

FOOD UTILIZATION AND EGESTION RATES OF THE PREDATOR *GEOCORIS PUNCTIPES* (HEMIPTERA: HETEROPTERA) FED ARTIFICIAL DIETS WITH RUTIN

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ABSTRACT

A predaceous hemipteran, *Geocoris punctipes* (Say) was fed an artificial diet containing 0, 0.01, 0.10, or 1.00% rutin, a plant secondary chemical known to affect adversely certain phytophagous insects. Survival rates, exuvial weights, egg weights, relative growth rates, consumption indices, growth efficiencies, metabolic efficiencies and digestive efficiencies were unaffected by any of the rutin concentrations tested. Frass production for nymphs and adults was decreased by all rutin treatments. Uric acid excretion was decreased by all rutin concentrations in adult *G. punctipes* but not in nymphal insects.

Key Words: Plant secondary compounds, rutin, insect nutrition, predator, *Geocoris punctipes*.

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INTRODUCTION

Because of their great economic importance, cotton its pests and their allelochemistry have received considerable attention (Jenkins et al. 1983; Lukefahr and Martin 1966; Bottger et al. 1964; Shaver et al. 1970). In several of these studies artificial diets for cotton pests have been used to evaluate the effects of allelochemicals. Many insects that succeed in ingesting allelochemicals are capable of tolerating these materials at low to moderate levels. Monarch butterflies (Danaiidae) and milkweed bugs *Oncopeltus fasciatus* (Dallas) (Lygaeidae) sequesters their host plant's cardiac glycosides and use them as defenses against vertebrate predators (Brower and Brower 1964). Similarly, Smiley et al. (1985) showed that a chrysomelid beetle sequestered from host willows salicin which was effective as a wasp deterrent.

Rutin, a flavenol glycoside found in cotton and many other plant species [Krewson and Naghski (1953) listed 32 families and 65 plant species that contain rutin]. It is toxic to *Heliothis virescens* (F.) and *H. zea* (Boddie) (Lukefahr and Martin 1966); more so to the former than to the latter. Isman and Duffey (1983) have shown that rutin ingested by *H. zea* is absorbed by the digestive system. They found that at least 5% of the radio-labelled compound crossed the gut and became incorporated in the tissues of the *H. zea* used in that study.

The presence of an alleged allelochemical in the tissues of a phytophagous insect raises questions as to the effect of that substance upon predators of the herbivore. An allelochemical accumulated by a herbivore could become a phagodeterrent or a toxin to predators. As a result it could become a defense against predators. This could hinder biological control of phytophagous pests by their natural or introduced enemies. It is therefore important to know the effects

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of any allelochemical that is a candidate for use in programs aimed at using plant resistance as an anti-herbivore strategy.

Geocoris punctipes (Say) is a predator of *Heliothis* spp. and other pests. As a subject for studies on the effects of allelochemicals on predators, it is an excellent choice for several reasons: 1) Details of its trophic physiology and nutrition have been investigated (Cohen 1984), 2) It can be reared for continuous generations on an artificial diet (Cohen 1985) which can be manipulated to accommodate any allelochemicals to be tested, 3) It is important in several types of agroecosystems, and 4) It is known to feed on many kinds of prey, many of which may accumulate plant chemicals. We report here the results of studies on the effects of the plant secondary compound, rutin, on the trophic physiology of *G. punctipes*.

MATERIALS AND METHODS

Geocoris punctipes used to initiate cultures for these tests were collected from alfalfa fields in the Avra Valley ca. 32 km northwest of Tucson, Pima County, AZ. Nymphs were kept in ca. 4 L cardboard cartons covered with organdy, and adults were kept in 32 × 24 × 15 cm plastic cages topped with organdy. Artificial diet consisted of 100 g beef hamburger, 100 g beef liver and 12 ml of 5% sucrose solution blended and placed in stretched Parafilm®¹ feeding units (Cohen 1985). Each culture feeding unit (weighing ca. 175 mg) was changed every 48 hours and was found to be adequate food for 20 - 30 *G. punctipes*. Water was provided *ad libitum* via compressed cellulose sponge fitted and seated in a 9 cm (diameter) Petri dish. Eggs were harvested from wadded cotton oviposition sheets (Cohen and Debolt 1983).

Limitations imposed by difficulty in producing small, well-sealed diet packets forced us to use collective rather than individual experimental units so that measurable amounts of food consumption could be detected against the background of variation and natural losses in feeding unit weight. We followed as carefully as possible Waldbauer's (1968) admonition to use aliquots of diet as small as possible that still allow *ad libitum* feeding.

Only newly molted 3rd instar nymphs were used in these experiments. Larger 2nd instar nymphs were removed from the main culture and held separate until they molted at which time they were weighed and placed in groups of 5 in experimental cages made of ca. 40 ml plastic containers covered with organdy. They were provided with specially made feeding units with either the control diet (described above) or diet with 0.01%, 0.10% or 1.00% rutin (wt/wt wet weight) and water provided via a cellulose sponge in a vial. Crumpled waxed paper was added to the cage to reduce cannibalism. Six replications were made for each treatment.

Test diet feeding units were made by stretching Parafilm over 0.5 ml plastic centrifuge tubes. The resulting conical structures were filled with diet via a 20 ml syringe without a needle and carefully sealed by warming with tap water (ca. 55°C) and finger pressure. These structures were 13 mm tall cones with a 7 mm diameter bottom and weighed about 175 mg each. They were changed every 48 hours and pre- and post-weighed to the nearest 10 µg on a Cahn 25® Microbalance.

Control diet units were kept under identical conditions with infested ones to allow for correction for natural losses as described by Waldbauer (1968). The

¹ Mention of a trade mark does not constitute a recommendation by the U.S. Department of Agriculture or any other commercial product.

indices used in this study AD (digestive efficiency), ECI (growth efficiency), ECD (metabolic efficiency), CI (consumption index) and GR (growth rate) are all from Waldbauer (1968).

After all *G. punctipes* in a cage molted, they were taken collectively and placed in a tared gelatin capsule, then weighed to the nearest μg . Food and control units were weighed and changed every other day.

Frass production was estimated by the procedure described by Cohen (1984). The dry atmosphere in the cage (ca. 40% RH) expedited drying of frass thereby reducing dry-matter weight loss due to decomposition. Cages were removed after the insects had molted to the adult stage and were stored at $< -20^{\circ}\text{C}$. For analysis, cages were dried at 80°C for 2 hours and cooled to room temperature in a desiccator over CaSO_4 . Frass samples in each treatment and age group (3rd, 4th, and 5th instar nymphs and adults) were scraped and analyzed for purines (Cohen 1983). The ratios of purine to total frass weight were used to estimate total fecal production/cage (i.e., per group of 5 *G. punctipes*). Purine analyses of the scraped samples and of washed cages were performed by HPLC as described by Cohen (1983). Total frass weight was estimated from ratios of uric acid to total frass as described by Cohen (1984).

RESULTS AND DISCUSSION

The diets supported 73 - 83% survival of adults from 3rd instar nymphs (Table 1). There were no significant differences in survival between any of the groups ($P > 0.05$ in an ANOVA). Food consumption per individual was significantly higher ($P < 0.05$) in controls than in *G. punctipes* fed .01% rutin diet. Consumption of the other 2 rutin diets did not differ significantly from the control or the .01% rutin diet. Mean survival and egg weights were not significantly different in the 4 groups (Table 1).

Table 1. Mean % survival, food eaten, exuvial weight and egg weight/♀ in *Geocrois punctipes* fed artificial diets with 3 concentrations of rutin.

Rutin (%)	% Survival*	Consumption (mg)/individual†	Exuviae (μg)*‡	Egg wt./♀ (μg)*
Control	76.7	36.2b	481.7	80.7
0.01	83.3	26.3a	445.3	60.3
0.10	73.3	27.8a	399.3	45.0
1.00	83.3	34.0b	438.8	80.8

* Means were not significantly different at $P = .05$ level by ANOVA.

† Means followed by same letter were not significantly different at $P = .05$ level by SNK test.

‡ Collective for group of 5 individuals.

Growth rates were nearly identical (ca. 0.060) for the 4 groups ($P > 0.05$ in an ANOVA). Consumption indices range from 0.452 to 0.559 with no significant differences.

Growth efficiencies (ECI) ranged from ca. 11 - 14%. Metabolic efficiencies (ECU) ranged from ca. 12.5 - 15.5%, and absorption efficiencies ranged from ca. 82.5 - 93.5%. There were no significant differences in any of these means ($P > 0.05$ in ANOVA). However, ADs for nymphs were significantly higher ($P < 0.001$ in an ANOVA) than ADs for adults in all treatments. There were no significant differences ($P > 0.05$) between adult and nymphal ECIs or ECDs.

Total frass production was significantly higher ($P < 0.025$ in an ANOVA) in controls than it was in any rutin diet-fed group for both nymphal or adults (Table 2). There were, however, no significant differences between mean frass production among the rutin diet-fed groups. The % purine content of the frass produced by adults was significantly higher ($P > 0.025$) than it was in any rutin diet-fed group. There were no significant differences in mean purine content in any nymphal treatment groups.

Table 2. Mean frass production mg/insect in nymphal and adult *Geocoris punctipes* fed artificial diets with 3 concentrations of rutin.

Stage	% Rutin*			
	Control	0.01	0.10	1.00
Nymphs	0.83a	0.53b	0.55b	0.60b
Adults	2.13a	1.50b	1.44b	1.78b

* Means with a stage followed by the same letter within a row are not significantly different from one another (SNK, $P = 0.05$).

Isman and Duffey (1983) reported that *Heliothis zea* absorbed 2 - 5% of ingested rutin into their hemolymph resulting in a concentration of 0.003 to 0.03% of their tissue wet weight. Thus as a predator of *H. zea*, *G. punctipes* in nature could be exposed trophically to such concentrations in their prey and to even higher concentrations through ingestion of plant material containing rutin. These authors cite several studies that demonstrate the activity of rutin as a growth inhibitor of several species of phytophagous insects. In contrast McFarlane and Distler (1982) demonstrated enhancement of growth and food utilization stimulated by rutin in the diet of the house cricket, *Acheta domesticus*. These facts and the fact that plant phenolics such as rutin are known to inhibit enzymes under certain conditions (Van Sumere et al. 1975), necessitate an expanded investigation of the trophic effects of a ubiquitous phenolic such as rutin on insects.

The low mortality in all treatments indicates that rutin is essentially harmless to *G. punctipes* within the range of concentrations tested. We found that mortality which did occur was almost invariably a result of one nymph maturing asynchronously with the others in the group. Such an individual was then invariably a victim of cannibalism.

It appears anomalous that there was a difference between the rate of food consumption in the control and 1.0% groups vs. the groups fed the lower concentrations of rutin, yet the weights of exuviae and eggs (Table 1) were unaffected by rutin concentration. However, the data on egg weights must be interpreted cautiously since it represents only newly-eclosed females (< 5 days old); and the peak egg production does not begin until days 5 - 40 after adult eclosion (Cohen and Debolt 1983).

The fact that relative growth rates and consumption indices in all treatments were nearly identical further substantiates that rutin is not harmful to *G. punctipes*, nor is it beneficial as was the case with crickets (McFarlane and Distler 1982).

Further evidence of the benign effect of rutin on *G. punctipes* is seen in comparison of ECIs, ECDs, and ADs for the insects fed various concentrations of the compound. Although it might be predicted that a dose-response inhibitive effect should be evident (because of phenolic-caused enzyme inhibition), no such effect is seen within the range of dietary concentrations tested. There was a

significant difference between ADs of nymphs vs. adults ($P > 0.001$ in an ANOVA). There were no differences between diet treatments groups in growth, metabolic or absorptive efficiencies.

Cohen (1984) pointed out that the piercing-sucking mouthparts of *G. punctipes* allow extensive pre-ingestive culling and selecting of highly nutritive food, possibly accounting for the relatively high AD in this species. This applies equally well to the artificial diet used in the present study and to natural diets. The low ECIs and ECDs in the artificial diet-fed insects (compared to those reported by Cohen 1984) suggest that while this food may be digested efficiently, it is not utilized for anabolism as efficiently as are insect eggs. Although efficiencies (ECIs and ECDs) were lower in this study than they were in the *G. punctipes* study reported earlier (Cohen 1984), they were comparable to values summarized by (Slansky and Scriber 1984). The higher efficiencies seen in experiments with the smaller *G. punctipes* had been observed in several studies (Walbauer 1968; Cohen and Patana 1983), and is attributable to greater selectivity of smaller mouthparts.

In this context, the rate of egestion (frass production) was significantly higher in controls than in any of the rutin diet test groups. Also controls produced frass with considerably higher purine (uric acid) content than that of the rutin diet-fed *G. punctipes*, possibly indicating greater protein utilization in controls. However, the results of the present study demonstrate neither a beneficial nor a deleterious character of rutin at levels tested.

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LITERATURE CITED

- Bottger, G. T., E. T. Sheehan, and M. J. Lukefahr. 1964. Relation of gossypol of cotton plants to insect resistance. *J. Econ. Entomol.* 57: 283-85.
- Brower, L. P., and J. V. Z. Brower. 1964. Birds, butterflies, and plant poisons: A study in ecological chemistry. *Zoologica* 49: 137-59.
- Cohen, A. C. 1983. A simple, rapid and highly sensitive method of separation and quantification of uric acid, hypoxanthine, and xanthine by HPLC. *Experientia*, 39: 435-36.
- Cohen, A. C. 1984. Food consumption, food utilization, and metabolic rates of *Geocoris punctipes* [Het.: Lygaeidae] fed *Heliothis virescens* [Lep.: Noctuidae] eggs. *Entomophaga* 29(4): 361-67.
- Cohen, A. C. 1985. Simple method for rearing the insect predator *Geocoris punctipes* on a meat diet. *J. Econ. Entomol.* 78: 1173-175.
- Cohen, A. C., and J. W. Debolt. 1983. Rearing *Geocoris punctipes* on insect eggs. *Southwest. Entomol.* 8: 61-4.
- Cohen, A. C., and R. Patana. 1983. Efficiency of food utilization by *Heliothis zea* fed artificial diets or green beans. *Can. Entomol.* 116: 139-46.
- Cohen, A. C., and N. M. Urias. 1986. Meat-based artificial diets for *Geocoris punctipes* (Say). *Southwest. Entomol.* 11(3): 171-76.
- Isman, M. B., and S. S. Duffey. 1983. Pharmacokinetics of chlorogenic acid and rutin in larvae of *Heliothis zea*. *J. Insect Physiol.* 29(4): 295-300.
- Jenkins, J. N., P. A. Hedin, W. L. Parrott, J. C. McCarty, Jr., and W. H. White. 1983. Cotton allelochemicals and growth of tobacco budworm larvae. *Crop Sci.* 23: 1195-198.
- Krewson, C. F., and J. Naghski. 1953. Occurrence of rutin in plants. *Amer. J. Pharm.* 117: 190-200.

- Lukefahr, M. J., and D. F. Martin. 1966. Cotton-plant pigments as a source of resistance to the bollworm and tobacco budworm. *J. Econ. Entomol.* 59(1): 176-79.
- McFarlane, J. E., and M. H. W. Distler. 1982. The effect of rutin on growth, fecundity and food utilization in *Acheta domesticus* (L.). *J. Insect Physiol.* 28(2): 85-8.
- Shaver, T. N., M. J. Lukefahr, and J. A. Garcia. 1970. Food utilization, ingestion and growth of larvae of the bollworm and tobacco budworm on diets containing gossypol. *J. Econ. Entomol.* 63: 1544-546.
- Slansky, Jr., F. 1979. Effect of the lichen chemicals atranorin and vulpinic acid upon feeding and growth of larvae of the yellow-striped armyworm, *Spodoptera ornithogalli*. *Environ. Entomol.* 8: 865-68.
- Slansky, Jr., F., and M. Scriber. 1981. The nutritional ecology of immature insects. *Ann. Rev. Entomol.* 26: 193-211.
- Smiley, J. T., J. M. Horn, and N. E. Rank. 1985. Ecological effects of salicin at three trophic levels: new problems from old adaptations. *Science* 229: 649-51.
- Van Sumere, C. F., J. Albrecht, A. Dedoner, H. DePooter, and I. Pe. 1975. Plant proteins and phenolics. In the chemistry and biochemistry of plant proteins (Ed. by Harborne, J. B., and C. F. Van Sumere) pp. 211-56. Academic Press, NY.
- Waldbauer, G. P. 1968. The consumption and utilization of food by insects. *Adv. Insect Physiol.* 5: 139-46.
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