European Arachnology 2000 (S. Toft & N. Scharff eds.), pp. 65-70.
 ISBN 87 7934 001 6
(Proceedings of the 19th European Colloquium of Arachnology, Århus 17-22 July 2000)

Responses of a detoxification enzyme to diet quality in the wolf spider, *Pardosa prativaga*

SØREN ACHIM NIELSEN[1] & SØREN TOFT[2]
[1]*Department of Life Sciences and Chemistry, Roskilde University, P.O. Box 260, DK-4000 Roskilde, Denmark* (san@ruc.dk)
[2]*Department of Zoology, University of Aarhus, Bldg. 135, DK-8000 Århus C, Denmark*
(soeren.toft@biology.au.dk)

Abstract
A previous study revealed a significantly increased respiration rate in wolf spiders, *Pardosa prativaga*, fed diets of low-quality prey through 2-4 weeks compared to spiders fed high-quality prey. We tested here the hypothesis that a higher metabolic rate was due to activity of detoxification enzymes, induced by presumed toxins in the low-quality prey. Two aspects of the activity of a detoxification enzyme (Glutathione S-transferase (GST) and its peroxidase activity (GSTpx)) were measured on the same individuals as in the previous study. The activity of both enzymes was significantly affected by the diet treatments, but in different ways. GSTpx activity was more or less reduced by all low-quality diets compared to high-quality diets and therefore showed a near-significant relationship with respiration rate and daily weight change over the experimental period. GST activity was reduced only by two aphid species and showed no correlation with the above-mentioned parameters. There was no relationship between the two enzyme activities. Since all significant responses to low-quality prey were inhibitive, the results did not confirm our hypothesis.

Key words: Araneae, biomarker enzymes, food, generalist predator, prey quality

INTRODUCTION

Insects vary in their qualitiy as food for generalist predators, including both high-quality, low-quality as well as toxic prey (Toft 1995; Toft & Wise 1999a,b; Marcussen et al. 1999; Bilde & Toft 2001). Quality statements are based here on feeding experiments determining the fitness consequences of keeping the spiders on diets of single prey species or specified mixed diets. Though in most cases the chemical identity is unknown, deterrents or toxins in the prey are most likely to be responsible for low quality. This may be true both when prey consumption is low or practically zero (in which case the prey is highly deterrent – a predigestive effect) and when consumption is substantial but food utilization is poor (in which case the prey is most likely to contain toxin(s) with postdigestive effects). However, metabolic effects on the predator must be very different in the two situations. Toxin containing prey should induce a variety of physiological responses depending on the chemistry of the toxin, whereas highly deterrent prey that are hardly eaten at all may have no other physiological effects than those of starvation. Most low-quality prey types are probably intermediate between these extremes, i.e. they are both deterrent and toxic to varying degrees, so starvation effects and responses to toxins will often

occur simultaneously. One effect may therefore mask the other.

Turnbull (1960) recorded 153 prey species eaten by *Linyphia triangularis* (Clerck) in the field. This exemplifies the high diversity of prey eaten by generalist predators such as spiders. In view of this, spiders should be well equipped with detoxifying enzymes to deal properly with the diversity of defensive compounds that many of these insects may contain. We have earlier reported on activities of Glutathione S-transferase (GST), and two peroxidases (GSTpx and Glutathione peroxidase (GPOX)) in *L. triangularis* and two *Pardosa* spp. (Nielsen et al. 1997, 1999; Nielsen & Toft 1999) and some of the factors influencing their magnitude, incl. feeding level and insecticide treatment. In another study (Toft & Nielsen 1997) we reported on the effects of various single- and mixed-species diets of different quality on weight change and respiratory rate in the wolf spider *Pardosa prativaga* (L. Koch), indicating an increased respiration rate when the spiders were fed low-quality prey. We hypothesized that the increased metabolism might be due to the handling of toxins by detoxification enzymes induced by the low-quality food. The individuals of this previous experiment were stored in a deep-freezer for later measurements of detoxification enzyme activity, the results of which are presented here.

GST is a ubiquitous family of isozymes that are able to handle natural toxins and xenobiotics via conjugation with glutathione (GSH), forming derivatives that are easily excreted (e.g. Timbrell 1991). GSTpx, which is the peroxidase activity of GST (Ahmad 1995), serves to eliminate peroxidised derivatives and superoxide anions formed during metabolic transformation of toxins or food (Ahmad 1992, 1995).

MATERIALS AND METHODS

Since all methods have previously been published we restrict ourselves here to presenting the basic details for understanding the new results, and refer to Toft & Nielsen (1997) for details about respiration measurements, and to Nielsen et al. (1998, 1999) regarding enzymatic measurements and determination of protein content. For the enzyme assays the following substrates were used: 1-chloro-2,4-dinitrobenzene (CDNB) for GST; and t-butyl-hydroxyperoxide (TBH) for GSTpx.

Procedures

Pardosa prativaga were collected as juveniles in spring and kept for 2-4 weeks on 9 prescribed diet treatments representing the full range of qualities from high-quality to toxic, and using starved animals as controls (Table 1). Low-quality prey types gave negative or only slightly positive growth rates (DWC in Table 1), not significantly different from that of the starvation group, while high-quality prey sup-

Table 1. Diet treatments to which *Pardosa prativaga* was subjected prior to measurements of detoxification enzyme activity. Quality statements are based on the mean daily specific weight change (DWC: Δmg/mg/day) during the experimental period (see Toft & Nielsen 1997). N: number of spiders in each treatment group.

Diets	DWC	Quality	Culture medium/host plant	N
Aphid *Rhopalosiphum padi* (L.)	-2.34	Low	Wheat seedlings (mixed cultivars)	6
Aphid *Sitobion avenae (F.)*	-0.72	Low	Wheat seedlings (mixed cultivars)	13
Aphid *Metopolophium dirhodum (Walker)*	3.55	Low	Wheat seedlings (mixed cultivars)	15
A mixture of aphids *R. padi, S, avenae, M. dirhodum*	1.18	Low	Wheat seedlings (mixed cultivars)	15
Aphid *Aphis nerii (B. de F.)*	-14.02	Low	*Asclepias curassavica*	8
Collembola *Folsomia candida (Willem)*	-6.50	Low	Baker's yeast	12
Collembola *Isotoma anglicana Lubbock*	22.72	High	From the field (fed Baker's yeast)	22
Fruit fly *Drosophila melanogaster (Meigen)* wild type	30.93	High	Carolina fruit fly medium	17
Mixture of *I. anglicana* and *D. melanogaster*	30.71	High	(See above)	17
Starvation (control)	-7.60	-	-	15

ported high growth rates (Toft & Nielsen 1997). From other studies we have evidence that *F. candida* is toxic but only little deterrent to wolf spiders (Toft & Wise 1999ab; Mayntz & Toft 2000), while the aphids are more deterrent and have no overall toxic effects, possibly because deterrency keeps the spiders' consumption rates low.

All prey types were raised in laboratory cultures, except *Isotoma anglicana* which was collected in the field. Prey was offered ad libitum (except *Sitobion avenae* due to problems with the culture). In the mixed diets each species was offered in approximately equal numbers, and in amounts so that all species were constantly available. The spiders were weighed at the start of the feeding period, and again when respiration measurements were made and the animals were sacrificed for enzymatic measurements. Percent daily weight change was used as an overall measure of the quality of the diets.

Statistical analyses

Variance homogeneity with respect to the ten diet treatments was obtained or approximated by squareroot-transformation for both series of specific enzyme activities (nmol/min/mg protein) (Levene's test; GST: P = 0.069, GSTpx: P = 0.026), and the data were analysed with parametric ANOVA. Because of the variation in food quality, the spiders of the various treatments grew to different final sizes. Size-adjusted activities were therefore obtained by entering an indicator of final spider size as covariate and presenting the results as least squares means. Final body weight and total protein content were strongly correlated (regression analysis, P < 0.001), and the same conclusions were reached whether one or the other served as the size-indicator. The results presented (Fig. 1) used protein content (mg protein/spider) as covariate. Pairwise post-hoc comparisons of treatments were made using the Tukey HSD test.

Regression analysis was used in search for relationships between enzyme activity, respiration rate, and diet quality (% weight change), using treatment-group means as data points (N = 10).

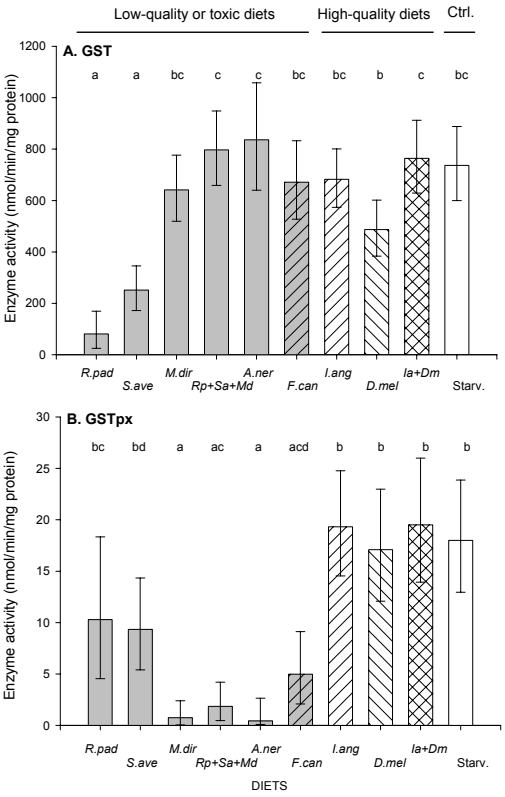

Fig. 1. Least squares means ± 95% confidence limits of detoxification enzyme activities (**A**: GST; **B**: GSTpx) of *Pardosa prativaga* under different diet treatments, adjusted for size (= protein content). Values were obtained by back-transforming means and lower/upper confidence limits of square-root transformed data, obtained by ANCOVA. Grey columns: low-quality prey; white column: high-quality prey and starvation. Left-hatched: Collembola; right-hatched: Diptera; cross-hatched: mixed Collembola/Diptera; unhatched-grey: aphids; unhatched-white: starvation. Letters above bars: bars indicated by the same letter are not significantly different (Tukey HSD).
Treatments: R.pad: *Rhopalosiphum padi*; S.ave: *Sitobion avenae*; M.dir: *Metopolophium dirhodum*; Rp+Sa+Md: mixed diet of *R. padi*, *S. avenae* and *M. dirhodum*; A.ner: *Aphis nerii*; F.can: *Folsomia candida*; I.ang: *Isotoma anglicana*; D.mel: *Drosophila melanogaster*; Ia+Dm: mixed diet of *I. anglicana* and *D. melanogaster*; Starv.: starvation.

RESULTS

GST

Spiders fed *R. padi* and *S. avenae* had significantly lower GST activity than spiders of all other diet treatments (Fig. 1A). Though the statistical analysis indicates significant variation among some of these latter treatments, the actual differences are small and not easily interpretable. They may be fortuitous.

Mean specific GST activity correlated with no other measured parameter, i.e. diet quality (P = 0.66) or respiration rate (P = 0.64). Also, there was no relationship between GST activity and GSTpx activity (P = 0.58).

GSTpx

The responses of GSTpx were very different from that of GST. Four low-quality treatments (*M. dirhodum*, 3-aphid mix, *A. nerii*, *F. candida*) showed reduced activity of this enzyme. *R. padi* and *S. avenae* treatments did not inhibit this enzyme significantly compared to the high-quality diets, but this may be due to the high variances of the measurements.

However, all low-quality diets had lower activity than all high-quality diets and starvation (to the right in Fig. 1B). As a result of this, specific GSTpx activity correlates positively with diet quality (P = 0.050) and negatively with specific respiration rate (P = 0.054).

DISCUSSION

GST showed no correlation with the overall measure of food quality (% weight change) over the range of diets investigated. The GST activity may depend not only on the presence of certain toxins in the food, but also on the general feeding condition of the spider. Thus, we have previously demonstrated an increased activity with degree of starvation (Nielsen & Toft 1998). This effect is not apparent in the present results. Anyway it is probably safe to conclude that the very low GST activity in the *R. padi* and *S. avenae* diet groups is not due to the starvation effect of these two low-quality prey, because the starvation group had high GST activity. More likely it is due to the specific chemical components of the two aphid species. Specificity of the effects on GST is underscored by the fact that other low-quality, whether deterrent or toxic, prey did not influence GST activity. GSTpx showed a more consistent reduction of activity with low-quality prey, though this was not significant in all cases. The results are surprising because it was not the same low-quality diets that inhibited the two enzyme activities the most. Also the GST and GSTpx activities did not correlate with each other. Both of these results may be due to their different substrate specificities, in spite of being mediated by the same enzyme. GSTpx activity was inhibited by three aphid treatments (*M. dirhodum*,mixed cereal aphids and *A. nerii*) and by the toxic Collembola *F. candida*, but only slightly (and non-significantly) by *R. padi* and *S. avenae*. Thus, the effects were not related to whether the low-quality prey were primarily deterrent or toxic.

The mixed-aphid diet contained prey species that as single-species diets were inhibitive to GST and others that were inhibitive to GSTpx. However, the mixed-aphid diet itself inhibited GSTpx but not GST. Both enzyme activities responded as the *M. dirhodum* single-species diet. Probably the spiders consumed more of *D. dirhodum* than of the two other species of the mixed diet, because of the three *M. dirhodum* is of the highest food quality for *P. prativaga* (Toft 2000). The spiders eat more *D. dirhodum* than *R. padi* or *S. avenae* before a prey aversion is developed (Toft 1997).

The similar effects of *M. dirhodum*, *A. nerii* and *F. candida* on the one hand, and *R. padi* and *S. avenae* on the other, are not easily reconcilable with other ecophysiological results. *F. candida* is toxic to wolf spiders (Toft & Wise 1999 a, b) and *S. avenae* was toxic to the linyphiid *Erigone atra* (Bl.) for some fitness parameters (Bilde & Toft 2001). In most studies, the aphids have turned out as low-quality but not toxic, e.g. *A. nerii* (Toft & Wise 1999a) and the three cereal aphids (Toft 1995, 2000). Mayntz & Toft (2000) found *R.padi* to be highly deterrent, whereas *F. candida* was less deterrent. When

presented together with high-quality insect prey in mixed diets, *R. padi* has even given positive fitness effects both in spiders (Toft 1995; Bilde & Toft 2001) and in partridge chicks (Borg & Toft 2000).

Also, the enzyme responses do not show any consistent pattern in relation to the systematic classification of the aphids. All are members of the Aphididae, but *R. padi* and *A. nerii* both belong to the tribe Aphidini, whereas *Sitobion* and *Metopolophium* belong to the tribe Macrosiphini (Heie 1986,1994).

Earlier we found that GST (but not GSTpx) correlated significantly with feeding level, with activity increasing with degree of starvation. Spiders on the low-quality diets grew little or not at all, reflecting their low consumption rates of these prey (Toft 1995, 1997; Bilde & Toft 1997; Toft & Wise 1999b). In the present study, GST activity of starved spiders did not differ significantly from groups fed high-quality prey ad libitum (i.e. kept at satiation level) and also did not differ from several low-quality treatments (which are likely to cause starvation in the spiders due to low consumption rates). The same can be said here about GSTpx, except that it was different types of low-quality treatments that resembled starvation in the two cases. One interpretation that may explain these results is that the enzyme responses are determined by specific chemical compounds of the different prey types and are unrelated to prey quality as such.

Toft & Nielsen (1997) hypothesized that the increased respiration rate found in the low-quality diet treatments might be due to increased metabolic costs of detoxifying supposed toxins in the low-quality prey. If that were true we would have expected to find increased activities of detoxification enzymes in the low-quality diet treatments due to induction (Terriere 1984), but all significant differences were in the opposite direction: the measured enzyme activities were either inhibited or unaffected by low-quality prey. It is possible, however, that other enzyme systems, not measured here, were induced by the low-quality prey.

While the effects of diets on detoxification enzyme activity may provide indirect confirmation that secondary substances are involved in determining the food quality of insects to spiders, they give no direct clue as to what compounds are involved. A full understanding of the effects of prey on generalist predators probably requires both direct chemical identification of prey toxins and analysis of a wider range of detoxifying enzyme systems.

ACKNOWLEDGEMENTS

We are indebted to Gitte Wikstrøm for doing the enzyme measurements, to Karl-Martin Vagn Jensen for useful discussions, and to David Mayntz and Trine Bilde for critical comments on the manuscript.

REFERENCES

Ahmad, S. 1992. Biochemical defence of pro-oxidant plant allelochemical by herbivorous insects. *Biochemical Systematics and Ecology* 20, 269-296.

Ahmad, S. 1995. Antioxidant mechanisms of enzymes and proteins. In: *Oxidative Stress and Antioxidant Defenses in Biology* (S. Ahmad ed.), pp. 238-272. Chapman & Hall, New York.

Bilde, T. & Toft, S. 1997. Consumption by carabid beetles of three cereal aphid species relative to other prey types. *Entomophaga* 42, 21-32.

Bilde, T. & Toft, S. 2001. Value of three cereal aphid species as food for a generalist predator. *Physiological Entomology* 26, 58-68.

Borg, C. & Toft, S. 2000. Importance of insect prey quality for grey partridge chicks (*Perdix perdix*): a self-selection experiment. *Journal of Applied Ecology* 37, 557-563

Heie, O.E. 1986. *The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. III.* Fauna Entomologica Scandinavica 17. E.J. Brill/ Scandinavian Science Press, Leiden.

Heie, O.E.1994. *The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. V.* Fauna Entomologica Scandinavica 28. E.J. Brill, Leiden.

Marcussen, B.M., Axelsen, J.A. & Toft, S. 1999.

The value of two collembola species as food for a cereal spider. *Entomologia Experimentalis et Applicata* 92, 29-36.

Mayntz, D. & Toft, S. 2000. Effects of nutrient balance on tolerance to low quality prey in a wolf spider. *Ekológia (Bratislava)* 19 Suppl. 3, 153-158.

Nielsen, S.A., Clausen, J. & Toft, S. 1997. Detoxification strategies of two types of spiders revealed by cypermethrin application. *Alternatives to Laboratory Animals* 25, 255-261.

Nielsen, S.A. & Toft, S. 1998. Responses of Glutathione S-transferase and Glutathione Peroxidases to feeding rate of a wolf spider *Pardosa prativaga*. *Alternatives to Laboratory Animals* 26, 399-403.

Nielsen, S.A., Toft, S. & Clausen, J. 1999. Cypermethrin effects on detoxification enzymes in active and hibernating wolf spiders (*Pardosa amentata*). *Ecological Applications* 9, 463-468.

Terriere, L.C. 1984. Induction of detoxication enzymes in insects. *Annual Review of Entomology* 29, 71-88.

Timbrell, J.A. 1991. *Principles of Biochemical Toxicology*. 2nd ed. Taylor & Francis, London.

Toft, S. 1995. Value of the aphid *Rhopalosiphum padi* as food for cereal spiders. *Journal of Applied Ecology* 32, 552-560.

Toft, S. 1997. Acquired food aversion of a wolf spider to three cereal aphids: intra- and interspecific effects. *Entomophaga* 42, 63-69.

Toft, S. 2000. Species and age effects in the value of cereal aphids as food for a spider. *Ekológia (Bratislava)* 19 Suppl. 3, 273-278.

Toft, S. & Nielsen, S.A. 1997. Influence of diet quality on the respiratory metabolism of a wolf spider *Pardosa prativaga*. In: *Proceedings of the 16th European Colloquium of Arachnology, Siedlce* (M. Zabka ed.), pp. 301-307. Wyższa Szkoła Rolniczo - Pedagogiczna, Siedlce.

Toft, S. & Wise, D.H. 1999a. Growth, development and survival of a generalist predator fed single- and mixed-species diets of different quality. *Oecologia* 119, 191-197.

Toft, S. & Wise, D.H. 1999b. Behavioral and ecophysiological responses of a generalist predator fed single- and mixed-species diets of different quality. *Oecologia* 119, 198-207.

Turnbull, A. 1960. The prey of the spider *Linyphia triangularis* (Clerck; Araneae, Linyphiidae). *Canadian Journal of Zoology* 38, 859-873.