Archives of Insect Biochemistry and Physiology 58:166–174 (2005)

Glutathione S-Transferases in the Adaptation to Plant Secondary Metabolites in the *Myzus persicae* Aphid

Frédéric Francis,* Nicolas Vanhaelen, and Eric Haubruge

Glutathione S-transferases (GST) in insects play an important role in the detoxification of many substances including allelochemicals from plants. Induction of GST activity in *Myzus persicae* in response to secondary metabolites from *Brassica* plants was determined using different host plant species and confirmed using artificial diet with pure allelochemicals added. The 2,4-dinitro-1-iodobenzene (DNIB) was found to be a useful substrate for identifying particular GSTs in insects. GSTs from *M. persicae* were purified using different affinity chromatography columns and related kinetic parameters were calculated. GST isoenzymes were characterised using electrophoretic methods. Although SDS-PAGE results indicated similarity among the purified enzymes from each affinity column, biochemical studies indicated significant differences in kinetic parameters. Finally, the GST pattern of *M. persicae* was discussed in terms of insect adaptation to the presence of plant secondary substances such as the glucosinolates and the isothiocyanates, from Brassicaceae host plants. Arch. Insect Biochem. Physiol. 58:166–174, 2005.

KEYWORDS: Brassicaceae; aphid; plant-insect co-evolution

INTRODUCTION

Adaptation to plant allelochemicals is one aspect of herbivore chemical ecology (Pickett et al., 1992). The effects of secondary plant metabolites from Brassicaceae on biological parameters of herbivorous insects (e.g., mortality rate, developmental rate, fecundity and egg viability) have been investigated in previous studies (Francis et al., 2000, 2001a–c). Two enzymatic detoxification systems were found to be involved in the adaptation of phytophagous insects to their host plants. One such enzyme, a myrosinase (β-thioglucosidase, E.C.3.2.3.1.) catalyses the degradation of glucosinolates (GLS) in products such as isothiocyanates (ITC), nitriles, thiocyanates, and oxazolidinethiones (Halkier and Du, 1997), and was characterised from *Brevicoryne brassicae*, a specialist on Brassica spp. (Francis et al., 2002a). A second system, the glutathione S-transferases (GST, EC 2.5.1.18), consists of phase II enzymes that play an important role in xenobiotic detoxification, and catalyzes the conjugation of electrophilic molecules with reduced glutathione (GSH) (Boyland and Chasseaud, 1969). GST activity can be induced by the administration of various xenobiotics (Pickett and Lu, 1989) and may confer resistances to these toxicants (Clark, 1990; Ottea and Plapp, 1984). In the generalist herbivore *Myzus persicae*, GST contributes to tolerance to ITC in brassicaceous plants. A multitrophic approach of plant-insect interactions allowed us to demonstrate that GST was also involved when entomophagous predators such as two spot ladybirds *Adalia bipunctata* L. (Francis et al., 2002b) and the hoverfly *Episyrphus balteatus* Degeer (Vanhaelen et al., 2001) were exposed to plant secondary substances. Most works on GST has focused on Lepidoptera (Yu, 1982, 1989, 1999) and Diptera (Clark and Sha-

Department of Pure and Applied Zoology, Gembloux Agricultural University, Gembloux, Belgium

Contact grant sponsor: Fond pour la formation à la Recherche dans l'Industrie et l'Agriculture (FRIA)

*Correspondence to: F. Francis, Dept. of Pure and Applied Zoology, Gembloux Agricultural University, Passage des Départés 2, b-5030 Gembloux, Belgium.
E-mail: francis.f@fsagx.ac.be

Received 24 February 2004, Accepted 13 November 2004

DOI: 10.1002/arch.20049
Published online in Wiley InterScience (www.interscience.wiley.com)

maan, 1984; Fournier et al., 1992; Prapanthadara et al., 1996, 2000). GST from hemipteran species were weakly investigated even though some of them are major pests that are difficult to control due to insecticide resistance (Devonshire et al., 1998).

Resistance to xenobiotics typically results from either a modification of the target site or to amplified production of a detoxification enzyme (Haubruge and Amichot, 1995). Inductions of GST were observed in generalist Lepidoptera species such as *Spodoptera frugiperda* fed on diet including xanthotoxin or indole 3-acetonitrile (Yu, 1984). In this work, the GST response of a polyphagous aphid species, *Myzus persicae*, was determined in relation to secondary substances from brassicaceous host plants. Purification and the characterisation of GST from *M. persicae* were performed using various affinity chromatography methods. The substrate specificity and related kinetic parameters were also determined to identify different GST isoenzymes in the aphid. The response of some GST isoenzymes in *M. persicae* are discussed in relation to aphid adaptation to secondary plant substances and feeding behaviour.

MATERIALS AND METHODS

Chemicals

Reduced glutathione (GSH) was purshased from Janssen Chimica. Sepharose 6B and 4B, Agarose, and PD10 columns including 10 ml of Sephadex G-25 were provided by Pharmacia (Piscataway, NJ). Additional reagents were purchased from Fluka or V.W.R.

Plants and Insects

Broad beans (*Vicia faba* L.) were planted in a mixture of perlite and vermiculite (v:v, 50:50) in 20- x 30-cm plastic trays and grown in a controlled environment room at 20 ± 2°C temperature and 16/8 photoperiod. Two Brassicacae species, *Brassica napus* L. and *Sinapis alba* L., were raised in an ordinary compost in the same environmental conditions as above. Both crucifers and bean plants were inoculated with *Myzus persicae* Sultzer at the 5–6 leaf stage. The aphids had been reared on broad bean plants in the laboratory for several years.

Effect of Plant Allelochemicals on Aphid Glutathione S-Transferase Activity

Aphids were reared for two weeks on each of the three host plant species: *V. faba* as a glucosinolate-free control, and *B. napus* and *S. alba* with low and high rates of glucosinolates, respectively, before being analysed biochemically. Samples of 20 mg of aphids were used for GST activity measurements and each experiment had 5 replicates. The glucosinolate (GLS) contents of each host plant species has been described in a previous report (Francis et al., 2001a).

Artificial diets (10% sucrose solution) including 0.2% sinigrin (allyl-glucosinolate), allyl-isothiocyanate (ITC), or benzyl-isothiocyanate (BITC) were used to feed the aphids for a week. Feeding solutions were prepared and provided fresh daily. The feeding system consisted of a glass tube (3 cm high x 2 cm diameter) containing the liquid diet covered with a double layer of Parafilm®. Twenty aphids were fed per tube and five replicates were performed with each glucosinolate dosage. All aphids from each tube (5 replicates) were pooled and analysed for GST activity measurements.

Purification of Enzyme

Control aphids reared on broad beans were homogenised in a blender in 3 times their volume of 22 mM sodium phosphate buffer, pH 7.0. The homogenate was then ultracentrifuged (1 h, 100,000*g*) and the supernatant applied to a PD10 column (Pharmacia) before application to the affinity column. Three kinds of affinity columns were used: (1) epoxy-activated Sepharose 6B reacted with gluthatione (GSH) as described by Simons and Vander jagt (1977), (2) epoxy-activated Sepharose 4B coupled to GSH (Amersham, Arlington Heights, IL), and (3) epoxy-activated Agarose coupled to GSH (Sigma, St. Louis, MO). The latter two were obtained as ready-to-use media from the manufacturer. Each column was eluted

with 20 mM phosphate buffer, pH 7.0, up to the end of protein detection in the elution buffer. Bound GST were then eluted with 50 mM Tris-HCl, pH 9.6, including 15 mM GSH. One-milliliter fractions were collected and GST activity was assessed. Only fractions with high GST activity were used for the electrophoretic and kinetic studies.

Enzyme Assays and Protein Determination

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 0.25% ethanol. Benzene substrate [either 1-Chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitro-1-iodobenzene (DNIB), or 1,2-dichloro-4-nitrobenzene (DCNB)] and GSH were used at final concentration of 0.5 and 1 mM, respectively. All enzyme activity values were corrected for non-enzymatic conversion rates. The protein concentration of homogenates was determined according to Lowry et al. (1951). Serial dilutions of bovine serum albumin were used for the construction of a standard curve to provide the extinction coefficient. GST activity was measured during the purification step using CDNB as the second substrate. A Shimadzu UV-160A spectrophotometer was used for protein and enzymatic measurements.

Enzyme Kinetics

The enzyme kinetics of purified GST from *M. persicae* were determined for GSH, and CDNB, DNIB, and DCNB substrates by recording activity toward a 0.1–1-mM range of GSH, keeping a 0.5-mM constant CDNB concentration, or a 0.05–0.5 mM range of either CDNB, DNIB, or DCNB, keeping a 1-mM GSH concentration. Maximal velocity V_{max} and Michaelis constant K_m values for each substrate were determined from Lineweaver-Burk plots.

Denaturing Polyacrylamide Gel Electrophoresis (SDS/PAGE)

For analytical SDS/PAGE, samples were diluted 1:4 with a solubilizer (1% SDS; 0.02% bromophenol; 1% b-mercaptoethanol in running buffer) and boiled for 3 min before electrophoresis. Separation gels were 10% acrylamide/0.01% SDS in 0.5M Tris-HCl, pH 8.8. Stacking gels were 3.5% of acrylamide in 1.5M Tris-HCl, pH 6.8. The Laemmli (1970) discontinuous buffer system was used; the 10x running buffer was 2M-glycine/0.1% SDS/0.4M Tris, pH 8.3. Electrophoresis was carried out at 100 V and 50 mA for 2 h in a S-lab gel system (Bio-Rad, Gaithersburg, MD). Due to the low amount of purified proteins, the gels were silver stained using the "Plus one Silver Staining kit" according to the manufacturer's protocol (Bio-rad).

Statistical Analysis

Results of the enzymatic activity measurements were analysed by ANOVA followed by mean separation by the Tukey method using MINITAB software (version 11.2). Means of GST activity were calculated on five replicates.

RESULTS

Effect of Plant Allelochemicals on Glutathione S-Transferase Activity

Plant GLS content (Table 1) influenced *M. persicae* GST activity (Fig. 1). GST activity from aphid reared on *S. alba* was significantly higher than that of aphids reared on the other two plant species ($2.39 < t < 3.12$ and $0.027 < P < 0.005$).

Glucosinolate (sinigrin) and isothiocyanate

TABLE 1. Glucosinolate Contents in Aphid Host Plant (in µmol/g of Fresh Material) by HPLC According to ISO 9167-1 Method (Francis et al., 2001a)

	Host plant leaf		
Glucosinolates	*Vicia faba*	*Brassica napus*	*Sinapis alba*
Brassicanapin	Nd	Nd	<0.01
Glucobrassicin	Nd	0.16 ± 0.01	Nd
Gluconasturtin	Nd	0.49 ± 0.04	1.82 ± 0.01
4OH-glucobrassicin	Nd	<0.01	<0.01
Progoitrin	Nd	0.57 ± 0.00	0.28 ± 0.06
Sinalbin	Nd	Nd	8.83 ± 0.15
Glucoraphanin	Nd	0.37 ± 0.01	Nd
Total	0.00 ± 0.00	1.59 ± 0.04	10.93 ± 0.13

Nd: non-detected glucosinolates.

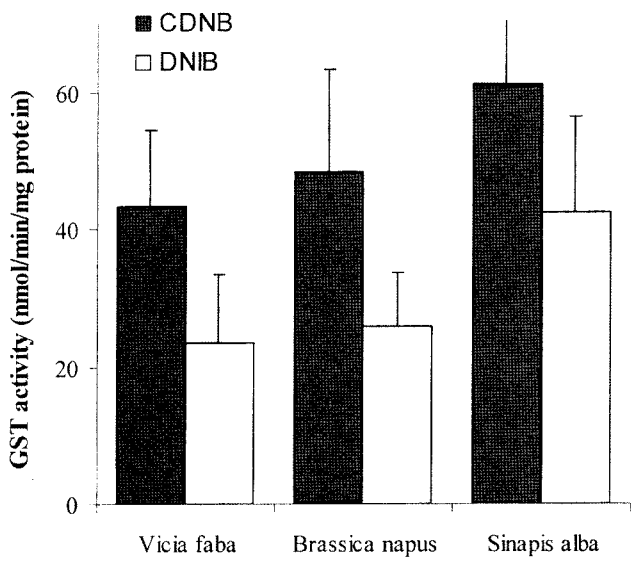

Fig. 1. Variation of gluthatione S-transferase activity of *Myzus persicae* depending on the host plant species. Error bars represented standard deviations of the means (n = 5 replicates). Free glucosinolate control plant was *Vicia faba*.

(AITC and BITC) induction of *M. persicae* GST activity was confirmed in the study employing an artificial diet (Fig. 2). Higher GST activities toward CDNB were observed with all substances tested in the diet ($13.14 < t < 6.45$ and $0.024 < P < 0.049$) whereas the presence of secondary metabolites in

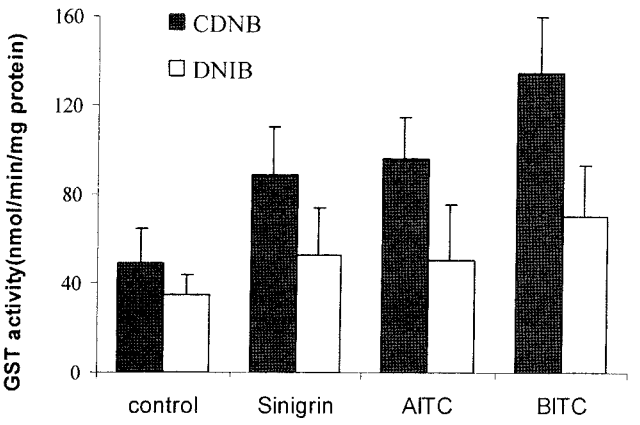

Fig. 2. Variation of gluthatione S-transferase activity of *Myzus persicae* fed with artificial diet including 0.2% of different Brassicaceae secondary substances: sinigrin (glucosinolate), allyl-isothiocyanate (AITC), or benzyl-isothiocyanate (BITC). Error bars represented standard deviations of the means (n = 5 replicates).

TABLE 2. Purification of the Glutathione S-Transferases From *Myzus persicae* Using Epoxy-Activated Agarose and Sepharose 4B Coupled to GSH Affinity Columns

	Proteins (mg/ml)	Specific activity (µmol/min.mg proteins)	Yield (%)	Purification fold
Agarose				
Homogenate	1.63 ± 0.09	0.42 ± 0.06	100.0	1.00
After PD10	0.72 ± 0.04	0.98 ± 0.12	114.1	2.33
Affinity bound fraction	0.22 ± 0.03	6.32 ± 0.68	14.3	15.05
Sepharose 4B				
Homogenate	1.02 ± 0.12	0.44 ± 0.03	100.0	1.00
After PD10	0.62 ± 0.08	0.76 ± 0.05	109.1	1.72
Affinity bound fraction	0.07 ± 0.00	9.72 ± 1.01	38.3	22.11

the artificial diet did not significantly affect aphid GST activity toward DNIB.

Affinity Chromatography

Purification yields varied with the selected affinity column. Epoxy-activated Sepharose 4B coupled to GSH was approximately 50% more efficient in GST purifiction as the epoxy-activated agarose coupled to GSH (Table 2). Due to the reduced GST activity and the very low amount of proteins in the purified GST fractions eluted from the Sepharose 6B column, GST characterisation focused on the GST purified by the two other affinity methods. The elution profiles of *M. persicae* homogenates on both epoxy-activated agarose and sepharose 4B coupled to GSH affinity columns are presented in Figure 3.

Denaturing Polyacrylamide Gel Electrophoresis

The bound GST fractions obtained from affinity chromatography were analysed on electrophoresis gel (Fig. 4). No band was observed using the purified GST fraction obtained from the epoxy-activated sepharose 6B coupled to the GSH affinity column. Purified GST fractions from the two other columns revealed the presence of one band with a molecular weight of 28 kDa.

Kinetic Study

Large differences in kinetic properties toward the tested substrates were observed between the *M.*

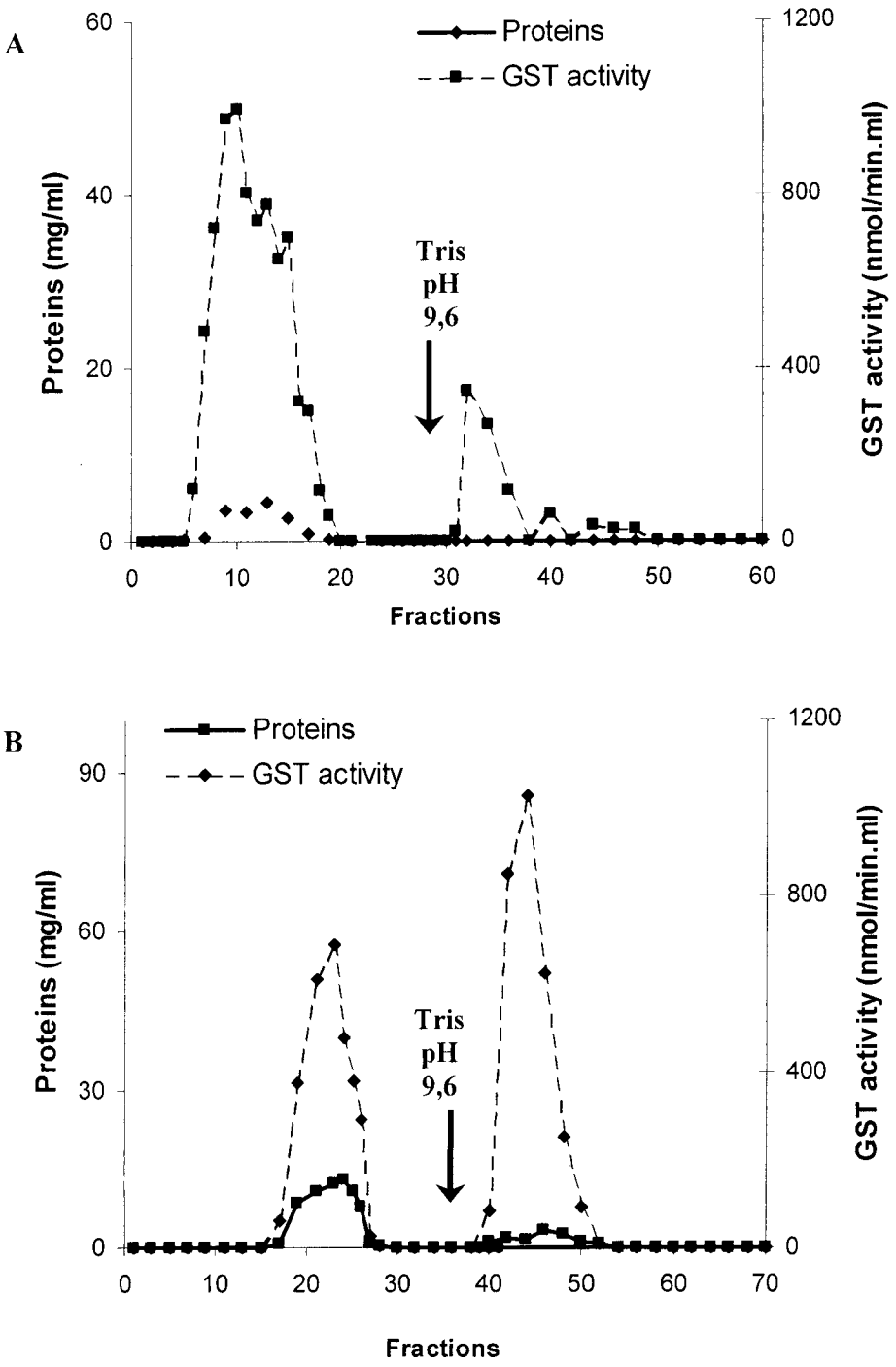

Fig. 3. Elution profile of the glutathione S-transferases from *Myzus persicae* on an epoxy-activated agarose coupled to GSH (A) and epoxy-activated sepharose 4B coupled to GSH (B) affinity column.

persicae GST purified with the epoxy-actived sepharose 4B column and that purified with agarose coupled to GSH (Fig. 5 and Table 3).

DISCUSSION

The presence of secondary plant compounds in particular plant families may be one factor mediating co-evolution between plants and related phytophagous insects (Berenbaum, 1995). The observation of GST inductions in response to the presence of plant allelochemicals in artificial diets has been previously reported in lepidopteran species such as *Sodoptera frugiperda* (Yu, 1984). Similar GST inductions were observed in *M. persicae*

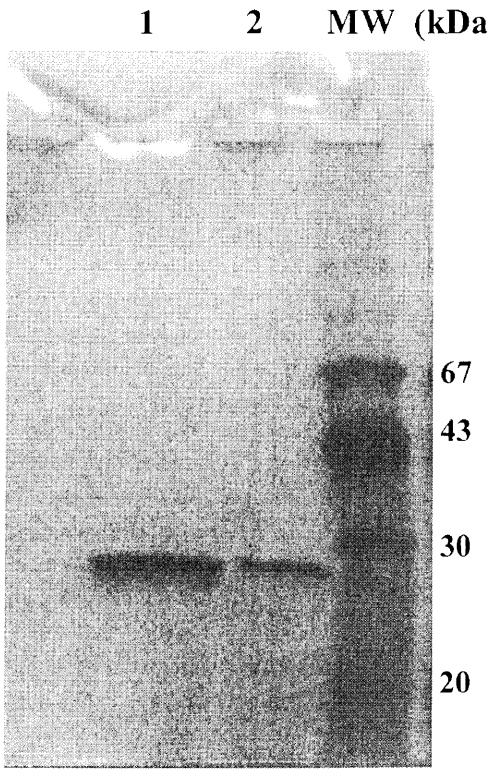

Fig. 4. Electrophoresis in SDS-PAGE of the glutathione S-transferases from *Myzus persicae* purified on epoxy-activated sepharose 4B coupled to GSH (**lane 1**) and epoxy-activated agarose coupled to GSH (**lane 2**) affinity columns. The sizes (kDa) of the molecular markers (MW) are presented.

in the present study when fed with Brassicaceae plants or directly exposed to GLS and ITC.

Different GSTs were purified with varying efficacy according to the kind of affinity chromatography support, sepharose or agarose, coupled to GSH. The specificity of purified GST from *M. persicae* differed depending on the substrate and was very low toward DCNB (unlike many other insects; Franciosa and Bergé, 1995) but high with DNIB, an original substrate for insect GST. Variations of GST specificity toward several substrates have been observed in *Musca domestica* strains and related to different levels of insecticide resistance (Clark et al., 1984). The differential responses of the *M. persicae* purified GST according to the tested substrates allowed us to conclude that at least two GST groups occur. Similar observations have been reported for GSTs from other aphid species, namely *Aulacorthum solani* and *Acyrthosiphon pisum* (Francis et al., 2001c).

The kinetic parameters of *M. persicae* GST varied according to the purification procedure. The K_m of GST eluted from the epoxy activated sepharose 4B affinity column were variable (from a factor 1.1 to 38.2) but were in accordance with those of GST from most insect species ($K_{m\,(CDNB)}$ of 0.025 to 0.294 mM; Prapanthadara et al., 1996). The K_m of GST purified on epoxy-activated agarose coupled to GHS were more stable (5.3 variation factor) independent of the substrate tested. The kinetic study of purified GST extracts confirmed the presence of qualitative differences and, in particular, the occurrence of several GST isoenzymes in *M. persicae.*

Our estimation of the molecular weight of GST from *M. persicae* on the SDS-PAGE gels was in accordance with the results of previous work, yielding sub-unit sizes between 20 and 30 kDa (Clark and Shamaan 1984; Grant and Matsumura, 1989; Fournier *et al.*, 1992). In *M. persicae*, the purified GST appears to be homodimeric, consisting of two 28-kDa sub-units.

GST forms purified from epoxy-activated sepharose coupled to GSH presented higher affinity and efficacy toward CDNB and seemed to be mainly involved in the aphid response to cope with the secondary substance presence in the host plants. The occurrence of increased numbers of GST isoenzymes and overproduced GST by polyphagous insects indicates two possible adaptations of generalist pests for coping with plant secondary metabolites. Whether qualitative and quantitative GST changes were related to the feeding behaviour of insects (depending on the allelochemical presence in diets and host plants), an other enzymatic detoxication system, cytochrome P-450, is involved in the metabolisation of a broad range of xenobiotics and secondary metabolites in herbivore host plants (Cohen et al, 1992). The furanocoumarins in *P. polyxenes* was detoxified by the expression of multiple enzymes of cytochrome P450 monooxygenase and also of GST (Hung et al., 1995). More generally, polyphagous insects can selectively express a broad range of enzymes that assist in the

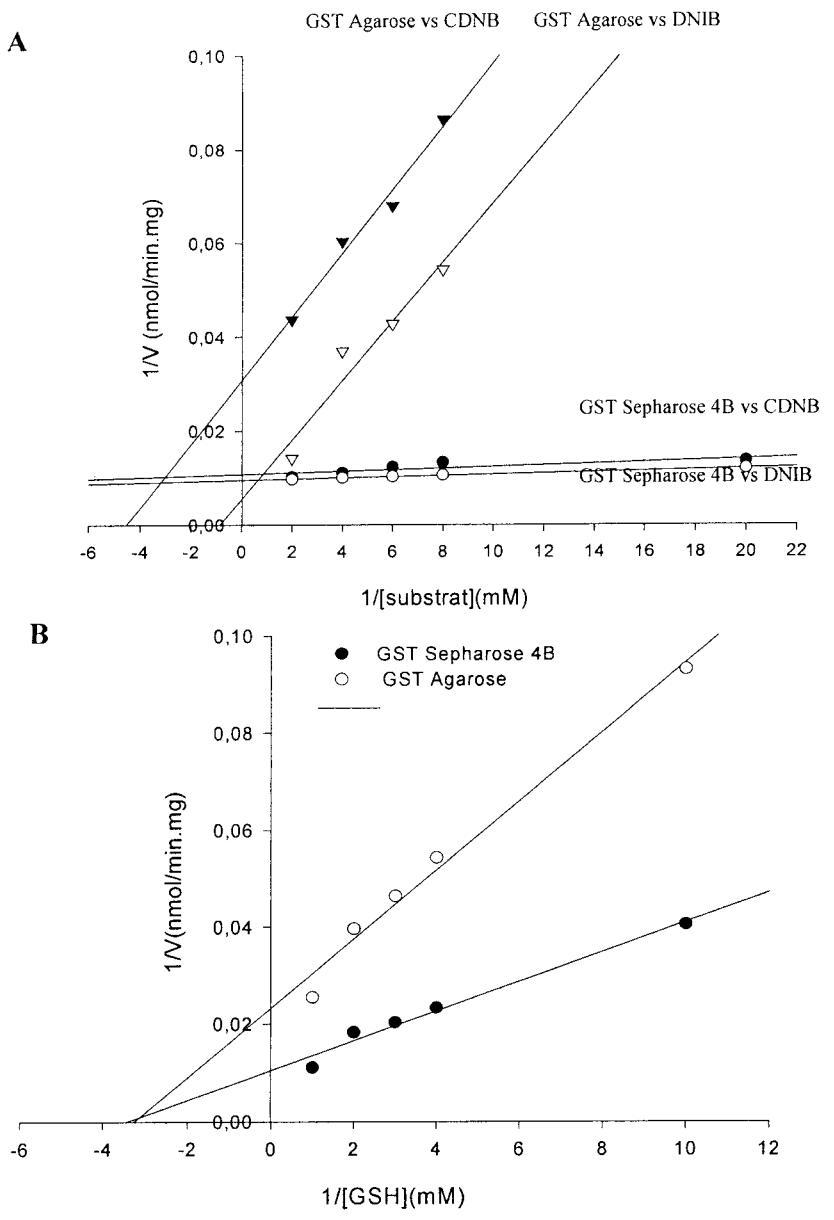

Fig. 5. Regressions according to Lineweaver-Burk of the conjugation of DNIB and CDNB (A) and GSH (B) by the GST from *Myzus persicae* purified using epoxy-activated sepharose 4B coupled to GSH and epoxy-activated agarose coupled to GSH affinity columns. To measure the enzymatic activity by changing the substrate amounts, the concentration of the other substrate was constant at 0.5 or 1 mM of CDNB or GSH, respectively. The curve related to the activity variation of GST purified with epoxy-activated sepharose 4B coupled to GSH depending on the rate of DCNB was not presented due to the too high difference of the Y axis scale.

TABLE 3. Kinetic Properties of *Myzus persicae* GST Purified on Epoxy-Activated Sepharose 4B and Agarose Coupled to GSH Affinity Columns*

Properties	Substrate	Sepharose 4B	Agarose
K_m (mM)	CDNB	0.015	0.221
	DNIB	0.013	1.163
	DCNB	0.497	—[a]
	GSH	0.291	0.307
V_{max} (nmol/min.mg)	CDNB	92.76	32.62
	DNIB	105.13	184.13
	DCNB	3.30	—[a]
	GSH	95.43	43.02

*V_{max} and K_m were calculated using a constant concentration of 1 mM GSH or benzene substrate.
[a]As the enzymatic activities from the first concentration reduction were nil, the kinetic parameters were not calculated.

detoxification of numerous xenobiotics including secondary metabolites from plants (Li et al., 2000).

ACKNOWLEDGMENTS

The authors thank Dr. Paul Dirieckx from the Scientific Institute of Public Health, Division Toxicology at Brussels, Belgium, for his collaboration in work on the GST purification. Prof J.P. Michaud from Kansas State University, Agricultural Research Centre in Hays, is gratefully acknowledged for his comments on the English language version of the manuscript. Nicolas Vanhaelen was funded by the

Fond pour la formation à la Recherche dans l'Industrie et l'Agricultrure (FRIA).

LITERATURE CITED

Berenbaum MR. 1995. The chemistry of defence: theory and practice. Proc Natl Acad Sci USA 92:2–8.

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

Clark AG. 1990. The glutathione S-transferases and resistance to insecticides. In: Hayes JD, Pickett CB, Mantle TJ, editors. Glutathione S-transferases and drug resistance. London: Taylor & Francis. p 369–379.

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

Clark AG, Shamaan NA, Dauterman WC, Hayaoka T. 1984. Characterisation of multiple gluthatione S-transferases from the house fly, *Musca domestica* (L.). Pestic Biochem Physiol 22:51–59.

Cohen MB, Schuler MA, Berenbaum MR. 1992. A host inducible cytochrome P-450 from a host specific caterpillar: molecular cloning and evolution. Proc Natl Acad Sci USA 89:10920–10924.

Devonshire RL, Field LM, Foster SP, Moores GD, Williamson MS, Blackman RL. 1998. The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*. Phil Trans R Soc Lond B 353:1677–1684.

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

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

Francis F, Haubruge E, Gaspar C. 2000. Influence of host plants on the development of the specialist/generalist aphid and on their natural predator *Adalia bipunctata*. Eur J Entomol 97:481–485.

Francis F, Lognay G, Wathelet JP, Haubruge E. 2001a. Effects of allelochemicals from first (Brassicaceae) and second (*Myzus persicae* and *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. J Chem Ecol 27:243–256.

Francis F, Haubruge E, Hastir P, Gaspar C. 2001b. Influence of the aphid host plant on the development and reproduction of the third trophic level, the predator *Adalia bipunctata* L. (Coleoptera:Coccinellidae). Env Entomol 30:947–952.

Francis F, Haubruge E, Gaspar C, Dierickx PJ. 2001c. Glutathione S-transferases of *Aulacorthum solani* and *Acyrthosiphon pisum*: partial purification and characterization. Comp Biochem Physiol 129:165–171.

Francis F, Lognay G, Wathelet JP, Haubruge E. 2002a. Characterisation of aphid myrosinase and degradation studies of glucosinolate. Arch Insect Biochem Physiol 50:173–182.

Francis F, Dierickx P, Haubruge E. 2002b. Isoenzymes of glutathione S-transferase from the two-spots ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). Arch Insect Biochem Physiol 49:158–166.

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

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases : the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139.

Halkier BA, Du L. 1997. The biosynthesis of glucosinolates. Trends Plant Sci 2:425–431.

Haubruge E, Amichot M. 1995. Les mécanismes responsables de la résistance aux insecticides chez les insectes et les acariens. *BASE* 2:161–174.

Hung CF, Prapaipong H, Berenbaum MR, Schuler MA. 1995. Differential induction of cytochrome P450 transcripts in *Papilio polyxenes* by linear and angular furanocoumarins. Insect Biochem Molec Biol 25:89–99.

Laemmli UK. 1970. Cleavage of structural proteins during assembly of head bacteriophage T4. Nature 227:680–695.

Li X, Berenbaum MR, Schuler MA. 2000. Molecular cloning and expression of CYP6B8: a xanthotoxin inducible cytochrome P450 cDNA from *Helicoverpa zea*. Insect Biochem Mol Biol 30:75-84.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275.

Ottea JA, Plapp FW. 1984. Glutathione S-transferase in the house fly: biochemical and genetic changes associated with induction and insecticide resistance. Pest Biochem Physiol 22:203–208.

Pickett CB, Lu AYH. 1989. Glutathione S-transferases: gene structure, regulation and biological function. Ann Rev Biochem 58:743–764.

Pickett JA, Wadhams LJ, Woodcock CM. 1992. The chemical ecology of aphids. Annu Rev Entomol 37:67–90.

Prapanthadara L, Koottathep S, Promtet N, Hemingway J, Ketterman AJ. 1996. Purification and characterization of a major glutathione S-transferase from the mosquito *Anopheles dirus* (species B). Insect Biochem Mol Biol 26:277–285.

Prapanthadara L, Promtete N, Koottathep S, Somboon P, Ketterman AJ. 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 PC, Vander jagt DL. 1977. Purification of glutathione S-transferases from human liver by glutathione affinity chromatography. Anal Biochem 82:334–341.

Vanhaelen N, Haubruge E, Francis F. 2001. Effects of Brassicaceae secondary metabolites on the Glutathione S-transferase activity from *Episyrphus balteatus* De Geer (Diptera: Syrphidae). Pest Biochem Physiol 71:170–177.

Yu SJ. 1982. Host plant induction of glutathione S-transferase in the fall armyworm. Pest Biochem Physiol 18:101–106.

Yu SJ. 1984. Interactions of allelochemicals with detoxification enzymes of insect-susceptible and resistant armyworm. Pest Biochem Physiol 22:60–68.

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

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