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Effect of diet on detoxification enzyme activity of *Platynota idaeusalis* (Walker) (Lepidoptera: Tortricidae) larvae strains

Efecto de la dieta en la actividad enzimática de detoxificación de larvas de *Platynota idaeusalis* (Walker) (Lepidoptera: Tortricidae)

O. E. Domínguez-Gil y B. A. McPheron

Abstract

The effect of diet on detoxification enzyme capabilities in two genetically similar strains (one susceptible and other resistant to azinphosmethyl) of the tufted apple bud moth, *Platynota idaeusalis* (Walker) (Lepidoptera: Tortricidae), was studied by biochemical methods. The larvae were fed with a synthetic diet and four different host plant species: apple, *Malus domestica* (Bork.) cv Red Yorking; black raspberry, *Rubus occidentalis* L.; broad-leaved plantain, *Plantago major* L.; and dandelion, *Taraxacum officinale* Wiggers. Host plant affected larval detoxification enzyme activity in both the resistant and susceptible strain. Glutathione transferase and esterase activities, both implicated in *P. idaeusalis* resistance to azinphosmethyl, varied significantly between strains and among hosts. Diets of apple and plantain appeared to inhibit both enzyme systems compared to artificial diet in both insect strains. However, patterns of enzyme activity and azinphosmethyl susceptibility are not clearly linked, reinforcing the complex relationship of the insect with the chemistry of its host.
Key words: diet-insect interaction, *Platynota idaeusalis*, resistance, detoxification enzyme activity.

Resumen

Se examinó el efecto de la dieta en la capacidad de la actividad enzimática de detoxificación a insecticida de dos razas similares genéticamente (una susceptible y una resistente a azinphosmethyl) de la "polilla de la manzana" *Platynota idaeusalis* (Walker) (Lepidoptera: Tortricidae), fue examinada. Las larvas objeto del estudio fueron alimentadas separadamente con una dieta artificial y cuatro diferentes especies de plantas hospederas: manzana, *Malus domestica* (Bork.) cv Red Yorking; mora, *Rubus occidentalis* L.; llantén, *Plantago major* L.; y diente

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de león, *Taraxacum officinale* Wiggers. Las plantas hospederas afectaron la actividad enzimática de detoxificación de las larvas tanto resistentes como susceptibles. La actividad de las enzimas glutationa transferasa y esterasas, ambas implicadas en la resistencia de *P. idaeusalis* a azinphosmethyl difirieron significativamente entre razas y hospederas. Las dietas de manzana y llantén parecen inhibir ambos sistemas de enzimas cuando se compararon con la dieta artificial en ambas razas del insecto. Sin embargo, la actividad enzimática y la susceptibilidad a azinphosmethyl no están claramente ligadas, indicando la compleja relación que existe entre el insecto y la química de su hospedero.
Palabras clave: interacción dieta-insecto, *Platynota idaeusalis,* resistencia, actividad enzimática de detoxificación.

Introduction

Currently, insecticide resistance is a serious problem in *Platynota idaeusalis* (Walker) control. This tortricid has developed resistance to many of the organophosphate (OP) insecticides most commonly used in apple orchards during the past 30 years (32, 23, 24). These increased resistance levels have resulted in field failures of OP insecticides (25). Fruit growers have increased applications of carbamates (22) and pyrethroid insecticides (23) in response to the development of OP resistance. The use of these alternative insecticides can disrupt carefully balanced integrated pest management (IPM) programs developed in apple orchards over the last 20 years (15, 7). Moreover, OPs, so-called IPM-compatible materials, have been used to selectively control up to 15 other pests that attack apples (Hull 1991, unpubl. data). Therefore, preserving *P. idaeusalis* susceptibility to currently available OP compounds is valuable until we have other IPM-compatible control measures. It is important to consider which factors influence the loss of *P. idaeusalis* susceptibility and to obtain a basic understanding of non-genetic influences (diet, age and deve-lopment, temperature) on the expre-ssion of insecticide resistance. Previous studies in some insect species have shown that host plants also affect le-vels of resistance (46, 6, 36, 41, 37, 43, 17). Even though some insects have genes for insecticide resistance, something in the plant appears to modify the expression of resistance. One important factor is the effect of larval host plant chemistry on detoxification capabilities. It has long been known that feeding on certain host plants can induce and/or suppress enzymes involved in the detoxification of pesticides. Detoxification of plant allelochemicals by enzyme systems has been shown to be one of the most important ways that insects deal with plant diets (27). At least 27 species of arthropods have been found to have detoxification systems inducible by plant chemicals (47). Because *P. idaeusalis* is a highly polyphagous species, there is a high probability for this insect to feed on a wide range of plants with unique se-condary compounds. *Platynota idaeusalis* experiences a wide array of different compounds in

its use of 17 plant families (30). Numerous studies with the highly polyphagous gypsy moth, which utilizes over 300 species of trees and shrubs from at least 14 plant families (16, 26), have shown that food selection and utilization of both hosts and nonhosts are related to plant allelochemicals (4, 38, 29). The same enzymes that are involved in metabolism and detoxification of pesticides are also involved in the metabolism of plant allelochemicals. Thus, it is reasonable to assume that OP insecticides and plant secondary chemicals may interact to produce positive or negative synergistic effects upon OP toxicity in *P. idaeusalis*. Hunter *et al.* (21) determined that an apple allelochemical, phloridzin, influenced detoxification activities of larval *P. idaeusalis*. Phloridzin decreased GST activity in both susceptible and resistant *P. idaeusalis*. Also, phloridzin inhibited esterase and aniline hydroxylation of the susceptible larvae, but induced higher esterase activity in resistant larvae. Therefore it is critical to investigate the effect of host plants on levels of some enzyme systems that are related with patterns of resistance to OPs. The interaction of *P. idaeusalis* with its host plants has not been extensively studied and affects many components of pest management. Natural enemies may use non-crop plant species to feed in the adult stage. Alternative hosts may harbor susceptible or resistant *P. idaeusalis* populations which can disperse into the orchard environment. Results of this study can be combined with other research results to help design the next generation of pest management strategies. They may also be used to preserve the effectiveness of currently recommended OP insecticides, which are IPM compatible materials, until these new strategies can be implemented. The present study measured detoxification enzyme activity of glutathione transferase and carboxylesterases (previously implicated in pesticide resistance (12, 13, 11) on susceptible and resistant *P. idaeusalis* larvae reared on four host plants and artificial diet.

Materials and methods

Chemicals. Glutathione (98-100% reduced), a-naphthyl acetate (a-NA), a-naphthyl butyrate (a-NB), and 1,2- dichloro-4-nitrobenzene (DCNB) (95% purity), were purchased from Sigma Chemical Co. (St. Louis, MO). 1-chloro-2,4-dinitrobenzene (CDNB) (99% purity) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Coomassie Blue G-250 was purchased from Serva Company. All other chemicals were of analytical quality and purchased from commercial suppliers.

Host Plants. Host plant species (table 1) were raised in the greenhouse at the Pennsylvania State University, University Park. Apple is the economically important host of *P. idaeusalis*; the other three species are present in the apple orchard and surrounding vegetation. The apple trees were from the cultivar Red Yorking on Emla 7 root stock. Black raspberries were started from cuttings. The remaining host species were grown from seed in potting soil. All plants were trans-

Table 1. Plant species used in fitness study for *Platynota idaeusalis*.

Common name	Scientific name	Source	Variety	Family
Apple	*Malus domestica* (Bork.).	Adams County Nursery, Aspers, PA.	Red Yorking	Rosaceae
Black raspberry	*Rubus occidentalis* L.	Miller Nurseries, Canandaigua, NY.	Allen	Rosaceae
Broad-leaved plantain	*Plantago major* L.	B. McPheron house, Port Matilda, PA.		Plantaginaceae
Dandelion	*Taraxacum officinale* Wiggers	Stokes Seeds, Inc., Fredonia, NY.		Asteraceae

planted to pots filled with Terra Lite Metro-Mix 250® Growing medium (E. C. Geiger, Harleysville, Pennsylvania). Plants were fertilized every two months with water-soluble 15-30-15 (N-P-K) fertilizer (4.0 g/l). Plants were watered as needed and grown under a 16-h photophase and ambient relative humidity. Whitefly, thrips, mite and aphid infestations in the greenhouse necessitated the use of 2.5% safer insecticidal soap (50.5% potassium salts of fatty acids, AgroChem, Jamul, California). As a precaution, all host species were treated at the same time. Test plants were selected arbitrarily from a group of individual plants that were similar in height and phenology. For comparison with previous studies, *P. idaeusalis* larvae were also reared on a lima bean based artificial diet (31) under identical environmental conditions.

Insect cultures. The genotype resistant to azinphosmethyl used in this experiment is a line originally derived from resistant larvae collected in an apple orchard in Adams County, Pennsylvania and made nearly isogenic relative to a laboratory susceptible line. The latter was accomplished by the protocol of backcrossing and selection detailed as followed: larvae were collected from apple orchard with a history of resistance in Adams County, Pennsylvania. Field-collected larvae were reared to pupation, sexed, and mixed in two reciprocal crosses (male field × female laboratory susceptible and male laboratory susceptible × female field). Progeny (F1) had, on average, 50% genetic background from the susceptible strain. This generation was interbreed for one generation to increase numbers (F2) and then selected (8.5 ppm azinphosmethyl (50WP) directed at neonates in a diet incorporation bioassay (7). Surviving larvae were reared to pupation, sexed, and two reciprocal crosses with the susceptible line were conducted. Progeny (F3) had, on average, 75% genetic background from the susceptible strain. This generation was interbreed for one generation to increase numbers (F4). Pupae were sexed, and two reciprocal crosses were conducted. Progeny (F5) had, on average, 87% genetic background from the susceptible strain. This generation was interbreed for one generation to increase numbers (F6) and then selected (8.5 ppm as in F2). A bioassay (neonate using diet-incorporated azinphosmethyl) at this generation showed an LC_{50} of 46 ppm. Surviving larvae were reared to pupation, sexed, and two reciprocal crosses with the susceptible line were conducted. Progeny (F7) had, on average, 94% genetic background from the susceptible strain. This generation was interbreed for one generation to increase numbers (F8). Pupae were sexed, and two reciprocal crosses were conducted. Progeny (F9) had, on average, 97% genetic backgrounds from the susceptible strain. This generation was interbreed to increase numbers (F10). The F10 neonates were selected (25 ppm azinphosmethyl (50 WP) directed at neonates in a diet incorporation bioassay). The survivors of this selection constituted the resistant strain colony. No further crosses to the susceptible line were made. The protocol for diet incorporated azinphosmethyl selection bioassay of neonates followed (7).

Briefly, formulated azinphosmethyl was applied (0.5 ml) in diluted aqueous solution to the surface of 7-10 ml of lima bean based synthetic diet in plastic cups and allowed to air-dry for 2-3 h. Test larvae were introduced into diet cups in groups of four. The larval exposure period to the treated diet was until pupation or death. Repeated backcrossing essentially creates an "isogenic" strain by gradually diluting the fraction of the genome coming from the resistant parent (39). This method is used to move a major resistance gene into a susceptible genetic background and thereby isolate it from other genes that affect the resistant phenotype.

Treatment of insects. Twelve day old fourth instar larvae were used for experiments. Larvae from the resistant strain were generation 16, except for the experiment on apple, where generation 18 was used. Larvae were maintained from hatching on a lima bean based artificial diet (31) at 26.7ºC, 60% relative humidity with a photoperiod of 16:8 (L:D) h in the Department of Entomology, Pennsylvania State University. Twelve day old larvae were removed from artificial diet and transferred to one of four host plants apple, black raspberry, plantain, or dandelion or artificial diet for seven days.

Sleeve cages (5-10 larvae per cage) made of fine pore nylon meshed and sealed with parafilm were used to prevent larval escape. Plants raised in the greenhouse were moved to a walk in type growth chamber at the Department of Entomology, Pennsylvania State University set at 26.7ºC, day length: 16:8 (L:D) h and 50-80% R.H.

After seven days on the host, larvae were removed, weighed individually, and frozen at -80ºC. These larvae and the larvae used in the previous bioassays were reared at the same time on the same plants.

Biochemical analyses. Detoxification enzyme activity was measured in fifth instar larvae (19-days old) of both azinphosmethyl-resistant and susceptible strains reared on the four plant species and artificial diet. Assays were conducted for two different enzyme systems. Glutathione transferase (GST) and carboxylesterase activities were assayed according to the protocols of Carlini (13). GST activity was determined because it is likely to be the most important enzyme in *P. idaeusalis* resistance to a azinphosmethyl (13, 30) in Pennsylvania. Carboxylesterase activity was measured because this enzyme system is a generally important detoxification mechanism in insects, including *P. idaeusalis* (12, 13, 11). Ten to thirty six individuals per treatment per enzyme substrate were used in each of the enzyme assays. In most cases, GST and esterase activities were assayed from single individuals.

Carboxylesterase and glutathione transferase enzyme preparations. Whole larvae were homogenized in 100 ml of 50 mM sodium phosphate buffer, pH 7.4, using a teflon conical homogenizer. The homogenate was centrifuged at 12,000 x g at 4ºC for 10 min in a Brinkman fixed angle Eppendorf 5415 centrifuge. The supernatant was used to determine carboxylesterase and glutathione transferase activity. Supernatant not used immediately was stored at -80ºC

until needed for analysis.

***In vitro* enzyme assays.** Carboxylesterase activity (3) was measured spectrophotometrically with a-naphthyl acetate and a-naphthyl butyrate as substrates according to a method described by Gomori (18). The incubation mixture (V_F=1 ml) contained 1 ml of supernatant, 1 ml of substrate (0.25 mM final concentration), and 998 ml of 50 mM sodium phosphate buffer, pH 7.4. The reaction mixture was incubated for 10 min at 37ºC and ended by the addition of 2 ml of aqueous solution containing 0.9 mg fast blue B salt and 7.5 mg sodium dodecyl sulfate. The absorbance of the reaction was read at 600 nm against a reference lacking enzyme with a Perkin-Elmer 3B Lambda spectrophotometer equipped with a computer interface to a Swan 286/12 and converted to concentration of a-naphthol using a standard curve.

Glutathione transferase activity was assayed spectrophotometrically with DCNB and CDNB as substrates. The procedure to determine GST activity using DCNB was based on a method reported by Siegfried and Mullin (41), modification of a method described originally from Booth *et al* (8). When DCNB was used as the substrate, the reaction mixture for larvae reared on plantain and artificial diet was 100 ml of homogenate added to a solution containing 100 ml glutathione at a concentration of 50mM in distilled water and 775 ml of 50mM sodium phosphate buffer, and incubated for 2 min at 37ºC. The incubation mixture for larvae reared on apple, black raspberry, and dandelion consisted of 50 ml of homogenate, 50 ml of 50 mM glutathione solution, and 387.5 ml of 50 mM sodium phosphate buffer. The reaction was started by adding 12.5-25 ml of DCNB at a concentration of 40 mM in ethanol to the solution (artificial diet and plantain: 12.5 ml; apple, black raspberry and dandelion: 25 ml). Absorbance change at 344 nm was monitored for 5 min.

The procedure to determine GST activity using CDNB was based on a method modified by Carlini (2, 13, 20). In the case of larvae reared on artificial diet and plantain, the reaction mixture consisted of 1 ml protein, 100 ml of 50 mM glutathione solution, and 874 ml of 50 mM sodium phosphate buffer, pH 7.4, which was incubated for 2 min at 37ºC. For larvae reared on black raspberry and dandelion, I used 1ml homogenate, 50 ml of 50 mM glutathione solution, 436.5 ml of 50 mM sodium phosphate buffer. The incubation mixture for apple was 10 ml homogenate, 100 ml of 50 mM glutathione solution, and 865 ml of 50 mM sodium phosphate buffer. The CDNB reaction was started by adding 12.5 to 25 ml CDNB at a concentration of 40 mM in ethanol to the solution (artificial diet and plantain: 25ml; black raspberry, apple, and dandelion: 12.5 ml). The reaction was monitored for 3 min at 340 nm by recording change in absorbance.

Enzyme activity (amount of conjugate formed) was converted to specific activity using a millimolar extinction coefficient of 8.5 cm^{-1} for DCNB and 9.6 cm^{-1} for CDNB (20).

Protein estimations. Protein content of samples was determined by the method of Bradford (9). Five ml of sample were added to 95 ml of 50mM

sodium phosphate buffer, pH 7.4, and 5 ml of coomassie blue solution G-250. The absorbance was read 2 min after addition of the dye at 595 nm against a blank lacking protein and compared to a bovine serum albumin (BSA) standard curve.

Statistical Analyses. Activity levels of detoxification enzymes among larvae fed different diets were analyzed by two way analysis of variance (ANOVA) using the Statview statistical program (1), followed by Fisher's protected least significant difference (PLSD) mean separation tests (33). Factors for the two-way analyses were diet and strain. Specific comparisons between the resistant and the susceptible strain within each host were determined by 2 tailed "t" tests.

Results

Bioassays in the laboratory confirmed that the resistant line was resistant to azinphosmethyl. Results of topical bioassays on the F_{16} generation indicated a 3.4-fold level of resistance to azinphosmethyl when compared with the LD_{50} of the susceptible laboratory strain. Host plant affected larval detoxification enzyme activity in both the resistant and susceptible strain. Glutathione transferase and carboxylesterase activities, both implicated in *P. idaeusalis* resistance to azinphosmethyl, varied significantly between strains and among hosts.

Glutathione transferase activity. Activity levels of GST using the model substrates DCNB and CDNB were significantly affected by diet, strain, and the interactions of these two factors (table 2). Mean enzyme activities of both the susceptible and resistant strain on each diet are presented in table 3.

Resistant larvae fed artificial diet and black raspberry exhibited significantly higher GST activity levels than their susceptible counterparts (table 3, figures 1 and 2), when DCNB was used as the substrate. Little or no difference in activities was detected between strains in larvae reared on dandelion, plantain, or apple (table 3, figures 1 and 2). Susceptible larvae from artificial diet and dandelion had significantly higher DCNB-specific GST activities than susceptible larvae on apple, plantain, or black raspberry (figure. 1). GST activity toward DCNB was highest in resistant larvae fed artificial diet, followed by black raspberry, dandelion, plantain, and apple (figure 1).

Resistant larvae reared on black raspberry, artificial diet, and plantain had significantly higher specific GST activities toward CDNB than did their susceptible counterparts in pairwise comparisons (table 3, figures 3 and 4). Resistant larvae fed apple had significantly reduced activity compared to susceptible larvae when CDNB was used as a substrate. Although not significantly different, resistant larvae on dandelion also had lower activity than susceptible larvae for CDNB conjugation (table 3, figures 3 and 4). Resistant larvae responded to a diet of black raspberry with the highest CDNB-specific GST enzyme activity and apple, the lowest, with the values for resistant larvae fed artificial diet, dande-

Table 2. Two-way analysis of variance indicating source of variation, degrees of freedom, F, and P values for data from enzyme analysis.

Detoxification Interaction enzyme (Strain × Diet)			Strain		Diet	
	df	F	df	F	df	F
Glutathione transferase-DCNB/Protein	1,250	52.8**	4,250	60.0**	4,250	36.7**
GST- CDNB/Protein	1,296	75.6**	4,296	77.5**	4,296	27.4**
EST- NA/Protein	1,278	27.2**	4,278	65.9**	4,278	10.9**
EST- NB/Protein	1,277	6.5*	4,277	29.9**	4,277	7.9**

*F is significant at P< 0.01,** P< 0.001

lion, and plantain intermediate (table 3, figure 3).

When susceptible larvae fed on dandelion, their GST activity toward CDNB was the highest, with apple and plantain the lowest and artificial diet and black raspberry intermediate (table 3, figures 3 and 4).

For CDNB conjugation, both the susceptible and resistant strain larvae reared on apple had the lowest activity levels among all treatments (table 3, figures 3 and 4).

Carboxylesterase activity. General carboxylesterase activities toward a-naphthyl acetate and a-naphthyl butyrate were significantly affected by diet, strain, and the interac-

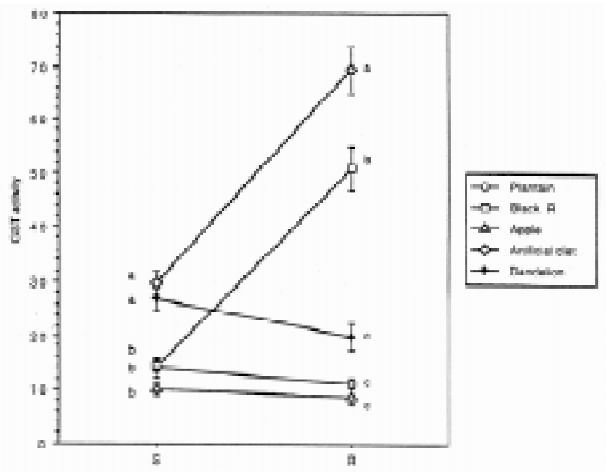

Figure 1. DCNB-specific glutathione transferase activity of both the susceptible (S) and resistant strain (R) larvae of *P. idaeusalis* fed different diets. Vertical lines indicate 1 SE. Means within each strain indicated by the same letter are not significantly different from each other (P<0.05, Fisher´s protected LSD).

Table 3. Mean enzyme activities [mM/min/mg] (± SEM; n) of susceptible (S) and resistant (R) *P. idaeusalis* strains fed different diets.

		Glutathione transferase	Carboxylesterase		
Diet	Strain	DCNB	CDNB	a-NA	a-NB
Artificial diet	S	29.7(2.3;16)	3,174(343;16)	935(159;16)	770(112;16)
Artificial diet	R	69.0(4.4;28)	6,113(526;27)	523(128;15)	556(108;15)
P-level (t-test)		0.0001	0.0003	0.055	0.1818
Apple	S	10.2(1.1;23)	717(62;33)	347(43;31)	261(54;31)
Apple	R	8.9(1.5;10)	363(33;31)	118(143;18)	87.6(9.3;18)
P-level (t-test)		0.5273	0.0001	0.0002	0.0194
Black raspberry	S	14.5(1.3;35)	2,998(241;36)	157(15;35)	134(16.0;35)
Black raspberry	R	50.9(4.0;32)	7,298(454;36)	340(31;35)	298(31.9;34)
P-level (t-test)		0.0001	0.0001	0.0001	0.0001
Plantain	S	13.9(1.6;24)	1,237(77;34)	271(34;36)	256(31.2;36)
Plantain	R	11.2(1.0;35)	4,952(482;28)	172(18.1;36)	198(26.4;36)
P-level (t-test)		0.1461	0.0001	0.011	0.1554
Dandelion	S	26.9(2.1;27)	6,157(395;30)	1,221(84;30)	567(53;30)
Dandelion	R	20.0(2.4;30)	5,124(368;35)	707(86;36)	341(44;36)
P-level (t-test)		0.034	0.0603	0.0001	0.0015

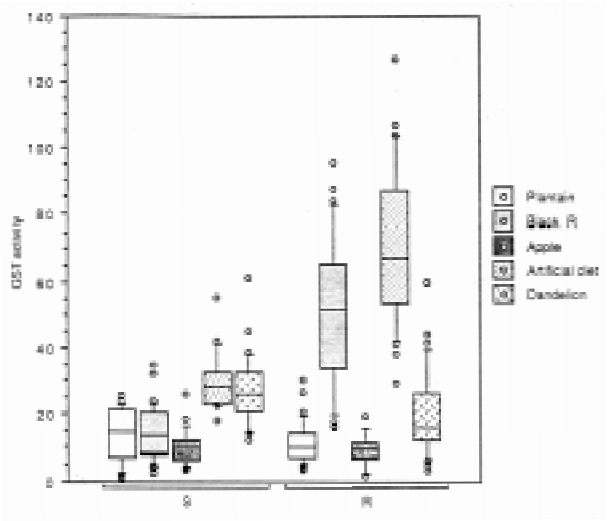

Figure 2. Boxplots of DCNB-specific glutathiones transferase activities of both the susceptible (S) and resistant strain (R) larvae of *P. idaeusalis* fed different diets. Box in the middle 50% of observations. The boxplot displays the 10th, 25th, 50th and 90th percentiles of a variable. All values for the variable above the 90th percentile and below the 10th percentile are plotted separately.

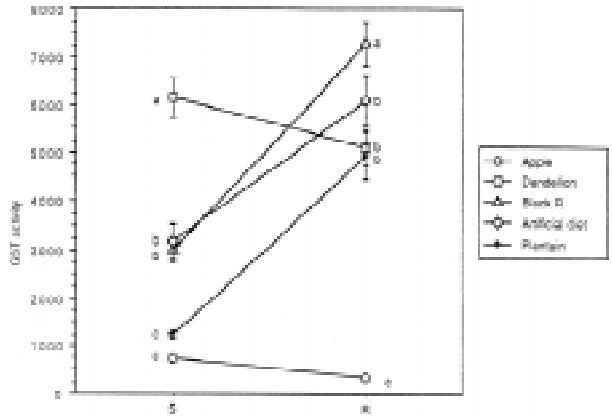

Figure 3. CDNB-specific glutathione transferase activity of both the susceptible (S) and resistant (R) larvae of *P. idaeusalis* fed different diets. Vertical lines indicate 1 SE. Means within each strain indicated by the same letter are not significantly different from each other (P < 0.05, Fisher´s protected LSD).

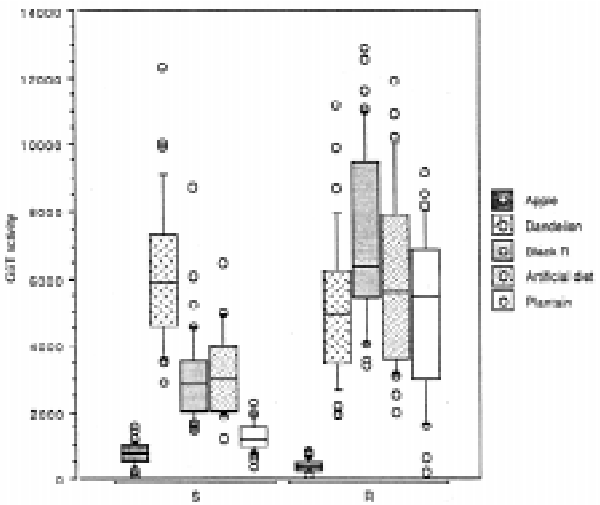

Figure 4. Boxplots of CDNB-specific glutathione transferase activities of both the susceptible (S) and resistant strain (R) larvae of *P. idaeusalis* fed different diets. Box is middle 50% of observations. The boxplot displays the 10th, 25th, 50th, 75th and 90th percentiles of a variable. All values for the variable above the 90th percentile and below the 10th percentile are plotted separately.

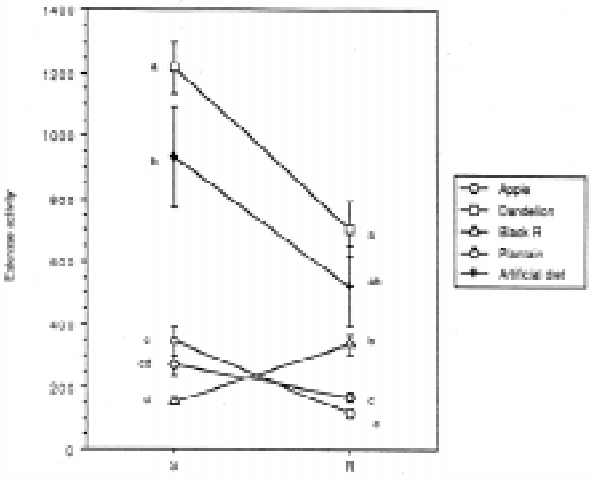

Figure 5. a-naphthyl acetate-specific esterase activity of both the susceptible (S) and the resistant strain (R) larvae of *P. idaeusalis* fed different diets. Vertical lines indicate 1 SE. Means within each strain indicated by the same letter are not significantly different from each other ($P < 0.05$, Fisher´s protected LSD).

tions of these two factors (table 2). Mean enzyme activities of both the susceptible and resistant strain on each diet are presented in table 3.

Resistant larvae reared on dandelion, apple, artificial diet, and plantain had significantly lower a-NA specific esterase activities than their susceptible counterparts in pairwise comparisons (table 3, figures 5 and 6), whereas resistant larvae on black raspberry exhibited an opposite trend in activity with higher values than susceptible larvae fed black raspberry (table 3, figures 5 and 6). Both resistant and susceptible larvae fed dandelion exhibited the highest esterase activity toward a-NA among all treatments (table 3, figures 5 and 6).

Resistant larvae fed dandelion and artificial diet had significantly higher levels of a-NA hydrolysis than resistant larvae reared on plantain and apple, while resistant larvae fed black raspberry had an intermediate value (figure 5). The lowest levels of a-NA hydrolysis in resistant larvae were observed in larvae fed on apple and plantain (table 3, figures 5 and 6). When susceptible larvae fed on dandelion, their levels of a-NA hydrolysis were the highest, with black raspberry, artificial diet, and apple following in decreasing level of activity.

Resistant larvae fed dandelion and apple had significantly reduced a-NB specific esterase activities compared to their susceptible counterparts in pairwise comparisons (table 3, figures 7 and 8), whereas resistant larvae on black raspberry showed an opposite trend in activity with higher activity than their susceptible counterpart in a pairwise comparison (table 3, Figures 7 and 8). Although not significantly different, resistant larvae fed artificial diet and plantain had lower levels than for the susceptible larvae, for a-NB hydrolysis. Both resistant and susceptible larvae reared on artificial diet exhibited the highest esterase activities toward a-NB among all treatments. Among natural hosts, dandelion had the esterase activities toward a-NB. The lowest levels of a-NB hydrolysis were observed in resistant larvae fed apple, while resistant larvae fed plantain and black raspberry had intermediate values (table 3, figures 7 and 8). When susceptible larvae ate artificial diet, their levels of a-NB hydrolysis were the highest, followed by dandelion, apple, plantain, and black raspberry (figure 7).

Discussion

Plant allelochemicals modify levels of detoxifying enzymes in herbivores and, therefore, their susceptibility to insecticides (6, 42, 10, 28, 40, 29, 34). Although plant allelochemicals may directly induce or inhibit detoxification enzymes, they may also impair the overall fitness of the herbivore thereby reducing its ability to cope with additional toxicant exposures (19, 5). Even a reduction in lipid reserves may increase the susceptibility of an insect to an insecticide such as DDT due to loss of sequestration sites (35).

Previous studies of the resistance of *P. idaeusalis* to azinphosmethyl have demonstrated that increased activities of both glutathione transferases

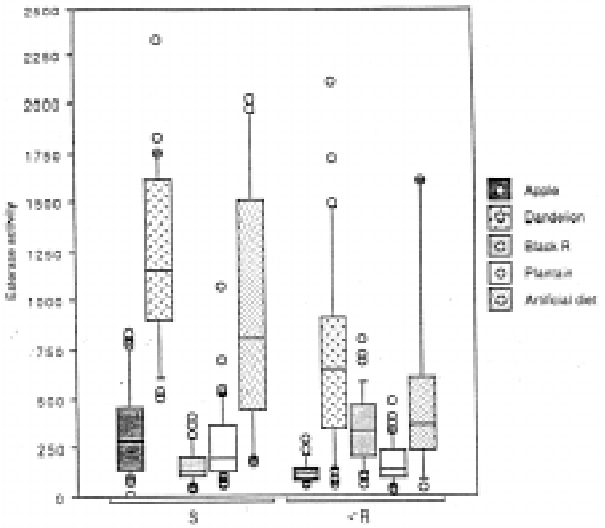

Figure 6. Boxplots of a-naphtyl acetate-specific esterase activity of both the susceptible (S) and the resistant strain (R) larvae of *P. idaeusalis* fed different diets. Box is middle 50% of observations. The boxplot displays the 10th, 25th, 50th, 75th, and 90th percentiles of a variable. All values for the variable above the 90th percentile and below the 10th percentile are plotted separately.

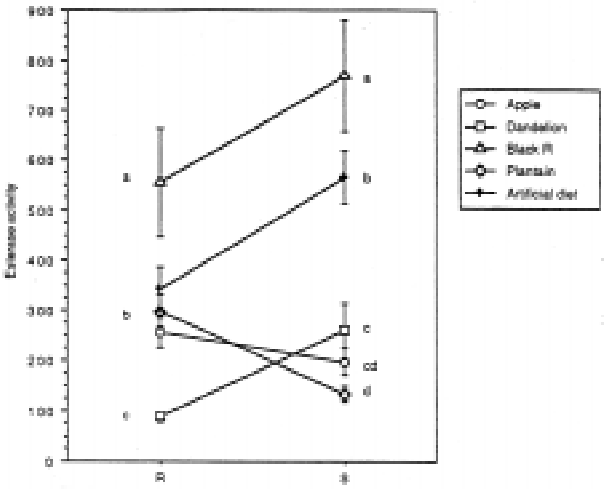

Figure 7. a-naphtyl butyrate-specific activity of both the susceptible (S) and resistant (R) larvae of *P. idaeusalis* fed different diets. Vertical lines indicate 1 SE. Means within each strain indicated by the same letter are not significantly different from each other (P < 0.05, Fisher´s protected LSD).

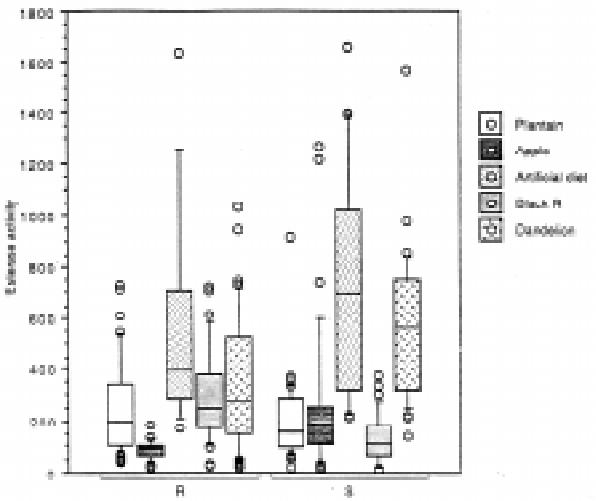

Figure 8. Boxplots of a-naphtyl butyrate-specific esterase activity of both the susceptible (S) and the resistant strain (R) larvae of *P. idaeusalis* fed different diets. Box is middle 50% of observations. The boxplot displays the 10th, 25th, 50th, 75th, and 90th percentiles of a variable. All values for the variable above the 90th percentile and below the 10th percentile are plotted separately.

(13, 14, 30) and general esterases (11, 12) are linked to azinphosmethyl resistance in adults of *P. idaeusalis*.

Hunter *et al* (21) reported that GST activity of both the susceptible and resistant strain *P. idaeusalis* larvae was decreased by the presence of an apple allelochemical, phloridzin. Also, phloridzin inhibited esterase activity and aniline hydroxylation in susceptible larvae. These previous studies were conducted with larvae reared on a lima-bean based synthetic diet with or without phloridzin (21) or with male adults collected on pheromone traps (13, 14, 30).

Although *P. idaeusalis* is a highly polyphagous insect, little is known about the effects of host plants on detoxification enzyme activity in larvae. The data reported here showed that diet, genotype, and the interactions of these two factors affect the activity levels of GST and carboxylesterases. These results mean that activity in a given strain depended on the diet upon which larvae were reared.

The use of two isogenic strains in this study, one susceptible to azinphosmethyl and the other resistant to azinphosmethyl, enable us to attribute any differences in response to azinphosmethyl to resistance mechanisms (or possibly, closely-linked genes) rather than unrelated strain differences.

Susceptible larvae fed dandelion had consistently high enzyme activities (GST-CDNB, GST-DCNB, EST-

NA) which correlates with the bioassay results. Susceptible larvae fed artificial diet tends to have high enzyme activities but a low $LD_{50.}$ Susceptible larvae fed apple, plantain, and black raspberry all have low enzyme activities.

Resistant larvae fed black raspberry are more susceptible to azinphosmethyl than on artificial diet, associated with similar GST activities but lower esterase activities. There was no consistent association between toxicity and enzyme activity on any of the other hosts.

Resistant larvae reared on apple had the lowest values of GST and carboxylesterase activities (table 2). The data support the studies of Hunter *et al* (21) using phloridzin. Hunter *et al* (21) suggested that resistant larvae did not have higher mortality on diet with phloridzin because the inhibition of GST activity in resistant larvae may have been counterbalanced by the induction of carboxylesterases. The inhibition observed was not clearly related to changes in azinphosmethyl sensitivity because of wide confidence limits. However, the LD_{50} of resistant larvae fed apple was 2.5 times lower compared to resistant larvae fed artificial diet. Robertson *et al* (37) also obtained wide confidence limits in the calculation of LD_{50} for both resistant and susceptible light brown apple moth larvae reared on apple.

Resistant larvae fed on apple or black raspberry might be erroneously classified as being genetically susceptible because they are more susceptible to azinphosmethyl compared with resistant larvae fed any other diets, and also because the mortality (LD_{50}) is not significantly different from susceptible larvae fed artificial diet. These results agree with the report of Robertson *et al* (37), who found that resistant light brown apple moth larvae reared on blackberry were significantly less resistant than resistant larvae reared on any other diet. Enzyme analyses showed that GST activities were relatively high resistant larvae fed black raspberry. That increased activity was not clearly related to changes in azinphosmethyl sensitivity. The likelyhood ratio results suggested that resistant larvae fed different diets might be responding to azinphosmethyl by different levels of the same mechanism. These enzyme assays suggested, however, that detoxification enzyme systems in addition to GST might be responsible for resistance, as previously suggested by Bush *et al* (11). for *P. idaeusalis*.

The data indicate that one cannot designate a population as resistant or susceptible without specifying the environment. The magnitude of resistance depends on the surrounding environment. Furthermore, the data show that there are not single mechanisms responsible for the effect of host plant on the toxicity of azinphosmethyl. Some other mechanisms should be examined to further explain the relationship between larvae *P. idaeusalis* resistance to azinphosmethyl and host plants including aniline hydroxylation activity (21), PSMO (44), existence of multiple forms of GST (45), and a decrease in the percentage of penetration (44).

No clear relationship between

toxicity and enzyme activity on different hosts was found. One possibility could be the complex chemistry of the plant could obscured the expression of the enzyme responsible for detoxifying the pesticide. It needs to look at the effect of other isolated major plant compounds like phloridzin on detoxification and susceptibility to azinphosmethyl in some of the other plant species. Our results suggest to study the combined effects of multiple plant compounds that could have antagonistic effect on the detoxification and susceptibility to azinphosmethyl. Additional work is needed to determine if there is any carry-over of induction or inhibition of detoxification abilities to other life stages. The fact that multiple forms of each enzyme respond to the model substrates could mean that the effects of an enzyme dealing with azinphosmethyl might be obscured by other enzymes measured by the assays. If we had specific identification of one or more detoxification enzymes, we could follow this in response to hosts. It is also necessary to study the relationship of enzyme detoxification abilities with adult susceptibility or neonate susceptibility. Finally, the results of the present study clearly suggest that host plants might affect the expression of resistance in *P. idaeusalis* populations.

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