Modification of the nutritional quality of nitrogen content of Leguminosae seed damaged by *Acanthoscelides obtectus* (Say), Coleoptera, Bruchidae

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Abstract

The nutritional quality of Leguminosae grain of worldwide economic importance (*Phaseolus vulgaris, Vigna unguiculata, Cicer arietinum* and *Vicia faba*) was studied. Studies related to the intensity of the attacks of a bruchid, *Acanthoscelides obtectus* (Say) (Coleoptera) an insect that can damage the seeds of its host plant (*P. vulgaris*) as well as of non-host plants.

The study involved the determination of total nitrogen by Kjeldahl method, total protein and uric acid. The nitrogen content of the grain was considerably altered. Total protein decreased whilst uric acid greatly increased, reducing the nutritional value of the grain with respect to nitrogen uptake. It appears that the estimation of crude protein by the determination of total nitrogen, generally considered to be a rapid and convenient to estimate the protein content of a food source, is not appropriate in the case of stored products damaged by pests.

Introduction

Leguminosae still play an important role in the diet of the populations in developing countries as they provide proteins and glucides in a form easily carried and used without complicated transformation. However, the crops are often attacked by insect and rodent pests: more than 40% of stored products are reported damaged in tropical areas (Huignard 1985). Earlier studies have determined the nutritional quality of the weakened seeds (Singh et al. 1982). In the study reported have we examined the nitrogen content of Leguminosae seeds infested by *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae).

A. obtectus is widespread all over the world because of its physiological adaptability. Oviposition and growth are continuous (without imaginal diapause) between 14° and 35°C (i.e. climates of tropical and temperate countries) and its development has been recorded in the field, in cultures and in stored seeds. The larvae develop within the seeds and this is the reason *A. obtectus* is one of the beetles least manageable by classical pesticides (Labeyrie 1962; 1977). Traditionally, farmers store their seed in lofts. The seeds contain developing larvae. The adults emerge, lay eggs to produce another generation and some of them, if the loft is not well sealed, escape to infest fresh beans in the fields (Jarry 1987). Moreover, this bruchid can live without food for 16 days at 23–25°C and for 42 days at 19–21°C. These facts perhaps explain why *A. obtectus* is a widespread cosmopolitan beetle. The host-plant of this insect is *Phaseolus vulgaris* but it has been recently observed that it can adapt to non-host plants as *Vicia faba*, *Vigna unguiculata* or *Cicer arietinum* (Hamraoui and Regnault-Roger, unpublished data). All these pulses play an important economic role in developing countries.

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Material and Methods

Biologicals and botanicals

The beetles, *Acanthoscelides obtectus* Say, and botanicals (*Phaseolus vulgaris, Vicia faba, Cicer arietinum* and *Vigna unguiculata*) were reared in our Institute. After the crops, the beetle was introduced into the stored seed stocks.

Bioassay

After 6 weeks, the seeds were separated into several groups according to their degree of infestation. The first level of infestation presented 1 to 4 holes in the seeds (i.e. imagos which emerged), the second level 5 to 9, the third level 10 to 14, and the fourth level 15 to 20. After this level, only dust could be collected. For *Cicer arietinum* and *Vigna unguiculata*, levels only up to 3 and 2, respectively, could be gathered as the size of the seeds was smaller. A control with sound seeds was included in the experimental series.

The following analyses were conducted on the different samples: dry weight, total nitrogen by Kjeldalh method, proteins by Biorad protocol according to Bradford (1976), uric acid by Biomérieux protocol according to Barham and Trinter (1972). Larvae and imagos within the seeds were separated from them before the analyses. Three replications were done for each series.

Statistical studies

Statistical studies were performed on a one factor variance analysis: Anova test (Sokal and Rohlf 1981) followed by a rank of the average by Newman-Keuls (N.K.) test and a t-test [STAT-ITCF computer program (Roux 1985)].

Results and Discussion

Water and volatile percentages varied between 10 and 13.5%. This parameter was not affected by the degree of seed attacks. The content of total nitrogen increased with the level of infestation, as did uric acid concentrations, whilst the protein content decreased. Variance analyses gave significant differences between the steps of infestation for all the Leguminosae species and N.K. test classified each step in one group apart.

For the same level of infestation a nearly equal concentration of uric acid, protein and total nitrogen could be observed in all the seeds (Table 1). The beetle developed in the same way in host and non-host plants and fed similarly on the nutritional components in each species. Proteins were consumed for half of them and uric acid concentrations increased five

Table 1. Nitrogen evaluation of Leguminosae damaged by *Acanthoscelides obtectus* at different steps.

Leguminosae	Infestation degree	%DW	Total nitrogen g% DW ± SD	Protein N g% DW ± SD	Uric acid N g% DW ± SD
Phaseoleus vulgaris	1	87.6	4.43 ± 0.05	4.06 ± 0.15	0.05 ± 0.009
	2	86.5	4.88 ± 0.08	3.68 ± 0.18	0.10 ± 0.02
	3	87.8	6.02 ± 0.13	2.74 ± 0.22	0. 165 ± 0.03
	4	87.5	7.92 ± 0.10	1.95 ± 0.10	0.25 ± 0.02
	5	90	8.69 ± 0.33	1.75 ± 0.12	0.34 ± 0.03
Vicia faba	1	88	5.00 ± 0.02	3.74 ± 0.22	0.04 ± 0.009
	2	87.4	5.54 ± 0.006	3.44 ± 0.22	0.08 ± 0.029
	3	90	6.22 ± 0.0	2.55 ± 0.11	0.14 ± 0.01
	4	89.5	7.00 ± 0.02	1.78 ± 0.53	0.23 ± 0.01
	5	90.7	8.04 ± 0.03	1.64 ± 0.14	0.31 ± 0. 009
Cicer arietinum	1	87	3.77 ± 0.02	3.26 ± 0.12	0.04 ± 0.006
	2	90	4.81 ± 0.04	2.97 ± 0.12	0.08 ± 0.003
	3	89.25	6.88 ± 0.18	2.20 ± 0.21	0.14 ± 0.01
	4	88.8	7.97 ± 0.12	1.54 ± 0.36	0.22 ± 0.01
Vigna unguiculata	1	89.7	3.77 ± 0.03	3.61 ± 0. 16	0.04 ± 0.006
	2	89.5	4.81 ± 0.02	3.36 ± 0.30	0.08 ± 0.009
	3	90	6.87 ± 0.02	2.20 ± 0.08	0.14 ± 0.013

times as compared with the control. As an insect, *Acanthoscelides obtectus* is an uricotelic organism. Leguminosae seeds are naturally rich in uric acid but the level increased with beetle excretion.

The nutritional quality of the seeds changed as the nitrogen content did not give the same pattern: the protein nitrogen was strongly affected and was partly transformed into uric acid. So the biological value of the seed was modified. At the first level of infestation, spoilage was not very great.

The amount of total nitrogen by Kjeldahl method increased with the level of infestation. This parameter was in a dynamic situation when the nitrogen content changed in regard to the biological metabolism of animals: it did not seem to be appropriate to estimate the nutritional value of the seeds as no more correlation with the protein content of the seeds existed and it did not reflect the deep modification of chemical structures including this element. However, the high increase of the total nitrogen appeared not to be only the result of the uric acid production. Moreover, the same phenomenon, with the same variation of nitrogen, occurred in all the experimental seeds. Besides the fact that Leguminosae included other non protein nitrogenous compounds, such as cyanide glucosides, several hypotheses could be made. They involved the beetle physiology and metabolism.

From a metabolic point of view, the transformation of nitrogenous compounds into uric acid required several steps and included precursors. These compounds were not estimated by the very specific method used in the experiment which determined only uric acid. It has also been observed that a tropical bruchid larvae, *Caryedes brasiliensis*, has been found to produce urea and ammonia during the detoxification process of L-canavanine (Rosenthal and Janzen, 1981).

From a physiological point of view, it is well-known that five larval steps of development occur before *A. obtectus* metamorphose to imagos. The phases develop inside the host-plant seed and involve a moult at each step. These changes could release some nitrogen from the chemical structural components of the organism and consequently improve the nitrogen content of the seeds.

Conclusion

The attack of bruchids on Leguminosae seeds markedly changes the pattern of nitrogen content. The proteins decrease and so does the biological value of the seed, whereas uric acid and total nitrogen increase. Consequently, the determination of total nitrogen by the Kjeldahl method which is usually practiced to estimate the nutritional value of the food does not appear to be appropriate in the case of stored seeds infested by pests.

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