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THE RESISTANCE OF SEEDS OF COWPEA *(VIGNA UNGUICULATA)* TO THE COWPEA WEEVIL *(CALLOSOBRUCHUS MACULATUS)*

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Cowpea (Vigna unguiculata) *seeds are heavily damaged during storage by the bruchid* Callosobruchus maculatus. *Seeds of some Nigerian varieties showed a strong resistance to this bruchid. By utilizing biochemical and entomological techniques we were able to rule out the participation of proteolytic enzyme (trypsin, chymotrypsin, subtilisin and papain) inhibitors, lectins, and tannins in the resistance mechanisms. Fractionation of the seed meal of a resistant variety suggests that the factor(s) responsible for the effect is (are) concentrated in the globulin fraction.*

Key words: cowpea seeds – *Vigna unguiculata* – resistance – *Callosobruchus maculatus* – bruchids

Cowpea *(Vigna unguiculata)* is a legume whose seeds are largely consumed by low-income populations in Northeastern Brazil (May et al., 1988). It is a culture which was originated in tropical and sub-tropical regions of the African continent and at present is distributed in similar regions all over the World (Ng & Marechal, 1985). The cowpea is one of the most important cultures of the Brazilian Northeast and is cultivated mainly by small farmers or as a subsistence crop (May et al., 1988).

The cowpea culture is attacked by a number of disease agents and several pests both in the field and during storage (Watt & Araujo, 1988). One of the main pests of the stored seeds is the bruchid beetle, known as the cowpea weevil, *Callosobruchus maculatus*. The damage caused by this insect can reach up to 70% of the grains produced in a given region if the necessary measures are not taken to check the attack. The damaged seeds are unsuitable for human or animal consumption and they can not be used for planting (Watt & Araujo, 1988).

Recently three varieties of cowpea that showed resistance to the cowpea weevil were discovered in Nigeria (Singh et al., 1985). In the case of one of these, TVu 2027, the resistance was thought to be due to the high levels of trypsin inhibitors found in these seeds (Gatehouse & Boulter, 1983). This idea seems to have been based on the old assumption that seed eating bruchids have trypsin like enzymes (serine proteinases) in their digestive tract (Applebaum, 1964).

When working with susceptible and resistant varieties and cultivars of cowpea it was shown, in the author's laboratory, that there was no correlation whatsoever between the levels of inhibitors of the serine proteinases trypsin, chymotrypsin, and subtilisin or the cysteine proteinase papain and the resistance/susceptibility to predation by the cowpea weevil (Xavier-Filho et al., 1989). It was also shown that the levels of lectin (hemagglutinating activity) found in the seeds studied did not also correlate with the resistance/susceptibility; this was the same with the levels of tannins (Xavier-Filho et al., 1989). Both lectins and tannins have been suggested as protective agents in some plants and seeds (Swain, 1979., Janzen et al., 1986).

Since none of the compounds tested did not correlate with the resistance/susceptibility we decided to fractionate the whole seed meal of one of the resistant cultivars (IT81D-1045) and incorporate the resulting fractions into artificial seeds. The fractionation was done by suspending the seed meal in water adjusted to pH 7.5, collecting the supernatant and dialysing against water to obtain albumins and globulins;

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the small molecular weight fraction was recovered from the dialysate by freeze drying; the residue of the extraction was also freeze dried. The artificial seeds were prepared by utilising gelatin capsules filled with the mixture to be tested. The fractions obtained, albumins, globulins, residue and the small molecular weight fraction, were mixed with the meal of a susceptible cultivar and pressed into gelatin capsules; these were offered to *C. maculatus* females for oviposition. The globulin fraction was the only one that did not promote larval development.

Although the above results are preliminary ones it is worthwhile to speculate about the reasons seed globulins of the resistant cowpea seeds seem to be detrimental to the cowpea weevil larval development.

The reserve proteins of cowpea are mainly of the vicilin type (Carasco et al., 1978). The vicilin molecule is a trimer formed by three different polypeptide chains of ca. 50 kD each (Derbyshire et al., 1976). They are deposited in specialized structures in cotyledonary cells known as the protein bodies. The genes coding for the synthesis of the three polypeptide chains are grouped into three families corresponding to each chain; each of these families is formed by six different genes (Higgins, 1984). This suggests a great potential for diversity of the individual polypeptide chains.

When we submitted crude seed meal extracts of one susceptible (CE-31, pitiuba) and one resistant cowpea cultivar (IT81D-1045) to hydroxyapatite chromatography we obtained profiles that suggest a more acidic character for the vicilin from the resistant cultivar as compared to that from the susceptible one. When both vicilin fractions were submitted to isoelectric focusing in thin layers of polyacrylamide gels the higher relative acidity of the vicilin from the resistant cultivar was clearly shown.

We have recently published results showing that another seed eating bruchid, *Zabrotes subfasciatus*, that can also infest cowpea seeds, survive in *C. maculatus* resistant cultivars (Xavier-Filho et al., 1989). We have also found that both bruchids rely on aspartic and cysteine classes proteinases for protein digestion (Campos et al., 1989; Lemos et al., 1990). Measurements of the levels of both type of proteinases in the larvae of these bruchids indicate that *Z. subfasciatus* has two times more cysteine proteinases and four times more aspartic proteinases than the larvae of *C. maculatus*.

In the light of the above results we advance the suggestion that the reason why some cowpea seeds are detrimental to the development of *C. maculatus* larvae lies in an inadequate provision of proteolytic enzymes to digest the modified forms of vicilin found in the resistant seeds. Experiments leading to an answer to this question are being carried out in the laboratory of the author.

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