were desiccated. Many of the agar plates contained fungal growth. Two embryos were found when ova dissections were conducted; both were from ova stored in the 4°C chamber. One of the embryos, as shown in Fig. 5, was almost completely developed and appendages were apparent. Upon dissection from the shell a bright red eyespot was apparent.

The chromosome sets along with the evidences of embryo development indicate that at least some of the ova are capable of development if placed under the proper environmental conditions.

Genetic recombination in sexual generations is a possible means of biotype development; however, information thus far is not conclusive enough to determine the significance of sexual generations in field or greenhouse populations.

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Feeding and Nutrition of Insects Associated with Soybeans. 2. Soybean Resistance and Host Preferences of the Mexican Bean Beetle, *Epilachna varivestis*[1,2]

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ABSTRACT

Dual choice tests were used to analyze food preferences by the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae). Seven series of tests including 26 comparisons of Clark normal, Harosoy normal, and Bragg soybeans with 6 Clark and Harosoy isolines, 9 resistant selections, and 5 other species of legumes were performed. It is suggested that there is no evidence for an influence of leaf pubescence on the acceptance of Clark and Harosoy soybeans. All Clark isolines were preferred to Harosoy normal, regardless of leaf pubescence. The selections PI's 227,687 and 171,451 were low in the food preference scale indicating a strong nonpreference type of resistance. PI's 229,358, 243,519, and 81,777 were partially rejected by adult beetles. F_1 cross of Bragg × PI 229,358 showed a preference index intermediate between that of the parent lines suggesting a semidominance type of inheritance of the resistance trait. Soybean-associated Mexican bean beetles do not seem to have drastically shifted their food preferences, as they prefer snap and lima beans even under action of possible induction effect of a continuous soybean diet. The paired comparison test seems to be a useful subsidiary bioassay in a breeding program for soybean insect resistance.

Early taxonomic literature on the Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant, made no reference to host plants (Mulsant 1850, Crotch 1874). The first host records appeared only after the insect was recognized as a pest on garden beans in the western United States (Riley 1883; Wielandy 1889, 1891; Gillette 1892). However, it has been suggested that native hosts might be species of *Phaseolus* and *Desmodium*, genera of Leguminosae with strong Neotropical representation (Howard 1924, Lippold 1957[3]). This assumption is supported by the present-day patterns of host preference and the relative frequency of occurrence of the beetle on plants of these genera.

The expansion of the food range and the distribution of the MBB have been recorded in detail during the past 75 years as its economic role increased. The association of the MBB with soybeans could have begun only after the introduction of this plant into the New World in the mid-1800's (Piper and Morse 1910). The association is therefore a rather recent occurrence, with MBB pest status on this crop showing considerable temporal and spatial variation. In general, the species causes significant damage to soybeans in the Atlantic Coast States, from Delaware to northern Florida, and west into Alabama. Soybeans in certain regional pockets in south and central Indiana suffer economic damage every year, and the species is found on soybeans in central and southern

[1] Coleoptera: Coccinellidae.

[2] Received for publication Nov. 19, 1971.

[3] P. C. Lippold. 1957. The history and physiological basis of host specificity of the Mexican bean beetle, *Epilachna varivestis* Muls. (Coleoptera: Coccinellidae). Ph.D. thesis, Univ. of Illinois, Urbana. 145 p.

Illinois and southern Ohio. It appears that MBB-soybean associations are in a state of flux, the causal factors for soybean field infestations remaining a problem for further investigation.

Host preferences of the MBB were observed in the field by Hinds (1921), Howard (1922, 1924), Thomas (1924), Friend and Turner (1931), White (1940), Elmore (1949), and Auclair (1959), among many others (for a more complete bibliography see Nichols and Kogan 1972). Wolfenbarger 1961[4] screened many lines and varieties of several species of beans, including soybeans, for resistance to insects. He concluded that, under Ohio field conditions, 154 varieties and lines of soybeans showed varying levels of resistance to the MBB. Lippold[3] tabulated published host records and preferences based on field and laboratory observations and performed a series of qualitative and quantitative comparative studies. These accounts indicate that the actual host range of the MBB is restricted to some species of *Phaseolus, Desmodium,* and *Glycine* with a few other genera of Leguminosae eliciting considerably lesser degrees of acceptance.

In my current studies on the behavioral and physiological basis of insect-soybean associations I use the MBB because it shows considerable host specificity. One objective of these studies is the understanding of the nature of soybean resistance to insect attack. I report here the results of a series of paired comparison preference tests designed to investigate: (1) the role of leaf pubescence of 4 near isogenic lines of each 'Clark' and 'Harosoy' soybeans in food acceptance by the beetles; (2) the patterns of relative preference of 9 plant introduction lines selected for insect resistance in Maryland and South Carolina[5] (Van Duyn et al. 1971); (3) the inheritance of the resistance trait in F_1 crosses of one resistant line as compared to both parent lines; (4) the hypothesis that soybean-feeding MBB represent an evolving biological race which is in the process of shifting its pattern of host preference. Concurrently, the usefulness of the paired comparison preference tests as an accessory bioassay for screening of resistance in breeding programs of soybeans has been evaluated.

MATERIALS AND METHODS

Insect Cultures.—Test insects were reared on Clark 63 and Harosoy soybeans. A rearing technique described previously (Kogan 1971) was mainly employed but later a new rearing method was adopted that greatly reduced handling time and was more productive. The latter method used rectangular, clear-plastic, refrigerator crispers with a bottom layer of 1-cm-thick plaster of paris which was kept moist and covered with a sheet of moist blotting paper. Soybean leaflets were layered in the box and hatching eggs were placed on top of the leaves. To renew the food supply, leaflets and larvae were covered with a sheet of perforated brown paper and fresh leaves were layered on it. Larvae moved upward to the fresh leaves within 3–6 hr, passing through the holes in the brown paper. The latter was then lifted and the old leaves were removed. The method allowed easy supply of fresh food without need for individual manipulation of larvae. The number of larvae per container varied with age and an average density of 1/10 cm^2 of box surface was achieved by the 3rd stage. Adults were kept on bouquets of soybean trifoliates in the manner described previously (Kogan 1971). Only females one week old or older were used in the tests.

Plant Materials.—Table 1 lists the species, varieties, lines, and one cross used in tests. All plants were grown in the greenhouse with supplementary lighting when needed to maintain at least a 14-hr

Table 1.—Plants used to test host preferences of Mexican bean beetle adult females.

Source and species	Variety	Line	Pubescence type	Maturity group
USDA Soybean Lab.—Urbana, Ill.				
Glycine max	Harosoy	L-2	Normal	2
	Harosoy	L62-561	Glabrous	2
	Harosoy	L63-1097	Curly	2
	Harosoy	L62-801	Dense	2
	Clark	L-1	Normal	4
	Clark	L62-1385	Glabrous	4
	Clark	L63-2435	Curly	4
	Clark	L62-1686	Dense	4
	Bragg	...	Normal	7
USDA—Stoneville, Miss.				
		PI 171,451	Normal	7
		PI 227,687	Dense	8
		PI 229,358	Normal	7
College Park, Md.				
		PI 80,837	Dense	4
		PI 81,777	Normal	4
		PI 89,784	Normal	3+
		PI 103,091	Appressed	4
		PI 157,482	Normal	4
		PI 243,519	Normal	4
USDA—Stoneville, Miss.				
	Bragg × 229,358[a]		Dense	7
Commercial varieties				
Phaseolus vulgaris	Burpee's stringless	no. 6163	...	...
P. lunatus	Fordhook	no. 6183	...	...
P. aureus	[b]	[b]	...	...
P. mungo	[b]	[b]	...	...
Illinois Natural History Survey, Urbana				
Medicago sativa	[b]	[b]	...	...

[a] F1 cross.
[b] Unidentified.

[4] D. Wolfenbarger. 1961. Resistance of beans (*Phaseolus, Glycine max, Vigna sinensis, Vicia fabae,* and *Dolichos lablab*) to the Mexican bean beetle and the potato leafhopper. Ph.D. thesis, Ohio State University, Columbus. 137 p.

[5] Thanks are due Drs. E. E. Hartwig, Delta Branch Experiment Station, Stoneville, Miss., and J. A. Schillinger, University of Maryland, College Park, for supplying the seeds of the resistant lines. Dr. Hartwig supplied seeds of the lines selected in South Carolina. Dr. R. L. Bernard, USDA Soybean Laboratory, Urbana, Ill., supplied the seeds of all other soybeans used in the experiments.

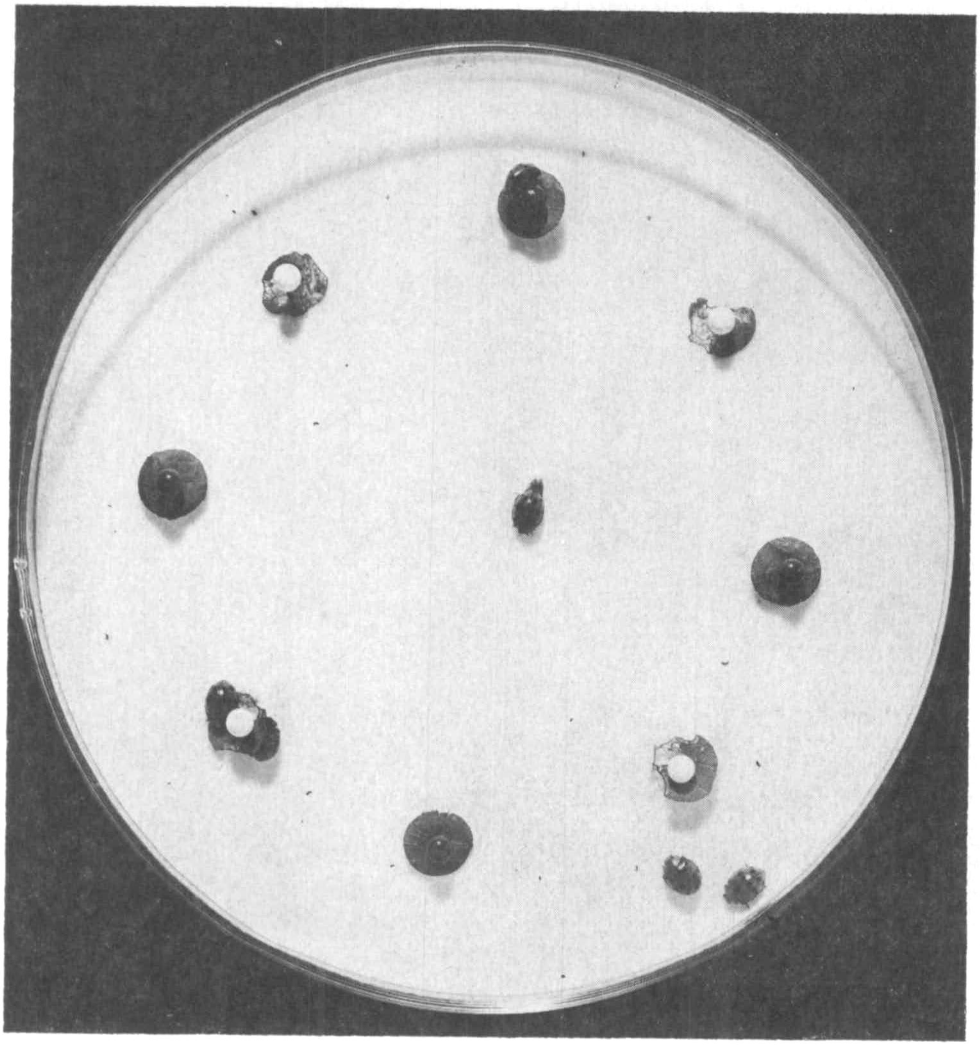

FIG. 1.—Dual choice preference test with standard plant leaf discs on white maptacks and test plant leaf discs on dark maptacks. Notice strong nonpreference to the test plant displayed by Mexican bean beetles.

photophase. Leaves were clipped from plants 6 weeks old or older, and effort was made to use leaves of uniform size, age, and similar state of turgidity. No attempt was made to compensate for differences in rates of development of plants in different maturity groups.

Testing Procedure.—Paired comparison tests, slightly modified from Kogan and Goeden (1970), were used. Tests were conducted in a 150×20-mm petri dish arena, the bottom of which was covered with a layer of plaster of paris and then with a disc of Whatman no. 1 filter paper. The bottom layer was saturated with distilled water prior to the tests. Four 11-mm-diam leaf discs, punched from freshly excised leaves of the standard plants (see hereinafter) and the test plants, were skewered on maptacks and positioned alternately near the perimeter of the arena (Fig. 1). Six beetles which were starved 12–16 hr were allowed to feed for 3 hr in each arena during which time they were kept in the dark at 27±2°C. Each paired comparison was replicated 20 times in 5 series of tests and 30 times in 2 others.

Test Series.—Harosoy normal, Clark normal, and 'Bragg' soybeans were used as the standards for comparison in 7 series of tests. These series were designed to permit analysis of the various aspects defined as objectives of this investigation (see introduction). The composition of each test series and its purpose are described in Table 2.

Quantitative Evaluation of Feeding.—Nonconsumed portions of leaf discs were photographed under UV light against a fluorescent background. The resulting picture of the spots was read with the aid of a photodensitometer. Photometer readings were used to calculate a host index used in the analyses of the results. The technique was described in detail by Kogan (1972).

Analytical Methods.—Results of dual choice pref-

Table 2.—Combinations of standard and test plants grouped in 7 series used to determine patterns of host preferences by Mexican bean beetle adult females.

Series	Standard plant	Test plant	Objectives of tests
I	Clark normal	Clark glabrous	The role of leaf pubescence in patterns of food preference.
		Clark curly	
		Clark dense	
II	Harosoy normal	Clark normal	
		Clark glabrous	
		Clark curly	
		Clark dense	
III	Harosoy normal	Harosoy glabrous	
		Harosoy curly	
		Harosoy dense	
IV	Harosoy normal	PI 171,451	Patterns of food preferences of lines selected for Mexican bean beetle resistance. Degree and nature of resistance.
		227,687	
		229,358	
		80,837	
		81,777	
		89,784	
		103,091	
		157,482	
		243,519	
V	Harosoy normal	*P. vulgaris*	Shifts of feeding habits of Mexican bean beetles in their association with soybeans. Induction effects.
		P. lunatus	
		P. aureus	
		P. mungo	
		M. sativa	
VI	Harosoy normal	Bragg	Inheritance of resistance characteristics.
		PI 229,358	
		Bragg × 229,358[a]	
VII	Bragg	PI 229,358	
		Bragg × 229,358[a]	

[a] F_1 cross.

erence tests were expressed as a host preference index (C) used by Kogan and Goeden (1970).[6] Theoretically, C varies between 0 and 2, with $C = 1$ denoting no preference of a test plant in comparison to the standard; $C>1$, preference for the test plant; and $C<1$, preference for the standard. Fig. 2 shows results of 3 comparisons exemplifying the types of feeding that would lead to obtaining C equal to, less than, or greater than 1.

For each test series, a control was run in which all 8 positions in the arena were occupied by the standard plant. If no factor other than random distribution of the beetles in the arena accounted for variations in patterns of feeding on the 8 discs, the mean value of $\overline{C}$ for all replicates of control (Co) should approximate 1. These values were used to calculate a correction factor (K) such that $K = (1 - \overline{C}o)/\overline{C}o$ which was then used to correct C values within each series of tests following an adaptation of Abbott's formula: $C_K = C(1 + K)$, where C_K is the corrected value of C.

[6] $C = \frac{2T}{T+S}$, where T = feeding on test plant discs and S = feeding on standard plant discs (in photometric units).

The null hypothesis established that treatments with mean values of $C_K \approx 1$ did not differ from the standard. The hypothesis was tested using a simple t test, following $t = (\bar{X}i - 1)/S_{\bar{x}}$. Significant differences at 0.05 level were used in the interpretation of results. An LSD test with t at 0.05 level was used to compare treatment means within each series. All series had a highly significant F for treatments.

RESULTS AND DISCUSSION

Results are graphically presented in Fig. 3, 4, and 5, including the means and 95% confidence limits (fine vertical lines) for all treatments with heavy vertical bars connecting treatments that are not significantly different at 0.05 levels using the t or LSD tests. Table 3 shows the values of C obtained for controls in all series of treatments.

Role of Leaf Pubescence in Patterns of Food Preference.—There is strong evidence that leaf pubescence is inversely correlated with hopperburn and leafhopper infestations. Wolfenbarger[4] found that resistance to hopperburn did not necessarily follow resistance to leafhoppers as some lines with irregular appressed pubescence were resistant to hopperburn but susceptible to leafhoppers. He concluded with Poos and Wheeler (1943) that factors other than leaf pubescence are responsible for resistance to hopperburn. Wolfenbarger's studies were based on comparisons of several different lines and varieties representative of many types of pubescence. These plants evidently differed genetically in many aspects other than leaf pubescence. The role of leaf pubescence on hopperburn and leafhopper infestation could be more precisely assessed after near isogenic lines of Clark and Harosoy soybeans were developed. These lines differ only in the type of pubescence, a character which is apparently controlled by single genes (Bernard and Singh 1969). Singh et al. (1971) described the morphology of pubescence and discussed its relationship to plant vigor, and concluded that leafhopper damage was possibly the causal factor in vigor reduction of glabrous and curly plants. Kogan and Armbrust (unpublished data) recorded, in experimental plots, populations of the potato leafhopper on normal, glabrous, curly, and dense pubescent types of both Clark and Harosoy. Results were coincident for both Clark and Harosoy isolines: populations of the potato leafhopper were 3–12 times larger on glabrous and curly than on normal or dense soybeans.

The effect of leaf pubescence on insects with chewing mouth parts is less clear. Kogan and Armbrust (unpublished data) found no apparent differences in field populations of *Cerotoma trifurcata* (Forster), *Diabrotica longicornis* (Say), and *D. undecimpunctata howardi* Barber on the 4 isolines of Clark and Harosoy. According to results of present tests (Fig. 3), female MBB seem to prefer all 4 types of Clark to Harosoy normal (Series II) regardless of leaf pubescence of the former variety. Compared within their own genetic backgrounds (Series I and III), curly and glabrous Harosoy were preferred to dense

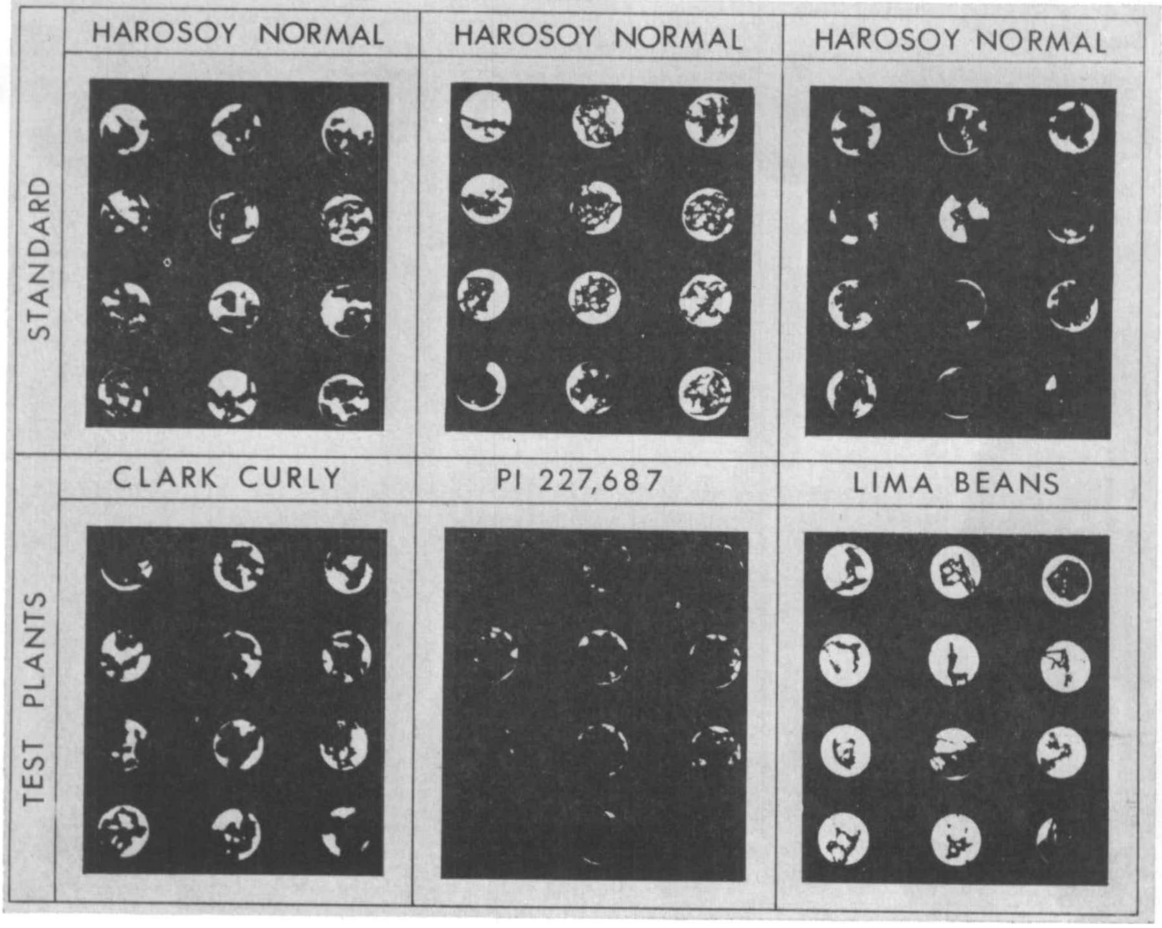

FIG. 2.—Response of Mexican bean beetles, illustrating typical examples of 3 ranges of preference index C. C≈1 (Harosoy normal × Clark curly, left); C<1 (Harosoy normal × PI 227,687, center); C>1 (Harosoy normal × lima beans, right).

and normal (Series III); however, dense and normal Clark were superior to glabrous and curly (Series I) (Fig. 3).

Despite all efforts to keep plants under uniform growing conditions, the possibility of occurrence of physiological variation among lines is not ruled out. This variation could probably account for the evident discrepancies of results of tests in Series I and III.

Table 3.—Mean C values of controls for the position of leaf discs in the arena in all series of tests, and calculated correction factors.

Test series[a]	Standard plant	$\bar{C}o \pm SD$	Correction factor ($K = 1 - \bar{C}/\bar{C}o$)
I, III, IV, V	Harosoy normal	1.0087±0.0883	−0.0086
II	Clark normal	1.0029± .0714	− .0028
VI	Harosoy normal	0.9583± .1170	.0435
VII	Bragg	1.0001± .1694	.0000

[a] See Table 2 for description of test series.

However, when interpretation is based on the combined results of Series I, II, and III, it seems licit to propose that leaf pubescence of Clark and Harosoy isolines had little or no influence in the patterns of acceptance of host plants by the female MBB.

Degree and Nature of Resistance of Selected Soybean Lines.—The bioassay used here was designed to test short-term responses expressed as comparative preference (C). The kind of resistance that was observed would fall within Painter's nonpreference modality (Painter 1968). The 9 plant introductions that were tested were originally selected in Maryland (J. A. Schillinger, personal communication) and in South Carolina based on field and laboratory observations (Van Duyn et al. 1971). The 3 southern selections were superior to those from Maryland in terms of resistance. In particular, PI's 171,451 and 227,687 were practically rejected by the beetles, resulting in the lowest C values obtained so far (0.689 and 0.601 respectively) (Fig. 4). The Maryland selection 243,519 showed reduced acceptance and was considered partially resistant to adult beetle feeding. Ranking of nonpreference under the conditions of these experiments must be interpreted with caution

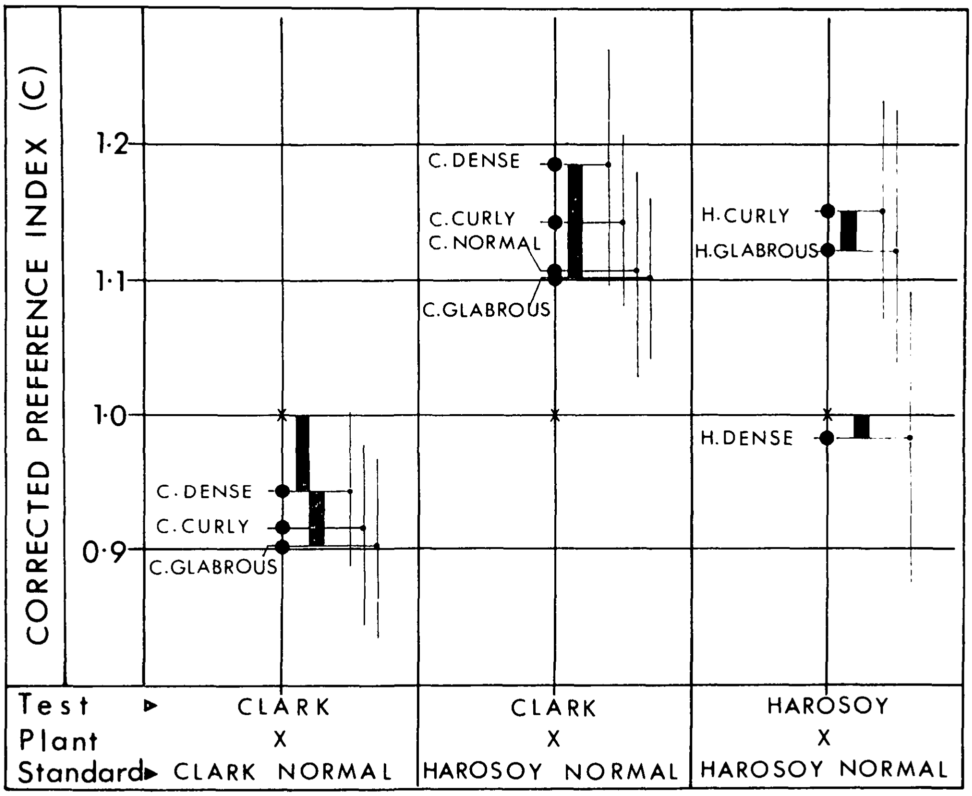

FIG. 3.—Graphic representation of results of test Series I, II, and III (from left to right). Vertical bars connect treatments that are not statistically different (LSD test, 0.05 level). Vertical lines indicate confidence limits at 0.05 level.

because the plants that ranked best belong to much later maturity groups than the standard. All Maryland selections are in group 3+ and 4 and the standard is in group 2, thus they were physiologically much more akin than the very late selections from the South which are in groups 7 and 8. However, tests in Series VII suggest that indeed the southern selection 229,358 is strongly nonpreferred even when compared to a standard in its own maturity group (Bragg, group 7). All these selections were included in studies of other modalities of resistance but further work on the chemical and physical factors associated with resistance concentrated on PI's 171,451, 227,687, 229,358, and 243,519. Results of these studies will be reported elsewhere.

Inheritance of Resistance in F_1 Crosses. — PI 229,358 was crossed with Bragg[7] in the initial steps of a breeding program for incorporating the resistance traits into varieties of acceptable commercial value. Even at this early stage, it was of interest to observe the response of the beetles to the cross. Tests in Series 6 and 7 were designed to compare the preferences of the F_1 and both parents to a standard (Harosoy normal) and to compare the resistant line and the F_1 to the susceptible parent (Bragg). Results (Fig. 5) show that the F_1 elicited an intermediate response between the 2 parents. Bragg and the F_1 did not significantly differ when compared to a Harosoy normal standard in Series 6, but when compared to Bragg as standard (Series 7) the difference was highly significant. The reduced acceptance of Bragg as compared to Harosoy, under the present conditions, could account for the elimination of difference between the F_1 and Bragg; however, the tendency is clearly demonstrated that the F_1 inherited part of the resistance factor and that no dominance is demonstrated to either resistance or susceptibility.

Evaluation of a Soybean-Preferring Mexican Bean Beetle Race.—Well-documented examples of dramatic shifts of insect-hostplant associations are not very numerous and several decades of active biological weed control practice support the assumption that those cases are rare indeed (Anonymous 1968). However, there are many instances of expansion of the host range of an insect at the expense of plants that are botanically and/or chemically related to the original host. The Colorado potato beetle is perhaps the best known of these cases. Early reports on the MBB were rather omissive about the beetle's relationship to soybeans. Wielandy (1891) wrote: "I have not been able to learn that it feeds upon any other plant except those of the *Phaseolus* family . . ." (sic). Still, in 1898, no particular reference is found of attacks to

[7] E. E. Hartwig performed the crosses and kindly supplied the seeds of the plants used in these tests.

soybeans (Chittenden 1898). Many varieties of *P. vulgaris* first, and of *P. lunatus* next, are repeatedly reported as preferred hosts (List 1921, Crawford 1924, Thomas 1924, Eddy and McAlister 1927, Howard and English 1924). Friend and Turner (1931) reported slight damage to soybeans in the field in Connecticut and rejection of soybean leaves by 1st- and 2nd-stage larvae in the laboratory. White (1940) reported that the insect does not live on young soybeans but will thrive on them later in the season. The current status of the species on soybeans is yet to be defined. It is a serious pest in many soybean-growing areas (Atlantic Coast States, south central Indiana) and it occurs in others but has only marginal importance (Alabama, central Illinois, Mississippi, for example).

The hypothesis of the MBB evolving a new "soybean-preferring" race was tested based on the so-called induction effect of a food plant on host preferences. The test beetles were collected on soybeans (Kogan 1971) and reared continuously on these plants for over 18 generations. Should a marked preference for soybeans be a characteristic of this population, it would be expected to evidence this

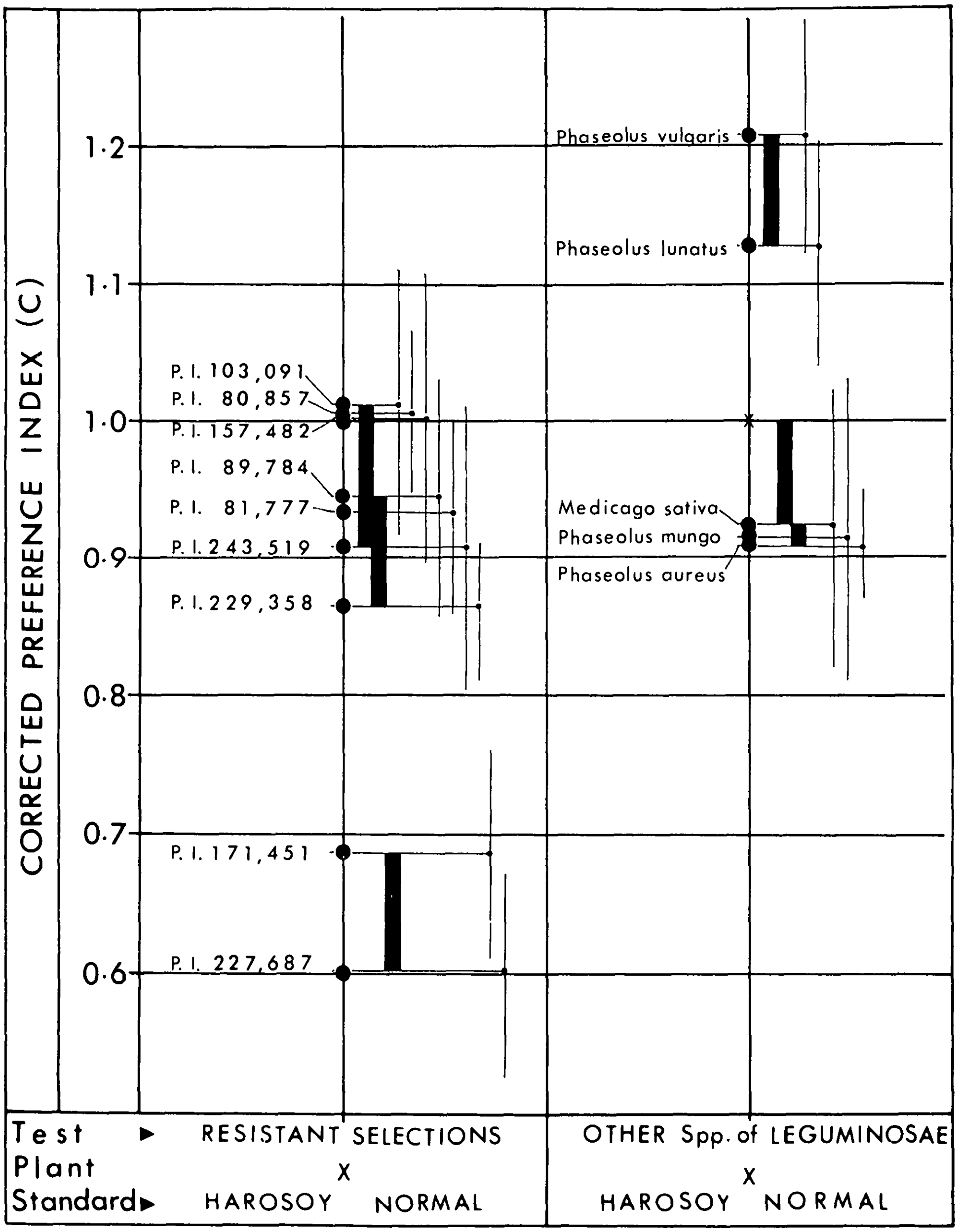

FIG. 4.—Graphic representation of results of test Series IV (left) and V (right). Vertical bars connect treatments that are not statistically different (LSD test, 0.05 level). Vertical lines indicate confidence limits at 0.05 level.

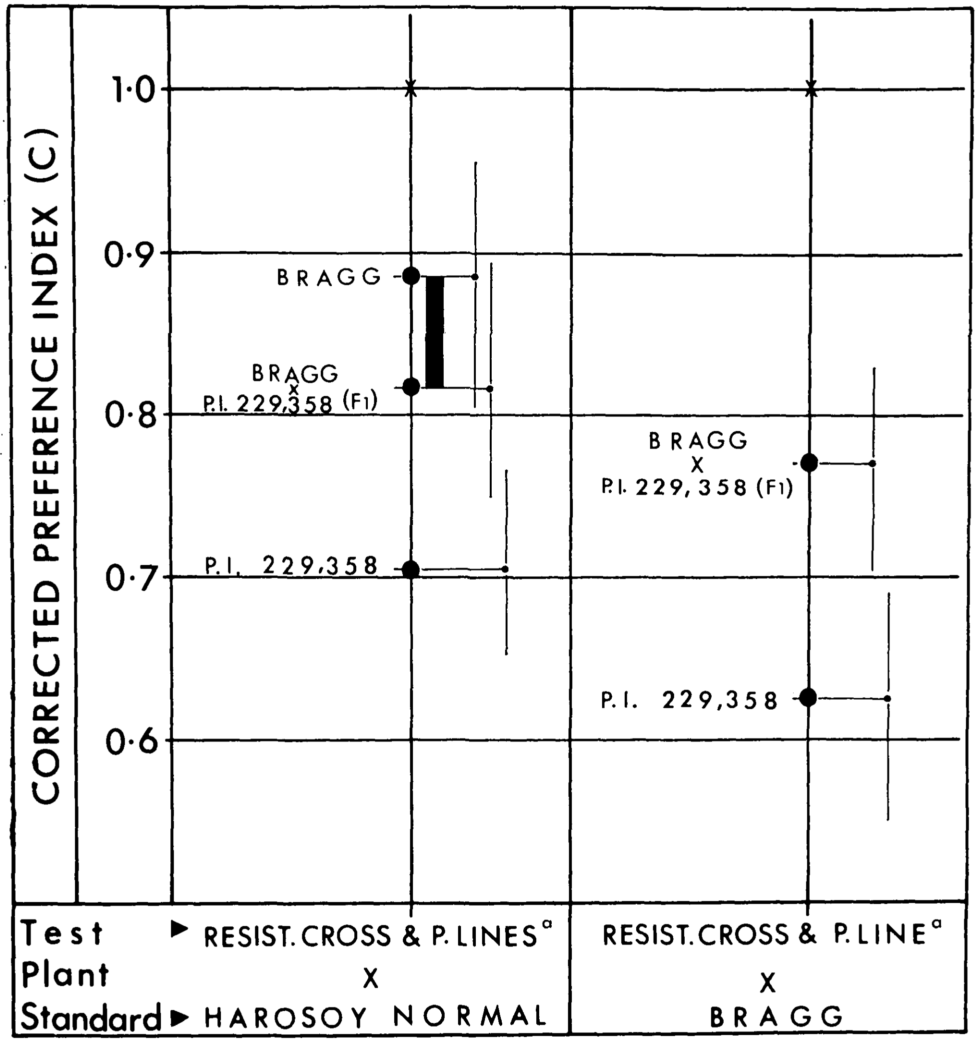

FIG. 5.—Graphic representation of results of test Series VI (left) and VII (right). Vertical bars connect treatments that are not statistically different (LSD test, 0.05 level). Vertical lines indicate confidence limits at 0.05 level. a, Resistant cross (F_1) and parent line (S).

preference in tests where common or lima beans were offered in choice. The induction effect (if prevalent) would further reinforce the natural tendency of the population to select soybeans as preferred food (Jermy et al. 1968). Fig. 4 shows results of tests in which 4 species of *Phaseolus* and alfalfa were compared to Harosoy normal. It is evident that when offered a choice, the beetle strongly prefers common and lima beans. This response may even be somewhat depressed by the induction effect. The other 2 species of *Phaseolus* and alfalfa were not preferred.

There is little evidence from these experiments to support the assumption that soybean-attacking MBB represent an evolving race displaying increased preference for the soybean plant. To further test this assumption, similar tests should be conducted with populations originating from common bean fields that have had no history of contact with soybeans—perhaps a native Mexican population. The possibility that soybean-field infestations may be related to the increased ability of certain beetle populations to use soybeans as their main food source is supported in part by studies on the nutritional value of soybeans to the MBB (Kogan, unpublished data).

Finally, it is suggested that the dual choice test is a useful method for screening for resistance and monitoring the inheritance of resistance in breeding for insect-resistant cultivars. Evidently the only type of resistance that is tested by this method is nonpreference. Thus, a poor performance of a selection in a preference test does not necessarily rule out its potential as a source of other type of resistance. The tolerance of soybeans to defoliation may render even a partial susceptibility to foliage-feeding insects perfectly acceptable.

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Colonization of *Lutzomyia trinidadensis* and *L. vespertilionis* (Diptera: Psychodidae)[1]

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ABSTRACT

The laboratory colonization of 2 species of New World phlebotomine sand flies, *Lutzomyia trinidadensis* (Newstead) and *L. vespertilionis* (Fairchild & Hertig) was accomplished through modifications of standard rearing techniques used at Gorgas Memorial Laboratory, Panama. The development of a new adult sand fly feeding and maintenance cage contributed to consistent host-feedings by females facilitating subsequent colonization. The colony of *L. trinidadensis* was terminated after 6 generations. The colony of *L. vespertilionis*, presently in its 6th generation, is being maintained.

Approximately 15% of the more than 250 described species of New World phlebotomine sand flies have been reared from immature stages to adults. Colonization of these flies, however, has been limited to only a few species. *Lutzomyia sanguinaria* (Fairchild & Hertig) and *L. gomezi* (Nitzulescu) colonies, initiated by Hertig and Johnson (1961) in January and February 1959 at Gorgas Memoral Laboratory in Panama, are presently in their 56th and

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