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The Performance of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) in Chitin Containing Artificial Seeds

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Desempenho de *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) em Sementes Artificiais Contendo Quitina

RESUMO - O efeito da adição de quitina sobre o desenvolvimento e a sobrevivência do bruquídeo *Callosobruchus maculatus* (F.) foi estudado empregando-se um sistema de sementes artificiais feitas com farinha de *Vigna unguiculata* (feijão-de-corda). O polissacarídeo interferiu no desenvolvimento do inseto causando uma diminuição na taxa de sobrevivência. O índice de Howe mostra que as dietas contendo quitina são nutricionalmente inadequadas.

PALAVRAS-CHAVE: Insecta, *Vigna unguiculata*, vicilinas, globulinas 7S.

The resistance of some Nigerian lines of cowpea (*Vigna unguiculata*) seeds to the bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) was found to be due to the presence of variant vicilins (7S storage proteins) in these seeds (Macedo *et al.* 1993). The mechanisms of resistance seem to involve lower rates of hydrolysis in the insect's midgut (Sales *et al.* 1992) coupled with a higher capacity of binding to chitin in midgut structures as compared to vicilins from susceptible seeds (Sales *et al.* 1996, Firmino *et al.* 1996). Proteins that bind to chitin, a β-1,4-linked biopolymer of N-acetyl-D-glucosamine, affect the growth of organisms that contain it either in their cell wall, like fungi or in structures of the digestive tract as in insects (Raikhel & Lee 1993).

In this note we report on the performance of *C. maculatus* in artificial seeds (Macedo *et al.* 1993) composed of meal of susceptible cowpea seeds containing chitin in increasing concentrations. Two different assays were employed and in both we utilized seeds (400 mg) prepared with the finely ground cowpea (EPACE-10) seed meal containing chitin at 0.0, 0.5, 1.0 and 2.0% final concentrations. The infestation of the artificial seeds was accomplished by placing 2-d-old gravid females in glass vials containing three seeds for 24 hours at 28º C, 80 % RH. The excess eggs laid were removed from the seeds leaving 3 eggs/seed. After 20 d infested seeds were opened and the weight and number of larvae

were counted. Dose-response curves were drawn and the half-weight-dose (WD_{50}) was calculated. Control artificial seeds were made with the meal of cowpea seeds (EPACE-10). All the experiments were run in triplicate and the mean (± SEM) was calculated.

In an alternate experiment after infestation, the artificial seeds were offered to insects in glass tubes (20 x 2 cm) covered with cotton plugs and placed in a growth chamber (28º C and 80 % RH). The number of surviving insects was recorded until 40 d, and the percentage survival to adult emergence (S) was calculated; the days adults emerged were also recorded to estimate the mean developmental period (T). In this test the number of replicates was nine and the mean (± SEM) was calculated. We also calculated the Howe's Index which is a measure of the suitability of a diet (Howe 1971).

A low concentration of chitin (0.42 %) in the seeds was capable of lowering the average mass of the larvae at 20 d but this had no effect on larvae survival (LD_{50} > 2 %). In the 40-d experiment with increasing chitin content in the artificial seeds, the developmental time did not increase with increasing chitin concentration, but the survival rate decreased significantly (Table 1). This seems to indicate that after an initial period (20 d), the effect of chitin is more pronounced on survivorship suggesting that some larvae do not survive to the exposure of the diet containing chitin, although their developmental time was not affected. The Howe's index decreased significantly as shown by the high negative correlation coefficient (r = - 0.935), reinforcing the idea that chitin was detrimental to the development of *C. maculatus* when incorporated in the diet. This effect may be due to vicilins from cowpea (and other leguminous seeds), which are strongly associated with chitin as a matrix in chromatographic experiments (Sales *et al.* 1996), in bruchid midguts (Firmino *et al.* 1996); filamentous fungi (Gomes *et al.* 1997); and yeast cells (V. M. Gomes *et al.*, unpublished).

Table 1. Performance of *Callosobruchus maculatus* fed on artificial seeds containing increasing amounts of chitin.

Concentration of Chitin (%)	Developmental Time (days)[1](X ± SEM)	Survival (%)[1] (X ±SEM)	Log $S \div T$ (Howe's index)
0	31.7 ± 2.9 a	93.8 ± 9.8 a	0.062
0.5	33.8 ± 1.8 a	91.3 ±17.4 a,b	0.058
1	33.0 ± 1.6 a	85.1 ± 17.6 b	0.058
2	34.1 ± 2.5 a	66.7 ± 29.9 c	0.053

[1]Values followed by the same letter in a column are not significantly different (Duncan's multiple range test, $P \leq 0.05$). Howe's index was analysed by correlation analysis. A highly significant correlation ($r = -0.935$; $P \leq 0.05$) was found between the indices and the chitin concentration; $n = 9$.

Literature Cited

Firmino, F., K. V. S. Fernandes, M. P. Sales, V. M. Gomes, M. R. M. Alcantara, S. J. S. Domingues & J. Xavier-Filho. 1996. Cowpea (*Vigna unguiculata*) vicilins associate with putative chitinous structures in midgut and feces of the bruchid beetles *Callosobruchus maculatus* and *Zabrotes subfasciatus.*

Braz. J. Med. Biol. Res. 29: 749-756.

Gomes, V. M., Mosqueda, M. I., Blanco-Labra, A., Sales, M. P., Fernandes, K. V. S., Cordeiro, R. A. & Xavier-Filho., J. 1997. Vicilin storage proteins from *Vigna unguiculata* (legume) seeds inhibit fungal development. J. Agric. Food Chem. 45: 4110-4115.

Howe, R. W. 1971. A parameter for expressing the suitability of an environment for insect development. J. Stored Prod. Res. 7: 63-65.

Macedo, M. L. R., L. B. S. Andrade, R. A. Moraes & J. Xavier-Filho. 1993. Vicilin variants and the resistance of cowpea (*Vigna unguiculata*) seeds to the cowpea weevil (*Callosobruchus maculatus*). Comp. Biochem. Physiol. 105C: 89-94.

Raikhel, N. V. & H. I. Lee. 1993. Structure and functions of chitin-binding proteins. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 591-615.

Sales, M. P., M. L. R. Macedo & J. Xavier-Filho. 1992. Digestibility of cowpea (*Vigna unguiculata*) vicilins by pepsin, papain and bruchid midgut proteinases. Comp. Biochem. Physiol. 103B: 945-950.

Sales, M. P., V. M. Gomes, K. V. S. Fernandes & J. Xavier-Filho. 1996. Chitin binding proteins from cowpea (*Vigna unguiculata*) seeds. Braz. J. Med. Biol. Res. 29: 319-326.

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