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Ecology, Vol. 69, No. 2. (Apr., 1988), pp. 427-433.

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Fri May 11 15:31:51 2007

Ecology, 69(2), 1988, pp. 427–433
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BIOCHEMICAL ECOLOGY OF CANAVANINE-EATING SEED PREDATORS[1]

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Abstract. *L*-canavanine, a nonprotein amino acid structurally similar to *L*-arginine, is potentially toxic to many insects. In northwestern Guanacaste Province in Costa Rica, most insect seed predators do not feed on seeds that contain canavanine. However, several larval Coleoptera have become specialists on canavanine-containing seeds. Three of these beetles were compared with a number of other insects with regard to their biochemical ability to deal with and utilize *L*-canavanine. Our analyses revealed that certain biochemical capacities required by canavanine-feeding insects may have existed prior to their exposure to dietary canavanine.

Key words: *arginase; biochemical ecology; Bruchidae; Curculionidae; L-canavanine; plant–herbivore interaction; urease.*

INTRODUCTION

The deciduous lowland forest of northwestern Costa Rica (Guanacaste Province) harbors ≈975 species of dicotyledenous plants (Janzen and Liesner 1980). More than 100 of these are subject to insect seed predation from the larvae of beetles in the Bruchidae, Curculionidae, and Cerambycidae. About 75% of these seed predators are extremely specialized herbivores, feeding on a single plant species. Of the remaining 25%, the majority prey on two plant species, with only 12% feeding on three or more host plants (Janzen 1980).

An important underlying reason for this high degree of host-plant specificity is undoubtedly the host-plant chemistry. The presence of secondary defensive compounds, including nonprotein amino acids, may play a decisive role in determining host-plant specificity (Rosenthal and Janzen 1979). Legumes are a particularly rich source of the ≈250 nonprotein amino acids that have been isolated from higher plants (Bell 1971, Rosenthal 1982).

One of these nonprotein amino acids is *L*-canavanine, which is structurally related to *L*-arginine and has been detected in 1200 species representing 240 genera of the Papilionoideae (Fabaceae), a subfamily of the Leguminosae (Bell et al. 1978).

$$H_2N{-}C(NH_2){=}N{-}O{-}CH_2{-}CH_2{-}CH(NH_2)COOH$$

L-canavanine

A number of these legumes store up to 13% canavanine (dry mass) in their seeds (Rosenthal et al. 1977). The toxicity of canavanine has been demonstrated in a diverse variety of organisms, including herbivorous insects (see Rosenthal 1977*a*). Many insect seed predators are likely to be exposed to high concentrations of nonprotein amino acids (Rosenthal 1982), and it has been proposed that seed nonprotein amino acids represent an allelochemical defense against seed predation (Janzen 1969, 1971, 1981, Bell 1971, Rosenthal and Bell 1979). Consistent with the idea that canavanine may act as an allelochemical defense are the demonstrations of its toxicity to insect seed predators (Rehr et al. 1973*a*, *b*, Janzen et al. 1977, Rosenthal 1977*b*), the high host specificity of insects that feed on canavanine-rich seeds (Janzen 1980), and the demonstration that insects able to consume these toxic allelochemicals have evolved specific mechanisms to deal with them (Rosenthal 1983).

Larvae of at least 11 species of bruchid beetles (Bruchidae: Coleoptera) consume the canavanine-rich seeds of certain leguminous species native to the lowlands of Costa Rica (Table 1; Bell et al. 1978, Janzen 1980). The bruchid beetle genus *Caryedes* contains 19 Central American species, several of which are seed predators on the canavanine-containing seeds of *Dioclea* and *Canavalia* (Kingsolver and Whitehead 1974). In northwestern Guanacaste Province, Costa Rica, larvae of *Caryedes brasiliensis* (Rolfe) feed solely upon the seeds of *Dioclea megacarpa* (Thunberg) (Janzen 1971), a woody vine containing up to 13% dry mass of its seeds as canavanine (Rosenthal 1977*c*). The means by which *C. brasiliensis* not only circumvents the toxicity of canavanine, but also metabolizes the nitrogen stored in the terminal portion (guanidinooxy moiety) of canavanine to support amino acid biosynthesis is beginning to be understood (Rosenthal 1983).

[1] Manuscript received 28 October 1986; revised 27 April 1987; accepted 26 May 1987.

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TABLE 1. A partial list of canavanine-containing seeds of Santa Rosa National Park (Northwestern Guanacaste Province, Costa Rica) and their associated insect seed predators.

Plant*	Insect†
	Coleoptera
	Bruchidae
Sesbania emerus	*Acanthoscelides griseolus*
Indigofera suffruticosa	*Acanthoscelides kingsolveri*
Calopogonium mucunoides	*Acanthoscelides puellus*
Calopogonium caeruleum	*Acanthoscelides hectori*
Dioclea megacarpa	*Caryedes brasiliensis*
Centrosema pubescens	*Caryedes helvinus*
Centrosema pubescens	*Caryedes incensus*
Galactia striata	*Caryedes juno*
Calopogonium caeruleum	*Caryedes paradisensis*
Centrosema plumieri	*Caryedes quadridens*
Sesbania emerus	*Stator pruininus*
	Curculionidae
Canavalia brasiliensis	*Sternechus tuberculatus*
	Lepidoptera
	Pyralidae
Canavalia brasiliensis	Unidentified
Canavalia maritimus	Unidentified

* Adapted from Bell et al. 1978.
† Adapted from Janzen 1980.

Nitrogen utilization from canavanine by this bruchid beetle commences with the enzyme arginase (EC [enzyme code] 3.5.3.1), which converts *L*-canavanine to *L*-canaline and urea.

$$H_2N{-}C(NH_2){=}N{-}O{-}CH_2{-}CH_2{-}CH(NH_2)COOH \rightarrow$$

L-canavanine

$$H_2N{-}O{-}CH_2{-}CH_2CH(NH_2)COOH +$$

L-canaline

$$NH_2{-}C(=O){-}NH_2$$

urea

In most organisms, canavanine is not an efficient substrate for arginase; this enzyme obtained from mammals typically exhibits limited catalytic activity with canavanine (Reddy and Campbell 1969). In contrast, canavanine-producing legumes such as the jack bean, *Canavalia ensiformis*, employ arginase both in canavanine and arginine degradation (Rosenthal 1970). This legume produces an arginase that is much more reactive with canavanine as a substrate than is that of the soybean, *Glycine max*, a canavanine-free legume. In other regards, however, the arginine-utilizing arginases of these two plants are similar biochemically (Downum et al. 1983). Canaline, the potentially deleterious product of arginase-mediated cleavage of canavanine, is converted by *C. brasiliensis* to the nontoxic nonprotein amino acid *L*-homoserine (Rosenthal et al. 1978).

The second step in the process of canavanine catabolism by *C. brasiliensis* involves the enzyme urease (EC 3.5.1.5), which degrades urea to carbon dioxide and ammonia.

$$H_2N{-}C(=O){-}NH_2 \rightarrow CO_2 + 2NH_3$$

Although this enzyme occurs only rarely in insects, Rosenthal et al. (1977) found extraordinary levels of urease activity in larval *C. brasiliensis*.

An understanding of the biochemical basis for canavanine's antimetabolic properties is emerging. Because of the similarity in structure between canavanine and arginine, canavanine is a substrate for arginyl tRNA synthetase (Allende and Allende 1964). This enzyme is responsible for linking arginine to the transfer RNA molecule that carries this amino acid to the protein assembly site. In all canavanine-sensitive insects studied to date, this enzyme possesses limited capacity to distinguish between arginine and canavanine (Rosenthal et al. 1987), and it attaches canavanine to the tRNA molecule that normally transports arginine. This error results in canavanine incorporation into newly formed protein; these canavanine-containing, structurally aberrant proteins exhibit biochemically impaired function (Rosenthal 1986). Other than the insects considered in this report, no data exist regarding modes of canavanine degradation in other canavanine-eating seed predators. Such information can provide an evolutionary perspective on how the ability to avoid canavanine toxicity may have developed.

In this study, we examined some of the canavanine-related biochemical capacities of three Central American beetles that feed on canavanine-containing seeds. The bruchid beetle *Caryedes brasiliensis* and its congener *Caryedes quadridens* (Jekel) spend their larval stages in canavanine-storing seeds. Because *Centrosema plumieri* seeds, the leguminous host of larval *C. quadridens*, have low levels of canavanine (<1% dry mass), whereas the host seeds of larval *C. brasiliensis* (*Dioclea megacarpa*) contain up to 13% stored canavanine, these two congeneric beetles are ideal for comparative biochemical studies. The larvae of a curculionid weevil, *Sternechus tuberculatus*, consume the seeds of *Canavalia brasiliensis* in Costa Rica. This legume contains 7 to 9% dry mass seed canavanine. Neto (1977) also reported the same species of weevil from the seed of a Brazilian *Canavalia* species. These three species of insects allow comparisons of biochemical abilities of seed predators to degrade or otherwise avoid the toxic nonprotein amino acid *L*-canavanine.

After the canavanine concentration (dry mass) for each of the host seeds of these three insects was determined, two comparisons were made.

1) We examined the relative abilities of *Caryedes brasiliensis*, *Caryedes quadridens*, and *Sternechus tuberculatus* to catabolize canavanine or arginine with arginase. Rate of product formation, under substrate saturation conditions, was the kinetic parameter examined. For comparative purposes, several other insect arginases were also assayed.

2) We examined the relative abilities of *C. brasiliensis*, *C. quadridens*, and *S. tuberculatus* to degrade, via urease, urea to ammonia and carbon dioxide. Once again, rate of product formation under substrate sat-

uration conditions was the kinetic parameter evaluated. For comparative purposes, several other insect species were also analyzed for their urease activity.

Materials and Methods

Enzyme sources.—Costa Rican seed-eating beetles were collected in Santa Rosa National Park, northwestern Guanacaste Province, Costa Rica. *Caryedes brasiliensis* or *Sternechus tuberculatus* larvae were obtained from *Dioclea megacarpa* or *Canavalia brasiliensis* seeds, respectively. They were collected in January 1984 and again in January and February of 1985. *Caryedes quadridens* larvae were collected from seeds of *Centrosema plumieri* in January and February of 1985. Adult *C. brasiliensis* were taken as they emerged from *D. megacarpa* seeds in May 1984. Breeding stock for all other Coleoptera were secured from the United States Department of Agriculture Insect Research Laboratory, Savannah, Georgia and were reared in our laboratory in Kentucky. *Manduca sexta* (Sphingidae: Lepidoptera) and *Heliothis virescens* (Noctuidae: Lepidoptera) larvae were obtained from a continuous colony maintained at the University of Kentucky. Larval *Ischnura verticalis* (Coenagrionidae: Odonata) were a gift of Mark McPeek, University of Kentucky. All animal tissues were stored at −60°C prior to enzyme extraction.

Enzyme preparation.—A whole-body insect extract was prepared by grinding the insects and a small quantity of acid-washed sea sand with a mortar and pestle in 20 mmol/L sodium tricine buffer (pH 8.2), centrifuging the resulting slurry at 18 000 *g* for 15 min, and passing the supernatant solution through several layers of cheesecloth to remove floating debris. The filtrate was divided into three equal parts: one-third was used for arginase assays with canavanine, one-third was employed for arginase assays with arginine, and the final third was prepared for urease assays. Each third was diluted with the appropriate buffer.

For arginase assays with canavanine as substrate, 200 mmol/L sodium tricine (pH 7.4) was used, while arginase assays with arginine substrate employed 200 mmol/L sodium tricine (pH 8.8). These pH values correspond to the established optimal pH values for arginase for these two substrates (Damodaran and Narayanan 1940, Reddy and Campbell 1969). In all arginase assays, $MnCl_2$ (2 mmol/L) and 10 mg urease (Sigma, 32 micromolar units per gram) were added per millilitre insect extract.

Urease assays were conducted in 100 mmol/L sodium tricine (pH 7.4) with 0.1% (volume/volume) 2-mercaptoethanol (Rosenthal et al. 1976).

Substrate preparation for arginase.—In arginase assays with arginine, the substrate solution contained 100 mmol/L *L*-arginine in sodium tricine (pH 8.8) and 92.5 Bq/μmol *L*-[guanidino-^{14}C]arginine (New England Nuclear, 2.0 MBq/μmol). When canavanine was the substrate, 200 mmol/L canavanine, isolated from jack bean seeds (Rosenthal 1977*a*) and 46 Bq/μmol of *L*-[guanidinooxy-^{14}C]canavanine (Rosenthal et al. 1983) were in sodium tricine (pH 7.4).

Substrate preparation for urease.—In urease assays, the substrate solution contained 200 mmol/L urea and ≈170 Bq/μmol [^{14}C]urea (New England Nuclear) in 100 mmol/L sodium tricine (pH 7.4).

Enzyme assays for arginase.—The appropriate enzyme solution (100 μL) was placed in the chamber of a 25 mL Erlenmeyer flask; the reaction vessel and the assay procedure have been described thoroughly (Rosenthal and Janzen 1983*a*, Rosenthal and Thomas 1985). The reaction was initiated with 200 μL substrate and terminated by the addition of 150 μL of 6mol/L HCl. All assays were conducted in triplicate, and product formation was determined after 10, 20, and 30 min. Zero time samples served as the controls. Care was taken to ensure that maximum reaction velocity (i.e., maximum product formation) was attained.

Enzyme assays for urease.—Urease was assayed essentially by the method of Rosenthal et al. (1976). Assays were conducted 1–3 times, in triplicate, and formation was monitored after 7, 14, and 21 min. Zero time samples served as the controls.

Seed canavanine concentration.—Seeds of Costa Rican legumes were collected from the same location as were the insects. Following removal of the testa, the seeds were dried to a constant mass at a temperature not exceeding 60°C. Seeds were pulverized to a fine powder, with either a mortar and pestle or a Wiley Mill. Seed powders were extracted for 18 h at 4° with 100 volumes of 55% aqueous ethanol containing 50 mmol/L H_2SO_4. The extracted solution was centrifuged at 18 000 *g* for 15 min, and the supernatant solution was filtered over Whatman Number 1 paper into a graduated cylinder. This extract was evaluated for canavanine by the PCAF colorimetric assay (Rosenthal 1977*a*).

Protein assay.—Protein concentration was determined by the method of Lowry et al. (1955) using bovine serum albumin as the standard. All values for enzymatic activity were normalized by comparing the amount of catalytic activity with the concentration of soluble insect protein.

Results

Seed canavanine concentration

The weighted mean percentage dry mass canavanine of four pooled samples (34–48 seeds per sample) of *Centrosema plumieri* seeds assayed at 0.64% (range 0.47–0.79%). *Canavalia brasiliensis* and *Dioclea megacarpa* seeds contained at least 10 times this amount of dry mass canavanine. The weighted mean percentage dry mass canavanine of four pooled samples (14–20 seeds per sample) of *Canavalia brasiliensis* was 7.7% (range 7.1–8.3%). Five pooled samples (one seed per sample) of *D. megacarpa* seeds contained a weighted mean of 9.0% (range 8.2–9.9%) dry mass canavanine.

TABLE 2. Maximum reaction velocity (rate of product formation) of canavanine-utilization and arginine-utilizing arginase.

Insect*	Maximum velocity† (μmol·min^{-1}·[mg soluble protein]$^{-1}$)		
	Arginine	Canavanine	Ratio
Canavanine-feeding insects			
Coleoptera			
Caryedes brasiliensis (A)	4.5	2.5	1.8:1
Caryedes brasiliensis (L)	4.8	1.8	2.7:1
Caryedes quadridens (L)	5.2	3.4	1.5:1
Sternechus tuberculatus (L)	2.7	1.8	1.5:1
Non–canavanine-feeding insects			
Coleoptera			
Callosobruchus chinensis (A)	2.8	1.4	2.0:1
Callosobruchus chinensis (L)	6.3	2.6	2.4:1
Callosobruchus maculatus (A)	4.5	3.3	1.4:1
Callosobruchus maculatus (L)	4.2	1.8	2.3:1
Acanthoscelides obtectus (A)	2.7	1.8	1.5:1
Acanthoscelides obtectus (L)	2.5	1.3	1.9:1
Lepidoptera			
Manduca sexta (L)	1.5	0.3	5.0:1
Heliothis virescens (L)	2.6	1.1	2.4:1
Odonata			
Ischnura verticalis (L)	8.8	4.0	2.2:1
Orthoptera			
Periplaneta americana (A)	9.0	3.0	3.0:1
Hymenoptera			
Polistes sp. (L)	0.5	0.2	2.5:1
Hemiptera			
Oncopeltis fasciatus (A)	6.1	1.6	3.8:1

* A = adult; L = larva.
† Each value represents the mean of three replicates of one determination.

Arginase determinations

The maximum velocity of product formation by arginase, with canavanine or arginine, was determined for the larvae and/or adults of 12 insect species. Insects examined represented six orders and included canavanine-consuming and non-canavanine-consuming species (Table 2).

When arginine was the substrate, the maximum velocity of product formation ranged from 0.5 to 9.0 μmol·min^{-1}·(mg soluble protein)$^{-1}$. In all insects examined, arginase activity with canavanine was less than with arginine as substrate. Rates of product formation with canavanine ranged from 0.2 to 4.0 μmol·min^{-1}·(mg soluble protein)$^{-1}$.

The average value (±SE) in all insects of arginine-utilizing arginase activity divided by canavanine-utilizing activity was 2.4 ± 0.2. This ratio represents the ability of arginase to catabolize arginine relative to its ability to degrade canavanine at substrate saturation (where product formation velocity is maximized) for each amino acid. The average ratio value in canavanine seed-feeding beetles was 1.9 ± 0.3, whereas this value in insects naive to canavanine was 2.5 ± 0.3. A Mann-Whitney U test comparing this ratio in canavanine-seed feeders and non–canavanine feeders detected no difference between these two categories ($P > .05$). Although *Caryedes quadridens* was considered to be a canavanine-seed eater, the seeds in which this insect develops contain $< 1/10$ the canavanine found in seeds of either *Dioclea megacarpa* or *Canavalia brasiliensis*. Therefore, *C. quadridens* was grouped with the non–canavanine-consuming species for a second analysis of these data. Again, no significant difference was observed between these two groups of insects ($P > .05$). It appears that all of the insects examined possess an ability to catabolize canavanine via arginase that is roughly 40–50% of their capacity to degrade arginine via arginase.

Urease determinations

The maximum velocity of product formation by urease was determined for the larvae and/or adults of 13 insect species, representing six orders. Examined insects included canavanine-eating and non–canavanine-eating species. The level of urease activity ranged from several insects in which urease activity was undetectable to a high, in larval *Caryedes brasiliensis*, of 35 815 pmol·min^{-1}·mg^{-1}. The adults of this species and the larvae of *Sternechus tuberculatus* are the only other insects examined with comparably high urease activity (Table 3).

A Mann-Whitney U test indicated that canavanine-seed-eating beetles possessed significantly higher urease activity than did non–canavanine-eating species ($P < .05$). *Caryedes quadridens* larvae possessed only low levels of urease activity. Since the seeds in which this insect spends its larval life contain $< 10\%$ of the canavanine found in seeds of either *Canavalia brasiliensis* or *D. megacarpa*, *C. quadridens* was grouped with non-canavanine-eating species, and a second analysis of these data was done. A higher significant difference was then observed between urease levels in the two groups of insects ($P < .01$).

DISCUSSION

We have demonstrated that a number of insects have the capacity to liberate the nitrogen contained in the canavanine molecule through their arginase activity. Statistical analyses indicated no significant difference in the efficiency of this canavanine-cleaving activity between canavanine-eating and non–canavanine-eating groups of insects. Thus, all of the insects examined, including non–canavanine-consuming organisms, have the enzymic ability to catabolize canavanine.

The maximum velocity of arginase with canavanine as the substrate (Table 2) is significantly higher than that reported in a prior study (Rosenthal and Janzen 1983*a*). The prior study indicated that the arginase of the tested insects possessed significantly higher activity

with arginine as substrate than with canavanine. Closer examination of the methodology of the study revealed that radiochemical decay of the frozen ^{14}C-labeled canavanine occurred between the time of substrate preparation and evaluation and its actual use. This artificially decreased the canavanine maximum velocity values and led to an erroneous conclusion. This finding helped to instigate development of a new method for radiochemical production of *L*-[guanidinooxy-^{14}C]canavanine (Ozinskas and Rosenthal 1986). We have been very careful to monitor the radiochemical degradation of *L*-[guanidinooxy-^{14}C]canavanine. Thus, this report supersedes the earlier study (Rosenthal and Janzen 1983*a*).

While the majority of insects assayed in this study have had no significant evolutionary or ecological exposure to canavanine, they have an arginase that can efficiently degrade canavanine. This trait is a fortuitous circumstance. Thus, the ability of the studied canavanine-consuming insects to degrade canavanine via arginase may not have been specifically selected for as part of their adaptation to canavanine-containing seeds. The only other study comparing the abilities of canavanine and arginine to function as substrates for arginase is that of Downum et al. (1983). They demonstrated that a canavanine-producing jack bean, but not the canavanine-free soybean, has an arginase that can efficiently degrade canavanine.

Larvae of both *Caryedes brasiliensis* and *Sternechus tuberculatus* can cleave *L*-canavanine to *L*-canaline and urea. Excretion of the urea produced from canavanine degradation would represent a substantial loss of nitrogen that could be used for amino acid biosynthesis. Instead of eliminating urea, larvae of both species have high urease activity. This pronounced catalytic ability enables them to effectively produce ammonia from urea. Ammonia, in turn, can then be fixed into newly synthesized amino acids. The enzyme urease has rarely been reported in insects (Rosenthal et al. 1977). In this study, those non–canavanine-feeding insects with urease show only limited activity. Therefore, possession of high urease activity in the larvae of *C. brasiliensis* and *S. tuberculatus* may represent an adaptation to dietary canavanine. Moreover, high levels of urease activity may be of critical importance in systems where canavanine serves as a primary nitrogenous resource.

Sternechus tuberculatus larvae have ≈40 times the urease activity of any other insect examined, with the exception of *Caryedes brasiliensis*, which has almost twice the urease activity of larval *S. tuberculatus*. Our analyses of seed canavanine concentration indicated that both of these seed predators must cope with ≈7–9% dietary canavanine. Rosenthal (1977*c*) reported that certain seeds of *D. megacarpa* may contain up to 13% dry mass canavanine. *Caryedes brasiliensis* larvae can therefore be exposed to almost twice the concentration of seed canavanine that *S. tuberculatus* encounters. It is possible that the higher urease activity in *C. brasiliensis* may be related to an enhanced canavanine concentration. In a prior study, Rosenthal (1974) demonstrated the existence of a distinct correlation between seed canavanine content and urease activity in a variety of legumes. It is not known presently whether an unusual amount of urease is present or whether its turnover number (i.e., moles of substrate converted to product per minute per mole of enzyme) is high. The adults of *C. brasiliensis*, which feed on pollen rather than canavanine-containing seeds, still have retained appreciable urease. Pollen is a rich source of amino acids, and developmental retention of urease by the adult would be valuable for utilization of pollen arginine.

Caryedes quadridens feeds on seeds containing <1.0% canavanine. This insect has a urease activity comparable to the non–canavanine-feeding insects in this study. Two possibilities may account for the lack of high urease activity in larval *C. quadridens*. First, unlike *D. megacarpa* seeds, where canavanine represents 95% of the free seed nitrogen (Rosenthal 1977*c*), canavanine represents only a small percentage of the free nitrogen pool of *Centrosema plumieri* seeds, the host

TABLE 3. Urease activity (velocity of product formation) in insects.

Insect*	Activity (pmol·min^{-1}·mg^{-1}) $\bar{X}$	(SE)	[N]†
Canavanine-feeding insects			
Coleoptera			
Caryedes brasiliensis (A)	15 620		
Caryedes brasiliensis (L)	35 815	(1745)	[3]
Caryedes quadridens (L)	215	(90)	[2]
Sternechus tuberculatus (L)	18 175	(1130)	[2]
Non–canavanine-feeding insects			
Coleoptera			
Callosobruchus chinensis (A)	Not detected		
Callosobruchus chinensis (L)	Not detected		
Callosobruchus maculatus (A)	65		
Callosobruchus maculatus (L)	340		
Acanthoscelides obtectus (A)	45		
Acenthoscelides obtectus (L)	40		
Lepidoptera			
Manduca sexta (L)	Not detected		[2]
Heliothis virescens (L)	435		
Estigmene acraea	Not detected		
Odonata			
Ischnura verticalis (L)	Not detected		
Orthoptera			
Periplaneta americana (A)	90		
Hymenoptera			
Polistes sp. (L)	Not detected		
Hemiptera			
Oncopeltis fasciatus (A)	Not detected		

* A = adult; L = larva.

† Unless otherwise noted in the bracket, all determinations were conducted once.

of the larvae of this insect. Alternative free nitrogen sources for amino acid production by developing *C. quadridens*, such as arginine, exist. Second, it is possible that larval *C. quadridens* do not base their amino acid metabolism on the nitrogen of free amino acids of the host seed, but rather on the protein nitrogen of host seeds. Sulfhydryl proteases are common in most bruchid beetle larvae (Murdock and Slade 1986). It is conceivable that *C. quadridens* has high gut proteolytic activity, thereby enabling it to degrade seed proteins into usable amino acids.

Recent investigations of *Caryedes brasiliensis* and *S. tuberculatus*, canavanine-utilizing insects; *Canavalia ensiformis*, a canavanine-storing plant; and *Heliothis virescens*, a canavanine-resistant generalist herbivore (Berge et al. 1986), show that these organisms do not accumulate significant canavanyl proteins. By contrast, *Manduca sexta*, an insect highly sensitive to the adverse effects of canavanine, and *Glycine max*, a canavanine-free legume, readily incorporate canavanine into newly synthesized proteins.

The substitution error frequency (SEF), a measure of how frequently canavanine replaces arginine in newly synthesized proteins, was determined in these organisms (Rosenthal et al. 1987). The SEF for *Caryedes brasiliensis* and *S. tuberculatus* is 1 in 360 and 1 in 500 (perhaps as low as 1 in 1000), respectively. *Heliothis virescens*, a canavanine-resistant insect, has a SEF of 1 in 75. Comparable analysis of *Manduca sexta*, an herbivore markedly sensitive to the potential insecticidal properties of canavanine (Dahlman and Rosenthal 1975) discloses an SEF of 1 in 6 for the proteins of a whole-body larval extract. This value falls to 2 in 5 for newly formed hemolymph proteins.

At least 100 seed-eating Coleoptera occur in the lowland neotropical habitat of *Dioclea megacarpa* and *Canavalia brasiliensis* (Janzen 1980). Yet, only the two beetles of our study are known to feed on the seeds of these legumes. Although nutritional deficiencies, other toxic allelochemicals, and physical factors such as seed size or hardness may be linked to this lack of predation (Janzen 1981), another compelling factor cannot be overlooked. In order to obtain the nitrogen from canavanine to support amino acid synthesis, an insect must circumvent the potential toxicity of canavanine. Neither having an arginase that can degrade canavanine nor the presence of high levels of urease activity is an adequate safeguard against canavanine toxicity. Since canavanine's antimetabolic properties result largely from the production of anomalous, canavanyl proteins, canavanine-consuming organisms must avoid their production. Through the discriminatory capacities of their protein synthesis system, both *Caryedes brasiliensis* and *Sternechus tuberculatus* can avoid aberrant protein production and thereby benefit from canavanine.

The above findings suggest that development of the biochemical ability to avoid canavanyl protein production must occur or be acquired early in the process of a seed predator's radiation onto plants containing significant canavanine. The genetic capacity to degrade canavanine via arginase, to use urease to obtain ammonia, to produce its amino acids from this ammonia (Rosenthal et al. 1982), and to efficiently process and eliminate this ammonia (Rosenthal and Janzen 1981, 1985) could have evolved subsequently. Much more importantly, this study suggests that some of these metabolic capacities may have existed prior to exposure to dietary canavanine.

Finally, the genus *Caryedes* has been subject to extensive taxonomic scrutiny (Kingsolver and Whitehead 1984). These researchers separated the 19 Middle American species of *Caryedes* into seven groups of species. Although most beetles in these groups feed on canavanine-containing seeds in the genera *Calopogonium, Galactia, Canavalia, Centrosema, Dioclea,* and *Pachyrhizus*, at least one species, *C. paradisensis*, feeds on the seeds of *Phaseolus vulgaris*. These seeds do not contain canavanine (Bell et al. 1978). Furthermore, both *C. cavatus* and *C. x-liturus* feed on the seeds of *Bauhinia glabra*, a non–canavanine-producing caesalpiniaceous legume. Examination of canavanine-related biochemical capacities in these insects would be intriguing, particularly because Kingsolver and Whitehead (1974) believe that the two species that feed on *B. glabra* probably are descended from ancestors that fed on the canavanine-containing seeds of *Dioclea*. In addition, *C. paradisensis* represents the most primitive of the 17 Middle American species and probably gave rise to the lineage that produced the group containing *C. brasiliensis*.

Acknowledgments

This study was supported by grants from the National Science Foundation (PCM-82-19721, BSR 84-0351, and BSR 83-08388), the Graduate School of the University of Kentucky, and by Servicio de Parques Nacionales de Costa Rica. Beetle identifications were done by D. Whitehead and J. Kingsolver. This manuscript profited from discussion with Dr. Philip Crowley and Dr. Andrew Sih of the University of Kentucky, and the comments of Dr. Paul Feeny and an anonymous reviewer.

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