EFFECT OF THE CARDENOLIDE DIGITOXIN ON PERFORMANCE OF GYPSY MOTH (LYMANTRIA DISPAR) (LEPIDOPTERA: LYMANTRIIDAE) CATERPILLARS

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ABSTRACT

Despite the central role of cardenolides in the classic milkweed-monarch-predator chemically-mediated multiple trophic level interaction, very few studies have actually demonstrated that cardenolides protect host plants against herbivores. We tested the hypothesis that cardenolides are an effective chemical defense against generalist insect herbivores by feeding fifth instar gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), larvae artificial diets containing low and high levels of digitoxin, a cardenolide found in leaves of purple foxglove, *Digitalis purpurea* L. (Scrophulariaceae). Relative to performance of larvae fed the control diet, consumption rate, conversion efficiency, and growth rate were all reduced by at least 60% for larvae fed the relatively low level of digitoxin found in young leaves, and by at least 90% for larvae fed the higher level found in older leaves. Digitoxin acted primarily as a feeding deterrent at the lower level, but was toxic at the higher level.

These results suggest that the cardenolide digitoxin is a highly effective plant defense against generalist herbivores.

It has been estimated that approximately 80% of insect herbivores of herbaceous plants are "specialists," feeding on three or fewer plant families (Schoonhoven et al. 1998). The evolution of feeding specialization among insect herbivores is often attributed primarily to interspecific variation in plant chemical defenses (Rausher 1983, Futuyma and Peterson 1985, Jaenike 1990).

Cardenolides (cardiac glycosides) play a central role in one of the most cited examples of chemically-driven feeding specialization among insect herbivores. Caterpillars of the monarch butterfly *Danaus plexippus* (L.) (Nymphalidae) feed almost exclusively on plants in the Asclepiadaceae (Ackery and Vane-Wright 1984) and sequester cardenolides from their *Asclepias* host plants (Malcolm and Brower 1989). As a consequence, monarch caterpillars and adults are unpalatable to a wide range of vertebrate predators, including birds (Brower 1984, Brower and Fink 1985, Brower et al. 1988) and mice (Glendinning and Brower 1990).

Surprisingly, despite their central role in this classic example of a chemically-mediated multiple trophic level interaction, very few studies have actually demonstrated that cardenolides protect host plants against insect herbivores. We are not aware of any study that has definitively demonstrated an effect of milkweed cardenolides on monarch caterpillars, possibly because it is difficult to separate their effect from that of the sticky latex in which they are concentrated (Zalucki et al. 2001). However, feeding by monarch caterpillars was deterred by one of three non-milkweed cardenolides, though not by two others (Vickerman and de Boer 2002). Cardenolides in members of the Brassicaceae genera *Cheiranthus* and *Erysimum* make these species unpalatable to larvae and ovipositing females of the cabbage butterfly, *Pieris rapae* L. (Pieridae)

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(Sachdev-Gupta et al. 1993; Chew and Renwick 1995). In the only other study of which we are aware, Akhtar and Isman (2004) demonstrated that, when added to an artificial diet, the cardenolides digitoxin (from foxglove) and cymarin (from pheasant's eye) reduce feeding and growth of the cabbage looper, *Trichoplusia ni* (Hübner) (Noctuidae).

In this study, we sought to augment the sparse information about the ability of cardenolides to defend plants by conducting quantitative feeding trials in which we exposed a generalist herbivore, gypsy moth, to concentrations of digitoxin that spanned the range observed in foxglove plants. We asked two related questions: 1) Does digitoxin reduce consumption, digestive efficiency, conversion efficiency, and/or growth of gypsy moth caterpillars? and 2) If so, is the effect of digitoxin dose-dependent?

MATERIALS AND METHODS

Gypsy moth caterpillars were reared from hatching through the end of the 4th instar on gypsy moth artificial diet purchased from BioServ (Frenchtown, NJ). Larvae that had stopped feeding and with protruding head capsules at the end of the 4th instar were placed in individual 25-ml polystyrene cups for molting. Upon molting into the 5th instar, 14-16 larvae per diet were weighed, placed in individual 25-ml polystyrene cups containing a preweighed amount of artificial diet containing no digitoxin (control), 70 µg/g dry weight digitoxin (low digitoxin), or 575 µg/g dry weight digitoxin (high digitoxin). Low and high digitoxin levels were chosen to represent the range found in *D. purpurea* leaves throughout the growing season (Evans and Cowley 1972). After 72 hours, each larva was removed from its diet, placed in an empty cup for two hours to allow gut clearing, frozen, dried to constant weight at 70 °C, and weighed. Uneaten food and frass were also dried to constant weight at 70 °C and weighed. Ten additional freshly molted 5th instar larvae were weighed, frozen, dried to constant weight at 70 °C and reweighed to provide a conversion factor for estimating the initial dry weight of test larvae. Digitoxin was purchased from Sigma Chemical Company (St. Louis).

Larval performance measures (Waldbauer 1968), based on dry weights, were calculated as follows:

Relative consumption rate (RCR) = food ingested/(average larval weight)(days)

Approximate digestibility (AD) = (food ingested – frass)/food ingested

Efficiency of Conversion of Digested Food (ECD) = weight gained/(food ingested – frass)

Relative growth rate (RGR) = weight gained/(average larval weight)(days)

Since data for all variables were not normally distributed, means were compared by a Kruskal-Wallis test followed by pairwise Kolmogorov-Smirnov tests. All statistical analyses were performed using SPSS v14.0.

RESULTS

Even at the lowest dose reported from *D. purpurea*, digitoxin acted as a powerful feeding deterrent for gypsy moth larvae (Table 1). Relative consumption rate (RCR) was reduced by 74% for larvae fed the low digitoxin diet (K-S = 2.78, $P < 0.001$), and by 90% for larvae fed the high digitoxin diet (K-S = 3.73, $P < 0.001$). The effect of digitoxin on consumption appeared to be dose-dependent, as larvae fed the high digitoxin diet consumed only 40% as much diet as larvae fed the low digitoxin diet (K-S = 2.15, $P < 0.001$).

Table 1. Effect of dietary digitoxin on performance of gypsy moth larvae. Means (± S.E.) followed by different letters are significantly different at $P < 0.005$ by Kruskal-Wallis tests followed by pairwise Kolmogorov-Smirnov tests. Sample sizes are 16, 15, and 14 for the control, low digitoxin, and high digitoxin diets, respectively.

Diet	RCR[1] (g/g/d)	AD[2] (%)	ECD[3] (%)	RGR[4] (g/g/d)	Weight Gained (mg)
Control	2.08 ± 0.06[a]	48.9 ± 1.8[a]	52.4 ± 2.5[a]	0.52 ± 0.02[a]	68.2 ± 3.9[a]
Low Digitoxin	0.55 ± 0.06[b]	68.6 ± 3.3[b]	21.5 ± 5.6[b]	0.10 ± 0.02[b]	6.6 ± 1.9[b]
High Digitoxin	0.22 ± 0.02[c]	70.6 ± 4.1[b]	-15.3 ± 9.2[c]	-0.02 ± 0.01[c]	-0.7± 0.9[c]

[1]RCR - relative consumption rate
[2]AD - approximate digestibility
[3]ECD - efficiency of conversion of digested food
[4]RGR - relative growth rate

Likely as a consequence of reduced consumption, and therefore increased residence time of food in the gut, approximate digestibility (AD) increased significantly for larvae fed the low (K-S = 2.25, $P < 0.001$) and high (K-S = 2.20, $P < 0.001$) diets. However, AD did not differ between larvae fed the two digitoxin diets (K-S = 1.03, $P = 0.24$).

Larvae fed digitoxin diets converted significantly less digested food into larval biomass. The efficiency of conversion of digested food (ECD) was reduced by 60% for larvae fed the low digitoxin diet, and was actually negative for larvae fed the high digitoxin diet (K-S = 2.39, $P < 0.001$). Again, the effect of digitoxin appeared to be dose dependent, as ECD was significantly lower for larvae fed the low digitoxin diet than for larvae fed the high digitoxin diet (K-S = 1.74, $P = 0.005$).

As a consequence of reduced consumption rate and conversion efficiency, larvae fed digitoxin diets grew significantly more slowly than larvae fed the control diet. Relative growth rate (RGR) was reduced by 81% on the low digitoxin diet (K-S = 2.78, $P < 0.001$), and weight gain was reduced by 90% (K-S = 2.78, $P < 0.001$). RGR and weight gain were both negative for larvae fed the high digitoxin diet (K-S = 2.73, $P < 0.001$ for both measures of growth). RGR and weight gain were both significantly lower for larvae fed the high digitoxin diet than for larvae fed the low digitoxin diet (K-S = 1.78, $P = 0.004$ and K-S = 1.74, $P = 0.005$, respectively).

Growth strongly depended on consumption for larvae fed the control and low digitoxin diets, but not for larvae fed the high digitoxin diet (Figure 1). On the control diet, larvae that consumed more grew more rapidly ($r^2 = 0.80$, $F = 56.1$, $P < 0.0001$); this relationship was also very strong for larvae fed the low digitoxin diet ($r^2 = 0.63$, $F = 22.3$, $P = 0.0004$). However, consumption was not correlated with growth on the high digitoxin diet ($r^2 = 0.05$, $F = 0.6$, $P = 0.5$).

DISCUSSION

The results of this study suggest that, even at the lowest levels reported by Evans and Cowley (1972) for *D. purpurea* leaves, digitoxin constitutes an effective chemical defense against gypsy moth larvae. Relative to performance of larvae fed the control diet, consumption rate, conversion efficiency, and growth rate were all reduced by at least 60% for larvae fed low levels of digitoxin, and by at least 90% for larvae fed higher levels.
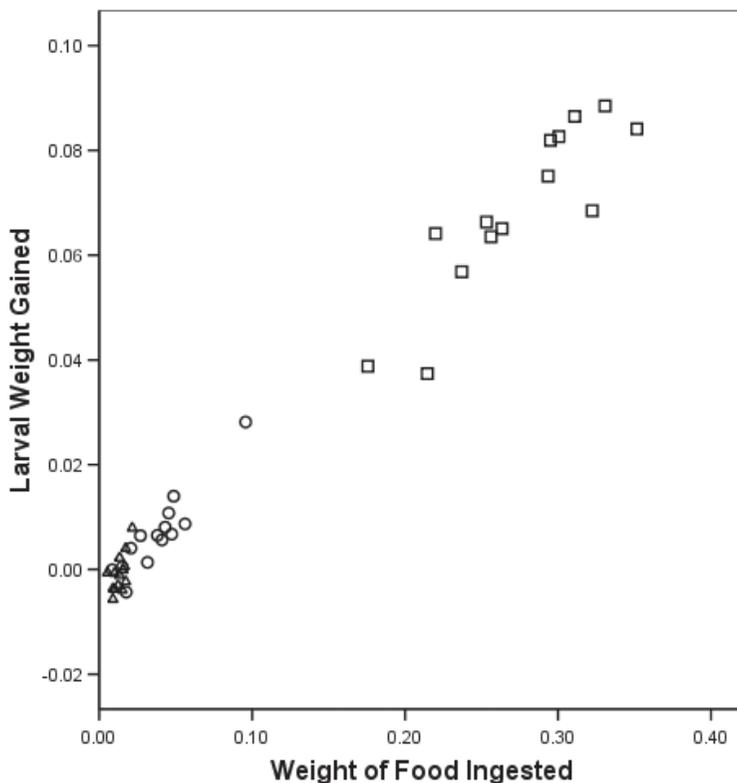
Figure 1. Correlation between weight gained and weight of food ingested (both in g dry) for gypsy moth larvae reared on the control diet (squares, $r^2 = 0.80$, $P < 0.0001$), low digitoxin diet (circles, $r^2 = 0.63$, $P = 0.0004$), and high digitoxin diet (triangles, $r^2 = 0.05$, $P = 0.5$).

Larvae fed the low digitoxin diet displayed positive growth, and likely would have completed development had they been allowed to feed for longer than 72 hours, albeit at a much slower rate than larvae that did not ingest digitoxin. The strong positive correlation between consumption and growth for larvae fed the low digitoxin diet suggests that, at the lower end of the range of concentrations reported from foxglove leaves, digitoxin acts primarily as a feeding deterrent rather than as a toxin. In contrast, larvae fed the high digitoxin diet displayed negative growth, and likely would not have completed development. Both negative growth and the lack of correlation between consumption and growth for larvae fed the high digitoxin diet suggests that, at levels near the high end of the range reported from foxglove leaves, digitoxin acts primarily as a toxin.

According to Evans and Cowley (1972), the low level of digitoxin used in this study is characteristic of young *D. purpurea* leaves, while the high level is characteristic of older leaves. The lower levels of defensive compounds in young leaves is often thought to arise from a trade-off between defense and autotoxicity and/or growth (e.g., Herms and Mattson 1992, van Dam et al. 1996, Iwasa 2000). If the response of gypsy moth larvae to digitoxin is characteristic of other non-adapted herbivores, then our results indicate that young *D. purpurea* leaves may contain little enough digitoxin to minimize autotoxicity and/or resource

allocation costs, yet enough to dramatically decrease consumption and thereby slow herbivore growth. As a consequence of their reduced development rate, herbivores that are sensitive to digitoxin would be forced to ingest foliage with high (and probably toxic) levels during their later instars. However, this hypothesis rests on the untested assumption that higher digitoxin levels would create substantial autotoxicity and/or resource allocation costs in young leaves.

Finally, it should be noted that, while the response of gypsy moth caterpillars may be representative of non-adapted herbivores in general, it is unlikely that even high levels of digitoxin would affect herbivores with peritrophic membranes that prevent absorption of this compound, such as the grasshopper, *Melanoplus sanguinipes* (Fab.) (Acrididae), and the caterpillar, *Manduca sexta* (L.) (Sphingidae), (Barbehenn 1999, 2001), or herbivores that can sequester cardenolides such as the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Lygaeidae), (Duffey et al. 1978, Scudder and Meredith 1982).

ACKNOWLEDGEMENTS

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