Cotton Allelochemics and Growth of Tobacco Budworm Larvae[1]

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ABSTRACT

Eight alleged allelochemics that naturally occur in cotton, *Gossypium hirsutum* L., or as a constituent part, were added to a commercial wheat-germ diet at various concentrations. Neonate larvae of tobacco budworm [*Heliothis virescens* (F.)] were allowed to feed for 5 days and then weighed to determine the effects of allelochemics on larval growth. Regression equations were fitted to the data. A linear regression in the form $Y = a + bX$ described the relationship for cyanidin, delphinidin, and gossypol up to 0.20% in the diet. A curvilinear regression equation in the general form of $Y = aX^b$ described the relationships for catechin, condensed tannin, chrysanthemin (cyanidin -3-β-glucoside), isoquercitrin, and quercetin. These were fed at higher levels (up to 1%) than the three allelochemics fitted with the linear regression equation. The level of each allelochemic in cotton required to reduce larval weight 90% is within the range of the amounts found in various cotton strains and primitive accessions. All except gossypol are found in several plant families and may thus be useful in developing plant resistance to tobacco budworm in several plant families. Several species of *Heliothis* are pests of economic importance worldwide.

Additional index words: Plant resistance, Genetics, *Heliothis virescens* F.

SEVERAL researchers have investigated the relationships between naturally occurring compounds in cotton, *Gossypium hirsutum* L., and growth of insects. As the age of larvae increases—in bollworm, [*Heliothis zea* (Boddie)], tobacco budworm, [*H. virescens* (F.)], and the pink bollworm, [*Pectinophora gossypiella* (Saunders)—their tolerance to gossypol also increases. The pink bollworm is the most tolerant (Shaver and Parrott, 1970). Reese et al. (1981) and Shaver et al. (1977) analyzed various tissues to determine a relationship between the concentration of allelochemics and feeding by larvae at a particular site. They reported that cotton anthers were important feeding sites. Shaver et al. (1977) found petals were the most toxic part of the square, but reported tobacco budworm larvae did not feed to any extent on petals until they had first consumed the anthers. The effect is that only larger larvae feed on petals.

Lukefahr and Martin (1966) found that gossypol was equally toxic to larvae of the bollworm and tobacco budworm. Quercetin was more toxic to tobacco budworm than bollworm larvae, and rutin was the least toxic of the three allelochemics, but was more toxic to tobacco budworm than bollworm. Quercetin, isoquercitrin, rutin, morin, quercitrin, and hesperidin were shown to be toxic to tobacco budworm and bollworm (Shaver and Lukefahr, 1969). These results supported and expanded the conclusions of Lukefahr and Martin (1966) that the naturally occurring flavonoid pigments of cotton may be sources of resistance to the tobacco budworm and bollworm.

Hedin et al. (1983b) have shown that cyanidin -3-β-glucoside (chrysanthemin), an anthocyanin pigment, is toxic to tobacco budworm larvae and have suggested that it may be a widely occurring plant chemical that serves as a natural defense mechanism against tobacco budworm larvae in a broad range of plant families.

Whittaker (1970) defined the term allelochemics as "chemicals significant to organisms of a species different from their source, for reasons other than food as such." Further subdivision of allelochemics includes allomones and kairomones. Brown et al. (1970) defines allomones as chemical substances produced or acquired by an organism, which, when contacting an individual of another species in the natural context, work in the receiver a behavioral or physiological reaction adaptively favorable to the emitter. A range of levels of various allelochemics (condensed tannin, flavonoids, terpene aldehydes, and cyclopropenoid fatty acids) were compared for their effects on bollworm and budworm larvae growth in vitro (Elliger et al., 1978; Chan et al., 1978a,b,c; and Hedin et al., 1981). In addition, they addressed the relationship of the sites of larval feeding to the localization of various allelochemics, but they have not adequately described the relationships between these allelochemics and larval growth.

The objectives of this study were 1) to identify the effects of eight naturally occurring cotton allelochemics added to artificial diet on the growth of neonate tobacco budworm larvae for 5 days and 2) to describe the relationships between allelochemic concentration and larval growth on artificial diet.

MATERIALS AND METHODS

Condensed tannin, cyanidin, delphinidin, chrysanthemin, quercetin, and isoquercitrin were isolated from cotton plant leaves approximatly 5 cm across by procedures previously described (Hedin et al., 1981). Catechin was procured commercially and gossypol was obtained as a gift from the Southern Regional Research Center, USDA, ARS, New Orleans, La. First instar larvae for the bioassay tests were obtained by the procedures of Jenkins et al. (1982). For the bioassay, we used a commercial wheat-germ based medium (742-A prepared by Bioserv, Inc., Frenchtown, N.J.[5]).

The allelochemics prepared from cotton were added as follows: 1) compounds soluble in organic solvent were diluted in either hexane or ethanol, added to the appropriate amount of dry diet mix and evaporated to dryness in a rotary evaporator under reduced pressure; and 2) water soluble compounds were dissolved in water and added to the diet in place of the prescribed amount of water.

Table 1. Effects on weight of tobacco budworm larvae grown for 5 days on diet with eight allelochemics.

Allelochemic	% allelochemic in diet									
	0.0063	0.02	0.05	0.063	0.10	0.15	0.20	0.50	0.63	1.0
	weight, % of control									
Catechin	92.5	90.2		37.4			17.7		0.9	
Chrysanthemin		116.2	81.3		36.8	30.5	28.1	20.1		7.7
Isoquercitrin		109.9	80.1		51.5	27.4	19.3	6.1		5.9
Quercetin	95.7	90.9		16.9			8.9		5.5	
Condensed tannin		94.7		22.9		7.5	6.6		5.1	
Cyanidin		96.6		88.7		53.3	40.8			
Delphinidin		113.6		92.6		42.3	20.1			
Gossypol		86.7	82.6		74.3	43.5	17.9			

Table 2. Regression equation for allelochemics effects on 1st instar tobacco budworm larvae grown on diet for 5 days.

Allelochemic	Regression form	a	b	Coefficient of determination r^2	Significance of	
					Regression	r
Catechin	$Y = aX^b$†	7.06	−0.562	0.90	**	**
Chrysanthemin	$Y = aX^b$	7.91	−0.707	0.81	**	**
Isoquercitrin	$Y = aX^b$	4.49	−0.888	0.85	**	**
Quercetin	$Y = aX^b$	3.29	−0.705	0.90	**	**
Condensed tannin	$Y = aX^b$	2.07	−0.880	0.90	**	**
Cyanidin	$Y = a + bX$	105.10	−332.9	0.71	**	**
Delphinidin	$Y = a + bX$	124.40	−540.4	0.89	**	**
Gossypol	$Y = a + bX$	101.61	−390.3	0.56	**	**

** Significant at 0.01 level.
† $Y = aX^b$ is same as $\log Y = \log a + b \log X$.

The concentrations of allelochemics fed varied from 0.0063% to 1% of the wet weight of the diet (Table 1). These are considered within the range of biological amounts of the allelochemics in plants. Higher concentrations of some allelochemics were not fed because quantities were insufficient. All diet ingredients were thoroughly mixed and poured into 10 cm petri plates to gel. Diet cylinders of 10 mm in dia by 5 mm in height were cut with a no. 9 cork borer and tranferred into 15 × 45 mm shell vials. One neonate larva was transferred to each vial with a camel hair brush and the vial was capped with aluminum foil and placed in an incubator at 26° C, 60% RH, 12 h light–12 h dark cycle for 5 days as our standard bioassay time and conditions. After 5 days, the larvae were weighed in groups of five. A randomized complete block design with eight replications of five larvae each was used. All replications and concentrations of a particular allelochemic were tested at the same time.

We used linear and curvilinear regresssion analyses to determine the best-fitting equation to describe the relationships between size of larvae and concentration of the allelochemic. To standardize the data, we included a control diet (with solvents but no allelochemic) in each experiment. The weights of larvae on the various concentrations of allelochemic were each expressed as a percentage of the mean weight of larvae on the control diet and used as data in the regression analyses. This standardization by use of a control diet allowed us to conduct the experiments with the eight allelochemics over several weeks. It was necessary since it was not possible to conduct the entire experiment involving all allelochemics at several concentrations as one experiment.

RESULTS AND DISCUSSION

The data for weights of 5-day old larvae expressed as a percentage of the control weight are shown in Table 1. The weights ranged from slightly greater than the control weight to less than 1% of control weight.

Regression analyses indicated a linear relationship between larval weight and levels of cyanidin, delphinidin, and gossypol up to the maximum level, 0.20% of the diet. On the other hand, the relationship was curvilinear between larval weight and levels of catechin, chrysanthemin, isoquercitrin, quercetin, and condensed tannin; however, each of these materials was fed at levels above 0.20% of the diet. The coefficient of determination (r^2) was calculated for each regression equation. Based upon r^2 values we considered the equations shown in Table 2 the best fit within the range of allelochemics fed.

We used the regression equations to determine the amount of each allelochemic that would be required to produce a given level of weight reduction (Table 3). Where the calculated amounts required are greater than actual levels fed in diets, we of course do not know if the regression relationships are valid. Validity was assumed, however, in order to calculate the amounts necessary to cause large weight reductions. The ED$_{50}$ (amount of allelochemic necessary to reduce size 50%) for each allelochemic was within the range of each allelochemic fed. The amount of each of the allelochemics required to reduce growth 50% is closer to being equal than at the 95% growth reduction range. Previous published research comparing toxicity of allelochemics has generally compared toxicity of allelochemics at the ED$_{50}$ (Elliger et al., 1978; Hedin et al., 1981, 1983a).

The age of the larvae is important; Reese (1983) found that neonate larvae of black cutworm, (*Agrotis ipsilon*), placed on corn (*Zea mays* L.) seedlings for 7 days weighed 1.7 mg; neonate larvae placed for 2 days on artificial diet, and then for 5 days on corn seedlings weighed 10.2 mg. He also found that neonate larvae of black cutworm placed on artificial diet for 48 h are far more sensitive to plant allelochemics and/or morphological defense than older larvae.

Table 3. Predicted amounts of allelochemics necessary in diet to achieve desired level of growth reduction in tobacco budworm. Based upon regression equations.

Allelochemic	Desired % of weight on control diet								
	100	75	50	25	10	5	2	1	0
	% allelochemic								
Catechin	0.009	0.015	0.031	0.105	0.538	1.848	9.430	32.360	--
Chrysanthemin	0.028	0.042	0.074	0.196	0.727	1.913	6.988	18.620	--
Isoquercitrin	0.030	0.042	0.066	0.145	0.406	0.886	2.485	5.425	--
Quercetin	0.008	0.012	0.021	0.056	0.207	0.552	2.025	5.412	--
Condensed tannin	0.012	0.017	0.027	0.059	0.167	0.367	1.041	2.285	--
Cyanidin	0.015	0.090	0.166	0.241	0.286	0.301	0.310	0.313	0.316
Delphinidin	0.045	0.091	0.138	0.184	0.212	0.221	0.227	0.228	0.230
Gossypol	0.004	0.068	0.132	0.196	0.235	0.248	0.255	0.258	0.260

Shaver and Parrott (1970) transferred larvae of corn earworm, tobacco budworm, and pink bollworm of different ages to diets containing different levels of gossypol. The detrimental effects of gossypol on development of the three species decreased with increasing larval age.

Although we have found (unpublished data) that larvae at 5 days of age are much larger when fed on the standard laboratory diet than when they are on terminals and squares of commercial cultivars of cotton, these larvae are still a pest with economic impact. Further increases in resistance to tobacco budworm would improve the economic return per unit input cost. One manner of accomplishing increased tobacco budworm resistance may be to increase the concentration of allelochemics in the plant. It will require considerably higher levels of allelochemics that have a curvilinear relationship, than those that are linear with proper consideration of the intercept and slope of the equations. We do not know if gossypol, cyanidin, and delphinidin are linear above 0.20% since we could not isolate sufficient quantities of them to feed at higher levels. At some levels added increments of several allelochemics elicit similar weight reductions; however, at other levels, the allelochemics act quite differently. We believe the levels of anthocyanin, isoquercitrin, quercetin, condensed tannin, and gossypol in cultivars of cotton are, in part, responsible for the "natural" reduction in size of tobacco budworm larvae. We also believe that it should be possible to increase anthocyanin, isoquercitrin, quercetin, and gossypol in cotton to levels that could achieve increased levels of plant resistance in new cotton strains.

We recognize that behavior of the young larvae is important (Parrott et al., 1983). Not only must the levels of the allelochemics be generally sufficient, but they must also be adequate in sites (tissues) where larvae feed. We have confirmed that several allelochemics are individually capable of reducing growth of tobacco budworm larvae in the laboratory and have derived regression equations to describe their effects on growth. In the cotton plant these allelochemics and perhaps others, as yet unidentified, may be acting additively, or in some other manner to further decrease larval growth. If so, several compounds in cotton could be increased by selection and may give increased levels of plant resistance (White et al., 1982). We should recognize, however, that while increased levels of allelochemics may increase resistance to some cotton pests, production of these compounds will require energy that the plant may presently be ex-

pending for fruit production. Also, very high levels of some allelochemics may actually be toxic to the plant.

There are several plant breeding implications from this study. Except for cyanidin and delphinidin, all of these allelochemics are naturally occurring compounds in cotton. Cyanidin and delphinidin were included in the study because of their model role relationships to other compounds occurring in cotton. The levels of the allelochemics in cotton required to reduce larval weight 90% are within the range of the amounts found in various cotton strains and primitive accessions (J. N. Jenkins, unpublished data). Additionally, all except gossypol, cyanidin, and delphinidin are known to occur in several plant families. Thus, these allelochemics may be useful in developing resistance to the tobacco budworm and perhaps related insect species in several plant families. The host range and pest status of *Heliothis* spp. cover numerous plant species of economic importance and the insects are worldwide in their distribution. We are planning additional studies to determine the relationships between the effects of these compounds on larval growth in diet studies and in whole plant studies. The behavior of larvae of various ages on host plants containing these allelochemics in various concentrations and the distribution of allelochemics in plant parts will certainly be important. This information will be needed in determining the effectiveness of these allelochemics as a basis for developing plant strains resistant to the tobacco budworm.

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