INORGANIC NUTRIENT ANALYSIS OF LEAF TISSUE FROM SOYBEAN LINES SCREENED FOR MEXICAN BEAN BEETLE RESISTANCE[1]

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ABSTRACT

Leaf tissue of 49 F_5 soybean [*Glycine max* (L). Merr.] lines derived from a cross between an experimental line, MBB 80-115, and the cultivar 'Ware' were fed to Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant, larvae in laboratory studies. The parents, Ware and MBB 80-115, and PI 171451 which was the original source of MBB resistance were included as checks in all studies. Laboratory tests suggested the presence of non-preference (antixenosis) and/or antibiosis in the resistant soybean genotypes and were efficient in identifying most resistant lines and mechanisms of resistance. The larvae reared on leaves of lines 119-2 and 119-11 took longer to pupate and reach the adult stage and had fewer numbers of emerging adults. Leaf tissue of selected lines was analyzed for the elements phosphorus, calcium, potassium, magnesium, and silicon to determine if any of these elements are associated with the feeding response and might serve as a quick index of selection for plant resistance. Calcium, phosphorus, and potassium content was each negatively correlated with pupal weight ($r = -0.23^{**}, -0.26^{**}, -0.20^{*}$, respectively). No significant correlations were observed between silicon and magnesium content and pupal weight. The progeny line 119-2 which demonstrated a high level of antibiosis had a higher calcium, phosphorus, and magnesium content than either parent or the resistant PI 171451 and also had a potassium content that was similar to that of the resistant PI. PI 171451 had the lowest silicon content of all lines tested.

Key Words: *Glycine max*, plant resistance, *Epilachna varivestis*, *plant nutrients*.

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INTRODUCTION

The Mexican bean beetle (MBB) *Epilachna varivestis* Mulsant, is an important defoliator of soybean, *Glycine max* (L.) Merr., in the eastern United States and portions of the midwest (Mellors et al. 1984). Sources of resistance have been identified (Van Duyn et al. 1971) and field or laboratory screening methods are effective in identifying resistant lines (Elden et al. 1974).

Several workers have investigated the chemical basis of insect resistance in soybean seeds and leaves, but a direct association between specific chemcials and resistance remains to be elucidated. Early reports by Van Duyn et al. (1971) and Kogan (1972) suggested that resistant genotypes may have less protein and reducing sugars in their foliage, but no clear associations could be established.

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Tester (1977) reported that resistant cultivars had lower nitrogen content, a larger amount of carbohydrates, and at flowering and pod fill more total sterols. Subsequent studies by Grunwald and Kogan (1981) on sterol composition of insect susceptible and resistant-soybean cultivars and lines indicated that resistance was not due to a sterol imbalance or unusual sterol content of the leaf. Other have looked at phytoalexins (Hart et al. 1983) and leaf extracts (Smith et al. 1979) for possible feeding deterrents or stimulants of MBB feeding.

Initial interest in the present study arose following the report by Johnson and Campbell (1982) that feeding damage of the twospotted spider mite, *Tetranychus urticae* Koch, on peanut, *Arachis hypogaea* L., was negatively correlated with calcium content of the leaves. No information on the effects of foliar nutrient concentrations in soybeans on MBB feeding was found in the literature. The objective of this study was to determine the calcium, phosphorus, potassium, magnesium, and silicon content of foliage from soybean breeding lines exhibiting a range in levels of resistance to the MBB to determine if a relationship exists between these elements and feeding reaction by the MBB. The identification of such a relationship could provide a useful selection criterion for MBB resistance.

MATERIALS AND METHODS

During the summer of 1980, a cross was made between the experimental line, MBB 80-115, and the cultivar 'Ware'. The experimental line was selected in Maryland from the cross 'Williams' × PI 171451 and exhibited moderate resistance to feeding by the MBB in previous field evaluations. The F_1 seeds were planted in Puerto Rico during the winter of 1980-1981 and the resulting F_2 seeds were grown in the field in the summer of 1981. In 1981, 87 F_2 plants were visually selected for desirable agronomic traits and harvested separately. A cage screening technique (Elden and Paz 1977) was used in 1982 to classify the 87 F_3 lines for reaction to MBB foliar feeding. Of the 87 lines, 50 lines which showed low leaf damage ratings and/or good seed yields and agronomic traits were selected for further evaluation. In 1983 these 50 F_4 lines were tested for seed yield and agronomic traits in row plots and in field cages for reaction to MBB feeding damage.

Seeds from plots of 49 of the F_4 lines evaluated in 1983 in the field, along with the parents and the resistant PI 171451 were planted in the greenhouse in 25-cm diameter pots at a rate of 6 seeds per pot, for use in laboratory screening tests. After germination, the pots were thinned to 4 plants per pot. At flowering, one soybean plant with good foliage growth was selected in each pot for use in the MBB larval feeding test. Leaflets of uniform size and age from the basal third trifoliolate upward were clipped from the selected plants, placed into plastic bags to maintain moisture, and brought into the laboratory.

A no-choice feeding procedure was used and larval development was monitored. The tests were conducted in plastic petri dishes 150 mm in diameter and 20 mm in depth. A disk of Whatman number 12 filter paper, which was uniformly saturated with water, was placed at the bottom of each petri dish and kept moist throughout the testing period. One leaflet from each soybean genotype was placed abaxial side up in individual dishes. Six second instar MBB larvae which had been reared on bush lima (*Phaseolus lunatus* L.) beans were transferred onto the exposed surface of the leaflet using a soft camel's hair brush. The petri dishes were covered with a perforated lid and placed in a growth chamber maintained at

27°C and 60% RH receiving 15 hours of light daily. Leaves were changed every 72 hours during the first 14 days of MBB development and every 24 hours thereafter until the larvae reached the pupal stage.

To determine the amount of larval leaf feeding and the effects of plant genotype on larval development, the following observations were taken: leaf damage rating, larval mortality, larval and pupal weights, duration of MBB developmental stages, and total number of larvae reaching each stage. The leaf damage rating was a visual rating based on a scale of 1 to 5, with 1 the least damaged and 5 the most damaged. The rating was taken as leaves were replaced in the petri dish. Larval mortality was an actual count of larvae which died before reaching the pupal stage. The duration of developmental stages was recorded as the number of days required for second instar larva to reach pupal and adult stages. Larvae were weighed 14 days after the test commenced and pupae were weighed 4 days after pupation. The experiment was a randomized complete block design with three replications of each of the 52 genotypes.

Foliage from the plants in each of the three replications used in the larval feeding test were also used for quantitative analyses of selected chemical constituents. Six leaflets per genotype were taken from trifoliolates three to five numbered from the apex (Smith 1985) approximately at the beginning of the pod developmental (R3) stage (Fehr et al. 1971). Leaf samples were oven dried at 70°C for 72 hours and ground in a Wiley Mill using a 1 mm sieve. Before weighing, the ground samples were again oven dried at 60°C for 24 hours to remove any moisture gained from atmospheric humidity. A 330 mg sample was transferred to a 100 ml kjeldahl flask. Three ml of concentrated HNO_3 were added to the flask, followed by 3 ml of 70% $HCLO_4$. The sample was heated on a micro-kjeldahl apparatus using a rotary digester until no traces of organic residue remained. The flask was then allowed to cool for 2 to 3 minutes followed by the addition of 5 ml of distilled water.

A 20 ml sample of this solution was then analyzed by the Soil Testing Laboratory, Department of Agronomy, University of Maryland for calcium, phosphorus, magnesium, and potassium concentration, using a Technicon Autoanalyzer. Observations were recorded as $\mu g\ g^{-1}$ leaf dry matter.

The silicon content of each of the genotypes was also determined. After digestion, 5 ml of distilled water were added to the flasks which were allowed to cool to room temperature. Flasks were reheated to allow the precipitated silicon to disperse. Samples were then transferred to a 50 ml plastic volumetric flask. Each digestion flask was then rinsed three times with distilled water and allowed to cool. Samples in the volumetric flasks were mixed well and transferred into plastic vials. The silicon content of the samples was determined by using an atomic absorption (AA) machine, Perkin-Elmer Model 5000. The data were recorded as $\mu g\ g^{-1}$ dried leaf tissue. All experiments were analyzed using the Statistical Analysis System (SAS). Mean separations were performed using the Least Significant Difference (LSD) test at the 5% level of significance.

RESULTS AND DISCUSSION

Significant differences in leaf damage in the larvae feeding test were observed among the genotypes (Table 1). Although there were no differences in leaf damage between the parent genotypes and PI 171451 which was the

Table 1. Mean MBB leaf damage and developmental stages of larvae reared on soybean leaves of the parents, the resistant PI, and 5 lines with the lowest and 5 lines with the highest leaf damage ratings.

			Pupae			Adults	
Entry	Leaf Damage	Surviving Larvae	Develop-ment	Total Survival	Weight	Develop-ment	Total Survival
	Score*	No.†	Days	No.	mg	Days	No.
PI 171451	2.5	3.7	23	2.7	20	32	3.0
MBB 80-115	2.8	4.0	20	3.7	33	28	3.0
Ware	2.8	3.3	20	2.7	32	28	2.0
119-2‡	1.6	1.7	27	1.0	27	36	1.0
118-10	1.8	3.3	25	1.7	30	34	1.7
126-8	2.0	3.7	20	2.7	30	30	2.0
119-11	2.1	2.0	25	2.0	28	32	1.0
121-5	2.3	3.0	22	2.7	30	31	2.3
119-13	2.7	3.7	21	2.0	33	30	1.3
123-7	2.7	3.7	22	2.3	33	30	2.0
124-5	3.2	4.3	21	4.0	32	28	2.3
121-1	3.2	5.3	22	5.0	31	30	3.0
120-12	3.2	4.3	23	4.3	32	30	3.7
LSD 0.05	0.9	2.0	4	1.9	6	4	1.9

* Score 1-5 Where 1 = least, and 5 = most damage.

† Infested with 6 larvae

‡ All numbered entries were drrived from the cross MBB 80-115 × Ware.

original source of resistance in this cross, the larval leaf damage of lines 119-2 and 118-10 was significantly lower than both parents. Leaf damage of 119-2 was also significantly lower than the resistant PI 171451.

The mean leaf damage rating of the 49 F_4 lines (2.7) was not significantly different from the mid-parent value (2.8). This was similar to results observed in previous field studies. Several lines demonstrated greater resistance to feeding damage in the laboratory than in the field. Leaf damage of lines 119-11 and 119-2 in the laboratory studies was similar to those of the preceding generation when tested in the field. The larvae reared on foliage of these lines took more days to pupate and reach the adult stage and had fewer numbers of emerging adults (Table 1). Hence, the laboratory tests suggest the presence of non-preference and/or antibiosis in several soybean genotypes tested. Conversely, the lines 124-5, 121-1, and 120-12 which demonstrated high levels of leaf damage in the laboratory screening test had moderate levels of leaf damage in the field study. The larvae reared on these lines had lower larval mortality and required fewer days to reach the pupal and adult stages. This indicated that these lines demonstrated a low level of non-preference and/or antibiosis.

In this study, the MBB-susceptible parent Ware had a similar leaf damage rating to that of the moderately resistant parent MBB 80-115. This differs from the results observed in three field trials where MBB 80-115 had significantly lower leaf damage than Ware. The dense leaf pubescence on greenhouse grown plants of the cultivar Ware precluded the use of first instar larvae in this study and may have been a factor in the overall reduction in leaf feeding on this cultivar.

Table 2. Simple linear correlation coefficients between MBB larvae leaf damage and insect developmental parameters.

	Surviving Larvae Total	Develop-ment Days to Pupae	Surviving Pupae Total	Pupae Weight	Develop-ment Days to Adult	Surviv-ing Adult Total
Leaf Damage Score	0.516**	-0.323**	0.496**	-0.133^{NS}	-0.257**	0.396*
Surviving Larvae Total		-0.284**	0.716**	0.058^{NS}	-0.294**	0.606*
Development Days to Pupae			-0.366**	-0.390**	0.653**	-0.329*
Surviving Pupae Total				0.166**	-0.381**	0.799*
Pupae Weight					-0.405**	0.160*
Development Days to Adult						-0.280*

NS, *, ** Correlation coefficient not significantly different or significantly different from zero at 0.05 and 0.01 level, respectively.

Table 3. Means of chemical analysis of soybean leaf tissue of the parents, the resistant PI, and 5 lines with the lowest and 5 lines with the highest leaf damage ratings.

Entry	Leaf damage	Calcium	Phosphorus	Potassium	Magnesium	Silicon
	Score*	----------μg g^{-1}leaf dry wt ----------				
PI 171451	2.5	18233	27867	21300	6037	1802
MBB 80-115	2.8	12267	20100	13633	4107	3303
Ware	2.8	13033	17100	13400	4887	2853
119-2†	1.6	21800	30433	19867	6860	3003
118-10	1.8	15577	19633	12167	4350	2553
126-8	2.0	15767	17667	13313	4450	3003
119-11	2.1	10900	18200	13100	3760	3003
121-5	2.3	15700	24200	17067	5263	3003
119-13	2.7	15267	15000	13633	4697	3103
123-7	2.7	10200	20167	15967	3427	1902
124-5	3.2	14567	26767	16633	4807	3904
121-1	3.2	14400	13533	11200	4943	3604
120-12	3.2	14533	24733	17200	4897	3003
LSD 0.05	0.9	1758	1268	2764	701	715
Mean of all 49 F_4 lines		13123	20119	15257	4777	2905

* Score: 1-5 where 1 = least, and 5 = most damage.
† All numbered entries were derived from the cross MBB 80-115 × Ware.

Significant negative correlations were observed between MBB larval leaf damage and both days to pupation and days to reach the adult stage (Table 2). Significant positive associations were detected between MBB larval leaf damage and number of surviving larvae, pupae and adults. These relationships concur with the results of Van Duyn et al. (1972) which demonstrated that marked non-preference reactions of MBB adult beetles existed when resistant soybean genotypes were used as the only food source. These reactions included reduced longevity and fecundity in adults and weight loss and high mortality in larvae.

The means of chemical analysis of soybean leaf tissue for each of the five elements sampled is presented in Table 3 for PI 171451, both parents, and 5 lines with the lowest and 5 lines with the highest leaf damage ratings. Large differences between lines were observed for each element. Calcium, potassium, and phosphorus content of foliage from the 49 F_4 lines was each negatively correlated (r = -0.23**, -0.26**, -0.20*, respectively) with pupal weight in the laboratory screening test. The concentration of each of these three elements was significantly higher in PI 171451 than in either parent. Foliage of PI 171451 which produced pupae with lower weights than those fed leaves of either cross parent contained higher levels of magnesium and lower levels of silicon than all but one other entry.

The resistant parent MBB 80-115 did not follow the pattern of the resistant PI 171451. MBB 80-115 had significantly more phosphorous and magnesium than Ware, but did not differ in calcium, potassium, or silicon. Whether the lack of a distinct separation between the parents indicates a weak association between the

chemical content and resistance is not clear. The level of resistance in our study for MBB 80-115 does not appear to be high since the larval feeding tests did not identify differences between the parents in feeding damage or larval development.

Interestingly, 119-2 which had demonstrated lower leaf damage, high mortality, took more days to reach pupal and adult stages, and achieved low pupal weights had the highest calcium, phosphorus, potassium, and magnesium levels. Conversely, lines 124-5, 121-1, and 120-12 which had higher leaf damage, lower larvae mortality, higher pupal weight and required fewer days for surviving larvae to reach pupal and adult stages had significantly less calcium, phosphorus, potassium and magnesium content than 119-2. Lines 124-5 and 121-1 also had the highest silicon content among those studied.

Management of soil fertility by application of fertilizers is a routine procedure in most crops and is practically an inherent part of the agroecosystem. Quantitative and qualitative variation in this environmental factor exerts dramatic influence on plant growth and development which frequently may lead to alterations in the behavioral and nutritional suitability of plant tissue for insects. The presence of certain nutritional elements in the host plant may in addition influence specific concentrations of organic compounds that act as a feeding stimulant or deterrent in the dietary substrate. These compounds may affect the various physiological processes related to growth, development, longevity, and fecundity of MBB's feeding on them.

The significance of macronutrients to the soybean plant has been reported by deMooy et al. (1973). They report that phosphorus is important for the formation and translocation of carbohydrates, fatty acids, glycerides, and essential intermediate products, that magnesium has a direct effect on dinitrogen fixation, and an indirect effect through increased photosynthesis and growth, and that potassium is important for all aspects of growth and has a large influence on the nutritional balance of the plant. On the other hand, calcium deficiency in the leaves raises the protein and nonprotein N contents, suggesting that protein synthesis is not affected.

The presence or absence of these nutritional elements probably does have a bearing on plant resistance as discussed by Scriber (1984) and references therein. In our study, the line 119-2 had the lowest larval damage score and also had high concentrations of calcium, phosphorus, potassium, and magnesium. Certain soybean genotypes genetically may have the ability to extract and utilize various chemicals from the soil with greater efficiency than other genotypes. If the increased or decreased concentration of certain elements contributes to resistance and this difference is genetically controlled, knowledge of such systems may provide a mechanism which can be used as a selection criterion for insect resistant genotypes. There is no previous information which attempts to show a relationship between the elements studied and the insect-plant relationship in soybean. This study represents the first attempt to do so.

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LITERATURE CITED

deMooy, C. J., J. Pesek, and E. Spalden. 1973. Mineral nutrition p. 267-334. *In* B. E. Caldwell, R. W. Howell, and R. W. Judd [ed.] Soybeans: improvement, production and uses. Agronomy monograph 16. Amer. Soc. of Agronomy, Madison, WI.

Elden, T. C., J. A. Schillinger, and A. L. Steinhauer. 1974. Field and laboratory selection for resistance in soybean to the Mexican bean beetle. Environ. Entomol. 3: 785-88.

Elden, T. C., and P. E. Paz. 1977. Field cage studies to determine effects of Mexican bean beetle resistance in soybeans. J. Econ. Entomol. 70: 26-29.

Fehr, W. E., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stage of development descriptions for soybean, *Glycine max* (L.) Merrill. Crop. Sci. 11: 929-31.

Grunwald, E., and M. Kogan. 1981. Sterols of soybeans differing in insect resistance and maturity group. Phytochemistry 20: 765-68.

Hart, S. V., M. Kogan, and J. D. Paxton. 1983. Effect of soybean phytoalexins on the herbivorous insects Mexican bean beetle and soybean looper. J. Chem. Ecol. 9: 657-72.

Johnson, D. R., and W. V. Campbell. 1982. Variation in the foliage nutrients of several peanut lines and their association with damage received by the two spotted spider mite *Tetranychus urticae*. J. Georgia Entomol. Soc. 17(1): 69-72.

Kogan, M. 1972. Feeding and nutrition of insects associated with soybeans. Soybean resistance and host preference of the Mexican been beetle; *Epilachna varivestis*. Ann. Entomol. Soc. Amer. 65: 675-83.

Mellors, W. K., A. Allegro, and S. E. Propts. 1984. Adult reproductive diapause in the Mexican been beetle (Coleoptera: Coccinellidae): Interaction of temperature with photoperiod. Environ. Entomol. 13: 409-14.

Scriber, J. M. 1984. Host plant suitability. p. 159-202. *In* W. J. Bell and R. T. Carde [ed.] Chemical ecology of insects. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Smith, C. M., R. F. Wilson, and C. A. Brim. 1979. Feeding behavior of Mexican been beetle on leaf extracts of resistant and susceptible soybean genotypes. J. Econ. Entomol. 72: 374-77.

Smith, C. M. 1985. Expressions, mechanisms and chemistry of resistance in soybean, *Glycine max* L. (Merr.) to the soybean looper, *Pseudoplusia includens* (Walker). Insect Sci. Applic. 6: 243-48.

Tester, C. F. 1977. Constituents of soybean cultivars differing in insect resistance. Phytochemistry 16: 1899-1901.

Van Duyn, J. W., S. G. Turnipseed, and J. D. Maxwell. 1971. Resistance in soybeans to Mexican been beetle. I. Source of resistance. Crop Sci. 11: 572-73.

Van Duyn, J. W., S. G. Turnipseed, and J. D. Maxwell 1972. Resistance in soybeans to the Mexican been beetle: II. Reactions of the beetle to resistant plants. Crop Sci. 12: 561-62.