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CONSTITUENTS OF SOYBEAN CULTIVARS DIFFERING IN INSECT RESISTANCE*

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Key Word Index—*Glycine max*; carbohydrate; sterols; organic acid; antibiosis.

Abstract—Leaf samples of two insect-resistant plant introductions (PI 227687 and PI 229358) and two susceptible (Ransom and Coker Hampton 266A) cultivars of soybeans, *Glycine max*, were analyzed at different growth stages for their contents of total nitrogen, carbohydrates, organic acids, and sterols. The two susceptible cultivars accumulated more total nitrogen and at a faster rate than did the two resistant plant introductions. At pod-filling, the two resistant cultivars had equivalent soluble carbohydrates and 33% more than the susceptible cultivars. The quantity of organic acids was essentially the same for the two susceptible cultivars. The resistant cultivars had distinctly different quantities from each other as well as from the susceptible cultivars. The quantity of total sterol of these soybean cultivars varied during the growth of the plant. The resistant cultivars accumulated sterol faster and by pod-filling contained from 20 to 50% more sterol than did the susceptible cultivars.

INTRODUCTION

The trend for combating phytophagous insects has shifted from the use of broad-spectrum insecticides to cultivating plant varieties that are highly resistant or immune to insect attack. Insecticides have a number of disadvantages; first, their effect is not specific; second, they are noxious to beneficial insects; third, massive applications have resulted in the emergence of resistant insects; and fourth, increasing restrictions are being placed on their continued use. Thus, a preferred method of preventing insect damage to agricultural crops would be to exploit the existing natural resistance in the plants. Pest populations could thereby be suppressed below the level of economic damage with no added pollution and no additional cost to the producer.

A wide range of natural resistance to insect damage exists in soybeans [1–5]. Several of the plant introduction cultivars express varying levels of resistance to a complex of insects [3–6]. One, PI 229358, exhibits a high level of resistance under intense population pressure to several insects, and this resistance approaches immunity in the case of the Mexican bean bettle, *Epilachna varivestis* Mulsant [7]. A high level of antibiosis was observed when the beetle consumed the resistant cultivar [8]. The symptoms were similar to starvation and mild toxication. Larvae did not complete metamorphosis, which suggests interference with the proper functioning of the insect hormones. The insect-moulting hormone, ecdysterone, is synthesized by the insects from their dietary plant sterols. In addition, dozens of insect-moulting hormones have been isolated from a wide variety of plants [9, 10]. These observations suggest that resistant soybean cultivars could have a chemical composition different from that of susceptible cultivars.

The objective of the present study was to analyze resistant and susceptible cultivars for plant chemical constituents that serve as nutrients for insects. If resistance could be related to a specific compound or class of compounds, then the breeder would have a tool for selecting for resistance at an accelerated rate.

RESULTS AND DISCUSSION

The characteristic patterns for total nitrogen in the leaves of the four cultivars during the entire growing season are shown in Fig. 1. The two susceptible cultivars accumulated more total nitrogen and faster than did the two resistant plant introductions. After flowering, the

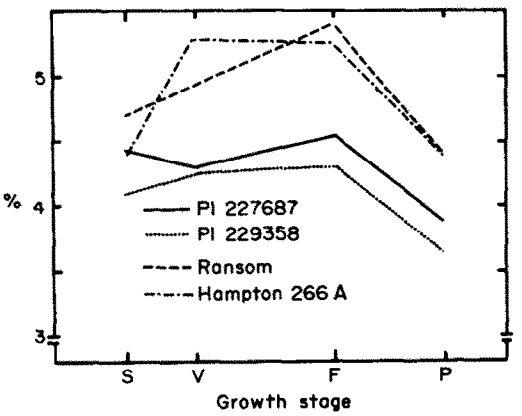

Fig. 1. Total nitrogen as percent dry weight of leaves of four cultivars of soybeans at four stages of growth. S = seedling, V = vegetative; F = flowering; and P = pod filling.

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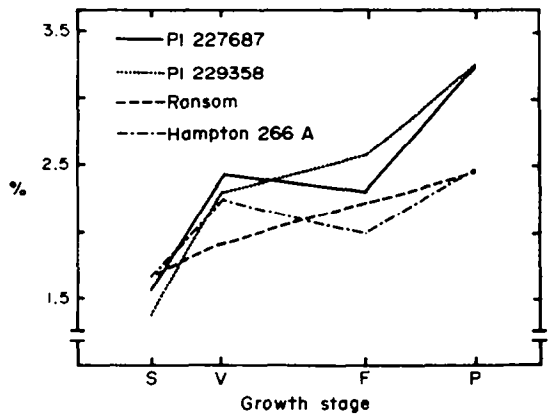

Fig. 2. Soluble carbohydrates as percent dry weight of leaves of four cultivars of soybeans at four stages of growth. S = seedling; V = vegetative; F = flowering; and P = pod filling.

percent nitrogen in leaves dropped as nitrogenous materials were redistributed to the developing pods. The plant introductions used in this study have a very limited agronomic potential [1]. The greater concentration of nitrogen in the susceptible cultivars may be related to their greater yields and higher protein content of the bean. In addition, the susceptible cultivars contained about 25% more soluble proteins and amino acids than the resistant cultivars (unpublished results). These extracted proteins and amino acids should be bioassayed to determine if the resistant cultivars are nutritionally deficient.

Soluble carbohydrates (starch plus soluble sugars) extracted in a 30% EtOH solution are shown in Fig. 2. The differences were not significant for the seedling and vegetative stages for three of the cultivars. Ransom accumulated carbohydrates at a constant rate, and contained significantly less carbohydrates than the other three cultivars at the vegetative stage. The contents for PI 227687 and Coker Hampton 266A decreased between the vegetative and flowering stages but increased substantially by the pod-filling stage. At pod filling, the two resistant cultivars had a 33% greater carbohydrate content

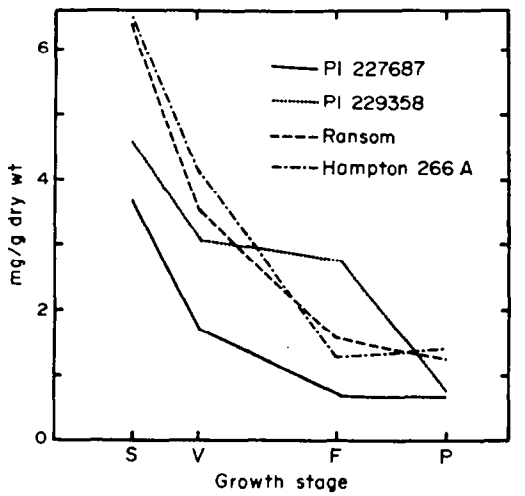

Fig. 3. Summation of malonic, fumaric, and succinic acids of leaves of four cultivars of soybeans at four stages of growth. S = seedling; V = vegetative; F = flowering; and P = pod filling.

than the susceptible cultivars ($p < 0.001$). Sucrose is the primary arrestant (chemical serving as effective phagostimulant and thereby maintaining prolonged feeding by the pest) for the Mexican bean beetle [11, 12]. In the present study, carbohydrates were not fractionated into the individual sugars. Sucrose and its hexose components could have been greater in the susceptible cultivars, but this awaits confirmation. Alternatively, it is known that superoptimal concentrations of certain otherwise attractive chemicals are avoided by insects [12]. If sucrose is superoptimal in the resistant cultivars, this could possibly contribute to their mode of resistance. The individual extracted sugars must be identified and subjected to bioassay before meaningful interpretations can be made.

The methylated organic acids were identified and quantified by GLC. The combined techniques for extraction and analysis gave superior resolutions with minimal interference by other substances. The values shown in Fig. 3 are the summation of malonic, fumaric, and succinic acids. These acids fluctuated during plant growth, but each acid gave the same type distribution curve. The two susceptible cultivars had essentially the same distribution, which was quite distinct from that of the resistant cultivars. The other acids such as malate, isocitrate, aconitate, and citrate varied during the growing season but were essentially the same for all four cultivars (unpublished data).

Solubility of the acids changed drastically at flowering and pod-filling; less than 5% were extractable by traditional means [13]. The leaf sample had to be exposed to 6N HCl to solubilize the acids. Whether they had become the salts of calcium or insoluble organic constituents was not determined.

One other acid varied dramatically between the resistant and susceptible cultivars. It eluted in the area of quinic acid. Its identity was not verified, but Clark [13] indicated its presence in soybeans. The alkaloid, quinine, and its many related compounds occur as salts of quinic acid. Alkaloids have been shown to be one of the primary group of chemicals involved in feeding and ovipositional stimulation and deterrence [14].

The total sterol, as determined by the digitonin procedure, in the leaves of these four soybean cultivars varied with growth stage (Fig. 4). Cultivars susceptible to Mexican

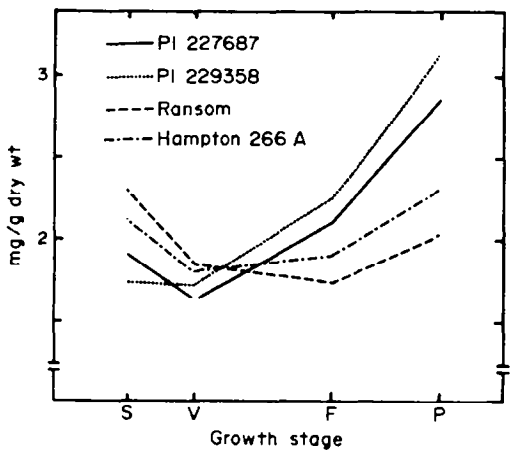

Fig. 4. Total sterol of leaves of four cultivars of soybeans at four stages of growth. S = seedling; V = vegetative; F = flowering; and P = pod filling.

bean beetle damage contained greater quantities of sterol during the seedling and vegetative growth stages than did the resistant cultivars. However, before flowering, the resistant cultivars accumulated sterol faster and by pod-filling contained from 20–50% more sterol than did the susceptible cultivars. These differences were significant ($p < 0.05$) at seedling and flowering and ($p < 0.01$) at pod-filling.

Insect-moulting hormones (derived from plant sterols) often occur in greater quantities in plants than they do in insects, and the plant compounds often exhibit more potent activity than those native to insects. Ajugalactone, a hormone analog, inhibits insect metamorphosis [15]. A plant sterol, nicandrenone, recently isolated from a Peruvian weed repels insects [16].

The juvenile hormone, in the presence of the moulting hormone prevents morphogenetic changes associated with metamorphosis. If later instar larvae are exposed to substances with juvenile hormone activity, they can not undergo further transformation into adults, resulting in the formation of supernumerary larvae that do not survive [17].

In one study [18], mortality of *Heliothis zea* (Boddie) larvae feeding on PI 229358 was 100% compared to 25% for those feeding on susceptible cultivars. Other expressions of antibiosis evident in the larvae included prolonged moulting, reduced weight, and prolonged development. In addition, the plant introduction cultivars elicited a high level of antibiosis to *H. virescens* (F) larvae [19]. These observations suggest that the mode of multiple insect resistance in these plant introductions may result from the presence of juvenile hormone analogs. Specific extracts from these resistant plant introduction cultivars should be subjected to bioassay to determine whether the higher level of antibiosis and larvae mortality results from either juvenile hormone analogs or other inhibitory compounds. A detailed analysis of specific compounds is prerequisite to identifying the basis for insect resistance in soybeans.

EXPERIMENTAL

Leaf samples of two resistant (PI 227687 and PI 229358) and two susceptible (Ransom and Coker Hampton 266A) cultivars [1] of soybeans were analyzed at different stages of physiological development for their content of total N, carbohydrates, organic acids, and sterol. The four cultivars were field-grown in four replicated plots and sampled at four growth stages: seedling two susceptible (Ransom and Coker Hampton 266A) cultivars tative (44 days after seeding), flowering (50% of the flowers opened), and pod-filling (pods approximately 50% mature). The penultimate fully-expanded trifoliate leaves of 100 plants of each cultivar were harvested at each sampling. Leaf samples (harvested at the same time each date to avoid diurnal phenomenon) were frozen in liquid N_2 and lyophilized to constant wt before being reduced to 60-mesh material with a Wiley mill.* The results reported are the means of four replicate determinations. Differences between the reported values were statistically analyzed by a *t*-test for significance and reported where appropriate. Total N was determined by a micro-Kjeldahl procedure [20], The samples were analyzed for soluble carbohydrates (starch plus soluble sugars) extracted in a 30% EtOH soln by an anthrone procedure [21]. Organic acids were extracted and analyzed by the procedure of Clark [13] except that Me derivatives were prepared with dry MeOH and HCl as catalyst. A modified extraction procedure was discussed under Results. The total sterol content of the moisture-free samples was determined by the method of Keller [22]. This procedure hydrolyzed both sterol esters and sterol glycosides and gave the total Me_2CO-soluble sterol present in the samples.

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