# Host-Plant Adaptation in Fall Armyworm Host Strains: Comparison of Food Consumption, Utilization, and Detoxication Enzyme Activities

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**ABSTRACT** The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), consists of two genetically different strains. The corn strain feeds primarily on corn, and the rice strain feeds primarily on forage grasses and rice. Possible causes for differences in larval performance between the strains were examined. The objectives were to establish whether behavioral or physiological factors could explain differences in performance on corn. A comparison between strains in food consumption and utilization indicated that poorer performance on corn by the rice strain is, in part, caused by a lower consumption rate. In addition, the rice strain has a lower efficiency of converting digested food into biomass than the corn strain when reared on corn. When larvae were reared on either corn or bermudagrass, the rice strain had lower mixed-function oxidase (MFO) activities than the corn strain. In the corn strain, MFO activity was higher when larvae were reared on corn than on bermudagrass. Corn and bermudagrass-reared larvae from the rice strain expressed similar MFO activities. Esterase activity did not differ between strains or between corn and bermudagrass-reared larvae. Thus, the results indicate that both behavioral and physiological factors associated with host use differ between the strains. Moreover, MFOs may play an important role in host-plant adaptation in the fall armyworm.

**KEY WORDS** *Spodoptera frugiperda*, detoxication enzymes, nutritional physiology

THE FALL ARMYWORM, *Spodoptera frugiperda* (J. E. Smith), (Lepidoptera: Noctuidae) consists of two genetically differentiated strains, which show differences in host use (Pashley 1986, 1988a). The corn strain feeds primarily on corn, *Zea mays* L., and the rice strain feeds on forage grasses and rice, *Oriza sativa* L. Caterpillars of both strains can easily be reared on each other's host plants in the laboratory (Pashley, 1988b), and some overlap in host-plant use occurs in the field (Pashley 1986; D.P.P, unpublished data). The fact that no consistent morphological differences have been found suggests that the strains are very closely related and probably of recent origin. These features make fall armyworm strains an ideal model for studying evolutionary aspects of host-plant adaptation.

Since the insect–plant coevolution model was first proposed by Ehrlich & Raven (1964), much emphasis has been placed on the toxicity of host-plant allelochemicals. Consequently, many view host-plant adaptation in terms of physiological and biochemical adaptations to host-plant allelochemicals (Ehrlich & Raven 1964, Ahmad et al. 1986, Caprio & Tabashnik 1992, Slansky 1992). From this perspective, populations are likely to show preferences for plants on which they are physiologically or biochemically best adapted (Thompson 1988, Via 1990, Rausher 1992). This phenomenon is especially likely to occur when specialists perform better than generalists.

In contrast, others stress the importance of ecological factors in determining host use. Some argue that populations are likely to evolve a preference for host plants on which larvae experience the lowest rates of predation (Bernays & Graham 1988) or for hosts that are locally abundant (Fox & Morrow 1981). Bernays & Graham (1988) argue that the most important effects of allelochemicals are their effects on feeding behavior, and that allelochemicals may well be more important as signals for host identification, than as actual toxins. In fact, allelochemicals from plants often seem to have little or no intrinsic toxicity but affect growth negatively by acting as feeding deterrents (Usher & Feeny 1983, Bernays & Graham 1988, Bernays & Cornelius 1992).

In the fall armyworm, both strains attain similar larval and pupal weights when reared on bermudagrass or rice. But, when reared on corn, the corn strain performs significantly better than the rice strain (Pashley 1988b, Whitford et al. 1988). Whereas the allelochemical content of forage grasses has only recently been studied (Dubis et

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al. 1992), corn is thought to contain a more active conglomerate of allelochemicals than forage grasses (Woodward et al. 1979, Elliger et al. 1980, Bernays & Barbehenn 1987). Thus, a possible explanation for the difference between the strains is that certain physiological or biochemical attributes of corn strain larvae result in lower toxicity of corn allelochemicals relative to the rice strain. Alternatively, if ecological factors are of primary importance, allelochemicals from corn might act mostly as feeding deterrents or stimulants. In that case, corn either contains feeding deterrents more effective toward the rice strain or feeding stimulants that have a greater effect on the corn strain.

In this study, we first examined differences between larvae of the two strains, when reared on corn, in consumption (i.e., feeding behavior) and nutritional physiology. We separated actual toxicity from effects of reduced consumption (Berenbaum 1986, Bernays & Cornelius 1992) by using an analysis of covariance approach (after Rausher 1981). Next, we examined activities of detoxication enzymes in both strains. Herbivorous insects possess a number of detoxication enzymes in their midguts that can metabolize potentially toxic compounds. We examined the possible role of detoxication enzymes in host-plant adaptation by measuring mixed-function oxidase (MFO) and general esterase activities in larvae. Enzyme activities were measured toward model substrates using midgut homogenates from last instars reared on artificial diet, bermudagrass, *Cynodon dactylon* (L.) Persoon, and corn. In addition, esterases were examined in more detail by slab gel electrophoresis.

## Materials and Methods

**Insects.** Fall armyworm larvae were collected on the Louisiana Agriculture Experimental Station research farm at Ben Hur (East Baton Rouge Parish) in August 1992. Caterpillars were collected from a cornfield and a nearby pasture (≈0.25 km apart) in which bermudagrass was the dominant grass. After field-collected individuals laid eggs in the laboratory, strain identity of each individual was determined from their esterase and mitochondrial DNA genotype (according to Pashley 1986, 1989). Offspring from field-collected insects were reared for another generation on artificial diet before experiments involving enzyme assays. Insects used for consumption and utilization determinations were reared for two generations before experiments. Inbreeding in the laboratory was avoided by recording ancestry of mating pairs.

All rearing took place in an environmental chamber at 25°C and at a photoperiod of 14:10 (L: D) h. A modified pinto bean diet was used (Quisenberry & Whitford 1988). All plants were grown in a greenhouse. Bermudagrass ('Comon') was grown from seeds, and corn plants ('Pioneer', hybrid 3165) were grown and used until the first signs of tasseling. Larvae reared on plant material were kept individually in 120-ml containers in which the bottom had been covered with a solution of 2% agar to avoid desiccation. Every other day, the plant material was replaced. Larvae were examined for molting every 12 h after day 8 of development and molting larvae were weighed and separated from the remaining larvae.

**Consumption and Utilization.** Forty larvae of each strain were reared individually on corn leaves (from first instar on). Larvae in the next to last instar that had started to shed their head capsules were weighed and transferred to containers with premeasured weights of corn leaves. Amount digested (approximate) was determined as consumption minus the amount of frass produced (dry weight). Approximately 12 h after pupation, pupae were sexed, frozen, dried in an oven, and weighed. The prepupal stage, when larvae cease feeding but have not yet pupated, was considered to be part of the last larval instar. To estimate the dry weight in molting larvae from wet weight, 8 rice strain and 13 corn strain larvae (all randomly selected) were killed. One estimate of dry weight was used for both strains because they did not differ.

Measurements of consumption and utilization were taken for 23 rice strain larvae and 21 corn strain larvae. These measurements were the basis for calculating the following parameters (modified after Waldbauer 1968): relative consumption (RC), consumption relative to the duration of the last instar; approximate digestibility (AD), the amount of food digested relative to consumption; efficiency of converting digested food (ECD), the efficiency of converting digested food into biomass; efficiency of converting ingested food (ECI), the efficiency of converting ingested food into biomass.

**Chemicals for Enzyme Assays.** *p*-Nitrophenol (PNP; spectrophotometric grade), β-NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase (type XI), α-naphthol acetate, α-naphthol (99+%), and Fast Blue B salt (90% dye content) were obtained from Sigma (St. Louis, MO). *p*-Nitroanisole (PNA) was obtained from Aldrich (Milwaukee, WI). Dichloromethane (spectrophotometric grade) was obtained from EM Industries (Gibbstown, NY). All other chemicals (highest possible purity) were purchased from commercial suppliers.

**Enzyme Assays.** All enzyme assays were performed with midgut tissues from last instars reared on artificial diet, corn, or bermudagrass. General esterase activity was determined in both strains and hybrids of the two strains, whereas MFO activity was determined in both strains. The same insect homogenates that were used for esterase assays were also used for gel electrophoresis. Insects used for the MFO assays had different parents than the insects used for esterase assays (i.e., MFO activity was determined in a separate experiment). The number of insects (determinations) in each treatment combination ranged from 9 to 14 for the

MFO assays and from 7 to 14 for the esterase assays.

For MFO assays, 24 larvae of each treatment combination (diet-by-strain) were reared and weighed on day 8 of larval development. Half of the larvae (randomly chosen) were kept for use in the MFO assays. The activity of MFO was estimated by measuring the enzymatic *O*-demethylation of PNA to form PNP by the method of Hansen & Hodgson (1971) as modified by Neal & Berenbaum (1989). Actively feeding last-instar larvae (316–555 mg) that had at least doubled their weight since the last molt were used. Larvae were weighed, chilled on ice, and then dissected under a stereo microscope. Tracheae and Malpighian tubules were separated from the midgut as much as possible, the midgut was cut open longitudinally, and the contents were removed. The midgut was rinsed twice with water and homogenized in 0.7 ml of ice-cold buffer (HEPES, pH 7.8, 0.1 M) in an all-glass homogenizer. The homogenate was centrifuged at 12,000 *g* at 0°C for 12 min. From the supernatant, 0.445 ml was used for one MFO assay. The amount of protein used in the reaction mixture ranged from 0.12 to 0.41 mg. Protein concentrations were estimated by the method of Bradford (1976) with bovine serum albumin as standard. From a NADPH generating system, 50 $\mu$l was added to the reaction mixture, so that the reaction mixture contained the following reagents (final concentrations in a final volume of 0.5 ml): HEPES buffer (pH 7.8, 0.1 M), $MgCl_2$ (7.5 mM), NADP (5 mM), glucose 6-phosphate (25 mM) and 0.4 units of glucose 6-phosphate dehydrogenase. Before incubation, the reaction mixture was placed in a water bath (30°C) for 2 min and the reaction was initiated by adding 5 $\mu$l of PNA (in ethanol, final concentration 1 mM). After 10 min, the reaction was stopped by adding 0.125 ml of 1 N HCl. Preliminary studies established that, under these conditions, product formation was linear with respect to tissue equivalents and incubation time used (data not shown). Product was extracted from the reaction mixture by adding 0.625 ml dichloromethane, followed by vigorous mixing for 1 min and centrifugation at 12,000 *g* for 5 min. An aliquot (0.5 ml) was taken from the organic layer, added to 0.5 ml of 0.5 N NaOH, then mixed and centrifuged as described above. Afterward, 0.35 ml was taken from the aqueous layer (which contains the ionized form of PNP) and transferred to a well of a microplate (flat bottomed, 96 well; Costar, Cambridge, MA). Absorbance was read at 405 *n*m in a THERMOmax microplate reader (Molecular Devices, Palo Alto, CA). Absorbance was compared with a blank containing 0.5 NaOH (no impurities were detected in the reaction mixture, which affect absorbance, and PNA is very stable at room temperature). Absorbance was converted to *p*moles produced using an experimentally derived extinction coefficient (19 $[350\ \mu l]^{-1}\ pM^{-1}$)

General esterase activity was measured using the assay of Gomori (1953) as modified by van Asperen (1962). Midgut homogenates were prepared as described above (but homogenized in HEPES buffer: pH 7.6, 0.1 M) and supernatants were stored at −70°C until used. HEPES buffer was used instead of phosphate buffer because phosphate buffers interact with SDS to produce turbid solutions. Just before assays, supernatants were thawed and diluted 50 times in buffer. From the diluted supernatants, 30 $\mu$l (containing 0.046–0.39 $\mu$g protein) was used as enzyme source for one assay. Reactions were performed in a water bath at 30°C in a total volume of 1 ml (=30 $\mu$l diluted homogenate + 960 $\mu$l HEPES buffer [pH 7.6, 0.1 M] + 10 $\mu$l substrate solution). Reactions were started by addition of substrate solution ($\alpha$-naphthyl acetate in ethanol; final concentration 1.35 mM $\approx$ 9 times the $K_m$ value, as was derived from a Lineweaver–Burk plot). After 10 min, the reaction was stopped by adding 2 ml of a solution containing Fast Blue B (0.2 mg/ml) and SDS (6 mg/ml). Exactly 20 min later, absorbance was read at 600 *n*m in 1-ml cuvets. Blanks were prepared by replacing the diluted supernatant with buffer. Conversion from absorbance to *n*moles of $\alpha$-naphthol produced was based on an experimentally derived extinction coefficient for $\alpha$-naphthol (13.7 $cm^{-1}\ mM^{-1}$). Esterase assays were done twice for each larva (using the same diluted homogenate), and means of the two replications were used in statistical analyses. Preliminary studies established that product formation was linear for at least 30 min and was also well within the linear range with respect to the amount of tissue equivalents (data not shown). A broad pH optimum in esterase activity was observed at pH 7.6.

Vertical polyacrylamide slab gels were prepared as described by Davis (1964) with the following modifications. Polymerization was initiated by using ammonium persulfate only and 7.5% acrylamide was used in the separating gel. In each well, a 20-$\mu$l sample was applied that contained 75% undiluted homogenate (prepared as described above) and 25% glycerol. Gels (16 × 16 × 0.15 cm) were run at 30 mA until the tracking dye (bromophenol blue) reached the end of the gel. After electrophoresis, gels were put in boric acid (250 ml, 0.2 M) and stored in a refrigerator for 30 min. Afterward, the gels were transferred to phosphate buffer (250 ml, pH 6.5) containing 40 mg $\alpha$-naphthol acetate. After soaking the gels in phosphate buffer for 30 min, 100 ml of Fast Blue B solution were added (75 mg/100 ml; filtered before use). After 15 min, the gels were removed and fixed.

**Data Analysis.** All statistical models were tested in PROC GLM of SAS (SAS Institute 1985). Normality of residuals were tested in PROC UNIVARIATE of SAS. If data were transformed for the analysis of variance (ANOVA), inverse transformations were applied to obtain means used in fig-

**Table 1. Development variables (mean ± SEM) of corn-reared larvae used in consumption and utilization experiments**

| Variable | Rice strain | Corn strain | $F$ | df | $P$ |
|---|---|---|---|---|---|
| Wt at day 8, mg | 38.3 ± 3.9 | 54.4 ± 3.7 | 8.93 | 1, 72 | 0.004 |
| Wt at last molt, mg | 146.0 ± 5.4 | 156.0 ± 5.1 | 2.10 | 1, 45 | 0.15 |
| Larval development time, d[a] | 16.0 ± 0.2 | 15.6 ± 0.2 | 1.69 | 1, 41 | 0.20 |
| Pupal wt mg[b] | 51.3 ± 1.2 | 57.4 ± 1.2 | 12.8 | 1, 41 | 0.0009 |

[a] Sex effect: $P = 0.92$, $F = 0.01$.
[b] Sex effect: $P = 0.17$, $F = 1.96$.

ures. Equality of variance in residuals between groups was tested in PROC TTEST.

All means are reported with their standard errors. Separation of means (in enzyme assays) was accomplished by a two-tailed $t$-test at $P < 0.05$. Adjustments for unplanned multiple comparisons were made according to Bonferroni (SAS Institute 1985). Planned comparisons, in the enzyme assays, for which Bonferroni's correction can be omitted included the following four contrasts: (1) larvae reared on corn, corn strain versus rice strain; (2) larvae reared on bermudagrass, corn strain versus rice strain; (3) within corn strain, bermudagrass versus corn reared; and (4) within rice strain, bermudagrass versus corn reared.

Strains were compared for relative consumption by an ANOVA with strain, sex, and instar duration (and interactions) as sources (interaction terms with $P > 0.3$ were removed from the models). The potential effect of larval weight on consumption was initially examined as the relationship between consumption and weight of larvae at the beginning of the last instar (see Farrar et al. [1989] for why the weight at the beginning of the instar was used, rather than an average weight during the instar). However, no effects of weight at the beginning of the last instar on consumption were detected. Therefore, larval weight was omitted from the analysis of consumption. To assess whether strains differ in their ability to digest corn (AD), the amount digested was regressed on consumption. By conducting this type of covariance analysis (ANCOVA; Neter et al. 1989), a possible correlation between the percentage digested and consumption (=covariate) can be avoided (Packard & Boardman 1987). If, for example, consumption increases, then food passes through the gut faster, potentially decreasing the proportion of ingested food that is digested (Slansky & Scriber 1985). For similar reasons, ECD and ECI were analyzed using an ANCOVA as well. For strain comparisons in ECD and ECI, the numerator of Waldbauer's original index (accumulated biomass) is regressed on its denominator (=covariate: amount digested in ECD, consumption in ECI). In the actual ANCOVA, regression lines of the different treatments (strain and sex) were compared. Significant treatment by covariate interactions indicated differences in slopes of regression lines, whereas significant main effects indicate differences in intercepts.

Linearity of regression lines was tested by adding quadratic terms of covariates to the model. Because significant quadratic terms were never obtained, they are not included in the final models. Interaction terms with $P > 0.3$ were removed from the models.

Data from enzyme assays were analyzed with ANCOVA as well. In this approach, activity differences between treatments (strain and diet) were compared at either the same larval weight or at the same larval weight and protein content, by using either weight or weight and protein as covariates. ANCOVA was used instead of ratios (i.e., activity per milligram of protein) because it is a more efficient method of taking effects of weight and protein on enzyme activity into account (Packard & Boardman 1987, Laurie-Ahlberg et al. 1980, Maroni et al. 1982). Least square means were calculated for activity levels at the average value of the covariates.

## Results

**Consumption and Utilization When Reared on Corn.** Corn strain larvae performed better on corn than rice strain larvae in that they attained greater larval and pupal weights (Table 1). Larval mortality in both strains was low (corn strain, = 10%; rice strain, 15%).

During the last instar, the corn strain consumed more on average (380 ± 6.0 mg) than the rice strain (337 ± 6.3 mg; $F = 24.9$; df = 1, 41; $P = 0.001$). Males and females of each strain did not differ significantly in total consumption ($F = 1.57$; df = 1, 41; $P = 0.21$). The strain difference in consumption was irrespective of instar duration. To determine whether duration of the last instar influenced differences in consumption between strains (RC), the above data were reanalyzed by taking last-instar duration into account (Fig. 1). Only observations with a last instar duration of 5.0 and 5.5 d were included in the ANOVA to obtain a more balanced design. This ANOVA contained a highly significant strain effect ($F = 21.9$; df = 1, 33; $P = 0.001$) indicating that corn strain larvae consumed more on a daily basis. There were no strain differences in the duration of the last instar ($F = 0.58$; df = 1, 40; $P = 0.45$). Thus, the corn strain consumed more than the rice strain in both total amount and on a daily basis.

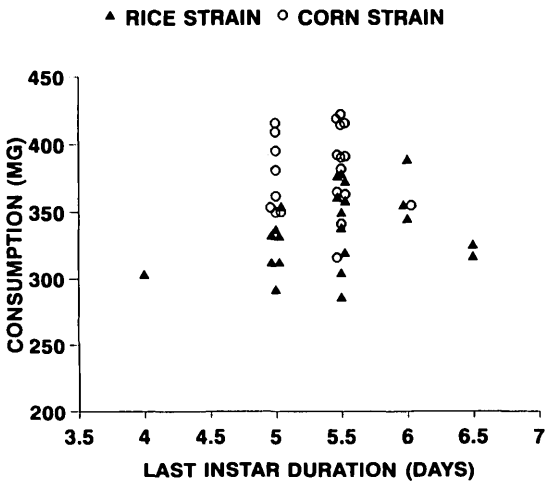


**Fig. 1.** Consumption in the two fall armyworm strains as a function of last instar duration (reared on corn).

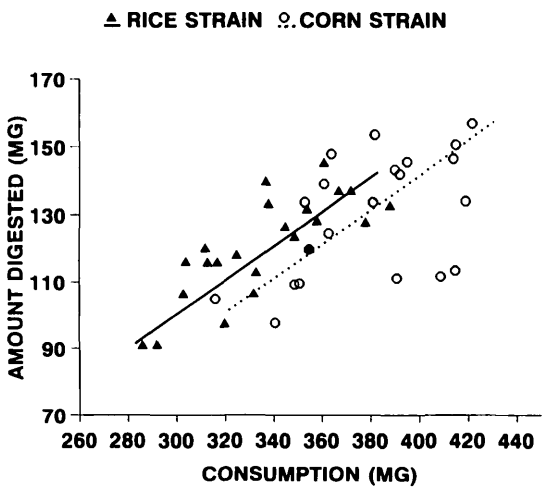


**Fig. 2.** Amount of food digested in the two fall armyworm strains as a function of consumption (reared on corn).

Strains did not differ significantly in their ability to digest corn (AD; Table 2) as determined by regressing the amount digested on consumption (Fig. 2). However, the corn strain was more variable in the amount it digested than the rice strain ($F$ = 2.77; df = 20, 22; $P$ = 0.02). The average total amounts digested were corn strain, 120.7 ± 3.4 mg and rice strain, 129.8 ± 3.5 mg.

Corn strain larvae accumulated significantly more biomass during the last instar (40.2 ± 0.97 mg compared with 35.0 ± 0.93 mg for the rice strain; $F$ = 15.0; df = 1, 41; $P$ = 0.0004). Sex effects were not significant ($F$ = 2.21; df = 1, 41; $P$ = 0.15).

The corn strain was more efficient in converting digested food into biomass (ECD). This was determined by regressing accumulated biomass on amount of food digested (Fig. 3). The analysis of covariance resulted in significant strain effects and strain-by-digestion interaction (Table 2). Because the strain-by-digestion interaction is significant, the difference in efficiency between the strains depended on the amount digested. At large amounts of digested food, the difference in efficiency is smaller than at small amounts digested (see Fig. 3). At the average amount digested by the corn strain (which is larger than the average for the rice strain), the corn strain is significantly more efficient ($t$ = 2.58, df = 39, $P$ = 0.014). Females were more efficient than males in converting digested food into biomass (Table 2).

Strains did not differ significantly in the efficiency of converting ingested food into biomass (ECI), although females were more efficient than males (Table 2). This was determined by regressing accumulated biomass on amount of food consumed (Fig. 4).

**Enzyme Assays.** When each strain was reared on their preferred host plant, for the mixed-function oxidase assays, they attained a higher larval weight than the other strain (Fig. 5). However, mean weights were only significantly different between strains when reared on artificial diet. The significant diet-by-strain interaction ($F$ = 4.05; df = 2, 125; $P$ = 0.020) indicated that the difference between strains depends on the diet on which lar-

**Table 2. Results of ANCOVA on utilization of consumed corn**

| Effect | Analysis | | | | | |
|---|---|---|---|---|---|---|
| | AD[a] | | ECD[b] | | ECI[c] | |
| | $F$ | $P$ | $F$ | $P$ | $F$ | $P$ |
| Strain | 1.78 | 0.19 | 6.67 | 0.013 | 0.09 | 0.77 |
| Sex | 0.03 | 0.86 | 1.25[c] | 0.27[c] | 11.2 | 0.002 |
| Covariate[d] | 27.0 | 0.0001 | 21.7 | 0.0001 | 43.8 | 0.0001 |
| Covariate*strain | | | 4.72 | 0.036 | | |
| Covariate*sex | | | 2.17[c] | 0.15[c] | | |

[a] Degrees of freedom for all effects 1, 40.
[b] Degrees of freedom for all effects 1, 38.
[c] If sex*digestion removed from model: $F$ = 6.51, $P$ = 0.015.
[d] Either digestion (ECD) or consumption (ECI).

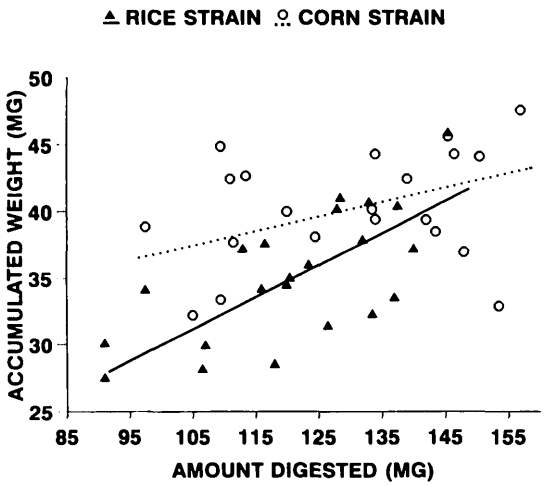


**Fig. 3.** Efficiency of converting digested food into biomass in the two fall armyworm strains (reared on corn). Biomass accumulation as a function of amount digested.

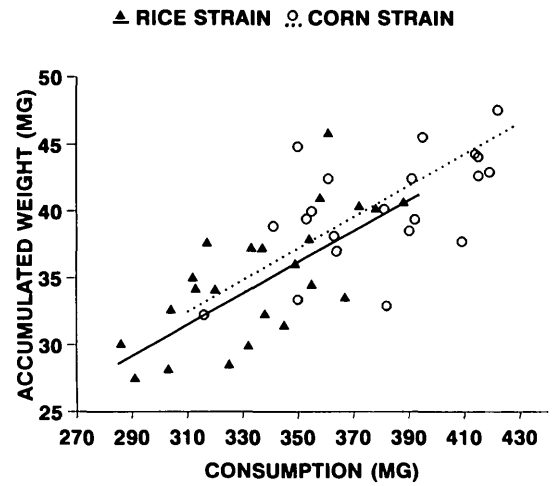


**Fig. 4.** Efficiency of converting ingested food into biomass in the two fall armyworm strains (reared on corn). Biomass accumulation as a function of consumption.

vae were reared. Thus, strains differed not just in overall average larval weight.

In artificial diet-reared larvae, MFO activity was high relative to plant-reared insects, but also significantly more variable (test of equal variance in residuals: $F = 2.96$; df = 19, 47; $P = 0.0025$, after a square root transformation). Therefore, observations on artificial diet reared insects were separated from those on other diets for analysis. The strains were not significantly different with respect to MFO activity when reared on artificial diet ($F = 3.78$; df = 1, 17; $P = 0.069$; corrected for weight & protein). Least square means (± SEM of MFO activity (in *p*mole per minute per midgut) for larvae reared on artificial diet were 44 ± 22 for the rice strain, and 105 ± 20 for the corn strain.

A comparison between strains when larvae were reared on either corn or bermudagrass showed that corn strain larvae expressed the highest MFO activity in both cases (Fig. 6; planned comparisons on bermudagrass: $P = 0.014$ [no protein correction]; $P = 0.021$ [corrected for protein]). Effects of larval host on MFO activity occurred in the corn strain (Fig. 6), but not in the rice strain (planned comparisons in rice strain: $P = 0.62$ [no protein correction]; $P = 0.98$ [corrected for protein]). The significant diet-by-strain effect in the ANCOVA, not corrected for protein differences (Table 3b), indicated that the effect of host plants on MFO activity differs (significantly) between the two strains. Thus, the effect of diet on MFO activity was significantly different between strains. If activity was corrected for protein, both strain and diet main effects were significant (Table 3a). Because differences in protein content between treatment combinations in the MFO assays were small and not significant (Table 3c), the difference between the two ways of expressing MFO activity was marginal. The manner in which activity was expressed caused a change in the significance of the diet-by-strain interaction to one of nonsignificance, but the overall pattern was the same (Fig. 6 A versus B).

There were no strain or diet differences in general esterase activity between larvae reared on either bermudagrass or corn (Fig. 7 A and B). No two-treatment combination means were significantly different from each other on these two hosts combined, even when corrections for multiple comparisons were omitted (in both Fig. 7 A and B). Therefore, strain and diet effects measured for general esterase activity (Table 4) seemed to result from artificial diet reared insects. The main difference between the two ways of expressing activity (Fig. 7 A versus B) was caused by the higher

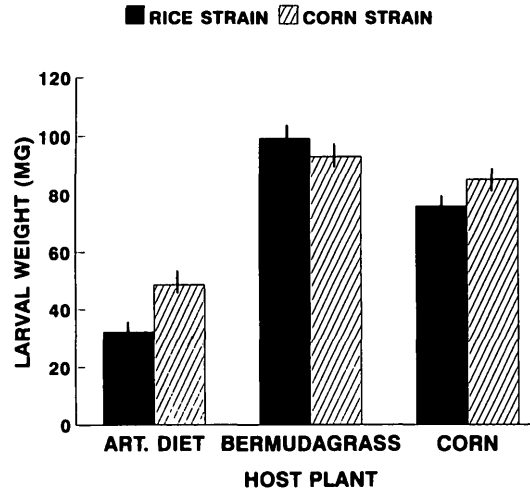


**Fig. 5.** Weights (mean ± SEM) on day 8 of development of larvae reared for mixed-function oxidase assays (134 observations total). Strains are significantly different from each other on artificial diet only ($P < 0.004$).

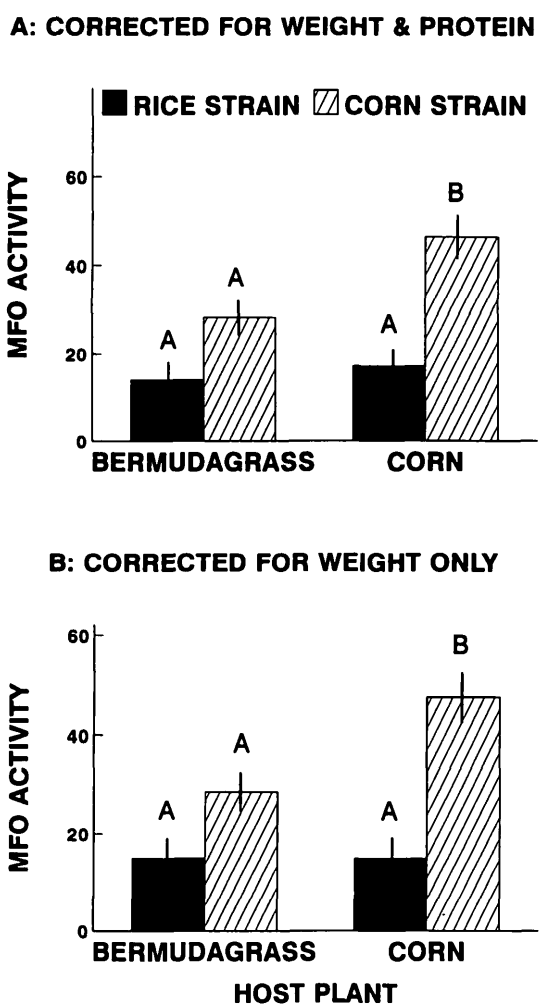


**Fig. 6.** Mixed-function oxidase activity (mean ± SEM; in *p*mole per midgut per minute) of both strains reared on bermudagrass or corn. Means are corrected for either weight and protein content (A), or for weight only (B). Means with no letter in common are significantly different ($P < 0.05$; Bonferroni's correction).

protein content in homogenates from larvae reared on artificial diet. A similar trend in protein content was also apparent in the MFO assays. There was a significant (overall) strain effect when esterase activity was corrected for protein (Table 4a). In addition, there was a trend toward a diet-by-strain interaction. The latter seemed to be caused by the rice strain's high activity on artificial diet (Fig. 7A). However, there were also significant differences between strains and diets in the protein content of homogenates (Table 4c). No strain or diet-by-strain effects were apparent when activity was corrected for weight only (Table 4b; Fig. 7B). Thus, the significant strain effect, when activity is corrected for weight and protein, could be explained by differences in protein content.

**Table 3. Results of ANCOVA on MFO activity**

| Effect | Mean square[a] | *F* | df | *P* |
|---|---|---|---|---|
| a. Corrected for wt and protein ($r^2$–model = 0.51) | | | | |
| Diet | 1,436 | 5.38 | 1, 41 | 0.025 |
| Wt | 485 | 1.82 | 1, 41 | 0.18 |
| Protein | 863 | 3.24 | 1, 41 | 0.079 |
| Strain | 6,993 | 26.2 | 1, 41 | 0.0001 |
| Strain*diet | 806 | 3.02 | 1, 41 | 0.0896 |
| Error | 267 | — | 41 | — |
| b. Corrected for weight only ($r^2$–model = 0.47) | | | | |
| Diet | 1,146 | 4.08 | 1, 42 | 0.050 |
| Wt | 981 | 3.49 | 1, 42 | 0.069 |
| Strain | 8,112 | 28.87 | 1, 42 | 0.0001 |
| Strain*diet | 1,387 | 4.94 | 1, 42 | 0.032 |
| Error | 281 | — | 42 | — |
| c. ANCOVA protein content midguts ($r^2$–model = 0.15)[b] | | | | |
| Diet | 4.75 | 0.98 | 1, 42 | 0.33 |
| Wt | 16.76 | 3.44 | 1, 42 | 0.071 |
| Strain | 6.31 | 1.30 | 1, 42 | 0.26 |
| Strain*diet | 14.56 | 2.99 | 1, 42 | 0.091 |
| Error | 4.86 | — | 42 | — |

[a] Multiply times 0.001 in protein content.
[b] Protein content was corrected for weight.

Banding patterns measured in electrophoretic gels stained for esterase activity were highly variable among individuals from both strains. Consequently, no diagnostic bands were found that could be used to separated strains or diets. A maximum of 12 bands could be distinguished per individual.

## Discussion

Our studies have contributed to a better understanding of the physiological and behavioral factors influencing differences in larval performance. Results indicated that differences between the strains in consumption rates can explain, in a large part, differences in performance on corn. However, differences in efficiency of converting digested food indicated that the corn strain is better able to utilize corn as a host because of physiological differences as well. In addition, results of the enzyme assays indicated that increased levels of MFO activity may contribute to better performance of the corn strain on corn.

We compared strains with respect to consumption rates, the ability to digest corn, and efficiencies of converting ingested and digested corn into biomass. Most studies in Lepidoptera do not examine directly the consequences of feeding behavior on nutritional physiology (but see Berenbaum 1986). Some justify this by arguing that behavior is not fundamentally different from physiology (Lockwood et al. 1984, Slansky 1990). However, most allelochemicals that reduce growth, reduce consumption as well (Bernays & Chapman 1987).

The conversion of consumed food into biomass can be viewed as a two-step process involving digestion and the conversion of digested food into biomass (Slansky & Scriber 1985). Host-plant al-

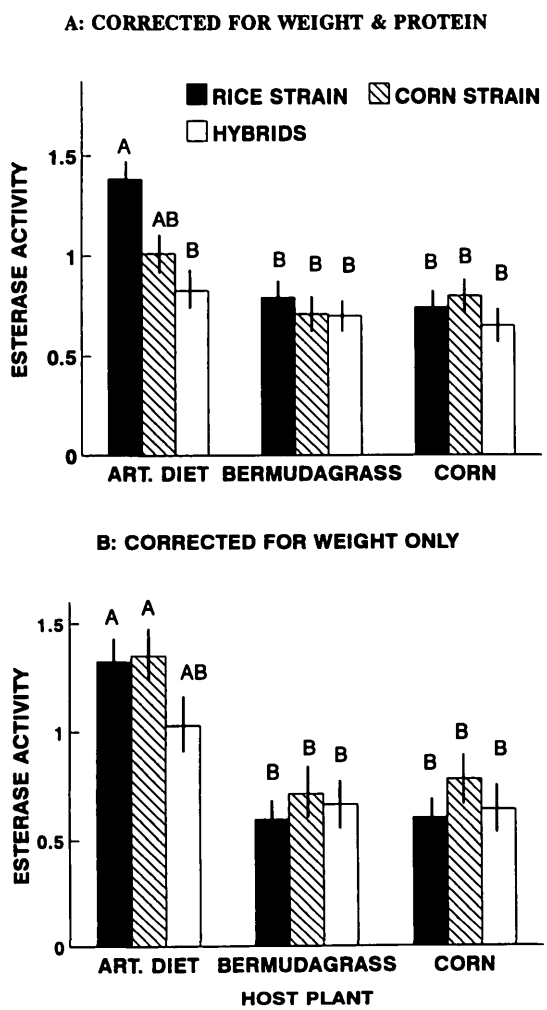


**Fig. 7.** General esterase activity (mean ± SEM; in μmole per midgut per minute) in both strains and hybrids. Means are corrected for either weight and protein content (A), or for weight only (B). Means with no letter in common are significantly different ($P < 0.05$; Bonferroni's correction).

**Table 4. Results of ANCOVA on general esterase activity**

| Effect | Mean square[a] | $F$ | df | $P$ |
|---|---|---|---|---|
| a. Corrected for wt and protein ($r^2$–model = 0.78)[b] | | | | |
| Diet | 211.4 | 12.19 | 2, 69 | 0.0001 |
| Wt | 15.3 | 0.88 | 1, 69 | 0.35 |
| Protein | 885.5 | 51.09 | 1, 69 | 0.0001 |
| Strain | 84.1 | 4.85 | 2, 69 | 0.011 |
| Strain*diet | 42.8 | 2.47 | 4, 69 | 0.053 |
| Error | 17.3 | — | 69 | — |
| b. Corrected for weight only ($r^2$–model = 0.61)[b] | | | | |
| Diet | 767.2 | 25.80 | 2, 70 | 0.0001 |
| Wt | 552.7 | 18.59 | 1, 70 | 0.0001 |
| Strain | 43.5 | 1.46 | 2, 70 | 0.24 |
| Strain*diet | 22.2 | 0.75 | 4, 70 | 0.56 |
| Error | 29.7 | — | 70 | — |
| c. ANCOVA protein content midguts ($r^2$–model = 0.61)[c] | | | | |
| Diet | 6.32 | 11.83 | 2, 70 | 0.0001 |
| Wt | 14.81 | 27.69 | 1, 70 | 0.0001 |
| Strain | 4.86 | 9.08 | 2, 70 | 0.0003 |
| Strain*diet | 0.70 | 1.30 | 4, 70 | 0.28 |
| Error | 0.53 | — | 70 | — |

[a] Multiply times 0.001.
[b] On square-root transformed data.
[c] On squared transformed data; protein content was corrected for weight.

lelochemicals can exert antibiotic effects before absorption into the midgut by reducing the digestibility of the ingested food (Slansky 1992). For example, Houseman et al. (1992) concluded that the allelochemical, DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), which has been isolated from corn, exerts its antibiotic effects in this way on the European corn borer, *Ostrinia nubilalis* (Hübner). The fall armyworm strains studied here digest similar amounts of ingested food when differences in consumption between strains are taken into account. Thus, interference of host-plant allelochemicals in the digestive process does not seem to be important in causing strain differences when reared on corn.

Differences between the sexes in efficiencies of conversion reported here for fall armyworm have been reported for other Lepidoptera as well (Slansky & Scriber 1985). Sexes are known to differ with respect to protein and lipid content of pupae (Lederhouse et al. 1982, Slansky & Scriber 1985). Therefore, sex differences likely reflect qualitative differences between the sexes in accumulated biomass.

The importance of the difference in consumption becomes evident from the fact that a 15% difference in biomass accumulation between the strains was matched by a 13% difference in consumption. The differences in consumption could be mediated through differential responses to feeding deterrents or stimulants. Thus, both behavioral and physiological differences seem to be important components influencing the difference between the strains in their performance on corn.

Because measurements of nutritional physiology are only indirect measures of physiological performance, it is difficult to determine the underlying causes of differences in physiology. Interpretation of these measurements can also be complicated by a possible correlation between feeding and digestive physiology. This complication is minimized here by using an ANCOVA approach. These limitations have led some to question the usefulness of these measurements for studying host-plant adaptations in insects (Berenbaum & Zangerl 1992). But the measurements of digestive physiology, as employed here, are quite useful for testing the hypothesis that allelochemicals affect only feeding behavior and have no intrinsic toxicity.

Among the detoxication enzymes in the midguts of herbivorous insects, MFOs, and general esterases are considered by many to be of primary im-

portance (Krieger et al. 1971, Dowd et al. 1983, Ahmad et al. 1986, Lindroth 1991). In our studies we observed differences in MFO, but not esterase, activities between fall armyworm strains. The corn strain expressed higher MFO activity when reared on corn, than when reared on bermudagrass. In contrast, no differences in MFO activity were apparent in rice strain larvae reared on these hosts. A possible explanation for this difference is that allelochemicals present in corn induce MFO activity in the corn strain, but not in the rice strain. If so, an advantage of high MFO activity in feeding on corn plants is suggested. Similar activity levels would have indicated no role of MFOs in host-plant adaptation. However, these data can not be directly related to host-plant allelochemical metabolism, because the metabolism of allelochemical substrates was not directly measured. Multiple forms of MFOs are known to exist that differ in substrate specificity (Soderlund & Bloomquist 1990). Thus, the corn strain could have different isozymes of MFOs, which metabolize PNA better than isozymes of the rice strain.

It has been argued that detoxication enzymes are energetically costly (Schoonhoven & Meerman 1978, Brattsten 1979, but see Neal [1987] and Appel & Martin [1992]). In addition, allelochemical content can be variable among and within host species. A strategy to deal with allelochemical variability is to produce detoxication enzymes in quantities that are proportional to the amount of host-plant allelochemicals encountered. This form of enzyme induction can be viewed as an adaptive form of phenotypic plasticity, and is considered to be a common strategy in herbivorous insects (Gould 1984). However, if insects are constantly exposed to inducing allelochemicals over evolutionary time, a canalization of the response could evolve (Weir 1992). If the response becomes canalized, enzyme activity is no longer a function of the amount of allelochemicals encountered.

Overall higher MFO oxidase activity in the corn strain compared with the rice strain on both host plants could indicate that some canalization had occurred in MFO activity levels. But, canalization was not of such magnitude that it led to the loss of inducibility. This could be interpreted as evidence that metabolic costs caused by the overproduction of detoxication enzymes are ecologically important.

A pattern similar to the one here in MFO oxidase activity was observed in a comparison of general esterase activities between two tiger swallowtail subspecies (Lindroth 1989). The two subspecies differ in their ability to use plants of Salicaceae as hosts, which is associated with a difference in esterase activity. In the subspecies that is able to utilize Salicaceae, higher general esterase activity is measured, and activity can be induced by allelochemicals from Salicaceae, whereas esterase activity in the other subspecies cannot be induced.

In our studies, esterase activities were similar in both strains when reared on both host plants, but were significantly higher in both strains when reared on artificial diet. Thus, esterase activity can be influenced (and possibly induced) by the diet on which larvae are reared. However, from the perspective of differences in host-plant adaptation between the fall armyworm strains, only diet effects in MFO oxidase activity seem to be relevant to host use patterns in nature.

An unexpected finding in our study was that the highest activity in both enzyme systems occurred on artificial diet. Although it is generally assumed that artificial diet contains no inducers for detoxication enzymes, Ahmad & Forgash (1978) also found higher MFO oxidase activity in larvae of the gypsy moth, *Lymantria dispar* (L.), reared on an artificial diet than in larvae reared on a plant material diet (oak). They speculated that insecticide residues in components of the artificial diet acted as MFO inducers. In the results reported here, contamination with xenobiotic compounds, or the presence of specific allelochemicals in the diet, could explain not just the higher enzyme activities of insects reared on artificial diet, but also their overall poor performance on artificial diet. Generally, fall armyworms grow as well or better on artificial diet than on plant material (Pencoe & Martin 1981, Pantoja et al. 1987; K.H.V., unpubished data).

An alternative explanation for the high enzyme activities in larvae reared on artificial diet is that enzyme activities were inhibited when reared on either corn or bermudagrass. If so, it implies that when larvae are reared on (real) host plants, the corn strain is less sensitive to enzyme inhibition than the rice strain. In that case, the results could imply that the corn strain is better adapted to corn because of its ability to overcome (some of) the inhibitory effects of allelochemicals from corn. But it would also suggest that enzyme activities are most inhibited when reared on bermudagrass. However, forage grasses, like bermudagrass, are thought to contain generally few active allelochemicals (Bernays & Barbehenn 1987). In a survey of hydroxamic acids, which include DIMBOA and MBOA (6-methoxybenzoxazolin-3-one) and is considered to be one of the most important groups of allelochemicals in the grass family, Zuniga et al. (1983) found no detectable amounts of these compounds in bermudagrass (see also Niemeyer 1988).

Results of esterase assays show the importance of considering differences in protein content between treatments when activity is corrected for protein. Because detoxication enzymes make up only a fraction of the proteins detected by the protein assay, differences in protein between diets and strains are most likely the result of differences in other proteins. Higher protein content measured from insects fed artificial diet, for example, is likely to be the result of high protein content of artificial diet, relative to corn or bermudagrass. Protein was,

however, a highly significant effect in the model for esterase activity, despite the fact that it is correlated with weight. This means that there is a strong (and positive) correlation within each treatment combination between the amount of protein in the homogenate and esterase activity. A likely explanation for this observation is that the protein content of the homogenate is an indicator of how much detoxication enzyme is actually liberated from the tissue during homogenization.

In our results of general esterase activity, strains were only significantly different if activity was corrected for protein. Because the difference in protein content between the strains was significant, we concluded that strains did not differ significantly in general esterase activity. The relevance of considering differences between treatments in protein content of homogenates was also shown by Lindroth et al. (1990, 1991) who examined the effect of dietary protein on esterase activity. When they reared larvae on a diet low in protein, esterase activity per milligrams of protein increased significantly. But given the results here of a higher protein concentration in homogenates from larvae fed a high protein diet, the higher esterase activity on a low protein diet could be caused by protein differences in homogenates. Strain differences in protein content were also detected by Grant et al. (1989) between an insecticide-resistant and susceptible strains of mosquitos.

In conclusion, the patterns of MFO activity indicated that enzymatic detoxication by these enzymes could play an important role in host-plant adaptation. However, we found no evidence that indicated the importance of general esterases in host-plant adaptation, although activity of these enzymes was affected by the diet on which they were reared.

Because differences between the two strains in performance are relatively minor, ecological factors could be important in determining host use in the fall armyworm. Research on the possible effect of ecological factors on host use patterns by phytophagous insects is undoubtedly too often neglected. Information on predation and parasitation rates for both strains in both cornfields and pastures would provide a more complete understanding of factors underlying host-plant specialization in fall armyworm strains. Our results, however, indicated that physiological and biochemical adaptations can provide at least part of the explanation for the patterns of host use in two strains of fall armyworm.

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