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Cyanidin-3-β-glucoside, a newly recognized basis for resistance in cotton to the tobacco budworm *Heliothis virescens* (Fab.) (lepidoptera: Noctuidae)[1]

P.A. Hedin, J.N. Jenkins, D.H. Collum, W.H. White, W.L. Parrott and M.W. MacGown

Agricultural Research Service, U.S. Dept. of Agriculture, P.O. Box 5367, Mississippi State (MS 39762, USA) and Dept. of Entomology, Mississippi State University, Mississippi State (MS 39762, USA), September 8, 1982

Summary. Cyanidin-3-β-glucoside was shown to be an important factor of resistance in cotton *Gossypium hirsutum* L. leaves to the tobacco budworm *Heliothis virescens* (Fab.). This provides a potential basis for achieving insect resistance in non-glanded cotton and other crops infested by *Heliothis.*

We report the identification of cyanidin-3-β-glucoside (chrysanthemin) as a major factor of resistance in cotton, *Gossypium hirsutum* L., to the tobacco budworm *Heliothis virescens* (Fab.) and we reaffirm the reported effectiveness of gossypol[2-4]. We also present data to show that the correlation between condensed tannins (proanthocyanidins) in terminal leaves and growth of larvae feeding on terminal leaves in the field was small and positive. Paradoxically, these 3 compounds when incorporated in diets are equally toxic for larvae. Two important implications of these findings are that a basis (anthocyanin content) is provided for achieving insect resistance in non-glanded low gossypol cotton, and potentially for selecting for resistance in crops, world-wide, to various *Heliothis.* The larvae of 3 additional *Heliothis* spp. are also important pests of cotton, tobacco, corn, and other food crops[5].

Anthocyanins were first found in cotton in the envelope of pigment glands by Stanford and Viehoever in 1918[6]. We identified the red pigment in the cotton flower as chrysanthemin in 1967[7]. Recently, Chan and Waiss[8] confirmed the presence of an anthocyanin in the pigment glands and identified it as the same pigment in cotton flowers, chrysanthemin. Gossypol occurs in association with a number of gossypol-related triterpenoids, sesquiterpenoid quinones, hemigossypols, and heliocides, all possessing comparable insect toxicity[9,10]. Condensed cotton tannin[11-13] and flavo-

Table 1. Inhibition of tobacco budworm larval growth by cotton constituents, ED_{50} as percent of diet

Constituent	ED_{50}, %[a]		
	Chan et al.	Stipanovic	Miss. State
Gossypol	0.12	0.05	0.113
Hemigossypolone	0.03	0.29	-
Heliocide H_1	0.12	0.10	-
Heliocide H_2	0.13	0.46	-
Methyl sterculate	0.41	-	N.T.
(+)-Catechin	0.13	-	0.052
Condensed tannin	0.15	-	0.063
Quercetin	0.05	-	0.042
Isoquercitrin	0.10	-	0.060
Cyanidin	-	-	0.166
Delphinidin	-	-	0.138
Chrysanthemin	-	-	0.070

[a]Percent of compound required to reduce weight gain by 50%.

Table 2. Relative effects of cotton flower petal allelochemics on tobacco budworm growth and survival

Cultivar	Petal color	Tannins (%)[a]	Gossypol (%)[a]	Chrysanthemin (%)[a]	Larval wt (mg)[d]	Insects surviving (%)
ST-7AGN (NG)[b]	W[c]	5.79	0.10	0.07	4.72[a]	39.5
	R	8.68	0.11	0.67	0.46	7.0
DH 66 (NG)	W	5.40	0.17	0.07	4.18[a]	31.0
	R	8.55	0.13	0.73	0.56	16.5
ST-213 (G)	W	3.49	0.52	0.13	1.52[b]	28.0
	R	8.75	0.79	0.59	0.41	8.5
DH 126 (G)	W	3.16	1.72	0.18	[e]	-
	R	6.25	2.46	0.65	[e]	-

[a]% of dry weight. [b]NG, nonglanded; G, glanded. [c]W, white: 1st day flower color; R, red; 2nd day flower color. Means of larvae fed on white petals not significantly different at 0.05 level if followed by same letter. [d]Average tobacco budworm weight after feeding 5 days on petals. [e]Not fed.

noids[14,15] also have been reported to be antibiotic chemicals for the tobacco budworm.

Table 1 gives ED_{50}-values (percent of compound required to reduce weight gain by 50%) obtained by us, Chan et al.[12], and Stipanovic (unpublished data) for a number of cotton constituents tested as inhibitors of tobacco budworm larval growth. A randomized complete block design with 8 replicates of 5 larvae each was used by us. Compounds were tested at 5–8 concentrations ranging from 0.006 to 0.6% of the diet on a dry weight basis. Regression equations were calculated for each and found to be different. The identity of gossypol was confirmed by comparison of the spectral (NMR, MS) and chromatographic properties with an authentic sample. The identity of the cotton leaf anthocyanin was confirmed by comparison with the chromatographic and spectral properties of the chrysanthemin isolated from cotton flowers, by ^1H NMR, and by procedures that we described previously[7]. The condensed tannins (polymeric proanthocyanidins) were characterized with regard to their stereochemistry, structural units, and molecular weight by the procedures of Czochanska et al.[16]. The condensed tannins were found (details to be published later) to consist of a mixture of related polymers, the molecular weight ranging from 1500 to 6000, the prodelphinidin:procyanidin ratio from 1.8 to 3.7, and the stereochemistry of the monomer units primarily cis (81–95%). Condensed tannin fractions of mol.wt 1500–6000 gave ED_{50}-values of 0.05 to 0.10% so it is deduced that the size of the molecule does not appreciably affect toxicity. There has been the expectation that some individual compound or group of related compounds could account for the resistance of cotton to this insect. However, this could not be demonstrated in laboratory feeding studies because the ED_{50}-values were essentially the same.

In a 2nd test, young tobacco budworm larvae were caged on the terminal leaves of plants growing in the field. Larval weights were recorded after 5 days, and plant tissue was harvested for determination of gossypol, chrysanthemin, and tannins.

Figure 1 gives percent concentrations of these 3 constituents in terminal leaves, and also gives tobacco budworm larval weights (4 replicates of 50 insects each) for the 20 cotton strains, 15 glanded and 5 non-glanded, grown on the Plant Science Farm at Mississippi State in 1980. The 5 non-glanded strains are DH-40, DH-36, DH-66, TXLY and ST-7AGN.

The chemical analyses reported were averages of 4 replicates of terminal leaves, 5–6 cm in diameter, that were collected weekly for 10 weeks from replicated field plots. Freeze-dehydrated tissue was analyzed for condensed tannin (heated n-BuOH-HCl), gossypol (phloroglucinol-HCl), and anthocyanins-anthocyanidins (alcohol HCl at 540 nm).

The percent contents of gossypol and chrysanthemin were negatively correlated with larval weights ($r = -0.38$ and -0.40) while the tannins were weakly positively correlated ($r = +0.16$); in fact, glandless lines that produced large larvae were as high in tannin as most of the lines that produced small larvae. Thus, these studies suggest that the absolute concentration of the tannins in the terminals does not necessarily govern the expected toxic effects of the insect feeding on intact tissue.

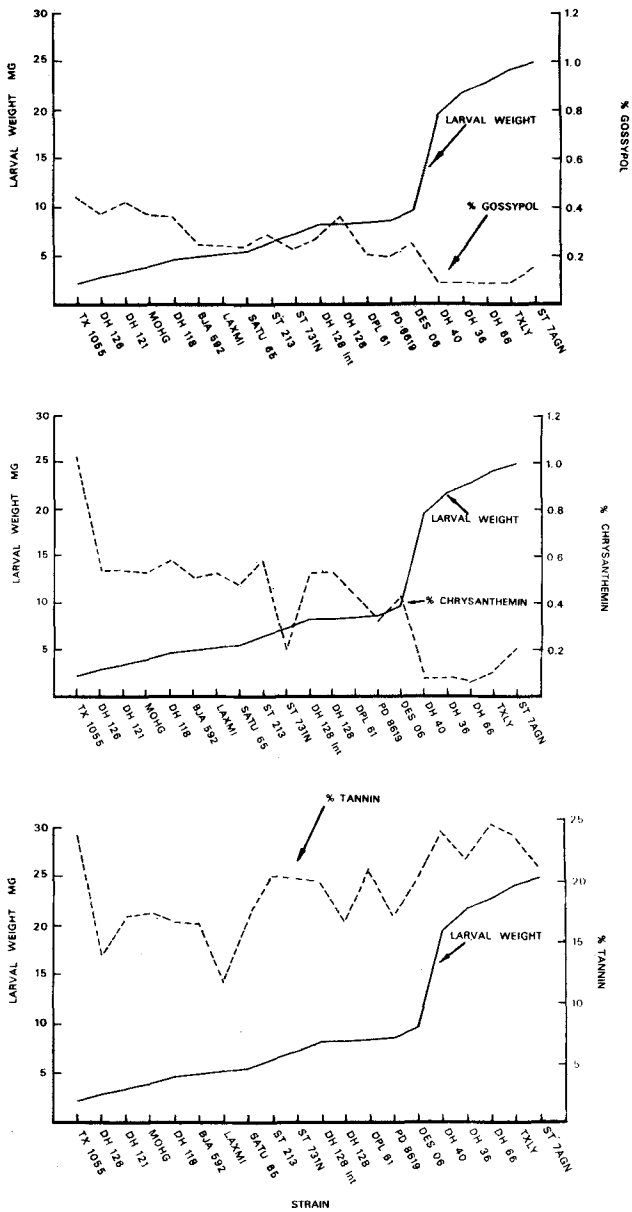

Figure 1. Percent gossypol, chrysanthemin, and condensed tannins in cotton terminal leaves and ranked tobacco budworm larval weights for 20 strains. The 5 non-glanded strains are on the right; DH-40, DH-36, DH-66, TXLY, and ST-7AGN.

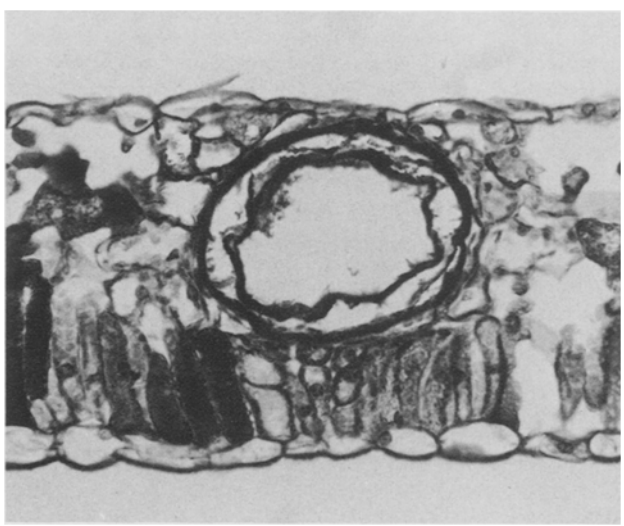

Figure 2. Paraplast section (5 µm) of a pigment gland and the anthocyanin envelope.

To further evaluate the hypothesized effects of anthocyanins, flower petals were collected, and young larvae were allowed to feed on them in the laboratory (4 replicates of 50 insects, 4 chemical analyses per data point). Table 2 presents tannin, gossypol, and chrysanthemin concentrations of white and red petals of 2 glanded and 2 non-glanded varieties, and for tobacco budworm 5-day larval weights and percent survival. Larvae fed more successfully on white, 1st day petals then on red, 2nd day petals, and also more successfully on non-glanded petals than on glanded petals. The tannin contents of the non-glanded white petals were higher than those of the glanded strains, but there was little difference in the red petal tannin contents. The gossypol content of the petals of the glanded strains was much higher than those of the non-glanded strains, and the chrysanthemin content of red petals was also much higher than those of white petals as expected. Based collectively on data in table 2 and figure 1, we suggest that chrysanthemin is more important than tannin for the reduced larval size and survival on glandless (gossypol-low) red petals. Gossypol and chrysanthemin contribute to the toxicity of glanded red petals and gossypol to that of glanded white petals. We now have preliminary data that larvae fed leaves and bracts of red cottons gained 20% less (statistically significant) than those fed leaves and bracts from comparable green strains. Thus, red coloration now appears to be a factor of considerable importance in insect feeding on both petals and leaves. There is still a residual mortality of insects feeding on white glandless petals (table 2). This can be attributed at least in part to the flavonoids, some of which we have previously identified[17] and demonstrated to be toxic to this insect (table 1).

We were able to observe by magnification that tobacco budworm larvae avoid pigment glands during feeding. We are also able to observe by means of 5 µm paraplast section through a young expanding cotton leaf of cultivar DH-126 at the pigment gland site, the outer anthocyanin containing envelope (halo) surrounding the pigment gland (fig. 2). In fresh tissue sections, this outer halo stains bright red in acid, and green when neutralized with base, verifying that it is anthocyanin. The feeding deterrence of the pigment gland which has been attributed to gossypol may therefore be due at least in part to the anthocyanin halo.

1 In cooperation with the Mississippi Agriculture and Forestry Experiment Station, Mississippi State. Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
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A technique for the comparison of biological distribution and solvent partition of drugs[1]

P.L. Gigon, H. van Hees and M.H. Bickel[2]

Department of Pharmacology, University of Bern, Friedbühlstrasse 49, CH-3010 Bern (Switzerland), November 3, 1982

Summary. A dialysis technique is described which allows the measurement of drug distribution between buffer and solvents as well as between buffer and biological preparations under identical experimental conditions. Partition and distribution coefficients of thiopental, pentobarbital, imipramine, and chlorpromazine were determined using octanol, other solvents, and tissue homogenates.

Permeation properties of a compound, its distribution in an organism, and its interactions with body constituents are known to be largely dependent on the lipophilicity of the compound. Among various molecular parameters which can be considered as quantitative criteria of lipophilicity, partition coefficients P have been most widely used. In particular, the partition coefficient in the system octanol/buffer has become recognized as an operational definition of relative lipophilic character. Modern views on partition coefficients have been discussed in recent work[3–5].

The concept of partition coefficients has been extended by several authors to systems in which the homogeneous organic phase is replaced by heterogeneous biological or supramolecular phases like membranes or liposomes[6–10], or subcellular fractions[11]. In these cases the concentrations in the separated biological structures and their aqueous environment are determined. Due to methodological differences, comparison with partition coefficients obtained with bulk solvents becomes questionable.

Distribution of drugs in biological preparations can be determined with equilibrium dialysis techniques[12]. If the biological material in the buffer of one dialysis chamber is replaced by a bulk solvent, then the solvent/buffer partition coefficients of a drug can be determined. In this study basic and acidic model drugs were used to demonstrate the feasibility of the dialysis technique for the determination of partition coefficients. The major advantage is that this unifying technique provides direct comparisons between