

EFFECT OF SOYBEAN PHYTOALEXINS ON THE HERBIVOROUS INSECTS MEXICAN BEAN BEETLE AND SOYBEAN LOOPER^{1,2}

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Abstract—Effects of soybean phytoalexins on the feeding of the soybean looper and Mexican bean beetle were investigated to test the hypothesis that phytoalexins might be a defense mechanism of plants against insects as well as against pathogens. Short-term behavioral responses to the phytoalexins were analyzed using dual-choice tests with phytoalexin-rich and phytoalexin-poor (control) tissues. Phytoalexin production was elicited with ultraviolet radiation. Results from the dual-choice tests indicated that 6th instar soybean looper larvae fed equally on the control and phytoalexin-rich tissues. Feeding by adult and 4th instar Mexican bean beetles, however, was strongly deterred by the phytoalexins as evidenced by “single-bite” mandible scars on the phytoalexin-rich cotyledon discs. Nutritional effects of the isoflavonoid phytoalexin glyceollin on early instar soybean looper larvae were tested by incorporating the phytoalexin into an artificial medium at a level of 1% dry weight (0.15% fresh weight). The larvae were reared for 7 days from emergence on diets of control and glyceollin-containing media. Although survival on the glyceollin diets was initially less than on the control diets, under the experimental conditions glyceollin had no significant effect on the growth, development, or subsequent survival of the larvae. Efficiency of food utilization (ECI) was reduced, indicating that the phytoalexins may be a mild digestibility-

¹Mexican bean beetle = *Epilachna varivestis* Mulsant, (Coleoptera: Coccinellidae); soybean looper = *Pseudoplusia includens* (Walker), (Lepidoptera: Noctuidae).

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reducing factor for the loopers. Implications of the results for host-plant resistance are discussed.

Key Words—*Pseudoplusia includens*, soybean looper, Lepidoptera, Noctuidae, *Epilachna varivestis*, Mexican bean beetle, Coleoptera, Coccinellidae, feeding preferences, nutrition, food utilization, host-plant resistance, induced resistance, glyceollin, isoflavonoids, soybean, phytoalexins.

INTRODUCTION

The literature on phytochemical defense against insect herbivores abounds with reports on secondary plant products that are present at potent levels prior to insect herbivory (Whittaker and Feeny, 1971; Levin, 1976; Kogan, 1977; Swain, 1977; Hedin, 1977; Rosenthal and Janzen, 1979; Schoonhoven, 1981). There are comparatively few examples of allelochemicals induced by herbivory (Ryan and Green, 1974; Ryan, 1978; Carroll and Hoffman, 1980), although induced substances, such as phytoalexins, are commonly considered to be involved in defenses of higher plants to microbes (Cruikshank, 1963; Kuc, 1972; Deverall, 1976; Swain, 1977). Phytoalexins may be defined as antibiotic metabolites which undergo enhanced or de novo synthesis and accumulate in plants following exposure to microorganisms (Van Etten and Pueppke, 1976; Paxton, 1980). Physiological stress from mechanical injury, UV radiation, and chemicals—including some pesticides—may also elicit phytoalexin production (Kuc et al., 1976). In some cases herbivores may be involved in eliciting phytoalexin production (Akazawa et al., 1960; Loper, 1968; Uritani et al., 1975). Recent work suggests that phytoalexins may be a causal factor in plant resistance to nematodes (Rich et al., 1977; Kaplan et al., 1980) and insects (Russell et al., 1978; Sutherland et al., 1980; McIntyre et al., 1981).

Given the theoretical and practical importance of knowing whether phytoalexins play a multiple role in plant defense, we studied their involvement in resistance of soybean to two herbivorous insects: the Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), and the soybean looper (SBL), *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae). The MBB is an oligophagous insect with a host range restricted to a few genera of Leguminosae (Kogan, 1972). The species causes significant damage to soybean in the Atlantic coastal states and certain regional pockets in the Midwest, but there is considerable temporal variation in its pest status on soybean (Turnipseed and Shepard, 1980). The SBL, a comparatively polyphagous insect (Herzog, 1980), is a serious pest of soybean in the southern United States (Burleigh, 1972).

With soybean, as with many plants, more than one phytoalexin accumulates after infection (Kuc et al., 1976). Glyceollin, an isoflavonoid with four isomeric forms (Figure 1) (Lyne et al., 1976; Lyne and Mulheirn, 1978),

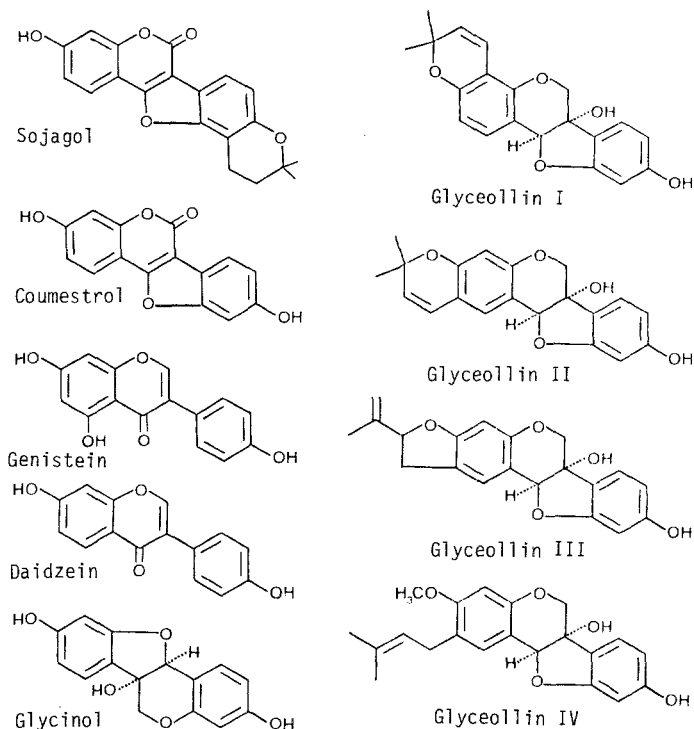


FIG. 1. Isoflavonoids commonly associated with the phytoalexin response in soybean plants.

has been the most-studied phytoalexin of soybean (Keen et al., 1972; Keen and Kennedy, 1974; Keen and Paxton, 1975). Until 1975 glyceollin was misnamed hydroxyphaseollin (Burden and Bailey, 1975). Recently another isoflavonoid phytoalexin, glycinol, was named (Figure 1) (Weinstein et al., 1981).

Chemical resistance of plants to insects either disrupts the behavioral sequence of host selection (antixenosis) or renders plants unfit for insect nutrition through antibiosis or both (Kogan, 1977). Hence we tested the effects of soybean phytoalexins on short-term host selection by the two insects using dual choice tests and assayed chronic effects of glyceollin on early instars of the soybean looper. The ecological implications of antiherbivory effects of soybean phytoalexins are discussed.

METHODS AND MATERIALS

Dual-Choice Tests. The two cotyledons from each 8- to 12-day-old soybean seedling, cultivar Clark 63, were excised and kept in two separate

sets. After all cotyledons were surface-sterilized for 5 min in 3% H₂O₂ and rinsed with sterile deionized water, 1-cm-diam. disks were cut from their centers. The first set of cotyledon disks was irradiated with a UV micro-bicidal lamp for 20 min at a distance of ca. 17 cm. UV light induces phytoalexin production, with significant accumulation occurring 12–24 hr after irradiation (Bridge and Klarman, 1973). Apparently, phytoalexin production mechanisms by UV stimulus are similar to those induced by biotic elicitors (Moesta and Grisebach, 1980).

The irradiated disks (designated hereafter as phytoalexin disks) were incubated in darkness at 24–25°C for 2 days to permit accumulation of phytoalexins. The other set of disks (designated hereafter as control disks) were kept under the same conditions as the phytoalexin disks and were irradiated for 20 min at the end of the 2-day incubation period.

Phytoalexin production may be elicited by physical injury such as cutting (Kuc et al., 1976). Thus, aliquots of control disks were analyzed to ascertain that the levels of glyceollin were very low relative to those in the phytoalexin disks.

For each replicate of the choice test, a control and a phytoalexin disk derived from the same seedling were placed in a 5-cm-diam. feeding arena (Figure 2). Tests were conducted with 4th instar MBB, 6-day-old adult MBB, and 6th instar SBL larvae. One insect was introduced into each dual-choice feeding arena within 2–4 hr after irradiation of the control disks. The MBB larvae were ca. the 3rd greenhouse-reared generation of beetles field-collected in North Carolina. The MBB adults were the 2nd greenhouse-reared generation of beetles collected in Tifton, Georgia. Larval and adult beetles were reared in the greenhouse on snap bean, *Phaseolus vulgaris*, foliage. The larvae, supplied with water from a cotton wick, were starved 1 day prior to the test. Adults were starved 2 days before testing. The SBL larvae came from a laboratory culture reared on an alfalfa meal-wheat germ medium adapted from Henneberry and Kishaba (1966). The genetic diversity and vigor of the stock culture were maintained by an annual addition of field-collected individuals. The SBL larvae were not starved prior to testing.

The MBB larvae ($N = 22$) were permitted to feed for 18 hr and the adults ($N = 30$), for 21 hr, at 27°C. Although mean feeding on the most-consumed disk in each replicate was only 35% (visual estimate) in the larval test and 20% in the adult test, the tests had to be terminated before phytoalexins reached significant levels in the controls. The tests were conducted in darkness to eliminate visual responses to the reddish phytoalexin disks or the green control disks. Feeding was measured as mean No. of feeding ridges/disk (see Figure 3).

The SBL larvae ($N = 40$) were allowed to feed 5 hr at 27°C in darkness. After 5 hr, mean feeding on the most consumed disk in each replicate was

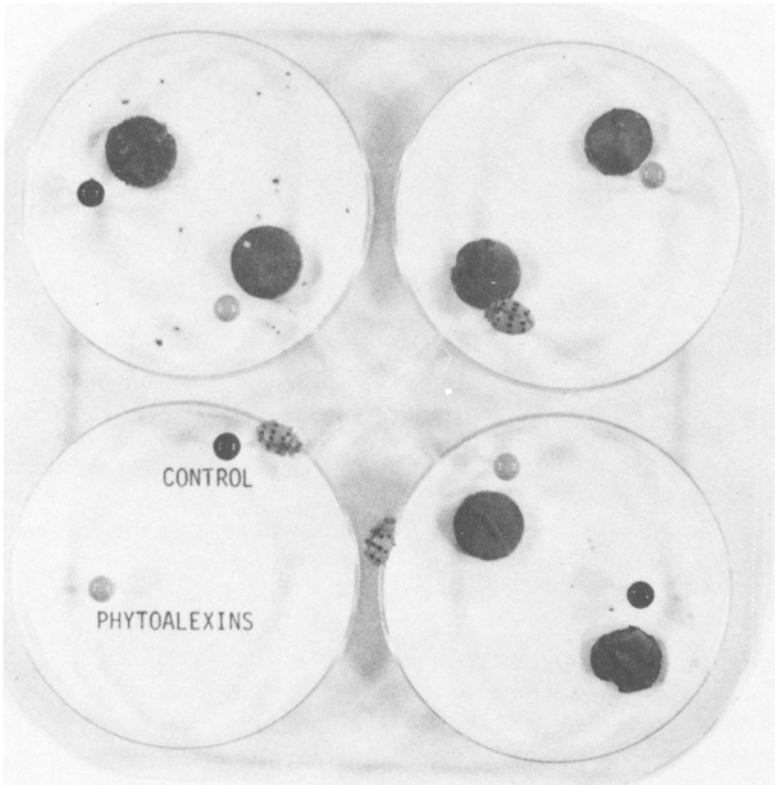


FIG. 2. Clustered plastic Petri dishes used as feeding arenas in dual-choice experiments. Each of the four round cells is a separate arena and contains a control and a phytoalexin cotyledon disk. For this photo the lid was removed and 3 adult Mexican bean beetles are standing on a cotyledon disk or on the rim of the arena.

ca. 50% (visual estimate). The mean fresh weight (FW) and mean dry weight (DW) of the disks consumed during the test were evaluated. An initial DW for the disks was obtained by multiplying their initial FW by a DW:FW ratio. This ratio (pooled mean) was calculated from the initial FW and final DW of 16 randomly selected pairs of phytoalexin and control disks. Weight loss due to evaporation was similar in control and in phytoalexin disks ($P < 0.05$).

For both the MBB and SBL choice tests, differences between feeding on control and phytoalexin disks were analyzed with a paired t test ($P < 0.05$). The preference index, C , was used to show the direction and magnitude of feeding preference on a scale of 0 to +2 (Kogan and Goeden 1970). Computation is based on the relation $C = 2P/(S + P)$, where S is the amount of

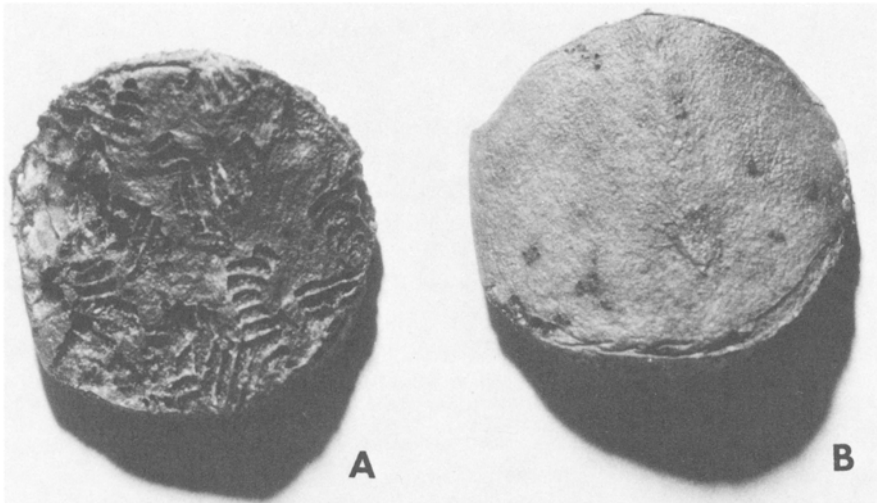


FIG. 3. Feeding patterns on cotyledon disks from Mexican bean beetle dual-choice tests. (A) Feeding ridges observed on the control disks. (B) Mandible holes (probing) observed on the phytoalexin disks.

feeding on the standard control disk and P denotes feeding on the corresponding phytoalexin disk. $C = 1$ indicates no preference for the control disk relative to the phytoalexin disk in an arena; $C < 1$ indicates a preference for the control disk vis-à-vis the phytoalexin disk. The hypothesis that the mean values of C were less than 1 was tested with a one-tailed t test ($P < 0.05$).

Random samples of 6–10 phytoalexin and control disks were frozen in 80% EtOH at the onset of the test for glyceollin analysis. Three of these samples, one from each of the choice tests, were later extracted in hexane and ethyl acetate using the first extraction procedure in Keen et al. (1971). The EtOAc fractions and glyceollin standards were applied to TLC plates (250 μm thick) of silica gel GF₂₅₄ (E. Merck). The plates were developed in 60 hexane–40 EtOAc–1 MeOH (v/v). Bands at the R_f values of the glyceollin standards were eluted in EtOH. The identity of the eluates was confirmed by their UV absorption spectra. Glyceollin concentration was measured at the absorption maximum of 287 nm. The concentrations of glyceollin were adjusted for a mean ($N = 3$) recovery from the TLC plates (250 μm thick) of 44% ($s = 8.1$). No attempt was made to measure total recovery of glyceollin during tissue extraction. Concentrations of induced compounds other than glyceollin were not determined.

Nutrition Test. Using an artificial medium, the chronic effects of glyceollin were tested at the high but physiological level of 1% DW (0.15 FW)

(Keen and Kennedy, 1974). Glyceollin was obtained by excising soybean cotyledons from week-old seedlings (cv. Clark 63) and inoculating them with an aqueous extract of *Phytophthora megasperma* var. *sojae*, race 1, as described by Frank and Paxton (1971).

The inoculated cotyledons were incubated at 25°C for 2 days, permitting high levels of glyceollin to accumulate. The cotyledons and diffusates were subsequently freeze-dried and ground, yielding 5.6 g of powder from which 11.5 mg of glyceollin was extracted. Extraction followed the same procedure described for the analysis of the choice-test cotyledons, but silica gel 60F₂₅₄ TLC plates, 2 mm thick (EM Reagents®), were used. For the control diets, an eluate (EtOH) was prepared using a quantity of blank silica gel equivalent (by weight) to that providing the glyceollin.

Since the quantity of glyceollin available was relatively small, we devised a method for making small volumes of diets. Five mg of glyceollin in 6 ml EtOH was added to the alphacel component of the alfalfa meal-wheat germ medium (AM) in each of two 5-cm-diam. Petri dishes. The EtOH was evaporated from the alphacel under vacuum. Media containing 1% glyceollin (DW) were prepared by pipetting 3.5 g of warm AM (15% DW) minus alphacel into each Petri dish. While a warm water bath prevented solidification of the media, the alphacel and extract residues were thoroughly mixed into the AM. The media without glyceollin were prepared using the same procedures, but the solution of glyceollin was replaced with a 6-ml solution of the control eluate.

Disks of media, 9 mm in diam. and 1–3 mm thick, were placed at the bottom of 9 × 30-mm shell vials ($\frac{1}{4}$ dr). The vials were sealed with Saran® film. Thirty of the diets were randomly selected from each treatment and inoculated with 2 newly emerged SBL larvae. The larvae were derived from the same stock as that used in the choice tests. The diets were incubated for 7 days at 27 ± 2°C, 80 ± 5% relative humidity, under a 15:9 hr light-dark photoperiod. At the start and end of the test, four diets were randomly selected from each treatment and analyzed to determine if glyceollin decomposed during the test.

The first day after inoculation of the diets (day 2), dead larvae were replaced with newly emerged larvae, and the Saran seals were pierced with an insect pin for ventilation. On day 3 the smaller larva was removed from each vial so that there remained one larva per vial. When both larvae appeared similar in length, removal was random.

Larval survival was recorded every other day. On day 7, the stadia of the larvae were determined using head capsule measurements (Jackai, 1978). Differences in survival were tested using contingency tables ($P < 0.05$). Insect growth and food utilization during the 7-day period were appraised by the mean DW gain of the larvae (μg), mean DW of the diet removed (μg), mean efficiency of conversion of ingested food (ECI), mean

consumption index (CI), and mean relative growth rate (RGR) (Wald-bauer, 1968; Klein and Kogan, 1974). Larval weight gain was calculated by subtracting the initial from the final larval weight. Initial DW was obtained from the mean of a 15-larvae aliquot from the same original population. For the determination of DW diet removed, the initial fresh weights of the diets were multiplied by a DW:FW ratio to obtain an estimate of initial DW. This DW:FW ratio (pooled mean) was calculated from the initial DWs and FWs of an aliquot ($N = 20$) of diets from each treatment. The quantity of diet consumed by larvae through the 3rd day of the test was assumed to be negligible. Dry weights were measured on a Cahn 21/Ventron® automatic electrobalance. Significance of differences between means was tested using one-way analysis of variance ($P < 0.05$).

The diets analyzed for glyceollin were extracted with EtOAc that had been treated with 0.01 M K_2HPO_4 (10:1) to reduce acidity. Analytical TLC of glyceollin was similar to that described for the choice tests, but TLC plates spotted with final extractions of the diets were developed in 85 benzene–15 MeOH (v/v), and glyceollin was eluted in MeOH or EtOAc. The EtOAc eluates were dried under air and reconstituted with MeOH before spectrophotometric analysis.

RESULTS

Choice Tests. The UV absorption spectra of glyceollin extracts matched those reported by Burden and Bailey (1975). The mean ($N = 3$) concentration of glyceollin was determined to be 1.82 ($s = 1.031$) mg/g DW [0.20 ($s = 0.101$) mg/g FW] for the phytoalexin disks and 0.13 ($s = 0.119$) mg/g DW [0.01 ($s = 0.013$) mg/g FW] for the control disks. Glyceollin production in the control cotyledons may have been induced when the tissue was cut into disks (Kuc et al., 1976).

The mean number of feeding ridges (Figure 3) made by the MBB larvae and adults of both sexes was significantly greater on the control disks than on the phytoalexin disks (Table 1). The mean preference indices (C), 0.22 ± 0.05 for the MBB larvae and 0.02 ± 0.02 for the adults, were both significantly less than 1 ($P < 0.05$) showing that these insects ate little of the phytoalexin tissue compared to the control tissue (Figure 4). Only one of the 30 adult beetles fed on a phytoalexin disk. While there were few feeding ridges on phytoalexin disks in the MBB tests, numerous mandible holes were observed where the insects bit the phytoalexin tissue but did not proceed to feed (Figure 3). Sixty-eight percent (30/44) of the phytoalexin disks without feeding ridges had mandible holes. All but one of the control disks had feeding ridges.

For the SBL test in which feeding was measured as both fresh and dry

TABLE 1. MEAN DIFFERENCE (X_d) IN FEEDING ON PAIRED CONTROL PHYTOALEXIN DISKS^a

Insect test	<i>N</i>	X_d	(SE)	
MBB				
Larvae	22	+33.1	(5.41)	* ^b
Adults				
♀♀	16	+10.8	(2.35)	*
♂♂	14	+14.9	(3.02)	*
♀♀ + ♂♂	30	+12.7	(1.89)	*
SBL larvae	40	+ 0.1	(0.87)	NS

^aFeeding was measured as number of feeding ridges for the Mexican bean beetle (MBB) and in estimated mg dry weight eaten for the soybean looper (SBL). Positive values indicate greater consumption of control disks than of phytoalexin disks.

^bThe mean difference in feeding on pairs of control and phytoalexin disks in the same arena is significantly greater than 0 at the 0.05 level (paired *t* test).

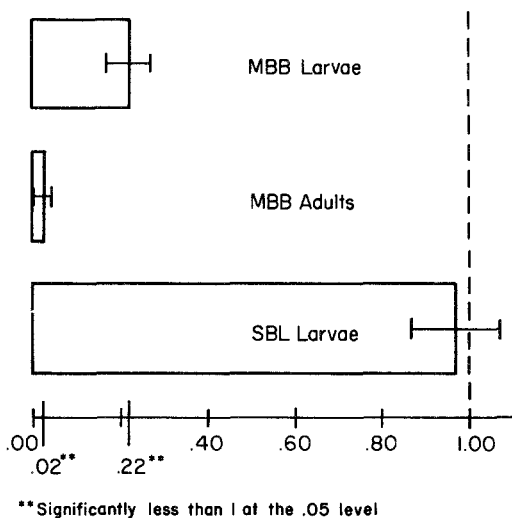


FIG. 4. Mean preference indices (*C*) for feeding on control disks (*S*) and on phytoalexin disks (*P*). MBB = Mexican bean beetle; SBL = soybean looper. Feeding was measured as number of feeding ridges for the MBB, and in estimated mean dry weight for the SBL.

TABLE 2. SURVIVAL OF NEONATE SOYBEAN LOOPER LARVAE REARED ON ARTIFICIAL DIETS OF 0% AND 1% GLYCEOLLIN (DRY WEIGHT) FOR 7 DAYS

Day of test	No. live larvae (% survival)	
	Control diet	1% Glyceollin diet
1	60	60
2 (before replacement of dead larvae)	55 (92%)	47 (78%)
3 (before thinning)	33 (51%)	40 (55%)
3 (after thinning)	30	30
7	19 (63%)	21 (70%)

weight of disk consumed, the differences between feeding on control disks and phytoalexin disks were not significant (Table 1). Likewise the mean preference indices (C), calculated from both the FW and DW data, were 1.00 ± 0.12 and 0.97 ± 0.10 , respectively, and not significantly less than 1 (Figure 4). Hence at the test concentrations, glyceollin and other phytoalexins present did not affect the SBL larvae's choice of food.

Nutrition Test. Survival of SBL larvae on day 2 of the nutrition test was significantly greater ($P < 0.05$) on the control diets (92%) than on the glyceollin diets (78%) (Table 2). Such effect, however, was not observed among the batch of newly emerged larvae that replaced the dead larvae on

TABLE 3. GROWTH AND FOOD UTILIZATION OF SOYBEAN LOOPER LARVAE FED ARTIFICIAL DIETS OF 0% AND 1% GLYCEOLLIN (DRY WEIGHT) FOR 7 DAYS AFTER EMERGENCE

Food utilization index	Mean (SE) ^a	
	Control diet ($N = 19$)	1% Glyceollin diet ($N = 20$)
Larvae dry weight gain (μg) (estimate)	141 (26.8)	110 (21.0) NS
Dry weight of diet consumed (μg) (estimate)	827 (160.0)	933 (121.5) NS
Efficiency of conversion of ingested food	39 (11.9)	15 (3.3) *
Consumption index	2.0 (0.51)	2.3 (0.26) NS
Relative growth rate ^b	0.35 (0.022)	0.31 (0.022) NS
Relative growth rate ^c	0.23 (0.007)	0.22 (0.009) NS

^aNS, differences between means not statistically significant; *, $P < 0.05$, $df = 37$, Student's t test.

^bKlein and Kogan (1974).

^cWaldbauer (1968).

day 2. On day 3, larval survival on those diets with replaced larvae was 60% (6/10) for the control diets and 79% (19/24) for the glyceollin diets. No differences were subsequently apparent between survival (Table 2), development, or growth of larvae reared on the control and glyceollin diets during the 7-day test. The mean DW gain of larvae fed the glyceollin diets was less, although not significantly different, than that of larvae on the control diets (Table 3). However, ECI was more than two times greater with the control than with the phytoalexin diet, although there were no significant differences in CI or RGR (Table 3). Hence the results of the nutrition study indicate that the survival, development, and growth of early SBL instars were not greatly affected by the 1% DW concentration of glyceollin, but food utilization was reduced. Glyceollin levels remained constant during the experiment; the mean concentration of glyceollin at the end of the test—6.1 $\mu\text{g}/\text{mg DW}$, $s = 4.02$ ($N = 4$)—was not significantly different from the mean concentration at the start of the test—5.2 $\mu\text{g}/\text{mg DW}$, $s = 2.45$ ($N = 4$).

DISCUSSION

Many of the isoflavonoids and related flavonoids possessing reactive free hydroxyl groups are biologically active (Harborne, 1979). Some of the flavonoids are attractant, deterrent, repellent, antibiotic, or toxic to insects (Shaver and Lukefahr, 1969; Todd et al., 1971; Harborne, 1979; Jones and Firn, 1979; Elliger et al., 1980). Many of the isoflavonoids are fungistatic and have estrogenic effects on mammals (Harborne, 1979; Mabry and Ulubelen, 1980). The insecticidal activity of the isoflavonoid rotenone is well-known (Fukami and Nakajima, 1971). Several isoflavonoid phytoalexins including coumestrol and genistein (Figure 1) deter feeding of two beetle species (Russell et al., 1978; Sutherland et al., 1980). Hence it was no surprise that cotyledons containing an isoflavonoid phytoalexin(s) had feeding-deterrent properties as defined by Dethier et al. (1960) and Beck (1965) towards the Mexican bean beetle. Deterrency is assumed to be due to the phytoalexins in the cotyledons, although other metabolites may also be involved.

At the physiological level of 1% DW (Keen and Kennedy, 1974), glyceollin had some effect on survival of 1-day-old SBL larvae. Subsequent survival and development were not markedly affected. The substantial reduction in ECI suggests that the isoflavonoid phytoalexins may be a digestibility-reduction factor even for a rather polyphagous species such as the SBL. No other chronic symptoms were detected, probably due to a compensatory increase in food consumption.

Plants may use substances for protection which have relatively subtle effects on attacking herbivores (Rosenthal and Janzen, 1979). Although

the differences in mean larval DW gain and mean DW of diet consumed for the SBL larvae on the control diets and on the 1% glyceollin diets were not statistically different, the effect on ECI was. There seem to be subtle differences between the two treatments that may have been obscured by several factors. The mean initial DW of control diet/vial as estimated from the FW was 15.5 mg. On the average only 0.50 mg DW or 3.3% of this initial quantity was consumed, whereas the mean absolute value of the differences between the estimated DW and the actual DW was 0.42 mg or 2.8% as determined from standard control diets. Hence the error in the estimate of initial DW was large relative to the DW quantity of diet eaten/vial. The relative size of the error in the estimate of DW of food eaten increases with the proportion of uneaten food when the initial dry weight of food is estimated from a DW:FW ratio (Waldbauer, 1968).

Error in measurements of weight gain may also have resulted because we did not account for the gut contents of the larvae. The amount of food in the gut of an insect varies within an instar, the gut being more or less empty just prior to and after a molt. However, on the basis of head capsule coloration, we detected no differences in within-stadium age of the 3rd instar larvae which represented over 80% of the larvae from both treatments.

Conditions of the miniature diets may have been stressful to the larvae, possibly masking effects of glyceollin. The DW gain and DW food consumed by the insects was only one tenth that of larvae reared on Harosoy soybean (Kogan and Cope, 1974), but the developmental rates of the larvae were similar to those reported by Kogan and Cope (1974) and to those for SBL reared on a lima bean-pinto bean artificial diet (Mitchell, 1967).

Although no large differences in glyceollin content were detected among diets analyzed for levels of glyceollin ($N = 8$), small variations in the distribution of the phytoalexin within diets may have contributed to variability in insect response on the glyceollin diets. The small larvae may have eaten the diet but avoided the points of greater glyceollin concentration.

Although glyceollin at 1% DW did not appear to affect the growth and development of young SBL larvae, perhaps glyceollin affects later stages of the insect. Another consideration is that the synthesis of glyceollin is accompanied by the accumulation of the isoflavonoids glycinol, coumestrol, sojagol, daidzein, and genistein (Keen et al., 1972; Keen and Kennedy, 1974; Keen and Paxton, 1975; Weinstein et al., 1981). The combined action of these phytoalexins may be needed to affect herbivory. In addition, since phytoalexin production in soybean appears to be a local phenomenon (Keen and Bruegger, 1977; Yoshikawa et al., 1978), the in situ concentration may exert effects not evident when the compounds are dispersed, such as in an artificial medium.

Our results show that the oligophagous MBB is clearly deterred from feeding on phytoalexin-rich tissue. MBB is not easily reared on an artificial

medium; therefore, we were unable to measure metabolic effects of glyceollin. The rather polyphagous SBL, however, readily accepted phytoalexin-rich soybean cotyledons. This insect is obviously endowed with the ability to handle a wide variety of plant defensive chemistry, as indicated by its broad host range (Herzog, 1980). Thus phytoalexin production by soybean does have an acute allomonal effect against the specialist MBB, but against the generalist SBL the effect is mildly chronic. The dual role as feeding deterrent to certain insects and as an antimicrobial factor reinforces the view that the postchallenge accumulation of phytoalexins is an important mechanism in plant strategies, one that may be more ubiquitous than has been reported so far.

Knowledge of the mode of action of phytoalexins against insects is important. In view of their microbiocidal properties, perhaps phytoalexins would be deleterious to the insect by harming microbial symbionts that aid nutrition or digestion (Mittler, 1971; Scriber and Slansky, 1981). On the other hand, phytoalexins might conceivably afford the insect some protection against entomopathogens (Shirata, 1978).

To determine whether phytoalexins increase a plant's fitness through a role in defense against insect herbivores, it is necessary to ascertain the level of contact of specific herbivores with potent concentrations of phytoalexins in a natural ecological context. Since phytoalexin production is most often a localized and delayed reaction, phytoalexins may only have a significant impact on sessile or confined herbivores, or perhaps phytoalexins cause feeding to be distributed over the total surface area of the plant in such a manner that the reduction in the plant's fitness is minimized (Janzen, 1979).

Requisite to a sound understanding of the interaction between phytoalexins and insects would be the identification of the insect-related elicitors of phytoalexin production—be they feeding by the herbivores themselves, secondary microbial infection of the insect-inflicted wounds, primary microbial infection, or chemicals.

If phytoalexins prove to be a facultative defense against herbivorous insects as well as against potential pathogens, the knowledge, adding a new dimension to our understanding of insect-plant-microbe interactions, would find valuable application in integrated pest management of crops. Unless yield is greatly reduced, crops might conceivably be temporarily or protractedly immunized by phytoalexin elicitors or nonpathogens when pest populations reach economic thresholds. More conventional applications might include breeding cultivars whose resistance to certain pathogens also may impart resistance to herbivores.

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