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PLANT SUSCEPTIBILITY TO INSECTS

Chemical Factors Influencing Host Selection by the Mexican Bean Beetle *Epilachna varivestis* Muls

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Studies of extracts of the seeds of *Phaseolus vulgaris* (L) indicated a factor or factors influencing feeding behavior of the Mexican bean beetle (*Epilachna varivestis* Muls). Sucrose was isolated and identified and shown to be the factor responsible for selectivity in the bioassay used. Quantitative sugar determinations on the seeds of a series of resistant and nonresistant plants showed that the concentration of nonreducing sugars is significantly higher in the seeds of nonresistant plants. Sucrose concentration in seeds is suggested as an index of resistance to attack by the Mexican bean beetle. In addition, the importance of sucrose in bioassay involving feeding behavior is discussed.

Certain varieties of bean plants are more susceptible to attack by the Mexican bean beetle than are others. The problem of determining the reason for this susceptibility may be approached from a number of points of view. If a particular variety of plant is preferentially attacked by insects, it may be that this variety possesses a factor which attracts the insect to the plant. Many host-plant relationships have been investigated from this point of view and, in several cases, chemicals have been isolated which function as attractants (*6–11,16,17*). On the other hand, the lack of a repellant or resistance factor in susceptible plants may be involved in determining host-plant specificity (*12–15*). The authors decided to look for a chemical factor that would act as an attractant for these insects, and that would be present only in the susceptible varieties.

Leaf material from *Phaseolus vulgaris* (L) was lyophilized and extracted with a series of solvents of varying polarity. These extracts were then tested by saturating filter paper disks with the extract and exposing these disks, along with control disks containing no extracted material, to the beetles. Details of this procedure have been reported elsewhere (*5*).

The criterion of attractiveness was the number of feeding marks or ridges the beetles made on the filter paper disks.

Aqueous or alcoholic extracts of either leaves or seeds gave a positive response in the bioassay procedure. Since seeds are easier to store and extract, and since bioassay indicated activity in this plant portion, seeds were used for the investigations.

The alcoholic extract from a 50-gram sample of ground seeds was filtered and the solvent removed. The dried residue was then dissolved in distilled water. A portion of this aqueous solution, after being heated on a water bath for 30 minutes, gave a negative Benedict's test for reducing sugars. The remaining sample was made acidic (pH 4.5) with dilute HCl and subjected to the same treatment. After neutralization of the solution, a positive Benedict's test was obtained.

Table I. R_g Values in Various Solvents[a]

Sample	A	B	C	D	Color
Sorbose	1.01	1.19	1.01	1.02	Green
Cellobiose	0.44	0.53	0.59	0.66	Blue
Fucose	1.34	1.46	1.13	1.16	Grey-brown
Galactose	0.80	0.81	0.87	0.89	Blue
Glucose	1.00	1.00	1.00	1.00	Blue
Mannose	1.05	1.20	1.06	1.10	Grey-blue
Fructose	1.05	1.18	1.08	1.08	Brown
Arabinose	1.12	1.32	1.03	1.03	Grey-brown
Sucrose	0.80	0.72	0.93	0.93	Brown
Ribose	1.37	1.89	1.22	1.21	Grey-brown
Hydrolyzed spot 1	1.03	1.17	1.05	1.08	Brown
Hydrolyzed spot 2	1.00	1.03	1.01	1.10	Blue
Unhydrolyzed	0.75	0.70	0.91	0.91	Grey-brown

[a] Solvent A: isopropanol–H_2O (16:4); solvent B: ethyl acetate–pyridine–H_2O (12:5:4); solvent C: *n*-butanol–pyridine–H_2O (8:8:4); solvent D: isopropanol–pyridine–H_2O (12:4:4).

To obtain some preliminary information about the nature of the sugars involved, samples prepared as above were chromatographed on $5^1/_2$- × 14-inch Whatman No. 1 paper strips according to the method of Bailey and Bourne (*3*). One spot was observed with the unhydrolyzed sample, and two spots with the hydrolyzed sample. R_g values were obtained and compared with other sugars as shown in Table I.

These results strongly indicated that sucrose was the nonreducing sugar present. This was proved by isolation of sucrose from the bean seeds.

The seeds were extracted in a soxhlet apparatus with ethanol, and magnesium oxide was added to the alcoholic extract. After removal of the solvent, the magnesium oxide (containing adsorbed material) was re-extracted with absolute alcohol. The alcohol extract was treated with charcoal, reduced in volume, and added to a fivefold excess of anhydrous ethyl ether. The flocculent precipitate which appeared was recrystallized in the same manner ten times to give finally a white crystalline material melting at 175° to 185° C. This material was identified as sucrose by comparison with a known sample of sucrose. Refractive indices, infrared spectra, R_f values in four solvent systems, and the melting point of the octa-acetate derivative all confirmed this identification.

Sucrose, either extracted from bean seeds or obtained commercially, gave positive results in the bioassay procedure mentioned above. With this in mind, the following sugars were subjected to bioassay: ribose, xylose, arabinose, rhamnose, galactose, mannose, sucrose, maltose, lactose, cellobiose, raffinose, glucose, and fructose. Only glucose, fructose, and sucrose gave positive results.

These results indicate that beetles are quite selective in their choice of sucrose and its component monosaccharides. Sucrose can hardly be considered to be an attractant in the sense that a volatile material might be, but it can be considered to be a feeding factor. Beck (*4*) has coined the term saccharotropism to describe selective feeding on diets or tissues containing high concentrations of sugars. Apparently, this effect has been encountered in the present studies.

With this saccharotropic effect in mind, bioassay procedures designed to indicate the presence of attractants must be modified to eliminate sugar concentration as a factor. This is most easily done by including sucrose in each sample to be tested. If sucrose is not included, the test insect may be attracted to a given sample, but may not feed. This would show up as a negative result in a bioassay based on feeding behavior.

To determine if a difference in sucrose concentration might be involved in host selection, quantitative determinations of reducing and nonreducing substances in a series of resistant and nonresistant bean seeds were performed. The results are set forth in Table II. A.O.A.C. methods were used (*1*, *2*). It appears that high sucrose levels in the seeds result in plants that are more suceptible to attack by the Mexican bean beetle. This should provide a rapid and convenient method for determining whether a given plant is resistant or nonresistant.

Since the beetles feed on the leaves and not the seeds, these observations do not necessarily indicate that sucrose is the factor present in leaf material that affects feeding behavior. Analyses are now being carried out on leaf material to determine the relationship of leaf sucrose concentration to resistance.

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Table II. Percent Dry Weight of Reducing and Nonreducing Substances in Resistant and Nonresistant Plants[a]

Resistant Plants	Reducing Sugars, %	Nonreducing Sugars, %
Phaseolus mungo	0.02	0.8
Phaseolus aureus	0.01	0.9
Phaseolus sp.	0.02	0.6
Phaseolus atropurpureus	0.01	0.9
Glycine max.	0.01	0.9
Nonresistant Plants		
Phaseolus vulgaris	0.02	1.9
Phaseolus lunatus	0.03	2.0
Phaseolus acutifolius	0.02	1.9
Phaseolus coccineus	0.02	2.0

[a] Classification as to resistance and nonresistance was made by R. H. Davidson and Dan Wolfenbarger (*18*, *19*), who also supplied the seeds.

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