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STEROLS OF SOYBEANS DIFFERING IN INSECT RESISTANCE AND MATURITY GROUP

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Key Word Index—*Glycine max*; sitosterol; stigmasterol; campesterol; host plant resistance; plant maturity.

Abstract—Eight soybean varieties and lines representing insect-susceptible and -resistant genotypes, and differing in plant maturity group, showed no difference in leaf dry wt. Over the growing season the total and bound sterols increased while the free sterols decreased. Sitosterol, stigmasterol and campesterol were the major sterols. Over the season sitosterol increased while stigmasterol decreased. No difference due to insect resistance could be established but early maturing plants showed a larger change in sterols.

INTRODUCTION

High levels of soybean resistance to the Mexican bean beetle, *Epilachna varivestis* Mulsant, were found in plant introductions (PIs) 171451, 227687 and 229358 [1]. Resistance to other foliage-feeding insect species was later detected in these same lines [2]. Moderate levels of resistance occur also in other lines and a range of resistance levels was determined by paired comparison preference and by weight gain tests [3,4]. The resistance mechanisms seem to be complex and there is evidence that both antixenotic and antibiotic factors are involved [4]. When the Mexican bean beatle is offered a choice between a resistant PI and a susceptible variety, the resistant PI is rejected. When forced to feed on the resistant lines, larvae grow very slowly and seldom complete their development.

The chemical factors of resistance in soybean have not been totally elucidated. However, it seems that both primary and secondary metabolites are involved. Nutritional imbalances may account for some of the abnormal development that has been observed. Recently an inositol analog, pinatol (3-*O*-methylchiroinositol), was reported as a possible resistance factor [5], with some antibiotic effect.

The Mexican bean beetle, like all phytophagous insects, lacks the ability for *de novo* synthesis of the steroid skeleton. Thus, for normal growth the beetle requires exogenous sterols to produce the moulting hormone, ecdysone, and also the structural steroidal complexes [6,7]. In a recent investigation, the total sterol content was determined for two susceptible and two resistant soybean cultivars at different stages of physiological development [8]. The cultivars susceptible to Mexican bean beetle attack contained larger quantities of sterol during the seedling and vegetative growth stages than did the resistant lines. At the flowering stage, however, the resistant lines had larger quantities of sterol, and at the pod-filling stage the resistant soybeans contained 20–50% more sterols than the susceptible cultivars. Tester [8] did not attempt to follow the individual sterols during physiological development of the various soybean cultivars.

The objective of the present study was to use a set of four resistant and four susceptible soybean lines and varieties for analysis of individual sterols over the growing season. Since the importance of physiological age as to sterol composition had been demonstrated [9–11], both early maturing and late maturing soybean genotypes were included.

RESULTS AND DISCUSSION

The eight soybean varieties and lines which were used in this experiment represented insect-susceptible and -resistant genotypes within maturity groups from II–IV (early) to VII–VIII (late) (Table 1). For comparison, Table 1 also gives the origin and parentage of the various genotypes.

The early maturing soybeans reached full bloom on 28 July while the late maturing soybeans attained this physiological stage 1 week later. Pod set by the early maturing plants occurred during the last week of August, while the late maturing plants started to set pods during the second week of September. Pod set was the last sampling date. The early maturing developed mature seeds but because of the latitude of the experimental site the late maturing soybeans did not develop mature seeds before the first frost. The per cent of leaf moisture for all eight soybean genotypes at any particular harvest date was very similar; however, it decreased slightly with harvest date. Leaf moisture was 77–79% before bloom and dropped to 73–76% at pod set, and no difference could be established for either maturity group or insect resistance.

The total, free and bound sterol levels of early and late maturing, and resistant and susceptible plants are given in Fig. 1. In general, the total sterol content of all eight lines increased with plant age which is in agreement with previously published results [10, 11]; however, the early maturing soybeans showed a significantly larger increase

Table 1. Soybean varieties and lines used in the comparative analysis of sterols

Variety or line	Resistance to insect attack	Maturity group	Origin or parentage
68692-2	Susceptible	Early (III)	Manchuria
Clark 63	Susceptible	Early (IV)	(Clark[7] × CNS) × (Clark[6] × Blackhawk)
68778	Resistant	Early (II)	Manchuria
54615	Resistant	Early (III)	Manchuria
Bragg	Susceptible	Late (VII)	Jackson × D49-2491
274507	Susceptible	Late (VIII)	Taiwan
171451	Resistant	Late (VII)	Japan
229358	Resistant	Late (VII)	Japan

in total sterol content, especially at pod set. In the present investigation, whenever late or early maturing plants are compared separately, the insect-resistant lines and varieties showed the same total sterol level as the susceptible genotype. This is contrary to Tester's [8] observation. He found a higher sterol level in the insect-resistant genotypes, especially during later stages of plant development.

The early maturing soybeans, at the 27 August harvest date, had set pods while the late maturing soybeans, at the 15 September harvest date, had just started to set pods and physiologically were not as far advanced. This difference in plant maturity may account partly for the difference in total sterols but certainly not for the total difference. Comparison of leaf maturity is not a simple process since the early maturing soybeans have indeterminate growth while the later maturing have a determinate pattern. Thus, the early maturing plants continued to produce new leaves longer than the late maturing. Since the chemical analysis was performed on leaves three through five from the top, the leaves from the early maturing plants were chronologically younger, even though the plants are physiologically more mature. This difference in chronological and physiological age is most pronounced late in the growing season. Thus, at the final harvest date the leaves of the late genotypes were significantly more mature than the leaves from the early genotypes. Looking at the sterol content, however, the physiologically younger leaves from the early maturing plants had higher levels. Thus, while leaf age and sterol level are directly related, the maturation rate of the plant also appears to control the sterol level. This point is further demonstrated if one considers the early maturing plants only. Soybean line 68778 belongs to maturity group II and has a higher sterol level than Clark 63 which belongs to maturity group IV. But lines 68692-2 and 54615, both belonging to maturity group III, had sterol values as high as 68778 and as low as Clark 63, respectively, and thus did not follow the pattern in absolute terms.

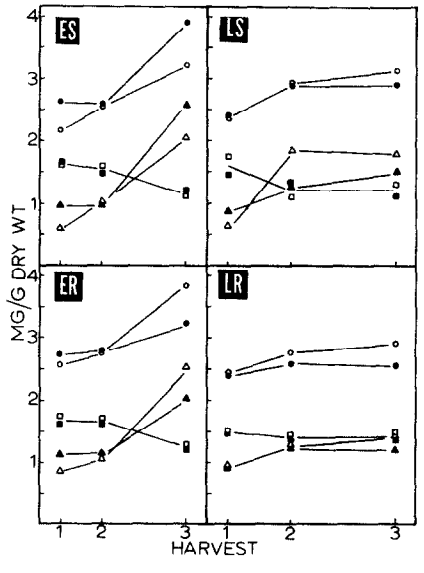

Fig. 1. Changes in total (circles), free (squares), and bound (triangles) sterol over the growing season of eight soybean genotypes differing in insect resistance and maturity group. ES: Early maturing, insect-susceptible symbols, open Clark, closed 68692-2. LS: Late maturing, insect-susceptible symbols, open Bragg, closed 274507. ER: Early maturing, insect-resistant symbols, open 68778, closed 54615. LR: Late maturing, insect-resistant symbols, open 229358, closed 171451.

The free and bound sterol change over the growing season of all eight soybean lines showed a very similar pattern, that is, the free sterols decreased while the bound sterols increased (Fig. 1). No differences could be established for insect-resistant and -susceptible soybeans, but again the early maturing plants showed a more dramatic change in sterol. Certainly resistance to insect attack cannot be explained on the basis of low levels of free sterols even though the free sterol content decreased with plant maturity. This latter point is contrary to previous experiments with *Solanum andigena* [12] and *Nicotiana tabacum* [10, 11] in which it was found that the free sterols increased with tissue maturity. The increase in bound sterols occurred late in the growing season with the early maturing soybeans but with the late maturing the increase occurred early. A conversion of free sterol to steryl ester and steryl glycoside can account for part of the increase in bound sterol but certainly not for all.

The individual sterol composition of the free and bound sterols is given in Figs. 2 and 3, respectively. As previously

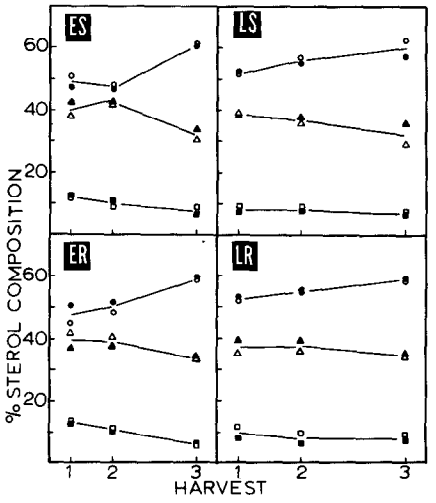

Fig. 2. Changes in free sitosterol (circles), free stigmasterol (triangles), and free campesterol (squares) over the growing season of eight soybean genotypes differing in insect resistance and maturity. ES: Early maturing, insect-susceptible symbols, open Clark, closed 68692-2. LS: Late maturing, insect-susceptible symbols, open Bragg, closed 274507. ER: Early maturing, insect-resistant symbols, open 68778, closed 54615. LR: Late maturing, insect-resistant symbols, open 229358, closed 171451.

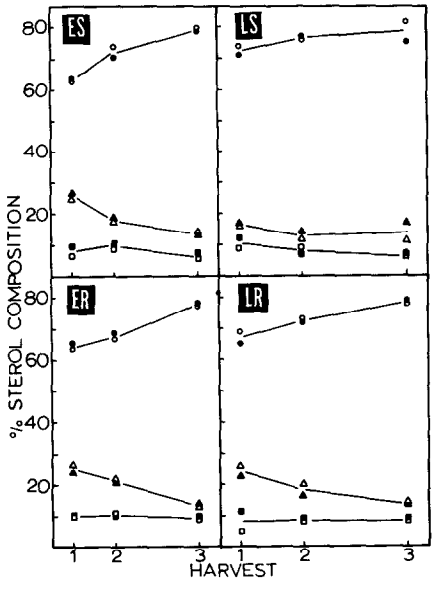

Fig. 3. Changes in bound sitosterol (circles), bound stigmasterol (triangles), and bound campesterol (squares) over the growing season of eight soybean genotypes differing in insect resistance and maturity. ES: Early maturing, insect-susceptible symbols, open Clark, closed 68692-2. LS: Late maturing, insect-susceptible symbols, open Bragg, closed 274507. ER: Early maturing, insect-resistant symbols, open 68778, closed 54615. LR: Late maturing, insect-resistant symbols, open 229358, closed 171451.

reported [13], soybean has three major 4-demethyl sterols (sitosterol, stigmasterol, campesterol). Sitosterol was the most dominant sterol both in the free and bound fraction. In the free fraction before bloom, sitosterol accounted for 50% of the sterols and increased to *ca* 60% at pod set. This general increase in sitosterol occurred in all eight lines irrespective of insect resistance or maturity group. In the bound sterol fraction, sitosterol increased from *ca* 65% before bloom to *ca* 80% at pod set. Stigmasterol, the second most dominant sterol, decreased from *ca* 40% of free sterols before bloom to *ca* 30% at pod set, and from *ca* 30% of bound sterols before bloom to 15% at pod set. Free campesterol also decreased in all eight lines over the growing season. For all sterols the general trend was the same whether or not the soybean variety or line was classified as resistant to insect attack or differing in maturity group. The observed changes in sterol composition with maturity agree with those found for *S. andigena* [12] but disagree with those found for *N. tabacum* [10, 11]. In the leaves of *N. tabacum*, sitosterol was the major sterol but it decreased with ageing while stigmasterol increased. At present these inconsistencies are difficult to explain and it is hard to believe that species difference is the only factor. While it is known that insects for normal growth require a dietary or exogenous source of sterols [7], the present data clearly demonstrate that resistance to insect attack is not due to a sterol imbalance or unusual sterol make-up of the soybean leaf. Surprisingly all eight soybean lines, even though they differed in origin or parentage (Table 1), when grown under similar conditions, showed similar changes in sterol composition over the growing season. The early maturing lines in general showed a larger response but in no way different from the later maturing lines.

EXPERIMENTAL

Plant material. Eight soybean (*Glycine max* (L.) Merrill) varieties ('Clark 63', 'Bragg') and lines (PIs 68692-2, 54615, 68778, 274507, 171451, 229358) were planted at the University of Illinois Vegetable Crops Farm, 40°5'N latitude, 88°15'W longitude, in 3-row plots of 10 m each. Each plant type was replicated 3 × in a randomized block design. Leaves three to five from the top were harvested in the early morning hr of 13 and 28 July and 27 August when the plants in early maturing groups were at full vegetation growth before bloom, at full bloom, and at pod set, respectively. Later maturing plants were harvested on 13 July, 5 August, and 15 September when these plants had reached similar physiological stages. At each harvest date one-third of each row (3.3 m) was harvested in a Latin square pattern to avoid possible changes in plant development due to defoliation. The harvested leaves were frozen in liquid N_2 in the field, placed in plastic air-tight containers and transferred to a freezer maintained at −65°. All samples were well mixed before any aliquots were removed for chemical analysis. Dry wt was determined by bringing two 1 g aliquots of each replication to constant wt at 100°.

Sterol analysis. A 10 g leaf sample was homogenized in 100 ml Me_2CO and extracted in a Soxhlet apparatus for 24 hr. A known amount of cholesterol was added as int. standard. The extract was taken to dryness, redissolved in 95% EtOH and one-half of the sample was removed for free sterol determination. The remaining fraction was used for total sterol analysis. Hydrolysis was with 0.5% H_2SO_4 in 95% EtOH for 12 hr, followed by saponification for 30 min in 5% KOH in 95% EtOH. The mixture was neutralized, filtered, extracted 3 × with hexane,

dried and redissolved in 95% EtOH. The sterols were precipitated with digitonin in 80% aq. EtOH. The ppt. was washed 3× with 80% aq. EtOH, 3× with Et_2O and dried. The sterol–digitonide was dissolved in 3 ml pyridine, heated at 100° for 30 min and subsequently left at room temp. for 12 hr. The digitonin was precipitated with 10 ml Et_2O. The Et_2O was dried and redissolved in EtOAc for GLC analysis [14]. The GLC was equipped with a FID connected to an electronic integrator. The column was glass, 1.80 m × 4 mm, packed with 5% OV-101. Column temp. isothermic 245°, with injector and detector temp. at 300°, carrier gas He at 80 ml/min [15]. Corrections were made for differences in detector response.

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