Chemical Basis of Resistance in Soya Bean to Cabbage Looper, *Trichoplusia ni*

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ABSTRACT

Thin layer chromatography resolved nine major compounds from the 60%-methanol extractables from PI 227687 soya bean (Glycine max (*L*) *Merrill*) *leaves. The flavonoids, daidzein, an unidentified flavonoid X2* (R_f *0·19*), *glyceollins, sojagol and coumestrol exhibited antifeedant and/or antibiotic effects against the larvae of cabbage looper,* Trichoplusia ni *Hb. The results indicate that several compounds in PI 227687 soya beans contribute to its antifeedant and/or antibiotic effects against* T ni. *The role of these compounds in plant resistance to insects is discussed.*

Key words: Plant resistance, flavonoids, cabbage looper, *Trichoplusia ni*, soya beans, *Glycine max*, antifeedants, antibiosis, phytoalexins.

INTRODUCTION

Knowledge concerning the role and significance of allelochemicals in plant defence against herbivory is of considerable importance. Phytochemicals, especially acetogenins, tannins, flavonoids, terpenoids and alkaloids, exhibit antifeedant, antibiotic or insecticidal properties towards insects (Harborne 1982). In field crops, the antiherbivory role of secondary plant substances has been analysed in only a few cases to elucidate the mechanisms of such plant resistance as a basis for breeding crop plants resistant to insects.

Different solvent extractables from insect-resistant soya beans have been reported to be biologically active against stink bug, *Nezara viridula* L (Jones and

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Sullivan 1979; Kester *et al* 1984); Mexican bean beetle, *Epilachna varivestis* Muls (Tester 1977; Smith *et al* 1979; Chiang *et al* 1986, 1987); corn earworm, *Heliothis zea* Boddie (Panda and Dougherty 1975; Dreyer *et al* 1979; Binder and Waiss 1984); soya bean looper, *Pseudoplusia includens* Walker (Hart *et al* 1983); and cabbage looper, *Trichoplusia ni* (Hubner) (Leudders and Dickerson 1977; Khan *et al* 1986a,b, 1987). Caballero and Smith (1986) reported that coumestrol, phaseol and afromosin in methanol extract of PI 227687 leaves show antibiotic effects against *T includens*. Liu *et al* (1988) found that insect-susceptible Davis soya bean contains greater amounts of 4-hexen-1-ol acetate, 2,2-dimethyl hexanal and 2-hexanal than PI 227687. Tetradecene was identified as a major repellant in the leaves of insect-resistant soya bean PI 227687 against the female moths of *T ni*.

As a part of our continuing efforts to elucidate the holistic nature and mechanisms of soya bean resistance to insects, the present studies report the extraction, separation and identification of flavonoids and other compounds responsible for antifeedant or antibiotic properties in the insect-resistant cultivar PI 227687 against the polyphagous pest, cabbage looper, *T ni*.

EXPERIMENTAL

Plants

Plants of the insect-resistant soya bean (*Glycine max* (L) Merrill) cultivar (PI) 227687 were grown in a greenhouse at the US Dairy Forage Research Center, University of Wisconsin, Madison. Thiram-treated seeds were germinated in sterilised and moistened vermiculite in aluminium trays in an incubator at 27 ± 1 C under a 16-h photophase. Seedlings were transplanted into earthen pots (20-cm dia) containing a sterilised mixture of soil, sand and vermiculite (2:1:1 v/v). Plants were watered once every 2 days for the first 4 weeks, and daily thereafter until harvest. Natural light was supplemented with high-intensity (100-W) Metalarc lighting in a 16-h photophase.

PI 227687 plants were grown to the V8 stage of development (Fehr *et al* 1971). Trifoliate leaves TL 3 to TL 7 were harvested and lyophilised. Dried leaves were powdered using a pestle and mortar, and the powder was sieved through a 24-mesh screen. The resultant leaf powder was stored in sealed glass bottles in desiccators until extracted.

Lima bean (*Phaseolus lunatus*) plants were raised in a Biotron (14 h photophase, 12 h full-light intensity, ie 300–500 μE m^{-2} s^{-1}; day temperature, 27 ± 1 C; night temperature, 20 ± 1 C; relative humidity, 65 ± 5%) for use as a susceptible host for the cabbage looper. Plants were provided with 30–40 ml of one-half strength Hoagland's nutrient solution (Hammer *et al* 1978) four times (each 6 h) per day.

Insects

Larvae of the cabbage looper *T ni* were used to evaluate the antifeedant and/or antibiotic properties of the compounds isolated from the leaves of PI 227687. Larvae were reared on a pinto bean based artificial diet (Shorey and Hale 1965) under laboratory conditions (27 ± 2°C, 12 h daylight and 60 ± 5% RH).

Extraction and fractionation

Lyophilised leaf powder (50 g) was homogenised in 850 ml 60% methanol in an ice-cooled Waring blender for 5 min. Homogenate was filtered through Whatman No 1 filter paper, and the residue was re-extracted five times with 500 ml 60% aqueous methanol for 8 h on an automatic shaker and then filtered. The residue was then extracted thrice with ethyl acetate. Combined extractables were rotoevaporated to dryness at 50 ± 1°C.

Resultant methanol extractables from each 50 g leaf powder were dissolved in 200 ml distilled water + 200 ml ethyl acetate, and then transferred into a separatory funnel. Ethanol (50 ml) was added to the above mixture, and the resultant ethyl acetate and aqueous phases were separated. The aqueous phase was next extracted five times with 200 ml ethyl acetate + 50 ml ethanol. The volume of the aqueous phase was maintained at about 200 ml. Ethyl acetate and water fractions were rotoevaporated to dryness, and then dissolved in ethyl acetate/ethanol/water (2:1:1) and 40% ethanol, respectively, to yield 10% stock solutions. These were stored at 4 C.

Thin layer chromatography (TLC)

Most studied compounds were isolated from the ethyl acetate extracted fraction by TLC on silica gel plates (250 μm thick and 20 × 20 cm with a 254-nm fluorescent indicator) (Sigma Chemicals, St Louis, Mo). Other leaf extractables and their fractions, obtained through other solvent systems, were subjected to similar TLC separations. Each (50 μg) fraction (sample) was spotted with a thin capillary tube on a TLC plate. Plates were developed in chloroform/acetone/acetic acid (90:10:1) in closed glass chambers saturated with solvent vapours. Plates were removed when the solvent front had moved 80% of the plate length, and were allowed to dry at room temperature. Resolved fluorescent spots were detected under 254 nm light (Mineral Light Lamp, UVSL-25, Ultra Violet Products, San Gabriel, CA). The R_f values of the fluorescent spots were calculated in relation to the solvent front.

A commercial sample of coumestrol, and glyceollin samples obtained from Dr N T Keen (University of California, Riverside) and Dr J Ebel (Biologisches Institut II der Universität, Freiburg in Briesgau, FRG), were co-chromatographed with plant extractables. TLC spots from soya bean leaf extractables which had R_f values corresponding to known flavonoids or related compounds in chloroform/acetone/acetic acid (Keen *et al* 1972) or in chloroform/acetone/ammonia (Ingham *et al* 1981) were especially investigated further. After initial analytical separations of flavonoids and related compounds on TLC plates, large-scale preparative TLC of leaf extractables was conducted after a thin-band (0·3–0·5 mm thick) application of the sample. One millilitre of a 10% sample solution was applied preparatively on each 20 × 20 cm silica gel plate. The solution in this case was applied with a glass Pasteur pipette (23 cm long) which had been drawn into a thin capillary at one end. After each application of sample the leaf extractables were dried under an air stream from a hair drier.

Preparative plates were developed in the solvent systems described above. Each

plate was removed from the glass chamber after 5–7 cm movement of the solvent front and allowed to dry in the air, and was then redeveloped to obtain better separation of the flavonoid bands.

With chloroform/acetone/acetic acid (90:10:1) the flavonoids and related compounds were first harvested in six bands (R_f <0·10, 0·11–0·25, 0·26–0·35, 0·36–0·50, 0·51–0·75 and >0·81). Silica gel was scraped with a clean microscope slide (2·5 × 7·5 cm). Compounds were extracted from the silica gel with 50–100 ml of ethanol or acetone, dried to about 1 ml in a rotoevaporator at 40 ± 1 C, and re-chromatographed preparatively. Resolved bands from re-chromatography were harvested individually and extracted with 50–100 ml ethanol or acetone as described above. Solvent was removed under vacuum. Each band obtained above was re-chromatographed twice in chloroform–2-propanol (90:10). Such purified bands were re-chromatographed in chloroform–acetone–acetic acid, scraped as above and extracted with HPLC reagent-grade ethanol. Compounds with R_f 0·25, 0·56 or 0·71 were resolved by TLC in chloroform–acetone–ammonia (50:50:1 v/v). The preparative bands were re-chromatographed twice in the above solvent system, and then once in chloroform–acetone–acetic acid (90:10:1 v/v). Solvent was evaporated from each purified sample under a stream of nitrogen, and each was finally dissolved as a 1% solution in ethanol (reagent grade) in a sealed glass vial and used in HPLC analyses. Major compounds were obtained in milligram amounts, and were used for bioassays and structural determinations. Sample purity was checked especially by high performance liquid (hexane/2-propanol, 90:10).

Antifeedant and/or antibiotic effects on first-instar larvae

Antifeedant and/or antibiotic activity was evaluated against 4- to 6-hour-old first-instar larvae. Antifeedant activity was bioassayed on leaf discs from lima bean. Fully expanded and mature leaves (4th leaf from the bud) were held between folds of water-soaked filter paper in an ice box while being brought to the laboratory. Leaf discs (18 mm dia) were cut with a No 7 corkborer. Discs for the feeding assay were mounted individually on insect pins. Two dosages of each sample (250 or 500 μg in 30 μl ethanol per disc) were tested, and there were three replications. Solvent was allowed to dry under a slow stream of air from a table fan for 1 h. Control discs were treated only with 30 μl of ethanol. Each treated lima bean disc was positioned individually over a filter paper in a 30-ml plastic cup in a no-choice test. Ten first-instar larvae were released on each disc in the cup. Each cup was covered with a lid having a water-soaked tissue paper on its inner surface to maintain the leaf disc in a turgid condition.

Discs treated with 250 μg were rated for insect feeding at 24 h after assay initiation. The number of larvae that survived was recorded 96 h after treatment.

Discs treated with 500 μg per disc were rated for insect feeding 36 h after treatment. After 4 days the larvae were transferred individually to a standard artificial diet in a rearing cup. The number of surviving larvae and larval weights were recorded at 2, 4 and 10 days after treatment. Data were also recorded on pupal weight, larval survival, adult emergence and duration of postembryonic development.

Antifeedant and/or antibiotic effects on third-instar larvae

Antifeedant and/or antibiotic activity of known compounds or fractions from PI 227687 soya bean leaves to third-instar *T ni* larvae was evaluated on 18-mm-dia, 400-μm-thick elderberry-pith discs in a double-choice assay. Elderberry pith discs were prepared using the modified method by Norris and Baker (1967). Each air-dried disc was first treated with 800 μg (40 μl of a 2% solution) of sucrose in 40% ethanol. Sucrose was used as a standard phagostimulant. Sucrose-treated discs were dried for 1 h under a slow air stream from a table fan. Such dried discs were then treated individually with 300 μg (30 μl of a 1% solution in ethanol) of a given plant compound or fraction, or with just solvent as a control. All discs were finally dried under a slow stream of air from a table fan. There were 5–20 replications per treatment.

One treated and one control (sucrose only) disc were offered in opposed positions to a larva in a double-choice assay. The two discs were placed 5 mm apart and anchored uniformly into an underlying but filter-paper-covered wax layer with an insect pin. A filter paper soaked with 2 ml water was attached to the inner surface of the top of the petri dish arena to keep the discs moistened. One newly moulted, 4-h-starved but water-satiated, larva was released in each petri dish arena. After 20 h exposure to the larva, each disc was measured (cm^2) for the remaining area using an automatic leaf area meter (LI 3100, LI-COR Inc, Lincoln, NE). After the disc area measurement, the control discs were removed from the petri dishes and the larva was confined with only the treated disc. A larva was allowed to consume about 90% of such a treated disc before being transferred into a 30-ml covered plastic cup containing 20 ml artificial diet to study the antibiotic effects. Time taken by the larvae to consume the treated discs was recorded every 4 h.

Larvae were weighed on a Mettler microbalance before and 5 days after initiation of an experiment. Once on artificial diet, times to complete larval and pupal development and the pupal weight were recorded. Growth rates of the larvae were computed as described by Waldbauer (1968).

Statistical analysis

Treatment means were compared using a two-sample *t*-test, or the least significant difference (LSD).

RESULTS

TLC resolution of leaf extractables

Sixteen fluorescent spots were detected analytically from the ethyl acetate fraction of methanol extract. Compounds resolved in solvent system 1 (Table 1) at R_f 0·10 (daidzein), 0·16 (flavonoid X1), 0·19 (flavonoid X2), 0·23 (chlorophyll A), 0·27 (coumestrol), 0·30 (carotenoid A), 0·41 (sojagol), 0·47 (glyceollins) and 0·81 (carotenoid B) were the major components in PI 227687 soya bean. They were purified in several-milligram amounts by TLC to study their biological and chemical properties. Less abundant flavonoids resolved at R_f 0·25 (glyceofuran +

TABLE 1

TLC R_f values of major resolved components in the 60% methanol extractables[a] from PI 227687 soya bean leaves, or authentic compounds,[b] in five solvent systems

Compound (and prep R_f)	R_f *value in indicated solvent system*[c]				
	1	*2*	*3*	*4*	*5*
Daidzein[a]	0·10	—	0·26	0·05	0·67
Unidentified flavonoid X1[a]	0·16	0·26	0·32	0·13	0·64
Unidentified flavonoid X2[a]	0·19	0·09			
Chlorophyll A[a]	0·23	0·65	0·72		0·76
Coumestrol[a]	0·27	0·43	0·41	0·21	0·69
Coumestrol[b]	0·27	—	0·39	0·21	—
Carotenoid A[a]	0·30	0·50	0·52	0·24	0·75
Sojagol[a]	0·41	0·59	0·55	0·05	0·76
Glyceollins[a]	0·47	—	0·41, 0·55	0·24, 0·35	—
Glyceollin III[b]	0·27	—	0·41, 0·17	0·25	—
Glyceollins[b]	0·27, 0·31		0·41, 0·17, 0·45	0·28	—
Carotenoid B[a]	0·77	0·79	0·78	0·71	0·76

[a] See 'Compound' column.
[b] See 'Thin layer chromatography (TLC)' in 'Methods and materials'.
[c] System 1 (chloroform acetone acetic acid, 90:10:1 v/v); system 2 (chloroform acetone ammonia, 50:50:1 v/v); system 3 (chloroform 2-propanol, 90:10 v/v); system 4 (hexane ethyl acetate methanol, 60:40:2 v/v); and system 5 (benzene ethyl acetate methanol, 40:30:30 v/v).

glyceocarpin), 0·56 (isoformononetin) and 0·71 (9-*O*-methyl glyceofuran) in chloroform acetone ammonia (50:50:1 v/v) (Ingham *et al* 1981) were also purified and studied for their biological effects on *T ni*.

Antifeedant and/or antibiotic effects of isolated compounds to first-instar larvae

Leaf discs treated with 250 μg of daidzein, coumestrol, flavonoid X2 and glyceollins reduced feeding ($P<0·05$) by the first-instar larvae under a no-choice condition (Table 2). Eighty-three per cent or less of the larvae survived on leaf discs treated with flavonoid X2, coumestrol, glyceollins and sojagol as compared with 97% survival on the control leaf disc.

At 500 μg per leaf disc, feeding at 36 h was significantly ($P<0·05$) reduced by daidzein, flavonoid X2, sojagol and glyceollins. Larvae weighed significantly ($P<0·05$) less when they were fed on a disc treated with daidzein, flavonoid X2 and coumestrol for 48 or 96 h. Weight gain by the larvae was lower when they were fed on discs treated with flavonoid X2 and sojagol. Daidzein, flavonoid X1, chlorophyll A, coumestrol, sojagol, glyceollins and carotenoid B increased the larval period as compared with the sucrose control (Table 3). In this no-choice situation, daidzein, flavonoid X2 and carotenoid A significantly ($P<0·05$) reduced pupation. Flavonoid X2 significantly ($P<0·05$) reduced pupal weight. No treatment significantly altered the pupal period. Daidzein, chlorophyll A and

TABLE 2

Antifeedant and antibiotic activities of TLC-resolved compounds or fractions in 60% methanol extractables from PI 227687 soya bean leaves against first-instar larvae of cabbage looper *T ni*

Compound	*250 μg disc*		*500 μg disc*			
	Damage rating	*Larval survival*	*Damage rating*	*Larval weight (mg)*		*Weight gain (mg)*
			36 h	*2 days*	*4 days*	
Daidzein	3·50[a,b]	97[c]	3·17[a]	0·36[a]	0·84[a,b]	0·48
Unidentified flavonoid X1	3·83[a,b,c]	97[c]	3·83[a,b]	0·41[a,b]	0·93[b,c]	0·52
Unidentified flavonoid X2	3·17[a]	80[a,b]	3·50[a]	0·35[a]	0·71[a]	0·36
Chlorophyll A	3·96[b,c]	97[c]	4·67[c]	0·41[a,b]	0·89[a,b,c]	0·48
Coumestrol	3·00[a]	73[a]	3·67[a,b]	0·33[a]	0·81[a,b]	0·48
Carotenoid A	3·83[a,b,c]	90[b,c]	4·00[b,c]	0·43[a,b]	0·89[a,b,c]	0·46
Sojagol	4·50[c]	77[a,b]	3·17[a]	0·39[a,b]	0·77[a,b]	0·38
Glyceollins	3·67[a,b]	83[b]	3·50[a]	0·50[b]	0·91[b,c]	0·41
Carotenoid B	4·17[b,c]	90[b,c]	3·83[a,b]	0·41[a,b]	0·87[a,b,c]	0·46
Control (sucrose)	4·67[c]	97[c]	4·50[b,c]	0·49[b]	0·91[b,c]	0·42
±SE (pooled)	±0·37	±6·4	±0·33	±0·04	±0·09	

Each lima bean leaf disc was treated with 250 or 500 μg of each compound or fraction. Ten first-instar larvae were confined in a no-choice assay with the treated disc in a 30-ml plastic cup for 96 h.
Damage ratings: 1, <10% disc area consumed; 2, 11–25%; 3, 26–40%; 4, 41–60% and 5, >60% disc area consumed.
[a–c] Values in a column followed by the same superscript letter are not significantly different at $P<0·05$.

sojagol lengthened the post-embryonic developmental period. Adult emergence was significantly ($P<0·05$) reduced by daidzein, flavonoid X1 and X2, carotenoid A, coumestrol, glyceollins and sojagol. All compounds caused a significant increase in abnormal adults with deformed wings as compared with the sucrose control.

Antifeedant and/or antibiotic effects of isolated compounds to third-instar larvae

Daidzein, flavonoid X2, coumestrol, carotenoid A, chlorophyll A, and sojagol showed antifeedant activity towards third-instar larvae in one or both sets of similar experiments (Table 4). Chlorophyll B, carotenoid B, isoformononetin and glyceofuran + glyceocarpin showed phagostimulant activity. Larvae took significantly more time to consume discs treated with flavonoids X1 and X2, coumestrol, carotenoid A, sojagol, glyceollins and carotenoid B.

Larval weights at 5 days were significantly ($P<0·05$) reduced by sojagol and carotenoid B (Table 5). Glyceofuran + glyceocarpin and isoformononetin significantly ($P<0·05$) increased the mass of 5-day-old larvae. Growth rate of larvae was slower ($P<0·05$) when fed in a no-choice situation on a disc treated

TABLE 3

Antibiotic effects of TLC-resolved compounds or fractions in 60% methanol extractables from PI 227687 soya bean leaves on first-instar larvae of *T ni*[1]

Compound	*Larval period (days)*	*Pupal period (days)*	*Post-embryonic developmental period (days)*	*Pupal wt (mg)*	*Pupation (%)*	*Adult emergence (%)*	*Abnormal adults (%)*
Daidzein	19·9^{cd}	11·2	31·1^{bc}	260·4^{c}	70·0^{a}	65·0^{a}	15·0
Unidentified flavonoid X1	19·6^{c}	11·0	30·6^{ab}	243·6^{ab}	76·7^{ab}	73·0^{ab}	16·7
Unidentified flavonoid X2	18·6^{ab}	11·0	29·6^{a}	239·5^{a}	70·0^{a}	70·0^{a}	20·0
Chlorophyll A	20·1de	11·6	31·7^{c}	252·1^{b}	86·7^{ab}	80·0^{abc}	13·3
Coumestrol	19·8^{cd}	10·6	30·4^{a}	248·1^{ab}	80·0^{ab}	76·7^{ab}	30·0
Carotenoid A	18·7^{ab}	10·8	29·5^{a}	259·2^{c}	66·7^{a}	66·7^{a}	20·0
Sojagol	20·4de	10·8	31·2^{a}	249·3^{ab}	73·3^{ab}	63·3^{a}	33·3
Glyceollins	19·6^{c}	10·7	30·3^{bc}	264·2^{c}	83·3^{ab}	73·3^{ab}	30·0
Carotenoid B	19·5^{bc}	11·2	30·7^{a}	255·0^{bc}	96·7^{b}	93·3^{c}	30·0
Control (sucrose)	18·1^{a}	11·3	29·4^{ab}	254·3^{bc}	93·3^{b}	93·3^{c}	3·3
+ SE (pooled)	± 0·3		± 0·4^{a}	± 3·9	± 8·9	± 7·9	—

[1] Ten larvae were fed in a no-choice assay on a lima bean leaf disc treated with 500 μg of one compound for 4 days, and then reared on artificial diet.

[a–e] Values in a column followed by the same superscript letter are not significantly different at $P < 0{\cdot}05$.

with flavonoid X2, chlorophyll A, coumestrol, carotenoid A and sojagol. The flavonoid 9-*O*-methyl glyceofuran also significantly ($P < 0{\cdot}05$) reduced the larval growth rate. The larval period of those fed on a disc treated with chlorophyll A, coumestrol or carotenoid B was significantly ($P < 0{\cdot}05$) lengthened. Pupal weight was significantly reduced on chlorophyll A, coumestrol, and chlorophyll B. The pupal period (Table 5) was lengthened by daidzein, flavonoid X2, chlorophyll A, carotenoid A, sojagol and carotenoid B.

DISCUSSION

The results indicate that several compounds contribute towards antifeedant and/or antibiosis activity in PI 227687 to the cabbage looper, *T ni*. Some fractions also showed phagostimulant activity. TLC results for PI 227687 leaf extractables in five solvent systems (Table 1), including ones used in previous classical analyses of such chemicals from soya beans (Keen *et al* 1972; Ingham *et al* 1981) and the overall bioassay results (Tables 2–5) support the concept that specific flavonoids, daidzein, the unidentified flavonoid X2 (R_f 0·19), coumestrol, sojagol and the glyceollins, act as antifeedants and/or antibiotics against this insect. Carotenoid A and chlorophyll A fractions also showed some antifeedant and/or antibiotic effects at the dosages tested. Chlorophyll B, carotenoid B, isoformononetin, and glyceofuran + glyceocarpan showed some phagostimulant effects towards the third-instar *T ni* larvae.

TABLE 4

Antifeedant activity of TLC-resolved compounds or fractions in 60% methanol extractables from PI 227687 soya bean leaves against third-instar larvae of cabbage looper, *T ni*

Compound	*Unconsumed disc area (cm^2)*				*Time taken to consume treated disc (h)*
	Experiment I		*Experiment II*		
	Treated	*Control*	*Treated*	*Control*	
	Chloroform/acetone/acetic acid (90:10:1)				
Daidzein	1·28 ± 0·05^{a}	1·11 ± 0·05^{b}	1·14 ± 0·07^{a}	1·10 ± 0·07^{a}	44^{cde}
Unidentified flavonoid X1	1·10 ± 0·06^{a}	1·06 ± 0·08^{a}	1·24 ± 0·05^{a}	1·26 ± 0·08^{a}	51^{h}
Unidentified flavonoid X2	1·21 ± 0·08^{a}	1·02 ± 0·07^{b}	1·30 ± 0·06^{a}	1·14 ± 0·08^{b}	55^{i}
Chlorophyll A	1·44 ± 0·04^{a}	1·32 ± 0·11^{a}	1·34 ± 0·06^{a}	1·07 ± 0·12^{b}	43^{cd}
Coumestrol	1·22 ± 0·06^{a}	1·07 ± 0·05^{b}	1·31 ± 0·06^{a}	0·97 ± 0·10^{b}	49^{fg}
Carotenoid A	1·59 ± 0·01^{a}	1·19 ± 0·06^{b}	1·33 ± 0·05^{a}	1·16 ± 0·07^{b}	57^{j}
Sojagol	1·19 ± 0·05^{a}	0·56 ± 0·23^{b}	1·38 ± 0·02^{a}	1·05 ± 0·09^{b}	47^{ef}
Glyceollins	0·96 ± 0·13^{a}	1·12 ± 0·13^{a}	1·20 ± 0·13^{a}	1·00 ± 0·12^{a}	45de
Chlorophyll B	0·96 ± 0·11^{b}	1·38 ± 0·06^{a}	1·30 ± 0·30^{a}	0·99 ± 0·05^{a}	43^{cd}
Carotenoid B	0·67 ± 0·11^{b}	1·38 ± 0·05^{a}	1·30 ± 0·18^{a}	1·23 ± 0·16^{a}	53^{hi}
	Chloroform/acetone/ammonia (50:50:1)				
Glyceofuran + glyceocarpin	—	—	1·14 ± 0·09^{a}	1·24 ± 0·06^{a}	37^{b}
Isoformononetin	—	—	0·90 ± 0·08^{a}	1·28 ± 0·07^{a}	33^{a}
9-*O*-Methyl glyceofuran	—	—	1·26 ± 0·04^{a}	1·16 ± 0·06^{a}	43^{cd}
Untreated (sucrose) control	1·28 ± 0·08^{a}	1·24 ± 0·10^{a}	1·26 ± 0·05^{b}	1·49 ± 0·02^{a}	41^{c}
SE					± 1·1

Each elderberry-pith disc was treated with 300 μg of the indicated R_f compound or fraction + 400 μg sucrose. Control discs were treated only with sucrose. Each experiment was conducted as a two-choice assay, and there were 5–10 replications. Disc area was measured 12 h after experiment initiation.

[a–j] Paired values in a column (within an experiment) followed by the same letter are not significantly different at $P < 0{\cdot}05$.

Flavonoids in legumes have previously been shown to reduce feeding by insects (Russell *et al* 1978; Sutherland *et al* 1980; Chiang and Norris 1983). Isoflavonoids phaseol, coumestrol and afromosin have been reported to be antibiotic toward larvae of *P includens* (Caballero and Smith 1986). The concentration of the phytoalexins glyceollins in soya bean tissue has been correlated with antifeedant activity against the Mexican bean beetle (Hart *et al* 1983). A flavonoid fraction of leaf extractables from PI 227687 has been reported to reduce feeding by *T ni* (Khan *et al* 1986a).

Maximal antibiotic effects of given compounds may not have been observed in these experiments because the larvae were exposed to 250–500 μg of these compounds for 2–4 days whereas under natural conditions a larva usually feeds

TABLE 5

Antibiotic effects[1] of 300 μg per disc of TLC-resolved compounds or fractions in 60% methanol. Extractable from PI 227687 soya bean leaves on third-instar larvae of cabbage looper, *T ni*

Compound	*Initial larval wt (mg)*	*Wt of feeding larva after 5 days*	*Larval growth rate*	*Larval period (days)*	*Pupal wt (mg)*	*Pupal period (days)*
Chloroform acetone acetic acid (90:10:1)						
Fraction including the flavonoid daidzein	$24{\cdot}3^{abcd}$	$59{\cdot}2^{abc}$	$0{\cdot}183^{cf}$	$20{\cdot}5^{ab}$	$190{\cdot}8^{bcd}$	$9{\cdot}0^{b}$
Unidentified flavonoid X1	$19{\cdot}4^{a}$	$52{\cdot}5^{ab}$	$0{\cdot}186^{cf}$	$22{\cdot}9^{cde}$	$199{\cdot}6^{bcd}$	$8{\cdot}3^{a}$
Unidentified flavonoid X2	$27{\cdot}4^{cde}$	$44{\cdot}4^{ab}$	$0{\cdot}089^{a}$	$23{\cdot}4^{cde}$	$242{\cdot}7^{ef}$	$10{\cdot}3^{c}$
Chlorophyll A	$26{\cdot}7^{bcde}$	$46{\cdot}4^{ab}$	$0{\cdot}095^{ab}$	$24{\cdot}0^{de}$	$120{\cdot}4^{a}$	$10{\cdot}0^{c}$
Coumestrol	$23{\cdot}7^{abc}$	$67{\cdot}4^{bcd}$	$0{\cdot}119^{abc}$	$24{\cdot}8^{e}$	$126{\cdot}0^{a}$	$7{\cdot}8^{a}$
Carotenoid A	$25{\cdot}1^{abcde}$	$44{\cdot}7^{ab}$	$0{\cdot}088^{ab}$	$23{\cdot}5^{cde}$	$206{\cdot}3^{bcde}$	$10{\cdot}0^{c}$
Sojagol	$34{\cdot}5^{f}$	$40{\cdot}6^{a}$	$0{\cdot}117^{abc}$	$21{\cdot}0^{abc}$	$212{\cdot}5^{de}$	$10{\cdot}0^{c}$
Glyceollins	$29{\cdot}8^{def}$	$84{\cdot}3^{cde}$	$0{\cdot}171^{def}$	$21{\cdot}3^{bc}$	$213{\cdot}4^{de}$	$8{\cdot}5^{a}$
Chlorophyll B	$26{\cdot}5^{bcde}$	$64{\cdot}6^{abcd}$	$0{\cdot}145^{bcde}$	$21{\cdot}5^{bcd}$	$159{\cdot}2^{ab}$	$8{\cdot}0^{a}$
Carotenoid B	$21{\cdot}1^{ab}$	$40{\cdot}2^{a}$	$0{\cdot}125^{abcd}$	$27{\cdot}0^{f}$	$208{\cdot}3^{cde}$	$11{\cdot}0^{d}$
Chloroform acetone ammonia (59:50:1)						
Glyceofuran + glyceocarpin	$30{\cdot}7^{ef}$	$106{\cdot}2^{e}$	$0{\cdot}212^{f}$	$19{\cdot}0^{a}$	$185{\cdot}7^{bcd}$	$8{\cdot}0^{a}$
Isoformononetin	$29{\cdot}8^{def}$	$108{\cdot}9^{e}$	$0{\cdot}221^{f}$	$21{\cdot}8^{bcd}$	$185{\cdot}9^{bc}$	$8{\cdot}7^{a}$
9-*O*-Methylglyceofuran	$27{\cdot}5^{cde}$	$50{\cdot}1^{ab}$	$0{\cdot}103^{ab}$	$22{\cdot}5^{bcd}$	$200{\cdot}3^{cd}$	$8{\cdot}0^{a}$
Control (sucrose)	$26{\cdot}9^{bcde}$	$68{\cdot}3^{cd}$	$0{\cdot}174^{de}$	$21{\cdot}0^{abc}$	$204{\cdot}9^{cde}$	$8{\cdot}0^{a}$
± SE (pooled)	±2·12	±9·45	±0·018	±0·72	±14·45	±0·29

[1] Each larva fed in a no-choice assay on 300 μg of a given compound or fraction applied to a disc.

[a–f] Values followed by the same letter in a column are not statistically different at $P < 0{\cdot}05$.

exclusively on a given cultivar. Highly insect-resistant cultivars such as PI 227687 may thus produce more pronounced antibiotic effects on the insect than were observed with individual compounds, fractions in these experiments. In the present limited-exposure experiments, the antibiotic effects of some compounds continued through the entire insect-developmental period, even after the larvae were transferred to the control artificial diet. Rearing of insects on such an artificial diet after their feeding on chemically treated discs may also cause a reversal of some antibiotic effects. However, the observed antifeedant and antibiotic effects on the larvae strongly implicate these specific compounds as components in the soya bean plant's defence mechanism against herbivorous insects.

Synthesis and production of individual flavonoids such as daidzein, glyceollins, coumestrol, sojagol, isoformononetin, glyceofuran, glyceocarpin and 9-*O*-methyl glyceofuran are interdependent (Keen *et al* 1972; Ingham *et al* 1981). Some of these compounds are precursors or metabolites of others in the general flavonoid biosynthetic process in plants (Keen *et al* 1972; Keen and Paxton 1975; Ebel 1986).

Flavonoids appear to be major contributors to holistic chemical defences against insect pests in soya bean and apparently other plants. Their structural

identification, location within a given plant, relative proportions in specific plant tissues, biological activities and inducibilities by given environmental stresses need much further investigation to utilise their potential as a mechanism of resistance to herbivores in field crops.

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Monomeric and Dimeric Phenolic Acids Released from Cell Walls of Grasses by Sequential Treatment with Sodium Hydroxide

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ABSTRACT

Cell walls of tall fescue Festuca arundinacea *Schreb and coastal bermudagrass* Cynodon dactylon *L Pers were treated sequentially with increasing concentrations of sodium hydroxide (0·1 M to 10 M) to release monomeric and dimeric phenolic acids. (E)-*p*-Coumaric and (E)-ferulic acids were the major monomers released. Most of the saponifiable feruloyl groups (97% for tall fescue, 89% for coastal bermudagrass) were released with 0·1 M sodium hydroxide. Much lower proportions of saponifiable* p*-coumaroyl groups (67% for tall fescue, 46% for coastal bermudagrass) were released with this treatment. The major dimers from both grasses were 4,4'-dihydroxy-α-truxillic, 4,4'-dihydroxy-3-methoxy-α-truxillic, and 4,4'-dihydroxy-3,3'-dimethoxy-α-truxillic acids, and were mainly released with 0·1 M sodium hydroxide. Similar proportions of the monomers and dimers were released from the cell walls of each grass with the 0·1 M and 1 M sodium hydroxide sequential treatments. It is probable that most if not all of the monomers and dimers released by the sequential alkali treatments were originally ester linked to the cell walls. If it is assumed that the cell wall bound dimers are formed photochemically from* p*-coumaroyl and feruloyl groups during plant growth, it is calculated that, for the two grasses, between 12 and 17% of the monomer units were converted to dimers.*

Key words: Festuca arundinacea, tall fescue, *Cynodon dactylon*, coastal bermudagrass, cell walls, *p*-coumaric acid, ferulic acid, substituted

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