Journal of Research on the Lepidoptera 1(3):169-182, 1963

1140 *W. Orange Grove Ave., Arcadia, California, U.S.A.*

SELECTION OF ALLYL ISOTHIOCYANATE BY LARVAE OF PIERIS RAPAE AND THE INHERITANCE OF THIS TRAIT[1]

WILLLIAM HOVANITZ and VINCENT C. S. CHANG

California Arboretum Foundation, Inc., Arcadia,
Los Angeles State College and
University of California, Riverside

LARVAE WHICH HAVE PREVIOUSLY BEEN FED on a particular food plant are more likely to select that plant than if they had not previously been fed on it (Hovanitz and Chang, 1962*a* and *b*). It was shown that larvae which were fed on kale are more likely to select kale, and larvae which were fed on mustard are more likely to select mustard. Also, larvae which have previously been fed nasturtium have a higher selection on that plant than if they had not been fed on it. This change in food plant selection operates both if the adaptation comes over a period of several generations, or if only for a short time in the individual larval life. Later, it was shown that strains selected for several generations on each of two plants, kale and mustard, had strong selection for their respective food plants (Hovanitz and Chang, 1963*a* and *b*). Individuals from these two strains were then crossed to get the F_1 generation, and these were crossed to get the F_2 generation. The inheritance of this trait appears to be multifactorial but because of the nature of the testing, this point may be open to question.

The data to be presented in this paper were obtained in much the same manner as in the previously mentioned papers (Hovanitz and Chang, 1963*a* and *b*) with the exception that instead of testing the selection of the larvae for the plants themselves, they were tested for their selection of various concentrations of black mustard oil (allyl isothiocyanate). Tests were made on the parental larvae as well as the F_1 and the F_2.

THE STRAINS

The strains of *Pieris rapae* used in these experiments were derived from two original sources. The first, the kale strain, originated with wild females obtained in a cabbage field (*Brassica oleracea* var. *capitata*) in a truck crop growing area near Huntington Beach in Orange County, California. The other, the mustard strain, originated in the fields of the

[1]Aided by a grant from the National Science Foundation.

Los Angeles State and County Arboretum where *P. rapae* is found on black mustard (*Brassica nigra*). Before testing in the experiments here described, the kale strain had passed through more than ten generations in the laboratory on kale (*Brassica oleracea* var. *acephala*) and the mustard strain had passed through more than six generations in the laboratory on black mustard.

EXPERIMENTAL SET-UP

The tests of the larvae were made in much the same manner as has previously been described (Hovanitz and Chang, 1962b) except that in place of the plants to be selected, at various points surrounding the flat, filter papers wetted with various solutions of allyl-isothiocyanate were placed. Preliminary tests had indicated that the larvae were attracted to dilutions of the mustard oil at 10^{-5} to 10^{-9} so the tests were made at these dilutions. Metal mosquito netting was cut in a circular arrangement to serve as a base for the filter paper. These discs of metal netting were placed on top of glass flasks partially buried in soil, secured to corks placed in the flasks and arranged in various parts of the flat according to the disposition shown in Figure 1. The filter paper was then set up top of the metal netting and wetted with distilled water solutions as shown. On one of these, distilled water only was placed so as to serve as a control for the others. To prevent the accumulation of mustard oil concentrations, the solutions were alternated around the flat. The set-up of the experiments is shown diagrammatically in Figure 1 and photographically in Figure 2. The larvae were set in the center of the flat and allowed to select any of the six test papers or to leave the flat without selection. It should be noted that the test papers were above the level of the larvae and therefore that the heavier-than-air mustard oils would drop down to their level. After each trial, the soil in the flat was mixed to prevent the origin of "trails." It should also be noted that it would be much easier for the larvae to leave the flat without any selection except for one factor, namely, that these larvae have a tendency to climb upwards when at all possible. Experience with lepidopterous larvae has shown that descent is usually made by means of dropping down on a silken thread, rather than by walking while ascent is made by walking. This accounts for the selection of the "water" alone just as frequently as they leave the flat without any selection at all.

The mustard oil used in these experiments was a natural product obtained from black mustard seed by compression and distillation. It is available commercially from the Fritsche Brothers firm. Allyl isothiocyanate is the principal component of the oil obtained from *Brassica nigra;* other mustard oils are found in other plants and all are esters of isothyocyanic acid. *Brassica nigra* seeds contain a glucoside, potassium myronate or sinigrin, which undergoes fermentation change due to the enzyme myrosin present in the seeds.

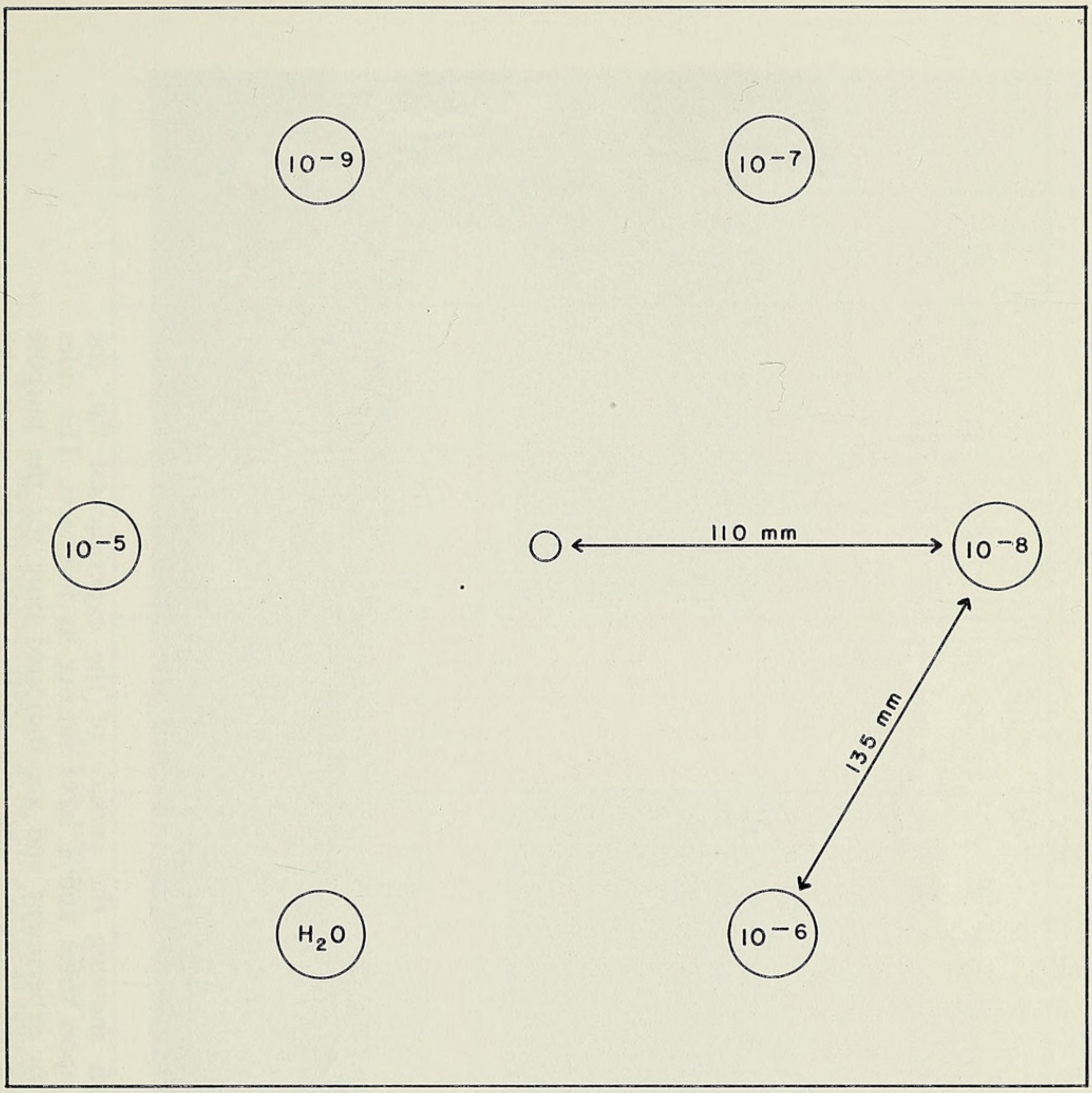

Fig. 1. The set-up for the larval tests. The larvae are placed in the center of the flat from where they are enabled to go in any direction toward one of the dilutions of mustard oil, or to leave the flat. The circles show the disposition of the filter papers which have upon them dilutions of mustard oil as indicated.

THE TEST SELECTIONS

Test selections were made by using a number of larvae for several times to get a combined sample. The number of individuals used depended in part on those available, but ranged from fifteen to forty-five giving a total number of tests from three hundred to nine hundred. These data are given in Table 1.

The parent strains were tested first. The mustard strain was tested with twenty-five larvae 20 or 40 times each giving a total of seven hundred trials. Of these, only nineteen or 2.7 percent made no selection and left the flat. Only twenty-four or 3.4 percent selected the filter paper with distilled water only on it. The others all selected the mustard oils, from a low of nearly 10 percent for the 10^{-9} to a high of nearly thirty percent for the 10^{-6}. These data are shown diagrammatically in Figure 3.

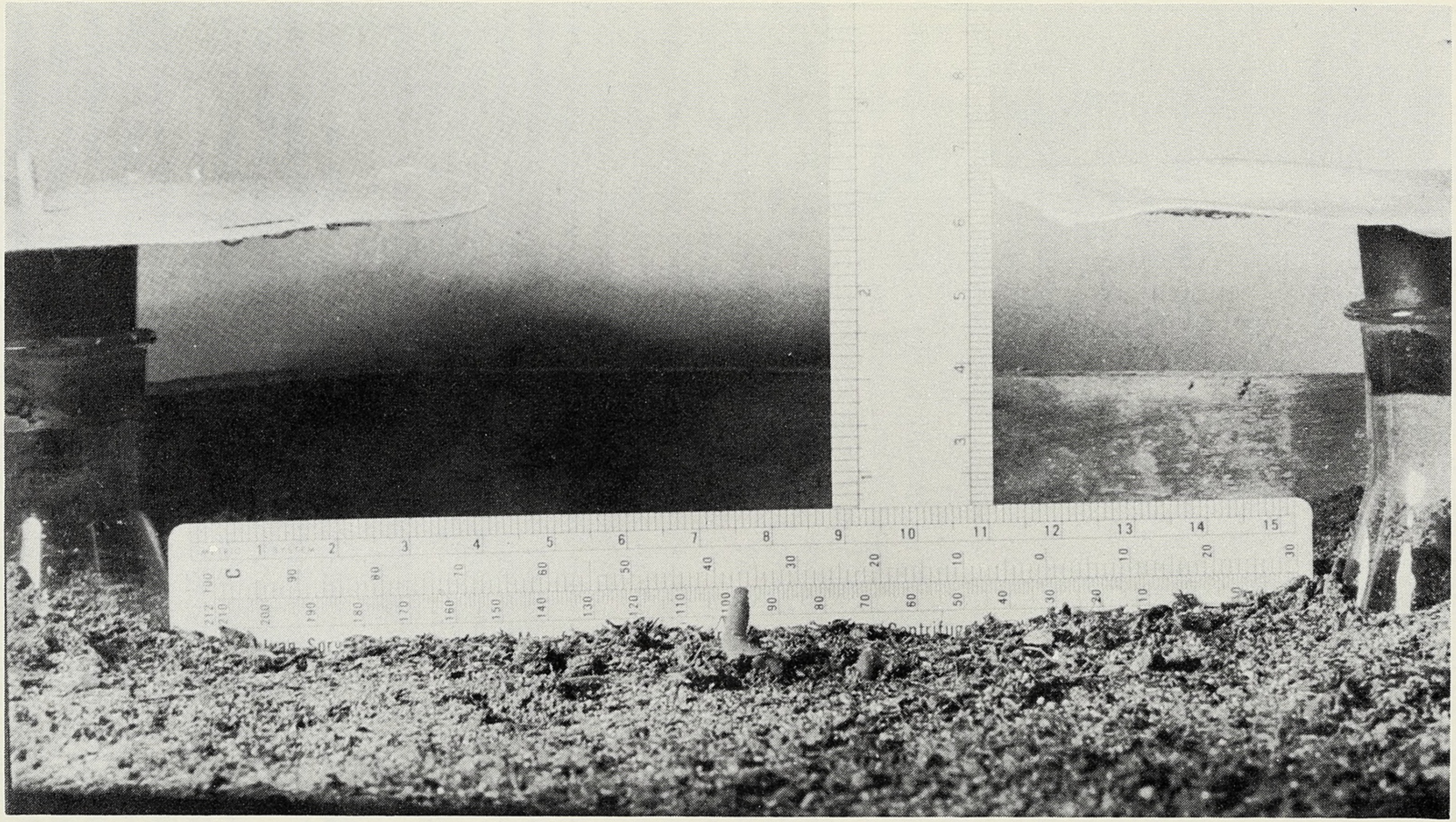

Fig. 2: Photograph showing the surface of the experimental flat, the flasks with filter paper upon them and a larva raising its foreparts. The rules are not present during an experiment and are indicated only for the purpose of illustrating the dimensions between the soil surface, the filter paper and the larvae.

TABLE 1 . The selection by larvae of different strains of *Pieris rapae* for different concentrations of allyl isothiothiocyanate (black mustard oil).

	10^{-5}		10^{-6}		10^{-7}		10^{-8}		10^{-9}		water		none		no. of larvae	total test times
PARENTS																
On mustard	109	15.57%	207	29.57%	141	20.14%	133	19.00%	67	9.57%	24	3.42%	19	2.7%	25	700
On kale	70	12.07%	88	15.17%	86	14.82%	198	34.13%	68	10.34%	19	3.27%	51	8.79%	25	580
F_1																
M♀ x K♂ (on mustard)	58	11.6 %	99	19.8 %	152	30.2 %	99	19.8%	57	11.4 %	28	5.6 %	7	1.4 %	25	500
K♀ x M♂ (on kale)	19	6.33%	55	18.33%	102	34.00%	69	23.0%	34	11.33%	11	3.66%	10	3.33%	15	300
F_2																
M♀ x K♂ (on mustard)	112	12.44%	211	23.44%	231	25.66%	187	20.77%	91	10.11%	42	4.66%	26	2.88%	45	900
K♀ x M♂ (on kale)	114	16.29%	146	20.86%	150	21.43%	124	17.71%	99	14.14%	41	5.86%	26	3.71%	35	700

The kale strain was also tested with twenty-five larvae for a total of five hundred eighty trials. The highest selection of these larvae was toward the concentration of 10^{-8}, larvae going to this concentration in thirty-four percent of the trials. This result is significantly different from the result for the kale larvae indicated above, in which the highest selection was toward the 10^{-6} concentration. These data can be compared diagrammatically in Figure 3. There is little doubt that the larvae of the kale-bred stock prefer the mustard oil at a lower concentration than do those of the mustard strain. It is probably not coincidental that the taste of the leaves of mustard is stronger than is that of kale (or cabbage) since the significant taste of these plants is due to allyl or other isothiocyanates. These data would seem to indicate (1) that the concentration of taste or smell-detectable mustard oils in the leaves of mustard is higher than that in kale, (2) that the larvae feeding on mustard have become adapted in some way to a higher concentration of these oils, and (3) that due to this adaptation, the "mustard" larvae have a greater selection of the higher concentration of mustard oil than the lower. It has been shown in another paper (Hovanitz and Chang, 1963b) that these two strains also select their own plants over the others when given a free choice. This preferred selection may be due wholly or in part to the difference in available allyl or other isothiocyanate in their leaves.

THE CROSSES

The crosses between the two food plant strains were carried out by making reciprocal hybrids. In the one case, the kale strain was used as the female parent and in the other case, the mustard strain. The hybrid larvae resulting from these crosses were fed on the food plant of the mother, since the mother was allowed to lay on its own preferred plant.

The selections made by the hybrid larvae in both crosses favored the concentration 10^{-7}, exactly half way between the selections made by the respective parent strains (Fig. 4). The frequency of selections for the kale female F_1 toward the 10^{-7} concentration was 34 percent as compared with 30 percent for the mustard female F_1.

Previous data have indicated that the immediate larval food plant eaten by the larvae had an effect on the food plant selections made by these larvae. If this held true for the selections of the mustard oils also, then the larvae of the kale female F_1, having been fed on kale during their life, and the larvae of the mustard female F_1, having been fed on mustard during their life, should have a preference for their respective plant. Data from Table 1 (Hovanitz and Chang, 1963b) indicate that this is so. Mustard female F_1 larvae selected mustard nearly 60 percent of the total selections as compared with nearly 50 percent selections for the kale female F_1 larval selections. It should follow, if our

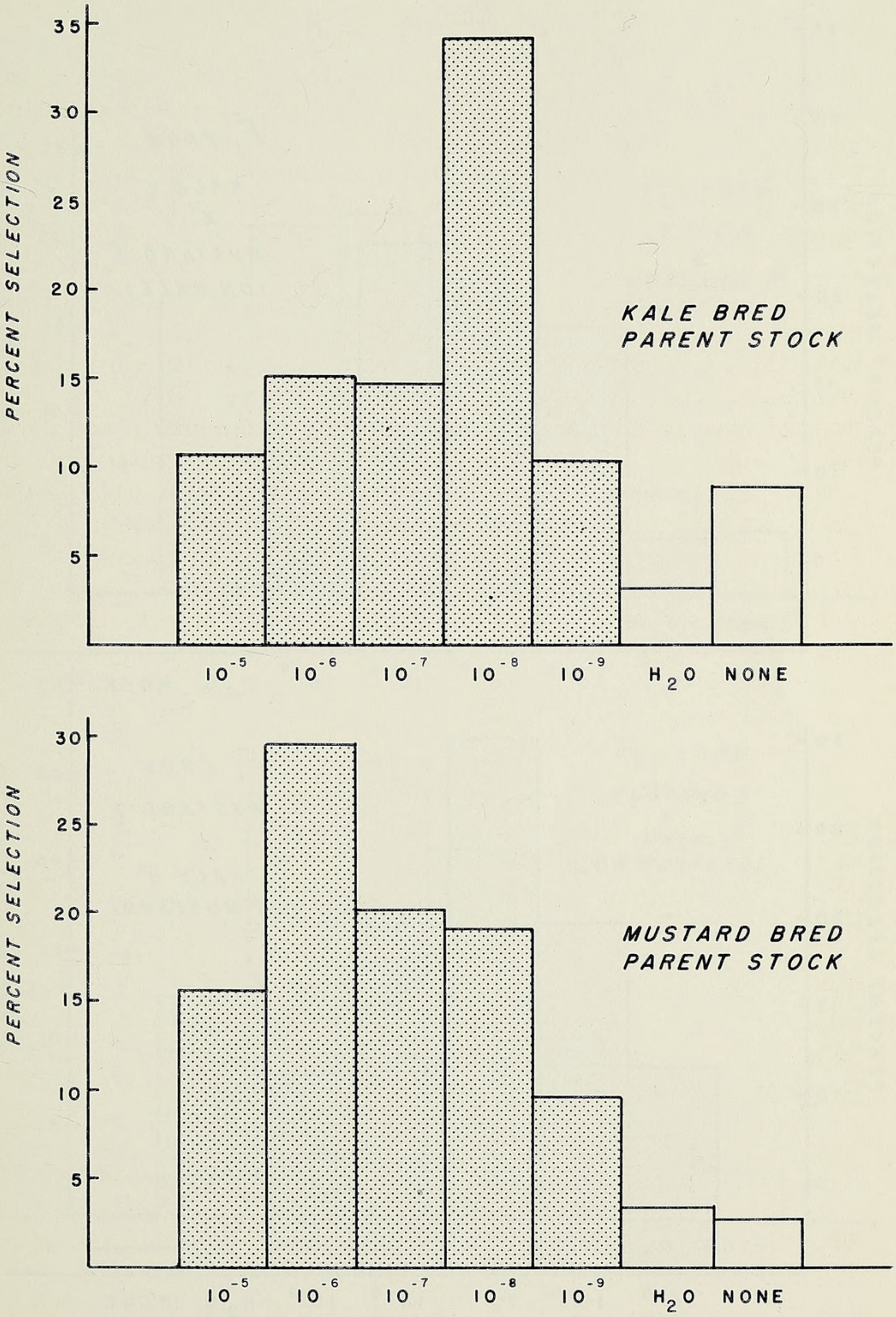

Fig. 3: Histograms illustrating the selections made by the larvae from the parental strains, comparing the kale and the mustard strains.

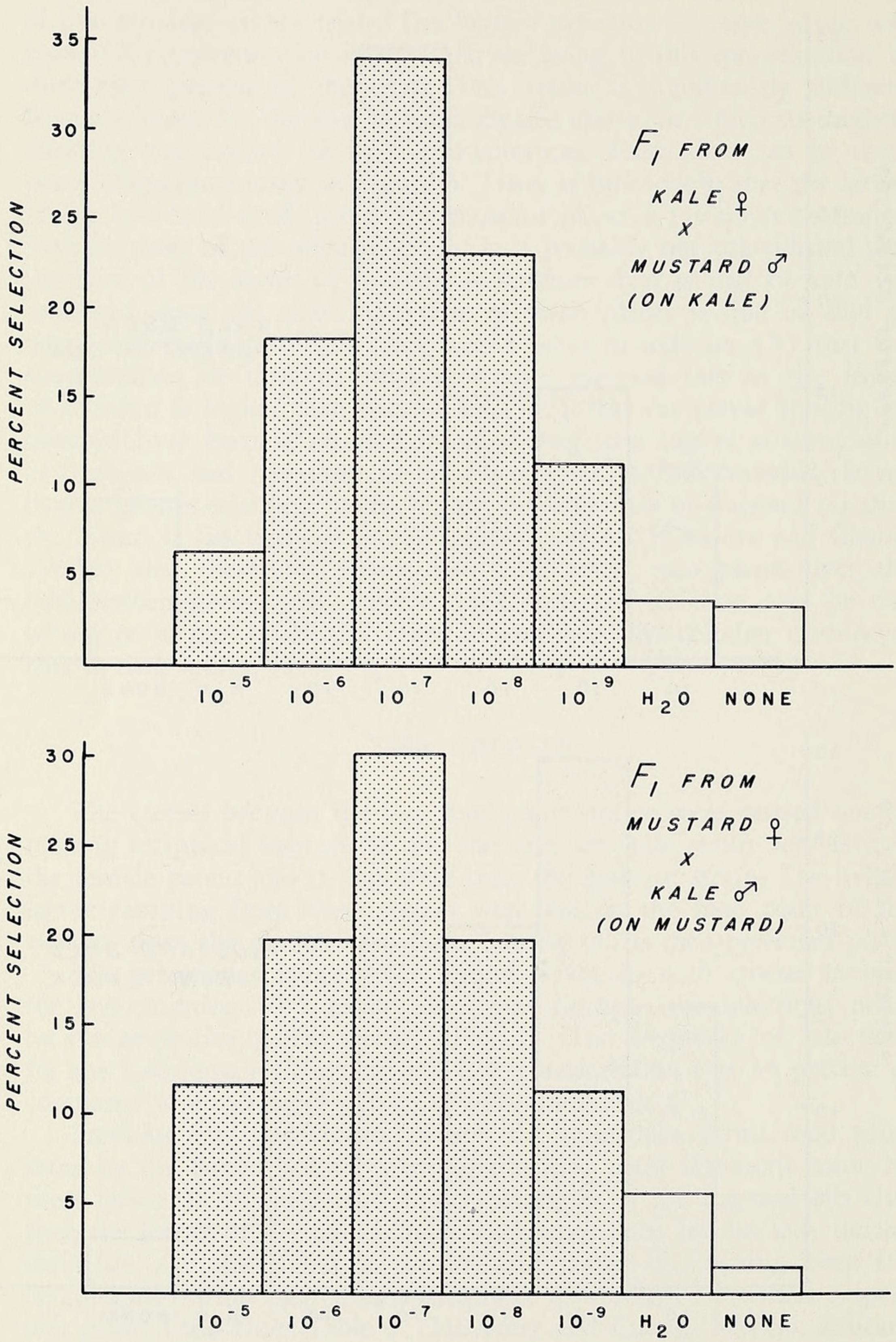

Fig. 4: Histograms illustrating the selections made by the F_1 larvae from the reciprocal crosses between the kale and mustard strains.

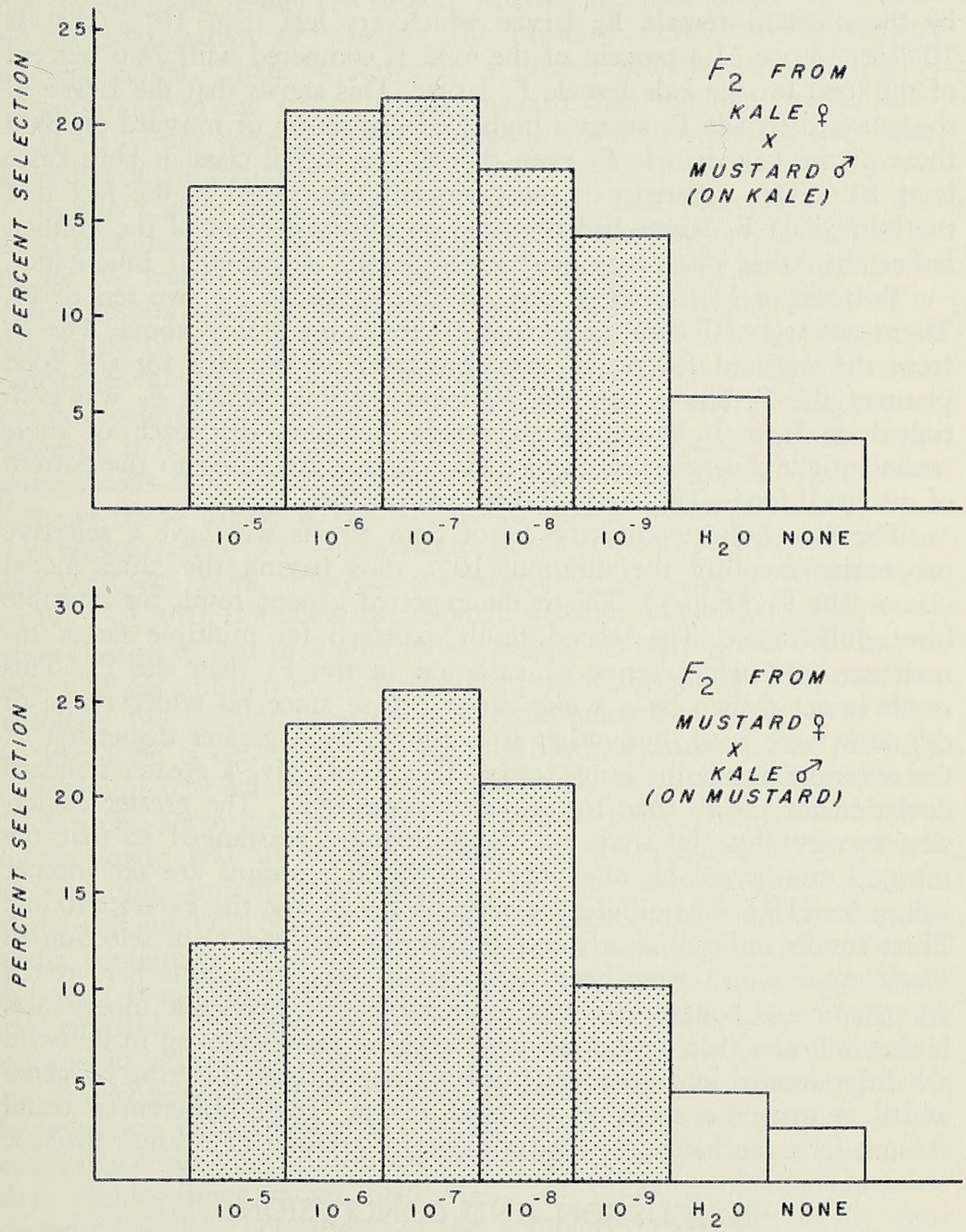

Fig. 5. Histograms illustrating the selections made by the F_2 larvae from the reciprocal crosses between the kale and the mustard strains.

assumptions are correct concerning the relationship between mustard oil concentration and food plant selectivity, that the mustard female F_1 larvae should select mustard oil concentrations at a higher level than the kale female F_1 larvae. That this is so can be seen by study of the histograms (Fig. 4) and the following calculations. Selections made by the mustard female F_1 larvae which are less than 10^{-7} (that is 10^{-5} or $^{-6}$) are 31.4 percent of the total as compared with 24.6 percent of the total for the kale female F_1 larvae. This shows that the larvae of the mustard female F_1 select a higher concentration of mustard oil than those of the kale female F_1 even though the modal class in both cases is at 10^{-7}. This difference is most probably attributed to the fact that the immediate F_1 larvae had been fed on the food plant of the mother, rather than that there was any genetic effect of maternal inheritance.

Two sets of F_2 have been bred, corresponding to the two sets of F_1. These two sets of F_1 are the reciprocal crosses mentioned above. The F_2 from the mustard female F_1 was continued on mustard for the food plant of the F_2 larvae, and the F_2 from the kale female F_1 was continued on kale. It would be expected, therefore, that each of these strains might diverge slightly in their selection according to the pattern of the larval foods. This result did not materialize.

The data indicate that the F_2 of both strains still have a selective propensity favoring the dilution, 10^{-7}, thus having the same modal class as the F_1 (Fig. 5). This is the expected genetic result for multiple factor inheritance. The second result expected for multiple factor inheritance is a wider range of variation in the F_2 than the F_1. This result is not shown by a wider range per se since no wider range of dilutions were used, but rather it is shown by a greater dispersion of the selections inside the range tested. This would give a greater standard deviation for the F_2 than for the corresponding F_1. The greater dispersion is very clear by study of the histograms, rearranged so that the mustard strains are on one page and the kale strains are on another (Figs. 6 and 7). The differences between the F_1 and the F_2 are striking. These results indicate clearly that the factors for food plant selection in *Pieris rapae* which have been separated in the two strains here tested are genetic and behave in a way comparable with polygenic inheritance. It also indicates that the populations of *Pieris rapae* existing in the wild are differentiated into genetically distinct populations, differing by genes which control the selection of food plants. These differences could account for a mechanism needed for species isolation (Hovanitz 1963c).

DISCUSSION AND CONCLUSIONS

The data indicated in this paper show that the strains which have been selected for particular food plant preferences over a period of several generations, and which when tested prefer the food plant upon which they have fed, also differ in their selection (preference) for various concentrations of mustard oil (allyl isothiocyanate). That these differences are genetic is apparent not only from the preceding data on food plant preferences but also on the data on mustard oil preferences introduced in this paper. Crossing of the two strains discussed here gives an F_1 which has a selection exactly intermediate between the two parental strains. The results are similar whether the kale strain contributes the maternal parent or whether the mustard strain contributes the maternal parent. There is, however, a greater selection of the higher concentrations in the strain fed on mustard than the one fed on kale.

The F_2 from these strains is similar to the F_1 except for the wider dispersion of the selections.

In the testing of these strains for food plant preferences, it was found that the larvae developed a preference for nasturtium in the F_1 and F_2. No reason can be deduced for this strange event based upon the present results unless it be that nasturtium emits at a concentration of mustard oil of the same attractive power as 10^{-7} allyl isothiocyanate or some other mustard oil of attractive significance. The odor of nasturtium does not seem to the human to be the same as that of allyl iosthiocyanate. However, it is possible that to the human, the odor of some other substance masks the odor of the attractive mustard oil, but that this odor does not do so in the larvae which are able to detect it.

Thorsteinsen (1953) has made the statement that larvae of the diamond-back moth, *Plutella maculipennis* (Cut.) are attracted to the odor of allyl isothiocyanate from a distance but are repelled by it when closeup so that they will not eat substances having this oil. Instead, they will eat substances with the corresponding glucoside, potassium myronate, but are not attracted to this substance from a distance. Since the glucoside is converted into the allyl isothiocyanate in the plant by a process of fermentation, it is probable that the attraction is really for the isothiocyanate not the glucoside, but that the isothiocyanate is produced slowly and in small quantities by the process of enzymatic fermentation. Indeed, Thorsteinson even indicates "in some experiments the addition of allyl mustard oils slightly increased feeding on media containing sinigrin." Wolfrom (1960) points out that "while the exact biological function of the plant glycosides is not established, it is probable that their formation provides the plant with a means of storing, in a harmless form, toxic and physiologically active materials which may be liberated by enzymes, in small quantities, when required." It seems not only possible but highly likely that the attraction differences noted by Thorsteinson are based upon the low concentration of allyl isothiocyanate rather than upon the presence of the glucoside sinigrin directly. Thorsteinson in fact admits this possibility in his comment, "On the other hand, it is possible that infinitesimal amounts of mustard oil vapor emanating from the leaves [by enzymatic action of myrosin on sinigrin] may stimulate the olfactory sense which is characteristically extremely sensitive in insects." Section in brackets not

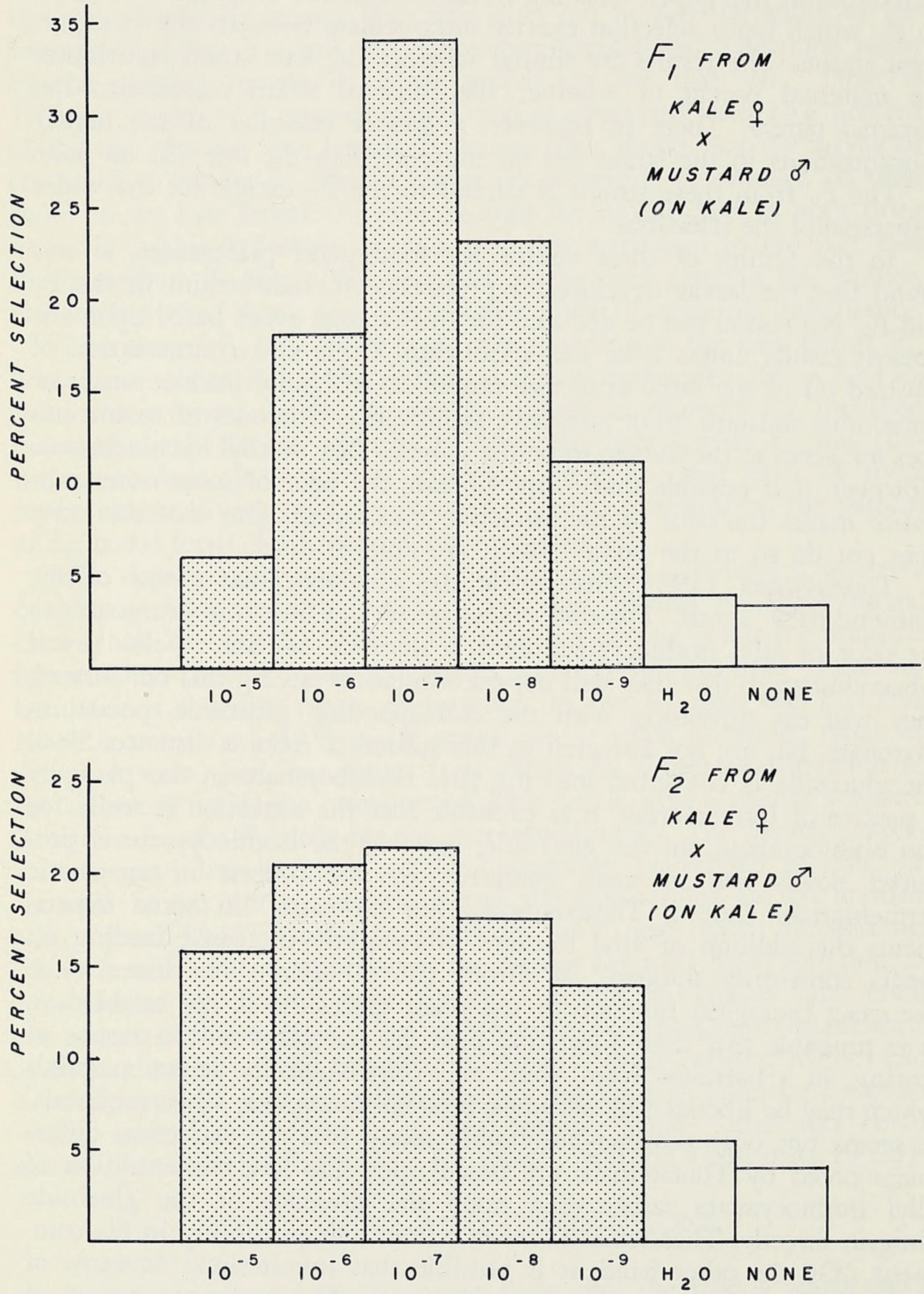

Fig 6. Histograms illustrating the selections made by the F_1 and F_2 larvae, comparing these larvae of the kale female crosses.

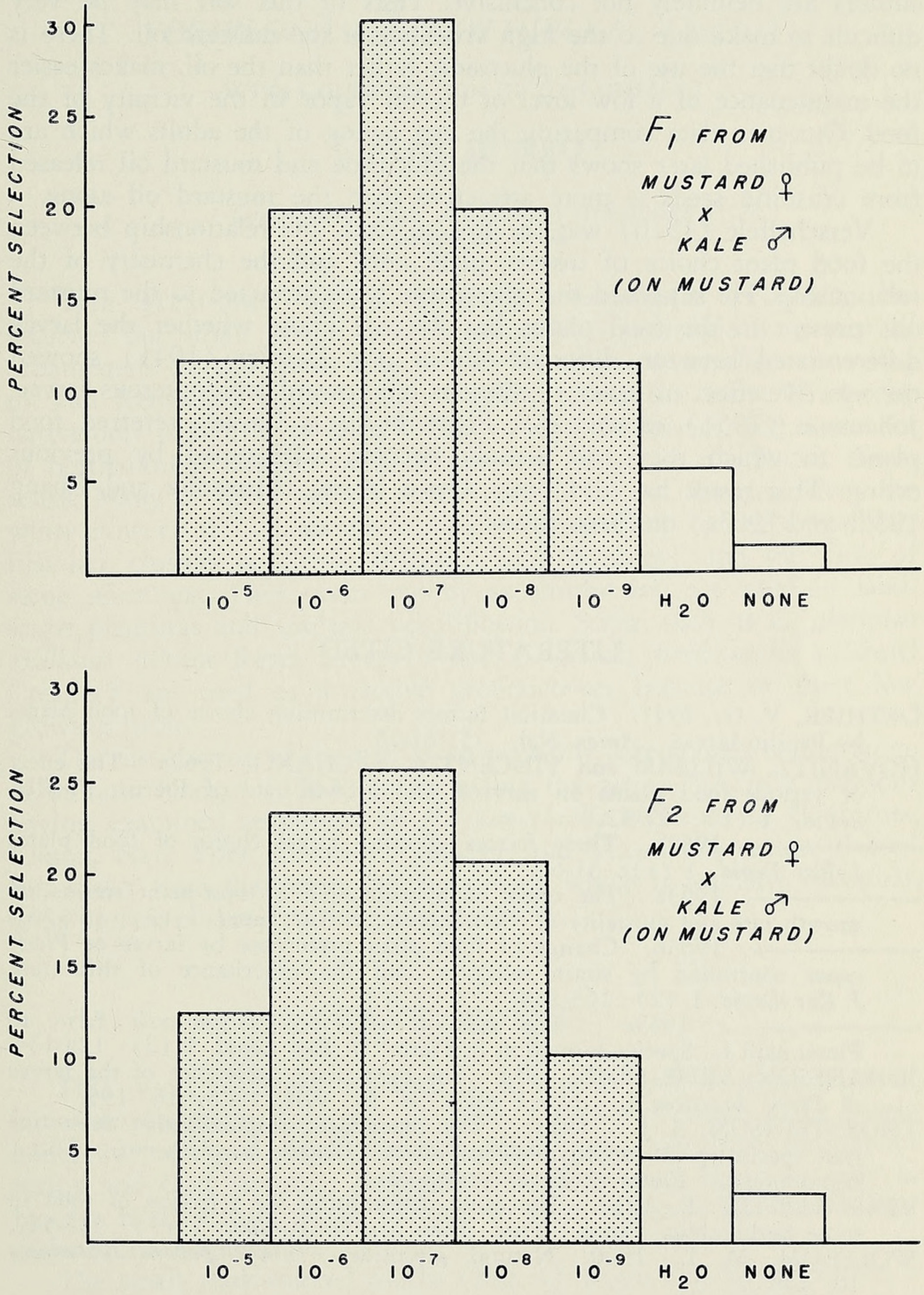

Fig. 7. Histograms illustrating the selections made by the F_1 and F_2 larvae, comparing these larvae of the mustard female crosses.

Thorsteinson's. Further testing of feeding responses of *Plutella* or *Pieris* larvae at a low mustard oil level are required to be certain of this differentiation. Presently published data of the present, or of other, authors are definitely not conclusive. Tests of this sort may be very difficult to make due to the high volatility of the mustard oil. There is no doubt that the use of the glucoside, rather than the oil, makes easier the maintenance of a low level of the oil vapor in the vicinity of the food. Our own data comparing the egg laying of the adults which are to be published later shows that the glucoside and mustard oil released from crushing seeds is more attractive than the mustard oil alone.

Verschaffelt (1910) was the first to study the relationship between the food plant choice of insects, plant odor and the chemistry of the relationship. He supposed that the larvae were attracted to the mustard oils present in the food plants but did not know whether the larvae differentiated between different oils or not. Dethier (1941) showed the selective effect of various odiferous oils toward lepidopterous larvae. Johansson (1951) showed that *Pieris brassicae* larvae preferred food plants to which they had already become accustomed by previous eating. This result has also been shown by us (Hovanitz and Chang 1962b and 1963a) on *Pieris rapae.*

LITERATURE CITED

DETHIER, V. G. 1941. Chemical factors determining choice of food plants by Papilio larvae. *Amer. Nat.* 75: 61-75.

HOVANITZ, WILLIAM and VINCENT C. S. CHANG. 1962a. The effect of various food plants on survival and growth rate of Pieris. *J. Res. Lepid.* 1 (1): 21-42.

————. 1962b. Three factors affecting larval choice of food plant. *J. Res. Lepid.* 1 (1): 51-62.

————. 1963a. The effect of hybridization of host-plant strains on growth rate and mortality of *Pieris rapae. J. Res. Lepid.* 1 (2): 157-161.

————. 1963b. Change of food plant preference by larvae of *Pieris rapae* controlled by strain selection, and the inheritance of this trait. *J. Res. Lepid.* 1 (2): 163-168.

————. 1963c. The relation of *Pieris virginiensis* Edw. to Pieris napi L. Species formation in Pieris? *J. Res. Lepid.* 1 (2): 124-134.

JOHANSSON, ARNE SEMB. 1951 The food plant preference of the larvae of *Pieris brassicae* L. *Norsk Ent. Tid. B.* VIII, 2.4-5: 187-195.

THORSTEINSON, A. J. 1953. The chemotactic responses that determine host specificity in an oligophagous insect (*Plutella maculipennis* (Curt.) Lepidoptera). *Canad. J. Zool.* 31: 52-72.

VERSCHAFFELT, E. 1910. The cause determining the selection of food in some herbivorous insects. *Proc. Acad. Sci. Amsterdam* 13(1): 536-542.

WOLFROM, M. L. 1960. Natural glycosides. *Encyclopaedia Britannica* 10: 448.