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Glutathione S-transferases of *Aulacorthum solani* and *Acyrthosiphon pisum*: partial purification and characterization

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Abstract

Glutathione *S*-transferases (GST) play an important role in the detoxification of many substances including allelochemicals from plants. Brassicaceae plants contain glucosinolates and emit volatile isothiocyanates which affect the GST system. A comparison of the GST of two aphid species, the generalist *Aulacorthum solani* found on Brassicaceae and the Fabaceae specialist *Acyrthosiphon pisum*, was made to try to explain their respective feeding behaviour. Differences of GST were determined among the two aphid species based on purification by affinity chromatography, SDS-PAGE and on kinetic studies. Purification yields using an epoxy-activated Sepharose 6B column were highly different for the two aphid species (18% and 34% for *A. solani* and *A. pisum*, respectively). These variations were confirmed by SDS-PAGE. While only a 27-kDa band was observed for *A. pisum*, two bands of approximately 25-kDa were visualized for the generalist aphid, *A. solani*. Considering the kinetic results, differences of K_m and V_{max} were observed following the aphid species when a range of substrates (CDNB and DCNB) and GSH concentrations were tested. Studies on the detoxification enzymes of generalist and specialist herbivores would be undertaken to determine accurately the effect of the host plant on the organisms eating them, particularly in terms of biochemical and ecological advantages.

Keywords: Glutathione S-transferase; Aulacorthum solani; Acyrthosiphon pisum; Allelochemicals; Detoxification

1. Introduction

Insects developed adaptations to protect themselves against potentially toxic compounds such as pesticides and plant metabolites. Several defensive enzyme systems, also called xenobiotic metabolizing enzymes (XME), can be induced to overcome the toxicity of these chemicals. The main pathways of enzymatic detoxification in animals are the so called phase I and phase II reactions leading to compounds with higher hydrophilicy and excretability. Glutathione *S*-transferases (GST) belong to the last category and play an important role in the xenobiotic detoxification. Indeed, they catalyze the conjugation of electrophilic molecules with reduced glutathione (GSH) (Boyland and Chasscaud, 1969). Most of the studies on glutathione transferases were fo-

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cused on Lepidotera (Yu, 1982, 1989) or Diptera (Clark and Shamaan, 1984; Fournier et al., 1992; Prapanthadara et al., 2000). Hemiptera and more particularly aphid species are poorly studied even if many species are economically important and cause problems to be controlled, partially due to insecticide resistance (Devonshire et al., 1998).

Several compounds as insecticides, herbicides or allelochemicals from different crop plants have been shown to be inducers or inhibitors of several of the XME in insects (Yu, 1982; Clark, 1989; Dauterman, 1989). The GST system is affected by secondary substances from Brassicaceae species (Bogaards et al., 1990; Egaas et al., 1991; Francis, 1999). The latter include glucosinolates, a group of thioglucoside compounds, which are hydrolysed when the plant tissues are damaged, e.g. during herbivore pest attacks (Heaney and Fenwick, 1995). These degradation compounds include mainly thiocyanates, nitriles and isothiocyanates. They are thought to constitute part of the plant's defence system (Porter et al., 1991).The adaptation to these plant allelochemicals is one aspect of the chemical ecology of herbivore pest behaviour and infestation (Pickett et al., 1992).

Two aphid species infesting different host plants were used in this work. The first one, *Aulacorthum solani* (Kaltenbach) is a generalist herbivore which is found on many botanical families including Brassicaceae species. The other one, *Acyrthosiphon pisum* (Harris), is a Fabaceae specialist and is unable to survive on crucifer plants. The pea aphid is apparently not adapted to this kind of plant allelochemicals. The aim of this work was to partially purify and characterize the GST of both herbivores. These enzymes could be the way to cope with secondary plant substances for the generalist pest and not for the other aphid species. Indeed, allyl-, phenyl- and benzyl isothiocyanates were found to be metabolized by GST from generalist phytophagous Lepidoptera (Wadleigh and Yu, 1988).

2. Materials and methods

2.1. Plants and insects

White mustard (*Sinapis alba* L.), and broad bean (*Vicia faba* L.) were raised in 20 cm × 30 cm plastic trays in a controlled environment room at 20 ± 2°C temperature and 16 h daylight photoperiod. Brassicaceae species was first sown in an incubator at 16°C in a plastic tray containing ordinary compost and placed in plastic pots with the same substrate when the plants had two true leaves. Broad beans were sown directly in a mixture of perlite and vermiculite.

Acyrthosiphon pisum had been reared in the laboratory for several years whereas *Aulacorthum solani* was collected from a white mustard field in September 1999. Pea aphid and *A. solani* were mass reared on bean and mustard, respectively.

2.2. Purification of enzyme

Whole aphids were homogenized in a blender in three times their volume of 22 mM sodium phosphate buffer (pH 7.0). The homogenate was ultracentrifuged (1 h, 100 000 × *g*) and the supernatant was applied to a PD10 column (Pharmacia) before an affinity column. The latter was packed with epoxy-activated Sepharose 6B that had been reacted with GSH as described by Simons and Vander jagt (1977). The column was eluted with 11 ml of 22 mM sodium phosphate (pH 7.0), then with 5 mM GSH (0.5 ml followed by 1.5 ml) in 50 mM Tris–HCl buffer (pH 9.6). The 1.5 ml fraction with GST activity was used for the electrophoretic and the kinetic studies.

2.3. Enzyme assays and protein determination

The GST activity was determined according to Habig et al. (1974) using a 100 mM Sorensen phosphate buffer (pH 6.5), containing organic solvent at an end concentration of, respectively, 0.25% ethanol. Benzene substrate (either CDNB, DCNB or DNIB) and GSH were used at a 0.5 mM and 1 mM final concentrations respectively. The protein concentration of homogenates was determined by the method of Lowry et al. (1951). Serial dilutions of bovine serum albumin were used for the construction of a standard curve that provided the extinction coefficient. During the purification step, the GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as the second substrate. A Shimadzu UV-160A spectrophotometer was used for protein and enzymatic measurements.

1-Chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitro-1-iodobenzene (DNIB), 1,2-dichloro-4-

nitrobenzene (DCNB) were purchased commercially from Fluka Chemical or Merck-Belgolabo corporations.

2.4. Enzyme kinetics

Enzyme kinetics of purified GST from *Aulacorthum solani* and *Acyrthosiphon pisum* aphids were determined for GSH and benzene substrates (CDNB and DCNB) by recording the activity toward a range of concentrations of GSH (0.1–1 mM) or benzene substrate (0.5–0.05 mM) while the concentration of the other substrate was kept constant at 0.5 mM or 1 mM of CDNB and GSH, respectively. Maximal velocity V_{max} and Michaelis constant K_m values for each substrates were determined from Lineweaver–Burk plots.

2.5. Denaturing polyacrylamide gel electrophoresis (SDS / PAGE)

For analytical SDS/PAGE, samples were diluted 1:4 with a solubilizer (1% SDS; 0.02% bromophenol; 1% β-mercaptoethanol in running buffer) and boiled for 3 min before electrophoresis. Separation gels were 12% acrylamide/0.01% SDS in 0.5 M Tris–HCl (pH 8.8). Stacking gels were 3.5% of acrylamide in 1.5 M Tris–HCl (pH 6.8). The Laemmli (1970) discontinuous buffer system was used; 10× running buffer is 2 M glycine/0.1% SDS/0.4 M Tris (pH 8.3). Electrophoresis was carried out at 45 V and 20 mA overnight in a Hoeffer vertical electrophoresis. Gels were stained with Coomassic Brilliant Blue.

3. Results

The purification yield of the affinity chromatography was very different for the two aphid species (Table 1): respectively, 18% (*Aulacorthum solani*) and 34% (*Acyrthosiphon pisum*) of the total CDNB-GST activity in the crude homogenate was desorbed with 15 mM GSH. The GSH- affinity chromatography eluates were used without further purification for SDS-PAGE. Studying the substrate specificity (using a 0.5 mM final concentration of CDNB, DCNB or DNIB) of the GST affinity bound fraction, the specific activities of *A. pisum* were 1.384 ± 0.045 μmol/min per mg, 0.006 ± 0.001 μmol/min per mg and 0.494 ± 0.069 μmol/min per mg with CDNB, DCNB and DNIB, respectively. The *A. solani* GST activities corresponding to CDNB, DCNB and DNIB were 0.481 ± 0.054 μmol/min per mg, 0.010 ± 0.001 μmol/min per mg and 0.002 ± 0.000 μmol/min per mg, respectively.

The electrophoretograms related to *A. pisum* and *A. solani* revealed the presence of 1 and 2 GST band(s), respectively (Fig. 1). The molecular size of the bands are estimated at approximately 25 kDa for *A. pisum* and 27 kDa for *A. solani* when compared to the molecular marker which was used in the gel.

The GST differences among the two aphid species were also observed for kinetic parameters (Fig. 2). At fixed GSH concentrations, the K_m and V_{max} values were 0.069 mM and 0.546 μmol/min per mg (towards CDNB); 0.798 mM and 0.029 μmol/min per mg (towards DCNB) for *A. solani* and were 0.199 mM and 1.710 μmol/min per mg (towards CDNB); 6.207 mM and 0.155

Table 1
Purification of glutathione S-transferases from *Aulacorthum solani* and *Acyrthosiphon pisum*

	Protein (mg/ml)	Activity (μmol/min per ml)	Specific activity (μmol/min per mg)	% Recovery	Fold purification
Aulacorthum solani					
Homogenate	3.26 ± 0.04	0.19 ± 0.01	0.06 ± 0.00	100	1
After PD10	2.14 ± 0.05	0.81 ± 0.09	0.38 ± 0.06	98.4	6.33
Affinity chrom. bound fraction	1.25 ± 0.19	0.76 ± 0.14	0.61 ± 0.11	18	10.11
Acyrthosiphon pisum					
Homogenate	4.02 ± 0.11	0.47 ± 0.04	0.12 ± 0.01	100	1
After PD10	1.82 ± 0.08	0.96 ± 0.10	0.52 ± 0.05	99.1	4.58
Affinity chrom. bound fraction	1.03 ± 0.03	1.61 ± 0.22	1.55 ± 0.21	34	13.27

μmol/min per mg (towards DCNB) for *A. pisum* respectively. At fixed CDNB concentrations, the K_m and V_{max} values were 0.144 mM and 0.478 μmol/min per mg (towards GSH) for *A. solani* and were 1.470 mM and 0.119 μmol/min per mg (towards GSH) for *A. pisum*, respectively.

4. Discussion

Only very few studies involving GST purification were carried out on aphid species even if they are ecologically and economically very important. After ultracentrifugation and before sample elution on the affinity column, the supernatant was applied to a PD10 column. Indeed, our previous purification's of aphid species revealed the presence of endogenous low molecular weight inhibitors in the insect homogenates. This step allowed to suppress the inhibitor presence keeping the nearly total GST activity of the aphid homogenate sample. The affinity chromatography revealed differences between GST from the two aphid species. Indeed, the amount of enzymes which was bound on the column was twice higher for *A. pisum*. The specific activities of the affinity purified GST from *A. solani* was twice lower than the one from *A. pisum*. These activities were both lower than the ones from several lepidopterous species (Yu, 1989).

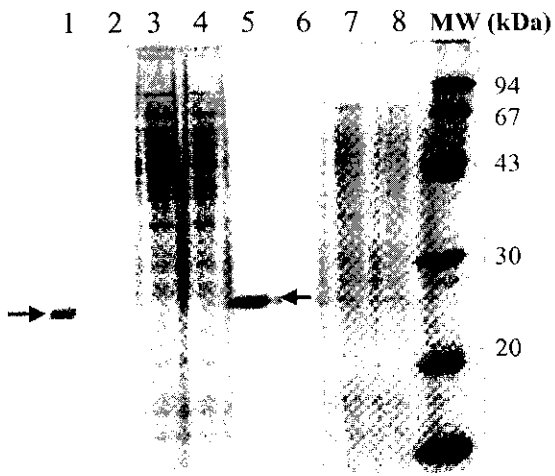

Fig. 1. SDS-PAGE of whole insect homogenates and purified GSTs from *Acyrthosiphon pisum* and *Aulacorthum solani* aphids on 12% polyacrylamide gels. The sizes (kDa) of molecular weight markers (MW) are indicated. Affinity chromatography bound fractions of *A. solani* (lane 1) and *A. pisum* (lane 5) as well as samples of homogenate and after PD10 of *A. solani* (respectively, lanes 3–4) and *A. pisum* (respectively, lanes 7–8) are also represented.

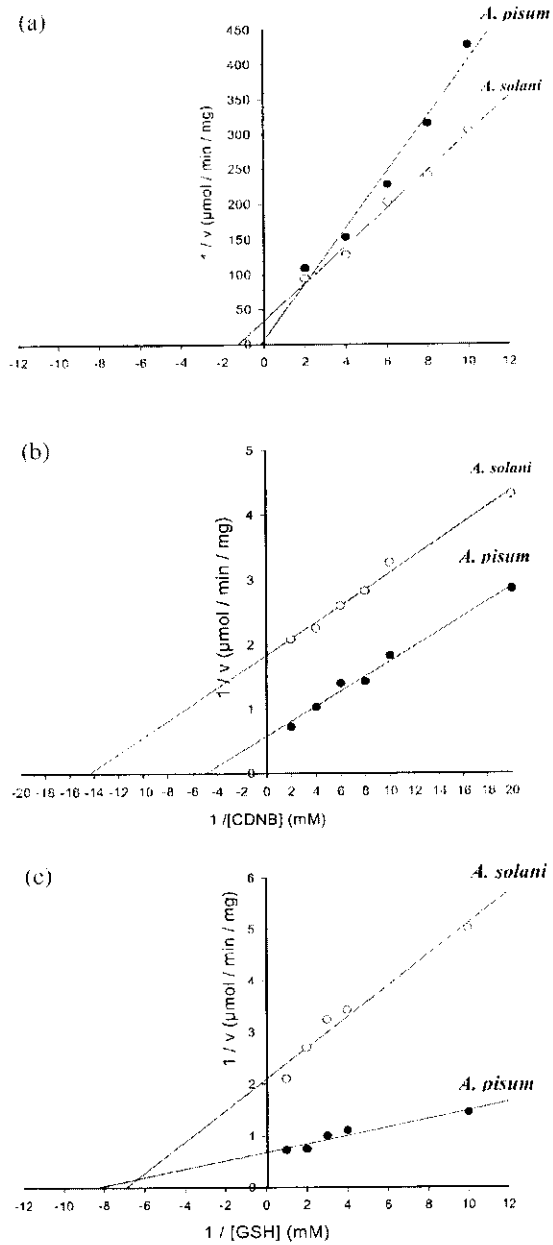

Fig. 2. Lineweaver–Burk plots of DCNB (a), CDNB (b) and GSH (c) conjugation by GST of *Aulacorthum solani* and *Acyrthosiphon pisum*. For measurements related to GSH and benzene substrates (CDNB or DCNB), varying concentrations of GSH (0.1–1 mM) or benzene substrate (0.5–0.05 mM) were used while the concentration of the other substrate was kept constant at 0.5 mM or 1 mM of CDNB and GSH final concentration, respectively.

Differences of specific activities were observed when using three benzene substrates. Whether highest activities were found with CDNB for the two aphid species, GST responses varied when switching from CDNB to DCNB: the specific activities were 220-fold and 50-fold lower with the latter substrate for *A. pisum* and *A. solani* respectively. Changing the optimal CDNB to DNIB also provided differential responses of specific activities: a three- and a twofold decrease for *A. pisum* and *A. solani*, respectively. Finally, comparisons of specific activities in response to the same substrate demonstrated differences between the two aphid species. Calculated ratios between *A. pisum* and *A. solani* activities were 3, 0.66 and 2 for CDNB, DCNB and DNIB substrates, respectively. Most of the GST studies in insects used CDNB or DCNB as benzene substrates (Franciosa and Bergé, 1995). Here, DCNB was shown to not be a sensitive substrate for these GST while DNIB corresponded to interesting results. The same observations were already made with GST from *Adalia bipunctata*, the two-spot ladybird beetle (Francis et al., 1999).

Considering the kinetic assays, important differences were also observed according to the substrate which was used. Indeed, while the K_m value related to *A. solani* toward CDNB was 2.9-fold lower than the one of *A. pisum*, K_m calculated for *A. solani* toward DCNB and GSH variations were 0.13- and 1.21-fold the values corresponding to the ones related to the pea aphid. Changes of V_{max} values were more constant: V_{max} values related to *A. pisum* were always higher than the ones from the other aphid species (3.0-, 3.1- and 3.0-fold higher toward CDNB, DCNB and GSH variations). The use of DNIB substrate induced enzymatic inhibition at optimal CDND concentration. It was the reason why it was not possible to determine the K_m and V_{max} values related to the DNIB substrate.

Glutathione S-transferases on SDS-PAGE electrophoretograms confirmed previous observed differences depending on the aphid species. While two bands at approximately 25 kDa were observed with the affinity bound fraction of *A. solani*, only one band of approximately 27 kDa was visualized for *A. pisum*. According to previous studies on GST isozymes, the molecular weight values are in the same range as the ones studied from several domestic and fruit flies (Clark and Shamaan 1984; Grant and Matsumura, 1989; Fournier et al., 1992). The presence of two distinctly different GST isoenzymes in *A. solani* aphid was in concordance with the observation of Egaas et al. (1991). Indeed, they found two different GST homomers in *Myzus persicae* by SDS-PAGE. The GST subunit molecular weights were 23 and 24 kDa. However, only one band was observed in *A. pisum*. This change can be explained by the feeding behaviour of the aphid species. While the two polyphagous *A. solani* and *M. persicae* are generalist herbivores, *A. pisum* is a Fabaceae specialist. The two generalist pests are commonly found on plants which include soluble secondary compounds or emit volatile allelochemicals. Brassicaceae plant species which include glucosinolates and emit isothiocyanates when infested by herbivore pests are usual host plant of *A. solani* and *M. persicae*. GST system of aphid or Brassicaceae related insects are induced by glucosinolates and isothiocyanates (Wadleigh and Yu, 1988; Weinhold et al., 1990; Francis, 1999) from crucifer plants such as rapes, mustards or cabbages. The gradual decrease in the GST activities observed when aphids were transferred from Brassicaceae plant to non crucifer species might reflect the inducing effect of allelochemical from plant. Following the characterization of these GST from *M. persicae* reared on Brassicaceae or not, Egaas et al. (1991) suggested that there were at least two host plant dependent GST isoenzymes. In contrast, Yu (1982) found only indications of increased levels of existing isoenzymes when various insect species were given allelochemicals. In a more recent work, Yu (1999) reported the induction of new GST isoenzymes in fat bodies of fall armyworm larvae in response to the presence of several allelochemicals.

Altogether, important GST differences were observed for *A. solani* and *A. pisum*. Both aphid species seem to be differently adapted to cope with allelochemicals from Brassicaceae plants. This kind of enzyme variations was already observed in other insects and was correlated with the feeding behaviour of herbivore pests. Yu (1989) demonstrated that the highly polyphagous Lepidoptera (*Spodoptera frugiperda* and *Heliothis zea*) possess multiple GST whereas the more specialized insects (*Heliothis virescens, Trichoplusia ni* and *Anticarsia gemmatalis*) have only a single form of the enzyme. The multiplicity of GST likely plays an important role in the feeding strategies of phytophagous insects. The highly

polyphagous insects may have evolved multiple GST to cope with the diverse toxic allelochemicals encountered in their host plants (Yu, 1989). More specialized insects, feeding on a narrow range of host plants and encountering more specific allelochemicals, have as few as one form of GST. The isozyme composition of GST in herbivore insects may be related to the host plant spectrum (Yu, 1989).

Studies on the XME of generalist herbivores as *A. solani* aphid reared on several plants including allelochemicals would be undertaken to determine accurately the effect of the host plant on the organisms eating them, particularly the characteristics of detoxification enzymes as the GST and probably also other enzymes. Yu (1982) demonstrated that GST induction by allelochemicals increased the tolerance of *Spodoptera frugiperda* to several organophosphate insecticides. As aphid species are important pests in crop cultures, it would be interesting to determine if there is a correlation between induced detoxification enzymes and a potential capability to resist to pesticides. More than an ecological advantage, some herbivore pests might display increasing economical interests only due to their host plant impact.

References

Bogaards, J.J.P., Van Ommen, B., Falke, H.E., Willems, M.I., Van Bladeren, P.J., 1990. Glutathione S-transferases subunit induction patterns of Brussels sprouts, allyl isothiocyanate and goitrin in rat liver and small intestinal mucosa: a new approach for the identification of inducing xenobiotics. Fd. Chem. Toxicol. 28, 81–88.

Boyland, E., Chasseaud, L.F., 1969. The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. Adv. Enzymol. 32, 173–219.

Clark, A.G., 1989. The glutathione S-transferases and resistance to insecticides. In: Hayes, J.D., Pickett, C.B., Mantle, T.J. (Eds.), Glutathione S-transferases and Drug Resistance. Taylor & Francis, London, pp. 369–379.

Clark, A.G., Shamaan, N.A., 1984. Evidence that DDT-Dehydrochlorinase from the house fly is a glutathione S-transferases. Pestic. Biochem. Physiol. 22, 249–261.

Dauterman, W.C., 1989. The role of glutathione S-transferases in herbicide tolerance and resistance. In: Hayes, J.D., Pickett, C.B., Mantle, T.J. (Eds.), Glutathione S-transferases and Drug Resistance. Taylor & Francis, London, pp. 347–357.

Devonshire, A.L., Field, L.M., Foster, S.P., Moores, G.D., Williamson, M.S., Blackman, R.L., 1998. The evolution of insecticide resistance in the peach-potato aphid, Myzus persicae. Phil. Trans. R. Soc. Lond. B 353, 1677–1684.

Egaas, E., Svendsen, N.O., Kobro, S., Skaare, J.U., Jensen, E.G., 1991. Activities and properties of xenobiotic metabolizing enzymes in the peach-potato aphid (Myzus persicae Sultzer) feeding on paprika (Capsicum annuun L.) or swedes (Brassica napus rapifera Metzer). Comp. Biochem. Physiol. 99, 105–110.

Fournier, D., Bride, J.M., Poirié, M., Bergé, J.B., Plapp, F.W., 1992. Insect glutathione S-transferases. Biochemical characteristics of the major forms from houseflies susceptible and resistant to insecticides. J. Biol. Chem.267 3, 1840–1845.

Franciosa, H., Bergé, J.B., 1995. Glutathione S-transferases in housefly (Musca domestica): location of GST-1 and GST-2 families. Insect Biochem. Molec. Biol. 25 (3), 311–317.

Francis, F., 1999. Conséquences évolutives des relations entre le puceron et son prédateur en présence de substances allélochimiques chez les Brassicacae. 5ème Conférence Internationale sur les Ravageurs en Agriculture, Annales ANPP, II, 503–510.

Francis, F., Broon, D., Haubruge, E., 1999. Studies of the glutathione S-transferases activities and their localisation in Adalia bipunctata L. (Coleoptera: Coccinellidae). Med. Fac. Landbouw. Univ. Gent. 64 (3), 349–355.

Grant, D.F., Matsumura, F., 1989. Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant Aedes aegypti. Pestic. Biochem. Physiol. 33, 132–143.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Heaney, R.K., Fenwick, G.R., 1995. Natural toxins and protective factors in Brassica species, including rapeseed. Natural Toxins 3, 233–237.

Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of head bacteriophage T4. Nature 227, 680–685.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent, J. Biol. Chem. 188, 193–265.

Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 1992. The chemical ecology of aphids. Annu. Rev. Entomol. 37, 67–90.

Porter, J.R., Morton, A.M., Kiddle, G., Doughty, K.J., Wallsgrove, R.M., 1991. Variation in the glucosinolates content of oilseed rape (Brassica napus L.) leaves. Effect of leaf age and position. Ann. Appl. Biol. 118, 461–467.

Prapanthadara, L., Promtete, N., Koottathep, S., Somboon, P., Ketterman, A.J., 2000. Isoenzymes of glutathione S-transferase from the mosquito Anopheles dirus species B: the purification, partial characterization and interaction with various insecticides. Insect Biochem. Mol. Biol. 30, 395–403.

Simons, P.C., Vander jagt, D.L., 1977. Purification of glutathione S-transferases from human liver by glutathione affinity chromatography. Analyt. Biochem. 82, 334–341.

Yu, S.J., 1982. Host plant induction of glutathione S-transferases in the fall armyworm. Pest. Biochem. Physiol. 19, 101–106.

Yu, S.J., 1989. Purification and characterization of glutathione S-transferases from five phytophagous Lepidoptera. Pest. Biochem. Physiol. 35, 97–105.

Yu, S.J., 1999. Induction of new glutathione S-transferase isozymes by allelochemicals in the fall armyworm. Pest. Biochem. Physiol. 63, 163–171.

Wadleigh, R., Yu, S.J., 1988. Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. J. Chem. Ecol. 14, 1279–1291.

Weinhold, L.C., Ahmad, S., Pardini, R.S., 1990. Insect glutathione-S-transferase: a predictor of allelochemical and oxidative stress. Comp. Biochem. Physiol. 95B, 355–363.