EFFECTS OF ISOTHIOCYANATES ON THE GLUTATHIONE S-TRANSFERASES ACTIVITY FROM *ADALIA BIPUNCTATA* L. (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

Insects are sensitive to biochemical aspects of their environment, chiefly to their host plants. Glucosinolates and their volatile metabolites (isothiocyanates, ITC) have been implicated for pests infestation on Brassicaceae plants (David and Garnier, 1966). Moreover, the cultivation of low glucosides rape varieties has not lead to differences in crucifer pests attack levels (Lamb, 1989). Derivative compounds from glucosinolates are powerful stimulants for Brassicacae specialist aphids but they could be tolerated by the polyphagous *Myzus persicae* Sulzer (Nault and Stayer, 1972). Induction of glutathione S-transferase (GST) has been observed in *M. persicae* infested Brassica species (Egaas and al, 1991). These enzymes induction is considered as a response to the presence of several inducers/inhibitors, changing the insects susceptibility to insecticides (Yu, 1982).
Ladybirds are in contact with these ITC compounds when they search or they consume aphids on Brassicacae species plants. In this study, the evolution of GST induction in relation to increasing concentrations of some ITC in *Adalia bipunctata* L. are reported. Mortality curves are also established for glucosinolates metabolites or ITC compounds. Effects of isothiocyanates compounds on the enzymatic responses in ladybirds are discussed.

INTRODUCTION

Glucosinolates are secondary plant substances found in several plant families, mainly in Brassicacae species. Degradation of these glucosides by myrosinase enzyme leads to isothiocyanates (ITC) releases. These compounds display toxic effects and constitute part of plant's defence against fungal and insect infestation (Heaney and Fenwick, 1995). ITC can play a role as repellents or attractants depending of the insect pest specificity. While Brassicacae specialist are stimulated to feed and oviposit by these substances, generalist herbivores are generally deterred. Due to its high polyphagy, *Myzus persicae* Sultzer is found on Brassicacae plant and tolerates the glucosinolates and their degradative compounds (Nault and Stayer, 1972).
Glutathion S-transferases (GST) exhibit a broad substrate specificity and catalyse the conjugation of reduced glutathione (GSH), via the formation of a thioether bond, with numerous electrophiles (Hayes and al, 1990). This increased ability to metabolize many drugs and toxic substances imply important detoxication and may lead to insecticides resistance (Clark, 1990). This system of metabolization seems to be affected by the glucosinolates/ITC presence. Indeed, Egaas *et al.* (1991) demonstrated the GST induction in aphids exposed to ITC compounds.
Induction of GST activity can have significant effect on the herbivores pest populations. New opportunities to cope with natural toxins or chemical

products could be induced in insects by these higher enzymatic activities. Selective advantage of populations from crucifer host plant could be observed. In the same way, the natural predators of phytophagous insects are also in touch with the volatile plant substances when they search or consume their preys. Evolutive and chemical approaches of this trtitrophic model (plant-prey-predator) is essential to understand the relations between each level. Whether secondary plant metabolites (called allelochemicals) display major role in phytophagous pests infestation (Lerin, 1980; Brattsten, 1988; Yu & Hsu, 1993), they also can have effects on biological and biochemical performances of the predator. Signals from second trophic level are generally weaker than these occurred from plants. Indeed, plant biomass is larger and the volatile compounds production is generally much higher. Moreover, minimization of odor emission by herbivores is one way to escape paratization and predation. To identify their prey, parasitoids and predators rely on molecules emitted by host plant rather than coming from their prey. Many factors can also influence odors from predator preys : the nutritional diet, the insect physiology...(Vet and Dicke, 1992).

Changes in GST activities in insects can correspond to different chemicals susceptibilities. Whether enzymatic system of aphids species are induced on Brassicaceae, it can be correlated to higher resistance to insecticides and thus to lower pest control. As ladybirds are natural predators of aphids, they can be used as biological control agent. In this way, beetles are in closed contact with the aphid host plant and are submitted to volatile plant compounds. What is the ITC efficacy on *Adalia bipunctata* L. behaviour? Are these substances responsible of biochemical changes in the third trophic level? Some ITC molecules are known to be used as fumigants and displayed strong toxic effects to insects and mammals. The aim of this study is to determine the effect of such compounds on ladybirds. When exposed in their natural environment, are GST activity induced ? From which concentration is there enzymatic changes? Is there important mortality rates corresponding to such GST increasing activity and ITC concentrations ?

MATERIAL AND METHODS

Rearing of ladybirds

Broad beans (*Vicia fabae*) were raised in 20 x 30 cm tray including a mixture of perlite/vermiculite in a controlled environment room at 20±2°C temperature and 16 hours daylight photoperiod. Plants were only watered when the substrate was dry. *Acyrthosiphon pisum* (Harris) were mass reared on bean which were inoculated when they had 2-3 true leaves.

Larvae of *Adalia bipunctata* were raised in rectangular plastic boxes at 15±1°C and 16 hours daylight photoperiod. After hatching, 15 first instar larvae were placed per box. At emergence, 25 to 30 adults of ladybirds were pooled in larger plastic boxes (3,5 litres). Every other day, the ladybirds were fed with plenty of fresh aphids. Section of a stem of broad bean was put in each rearing box to keep the aphids alive as long as possible and to provide a humidity source.

Filter papers were used in plastic boxes to increase contact areas and to receive layings of eggs once at the adult stage. Every day, the filter papers were removed to collect the eggs layings. The latter were placed at 15±1°C in 5 cm Petri dish to allow hatching. Plastic boxes were changed every week.

Chemicals

Methyl-isothiocyanate (MITC), allyl-isothiocyanate (AITC) were purchased from Aldrich Chemical company and prepared in ethanol solution.
1-Chloro-2,4-dichloro-4-nitrobenzene (CDNB) was prepared in ethanol at 200 mM solutions. Reduced Glutathione (GSH) was used as distilled water solution at 100 mM concentration. All products were purchased commercially from Fluka Chemical or Vel company.

Toxicity test and LC_{50} determination

Ladybirds exposure to volatile ITC were held in 800 ml glass dessicator. Twenty adults of *Adalia bipunctata* were placed in the container and the test was repeated twice for each concentration. Ten micro-litres of ethanol ITC solution were applied before closing the hermetic containers. Dead numbers were assessed after 24 hours. Mortality curves and LC_{50} were determined for each studied substance using the log-probit (vs 2) software.

Determination of Glutathione S-transferase activity

Surviving individuals of *Adalia bipunctata* were crushed in 1,5 ml eppendorf tube containing 600 ml of phosphate buffer (pH 7.0). Homogenates centrifugation were carried out in a Rotor Sigma 2K15 for 15 minutes at 4°C and 15000 g. Fifty micro-litres of supernatant were added to 930 ml of phosphate buffer, 10 ml of GSH solution and 10ml of substrate (CDNB or DNIB). Control were composed of 950 ml of buffer and 50 ml of supernatant.
Protein concentration was determined by the Bio-Rad method (Bradford method) with bovine serum albumin as reference. A Shimadzu UV-160A spectrophotometer was used for enzyme activity (at 340 nm) and protein concentration (at 750 nm) measurements.

RESULTS

Toxicity tests

No toxic effects on ladybirds were observed before 0.05 ppm and 0.075 ppm respectively for AITC and MITC (Figure 1). Evolution of mortality following increasing ITC concentrations corresponded to a sigmoïd shape for both ITC compounds. Mortality rates due to MITC seemed to be lightly higher than those induced by similar concentrations of AITC. No ladybirds survived to 1.5 ppm ITC exposure. LC_{50} and LC_{90} of AITC and MITC were reported in table 1.

a)

b)

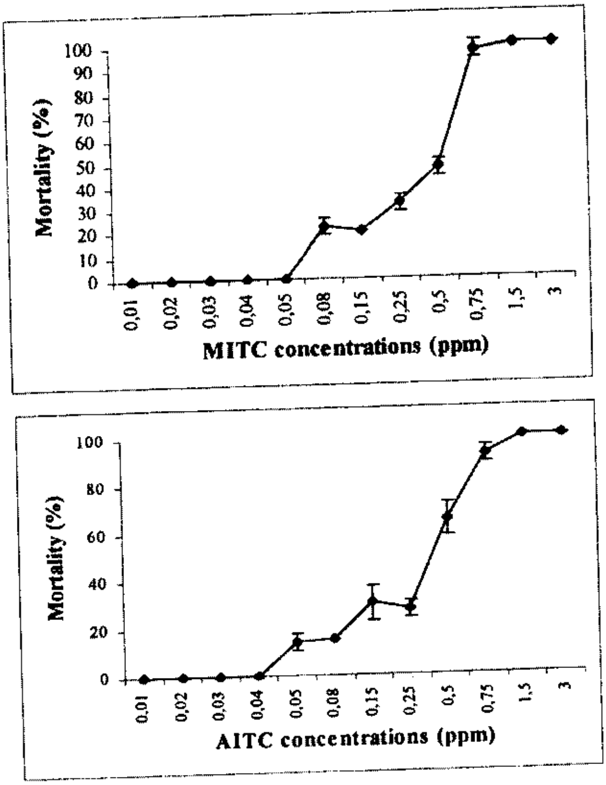


Figure 1 : Fumigant effects (% mortality) on adults of *Adalia bipunctata* exposed to several MITC (a) and AITC (b) concentrations

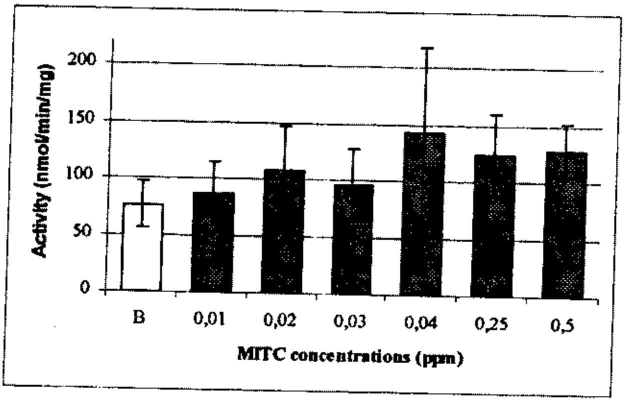
Table 1 : Toxicity of isothiocyanates on adults of *Adalia bipunctata*

Substance	LC_{50} (ppm)	LC_{90} (ppm)	χ^2 value
AITC	0.189<LC<0.308	0.720<LC<1.864	4.85
MITC	0.218<LC<0.551	0.385<LC<2.466	14.18

Enzyme activity

Increasing GST activity toward CDNB was observed following the increased concentration of ITC which was used for the toxic exposure (Figure 2). While inductions were underlined from very low concentration (0.01 ppm), a constant level of activity was obtained from 0.25 ppm. At this stage, higher mortality were related to higher ITC exposure without increased enzymatic activities. When comparisons of mortality observations and GST activites were made, enzymatic activity increased until a threshold without mortality. When this limit amount of GST was reached, higher ITC concentrations induced significant mortality. From 0.5 ppm of ITC, important lethal effects were rapidly obtained.

a)



b)

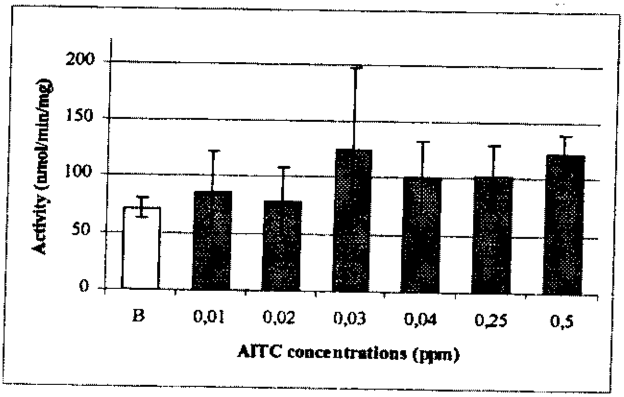


Figure 2 : Glutathione S-transferase activity of *Adalia bipunctata* adults following MITC (a) and AITC (b) exposures

DISCUSSION - CONCLUSION

Glucosinolates metabolites displayed strong toxic effects on the ladybirds from 0.5 ppm of AITC or MITC concentrations. AITC are released from plants which include sinigrin (as mustards, oilseed rape). Are these concentrations occurring in the natural environment of the aphid predator or not? Anyway, this experiment showed that enzymatic changes were related to ITC presence even at very low concentration. This phenomenon was already observed in aphids population (Egaas *et al*, 1991) and could be associated with potential insectides resistance. GST belong to the enzymes which detoxify xenobiotics and can therefore cause insect resistance to insecticides. Some insects species possess sets of GST enzymes with different substrate specificity but other species seem to produce only a single GST (Schoknecht 1992). At this stage, some relevant questions must be raised: is the occurrence of isoenzymes connected with a shift in substrates specificity and which isoenzymes conjugating insecticides of different chemical structures ?

Is there a relationship between the expression of GST enzymes and an evolutionnary process linked to the insect environment?

A co-evolution of prey-predator biochemical properties seems to be happened. In this experiment, GST activity of *Adalia bipunctata* is linked to the allelochemicals presence. The ladybirds sensitivity to these volatile compounds could be explained if these substances played a role in their prey detection. The release of herbivore induced plant volatiles that act as reliable and detectable host-location cues for parasitoids and predators has previously been demonstrated for several tritrophic systems. Weak responses observed to undamaged plants and to host aphids in the absence of the plant suggest that chemicals emitted from these sources were not efficient cues for foraging *Aphidius ervi* (Aphidiidae) (Du *et al*, 1990).

Beetles as *Meligethes aeneus* and *Ceuthorrynchus assimilis* displayed positive a response to Brassicacae ITC (illustrated by a 8 to 23 higher factor captures when allyl-isothiocyanate was used with yellow trap) (Lerin, 1980). GST activity was inducible by dietary allelochemicals which also stimulate other detoxification enzymes such as hydrolases and reductases (Wadleigh & Yu, 1988). The presence of high level of GST in *Papilio glaucus* was coupled with large amount of esterase (Lindroth, 1989). Nevertheless, the tolerance of plant allelochemicals does not necessary implicate cross-resistance with insecticides. Adaptation of insects to natural toxic substances may involve the evolution of particular isoenzyme forms that exhibit little activity against insecticides. Inhibition of insect enzyme by allelochemicals in the gut can also reduce their activity towards some agricultural chemicals.

Derivation of known biocidal compounds is a promising way to search for novel insecticides. Mustard oil derivatives of plant origin displayed interesting insecticidal or insects growth regulators properties (Weber *et al*, 1992). Acurate knowledges of biological effects of glucosinolates and their derivatives must be determined not only for insect pests but also for their natural predators or parasitoïds. Indeed, integrated pest management includes their selection to have efficient way of pest control. Comprehension of infochemical production by both the host plant and the pest population is the only way to ensure successful biological control and enhance the selection of plant cultivars which lead to efficient behaviour of natural enemies. Thus, the efficacy of pests control may be strongly influenced by insect host plant.

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