Host-Plant Selection by the Mexican Bean Beetle, *Epilachna varivestis*[1]

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ABSTRACT

The Mexican bean beetle, *Epilachna varivestis* Mulsant, feeds selectively on certain species of *Phaseolus* to the exclusion of the remaining representatives of this and other genera of beans. In a study of the chemical factors underlying this selective feeding, the phagostimulant fraction in the seeds of *P. vulgaris* was isolated and identified as sucrose. Bioassay of 13 sugars and 15 amino acids showed that only sucrose and, to a lesser degree, its hexose components induced feeding by the beetle. The seeds as well as leaves of the nonresistant species of *Phaseolus* were found to have a higher concentration of sucrose, glucose, and fructose, thereby revealing the role these sugars play as arrestants in host-plant selection by the beetle. Bioassay of the volatile fraction from foliage of host-plants yielded positive olfactory responses from Mexican bean beetle larvae, and only indirect evidence of similar responses from the adults. These findings indicate the presence of a short-range attractant in the volatile constituents of host-plants. The influence of both attractants and arrestants in host-plant selection by insect pests is discussed, and the significance of quantitative differences in essential nutrients in host-plants is stressed. Phytogeographical factors relating to the 10 common species of *Phaseolus* also are discussed, emphasizing that in host-plant selection their role, if any, is only subordinate to stimulant chemicals in plants.

Host preference in many phytophagous species of insects is well known, and the factors, in the host and of the pest, that make possible this preference are currently receiving an extensive probe from several investigators. Factors in the plant concern its physical and chemical makeup, while those of the pest concern its perceptive powers. Recognition and subsequent selection of the food-plant by the insect call into play a harmonious coordination of all these factors. Several of the easily discernible physical attributes of plants, size, shape, and color, are too variable and possess no uniqueness that could possibly aid their recognition by the pest. Vision, phototaxis, geotaxis, and hygrotaxis play a part in directing insects to the proper environment for feeding and oviposition: but the ultimate close-range forces, operating in the food-plant selection by the host-specific pests, are presumed to be largely chemical—regulated for the most part by smell and taste.

Ever since the first accounts by Verschaffelt (1910) and McIndoo (1919) invited attention to the possible relationships between the chemical constituents of plants and host-specific phytophagous insects, considerable progress has been made in unravelling some of the causal factors underlying this phenomenal host-specificity of certain insects. Dethier (1953), Kennedy (1953), Lipke and Fraenkel (1956), Beck (1956), and Thorsteinson (1960) provide some of the very helpful reports in this field of study. These reports give an adequate insight into the intricacies of the problem and list an extensive bibliography to the work of other investigators since Verschaffelt. A survey of this literature reveals emphasis along 3 basic lines of investigation: (i) consideration of the nutritional and other factors resident in plants and leading to the insect's "passive" selection of host-plants; (ii) search for nonnutritious, attractive or repellent factors responsible for "active" selection or rejection by phytophagous insects; and (iii) physiological changes in the plant and their impact on the food-finding behavioral pattern of the pest. Despite these 3 different approaches—nutrients, secondary chemicals, and physiological changes within the plant—it is obvious that the emphasis in all these investigations of host-plant selection in phytophagous insects remains essentially one of finding specific chemotactic stimuli resting in or emanating from the plants.

The Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), exhibits host-specificity. Of the 5 genera of beans, *Vigna, Phaseolus, Dolichos, Glycine, Vicia,* the beetle shows marked preference to only certain species of *Phaseolus.* All others (barring a negligible number of interspecific crosses showing "slight to intermediate" susceptibility, but never preferred) are categorized as resistant to the pest (Wolfenbarger 1961). The insect may, under experimental and occasionally even field conditions, live on certain plants totally unrelated to legumes (Auclair 1959). Elmore (1949) provides a list of plants the pest did not accept under experimental conditions, and a few it fed upon but without subsequent reproduction. The investigation reported in this paper was made to identify the plant constituents eliciting those chemotactic responses in the beetle that aid its unerring recognition and discreet acceptance of its preferred hosts.

MATERIALS

Throughout the period of this study, a large culture of Mexican bean beetles was maintained in the greenhouse. The beetles were reared mostly on red kidney and lima varieties of bean plants. Separate cages for adults, larvae, pupae, and newly emerged adults facilitated the use of samples of uniform age in all bioassays. Where feeding activity was the criterion sought, young adults (not older than 8–10 days) and early second- and third-instar larvae were found to yield more consistent results than other stages of the beetle.

Seeds for analysis were secured from commercial as well as various other sources (Augustine 1962).

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Plantings for foliage analysis were at the rate of 40 to 45 seeds per each 9-inch pot. Agrico water-soluble plant food 17-17-17 was mixed to half strength, and each pot received 16 oz of the solution. The water supply in the saucers holding the pots was replenished every other day, and the foliage for analysis was picked always from plants about the same stage of growth—when the trifoliate leaves were just unfolding.

METHODS AND RESULTS

Most of the damage done by the Mexican bean beetle, *E. varivestis*, to the susceptible bean plants is defoliation, although both larvae and adults are known to feed on pods of some varieties after foliage is destroyed. Adults or larvae, feeding from below, eat ragged areas in the lower surface of the leaf, often also cutting through the upper surface. The damaged leaf is a peculiar network which characterizes the attack of this pest (Howard 1941). Because of the pest's preferential feeding on leaf juices, it was postulated that the insect may attack filter paper treated with any attractant fraction from its host-plant tissues. Initial investigations (Byers 1961) using the leaf juices obtained by mincing leaves of *Phaseolus vulgaris* L. in a Waring blendor proved the procedure satisfactory. A damaged leaf and the pattern of feeding on filter paper with leaf juices are shown in Fig. 1.

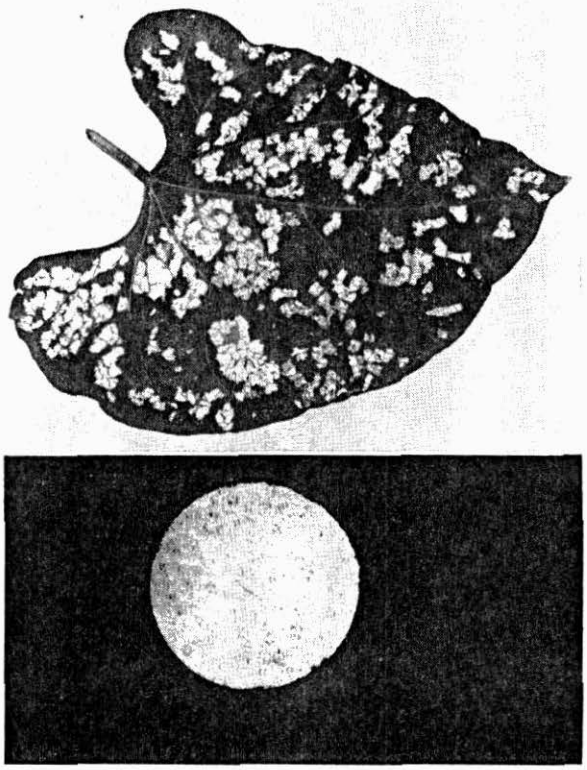

FIG. 1.—Pattern of feeding on leaves and filter paper.

Extraction of seeds.—Storage of seeds is easier than that of foliage, and seeds also assure a ready supply of uniform material for chemical analysis. All varieties of *P. vulgaris* are known to be susceptible to severe attack by the beetle and, therefore, seeds of one of its common varieties (red kidney bean) were chosen for thorough fractionation to isolate the principal feeding stimulant for the Mexican bean beetle.

A mixture of ground seeds of *P. vulgaris* and a small quantity of sea sand was placed in a sintered glass-bottom vessel and extracted in a soxhlet apparatus: first with ether absolute and then with 95% ethyl alcohol. Each sample was extracted for 20 to 22 hours. The extract fractions, after the removal of the solvent in vacuo, were dried in a vacuum desiccator and bioassayed. The fractionation scheme and the results of the bioassay of the fractions are found in Fig. 2. By techniques listed in Table 1, the active ingredient in the ethanolic fraction was identified as sucrose.

Bioassay.—The seed extracts were dissolved in known volumes of the solvent, and Whatman No. 1 (4.25 cm) filter paper discs were treated with meas-

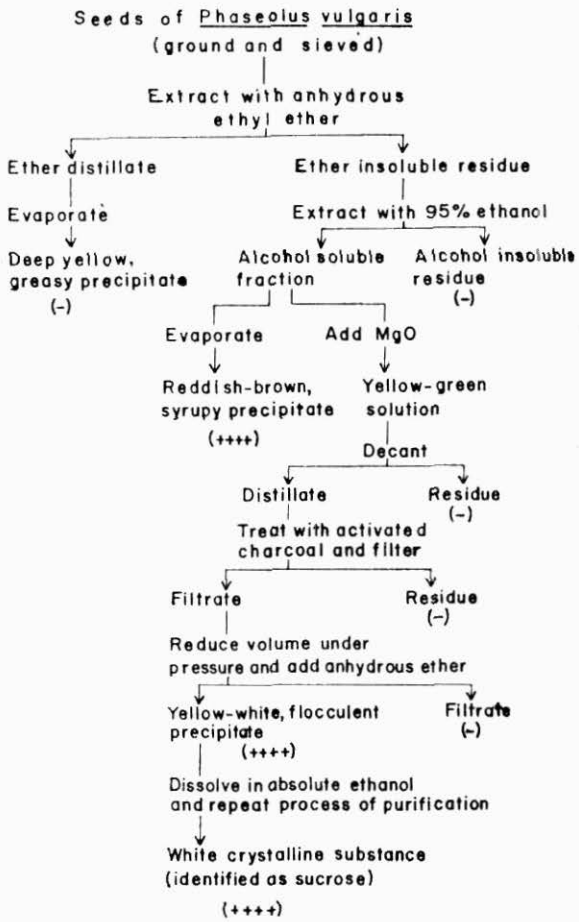

FIG. 2.—Fractionation scheme of seeds of *Phaseolus vulgaris*, and bioassay of the fractions. (−), inactive as feeding stimulant; (++++), very active feeding stimulant.

Table 1.—Identification of the phagostimulant crystalline material from the seeds of *Phaseolus vulgaris*.

Test	Results	
	Unknown crystals	Sucrose
Melting point	185° C. with decomposition	185° C. with decomposition
Refractive index	alpha —1.5374 beta —1.5649	alpha —1.5376 beta —1.5651
Infrared spectra	Identical with sucrose	
Chromatography, unhydrolyzed	R_f value (0.71) and spot color (brown) as of sucrose	
hydrolyzed (with dil. HCl)	R_f values (0.76 and 0.8) and colors of spots (slate blue and pale brown) as of glucose and fructose respectively	
Octa-acetate[a]	71°–72° C.	70°–72° C.

[a] Uncorrected values (Cleary 1962).

ured amounts of these solutions. These discs and the "controls" were then placed in a vacuum oven for 20 to 30 minutes to remove all traces of the solvent. Placed in a petri dish, the dry discs were moistened with a few drops of water and exposed to one or more beetles starved earlier for 20 to 24 hours. The petri dishes were then transferred to Boekel desiccators. Feeding marks of the beetles on extract-treated discs was considered as suggestive of the presence of a host-plant fraction serving as a feeding stimulant for the insects.

Keeping treated discs for intervals longer than 1 to 2 hours and adequate ventilation are essential to satisfactory testing conditions. Use of an air pump and a water-filled gas-bubbler sending a continuous, fine stream of humid air through Boekel desiccators facilitated prolonged periods of testing. Six to eight small holes in the lids of the sterile plastic petri dishes assured adequate ventilation. At a temperature of 82° to 84°F and relative humidity of 70% to 76%, discs moistened with 0.2 ml of water were found to retain 60% to 70% of the moisture even after being in the desiccators for 12 hours.

Testing "sugar and amino acid preferences" of the beetle.—Identification of the phagostimulant fraction as sucrose and the knowledge of the growing recognition among researchers in the field of insect nutrition (Ito 1960; Thorsteinson 1960) of the significance of nutrients as feeding stimulants for phytophagous insects necessitated testing the sugar preferences of the Mexican bean beetle. Solutions of the following sugars were prepared at varying concentrations: glucose, fructose, galactose, mannose, arabinose, ribose, rhamnose, xylose, sucrose, maltose, lactose, cellobiose, and raffinose. In each large petri dish 7 filter paper discs, each treated with a different sugar, were exposed to 5 to 6 starved beetles for periods ranging from 12 to 18 hours. The sugar solutions were applied in 0.2 ml portions to each disc. Only 3 of the 13 sugars tested stimulated feeding by the beetle. (LaPidus et al., in press). The degree of the insect's preference for these three sugars may be indicated as:

$$\text{sucrose} \geq \text{fructose} > \text{glucose}$$

A minimum concentration of 0.05M sucrose gave optimum results (symbolically shown as +++ and ++++ in the results of the bioassay).

Ethanolic extracts of plant tissues contain, besides traces of several chemical constituents, chiefly carbohydrates (free sugars in particular) and amino acids. The purified fraction (containing only sugar) as well as the crude ethanolic extract served as equally effective phagostimulants for the beetle. The activity, if any, of the amino acid fraction in initiating feeding by the beetle, therefore, had to be evaluated. Bioassay of varying concentrations of each of 15 selected amino acids (glycine, alanine, valine, leucine, proline, phenylalanine, aspartic acid, glutamic acid, serine, cystine, tyrosine, arginine, histidine, lysine, and tryptophan) gave negative results.

Quantitative estimation of sugars in seeds.—Following the finding that sucrose and its hexose components were phagostimulants of considerable importance for the beetle, the amounts of these sugars in seeds of resistant and nonresistant species of *Phaseolus* were determined by the Munson-Walker procedure (Horwitz 1960). The results are shown in Table 2.

Extraction of leaves.—The Mexican bean beetle feeds only on the foliage of its hosts, not the seeds. The estimation and comparison of the amounts of preferred sugars, therefore, was extended to the leaves of the resistant and nonresistant species of *Phaseolus*. Samples of fresh foliage (primary leaves from plants with trifoliate leaves just unfolding) were rapidly picked, weighed (10 g), and immediately plunged into a Waring blendor containing 150 ml of 80% ethanol at 70° to 75°C, and lacerated with scissors

Table 2.—Percentage dry weight of reducing and nonreducing sugars in seeds of resistant and nonresistant species of *Phaseolus*. (Modified from LaPidus 1963.)

Species	Percentage of:		
	Reducing sugars	Non-reducing sugars	Total "preferred sugars"
Resistant:			
P. mungo	0.02	0.8	0.82
P. aureus	.01	.9	.91
P. atropurpureus	.01	.9	.91
Nonresistant:			
P. vulgaris	.03	2.0	2.03
P. lunatus	.02	1.9	1.92
P. coccineus	.02	2.0	2.02
P. acutifolius	.02	2.0	2.02

beneath the surface of the alcohol. This very rapid processing of the leaves was to inactivate enzymatic reactions, especially the hydrolysis of sucrose. Each sample was blended for 6 to 7 minutes and the mixture, together with another sample of 60 ml of hot 80% ethanol used for rinsing the blendor, was transferred in a stoppered flask to an electric oven at 80°C. The extracts were maintained at this temperature for 1½ to 2 hours. Extraction of free sugars, amino acids, and plant acids is essentially completed when chlorophyll appears extracted. The samples were allowed to cool, filtered using a buchner funnel, and each residue washed with 60 ml of hot 80% ethanol. Equal amounts of activated charcoal were added to the filtrates, and the samples were filtered after 15 minutes. The clear ethanolic extracts were then made up to 250 ml with 80% ethanol (extraction procedure had resulted in a slight loss in the 260 ml of ethanol used in earlier steps), and refrigerated.

From the same plants, at the same time, duplicate samples of foliage were picked, weighed, and dried to constant weight in an electric oven at 80°C. Dry weights of the samples were determined by this treatment.

Quantitative estimation of sugars in the leaf extracts.—The colorimetric method of Nelson (1944), although essentially for reducing sugars, was used to advantage in this study for also estimating nonreducing sugars following their hydrolysis by 5% oxalic acid.

Standard sugar solutions (glucose, fructose, and a mixture of glucose, fructose, and sucrose) of varying strength, and in 2 sets, were prepared. One set was treated with activated charcoal. Using copper reagents and arsenomolybdate solution, color densities were read in a Bausch and Lomb Spectronic-20 colorimeter at a wave length of 520 mμ (Augustine 1962). Samples containing sucrose were hydrolyzed with 5% oxalic acid, and for these samples the volume of copper reagent was doubled to compensate for the pH change caused by the acid. Plotting density against concentration, standard curves (straight lines) were drawn for the 2 sets of standards. Overall percentage loss of sugars due to charcoal treatment was 10%.

One-ml samples of the ethanolic extracts of the foliage were first read for reducing sugars, and then the hydrolyzed samples for the total sugar content. The difference was taken to indicate the amount of sucrose. Paper chromatography of the leaf extracts revealed glucose, fructose, and sucrose, thereby confirming that the colorimetric estimations were predominantly of these 3 sugars. Results, after correcting for percentage losses due to charcoal treatment, are shown in Table 3.

Extraction and bioassay of the volatile constituents from bean foliage.—Volatile fractions from the foliage of resistant and nonresistant species of beans were extracted with a Virtis freeze-drying apparatus. A mixture of Cellosolve (ethylene glycol monomethyl ether) and dry ice (solid carbon dioxide) provided refrigeration to the vacuum chamber, and an infrared lamp (controlled by a variable transformer) outside the freeze-drying flask helped to maintain the temperature inside the flask at 30° to 32°C. Under these conditions, 70% to 75% dehydration of previously frozen foliage samples of 25 g was possible in about 20 minutes. After each extraction (or after continuous extraction of several samples of foliage of the same species), the mixture of water vapor and the volatile fraction frozen on the outer surface of the "cold finger" was allowed to collect in the bottom flask. Samples collected were immediately bioassayed. Extracted constituents refrigerated for 20 to 24 hours proved ineffective in bioassay.

Table 3.—Percentage of reducing and non-reducing sugars in the leaves of resistant and non-resistant species of *Phaseolus*.

Species	Percentage dry wt. of: Reducing sugars	Non-reducing sugars	Total "preferred sugars"	Percentage fresh wt. of "preferred sugars"
Resistant:				
P. mungo	2.6	5.4	8.0	0.602
P. aureus	1.55	0.61	2.16	0.342
P. atropurpureus	1.3	2.16	3.46	0.43
Nonresistant:				
P. vulgaris				
i) Golden Wax	11.48	8.0	19.48	1.35
ii) Stringless Greenpod	6.2	6.1	12.3	1.6
iii) Red Kidney	6.0	4.6	10.6	1.0
P. lunatus	10.6	6.1	16.7	1.33
P. coccineus	9.6	6.2	15.8	0.8

The aqueous extracts containing the volatile fractions from the foliage were tested for their attractiveness to the Mexican bean beetle by 3 methods.

Method 1.—Free exposure of beetles to extract samples: Filter paper discs were treated with solutions of either sucrose, fructose or glucose, and allowed to dry. Some of these discs were moistened with 0.2-ml portions of extracts containing the volatile constituents, and the others with water. The moist discs were placed in petri dishes and exposed to starved beetles for periods ranging 10 to 12 hours. Discs treated with only volatile constituents from host plants had no feeding marks; those with only sugar solution had few feeding marks; those with sugar solution plus volatile constituents of resistant beans had few feeding marks which, at best, could be described as "nibbling" marks as is evident in Fig. 4, while the discs treated with sugar volatiles from host species bore extensive "biting" marks indicating clearly the participation of a preference in the feeding behaviour of the beetles (Figs. 3 and 4). Under the test conditions, this preference can be ascribed only to the volatile fraction added to the sugar treated disc. The volatile fraction by itself did not induce feeding and, therefore, has no independent phagostimulant activity. Preferential feeding on discs

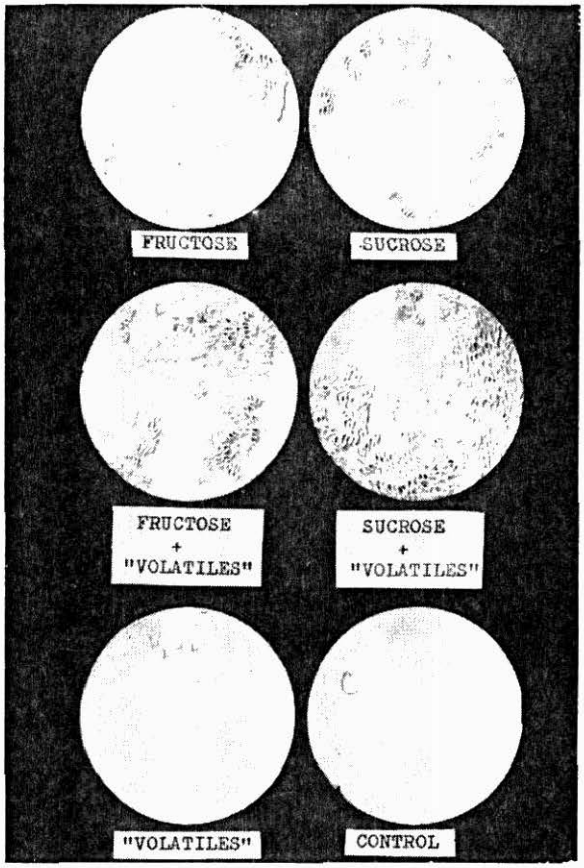

FIG. 3.—Attraction of standard sugars and host-plant "volatiles" to the Mexican bean beetle.

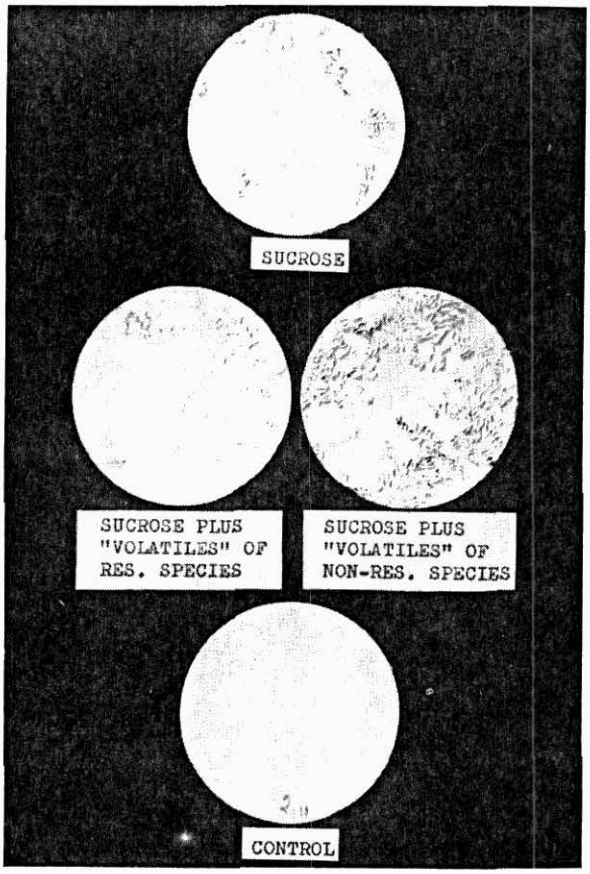

FIG. 4.—Attraction of "volatiles" from resistant and non-resistant species of beans to the Mexican bean beetle.

having both sugar solution and volatile fraction from leaves of host species can be explained as an effect of both olfactory and gustatory responses of the beetle.

Method 2.—Exposure of beetles to sucrose-treated filter paper discs placed over crucibles containing leaf extract samples: Volatile fractions of *P. vulgaris* and *P. lunatus* were placed in small crucibles, and filter paper discs treated with sucrose solution were kept over the crucibles. A choice of four crucibles (Table 4) was placed in each crystallization dish and exposed to starved beetles. The crystallization dishes permitted more freedom of movement to the insects, and at the same time also offered them choices over which they would not be "forced" to crawl as when confined to the limited space inside petri dishes. Sugar-treated discs over crucibles containing volatile fraction from host plants had more feeding marks than those similarly placed over crucibles containing water or volatile fraction from resistant species. Although this too is indirect evidence of the olfactorily attractant properties of the volatile fraction from host plants, it is better than the evidence obtained by Method 1.

Method 3.—Tests in olfactometers: Olfactometers of the Y-tube type as well as modified forms of it were used for testing the olfactory responses of the beetle to volatile fractions from the bean foliage. Results were mostly inconclusive. Some of the observations, when analyzed by the lenient measure of Relative Dispersion, seemed to indicate the presence of an olfactory stimulus in the volatile fraction. This indication was rendered void by the more stringent t-test, at the 5% significance level (Augustine 1962).

Olfactory responses of the larvae.—Third-instar larvae, previously starved for 8 to 10 hours, were offered the choice of 2 small petri dishes—one containing 5 ml of the aqueous extract obtained by freeze-drying leaves of *P. vulgaris*, and the other containing water. The petri dishes were placed 5 cm apart inside a large crystallization dish. With very little wandering between the petri dishes, the larvae soon clambered on and into the petri dish containing the extract sample. Results of 2 such tests are shown in Table 5.

Gas chromatography of volatile fractions from foliage.—Aqueous fractions obtained by freeze-drying bean foliage were extracted with either petroleum ether or ethyl ether, excess solvent removed using a rotary film evaporator, and chromatographic runs were made of the "odor fraction" of the plants. Aerograph Hi-Fi Model-600 was used with hydrogen

Table 4.—Bioassay with the volatile fractions in crucibles covered with 0.05M sucrose-treated filter paper discs.

"Volatiles" in crucibles	Volume	Number of assays[a] 1	2	3	4
P. vulgaris	3 ml.	+++	++++	++	+++
P. lunatus	3 ml.	+++	++++	++++	+++
Control 1 (water in crucible with sucrose-treated disc as cover)		++	++	++	+
Control 2 (water in crucible with water-treated disc as cover)		—	—	—	—

[a] — Indicates no feeding marks on disc.
+ Indicates the intensity of feeding on disc.

flame ionization detector and nitrogen as the carrier gas. The 10'x⅛" columns (Wilkens Instrument & Research, Inc. California) contained either 20% LAC-2-R446 (adipate polyester of diethylene glycol partially crosslinked with pentaerythritol) or 20% Carbowax 6000 on 60/80 firebrick. Optimum range of column temperature was 70°–75°C, flow rate of carrier gas 30 ml per minute, and aliquots of 1.0 μl were injected. Although the chromatograms of the different host species compare favorably (suggesting identical volatile constituents in injected samples), low percentage impurities in the commercial solvents used (petroleum ether and ethyl ether) were found to be definitely interfering with these chromatographic runs. Work is now in progress to purify the solvents and to obtain chromatograms yielding to precise interpretation of peaks.

Table 5.—Results of tests exposing larvae to host-plant volatile fractions.

Host-plant	Vol. of volatile fraction, and number of larvae	Interval in minutes	Number of larvae on or in[a] Extract container (x)	"Control" container (y)
P. vulgaris	5 ml. 35 larvae	5	2	–
		10	6	–
		15	7	3
		25	9	6
		30	14	4
		32	13	–
		45	15	5
		50	20	3
		55	21	3
		75	32	3
		120	34	1
P. vulgaris	5 ml. 19 larvae	5	3	1
		15	6	1
		30	5	2
		35	10	–
		45	9	1
		75	12	4
		105	14	1
		110	15	1
		115	17	1

[a] Hypothesis: $\bar{x} = \bar{y}$; Significance level: 5%; t-calculated: 4.5822; t-tabulated: 2.03.

PHYTOGEOGRAPHICAL CONSIDERATIONS

It is assumed that, during evolution, phytophagous insects became adapted to their environment in a many-sided manner. Such an adaptation, instead of being exclusively confined to nutritional requirements of the insect, might have been one of selecting a whole biotope, its ecoclimate, its natural enemies, and so on, as well as its food (Kennedy 1953). It is tempting to interpret this very reasonable assumption as suggesting that host-specificity of phytophagous insects is not because of any special stimuli emanating from the food plants, but is mostly a recognition of a particular species of plant and all that goes with it ecologically. Species of beans belonging to *Vigna*, *Dolichos*, and *Glycine* are essentially Asiatic in origin, and those of *Vicia* are native to the Mediterranean region. The genus *Phaseolus*, to some species of which the Mexican bean beetle is unmistakably partial, includes representatives native to the Old World as well as the New World (Hedrick et al. 1931). The Mexican bean beetle is a New-World species, and most of the resistant species of beans are native to the Old World. This difference in place of origin, at first sight, seems to subordinate or contradict the chemotactic basis of host-plant selection, and lay emphasis on phytogeography.

A closer study of the ten common species of *Phaseolus* (*P. vulgaris*, *P. lunatus*, *P. coccineus*, *P. acutifolius*, *P. mungo*, *P. aureus*, *P. acontifolius*, *P. calcaratus*, *P. angularis*, and *P. atropurpureus*) reveals that *P. atropurpureus*, which is native to South America and occurs abundantly throughout Mexico, Guatemala and Salvador (Piper 1926), is resistant to the Mexican bean beetle. At least 3 of the Oriental species of *Phaseolus* (*P. aureus*, *P. calcaratus*, and *P. angularis*), as well as several species of other genera of beans, have long been widely cultivated in parts of the New World. The absence of any nutritional correlation between the places of origin and spread of the plant species and the place of origin of the Mexican bean beetle is obvious. There should, therefore, be a factor or factors far more significant than any which phytogeographical records indicate of the host species, that contribute to food-plant specificity of the insect pest. The evolutionary processes may have equipped the insect with its "powers of chemotactic perception and evaluation of the nutrients in specific food plants" and not with "ability to distinguish native species of plants from closely related introduced species." In short, host-plant selection is largely dependent on the chemotactic perception by the host-specific insect pest, and all other factors, known and unknown, are only of secondary importance.

DISCUSSION

The Mexican bean beetle, *Epilachna varivestis*, shows in its food-plant selection a marked preference to only certain species of *Phascolus*. In a search for the chemotactic stimulants resident in the host species, ethanolic extraction and purification of fractions from the seeds of *Phascolus vulgaris* L. revealed the gustatory stimulant fraction to be sucrose. Bioassay of 13 selected sugars and, likewise, separately 15 amino acids also ranked sucrose as the most potent gustatory stimulant, followed in order by fructose and glucose. A comparison of the amounts of these 3 sugars in the seeds of resistant and nonresistant species of *Phascolus* showed a higher percentage of sucrose in host species. Since the beetle feeds on only the foliage of its hosts, the comparison was extended to the leaves of the resistant and nonresistant species of this genus. Foliage of the susceptible species was found to have a higher percentage of the 3 sugars—sucrose, glucose and fructose. These findings amply justify the conclusion that in nature these 3 sugars are the principal gustatory stimulants for the Mexican bean beetle. Also based on the findings of this study, one might venture to suggest that where certain sugars (or any other chemical constituents in host-plants) are known to serve as primary phagostimulants for an insect pest, quantitative estimation of such constituents in the seeds of the host and related species of plants will serve as a quick index of food-plant acceptability or, conversely, resistance (LaPidus et al. 1963).

Comparison of the percentages of the nonreducing sugars (mostly sucrose) in seeds and leaves (Tables 2 and 3) may pose a question as to why the figures obtained for leaves do not reflect the same clear-cut relationship obtained for seeds, i.e., percentage dry weight of sucrose in seeds of susceptible species was twice its percentage in resistant species; or why percentage of reducing sugars (particularly glucose) is higher in leaves than in seeds. Sucrose is essentially a storage sugar, and it is reasonable to assume that its proportions in seeds would exhibit a more stable pattern than in leaves. The relative proportions of glucose, fructose, and sucrose in leaves must always be in a state of flux, recognizing the fact that biosynthesis of these sugars is an immediate consequence of photosynthesis. Despite the apparent disparity in some details of the quantitative estimations of the 3 sugars in leaves as compared to seeds of *Phascolus* spp., it is obvious that the sum total of these 3 sugars in the plant tissues does greatly influence the latter's acceptability to the Mexican bean beetle.

Search for the plant fraction responsible for a possible olfactory stimulus for the beetles has not yielded conclusive results. Results obtained using adult beetles and volatile fractions of *P. vulgaris* in 2 types of olfactometers fell short of the ideal standards enunciated by Hoskins and Craig (1934) and customarily accepted by researchers in the field (Dethier 1947). However, indirect evidence of the participation of an olfactory stimulus in the food selection of the Mexican bean beetle was seen in the more intense feeding on filter paper discs treated with both sugar solution and the volatile fraction from the leaves of host-plants, than on discs treated with sugar solution alone. Open exposure of third-instar larvae to petri dishes containing aqueous extracts of the volatile constituents provided unmistakable evidence of olfactory signals reaching the insect at a close range of 3 to 5 cm. This finding, incidentally, is in general agreement with similar reports (Dethier 1937; Chin 1950; Munakata et al. 1959) giving positive evidence of olfactory responses from larvae of certain phytophagous insects, and inconclusive results with adults of the same species. It is entirely possible that this difference in the behaviour of larvae and adults may be due to the unknown complex of waxing and waning threshold levels involved in the overall behavioral pattern of the two stages. Feeding being a prime function of larvae, it is reasonable to assume that, in this stage, there would be a minimum interference by excitation thresholds other than those initiating and maintaining feeding.

In striking contrast to the frequent failures reported by investigators employing complex olfactometers, the "screen tests" of Dethier (1937) and Chin (1950), the "tube tests" devised by Munakata and his associates (1959), and the ordinary "petri dish" tests successfully employed in the present study with the larvae of the Mexican bean beetle, suggest the inappropriateness of combining highly complex olfactometers with the subtleties characteristic of olfactory signals. Thorsteinson's (1960) reflection that the unnatural restrictions imposed by the apparatus on freedom of movement, or the introduction of extraneous thigmotactic stimuli interfering with normal behaviour of the insects may also be responsible for the frequent sorry results encountered in the experiments employing highly complex olfactometers, has a special appeal to the present authors. The apparent "increased efficiency" suggested by the complexity of an olfactometer may in practice prove to be "enhanced unnaturalness" to the insect, interfering with the coordinated reflexes involved in its orientation.

As to the chemotactic stimulants (arrestants, attractants, and repellents) in plants, two possibilities exist: the specific stimulant constituent may be *qualitatively* different from the more common chemicals in plants, or may be the effect only of *quantitative* differences in one or more of the common chemicals in plants. The first stresses the need and, therefore, the presence of "odd" or secondary chemicals in plants; the second recognizes the possible influence of quantitative differences in the essential nutrient constituents of plants in food-plant selection of host-specific pests. Strict adherence to the first possibility would seem more instinctive than logical. It is known that superoptimal or hyperoptimal concentrations of certain otherwise attractive chemicals are avoided by insects. Observations of this nature decidedly reflect the stimulant-role of quantitative differences in the chemical constituents of the food material.

Preferred and prolonged feeding on specific plants is the essence of host-specificity exhibited by certain phytophagous pests. Olfactory and gustatory faculties of the pest are involved chiefly in the recognition and attack of its choice food plants. These two chemotactic senses necessarily depend on *attractant(s)* in plants—specific chemicals drawing the pest to the plant, as well as *arrestant(s)*—chemicals serving as effective phagostimulants and thereby maintaining prolonged feeding by the pest. An attractant-arrestant combination, where present, would naturally be more preferred than any one alone. Recent studies on a boll weevil arrestant extractant from cotton (Keller et al. 1963) provide a good example of the increased preference to such a combination. The studies on host plant selection of the Mexican bean beetle, *E. varivestis*, show that susceptible species of *Phaseolus* have a higher concentration of the preferred sugars. This finding, therefore, does not favor a summary exclusion of nutrients as potential feeding stimulants and regulators of food-plant selection by phytophagous insects. On the contrary, in the *Phaseolus* spp. the concentration of sucrose and its hexose components is seen to play the role of the *arrestant* for the Mexican bean beetle. The short-range olfactory sense exhibited by the larvae, and also evident in adult beetles, suggests the presence of an *attractant* chemical or chemicals in the volatile fraction of the host plants. The identity of the attractant is yet to be established.[5]

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[5] Note added in proof. Recently Nayar and Fraenkel (1963) reported that the cyanogenetic glycoside, phaseolunatin, noted from nonresistant and resistant bean varieties as well as flax and clover, "acts as a phagostimulant for the Mexican bean beetle larvae at lower concentrations, but at higher concentrations it acts as a deterrent or inhibitor." This compound, like our "volatiles" fraction, never promoted feeding by itself, but only in combination with a sugar (they employed glucose).