Journal of Chemical Ecology, Vol. 31, No. 7, July 2005 (©2005)
DOI: 10.1007/s10886-005-5794-z

ARE CHEMICAL COMPOUNDS IMPORTANT FOR SOYBEAN RESISTANCE TO *Anticarsia gemmatalis*?

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(Received September 23, 2004; revised February 24, 2005; accepted March 15, 2005)

Abstract—The identification and quantification of flavonoids (rutin and genistin) present in extracts of soybean genotypes, and their effects on the biology and physiology of *Anticarsia gemmatalis* Hübner (Lep.: Noctuidae) were studied. Analysis of covariance and bicoordinate utilization plots were used to remove the effect of feeding time from pupal weight and consumption as well as to separate pre- and postingestive effects of treatment on *A. gemmatalis* growth. Genotypes PI 274454, PI 227687, and "IAC-100" extracts in general, caused higher mortality, negatively influenced initial larval and pupal weight, and elongated larval cycle. Larvae fed on the "IAC-100" extract diet ingested larger amounts of food per unit of time, but were less efficient in its conversion to biomass. Leaf extracts of PI 227687 had the largest concentration of rutin (quercitin 3-*O*-rhamnosylglucoside), followed by PI 274454, and "IAC-100"; PI 74454 also had the highest genistin (genistein 7-*O*-glucoside) content. The susceptible cultivar "BR-16" showed only a kaempferol-based flavonoid in its chemical profile, indicating that after successive crosses, secondary compounds responsible for plant defenses were eliminated. Genotypes PI 274454, PI 227687, and "IAC-100" showed accentuated resistance characteristics and were considered inadequate sources for the development of *A. gemmatalis*. Considering rutin and genistin concentration in these genotypes, it is suggested that flavonoids are important factors conferring resistance to *A. gemmatalis*.

Key Words—Resistance, velvet bean caterpillar, rutin, genistin.

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INTRODUCTION

The velvet bean caterpillar (*Anticarsia gemmatalis* Hübner) (Lep.: Noctuidae) is considered the main defoliator pest of soybean [*Glycine max* (L.) Merrill] in Brazil. Utilization of insect-resistant cultivars have advantage of being less harmful to the environment and, at the same time, do not require adoption of complex technologies by growers. Resistance to insect attack is due largely to chemicals present in the host plant (Kubo and Hanke, 1986). These compounds, which generally are secondary metabolites, can have behavioral (preingestive) and physiological (postingestive) effects on insects (Berenbaum, 1986).

Efforts have been made with many crops to obtain lines and cultivars with moderate levels of resistance to insects. Wild soybean introductions PI 171451, PI 227658, and PI 229358 have been used since the early 1970s as sources of resistance to defoliator insects such as *Epilachna varivestis* (Van Duyn et al., 1971, 1972), *Trichoplusia ni* (Luedders and Dickerson, 1977), *Diabrotica speciosa*, and *Colaspis* sp. (Rezende and de Miranda, 1980), *Pseudoplusia includens* (Killen et al., 1977; Beach and Todd, 1988), *Spodoptera* spp. (Beach and Todd, 1987), and *A. gemmatalis* (Beach and Todd, 1988; Lambert and Killen, 1984; Oliveira et al., 1993). In addition, these PIs were considered moderately resistant to some seed-sucking insects (Jones and Sullivan, 1979; Piubelli et al., 2003a,b). In field trials, breeding lines with PI 274454 in their genealogy were less defoliated than cultivars used as susceptible controls (Rezende et al., 1980). Cultivar IAC-100, which in addition to other sources of resistance, has PI 274454 and PI 229358 in its genealogy, was released by the breeding program of the Instituto Agronômico (Campinas, São Paulo State, Brazil) because of its resistance to leaf feeders and stink bugs (Rossetto et al., 1995; Veiga et al., 1999).

Few attempts have been made to investigate the chemical basis for insect resistance in soybean. Consumption by *P. includens* larvae of an artificial diet, in which crude CH_2Cl_2 (methylene dichloride) leaf extracts of PI 227687 had been incorporated, caused strong allelochemical effects, such as weight reduction and increased mortality (Smith and Fischer, 1983). Sharma and Norris (1991) reported the extraction, separation, and identification of isoflavonoids of PI 227687 foliage as well as their antifeedant and/or antibiotic properties against *T. ni*.

In general, soybean and other leguminous species lack some potent secondary metabolites that are present in other plant families, such as Cruciferae and Solanaceae (Kogan, 1986). Nevertheless, some constitutive (Hoffmann-Campo, 1995) or induced flavonoids (Kogan and Fisher, 1991) were identified in diverse soybean organs. Those phenolic compounds also play important functions in defense against microorganisms and insects (Dixon and

Steele, 1999). Some of them may be antifeedant and/or antibiotic to soybean pests (Neupane and Norris, 1991; Sharma and Norris, 1991, 1994; Hoffmann-Campo et al., 2001).

Extracts from leaves of the insect-resistant genotype PI 227687 negatively affected the physiology and behavior of *Heliothis virescens* larvae (Hoffmann-Campo, 1995) and *T. ni* (Hoffmann-Campo, 1995; Hoffmann-Campo et al., 2001). The effects persisted when the most polar fraction, named fraction A, was added to the artificial diet. This fraction was composed mainly of rutin (quercitin 3-*O*-rhamnosylglucoside), quercitin 3-*O*-glucosylgalactoside, and genistin (genistein 7-*O*-glucoside). In further studies, rutin negatively affected the development of *H. virescens* (Hoffmann-Campo, 1995) and *T. ni* (Hoffmann-Campo et al., 2001). These insects are occasional and none of them are main pests of soybean (Kogan and Turnipseed, 1987) and, thus, likely not exposed to compounds of this plant. The aglycone quercitin and its glycoside rutin increased mortality and elongated larval period of *A. gemmatalis* (Gazzoni et al., 1997), although the physiological effect of flavonol was not investigated.

This study was conducted to evaluate the effects of different soybean genotype extracts with respect to the biology and physiology of *A. gemmatalis*, and also to quantify the flavonoids rutin and genistin present in these genotypes that are used as sources of resistance to insect pests in the breeding programs of Embrapa Soybean. Furthermore, we tested the hypothesis that, as a leguminous specialist and major defoliator, *A. gemmatalis* could cope with the mixture of allelochemicals present in soybean leaves by postingestive mechanisms.

METHODS AND MATERIALS

Plant Material. Soybean leaves of the genotypes "BR-16," PI 229358, PI 227687, PI 274454, and "IAC-100," produced under greenhouse conditions ($T = 23 \pm 2°C$, RH = 78%), were harvested at growth stage V6 (Fehr and Caviness, 1977) to carry out bioassays and chromatographic analyses. The bulk leaves were collected to perform bioassays, and samples of the fourth trifoliolate were taken to the Phytochemistry Laboratory to be analyzed by high-performance liquid chromatography.

Plant Extract for Diet Incorporation. To carry out the feeding experiments, 50g of dried leaves of each genotype were ground, mixed with 40% aqueous ethanol (EtOH), and left overnight (approximately 18 hr) in a shaker at 100 rpm. The extract was filtered through Framex paper and reduced by rotary evaporator. The aqueous extract was passed into a glass-wool plugged column (3×38 cm), with a nonionic Amberlite XAD-4 resin as adsorbent. Ultrapure water (pH 2.0 and 7.0) was passed through the column to eliminate the bulk of

non-flavonoid compounds. Methanol (MeOH; 80%) was used for flavonoid elution. The resulting extract was concentrated on a rotary evaporator, maintained frozen (−17°C) until addition to the artificial diet of *A. gemmatalis* (Greene et al., 1976, modified by Hoffmann-Campo et al., 1985), and offered to the larvae. A diet without addition of extract was used as control.

Feeding Experiments. A. gemmatalis larvae were obtained from the Embrapa Soybean Laboratory of Insect Mass Rearing, located in Londrina, PR, Brazil. Since eclosion, the larvae were reared on the diet containing each soybean extract. From late second and early third instars, larvae were weighed and housed individually in 30-ml acrylic cups (Fill-rite Corp., Newark, NJ, USA). Insects were maintained in controlled environmental chambers (25 ± 2°C; RH: 70 ± 10%; 14 L:10 D photoperiod) and observed daily to evaluate the average duration of each larval stage, until reaching the prepupal stage; numbers of dead insects were recorded to calculate percent mortality. Pupae were frozen (−50°C), oven-dried (60°C, 72 hr), and weighed to obtain dry pupal mass. Remaining food and frass were placed into tubes, oven-dried (60°C, 72 hr), and weighed. To estimate the initial dry weight of the larvae, five (second/third instar) were taken from each treatment, weighed, frozen (−50°C), oven-dried (60°C, 72 hr), and reweighed. The correction factor for initial fresh to dry weight was calculated. Values obtained were multiplied by the fresh weight of each set of experimental larvae. The same procedure was used to calculate the dry weight of the food.

Plant Extract Chemical Analysis. The fourth trifoliolate leaves of each genotype were excised and cut into small pieces. Aliquots of each sample (500 mg) were placed into glass tubes, macerated, and homogenized with 5.0 ml of 80% MeOH. After heating (50°C), extracts were maintained with constant shaking (150 rpm) for approximately 18 hr, and the supernatants were transferred into 50-ml beakers, and kept in fume cupboards until dryness (±96 hr). Samples were redissolved in 1.0 ml of 80% MeOH, and 20-µl aliquots were injected into an HPLC (Shimadzu, model SPD-M10A VP) and developed on a reverse-phase column (CLS-ODS-C18-M, 4.6-mm internal diam × 250 mm in length). Flavonoids were eluted with a linear gradient system (Hoffmann-Campo, 1995) composed of two solvents: (A) 2% acetic acid (HOAc, Vetec, UV/spectrometry grade) and (B) a solution composed of MeOH (J.T. Baker, HPLC grade), HOAc, and water (18:1:1). The initial gradient consisted of 75% solvent A and 25% B, which changed to 35% A and 65% B in 23 min, and returned to the initial condition at 25 min. This condition was maintained for 5 min for column cleaning. Solvent flow rate was 1.0 ml/min, and absorption was measured at 260 nm.

Flavonoid concentrations in the genotypes were estimated by comparing the concentration of rutin ($C_{27}H_{30}O_{16}$, quercitin 3-*O*-rhamnosylglucoside) and genistin ($C_{21}H_{20}O_{10}$, genistein 7-*O*-glucoside) in the genotype samples with

those obtained from HPLC-injected standards (Sigma). Standard concentrations of rutin (0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 mg/ml) and of genistin (0.01, 0.02, 0.04, and 0.08 mg/ml) were injected onto the HPLC to construct the compound equations. After the injection of samples, scanning by photodiode array was performed to compare HPLC trace spectra and retention times with those of the standards. The area corresponding to each compound trace was calculated and applied to the equation (standards) to estimate the concentrations of rutin and genistin in each genotype.

Statistical Procedures, Feeding Experiments. Bioassays were developed in a completely randomized design, with six treatments and 30 replicates. Mortality in each treatment was compared by using a χ^2 test of homogeneity, according to Banzatto and Kronka (1992), at a 5% probability. All other data were analyzed using the SAS statistical package for PC (SAS Institute, 1996). The effects of treatments on larval initial weight, pupal weight, food consumption, frass production, and feeding time (days) were analyzed through ANOVA, performed by the general linear model (GLM) procedure. If a significant effect of treatments at minimal 5% of probability was observed, the means were compared by least significant difference (LSD). Analysis of covariance (ANCOVA), proposed by Raubenheimer and Simpson (1992), followed by bicoordinate utilization plots (Raubenheimer and Simpson, 1994), was used to remove the effect of covariate feeding time from consumption and pupal weight. This statistical procedure was also used to separate pre- and postingestive effects of treatment on *A. gemmatalis* growth. Thus, differences in pupal weight were adjusted for covariates consumption and digested food (consumption − frass produced). When the interaction among covariate and treatments was not significant, the parallel line model was used, and the main effect of treatment and/or covariate was considered. If the effect of treatment was significant at a minimal 5% probability, means were compared by ANCOVA means (least square means).

Statistical Procedures, HPLC Analyses. A completely randomized design was used in the HPLC data analyses, with four genotypes and five replicates, injected in duplicate. Data were analyzed by ANOVA, and the means of each genotype were compared by Tukey's test at a 5% probability, by using the SAS statistical package (SAS Institute, 1996).

RESULTS

Differences in larval mortality rates were dependent on the treatment as indicated by a significant χ^2 test. Mortality rates were higher when larvae were fed on PI 274454, "IAC-100," or PI 227687 extract diets compared with the control, PI 229358, and "BR-16" extract diets (Table 1).

TABLE 1. LARVAL MORTALITY, LARVAL DRY WEIGHT (2ND/3RD), PUPAL DRY WEIGHT, FOOD CONSUMPTION, FRASS PRODUCTION, AND FEEDING TIME (FROM 2ND/3RD TO PREPUPATION) OF *Anticarsia gemmatalis* FED ON ARTIFICIAL DIETS CONTAINING EXTRACTS OF DIFFERENT SOYBEAN GENOTYPES OR CONTROL DIET

Diet	Mortality (%)	Weight (mg) ± SEM				Feeding time (days) ± SEM
		Larvae DW	Pupae DW	Food consumption	Frass production	
Control	2.2	0.56 ± 0.01 a	87.9 ± 3.52 ab	279.1 ± 4.80 a	144.4 ± 3.29 ab	7.3 ± 0.06 c
"BR-16"	1.7	0.31 ± 0.01 bc	86.0 ± 5.58 abc	281.5 ± 8.06 a	161.1 ± 4.81 a	9.9 ± 0.28 b
PI 229358	5.1	0.41 ± 0.01 b	96.6 ± 5.73 a	258.4 ± 8.08 ab	145.1 ± 5.24 ab	10.7 ± 0.30 b
PI 227687	26.7	0.29 ± 0.01 d	64.4 ± 4.41 c	259.5 ± 8.41 ab	144.9 ± 5.15 ab	13.7 ± 0.42 a
PI 274454	33.4	0.36 ± 0.01 bc	68.3 ± 5.98 bc	235.3 ± 7.49 b	132.7 ± 5.37 b	14.0 ± 0.60 a
"IAC-100"	33.4	0.26 ± 0.01 d	74.6 ± 6.44 abc	289.7 ± 10.00 a	147.4 ± 6.33 ab	13.0 ± 0.57 a
F values	58.61[a]	59.14***	5.22***	5.55***	3.13***	75.46***

Means followed by the same letter are not significantly different by Tukey test at 5% probability level.
[a] $\chi^2_{(5;\ 0.05)}$.
***$P < 0.001$

There was a significant effect of diet, by ANOVA, on larval and pupal weight, consumption, frass produced, and feeding time (Table 1). Larval weight was negatively affected by extracts of any soybean genotype, including "BR-16" (our control cultivar) as compared with plain diet. The lowest pupal weight was observed for larvae fed on diet containing extracts of PI 227687 (64.4 ± 4.41 mg) and PI 274454 (68.3 ± 5.98 mg). When larvae fed on PI 229687 extract diets, pupal weight was 96.6 ± 5.73 mg. Larvae fed on "IAC-100," "BR-16" extract diets, and control diet consumed larger amounts of food than those fed on PI 274454 extract; larvae feeding on the latter diet also produced the lowest amount of frass, whereas larvae fed on "BR-16" produced the largest amount of frass. The feeding time of *A. gemmatalis* larvae was negatively affected by all diets containing soybean extracts. The larval cycle was more elongated

TABLE 2. ANCOVA TESTING FOR THE EFFECT OF DIETS CONTAINING EXTRACTS OF DIFFERENT SOYBEAN GENOTYPES OR CONTROL DIET ON THE FOOD EATEN ADJUSTED FOR FEEDING TIME (TIME) AS COVARIATE (A, B), ON THE WEIGHT OF PUPAE ADJUSTED FOR CONSUMPTION AS COVARIATE (C, D), AND ON THE WEIGHT OF PUPAE ADJUSTED FOR DIGESTED FOOD (FOOD EATEN – FRASS) AS COVARIATE (E)

Source of variation	*df*	*F* values	
		Pupae	Food
(a) Time (covariate)	1	34.0***	19.1***
Diet	5	3.15**	0.99, ns
Time × diet	5	3.00*	0.58, ns
Residual	290	–	
(b) Time (covariate)	1	–	19.2***
Diet	5	–	4.28**
Residual	295	–	–
(c) Consumption (covariate)	1	82.4***	–
Diet	5	0.73, ns	–
Consumption × Diet	5	1.67, ns	–
Residual	284	–	–
(d) Consumption (covariate)	1	81.5***	–
Diet	5	6.24***	–
Residual	289	–	–
(e) Digested food (covariate)	1	27.2***	–
Diet	5	1.76, ns	–
Digested food × diet	5	2.98*	–
Residual	278	–	–

ns, Not significant.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

when extracts of PI 274454, PI 227687, and "IAC-100" were added to the diet in comparison with the plain diet, "BR-16," and PI 229358 extract diets.

Feeding time (covariate) and diets containing soybean extracts (treatments) affected pupal weight (Table 2a), indicating that *A. gemmatalis* growth depended on an interactive effect of covariate and treatments. Positive slopes were observed in the adjusted lines for the control and PI 227687 extract diets (Figure 1). All other treatments yielded negative slopes, indicating that increases in feeding time did not contribute to increased insect growth.

The interaction of covariates feeding time (Table 2a) and consumption (Table 2c) with diets was not significant for their relationships with the amount of ingested food or weight of pupae. Thus, a parallel line model was fitted. The

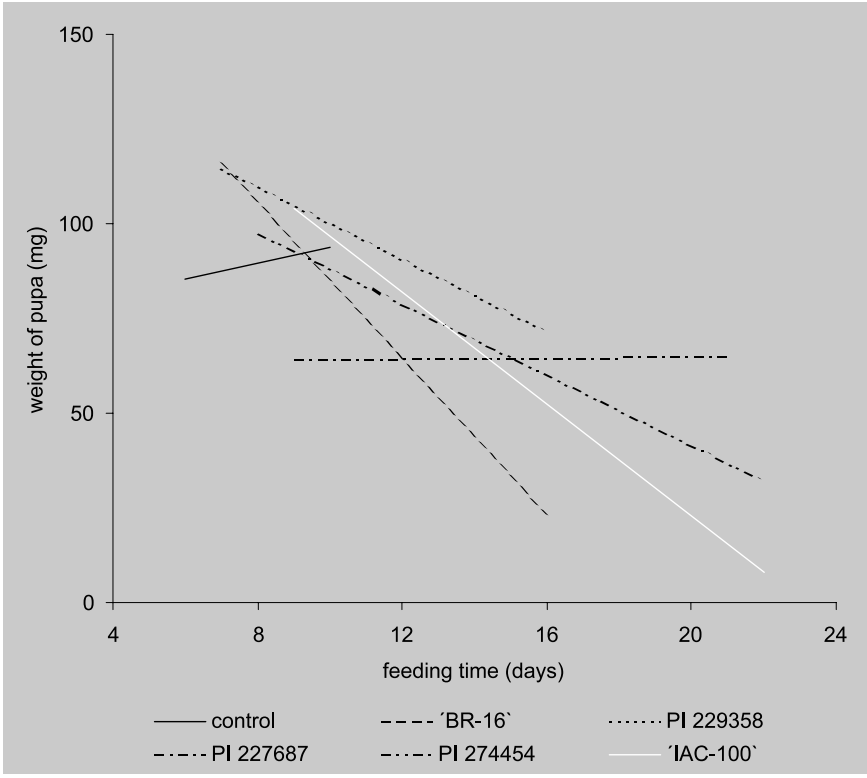

FIG. 1. Relationship between pupal weight and feeding time of *Anticarsia gemmatalis* fed on artificial diets containing extracts of different soybean genotypes or control diet. For statistical analysis, see Table 2a.

main effect of the diet and feeding time (Table 2b) or consumption (Table 2d) as covariate was significant, showing that the amount of ingested food depended on both factors, but not of the interaction between them. ANCOVA means of consumption adjusted by time (Figure 2) shows that larvae fed on "IAC-100" extract diet ingested a larger amount of food per unit of time, in comparison with the other treatments. Insects that fed on the PI 229358 extract diet converted more ingested food to biomass, as indicated by ANCOVA means of weight of pupae adjusted by consumption (Figure 3). Differently, lower conversion was observed when larvae were fed on extracts of "IAC-100" and PI 227687 compared with control diet, "BR-16," and PI 229358 extract diets.

The covariate digested food and diets significantly influenced pupal weight (Table 2e, Figure 4). Therefore, insect growth was dependent of the interactive effect between digested food and treatment. A positive relationship among the

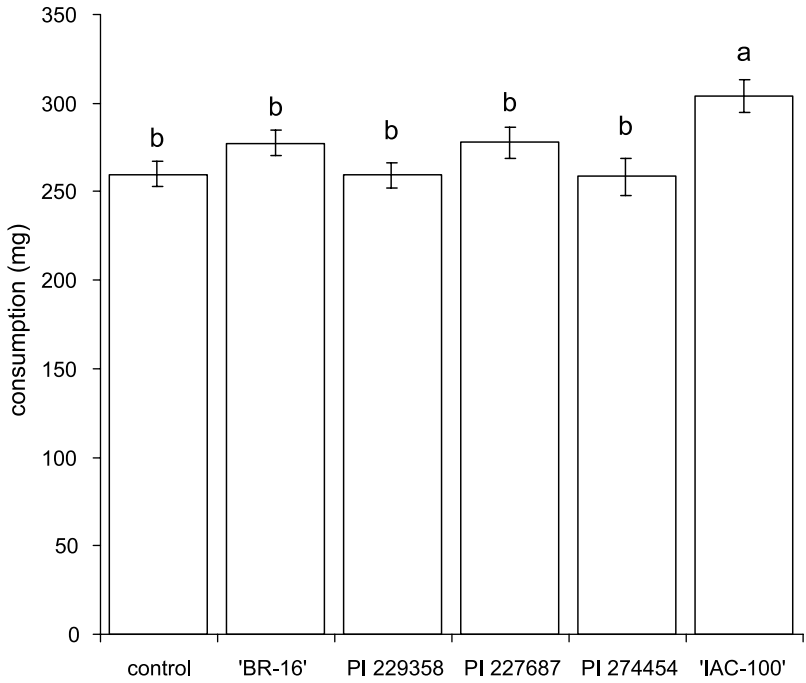

FIG. 2. ANCOVA means of consumption adjusted by feeding time of *A. gemmatalis* fed on artificial diets containing extracts of different soybean genotypes or control diet. For statistical analysis, see Table 2a and b. Columns followed by different letters are statistically significant.

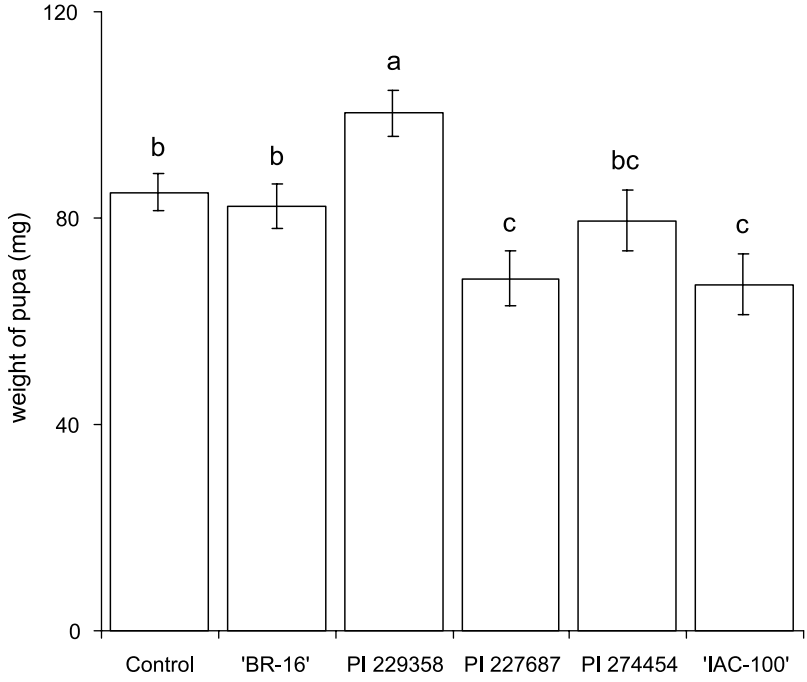

FIG. 3. ANCOVA means of weight of pupae adjusted by consumption of *A. gemmatalis* fed on artificial diets containing extracts of different soybean genotypes or control diet. For statistical analysis, see Table 2c and d. Columns followed by different letters are statistically significant.

factors is observed in Figure 4 for most of treatments, suggesting that those larvae that had higher amount of digested food (ingestion–excretion) became heavier. An exception was observed in larvae fed on the "IAC-100" extract diet, which presented a shallow negative slope, indicating that even with increased digested food, pupal weight remained at the same level.

The highest concentration of rutin (3.682 mg/g dried leaves) was observed in the extracts of PI 227687 (Table 3). PI 274454 and "IAC-100" presented intermediate, whereas PI 229358 had the lowest rutin content. The concentration of genistin in PI 227454 was higher than in the other tested genotypes. The susceptible cultivar BR-16 showed a smaller number of HPLC traces in comparison with the genotypes considered resistant. Rutin or genistin were not detected in its leaf extract, and, in fact, only a kaempferol-based compound was observed.

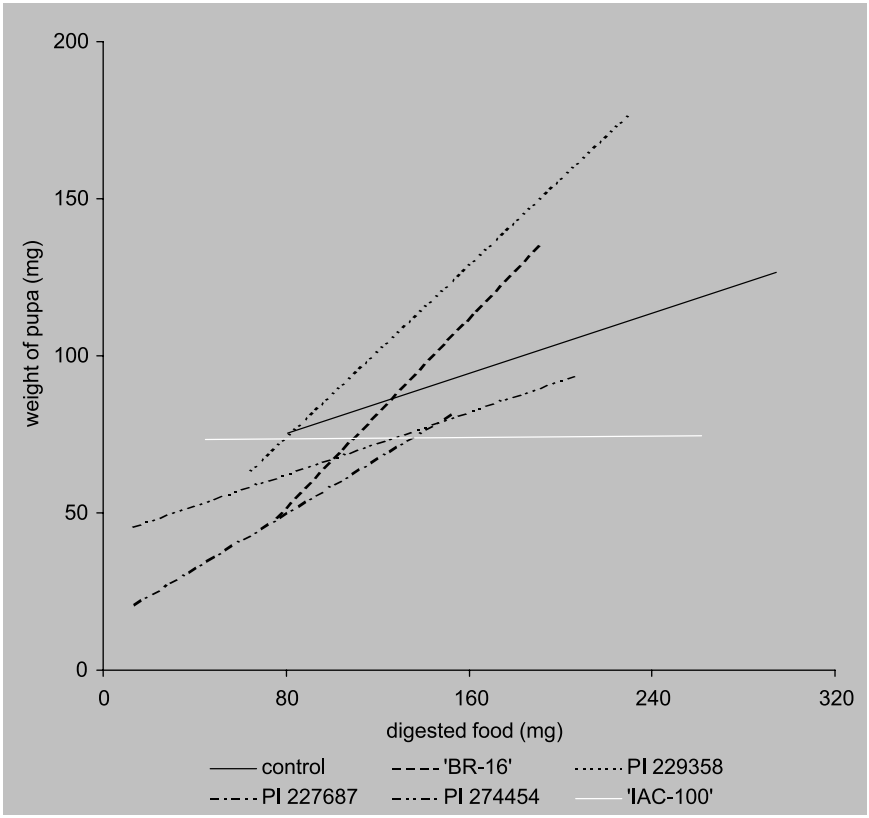

FIG. 4. Relationship between pupal weight and digested food of *A. gemmatalis* fed on artificial diets containing extracts of different soybean genotypes or control diet. For statistical analysis, see Table 2e.

TABLE 3. CONCENTRATION OF RUTIN AND GENISTIN IN SOYBEAN GENOTYPES WITH RESISTANCE CHARACTERISTIC TO INSECTS

	Concentration (mg/g)	
Genotypes	Rutin	Genistin
PI 227687	3.682 ± 0.416 a	0.122 ± 0.005 b
PI 274454	1.472 ± 0.282 b	0.258 ± 0.030 a
"IAC-100"	0.972 ± 0.082 bc	0.142 ± 0.011 b
PI 229358	0.212 ± 0.021 c	0.136 ± 0.005 b
F values	37.65***	13.28***

Means followed by the same letter are not significantly different by Tukey test at 5% probability level.
***$P < 0.001$.

DISCUSSION

For this study, we selected PI 227687, PI 229358, and PI 274454. The first two are those most used globally as sources of resistance to defoliating insects, and the latter is used similarly in Brazil. We also tested the cultivar IAC-100, which is regarded as possessing multiple insect-resistance characteristics (Rossetto et al., 1995), and "BR-16" as a nonresistant cultivar. Host-plant resistance can negatively affect insect physiology (antibiosis) or behavior (antixenosis). As morphological factors (trichomes, etc.) were ruled out, resistance was probably due to chemical factors (Soo Hoo and Fraenkel, 1966). These authors also stated that insects achieve appropriate growth when they have shorter development time (here we evaluated feeding time), heavier larva and/or pupa, and low mortality rate. Larvae fed on diet containing genotypes PI 227687, PI 274454, and "IAC-100" had protracted growth rates, higher mortality, and longer feeding times. The weights of pupae after adjustment by consumption were lower for insects fed on PI 227687 and "IAC-100" compared with other treatments, including the control and "BR-16," and PI 229358 extract diets, indicating that those insects were not able to efficiently transform food eaten in body mass.

In contrast, larvae fed on PI 229358 extract diet had lower mortality (5.1%), were heavier, and took the same time in the feeding period as those on "BR-16." When studying the biology of *H. virescens* in diets containing extracts of soybean genotypes, Hoffmann-Campo (1995) observed 45 and 10% mortalities when larvae were fed on PI 227687 and PI 229358 extracts, respectively. In field experiments, Hatchett et al. (1979) observed 87% (PI 227687) and 100% (PI 229358) larval mortalities. It should be pointed out that Hoffmann-Campo (1995) studied the effects of soybean crude extracts added to artificial diet, whereas Hatchett et al. (1979) evaluated the direct effects of insect feeding on resistant-genotype leaves. Thus, resistance of PI 229358 probably resulted from some morphologic factor than in this experiment, as in Hoffmann-Campo (1995), was removed by extraction, or else the whole plant has more resistant factors than could be detected with the extract provided in the present study.

An apparent contradiction was observed regarding the behavior of *A. gemmatalis* fed on the "IAC-100" extract diet. Larvae showed high mortality but consumed larger amounts of food, and pupal weight was similar to larvae fed on plain diet and on "BR-16" extract diet. In fact, this is not a completely atypical behavior for insects; they sometimes eat more to compensate for low nutrient levels (Slansky and Wheeler, 1989) and for intermediate level of allelochemicals in the diet (Paradise and Stamp, 1990). Consequently, mortality of larvae fed on the "IAC-100" extract did not result from preingestive, but likely postingestive effects. By ANOVA, larvae fed on such diet produced heavier pupae compared with those fed on PI 227687 extract diet, but after

adjustment by consumption, they showed lower ANCOVA means of pupal weight (similar to PI 227687). Additionally, despite retaining (digesting) large amounts of food, "IAC-100" extract diet was not able to transform this into heavier pupae, as indicated by the shallower slope, showed in Figure 4.

Higher consumption rates result in ingestion of greater doses of active compounds (Wheeler and Slansky, 1991), and *A. gemmatalis* probably failed to detect detrimental compounds present on "IAC-100" extract and, in the sequence, had to deal with their toxic effect in the diet. The same effect was also observed with *Helicoverpa* (*Heliothis*) *zea* (Duffey and Isman, 1981; Isman and Duffey, 1982a,b), *Manduca sexta* (Stamp and Scrobola, 1993), and *T. ni* (Hoffmann-Campo, 1995) when fed on rutin-enriched diet.

The highest concentration of rutin was found in PI 227687 compared with the other genotypes. Rutin concentration in "IAC-100" foliage was similar to PI 274454 and PI 229358, which are part of its genealogy (Veiga et al., 1999). Genistin was observed in all resistant genotypes, and its concentration in PI 274454 was nearly two times higher than in the other resistant genotypes. Before analysis, our crude leaf extracts were passed into a nonionic XAD-4 column to eliminate the bulk of nonflavonoid compounds, without any further fractionation. The identification of genistin and daidzin were performed by comparison of their ultraviolet spectra (from photodiode array), and retention time with authentic genistin and daidzin, and by coelution from HPLC. Neither daidzin nor daidzein (aglycone) were observed in our crude, unfractionated extracts of soybean leaf. Differently, Sharma and Norris (1991) reported the aglycone daidzein in methanolic extracts of PI 227687 after TLC fractionation in ethyl acetate. These authors did not refer to genistein (and its glycoside genistin) and rutin in PI 227687 leaf extracts. Also, there was no remark regarding how they identified daidzein.

The flavonoid aglycones, according to Markham (1982), are more reactive than the glycosides because the glycosylation makes possible their storage in the cell vacuoles. Flavonoid aglycones are rarely found as internal constituents, but regularly encountered on the external surface of leaves and fronds (Markham, 1989). In many years of analysis, we have not found isoflavone aglycones in healthy plants in our lab, even in pods/seeds. However, it is known that daidzein and genistein conjugates (7-*O*-glucosyl and 6″-*O*-malonyl-7-glucosyl) are constitutively present in large quantity in soybean seedlings after fungal infection (Graham et al., 1990) and in immature seeds after stinkbug damage (unpublished data). Graham et al. (1990) observed that daidzin conjugates can be rapidly hydrolyzed into free daidzein, which is the precursor of glyceollins, phytoalexins produced *de novo* in all soybean seedling organs.

Leaf extracts of genotype PI 227687 contain the isoflavone genistin and seven flavonol glycosides, i.e., three kaempferol-, two isorhamnetin-, and two quercitin-based compounds, one of them rutin (Hoffmann-Campo, 1995). This

compound was not observed in "BR-16," used as the susceptible control in the present study; kaempferol was the only flavonoid observed in other susceptible cultivars, such as Embrapa-1, Embrapa-4, IAS-5, and Davis (Hoffmann-Campo, 1995). Chan et al. (1978) observed that kaempferol was less toxic to *H. virescens*, *H.* (*Heliothis*) *zea*, and *Pectinophora gossypiella*, compared to quercitin-based compounds that possess a catechol group. According to Chan et al. (1978) and Elliger et al. (1980), there is a relationship between the presence of a catechol B-ring in a flavonoid and the inhibition of insect growth. This fact suggests that soybean-breeding programs usually do not allot priority to resistance to insects and, in practice, have eliminated secondary compounds otherwise responsible for plant defense.

Hoffmann-Campo (1995) qualitatively examined the flavonoid profile of 16 soybean genotypes. Rutin was found in one resistant cultivar (IAC-100), one breeding line (BR82-12547), and two wild soybean genotypes (PI 227687 and PI 229358). The present study reports that rutin concentration in PI 229358 was very low, which may explain the good performance of *A. gemmatalis* fed on diet containing this extract. Flavonoids are frequently used by monophagous and oligophagous insects to recognize their host plants (Harborne and Grayer, 1993), and, as *A. gemmatalis* is a leguminous specialist insect, it would be expected to be less affected by flavonoids from soybean leaves. However, this important growth inhibitor of lepidopterans is being removed after successive breeding crosses, and likely, *A. gemmatalis* has lost the ability to cope with this toxic compound.

The information obtained in the present work provides elements for future studies on the genetics of chemical compounds conferring resistance to defoliators. Consequently, the knowledge that PI 227687 and PI 274454 possess high rutin and genistin concentrations, respectively, and caused deleterious effects on *A. gemmatalis* physiology has various potential applications in IPM and in breeding programs, contributing to the sustainability of soybean-based agricultural systems. The identification of chemicals responsible for plant defense and their role in the interactions with insects can help breeders in the development of cultivars resistant to pests. Recent progress in biotechnology, especially in molecular biology, has opened new opportunities in developing host-plant resistance (Panda and Khush, 1995), and, according to Dixon and Steele (1999), flavonoids possess strong potential for metabolic engineering. In addition, with continual exposure to *Bacillus thuringiensis* (Bt) toxins, mainly in transgenic crops, resistance may develop in several insect pests (Tabashnik, 1994). Combining traditional chemically based breeding programs and genetically engineered *B*t toxin-based resistance has a potential to be much more sustainable and easily adopted than the usual higher dose/refuge strategies (Cooper et al., 2004). Thus, considering the concentrations of rutin and genistin in these genotypes, it is reasonable to suggest that flavonoids are important

factors in terms of resistance to *A. gemmatalis*. Effort is needed to maintain or increase flavonoid concentration in soybean cultivars. Finally, further studies regarding the effect of rutin, genistin alone, and interactively are necessary for a complete understanding of the role of flavonoids in the defense of soybean to *A. gemmatalis*.

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