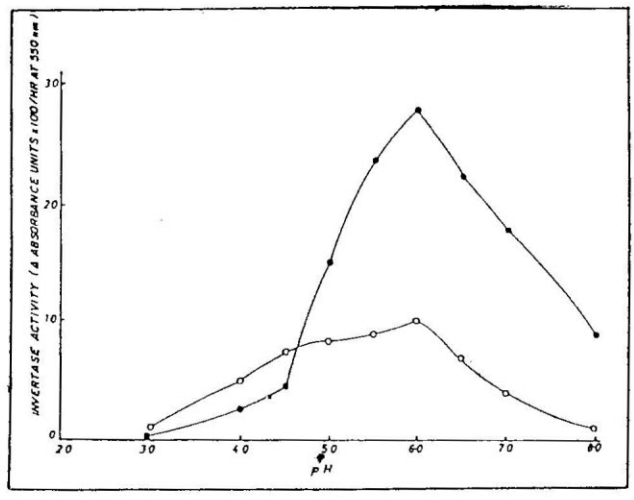

Fig.3: PH activity curves for sucrose hydrolysis by the kola weevil (S. insperatus) gut homogenates (●—●) larva (o—o) adult Each point is the mean of duplicate analyses of two separate whole gut preparations.

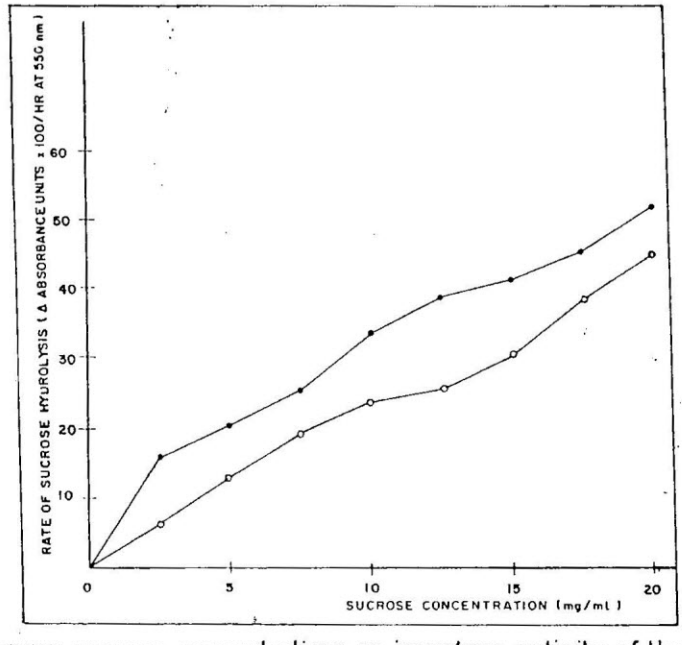

Fig.4: Effect of varying sucrose concentrations on invertase activity of the kola weevil (S. insperatus) gut homogenate (●—●) larva (o—o) adult. Each point is a mean of duplicate analyses of two separate whole gut preparations.

obtained over a broad pH range of 3.0 and 8.0. Considerable enzymatic activity occurred between pH 4.5 and 6.5 with a peak at pH 6.0 in both larvae and adults. In both stages of the weevil, sucrose hydrolysis increased with concentration up to 20mg/ml sucrose. The lineweaver - Burk plot (Figure 5) gave apparent km values of 9.09 and 52.63mg/ml for larval and adult weevils, respectively.

(c) *Effects of temperature on invertase activity*

Invertase activity was obtained between 20°C and 55°C with maximum activity at 45°C (Fig. 6). Activity was quite high between 30°C and 45°C in both stages although adult invertase activity was consistently lower than that of the larva.

(d) *Effect of kolanut extracts on invertase activity*

Incubation of the enzyme with the two different kola nut extracts decreased larval invertase activity (Table 1). The degree of enzyme inhibition was dosage dependent. The extracts of *Cola acuminata* inhibited invertase activity more than extracts of *C. nitida* in the larva.

DISCUSSION

In the present studies, enzymatic activity was consistently higher in the larva than adult weevils, thus suggesting that the larval stage is the more voracious feeder and destructive stage of the weevil. In *S. insperatus*, a digestion period of sixty minutes fitted well within the linear phase of the enzymic activity curve. Similarly, Ishaaya and Swirski (1970) reported a reaction period of 60 minutes as suitable for both Florida and California red scales. Ishaaya and Swirski (1976) reported a digestion period of 60 minutes as suitable for invertase activity in *Saissetia oleae.*

Invertase is very active in *S. insperatus* probably because of the large quantity of sucrose in the normal diet of the weevil. The optimum activity in both larva and adult *Sophrorhinus* occurred at pH 6.0. In the almond wasp, invertase optimum activity occurs at pH 5.6 (Ishaaya and Plaut, 1974) while in *Hodotermes mossambicus*, maximum activity of this enzyme is obtained at pH 6.0 (Retief and Hewitt, 1973). Evans and Payne (1964) also reported pH 6.0 for maximum invertase activity in the gut of *Schistocerca gregaria* L. However, pH 5.5 has been found to be the optimum for the invertases in *S. oleae* (Ishaaya and Swirski, 1976). It is obvious from the above reports that the optimum pH for *Sophrophinus* invertase is not very different from those of other insect species.

The temperature response curve obtained for invertase activity in the kola weevil showed maximum activity at 45°C for both larval and adult stages of the weevil. The apparently high optimum temperature obtained for *Sophrorhinus* invertase is in agreement with the data reported (Evans and Payne, 1964, Retief and Hewitt, 1973) for most other insects inhabiting tropical environments. Dixon and Webb (1965) remarked that optimum temperature cannot be regarded as a characteristic property of

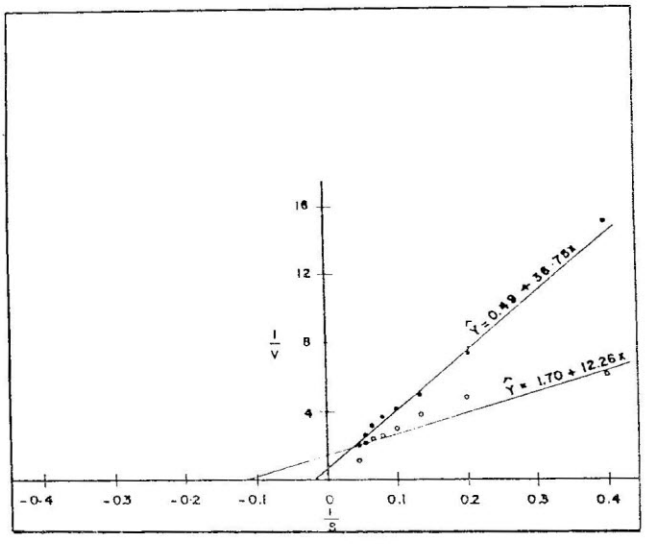

Fig. 5: Lineweaver-Burk plots for the hydrolysis of sucrose by invertase from larva(o) and adult (●) of S. insperatus

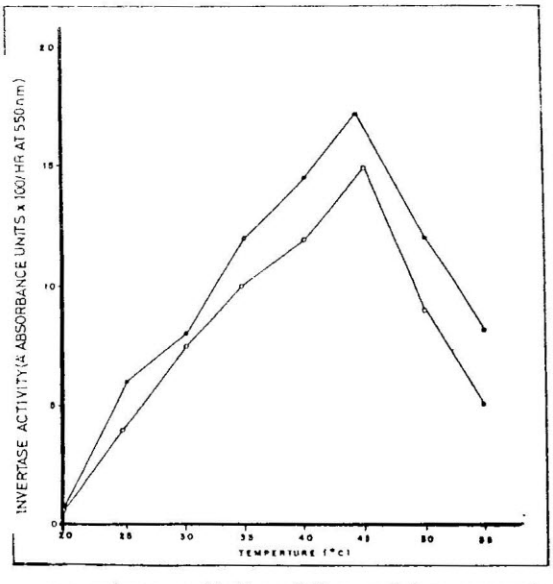

Fig.6: Effect of temperature on invertase activity of the gut homogenate of Sophrorhinus insperatus (●—●) larva;(o—o) adult. Each point is a mean of duplicate analyses of two separate whole gut preparations.

Table 1: Effect of varying concentrations of *Cola nitida* and *C. acuminata* extracts on the activity of invertase in the larvae of *Sophrorhinus insperatus.*

Extract concentration (mg/ml)	Enzyme activity (O.D. *+ S.E)	
	C. nitida	*C. acuminata*
0.00	0.386±0.0043	0.714±0.0152
1.00	0.360±0.0040	0.681±0.0188
2.00	0.334±0.0328	0.640±0.1826
3.00	0.306±0.0290	0.579±0.0166
4.00	0.263±0.0298	0.545±0.0131

*t = 12.40; significant at $p < 0.05$

Enzyme activity in *C. acuminata* extract was significantly higher than in *C. nitida.*

an enzyme, since it is influenced to a large extent by the purity of the enzyme, pH, incubation time and substrate concentration. However, an optimum temperature of 45°C has been obtained for invertase activity in the soldiers of H. *mossambicus* and 40°C for the larvae and workers (Retief and Hewitt, 1973) which agrees with the present finding. An optimum temperature of 50°C has also been reported for alpha-glucosidase of *S. gregaria* by Evans and Payne (1964).

The km values of 9.09 and 52.63mg/ml obtained for the invertase activity in the gut of *S. insperatus* are considered fairly high compared with those obtained for amlyase (Adedire, unpublished data). Retief and Hewitt (1973) also reported high km values for alpha-glucosidase activity in the various stages of termite and this was attributed to high concentration of carbohydrates in the gut and natural inhibitors. Similar factors may be responsible for the high km values of invertase in *S. insperatus.* This study revealed that water extracts of *C. acuminata* inhibited invertase activity more than that of *C. nitida* probably suggesting the observed high suceptibility of the latter to weevil attack on the field (Adedire, unpublished data). The occurrence of naturally occurring inhibitors in many host plants of insects have long been observed (Applebaum and Konijn, 1965; Ishaaya and Swirski, 1976; Gatehouse *et al.*, 1979). Previous studies by Ascher and Ishaaya (1973) and Ishaaya and Casida (1975) suggest that insect digestive enzymes could be used as an index for determining antifeeding activities while Ishaaya and Meisner (1973) opined that it could be used as a parameter for

phagostimulation. Although the role of digestive enzymes in plant resistance is still debatable, the results obtained from this study indicate that invertase activity could be taken as an index of the adaptability of the weevil to the host plant.

REFERENCES

ADEDIRE, C.O. 1990 Proteolytic activity in the gut homogenate of the kola weevil, *Sophrorhinus insperatus* Faust. Entomon (In Press).

ALIBERT, H. et MALLAMAIRE, A. 1955 Les characons de la noix de cola en Afrique; Moyens de le combattre. *Bull. Prof. Veg. Gouv. Gen Occ Frac. Dir. Gen. Serv Econ Insp. Gen. Agric. France* 29: 69-88.

APPLEBAUM, S.W. AND KONIJN A.M. 1965 The utilization of starch by larvae of the flour bettle, *Tribolium ocastaneum. Journal of Nutrition* 85; 275-282.

ASCHER, K.R.S. and ISHAAYA I. 1973 Antifeeding and protease and amylase inhibiting activity of fentin acetate in *Spodoptera littoralis larvae. Pestic. Biochem. Physiol.* 3: 326-336.

BAKER, J.E. 1983 Properties of amylases from midguts of larvae of *Sitophilus zeamis* and *Sitophilus granarius.* Insect *Biochem.* New York, 13940; 421-428.

DARAMOLA, A.M. 1974a A review on the pests of Cola species in West Africa. *Nigeria J. Entomol.* 1(1): 21-29.

DARAMOLA, A.M. 1974b Preliminary studies on the control of kola weevils by gamma radiation in Nigeria. I*SNA Newsletter* 3(2): 23 - 26.

DARAMOLA, A.M. 1983 Studies on the control of kola nut weevils (*Balanogestris kolae* and *Sophrorhinus spp.* (Coleoptera: Curculionidae) during storage in south-western Nigeria. *Tropical stored Prod. infor.* 46: 11-16.

DIXON, M. and WEBB, E.C. 1965 *Enzymes.* 2nd ed. Longmans. London.

EVANS, W.A.L. and PAYNE, D.W. 1964 Carbohydrases of the alimentary tract of desert locust, *Schistocerca gregaria* Forsk. *J. Insect Physiol.* 10: 657-674.

GATEHOUSE, A.M.R., GATEHOUSE, J.A., DOBIE, P., KILMINSTER, A.M. and BOULTVER, D. 1979 Biochemical basis of insect resistance in *Vigna unguiculata.J. Sc. Food Agric* **30:** 948-958.

GERARD, B.M. 1967 The control of *Balanogastris kolae* (Desbr.) and *Sophrorhinus insperatus* (Fst) Coleoptera: Curculionidae, in small samples of stored kola nuts using phosphine. *Bull. ent. Soc. Nigeria* **1:** 433-48.

ISHAAYA, I. and CASIDA, J.E. 1975 Phenyltin compounds inhibit digestive enzymews of *Tribolium confusum* larvae. *Pestic. Biochem. Physiol.* **5**: 350-358

ISHAAYA, I. and PLAUT, H.N. 1974 Digestive enzymes in *Eurytoma amygdali* and their relation to food digestion and to the boring processes of emergence holes in almond fruits. *Comp. Biochem. Physiol.* 48A: 37-44

ISHAAYA, I. and SWIRSKI, E. 1970 Invertase and amylase activity in the armoured scales *Chrysomphalus aonidum* and *Aonidiella auranti. J. Insect Phsysiol.* **16:** 1599 - 1606.

ISHAAYA, I and SWIRSKI, E. 1976 Trehalase, invertase and amylase activities in the black scales *Saissetia oleae* and their relation to host adaptability. *J. Insect Physiol.* **22:** 1025-1029.

ISHAAYA, I. and MEISNER, J. 1973 Physiological effect of sugars on various digestive enzymes of *Spodoptera littoralis* larvae. *J. Comp. Physiol.* 86: 117 - 124.

IVBIJARO, M.F. 1977 Gamma - BHC residues in kola nuts, *Cola nitida* and control of kola nut weevil, *Balanogastris kolae* (Desbr..). *Indian J. exp. Biol.* **15:** 1236-1238.

IVBIJARO, M.F. 1978 The susceptibilityof the immature and adult stages of the kola nut weevil, *Balanogastris kolae* (Desbr.) (Coleoptera; Curculionidae) to Phosphine. *Nigerian J. Entomol.* 1(3): 53-56.

NOELTING, G. and BERNFELD, P. 1948 Sur les enzymes amylolytiques - II La B - amylase: Dosage d'ativite et control de l'absence d- - amylase. *Helv. Chim. acta*. **31**: 286-290.

RETIEF, L.W. and HEWITT, P.H. 1973 Digestive carbohydrases of the harvester termite, *Hodotermes mossambicus* - glucosidase. *J. Insect Physiol.* **19:** 105-113.