Deterrency of Mexican Bean Beetle (Coleoptera: Coccinellidae) Feeding by Free Phenolic Acids[1]

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ABSTRACT We investigated the role of simple phenolic acid precursors of isoflavonoid phytoalexins, as free compounds, in food selection by the Mexican bean beetle, *Epilachna varivestis* Mulsant, a pest of soybean, *Glycine max* Merrill, and other legumes. An aerosol spray method was developed to apply compounds in a uniform and reproducible manner to leaf surfaces of common bean, *Phaseolus vulgaris* L., a preferred host. Twenty-three compounds were tested in dual choice preference tests. Included were benzoic acid, cinnamic acid, simple phenolic acids (hydroxylated benzoic and cinnamic acids), and their methoxylated analogs. Six compounds were found to cause rejection of leaves. All of these were hydroxylated acids with the hydroxyl group adjacent to the carboxylic acid group on the benzene ring. Two of these six (gentisic acid and salicylic acid) are reportedly produced in soybean leaves.

KEY WORDS *Glycine max,* soybean, *Phaseolus vulgaris,* insect/plant interactions, induction, allomones, phenolics, Mexican Bean Beetle, *Epilachna varivestis.*

Phenolic compounds are clearly involved in determining feeding preference in legume-leaf-eating insects. Vestitol, glyceollins, and other isoflavonoids have been identified or implicated as insect feeding deterrence factors in soybean and several other cultivated legumes (Russell et al. 1978; Hart et al. 1983; Lwande et al. 1985; Caballero et al., 1986; Chiang et al. 1987). These compounds arise from cinnamic acid or p-coumaric acid, which in turn, are derived from phenylalanine or tyrosine in biochemical pathways mediated by phenylalanine ammonia-lyase (PAL) or tyrosine ammonia-lyase (TAL). Those acids also give rise to other simple phenols that are used throughout the plant to form both structural and defensive compounds such as, flavones, lignin, soluble polyphenols, phenyl propanoids, quinones, tannins, and pterocarpan phytoalexins (Levin 1971). Several of the phytoalexins have been implicated in the resistance of soybean against fungal pathogens (Ingham 1982) and as possible feeding deterrents for insect herbivores (Kogan & Paxton 1983).

The assumption has been made that free phenolic acids are found only in woody tissue and that in most other tissues the acids are glycosylated or condensed to form more complex molecules (Robinson 1980). However, high levels of free phenolic acids have been reported in leaf tissue. For example, 40% and 46% of the ferulic acid in alfalfa and spinach leaves, respectively, is in the free form (Huang et al. 1986).

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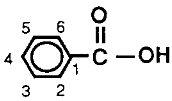
Elevated levels of phenolic acids can cause changes in food preference directly as free or esterified compounds (Woodhead & Bernays 1978). For example, high levels of free phenolic acids in genotypes of lettuce were associated with feeding deterrence of root aphids (Cole, 1984). Leszczynski et al. (1985) found that the addition of several free phenolic acids to the hydroponic solution used for wheat cultivars greatly reduced feeding by aphids. Simple phenolic acids in artificial diets caused rejection and/or weight loss in aphids (Todd et al. 1971). Tahvanainen et al. (1985) reported a strong correlation between high levels of phenolic glycosides in willow leaves and changes in feeding behavior of chrysomelid beetles. Hardin (1979) found that the soybean genotype (PI 171451) that causes feeding deterrence to the Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant, had higher levels of several simple phenolic acids than the variety 'Forrest' which was preferred by the MBB. Phenolic acid analysis in that study was preceded by acid hydrolysis; therefore, it is unknown whether the acids were free or esterified.

The MBB rejected soybean cotyledons following treatment with an elicitor (ultraviolet light, 254 nm) that induces production of isoflavonoid phytoalexins (including glyceollins) (Hart et al. 1983). However, those tests with the MBB could only correlate antixenosis with increased overall activity in the shikimic acid pathway that results in production of phenolic acids and eventually isoflavonoids (Floss 1977); deterrence to MBB feeding could not be assigned only to glyceollins. The previously cited work shows that some insects are sensitive to elevated levels of low molecular weight phenolic compounds. We attempted to assess the general "palatibility" and structure / activity aspects of a series of free phenolic acids on MBB feeding preference. We were interested (1) in the effect of the functional hydroxyl group, assuming that it would have a deterrent effect since it defines the chemistry of the phenolics which are generally deterrent to insects and (2) in the effect of position and number of hydroxyls in the basic phenolic acid molecule.

Materials and Methods

We tested 23 pure compounds including benzoic acid, cinnamic acid, simple phenolic acids (hydroxylated benzoic and cinnamic acids), and their methoxylated analogs. The list of tested compounds and their structural formulas are shown in Figure 1. Compounds were purchased in pure form from either Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO). All were 98.0 - 99.9% pure.

The compounds were dissolved in reagent-grade acetone (10 mM) and 2-ml of the solution was applied as an aerosol spray to the surface of excised common bean leaves (*Phaseolus vulgaris*, var. 'TopCrop'). Control leaves were sprayed with 2-ml reagent grade acetone. The aerosol was applied to leaves held flat against an 18.0-cm diam. circular wooden plate mounted on a shaft attached to an electric motor, forming a vertical turntable (see Fig. 2). An 18.0-cm diam. paper sheet covered the leaflet except for a 6.0-cm diam. area in the center. Two elastic bands helped secure the leaf between the paper mask and the wooden plate. The turntable was rotated at 300 rpm. The aerosol from a standard thin layer chromatography reagent sprayer (model K 422500, Kimax Co., Morton Grove, IL) was directed at the 6-cm circle of exposed leaf surface from a distance of 18.0 cm. The aerosol was created using pressurized nitrogen gas (flow rate 20 lpm).



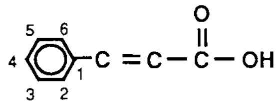
1. BENZOIC ACID

2. Hydroxy Benzoic Acids

2-HBA	(Salicylic Acid)
2,3-DHBA	
2,4-DHBA	
2,5-DHBA	(Gentisic Acid)
2,6-DHBA	
3,4-DHBA	(Protocatechuic Acid)
3,5-DHBA	
3,4,5-DHB	(Gallic Acid)
4-HBA	(p-Hydroxy Benzoic Acid)

3. Methoxy Benzoic Acids

2-MBA	
4-MBA	
3-H-4-MBA	
4-H-3,5-MBA	(Syringic Acid)
4-H-3-MBA	(Vanillic Acid)



4. CINNAMIC ACID

5. Hydroxy Cinnamic Acids

2-HCA	
3-HCA	
4-HCA	(p-Coumaric Acid)
3,4-DHCA	(Caffeic Acid)

6. Methoxy Cinnamic Acids

4-H-3,5-DMCA	(Sinapic Acid)
4-MCA	(p-Anisic Acid)
4-H-3-MCA	(Ferulic Acid)

Fig. 1. Phenolic compounds applied to snap bean leaves (BA=benzoic acid, CA=cinnamic acid, H=hydroxy, M=methoxy, D=di).

This arrangement permitted us to apply a uniform coating of test chemical to a circular 28-cm^2 area from which we removed three leaf discs (each 3 cm^2) to be used in feeding preference tests. Concentration and uniformity of application were calculated by cutting three discs from the coated area of each of five leaves sprayed with salicylic acid and extracting each with ethanol. The amount of salicylic acid extracted from each disc was measured by high performance liquid chromatography (HPLC) (C18 column, U. V. detector, 254 nm) against a standard solution. Concentration of salicylic acid on discs taken from sprayed leaves was analysed at 3, 6, 12, and 24 h after application.

Bean plants were grown in potting soil (soil: peat: sand: perlite; 1:1:1:1) under supplemental metal halide light (L:D14:10) in a greenhouse and fertilized once each week. Leaves from 4-week-old plants were sprayed with test solution immediately after excision from the plants. The lateral leaflets of a single trifoliolate were used as treatment and control, respectively.

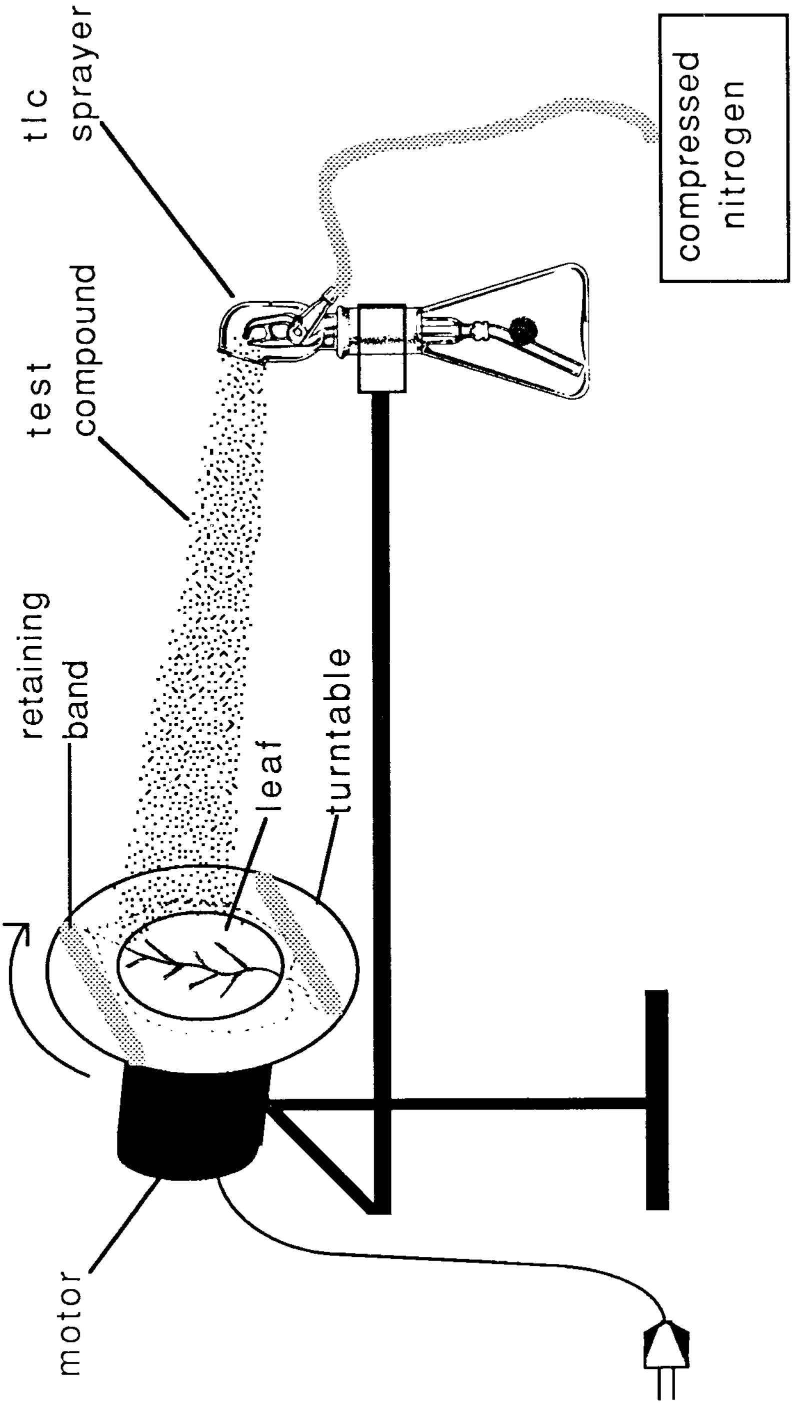


Fig. 2. Aerosol spray device used to coat leaves with test compounds.

Feeding preference tests were performed in arenas constructed from 18.0-cm glass petri dishes filled to a depth of 1 cm with hardened plaster of Paris, saturated with tap water, and covered with filter paper. For each test three treated discs and three acetone-sprayed control discs were arranged in an alternating manner in a circular pattern in the arena. MBB reared on common bean leaves in a greenhouse were used in all experiments. For each test four adult females MBB's, four to ten days old, were placed in the arena and allowed to eat until approximately half of the leaf material was consumed (usually within 6-8 hr). Test arenas were kept in the dark, in a rearing chamber, at 27° C ± 2°, and R. H. near saturation within the arenas. Beetles were starved for approximately 12 hr and used in no more than three tests on consecutive days. At the end of each test all discs were removed and their remaining area measured with a Licor (Model 3000) leaf-area meter. Leaf area eaten of each disc was calculated by subtracting the uneaten area from the original area of the discs. The test was repeated 16 times for each compound examined. Tests were conducted over a period of one month, generally 8 replicates for each of two compounds per day.

A preference index (PI) was calculated for each paired comparison according to the formula: $PI = 2T/(T+C)$, where, T = area eaten from the treatment disc; C = area eaten from the control disc. PI values, in this preference test, range from 0.0 to 2.0 with 0.0 indicating strongest possible preference for the control disc, 1.0 indicating no preference, and 2.0 indicating strongest preference for the treatment disc.

Results and Discussion

Common bean leaves are a more acceptable food to the MBB than soybean leaves. We selected common bean leaves as a substrate for the phenolic acids because we were seeking a rapid behavioral response. The activity of each phenolic acid could be easily measured by the degree of deterrency (antixenosis) it caused. The aerosol method used to coat leaves produced a uniform and reproducible film of each compound on the target leaf surface (mean concentration = 8.2 μmol/g fresh wt. ±5.8%). This concentration represented only 20.5% of the total volume sprayed, the remainder being lost in the air or deposited on the paper mask. There was no significant damage to leaf tissue from the acetone carrier since the acetone completely evaporated within a few seconds after application. The half-life of phenolic acids on leaf surfaces was approximately 12 h at concentrations used in this experiment, based on the salicylic acid tests.

Results of all preference tests are shown in Figs. 3 and 4. Some simple phenolic acids deterred feeding by the MBB on treated bean leaves ($PI << 1.0$) while others elicited a mildly excitatory effect ($PI > 1.0$). Effects varied with structure and substitution groups. The most pronounced effect was associated with location of hydroxyl groups on the ring. A very strong deterrent effect ($PI < 0.5$) was produced by compounds with hydroxyl groups in the 2 or 6 position, adjacent to the acid moiety. Thus, effective feeding deterrents were 2, 6-dihydroxy benzoic acid, 2, 3-dihydroxy benzoic acid, 2-hydroxy benzoic acid (salicylic acid), 2,5-dihydroxy benzoic acid (gentisic acid), and 2-hydroxy cinnamic acid.

Other factors had little effect on feeding. Increasing the number of hydroxyl groups did not increase deterrency except for 2,6-dihydroxy benzoic acid, which had two hydroxyl groups both adjacent to the acid moiety. For instance, 3,4,5-

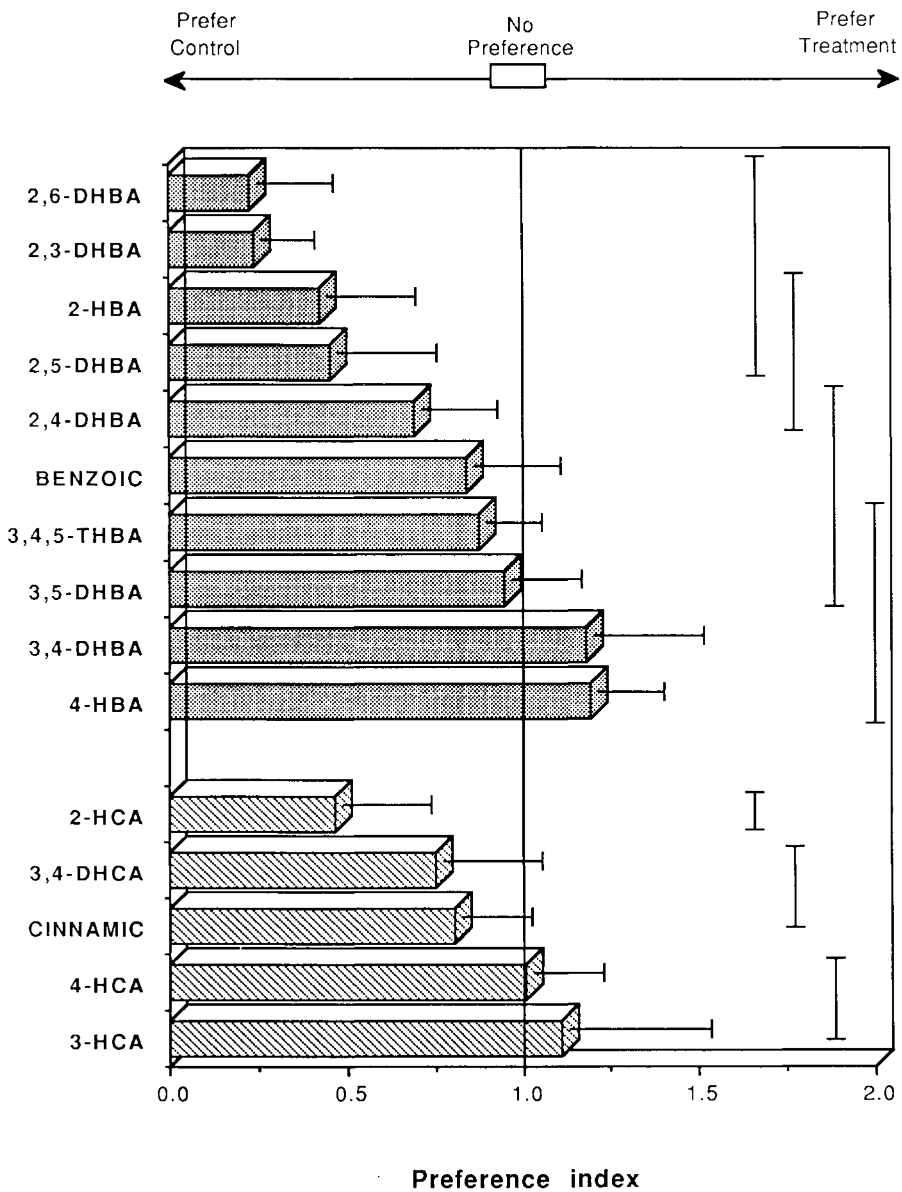


Fig. 3. Preference indices for phenolic acids with hydroxyl substitutions only. Upper, dark bars indicate benzoic acid based compounds. Lower lighter bars indicate cinnamic acid based compounds. Vertical bars indicate groups of compounds whose PI's were not significantly different (ANOVA and Scheffes f-test, $p < 0.05$).

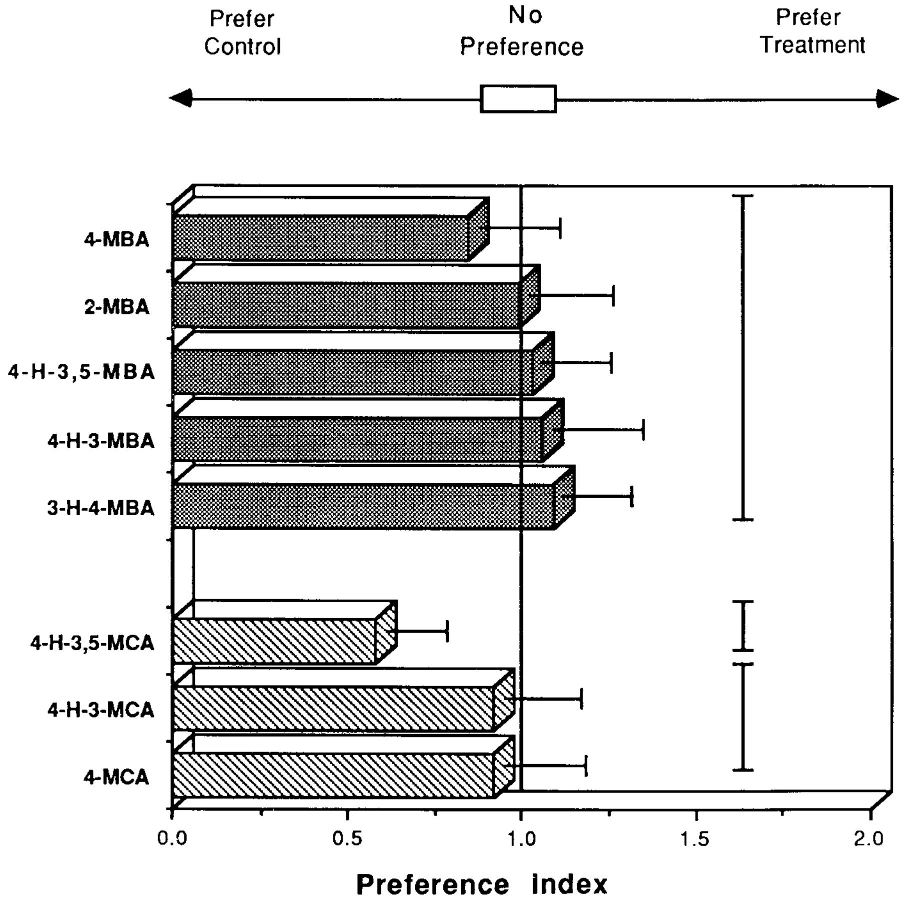


Fig. 4. Preference indices for phenolic acids with methoxyl substitutions. Upper, dark bars indicate benzoic acid based compounds. Lower, lighter bars indicate cinnamic acid-based compounds. Vertical bars indicate groups of compounds whose PI's were not significantly different (ANOVA and Scheffes f-test, $p < 0.05$).

hydroxy benzoic acid (gallic acid) was very near neutral (PI = 0.91); in fact, in some runs of the test it induced a mild feeding excitation (PI > 1.0) even though it has three hydroxyls. Methoxy groups on the ring, no matter what their position, did not affect feeding; for example, PI = 0.99 for 2-methoxy benzoic acid as compared with 0.42 for 2-hydroxy benzoic acid. Whether compounds were benzoic or cinnamic acid derivatives had no effect on feeding preference, and both cinnamic acid and benzoic acid themselves were weak deterrents.

Ten of the compounds tested occur naturally in soybean leaves at a combined concentration of approximately 1.0 μmol/g fresh weight (Hardin, 1979; Porter et al., 1986). Of these, 2-hydroxy benzoic and 2,5-dihydroxy benzoic acid (salicylic and gentisic acids, respectively) were found in our tests to be feeding deterrents in the free form to adult beetles. Our intent was to test the specific effects of individual compounds. Thus, we replaced the total concentration of all ten soybean phenolic acids with the equivalent amount of a single compound. Resulting treatment levels for individual compounds were 10 times higher than recorded total phenolic acid concentration in soybean leaves (Hardin, 1979). This additional tenfold increase was chosen for two reasons: (1) to compensate for the rate of disappearance of the compounds from the leaf surface within the duration of each experiment (half life 12h) and, (2) to override the effect of natural feeding excitants in common bean leaves. Common bean is the most preferred host of the MBB (Kogan 1972) probably because it contains effective levels of feeding excitants.

Extension of the findings from this series of experiments to soybean resistance to insect feeding is impossible because of the elevated concentrations tested. However, the results do yield useful information about the structure/activity relationships of simple phenolic acids for an oligophagous species of Coleoptera closely associated with grain legumes in North America.

Acknowledgments

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References Cited

Caballero, P., C. M. Smith, F. R. Fronczec, and N. H. Fischer. 1986. Isoflavones from an insect-resistant variety of soybean and the molecular structure of afrormosin. J. Nat. Prod. 49: 1126-1129.

Chiang, H. S., D. M. Norris, A. Ciepiela, A. Oosterwyk and P. Shapiro. 1987. Inducible versus constitutive PI 227687 soybean resistance to Mexican bean beetle, *Epilachna varivestis*. J. Chem. Ecol. 13: 741-749.

Cole, R. M. 1984. Phenolic acids associated with the resistance of lettuce cultivars to the lettuce root aphid. Ann. Appl. Biol. 105: 129-145.

Floss, H. G. 1977. The Shikimate Pathway. In recent advances in phytochemistry vol. 12, Biochemistry of Plant Phenolics (T. Swain, J. B. Harborne and C. F. Van Sumere, eds). Pleum Press. New York 651 pp.

Hardin, J. M. 1979. Phenolic acids of soybeans resistant and nonresistant to leaf feeding larvae. Masters Thesis, University of Arkansas, Fayetteville, Arkansas.

Hart, S. V., M. Kogan, and J. Paxton. 1983. Effect of soybean phytoalexins on the herbivorous insects Mexican bean beetle and soybean looper. J. Chem. Ecol. 9: 657-672.

Huang, H. M., G. L. Johanning, and B. L. Odell. 1986. Phenolic-acid content of food plants and possible nutritional implications. J. Agric. Food Chem. 34: 48-51.

Ingham, J. L. 1982. Phytoalexins of the Leguminosae. *In* Phytoalexins (J. A. Bailey. & J. W. Mansfield, eds.) John Wiley, New York. 334 pp.

Kogan, M. 1972. Feeding and nutrition of insects associated with soybeans. 2. Soybean resistance and host preference of the Mexican bean beetle, *Epilachna varivestis*. Ann. Entomol. Soc. Am. 65: 675-683.

Kogan, M. and J. Paxton. 1983. Natural inducers of plant resistance to insects. *In* Plant Resistance to Insects (P. A. Hedin, ed). Amer. Chem. Soc. Washington, D. C. 153 pp.

Leszczynski, B., J. Warchol, and S. Niraz. 1985. The influence of phenolic compounds on the preference of winter wheat cultivars by cereal aphids. Insect Sci. Applic. 6: 157-158.

Levin, D. A. 1971. Plant phenolics: An ecological perspective. Am. Nat. 105: 157-181.

Lwande, W., A. Hassanali, P. W. Njoroge, M. D. Bentley, F. DelleMonache, and J. I. Jondiko. 1985. A new 6a-hydroxypterocarpan with insect antifeedant and antifungal properties from the roots of *Tephrosia hildabrandtii* Vatke. Insect Sci. Applic. 6: 537-541.

Porter, P. M., W. L. Banwart, and J. J. Hassett. 1986. Phenolic acids and flavonoids in soybean root and leaf extracts. Environ. Exp. Bot. 26: 65-73.

Robinson, T. 1980. The organic constituents of higher plants Cordus Press, Amherst, Mass. 352 pp.

Russell, G. B., O. R. W. Sutherland, R. F. N. Hutchins, and P. E. Christmas. 1978. Vestitol: A phytoalexin with insect feeding-deterrent activity. J. Chem. Ecol. 4: 571-579.

Tahvanainen, J., R. Julkunen-Tiitto, and J. Kettunen. 1985. Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles. Oecologia 67: 52-56.

Todd, G. W., A. Getahun, and D. Cress. 1971. Resistance in barley to the greenbug, *Schizaphis graminum.* 1. Toxicity of phenolic and flavonoid compounds and related substances. Ann. Entomol. Soc. Am. 64: 718-722.

Woodhead, S. and E. A. Bernays. 1978. The chemical basis of resistance of *Sorghum bicolor* to attack by *Locusta migratorix.* Entomol. Exp. Appl. 24: 123-144.