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Energetic costs of detoxification systems in herbivores feeding on chemically defended host plants: a correlational study in the grain aphid, *Sitobion avenae*

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SUMMARY

Herbivorous insects have developed mechanisms to cope with plant barriers, including enzymatic systems to detoxify plant allelochemicals. Detoxification systems may be induced when insects are feeding on plants with increasing levels of allelochemicals. Increases in enzymatic activity have been related to energetic costs, and therefore less energy may be allocated to fitness-related traits. In this study, we explored the induction and energetic costs of detoxifying hydroxamic acids (Hx; a wheat allelochemical) in the grain aphid, *Sitobion avenae*. Aphids were reared on three wheat cultivars with different levels of Hx (0.26±0.08, 2.09±0.6 and 5.91±1.18 mmol kg^{-1} fresh mass). We performed a nested ANOVA to test the effect of Hx (main factor) and intrahost variation (nested factor) on body mass, standard metabolic rate (SMR) and the enzymatic activity of cytochrome P450s monooxygenases (P450s), glutathione *S*-transferases (GSTs) and esterases (ESTs). We found non-significant effects of Hx levels ($P>0.5$ for all tests), but there was significant intrahost variation ($P<0.05$ for all tests). In addition, we found a negative correlation between SMR and ESTs ($P=0.003$) and no correlation between SMR and GSTs or P450s (P=n.s after a Bonferroni correction). Multiple regression between SMR (dependent variable) and enzymatic activities (predictor variables) was significant ($P=0.007$), but detoxification enzymes only explained about 5% of the variation of SMR. Finally, we found a non-significant path coefficient between 'metabolism' and 'detoxifying capacity' ($P>0.05$). These results suggest that increased enzymatic activities do not entail increased metabolic rate. Therefore, low energetic costs in aphids would facilitate the use of different hosts and promote a wider ecological niche.

Key words: detoxification enzyme, hydroxamic acid, insect–plant interaction, metabolic rate, path analysis.

INTRODUCTION

Herbivorous insects need to cope with a number of barriers imposed by plants to avoid herbivory, such as chemical defences that provoke deleterious effects in insects, affecting their reproductive performance (Bernays and Chapman, 1994; Bennett and Walsgrove, 1994; Schoonhoven et al., 2005). However, insects have evolved behavioural, physiological and biochemical mechanisms, which allow them to feed on chemically defended plants (Berenbaum, 2001; Karban and Agrawal, 2002). Among the biochemical mechanisms are enzymatic detoxification systems, which are involved in degradation and neutralization of plant allelochemicals (Schoonhoven et al., 2005). The biosynthesis and metabolic activity of detoxification enzymes may entail significant energetic costs; more energy allocated to detoxification should reduce the amount of energy available for other biological functions, generating energetic tradeoffs between detoxification capacity and growth rate and/or reproduction (Cresswell et al., 1992; Karban and Agrawal, 2002). Therefore, increases in energetic costs could have important consequences for insect fitness.

In insects, several detoxification systems have been associated with allelochemical metabolism, the most important being the cytochrome P450s monooxygenases (P450s), glutathione *S*-transferases (GSTs) and the esterases (ESTs) (Yan et al., 1995; Scott et al., 1998; Salinas and Wong, 1999; Després et al., 2007). P450s oxidize a broad range of lipophilic xenobiotics, converting them into more polar compounds (Bergé et al., 1998; Feyereisen, 1999). GSTs conjugate reduced glutathione (GSH) to electrophilic substances, resulting in less toxic metabolites (Salinas and Wong, 1999; Enayati et al., 2005). Finally, ESTs hydrolyze esters and amides, converting them into more polar compounds (Brattsten, 1988). In all cases, the modified allelochemicals may be stored or excreted, reducing their toxicity. In the case of cereal aphids, these systems have been associated with the detoxification of allelochemicals present in their host plants (Lesczynski et al., 1994; Loayza-Muro et al., 2000). A good example is the grain aphid, *Sitobion avenae* (Fabricius), which thrives on wild and cultivated Poaceae. Many of the Poaceae contain hydroxamic acids (Hx), the main group of secondary metabolites involved in resistance against a wide variety of herbivores (Niemeyer and Perez, 1995; Sicker and Schultz, 2002). Hx are present in the intact plant as glucosides, which are hydrolyzed by β-glucosidases released upon tissue injury (Hofman and Hofmanova, 1969; Cambier et al., 1999). Hx produce behavioural, ecological and metabolic effects on *S. avenae*, including feeding deterrence (Niemeyer et al., 1989), decrease in performance (individual growth rates as well as intrinsic rates of population increase) (Thackray et al., 1990; Figueroa et al., 2004) and the induction of various detoxification systems (Leszczynski and Dixon, 1992; Leszczynski et al., 1994; Figueroa et al., 1999).

In the present study, we explored the energetic costs associated with the detoxification of host-plant defensive compounds by the

grain aphid. We carried out an experiment with aphids reared on three wheat cultivars with different levels of Hx (see Materials and methods for the Hx concentrations in the wheat cultivars) and measured the enzymatic activity of P450s, GSTs and ESTs as well as standard metabolic rate (SMR). The relationships between detoxification systems and metabolic rate were assessed through: (1) simple correlations between enzymatic activities and metabolic rate; (2) a multiple regression using the enzymatic activities as predictor variables, and metabolic rate as dependent variable; and (3) a structural equation model (i.e. path analysis) to estimate the association between variables from their covariance structure. With this experiment, we addressed the following questions: (1) are there energetic costs associated with detoxification systems in aphids and (2) do these costs vary with varying allelochemical levels (i.e. do they exhibit induction costs)?

MATERIALS AND METHODS

Study animals and maintenance

The grain aphid, *Sitobion avenae*, lives on several species of Poaceae, including cereals and pasture grasses from temperate climates. In Chile, this species was introduced in the early 1970s (Apablaza, 1974) and it is currently distributed between 32 deg. S and 41 deg. S. Living aphids were collected near Santiago (Chile, 33.3 deg. S.), from oat and wheat fields. A multiclonal aphid colony was reared in the laboratory under controlled conditions (20±1°C with a 16 h:8 h L:D photoperiod) that ensured parthenogenetic reproduction on seedlings of a low-Hx wheat cultivar (*Triticum aestivum* cv. Huayún; see Hx concentrations below). Seedlings were grown in plastic pots with sterile compost, caged with plastic cylinders and textile material. Experimental plants (i.e. wheat plants with aphids) were maintained under the same environmental conditions.

After at least five generations in the low-Hx cultivar, the stock colony was split into three groups, each assigned to one of the three wheat cultivars with different levels of Hx, as measured in 7-day-old seedlings (mean ± s.e.m., N=6): cv. Huayún (low Hx, 0.26±0.08 mmol kg^{-1} fresh mass), cv. Ciko (intermediate Hx, 2.09±0.6 mmol kg^{-1} fresh mass) and cv. Quelén (high Hx, 5.91±1.18 mmol kg^{-1} fresh mass). Five wingless female aphids were transferred to seedlings of each cultivar and, after 3 days, adult aphids were removed and the resulting nymphs were left on the plants. After 7 days, first-generation adult aphids on each cultivar were split into 16 groups and transferred to seedlings of the same cultivar on which they had been raised. After 3 days, adult aphids were removed and nymphs were left on the plants. Finally, adult aphids of this second generation were transferred to seedlings of the same cultivar and removed after 3 days. When the third generation of nymphs became adults, they were used for the experimental measurements. Aphids have telescopic reproduction, which means that adult females contain their daughters and granddaughters at the same time (Dixon, 2005). Therefore, only aphids of third generations have been entirely developed on a specific host, eliminating maternal and grandmaternal effects due to common environmental developing conditions.

Standard metabolic rate measurements

For each wheat seedling, 45 wingless adult female aphids of the third generation were separated into groups of nine individuals with similar body size. Each group of nine aphids was considered as a replicate, and a total of 240 replicates were measured (3 hosts × 16 seedlings per host × 5 replicates per seedling). However, the final sample size was equal to 185 because some replicates were lost during the experiment. Each replicate was placed in an Eppendorf tube, anesthetized on ice and weighed to the nearest 0.001 mg on a microbalance (Sartorius, Goettingen, Germany) before measuring metabolic rate.

Standard metabolic rate (SMR) was measured as the volume of CO_2 produced in a given period by each aphid replicate, using 'closed system' metabolic chambers (Vleck, 1987). Glass syringes were used as metabolic chambers, because this material is impermeable to CO_2. Each aphid replicate was placed in a 2 ml glass syringe, which was sealed, filled with CO_2-free air to a volume of 1.5 ml and placed in a dark climatic chamber at 20±1°C. After 3 h in the climatic chamber, 1 ml of air from each syringe was injected into a Bev-A-line tube connected to a Li-6262 CO_2 analyzer with a resolution of 1 p.p.m. of CO_2 in air (LI-COR Bioscience, Lincoln, NE, USA). Flow rates of CO_2-free air (ambient air was CO_2 scrubbed by passing it through a barium hydroxide column) were maintained at 100 ml min^{-1} (±1%) with a mass flow controller (Sierra Instruments, Monterey, CA, USA). The output from the CO_2 analyzer was recorded with Expedata software (Sable Systems International, Las Vegas, NV, USA). Rates of CO_2 production (in µl CO_2 h^{-1}) were calculated from the whole record and by transforming p.p.m. concentration of CO_2 to CO_2 fraction and then multiplying by the flow rate (i.e. 100 ml min^{-1}). From each CO_2 peak observed in the record, corresponding to each air injection, the area under the curve (integral of ml CO_2 min^{-1} *vs* min) was calculated. This area was equal to the volume of CO_2 produced by each replicate in the syringe, and this volume was divided by the total period of measurement (3 h), multiplied by 1000 to give µl CO_2 h^{-1} and finally divided by nine (assuming similar metabolic rates among individuals from the same replicate), to give the metabolic rate per aphid per hour. Fifteen empty syringes manipulated as described above were used as blanks for CO_2 concentrations.

Enzymatic determinations

Immediately after measuring metabolic rates, each replicate was separated into three sets of three individuals, and each set was used to determine the specific activity of one of the three detoxification enzymes: P450s, GSTs or ESTs.

P450s activity was determined by the fluorescence method described by de Sousa et al. with modifications (de Sousa et al., 1995). Three adult aphids were placed in one of the wells of a 96-well microplate and homogenized in 150 µl of 50 mmol l^{-1} pH 7.0 Hepes buffer using a pellet pestle. Then, 150 µl of substrate solution (0.4 mmol l^{-1} 7-ethoxycoumarin in 50 mmol l^{-1} pH 7.2 phosphate buffer) was added to each well. Plates were incubated for 4 h at 37°C in a thermoregulated bath and the reactions were stopped with 100 µl of 50% (v/v) glycin-ethanol buffer, pH 10.4. Fluorescence was measured using 390 nm excitation and 460 nm emission filters.

GSTs activity was determined following the methodology of Nauen and Stumpf with modifications (Nauen and Stumpf, 2002). Three aphids were placed in a microcentrifuge tube, homogenized in 300 µl of PBS pH 7.0 buffer and centrifuged at 9400 ***g*** for 5 min at 4°C. Then, 30 µl of supernatant was placed in one of the wells of a 96-well microplate, in which 30 µl of PBS pH 7.0 buffer, 150 µl of 3 mmol l^{-1} reduced glutathione (substrate) and 20 µl of 0.3 mmol l^{-1} monochlorobimane were added. Plates were incubated for 20 min at 22°C. Fluorescence was measured using 390 nm excitation and 465 nm emission filters.

ESTs activity was determined following the methodology of Devonshire et al. with modifications (Devonshire et al., 1992). Three aphids were homogenized in 100 µl of ice-cold PBS/Triton X-100 buffer. Twenty-five microlitres of homogenate was placed in one

of the wells of a 96-well microplate, and 25 µl of PBS/Triton X-100 buffer and 150 µl of 0.3 mmol l^{-1} α-naphthyl acetate (substrate) were added. Plates were incubated for 5 min at 22°C, and then 25 µl of Fast Blue B colorant solution was added. After 20 min at 22°C, absorbance was measured at 620 nm.

Fluorescence and absorbance measurements were performed in a Wallac 1420 Victor^3 microplate reader (Perkin-Elmer, Waltham, MA, USA). All enzymatic activities were expressed as U per aphid-equivalents and were calculated as the ratio between units of net fluorescence or absorbance (U) and aphid-equivalents (Nauen and Stumpf, 2002). Net fluorescence (for P450s and GSTs) or absorbance (for ESTs) corresponded to the difference between sample and blank, and aphid-equivalent was an estimation of the amount of aphid present in the supernatant [aphid-equivalent=number of homogenized aphids × (volume of supernatant analyzed/total volume of homogenizing buffer)]. For all enzymatic determinations, solutions without enzymes (i.e. aphid homogenate) were used as blanks. Determinations of GSTs and ESTs activity were performed twice, and the average of the two duplicates was used as raw data, whereas the determination of P450s was performed only once. To determinate the reliability of GSTs and ESTs activity, coefficient of variations (CV=s.d./mean) between duplicates was estimated. The average coefficient of variations for duplicates was 3.3% (N=185) for GSTs activity and 18.7% (N=185) for ESTs activity.

Statistical analyses

Some variables (except body mass) needed to be transformed in order to fulfil normality assumptions: P450s and GSTs were $\log_{10}$-transformed, whereas SMR and ESTs were square root-transformed. Although the distribution of ESTs was improved after transformation, according to the normality test (Kolmogorov-Smirnov) this variable still exhibited some departures from normality. Thus, for correlations of ESTs, Spearman's (r_S) correlations were performed; for other variables, Pearson's correlations (r_P) were conducted. Body mass was significantly correlated with standard metabolic rate (r_P=0.66, P<0.0001) and GSTs (r_P=–0.17, P=0.008) but not with P450s (r_P=–0.01, P=0.87) nor ESTs (r_S=0.06, P=0.88). To perform analyses with variables correlated with body mass, we used the residuals from a linear regression between mass-correlated variables and body mass. These linear regressions were significant between body mass and SMR ($F_{1,183}$=99.24, P<0.0001) and GSTs ($F_{1,183}$=22.38, P<0.0001), which justified the use of residuals as new mass-corrected variables.

In order to tests the effect of Hx levels (hosts) and seedlings (blocks) on body mass, mass-residuals of standard metabolic rate (resSMR), P450s activity and mass-residuals of GSTs activity (resGSTs), nested analyses of variances were performed with host as the fixed main effect and blocks as a random nested effect in host. In the case of ESTs activity, it fulfils the homocedasticity assumption but was not normally distributed; however, ANOVA are relatively robust to deviation from normality (Zar, 1999). Therefore, a nested ANOVA was also performed to test Hx and seedling effects. Unfortunately, the number of blocks was unbalanced between wheat hosts, thus the degrees of freedom for block effect 44=(16–1)+(15–1)+(16–1). For the correlational analyses, the conduct of simultaneous statistical tests could increase the probability of type I error (e.g. increase the probability of rejecting the null hypothesis when it is true). Thus, we applied the sequential Bonferroni correction to reduce the probability of type I error (Quinn and Keough, 2002).

Since there was no effect of wheat cultivar on any measured variable (see Results), the data were pooled to explore the relationship between variables. The relationships between enzymatic activities and SMR were assessed through: (1) a correlation analysis between resSMR, P450s, resGSTs and ESTs activity; (2), a multiple regression analysis using P450s, resGSTs and ESTs activity as predictor variables, and resSMR as the dependent variable; and (3) a structural equation model (SEM), for which two endogenous latent variables were created – 'detoxifying capacity' and 'metabolism' – the former related to P450s, resGSTs and ESTs activity, and the latter related to resSMR (Fig. 2). Estimations of standardized path coefficients (indicating the strength of the relationship between variables) and standard errors were estimated using maximum likelihood (Shipley, 2000). All statistical analyses were performed using Statistica 6.0® (Statsoft, v. 6.1; http://www.statsoft.com).

RESULTS

Descriptive statistics of body mass, SMR and P450s, GSTs and ESTs activity for aphids reared on different wheat hosts are shown in Table 1. We found significant effects of blocks (i.e. seedlings) but not host effects on the analyzed variables (Table 2).

Given the absence of cultivar effects, data set was pooled to perform correlation analyses between metabolic rate and enzymatic activities. First, we examined the simple linear correlation between the variables studied (Table 3). After a sequential Bonferroni correction, we only found a negative and significant correlation between resSMR and ESTs activity (Fig. 1C; Table 3). Additionally, resGSTs activity was negatively related to ESTs activity (Table 3). On the other hand, we found non-significant relationships between resSMR and P450s or between resSMR and resGSTs activity (Fig. 1A,B; Table 3). Second, we observed that a multiple regression analysis yielded a significant relationship between resSMR and enzymatic activities ($F_{3,182}$=4.22, P=0.007; $r^2_{adjusted}$=0.05). However, enzymatic activities only explained about 5% of the variance of resSMR ($r^2_{adjusted}$). In addition, only P450s activity ($r_{partial}$=–0.16, P=0.03) was significantly correlated with resSMR. Finally, the structural equation model proposed (Fig. 2) suggests that P450s,

Table 1. Body mass, standard metabolic rate (SMR), cytochrome P450s monooxygenase activity (P450s), glutathione *S*-transferase activity (GSTs) and esterase activity (ESTs) of aphids reared on wheat hosts differing in their levels of hydroxamic acids

	Levels of hydroxamic acids on wheat hosts		
	Low (N=62)	Intermediate (N=58)	High (N=65)
Body mass (mg)	0.447±0.011	0.440±0.012	0.452±0.011
SMR (µl CO_2 h^{-1})	0.2502±0.0080	0.2392±0.0091	0.2440±0.0091
P450s (U aphid-equiv.$^{-1}$)	29.35±2.04	28.34±1.77	27.60±1.32
GSTs (U aphid-equiv.$^{-1}$)	2384±145	2659±172	2491±141
ESTs (U aphid-equiv.$^{-1}$)	0.2774±0.0353	0.3337±0.0384	0.2485±0.0348

Values are means ± s.e.m. (sample size in parentheses; see Materials and methods for details of the sampling procedure).

Table 2. Nested ANOVA testing wheat host (fixed effect) and block nested in host effects (random effect) on body mass, mass-residuals of standard metabolic rate (resSMR), cytochrome P450s monooxygenase activity (P450s), mass-residuals of glutathione *S*-transferase activity (resGSTs) and esterase activity (ESTs)

Source	d.f.	MS	*F*-ratio	*P*
Body mass				
Host	2	0.00364	0.289	0.750
Block (host)	44	0.01256	1.949	0.002
Error	138	0.00645		
resSMR				
Host	2	0.0030	0.541	0.586
Block (host)	44	0.0056	2.624	< 0.0001
Error	138	0.0021		
P450s				
Host	2	0.0004	0.191	0.827
Block (host)	44	0.0020	2.628	< 0.0001
Error	138	0.0008		
resGSTs				
Host	2	0.0220	0.223	0.801
Block (host)	44	0.0985	3.871	< 0.0001
Error	138	0.0254		
ESTs				
Host	2	0.04568	0.458	0.636
Block (host)	44	0.09975	1.528	0.034
Error	138	0.06528		

Effects were considered significant at $P<0.05$.

resGSTs and ESTs activity were significantly related to 'detoxifying capacity'. However, this latent variable showed a non-significant association with 'metabolism' (Fig. 2). Finally, a high and significant path coefficient was estimated between 'metabolism' and resSMR (Fig. 2).

DISCUSSION

In the present study we found that the detoxification systems in the aphid *Sitobion avenae* were not induced when aphids were reared on host plants with different allelochemical levels. Consequently, the energetic costs of these systems did not increase simultaneously. We discuss our findings regarding evidence of the energetic costs of detoxification systems in phytophagous insects.

Induction and relationship between enzymatic activities

Detoxification enzymes (P450s, GSTs and ESTs) exhibited comparable activity in aphids reared on wheat cultivars with different Hx levels, suggesting that Hx did not induce differential responses in the detoxification systems of *S. avenae*. However, previous studies have found that detoxification enzymes in *S. avenae* could be induced when aphids were exposed to plants or artificial diets with different levels of defence compounds. For example, Leszczynski et al. found that aphids exposed to a moderately aphid-resistant wheat cultivar displayed higher GSTs activity compared to aphids reared on a low aphid-resistant wheat cultivar (Leszczynski et al., 1994). In addition, Loayza-Muro et al. found that aphids reared on high-Hx wheat exhibited lower activity for NADPH cytochrome reductase, GSTs, ESTs and catalase compared with aphids reared on low-Hx wheat cultivars (Loayza-Muro et al., 2000). However, these differences were detected in a long-term experiment (i.e. 10 generations), suggesting a probable mechanism of acquiring resistance to Hx that could be the end-product of clonal selection favouring high detoxification activity (Loayza-Muro et al., 2000). Thus, our study should be compared only with the first generations of this long-term experiment, where no difference can be observed between aphids reared on low and high Hx levels in the host plants.

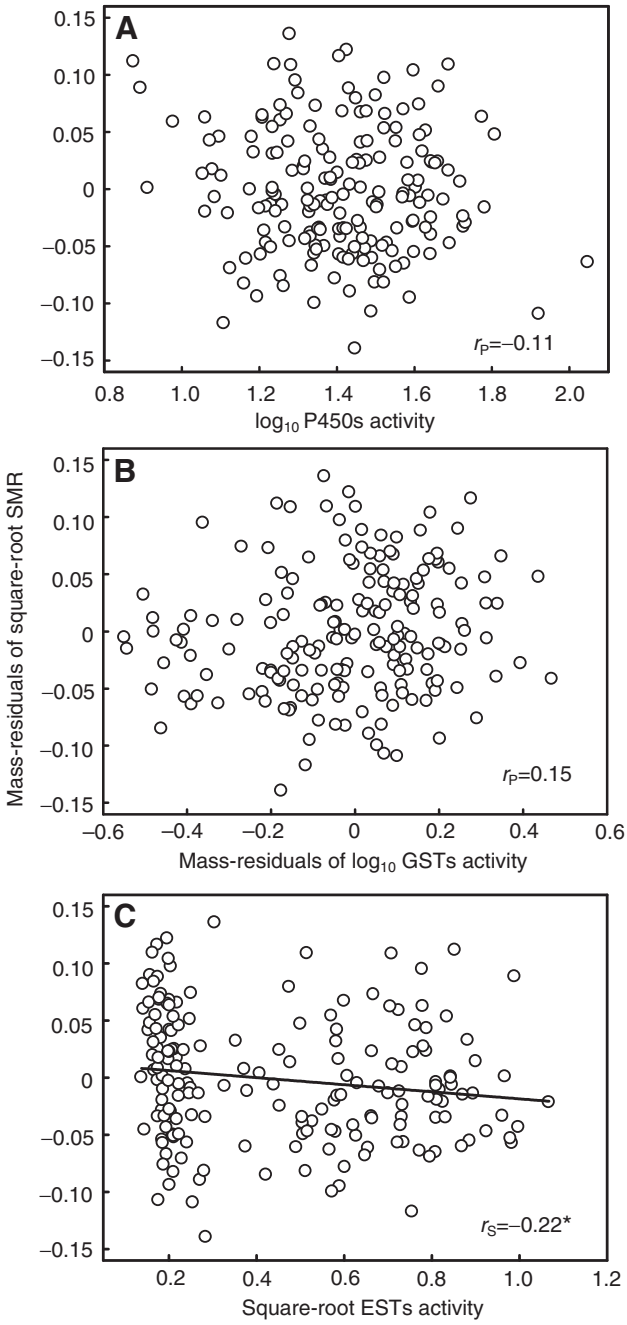

Fig. 1. Relationship between mass-residuals of square root-transformed standard metabolic rate (SMR; it was expressed as µl CO_2 h^{-1} $aphid^{-1}$) and (A) $\log_{10}$-transformed cytochrome P-450 monooxygenases activity (P450s; it was expressed as U aphid-equivalent^{-1}), (B) mass-residuals of $\log_{10}$-transformed glutathione S-transferases activity (GSTs; it was expressed as U aphid-equivalent^{-1}) and (C) square root-transformed esterases activity (ESTs; it was expressed as U aphid-equivalent^{-1}).

Moreover, non-plastic detoxification systems are in agreement with a parallel study by our research group, in which we evaluated the energetic and fitness costs in 'superclones' of *S. avenae*

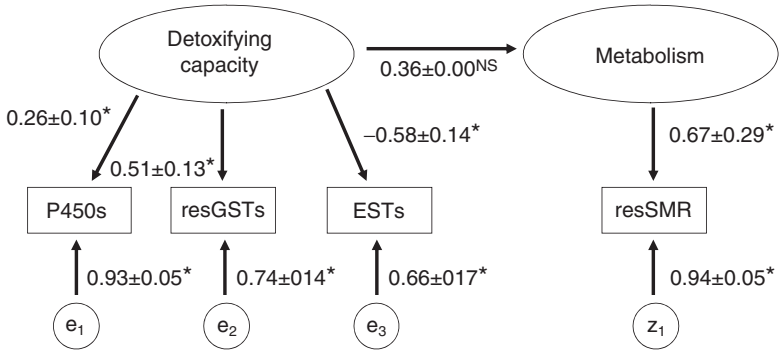

Fig. 2. Path diagram showing the proportional contributions (path coefficients ± standard errors) of enzymatic activities on metabolic rate. This diagram includes latent variables (i.e. variables that were not actually measured but statistically inferred from their relationships with measured variables; see text for details) within ellipses (detoxifying capacity and metabolism), manifest variables (i.e. variables that were measured, including measurement error; see text for details) within rectangles [enzymatic activity of cytochrome P-450s monooxygenases (P450s), mass-residuals of glutathione *S*-transferases (resGSTs), esterases (ESTs) and mass-residuals of standard metabolic rate (resSMR)], and residuals of enzymatic activities and metabolic rates within circles, represented by the letters 'e' and 'z', respectively (residuals indicate the variance not explained by the model). The path diagram had an associated chi-square equal to 7.39 with 2 d.f. (P=0.02). Asterisks and N.S. indicate significant (P<0.05) and non-significant path coefficients (P>0.05), respectively.

(L.E.C., C.C.F., E.F.-C., H.M.N. and R.F.N., submitted manuscript). Superclones are generalist genotypes, which exhibit high abundance and time-persistence in the field (Vorburger et al., 2003). In Chile, populations of *S. avenae* have predominantly asexual reproduction (Figueroa et al., 2005), which facilitates the maintenance of these genotypes over several years. In our parallel study, we found that these genotypes showed no effects of Hx on their detoxification systems, reflecting a rigid phenotype (i.e. similar to our multiclonal colony). A plausible explanation for the results shown here is that superclones were over-represented in our experimental multiclonal colony. The over-representation of superclones could have at least two causes: (1) our sampling could have been biased towards these genotypes because they were highly abundant at the moment of sampling and (2) we could have sampled a similar proportion of all genotypes in the field but, under favourable conditions for several generations, the advantageous characteristics of superclones allowed these genotypes to spread in the experimental multiclonal colony. Therefore, superclone-like detoxification systems could be expected in multiclonal colonies, as we found in the present study.

We found that increasing ESTs activity was negatively correlated with GSTs activity whereas the other relationships between enzymes were not significant. In the case of the negative correlation between enzymatic activities, this could suggest potential trade-offs between traits (Sgrò and Hoffmann, 2004). However, evidence of low correlations could not represent a 'real' trade-off between both enzymes, because it is necessary to estimate genetic correlations between traits. Even when phenotypic correlations are good predictors of genetic correlations, in most cases the latter are lower than the former (Roff, 1997). Hence, a low correlation between enzymes could indicate a lower (i.e. near to zero) genetic correlation, suggesting an independent relationship. Weak or non-significant correlations between enzymatic activities are evidence of the absence of trade-offs between them, which suggests that they could evolve independently.

Table 3. Correlations between mass-residuals of standard metabolic rate (resSMR), cytochrome P450s monooxygenase activity (P450s), mass-residuals of glutathione *S*-transferase activity (resGSTs) and esterase activity (ESTs)

	P450s	resGSTs	ESTs†
resSMR	–0.11 (0.15)	0.15 (0.04)	–0.22* (0.003)
P450s		0.17 (0.02)	–0.10 (0.17)
resGSTs			–0.32* (9×10^{-6})

Probability values for each correlation are shown in parentheses, and asterisks indicate significant correlations after a sequential Bonferroni correction. †Spearman's correlations were performed to test significant relationships between ESTs and the other variables.

Energetic demand of detoxification systems

Standard metabolic rate did not change in aphids exposed to different levels of Hx, suggesting that this allelochemical did not induce detectable energetic costs (O'Brien and Suarez, 2001). Our results, obtained from uni- and multivariate approaches, suggest that detoxification systems represent only a small fraction of the whole energetic budget of aphids. This conclusion is supported by: (1) a weak and negative correlation between metabolic rate and ESTs activity and the lack of correlation between metabolic rate and GSTs and P450s activity; (2) the variation in metabolic rate explaining only about 5% of the variation in the activity of detoxification enzymes; (3) a non-significant path coefficient between 'metabolism' and 'detoxifying capacity' in the path analysis; and (4) the large residual variation of metabolic rate, which suggests that metabolic rate variation is mostly explained by variation not accounted by the model.

Our findings suggest that levels of enzymatic activity are non-inducible by high levels of chemical defences and that detoxification of Hx did not entail high energetic costs. Similar results have been reported in other insect systems. For instance, Neal found that an increase in activity of polysubstrate monooxygenases was not accompanied by a metabolic cost on growth parameters in *Heliothis zea* (Lepidoptera) (Neal, 1987). Appel and Martin (Appel and Martin, 1992) found that *Manduca sexta* (Lepidoptera) changed neither metabolic rate nor growth variables when it was fed on artificial diets with different concentrations of nicotine. Finally, Berenbaum and Zangerl demonstrated that xanthotoxin induced the expression of detoxification systems in *Depressaria pastinacella* (Lepidoptera) but did not affect its growth parameters (Berenbaum and Zangerl, 1994). On the other hand, Cresswell et al. reported that an artificial diet containing nicotine produced an increase in metabolic rate

and a reduction of net growth efficiency in larvae of *Spodoptera eridania* (Lepidoptera) (Cresswell et al., 1992).

Finally, our results suggest that Hx did not affect body mass (i.e. a gravimetrical measure), which supports the assertion that allelochemical detoxification does not entail costs in aphids exposed to different concentrations of Hx. The lack of differences in body mass suggests that aphids were feeding on the wheat cultivars. To the best of our knowledge, most of the studies that have evaluated the costs of detoxification systems have used a gravimetric approach or have evaluated the metabolic rate associated with the ingestion of allelochemicals (see references above). Metabolic rate, however, is not usually evaluated with the activity of detoxification enzymes. Hence, this could be the first study that has evaluated the relationship between allelochemical detoxification systems and metabolic rate in insects.

Conclusions

Our results emphasize the need for increasing the number of experimental assessments of the metabolic costs of detoxification systems associated with plant allelochemical metabolism, including a wider range of insect species. This is especially important if energetic costs could impose significant physiological constraints on the evolution of herbivorous insects (Krieger et al., 1971; Després et al., 2007). The finding of low energetic costs of detoxification systems in invasive insects could have important consequences, facilitating the use of a wide range of host plants, thus favouring the expansion of their ecological niches. This information will assist in assessing their invasive potential.

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