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RHODANESE IN INSECTS

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Abstract—Forty-four species of insects were assayed for the presence of rhodanese, an enzyme generally considered to be responsible for the detoxification of cyanide. Rhodanese was found to be widely distributed in both adults and larvae and was not restricted to those species which encounter exogenous cyanide through feeding on cyanogenic plants. These results indicate that cyanide detoxification is unlikely to be the primary role for rhodanese in insects.

Key Words—Cyanide, rhodanese, cyanogenesis, detoxification, *Lotus corniculatus*, *Vicia sativa*, *Zygaena*, Insecta, chalk grassland.

INTRODUCTION

The ability of the enzyme rhodanese to detoxify cyanide has been known for many years (Lang, 1933). Rhodanese has a variety of other functions, however (reviewed by Westley, 1973), and Volini and Alexander (1981) have suggested that the traditional role of rhodanese as a sulfur donor to cyanide may merely be complementary to its routine function as a sulfur donor to proteins.

Insects, in general, are much less sensitive to cyanide than mammals, with certain groups being particularly resistant (Povolny and Weyda, 1981; Bernays, 1982; Brattsten et al., 1983). It has generally been assumed that this insensitivity is dependent upon rhodanese (e.g., Dowd et al., 1983), but there is evidence that alternative detoxification systems may also be important. Parsons and Rothschild (1964), for example, showed that the larvae and adults of *Malacosoma neustria* L. contained only trace quantities of rhodanese, and yet this species had the ability to feed on the highly cyanogenic leaves of laurel (*Prunus laurocerasus* L.). The larvae of *Zygaena* species are themselves cyanogenic and are extremely resistant to cyanide, yet contain little, if any, rhodanese (Jones et al., 1962). The source of this resistance may be a combina-

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tion of an insensitive cytochrome oxidase and the action of another cyanide detoxifying enzyme, β-cyanoalanine synthetase, the presence of which has been detected in *Zygaena* (R. Davis, personal communication). Long and Brattsten (1982) and Brattsten et al. (1983) have shown that the larvae of *Spodoptera eridania* Cramer can acquire a tolerance of cyanide and that the mechanism of this resistance is not based on rhodanese.

The ability of animals to feed on cyanogenic plants should be correlated with the amounts of detoxifying enzymes that they possess (Conn, 1979). In this paper we describe the results of a survey for the presence of rhodanese amongst a wide range of insects. These include species known to feed on *Lotus corniculatus* L. and *Vicia sativa* L., plants which possess cyanogenic leaves, petals and cyanogenic seeds, respectively.

METHODS AND MATERIALS

The insects studied are listed in Table 1. Most of them were collected from an area of chalk grassland at Wharram Quarry in North Yorkshire. Additional material was obtained from the following East Yorkshire locali-

TABLE 1. VARIATION IN LEVELS OF RHODANESE RECORDED IN INSECTS

Species	Stage[a]	No. of replicates	Rhodanese (units/g fresh wt)[b] Range	Mean
Orthoptera				
Omocestus viridulus (L.)	A	3	ND[c]–<0.01	<0.01
Myrmeleotettix maculatus (Thun.)	A	16	0.01–0.05	0.02
Chorthippus brunneus (Thun.)	A	20	0.01–0.05	0.03
Dermaptera				
Forficula auricularia L.	A	3	0.05–0.05	0.05
Heteroptera				
Myrmus miriformis (Fallen)	A	3	0.16–0.27	0.23
Plagiognathus chrysanthemi (Wolff)[d]	A	3	1.68–2.38	2.03
Orthotylus sp.	A	3	2.04–2.84	2.35
Homoptera				
Philaenus spumarius (L.)[d]	A	3	0.14–0.48	0.34
Neophilaenus lineatus (L.)	A	5	0.18–0.77	0.49
Megopthalmus sp.	N	5	0.05–0.23	0.19
Megopthalmus sp.	A	4	0.20–0.23	0.22
Agallia brachyptera (Boheman)	A	3	1.45–2.66	1.98
Agallia venosa (Fallen)	A	2	0.61–0.95	0.78
Aphrodes bicinctus (Schrank)[d]	N	3	0.11–0.11	0.11
Turrutulus socialis (Flor)	A	3	0.89–1.38	1.07
Arthaldens pascuellus (Fallen)	A	3	3.20–6.20	4.35

TABLE 1. Continued

Species	Stage[a]	No. of replicates	Rhodanese (units/g fresh wt)[b] Range	Mean
Paluda adumbrata Sahlberg.	A	3	0.27–0.95	0.67
Eupteryx notata Curtis	A	4	1.45–4.38	2.64
Aphis loti Kaltenbach[d]	A	3	0.73–0.93	0.84
Acyrthosiphon loti (Theobald)[d]	A	3	N.D.–0.09	0.03
Coleoptera				
Coccinella 7-punctata L.	A	2	0.05–0.07	0.06
Coccinella 11-punctata L.	A	3	0.07–0.14	0.11
Meligethes aeneus (Fabr.)	A	3	0.41–0.59	0.51
Bruchus atomarius (L.)[e]	L	6	0.09–0.41	0.20
Bruchus atomarius (L.)[e]	A	3	ND	
Bruchus loti Paykull	A	4	0.07–0.57	0.30
Crepidodera ferruginea (Scop.)	A	3	0.23–0.54	0.33
Aphthona atrovirens Foerst.	A	2	0.43–1.63	1.03
Altica sp.	A	3	0.05–0.18	0.11
Apion loti Kirby[d]	L	3	0.27–1.45	0.84
Apion loti Kirby[d]	A	8	1.63–4.63	3.37
Apion nigritarse Kirby	A	3	0.77–1.79	1.40
Apion assimile Kirby	A	5	0.77–5.11	2.57
Phyllobius roboretanus Gredler	A	7	0.14–0.64	0.28
Sitona striatellus Gyllenhal	A	3	0.02–0.18	0.09
Hypera plantaginis (Degeer)[d]	L	3	0.07–0.45	0.29
Hypera plantaginis (Degeer)[d]	A	3	0.45–0.61	0.51
Lepidoptera				
Micropterix aruncella (Scop.)	A	5	1.18–4.06	2.43
Zygaena filipendulae (L.)[d]	L	3	ND	
Zygaena filipendulae (L.)[d]	A	3	<0.01–0.01	<0.01
Cydia nigricana (Fabr.)[e]	L	7	0.84–2.07	1.30
Erynnis tages (L.)[d]	L	1	0.02	
Polyommatus icarus (Rott.)[d]	L	4	0.09–0.16	0.13
Polyommatus icarus (Rott.)[d]	A	1	0.11	
Coenonympha pamphilus (L.)	A	3	ND–0.02	<0.01
Tyria jacobaeae (L.)	L	1	0.01	
Diptera				
Contarinia loti (Degeer)[d]	L	6	0.59–1.62	0.88
Hymenoptera				
Tenthredo acerrima Benson[d]	L	3	<0.01–0.05	
Eurytoma platyptea (Walker)[d]	A	1	2.20	
Entedon diotimus (Walker)	A	1	ND	

[a] Nymph/larva or adult.
[b] As defined by Sorbo (1955).
[c] None detected.
[d] Species known to feed regularly on *Lotus corniculatus*.
[e] Species known to feed regularly on *Vicia sativa*.

ties: Hull (*Chorthippus brunneus*), Hessle (*Forficula auricularia*), Cottingham (*Aphis loti* and *Acyrthosiphon loti*), and Gilberdyke (*Coccinella 7-punctata* and *C. 11-punctata*). The specimens of *Bruchus atomarius* were obtained from Donnington Castle, Newbury, Berkshire, and the *Cydia nigricana* were from Raglan, Gwent.

The rhodanese content of the insects was assayed using the method of Sorbo (1955) modified for small volumes of homogenate. Insects were stored frozen for periods of up to one month before analysis. They were homogenized by an Araldite motor-driven pestle in 100–200 μl of chilled deionized water contained in a 1-ml polypropylene microcentrifuge tube. The crude homogenate was spun down for 10 min in a microcentrifuge and the supernatant assayed at room temperature (±21°C) for 10 min. Formaldehyde was used to terminate the reaction (Miller and Conn, 1980). The assay mixture was then centrifuged at 21,000 rpm for 30 min at 2°C and the supernatant read immediately at 460 nm in a 0.1-ml cuvette. Individual insects ranged in weight from 0.001 to 0.1 g and for most of the species it was necessary to combine several specimens within each replicate.

RESULTS

The results (Table 1) show that rhodanese is present in detectable quantities in both the larvae and adults of a wide variety of insects and is not restricted to those species that regularly feed on cyanogenic plants. Between-species comparisons of rhodanese levels can only be tentative because there is generally considerable variation in the activity recorded for replicates of the same species and because fresh weight comparisons are liable to be biased by variation in the water content of different species and different stages of the same species. Nonetheless, these results do suggest that the rhodanese levels of species which feed on cyanogenic plants are not appreciably greater than those of other species.

DISCUSSION

The rhodanese content of *Hypera plantaginis* and *Polyommatus icarus* has been assayed previously by Parsons and Rothschild (1964). The levels of rhodanese activity they recorded are considerably higher than those detected during the present study. In an earlier study, Jones et al. (1962) found that rhodanese was absent in *Zygaena filipendulae*. We have also failed to detect rhodanese activity in the larvae of this species, but did record very low levels in the adult. *Zygaena* spp. are themselves cyanogenic and synthesize their cyanogenic glucosides de novo (Jones et al., 1962; Davis and Nahrstedt, 1982). It could be argued that the presence of cyanide detoxifying enzymes would be disadvantageous for insects, like *Zygaena* spp., which employ cyanogenesis as a chemical defence. The presence of β-cyanoalanine synthetase in *Z. filipen-*

dulae (R. Davis, personal communication), however, does not support that argument. As the major food plant of *Z. filipendulae* is itself cyanogenic (Table 1) (Jones et al., 1962), it is perhaps not surprising to find that even these cyanogenic larvae have the capability for detoxifying exogenous HCN.

Rhodanese has been shown to be almost ubiquitous among a taxonomically varied sample of insects. Trace quantities of cyanogenic glucosides may be present in most plants (Jones, 1979), but these would seem unlikely to account for the quantities of rhodanese that were detected, and we conclude that insects may have a general requirement for rhodanese which is independent of their need to detoxify cyanide. These results also bring into question whether rhodanese is employed to detoxify cyanide in those insects which routinely feed on cyanogenic plants. These species do not appear to contain unusually high levels of rhodanese activity, which implies that either the basal rhodanese content of insects is able to cope with a cyanogenic diet or that other detoxifying enzymes are also involved. It is instructive to compare the rhodanese levels of *Cydia nigricana* (Lepidoptera) and *Bruchus atomarius* (Coleoptera), two species which feed on extremely cyanogenic seeds. Moderately high levels of rhodanese activity were recorded from *C. nigricana,* but very little from *B. atomarius* larvae and none from the adults. This may be an example of two species which share the same food item, but have evolved different methods of circumventing its secondary compounds. The β-cyanoalanine synthetase content of these species is currently being investigated.

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