Allylglucosinolate and Herbivorous Caterpillars: A Contrast in Toxicity and Tolerance

Abstract. *Allylglucosinolate, found in many cruciferous plants, is acutely toxic to* Papilio polyxenes *larvae, which do not normally attack crucifers. By contrast, larval growth of* Pieris rapae, *a crucifer specialist, is not affected even by artificially high concentrations of allylglucosinolate. Larval growth of* Spodoptera eridania, *a generalist feeder, is inhibited by high but not by low concentrations of the compound.*

Many plants contain repellents that inhibit feeding by herbivorous insects (*1*). Plants are also believed to contain compounds that would be toxic to insects that ingested them (*2*). Such adverse effects on metabolism presumably reinforce the selective advantage of the behavioral deterrence (*3*). Unequivocal demonstration of toxicity is often hampered, however, by the behavioral responses of experimental insects. Slow growth does not necessarily indicate adverse effects on metabolism since it can result simply from behavioral inhibition of feeding (*4*).

In an earlier study of the possible defensive function of glucosinolates in plants of the family Cruciferae, Erickson and Feeny (*5*) cultured leaves of celery (Umbelliferae: *Apium graveolens*) in aqueous solutions of allylglucosinolate (sinigrin); the treated leaves were then offered to larvae of the black swallowtail butterfly, *Papilio polyxenes*, that normally attack only plants of the family Umbelliferae. Slow growth and high mortality suggested that allylglucosinolate (AG) was toxic to the larvae, but no attempt was made to control experimentally for the effects of reduced feeding rate on larval growth.

Using a new technique that permits a clearer distinction between feeding inhibition and toxicity, we here confirm that natural levels of AG in crucifers are toxic to *P. polyxenes* larvae. Using the same technique, we show also that a substantial increase in the AG content of collards, *Brassica oleracea* var. *acephala*, has no detectable toxic effect on larvae of the cabbage butterfly *Pieris rapae*. Larvae of the southern armyworm, *Spodoptera eridania*, a generalist feeder, possess intermediate tolerance for the compound.

Our assay for toxicity of AG was the reduction in growth rate, at constant consumption rate, of larvae fed diets containing increasing amounts of the compound. Since AG may alter consumption rates, the effects of increasing AG concentration were compared by analysis of variance of the regression slopes of growth rate on consumption rate for each AG treatment.

A "calibration curve" was first obtained for the effect of varying consumption rate on larval growth rate in the absence of supplementary AG. We then reasoned that plots of growth rate against consumption rate for larvae feeding on diets containing supplementary AG would fall below the calibration line if AG was toxic. If the compound was not toxic, but inhibited feeding, the points should fall on the calibration line, although they would be concentrated in the range of low consumption rate.

We obtained calibration curves for final instar larvae of the three species by offering them different amounts of leaves, ranging from no food at all to a

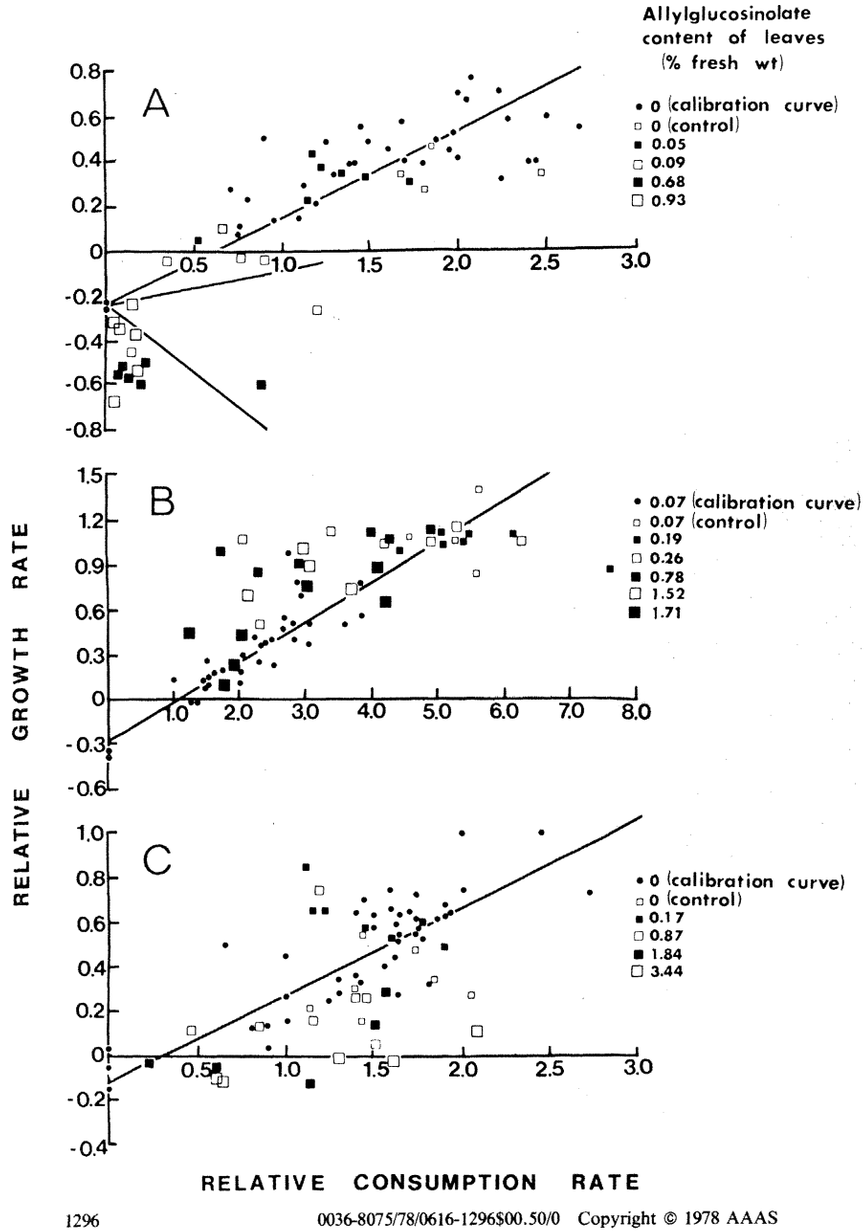

Fig. 1. Relative growth rates [expressed as milligrams of weight gain (dry weight) per milligram of larva (dry weight) per day] and relative consumption rates [expressed as milligrams of food eaten (dry weight) per milligram of larva (dry weight) per day] of larvae fed leaves containing various concentrations of allylglucosinolate (experiment 1). (A) *Papilio polyxenes* fed *Daucus carota* (Calibration: $y = 0.375x - 0.211$; $r = .81$). (B) *Pieris rapae* fed *Brassica oleracea* ($y = 0.275x - 0.286$; $r = .88$). (C) *Spodoptera eridania* fed *Phaseolus lunatus* ($y = 0.388x - 0.090$; $r = .82$). Calculated regression lines are shown only for calibration data and for *P. polyxenes* larvae fed on leaves containing 0.09 and 0.68 percent allylglucosinolate.

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surplus of food, from typical food plants for 24 hours. Larvae of *P. polyxenes* were offered carrot (Umbelliferae: *Daucus carota*), *P. rapae* larvae were offered collards, and *S. eridania* larvae were offered baby lima bean (Leguminosae: *Phaseolus lunatus*). The larvae had been reared from eggs on these plants and were placed in individual plastic petri dishes within a biochamber under controlled conditions (25°C day and 16°C night; a photoperiod of 16 hours of light and 8 hours of darkness; 60 percent relative humidity). Feeding and growth rates were measured by standard techniques (*6*) and converted to relative values so that results for the three species could be compared (Fig. 1).

Groups of freshly cut leaves of each food-plant species were then cultured for 12 hours in aqueous solutions containing various concentrations of AG (ICN Nutritional Biochemicals). Analysis by gas-liquid chromatography (GLC) of randomly selected leaf samples from each treatment revealed the concentrations of AG taken up into the leaves and confirmed that the compound had not been degraded within the cultured leaves (*7*). Under experimental conditions identical to those used in preparing the calibration curves, groups of four to seven final instar larvae of each species, in individual petri dishes, were then offered a surplus of treated leaves of the appropriate food plant and their rates of feeding and growth were measured for 24 hours (Fig. 1). For each species we also included control groups of larvae that were offered a surplus of untreated leaves.

Papilio polyxenes larvae fed more slowly as the AG content of their food increased; growth rates on most treatments were lower than the calibration line, however, indicating that declining food consumption was not the only reason for poor larval growth (Fig. 1A). On leaves containing 0.68 percent AG or more, larvae were atypically sluggish and excreted large quantities of fluid; after 24 hours, half of the larvae feeding on the leaves containing 0.93 percent AG had died.

Larval growth of *Pieris rapae*, by contrast, was evidently unimpaired by a substantial increase in the AG content of the food (Fig. 1B). *Spodoptera eridania* larvae seemed unaffected by low doses of the compound, but growth fell below the calibration line at higher AG concentrations (Fig. 1C).

For statistical analysis of the results for each species, we calculated a common intercept family of linear regressions of relative growth rate (RGR) on relative consumption rate (RCR), with one line for each AG treatment (including the calibration data). The regression lines shown in Fig. 1A, for example, are then but three rays of a family of six estimated lines converging to a common intercept (the RGR of starved larvae). Intercept and slopes were estimated and tested by standard statistical methodology for linear models (*8*). The slope of any ray represents growth efficiency of larvae (that is, their RGR, above that of starved larvae, divided by RCR) on that treatment.

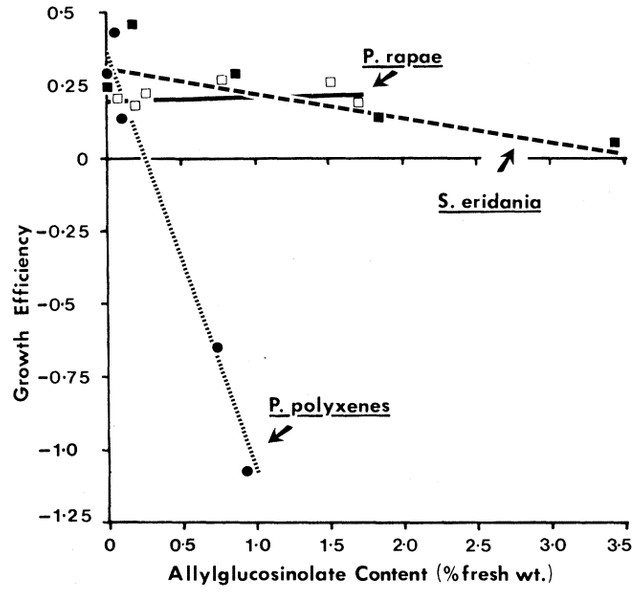

Fig. 2. Relation between larval growth efficiency and allylglucosinolate content of food leaves (experiment 1). For *P. polyxenes*, $y = 0.359 - 1.428x$; for *P. rapae*, $y = 0.194 + 0.017x$; for *S. eridania*, $y = 0.331 - 0.083x$.

The growth efficiency of *P. polyxenes* larvae varied significantly among treatments (excluding the calibration data, $F_{4,56} = 8.60$, $P < .001$) and declined with increasing AG content (Fig. 2; $F_{1,56} = 24.15$, $P < .0001$). The growth efficiency of *P. rapae* larvae, by contrast, did not differ significantly among treatments ($F_{5,60} = 2.28$; $.10 > P > .05$), nor was there a significant correlation with AG concentration (Fig. 2; $F_{1,60} = 0.86$, $P > .25$). Growth efficiency of *S. eridania* larvae differed among treatments ($F_{4,68} = 10.15$, $P < .0001$) and declined with increasing AG content ($F_{1,68} = 23.25$, $P < .0001$), though the slope was much less than for *P. polyxenes* (Fig. 2).

In a second experiment, ten freshly molted fifth instar *P. rapae* larvae, raised from eggs on collards, were reared throughout the fifth instar on each of four groups of collard leaves that had been cultured for 24 hours in aqueous solutions of AG. Larvae were placed in individual plastic petri dishes in a Twincubator (Scientific Systems, Baton Rouge) at 21° ± 1°C, 50 ± 1.5 percent relative humidity and 16 hours light, 8 hours dark. Leaves, with petioles extending through the walls of the dishes into Aquapics (Syndicate Sales, Kokomo, Indiana) containing distilled water (*9*), were replaced every 24 hours with new, weighed leaves that had been freshly cultured in the appropriate AG solutions. Larvae were weighed, and feces and unconsumed leaf were collected every 24 hours; growth parameters were determined by the usual methods (*6*). The AG concentrations of separate leaves, cultured alongside those fed to the larvae, were determined by high-pressure liquid chromatography (*10*). Increasing AG content of the leaves was found to have no significant effect on relative consumption rate (linear regression/analysis of variance: $r = .20$, $F_{1,37} = 1.59$, $P > .10$), relative growth rate ($r = .27$, $F_{1,37} = 2.84$, $P > .10$) or duration of the instar ($r = .04$, $F_{1,37} = .064$, $P > .25$) (*11*).

Reported AG levels in crucifers range up to 0.66 percent of fresh weight (*12*). Our results show that these levels would be toxic to *P. polyxenes* larvae that might attack such plants and they thus provide an effective defense against some nonadapted insects. Growth of *P. rapae* larvae, by contrast, is unaffected by variation in AG content. For this crucifer-adapted species, variation of plant nitrogen content is a dominant influence on larval growth (*13*).

Herbivorous insects that attack a diverse range of food plants might be less efficient at exploiting any one food plant than are specialized insects, which restrict their feeding to one or a few related plants (*14, 15*). In particular, the ability of generalists to tolerate a wide range of toxins could be correlated with reduced efficiency at detoxifying any one compound (*16*). Our results show that typical

natural levels of AG are unlikely to inhibit growth of *S. eridania*, although growth might be reduced on plants containing unusually high concentrations of the compound. This result is consistent with the finding that generalists exhibit great variation in growth rate on a range of food-plant species but that growth on some species is comparable to that of specialists feeding on similar plants (*15, 17*).

The mechanisms of toxicity of allylglucosinolate to *P. polyxenes* and, at higher concentrations, to *S. eridania* remain unknown although toxicity is likely to be a consequence of hydrolysis to allylisothiocyanate (*5*). We found this compound to be present in the gut contents, feces, and various body tissues of *P. polyxenes* larvae that had ingested AG (*18*). The mechanisms of counteradaptation in *P. rapae* and *S. eridania* are likewise unknown, although AG is known to stimulate induction of microsomal mixed-function oxidase enzymes in *S. eridania* larvae (*19*).

Feeny (*20*) has suggested that certain chemical compounds in plants may serve as qualitative barriers to herbivorous insects. Although effective even in small amounts against nonadapted herbivores, they are especially vulnerable to the evolution of counteradaptations and may have little or no dosage-dependent effect on the growth of insect species that are specialists at feeding on plants containing them. Allylglucosinolate seems to be an example of such a barrier.

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10. Leaves were extracted in methanol; AG was hydrolyzed to allylisothiocyanate (*7*). Ether extracts of the hydrolyzates were analyzed quantitatively on a high-pressure liquid chromatograph (Waters Associates) with a model 400 absorbance detector (254 nm). We used a Corasil II column (61 cm by 2 mm, inside diameter) with a chloroform, *n*-hexane solvent system (1 : 1, by volume) at a flow rate of 2.0 ml/min.
11. For the four AG concentrations, measured as 0.04 ± 0.01, 0.74 ± 0.05, 1.83 ± 0.15, and 2.39 ± 0.25 percent (fresh weight of leaf), values of relative consumption rate were, respectively, 1.09 ± 0.08, 1.76 ± 0.11, 1.32 ± 0.10, and 1.58 ± 0.15 mg (dry weight) per milligram of larval biomass (dry weight) per day; values of relative growth rate were, respectively, 0.18 ± 0.01, 0.18 ± 0.01, 0.17 ± 0.01, and 0.15 ± 0.01 mg (dry weight) per milligram of larval biomass (dry weight) per day; values of instar duration were, respectively, 5.0 ± 0.0, 4.6 ± 0.5, 4.7 ± 0.4, and 4.9 ± 0.3 days.
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18. Larvae were fed for 24 hours on leaves of *D. carota* that had been cultured in aqueous solutions of ^{14}C-labeled AG. Feces were collected, hemolymph was obtained from a snipped proleg, gut contents were removed, and remaining tissues (head capsule, cuticle, fat body, gonads, and Malpighian tubules) were combined. Extracts of each fraction were examined by scintillation counting, by thin-layer chromatography, and by paper chromatography. For techniques, see I. W. Grote, *J. Biol. Chem.* **93**, 25 (1931); K. G. Krebs, D. Huesser, H. Wimmer, in *Thin-Layer Chromatography*, E. Stahl, Ed. (Springer-Verlag, New York, 1969); O. E. Schultz and R. Gmelin, *Z. Naturforsch.* **7b** 500 (1953); E. Josefsson, *Phytochemistry* **6**, 1617 (1967).
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Prediction of Learning Rate from the Hippocampal Electroencephalogram

Abstract. *Samples of spontaneous electroencephalographic (EEG) activity from the dorsal hippocampus of rabbits were recorded immediately before classical conditioning of the nictitating membrane response. Computer analysis revealed a significant predictive relationship between EEG frequency characteristics and the subsequent rate of learning.*

The hippocampal region of the forebrain has been widely implicated in learning and related processes (*1–3*). In addition to lesion effects on learning (*2, 4*) and changes in neuronal unit activity during the course of conditioning (*5, 6*), the frequency characteristics of the hippocampal electroencephalogram (EEG) have been reported to be correlated with acquisition (*7*) or with other behavioral processes accompanying learning, such as attention, arousal, consolidation, or the motor response itself (*8–12*).

In the rabbit, the hippocampal EEG is dominated by rhythmic slow activity (RSA), a large-amplitude, almost sinusoidal waveform of approximately 3 to 7 Hz, which occurs (i) in the waking state, (ii) in response to many forms of stimulation, and (iii) during paradoxical sleep (*9, 13*). Previous research in our laboratory has demonstrated a significant positive relationship between increases in hippocampal unit activity and the development of classical conditioning of the nictitating membrane (NM) response [tone conditioned stimulus (CS); air puff unconditioned stimulus (UCS)] in the rabbit (*5*), but little is known about the relationship between hippocampal EEG and learning or between the EEG and unit activity under this paradigm. We now present an initial and unexpected result of our investigation of the relations among hippocampal EEG, neuronal unit activity, and learning: a significant and predictive correlation between pretraining frequency characteristics of the EEG in the dorsal hippocampus and the subsequent rate of classical conditioning.

Each of 16 New Zealand White rabbits (*Oryctolagus cuniculus*) was anesthetized with halothane (Fluothane) and implanted with one epoxylite insulated stainless-steel electrode having a record-

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