1970

An Experimental Study of the Food Plant Preferences of the Black Cutworm, Agrotis ypsilon (Hufnagel)

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An Experimental Study of the Food Plant Preferences of the Black Cutworm, Agrotis ypsilon (Hufnagel)
(TITLE)

BY

Alan C. York

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science in Education

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1970
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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ACKNOWLEDGMENT

I wish to acknowledge my appreciation to Dr. Ralph E. Sechriest for my introduction to the black cutworm, to Dr. Michael A. Goodrich for his interest and aid in this research, and most of all to my wife, Carol, without whose unending support, assistance, and typing skills, this project would not have been completed.

INTRODUCTION

The black cutworm is a most polyphagous insect. Its list of food plants includes apple, asparagus, bean, beets, cabbage, corn, cotton, grape, grass, potato, spinach, squash, strawberry, tobacco, tomato (Forbes, 1905), brinjal, celery, clove, gram, lucerne, marigold, mustard, plantain, radish, sunflower (Maxwell, 1908), Gynandropsis pentaphylla, Solanum xanthocarpum, Solanum nigrum, Trianthema monogyna, Chorozophora rotteri, Chenopodium album (Sen, 1942), onion (Lintner, 1893), castor bean, cauliflower, chick pea, clover, cucumber, cypress vine, lettuce, morning glory, orange seedlings, peach sprouts, pepper, and Russian thistle (Crumb, 1929).

In the laboratory it was found that the larvae will readily eat geranium, wheat, oats, corn, tomato, tobacco, rye grass, red clover, and sweet pea. It is likely that the list of food plants could be made much longer as the species is cosmopolitan, being found in most of Asia, Europe, New Zealand, Australia, Africa, and North America as far north as Manitoba and Hudson Bay (Forbes, 1905) and south to Uruguay. It is evident that the insect is not geographically isolated by a limited range of food plants.

This study was designed to investigate one aspect

of the feeding habits of the black cutworm: that of food plant preference. If food plant preferences exist in monophagous and oligophagous insects, then preferences might well exist, if to a lesser degree, in an insect with more catholic tastes. If such preferences do exist, they should be demonstrable. Such a demonstration was the purpose of this study.

HISTORICAL BACKGROUND

The study of host specificity and preferential feeding in Lepidoptera began in 1905 when Grevillius found that larvae of the browntail moth, Euproctes chrysorrhoea (L.), which commonly feeds solely on chick weed, could be induced to feed readily upon normally unacceptable plants by smearing the leaves of these plants with tannin, a chemical constituent of the chick weed.

Five years later Verschaffelt (1910) found that the range of acceptable host plants of the larvae of Pieris rapae (L.) and Pieris brassicae (L.) coincides with the distribution of plants containing mustard oil glucosides. With a technique similar to that of Grevillius, he found when he smeared a leaf not otherwise eaten with juice from a crucifer, feeding upon the leaf began immediately. Also wheat flour and filter paper moistened with water and a few drops of juice from a crucifer, Bunias orientalis, would be eaten by the larvae. From these experiments Verschaffelt concluded that the mustard oil

glucosides stimulate feeding in these insects and suggested that odor played a part in the process.

The hypothesis that insects were attracted by odor was substantiated by McIndoo (1926a) with a device he termed an olfactometer. This device was a Y-shaped tube through one arm of which passed a stream of ordinary air and through the other arm passed air bearing a test odor. The number of insects located in the arms at the end of the experiment classed the odor either as an attractant or a repellent. That same year he concluded that the Colorado potato beetle, Leptinotarsa decemlineata Say., is definitely attracted to plants by odor (McIndoo, 1926b).

In 1928 Moore discovered that the European corn borer, Pyrausta nubialis Hbn., is attracted to steam distillates and petroleum ether extracts of corn, smartweed, greater ragweed, and cockleburr.

Dethier (1937) established that odor is the most important property in food plant recognition. This was done in a series of classic experiments involving larvae of the milkweed butterfly, Danais plexippus L. One of these experiments, the "screen test," involved placing leaves from several different plants including milkweed on the floor of a cage. A wire screen was then laid on the leaves pressing them flat. Larvae were released onto the screen and could crawl about freely in close proximity to the leaves. They were, however, unable to

touch the leaves and could receive only olfactory stimuli and hygrostimuli. It was found when the larvae crawled about, their paths tended to be straight while passing over leaves not used as food. When passing over milkweed leaves, the larvae tended to follow zigzag paths and make exploratory movements with their heads. These head movements were not frequent when the larvae passed over the edge of a leaf. Fifty percent of the time the larvae would turn back over the edge of a milkweed leaf.

In another experiment Dethier put under the screen three leaves of plants not normally used as food: mullein, oak, and plantain. In addition, he put three of the same leaves coated with milkweed latex under the screen. When larvae were released on the screen and their paths plotted, it was shown that they recognized the milkweed leaves immediately. He further tested the responses of the larvae by coating filter paper with latex, and by making leaf sandwiches. The sandwiches were made by gluing with latex the epidermis of milkweed to the sides of leaves not normally eaten. Larvae readily ate both filter paper coated with latex and the foreign leaf sandwiches.

Raucourt and Trouvelot (1936) introduced a technique of using thin slices of elder pith as a mechanical support for plant extracts. They found this substance to be eaten much more readily than filter paper.

Dethier (1941) went further in the area of diet

selection in his work with the swallowtail larva, Papilio ajax L. In this work he demonstrated the importance of the odiferous essential oils as attractants of insects. When the chemicals which give rise to the odors of the essential oils (caraway odor arises from carvone; celery odor from sedanolid) are applied to filter paper, the filter paper is preferred to the plants normally containing the essential oils.

Chauvin (1945), using the elder pith method of Baucourt and Trouvelot, found that a glycosidic compound extracted from potato leaves proved to be a strong phagostimulant for the Colorado potato beetle.

The following year Brues (1946) published his Insect Dietary. This book, the most comprehensive volume on insect feeding, dealt with all types of insect dietary habits, and included a quite extensive bibliography.

Dethier (1947) produced his outstanding volume, Chemical Insect Attractants and Repellents. In this work he attempted to "bridge the borderline between chemoreception and the broader aspects of behavior based upon it." He proposes that insects are always attracted by odor, but that their choices may be modified by physical repellents, e.g. pubescence, or by undesirable taste.

Thorpe et al. (1947) proposed insect feeding as a cyclic sequence. Their sequence has since been modified (Thorsteinson, 1953; Dethier, 1954) to the following

four steps: (i) finding or orientation to the food source; (ii) initiation of feeding (biting or probing); (iii) maintenance of feeding (swallowing); (iv) cessation of feeding, most often followed by periods of rest, dispersal, or other locomotor activity.

Disagreeing with the token stimuli theory (insects are prompted to bite by a specific chemical or chemicals) of Dethier (1941), Pfadt (1949) believed that with most polyphagous insects, specific stimuli were not required to initiate biting. Instead an insect sampled plants at random until a satisfactory plant was found. He has since been supported in this idea of random sampling by Dadd (1960). Dadd reports that hungry grasshoppers will bite anything available, be it wood, glass, wax, etc.

Dethier (1953) maintained that often insects are stimulated to bite by the same chemical substances which cause their final orientation to the plant. Thorsteinson (1953) made it clear that the larva of the diamondback moth, Plutella maculipennis (Curtis), will not accept an artificial diet unless it contains a small amount of a specific phagostimulant (mustard-oil glucoside) found in its natural food plants. However, the insect will not give an appreciable response to the token stimulus (mustard-oil glucoside) in the absence of the proper nutrients (Thorsteinson, 1955).

Fraenkel (1959) maintained that food specificity in insects is based solely on the presence or absence of

token stimuli. Calling these token stimuli "secondary plant substances," he stated that they have no nutritional value *per se* (Fraenkel, 1953).

Fraenkel (1959) went on to state that the only purpose of these secondary plant substances is that of defense against insects and other organisms. This position tended to agree with that of Dethier (1947), who said that during the evolution of insects from polyphagous habits to monophagous or oligophagous habits, species not only overcame these plant defenses, but evolved the ability to utilize these chemical compounds as specific sensory cues.

The fact that compounds not essential nutritionally exercise an influence on insect feeding has been well documented (Thorsteinson, 1958; Gupta and Thorsteinson, 1960; Nayar and Thorsteinson, 1963; Hamamura and Naito, 1961; Hamamura et al. 1962). This side of the issue is further bolstered by the statement of Ehrlich and Raven (1964) as a conclusion from their extensive study of *Papilio* larvae and their food plants. They concluded, "A systematic evaluation of the kinds of plants fed upon by larvae of certain subgroups of butterflies leads unambiguously to the conclusion that secondary plant substances play the leading role in determining patterns of utilisation."

A somewhat different approach to insect feeding was the "dual discrimination" theory presented by Kennedy

and Booth (1951). This view of host selection was based upon two types of stimuli: "flavor" stimuli, which come from botanically specific substances such as alkaloids, glycosides, etc., and "nutrient" stimuli (carbohydrates, amino acids, etc.). These nutrient stimuli may or may not constitute the entire required nutrients of the insect (Kennedy, 1958).

That nutritional factors play a role in host plant selection cannot be dismissed. Beck (1956) has shown that the choice of feeding sites on corn plants by the European corn borer is determined by the highest concentration of sugars.

The principal phagostimulant for the Mexican bean beetle, Epilachna varivestis Mulsant, is sucrose (Dethier, 1966). Certain amino acids cause aggregation and biting in the case of wireworms (Thorpe et al., 1947). Various sugars and amino acids also play a part in the duration of feeding by the milkweed bug, Oncopeltus fasciatus (Dallas), (Feir and Beck, 1963). Ascorbic acid appears to be a phagostimulant to the diamondback moth larva, a grasshopper, Chorthippus longicornis Latreille, and the Colorado potato beetle (Thorsteinson, 1956).

It has been stated that the presence of inhibitors or deterrents largely determines the frequency of feeding that will occur on a particular plant species. (Fraenkel, 1958; Ito, Horie, and Fraenkel, 1959; Gupta and Thorsteinson, 1960). DeWilde (1958) demonstrated that

larvae of the Colorado potato beetle with palpi removed would eat plants which larvae with intact palpi rejected.

METHODS AND MATERIALS

The black cutworm larvae used in this study were laboratory reared, the original stock having been obtained from a culture maintained at the Illinois Natural History Survey. The original larvae were reared to adulthood fed upon corn and red clover. When these larvae reached maturity, they were mated and the eggs collected. The larvae hatching from these eggs were reared through pupation upon an artificial diet (Conterio, personal communication) designed for the corn earworm, Heliothis zea (Boddie), but which has been used very successfully in rearing the black cutworm (Sechriest, personal communication).

The components of the diet are as follows:

Agar-agar	6.4 g
Boiled pinto beans	106.0 g
Brewers yeast	16.0 g
Methyl p-hydroxy benzoate	1.0 g
Ascorbic Acid	1.6 g
Sorbic Acid	0.5 g
Formaldehyde (40%)	1.0 ml
Distilled water	320.0 ml

The above ingredients were mixed in a blender, autoclaved, and poured into sterile, screw top, baby food jars. The jars were then refrigerated at 4°C until needed.

Test I

The parents of all larvae used in the first feeding test were reared on an artificial diet. The larvae

were in the fifth instar, about twenty millimeters long, when the first test was conducted. All were offspring from a single female moth.

The test plants used in the first portion of the study were field corn, wheat, oats, tobacco, and tomato. All were grown from seed in the greenhouse and leaves were used from plants at various stages of development. Portions were cut from a leaf to weigh a total of 500 mg. The cut leaf was then placed in a 4½ inch diameter culture dish. A piece of absorbent cotton saturated with distilled water was placed in the center of the dish to keep the humidity at a high level. A 500 mg sample of a second food plant was then put into the dish with the first food plant. A fifth instar larva from which food had been withheld for twelve hours was placed upon the wad of cotton. A second dish was then placed upon the first and masking tape wrapped around the junction of the two dishes to seal in moisture, (see Fig. 1). The second dish was treated as the first. It contained one 500 mg sample of each of the two food plants used in the first dish, a saturated wad of cotton and a fifth instar larva. Twenty-five replicates of each combination were completed. The combinations in Test I were as follows: wheat/tobacco; wheat/tomato; wheat/corn; oats/tobacco; oats/corn; oats/wheat.

After allowing the larvae to feed in total darkness for twenty-four hours, the results were obtained by

weighing the remainder of each food plant not eaten. Included in each test were five control dishes containing food plants but no larvae. The weight of these food plants upon termination of the test gave an indication of weight loss due to loss of water from the cut leaf.

Test II

In the second test a mechanical support, chromatograph paper, was soaked in a plant extract and then offered to the larvae in test situations of two choice discrimination. The test included seven comparisons involving five different food plants. The plant combinations used in this test were oats/corn, tobacco/wheat, oats/tobacco, wheat/corn, oats/wheat, tomato/wheat, and corn/tomato. These plants were grown in the greenhouse, but were considered to be widely available to cutworms in the fields.

The corn (field corn) was about 10 inches high when cut. The oats and wheat were 6 to 8 inches high, and the tomato plants were about 12 inches high. The entire plant, excluding roots, of these four species was used in the preparation of the extracts. The tobacco plants were about 30 inches high, therefore only apical leaves and small portions of new stem were used in the tobacco extract.

In the preparation of the extracts, 100 grams of fresh plant material was blended with 250 ml of distilled water in a Waring blender at 18,000 RPM for 5 minutes. The resulting mixture was filtered three times through

several layers of glass wool with the aid of a vacuum flask and a filter pump (water aspirator). The filtrate was then divided into 50 ml portions and placed in sterile baby food jars. The jars were then immersed in a dry ice-acetone bath at -70°C and the plant materials quick frozen. The jars were then stored at -20°C until needed.

The next step in the preparation of the extracts was to put 50 ml of the material into a vacuum flask and evaporate the material to dryness at 0.2 mm of mercury. While the evaporation was taking place the flask was immersed in a water bath at 40°C to facilitate drying. When dryness was reached, the material in the flask was suspended in 20 ml of distilled water and allowed to soak for four hours. At the end of this period, the mixture was centrifuged at 450 x G for 15 minutes. The supernatant was placed in a flask and again evaporated to dryness at 40°C. At dryness the result was a golden film in the bottom of the flask. This material was suspended in 5.0 ml of distilled water and became the test extract.

Each extract had a noticable odor but all of the odors appeared similar. The liquids were clear, ranging from brown to yellow in color. The extracts were stored at 4°C, and when removed from refrigerator to set up a test were kept in an ice water bath.

The test chamber consisted of a plastic petri dish 100 x 15 mm with an "I" plastic divider which separated the dish into two compartments. The divider was sufficiently low (7.5 mm) to allow the larva easy access back and forth between the compartments, (see Fig. 2).

The extracts were presented to the larvae upon 1 x 4 cm strips of Whatman No. 1 chromatograph paper. In accordance with a technique described by Niimura and Ito (1964), the paper strips were heated in an electric oven for about ten hours at 170°C±10°. After this treatment the paper strips were a light brown color and would break when bent double.

Twenty paper strips were dipped into a plant extract and then laid upon a paper towel to air dry. A second set of twenty strips would then be dipped into a second plant extract and laid upon a second towel. When the strips were dry, approximately thirty minutes later, they were placed, one strip of each extract per dish, in their respective compartments of the dishes, the undersides of the dishes having been marked as to the particular extracts to be used in the test. After all strips were in the dishes, a single drop of water was dispensed onto each paper strip from a twenty-five gauge needle attached to a syringe.

A single larva, fifth instar, was placed in each dish. The larva of dish #1 was placed in the compartment with test extract A, the larva of dish #2 within the test

extract B compartment, the larva of dish #3 in test extract A compartment, larva of dish #4 in B, etc., until all dishes contained one larva. This procedure assured that each extract would be equally presented. The comparison of two food plants consisted of 100 replicates. When all dishes had been set up, they were placed in complete darkness for a period of six hours. The temperature was maintained at 20°C±2° and the relative humidity at 75%.

At the end of the six hour period the dishes were removed from the darkness and the larvae removed from the dishes. The paper strips or the remnants of the same were then measured and the results recorded as square millimeters eaten. Calculation of total amount eaten was accomplished by laying the portion of paper strip remaining after the test period upon graph paper marked with one hundred units to the square centimenter. Thus the area could be counted as millimeters square eaten. In the counting procedure, any area less than half a square millimeter was not counted while any area one half millimeter square or larger was counted as one (Hansberry, 1943). In the case that a larva ate all of one test strip the dish was not included in the results. If any larva ate less than a total of 30 mm^2 per dish, the dish was not counted.

The larvae used in the second testing program were reared from the eggs of three females all of whom

were reared from a single parent. Each larva was used in only one six hour test period and then discarded.

Test III

The third testing program was made possible through the development of a practical multiple discrimination test chamber. The test chamber was made from a standard petri dish, 100 mm x 20 mm. A quantity of melted paraffin was poured into the dish to a depth of two or three millimeters. As the paraffin cooled and became white, wooden splints, which had earlier been cut to the length of the radius of the petri dish, were inserted into the dish in spoke-like fashion to form 8 compartments. A small, 2 cm x 2 cm, square of heavy paper was placed at the junction of the splints, (see Fig. 3).

With the splints thus placed, the dish was placed on a warm top (warm enough to remelt the paraffin) and allowed to set for five to ten minutes. The paraffin coats the splints and when the dish cools the splints are fixed permanently in position. The paper square is also fixed to the splints by the action of the paraffin. This square offered a convenient platform for placing the larva.

The compartments were numbered one through eight for use with six test extracts and two distilled water controls. The extracts used in this test were those of corn, wheat, oats, tobacco, tomato, and ryegrass. On the dishes the compartments were numbered randomly in such a

way that the same extracts were not adjacent to one another in every dish. The extracts were prepared as in Test II.

In the third test the paper strips used were one centimeter wide by two centimeters long. They were treated as before: baked, dipped, air dried, placed in the proper compartment, and moistened with one drop of water. A larva was placed on the platform at the center of the compartments and the lid placed on the dish. The dishes were placed in total darkness for a period of two hours. The temperature was maintained at 20°C±2° and the relative humidity at 75%.

At the end of the two hour period the larvae were removed from the dishes. Any test strips which had been eaten from were measured in the same manner as in Test II. In any instance that a strip was completely eaten the dish was not included in the results. In this test there was no minimum amount to be eaten before the results were included. As in the second test, all larvae were used only once. Seventy-five replicates of this test were completed.

RESULTS

The results of Test I are shown in Table 1. Of the six combinations tested, only two displayed a significant difference between the mean amounts eaten. Tobacco was preferred over oats (significant at the 0.05 level)

and corn was preferred over oats (significant at the 0.05 level).

Table 2 contains the results of Test II. In this test a significant difference appears between the mean amounts eaten in each combination of food plants. In six of the seven combinations the difference of means is significant at the 0.01 level.

In Test II in addition to significance of means, the results tend also to be consistant. That is, where oats is preferred to tobacco and tobacco is preferred to wheat, oats also is preferred to wheat. Nowhere in the test is there a combination which reverses the preferences. It is interesting to note that in the cases of the two highest feeding rations, tomato was the less preferred in both instances. It is also noteworthy that the lowest feeding ratio occurs in the combination between the two food plants most often preferred: oats/corn.

It would appear that the two least preferred plants in this test were wheat and tomato. The total amount eaten by the larvae involved in this particular combination was the least of all combinations. In addition, the combinations resulting in the highest mean amounts eaten (oats/tobacco; oats/corn) both contained the apparently preferred oats.

In Table 3 can be seen the order of preference as determined by the amounts of paper eaten. Also given in this table is the mean amount of paper eaten of each test

extract. Table 4 shows the differences between the means of all of the extracts tested in the third test.

Table 1.--Feeding responses in milligrams eaten of black cutworm larvae to cut plant material. Each combination replicated 25 times with 1 larva per replicate.

Plant Combination	Amount Eaten (mg) by 25 Larvae	Avg./Larva (mg)
Wheat	2840	113
Oats	2380	95
Wheat	2400	96
Corn	2740	109
Wheat	2100	84
Tobacco	1740	69
Wheat	1620	65
Tomato	2360	94
Oats	990	39[a]
Corn	2780	111
Oats	1730	69[a]
Tobacco	3220	128

[a]Difference between means significant at .05 level.

Table 2.--Responses in mm^2 eaten of 700 black cutworm larvae to chromatograph paper treated with extracts of various plants in two-choice tests. Each combination replicated 100 times with 1 larva per replicate.

Plant Combination	Paper Eaten (mm^2)/100 Larvae	Avg./Larva
Oats	12455	124.55[a]
Corn	9067	90.67
Tobacco	11285	112.85[b]
Wheat	6268	62.68
Oats	16403	164.03[b]
Tobacco	8278	82.78
Wheat	4073	40.73[b]
Corn	17122	171.22
Oats	17971	179.71[b]
Wheat	2040	20.40
Wheat	13504	135.04[b]
Tomato	486	4.86
Corn	17414	174.14[b]
Tomato	501	5.01

[a]Difference of means is significant at .05 level.

[b]Difference of means is significant at .01 level.

Table 3.--Feeding responses of 75 black cutworm larvae to chromatograph paper treated with plant extracts and to control strips treated with distilled water. Extracts presented simultaneously in multiple choice test chamber.

Plant or Other Material	Paper Eaten (mm^2)	Avg./Larva (mm^2)
Oats	4455	59.40
Corn	3051	40.68
Tobacco	2005	26.73
Wheat	1166	15.55
Rye Grass	269	3.59
Tomato	4	.05
Control	3	.04
Control	2	.03

Table 4.--Differences (mm^2) between mean amounts of paper eaten by 75 black cutworm larvae during 8-choice test.

Plant	Difference of Means (mm^2)						
	Control	Control	Tomato	Grass	Wheat	Tobacco	Corn
Oats	59.37[a]	59.36[a]	59.35[a]	55.81[a]	43.85[a]	32.67[a]	18.72[b]
Corn	40.65[a]	40.64[a]	40.63[a]	37.09[a]	25.13[b]	13.95[b]	
Tobacco	26.70[a]	26.69[a]	26.68[a]	23.14[a]	11.18		
Wheat	15.52[b]	15.51[b]	15.50	11.96			
Grass	3.56	3.55	3.54				
Tomato	0.02	0.01					
Control	0.01						

[a]Difference significant at .01 level.
[b]Difference significant at .05 level.

DISCUSSION

Although Test I was conducted using plant tissues, it is not felt that the results give a valid picture of the preferences of the larvae. The results conflict in part with the results of Tests II and III. Part of this conflict must be attributed to the changes that begin taking place immediately after removal of the plant parts from the parent plant (Bonner, 1950).

Mulkern (1967) emphasized that due to varying rates of maturation, dessication, and decomposition, the use of actual plant parts in studies of food plant selection is ill advised. In addition, with this technique there is the occasional confusion as to which food plant the remnants belong.

The worth of the extract technique of testing feeding responses is well supported by a number of authors (Thorsteinson, 1955; Keller, Maxwell, and Jenkins, 1962; Maxwell et al. 1963; Loschiavo, Beck, and Norris, 1963; Soo Hoo and Fraenkel, 1964; Starks et al., 1965; McMillian and Starks, 1966; Mulkern, 1967; Guerra and Shaver, 1968). A primary advantage of the extract procedure lies in the elimination of those physical inhibitors or deterrents such as pubescence which Painter (1951) says play such an important part in insect feeding.

In the past the main objection of applying the extracts to paper substrates has been the toughness of the

paper fibers (Thorsteinson, 1955). Larvae tend to eat from the surface of the paper rather than from the edges. This makes an accurate measurement of the amount of paper eaten impossible.

I found that after baking the paper for ten hours at 170°C±10°, it was eaten readily with no surface feeding taking place. The paper when dry is brittle, but when moist it will bend easily without breaking. The feces resulting from this chromatograph paper diet is very similar in shape and consistency to normal feces of this insect.

The extract/paper technique is quick and convenient. The results can be read quickly and accurately with little experience. About thirty minutes are spent recording the results of twenty dishes. In addition, the remnants of the paper may, if one is pressed for time, be recorded anytime in the future or taped to a card for future reference.

I do not think it necessary to apply a specific quantity of extract to each strip of paper. Chromatograph paper is characterized by its constant absorbency rate. Each strip, therefore, should absorb nearly identical amounts of extract when dipped in the liquids. The paper, purchased in rolls with a width of four centimeters, can be cut into the desired size sample quickly and accurately.

Laboratory reared larvae prevented the possibility of pre-conditioned larvae (Johansson, 1951, from Dethier,

1966; Hovanitz and Chang, 1962; Stride and Straatman, 1962).

A comparison of the means of the combinations in Test II shows all to be significantly different. In each combination that oats appeared, it was preferred over the alternative choice. With the exception of oats/corn, corn was preferred in each of its appearances.

The creation of the multiple choice test chamber makes it possible to submit the larvae to a random selection of all experimental food plants simultaneously, and quantitatively measure their responses. The test chambers are easily and cheaply made, and work most satisfactorily. When the larva is placed on the platform at the hub of the chamber there is equal opportunity to go first to any extract.

The results of Test III are significant not only in themselves but also in correlation with the results of Test II. Oats is again the leader in amount of paper eaten and corn secondly preferred as it was in Test II.

When the results of Test III are analyzed in accordance with a Duncan Multiple Range Test (Walpole, 1968) and a Multiple Comparison Among Means (Dunn, 1961), a significant difference between means is found in most of the comparisons (see Table 4).

While only two combinations in Test I showed significance between means, it should be brought out that both of these combinations (tobacco/oats and corn/oats)

are reversed in Tests II and III. This makes it necessary to make a judgement in favor of the tests involving the extracts. It can only be said, however, that the black cutworm larva prefers the extract of oats to the extract of corn, the extract of corn to the extract of tobacco, etc.

Further testing is needed to state that the larva prefers oats to corn. Two choice discrimination tests with living corn plants versus living oat plants might establish the validity of such a statement.

Fig. 1.--Arrangement of test dishes in Test I.

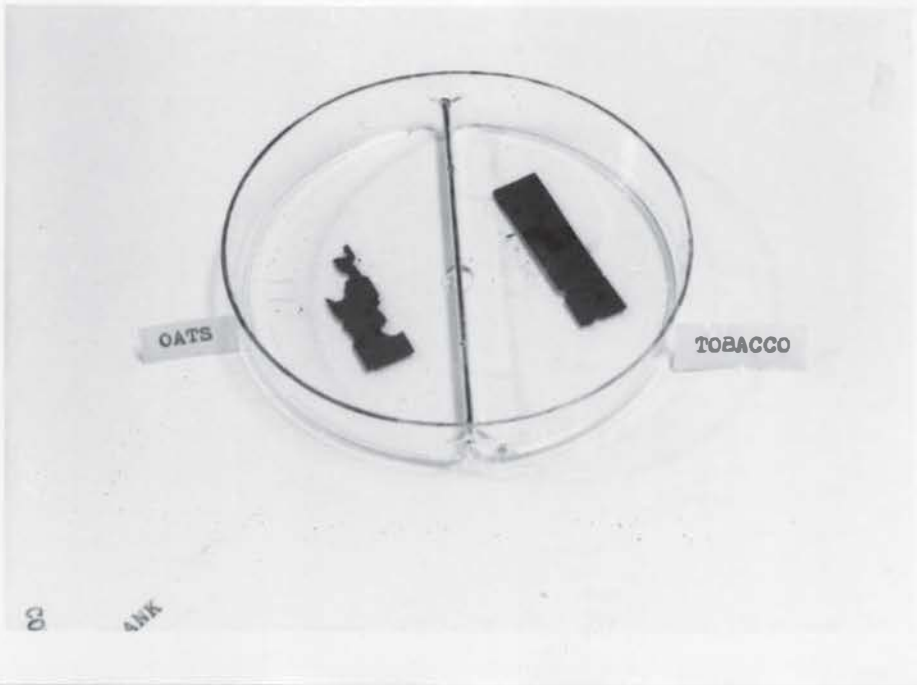

Fig. 2.--Chamber apparatus used in Test II.

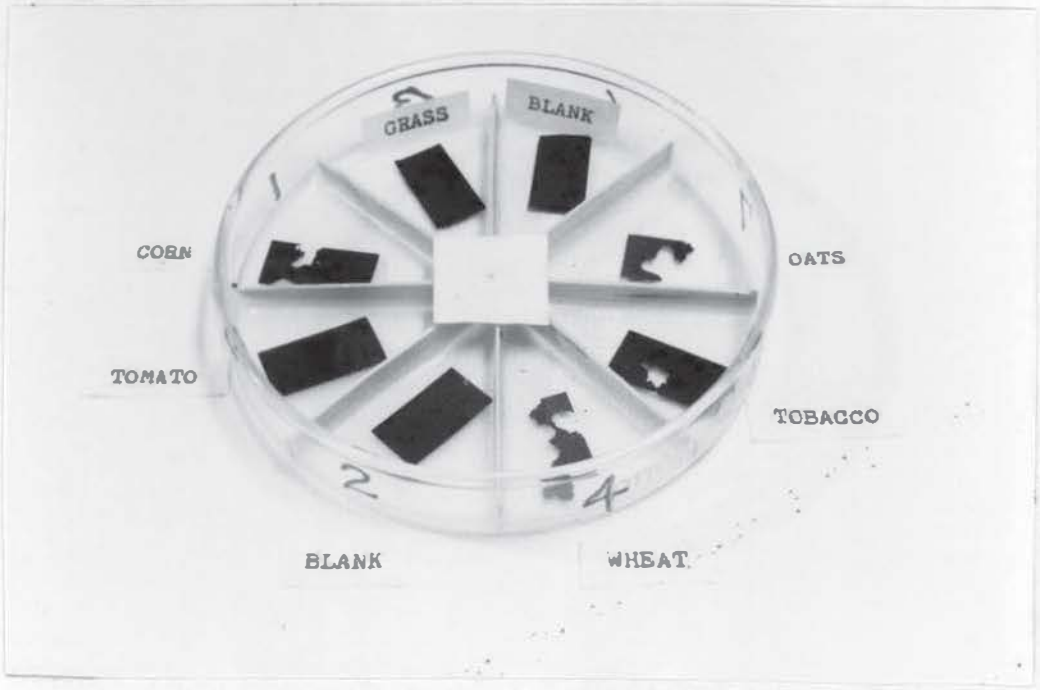

Fig. 3.--Multiple choice chamber used in Test III.

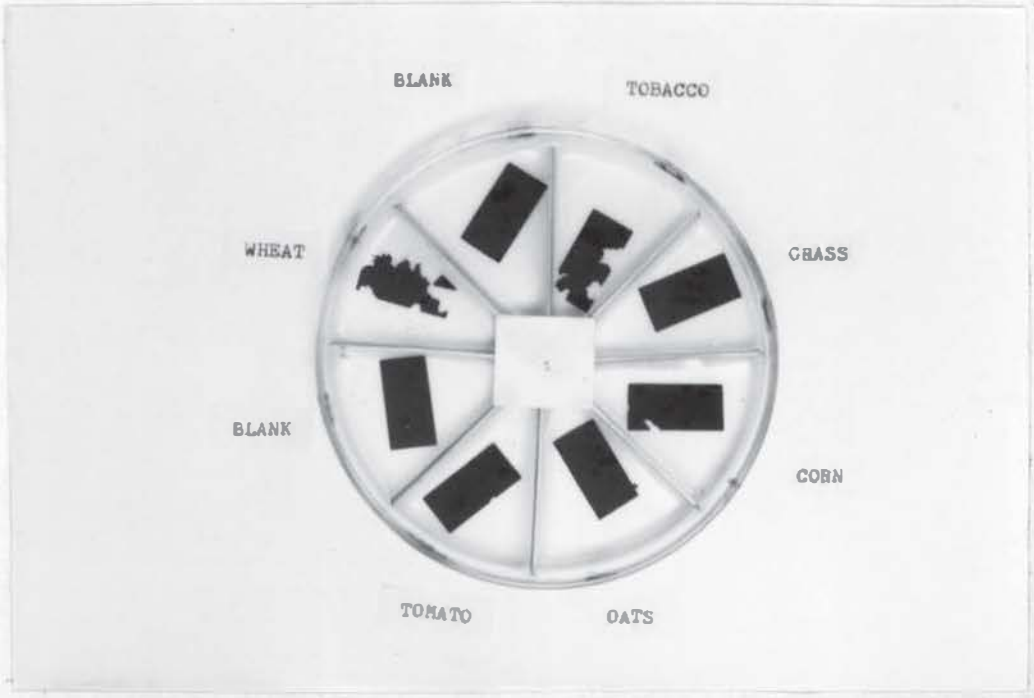

Fig. 4.--Overhead view of chamber used in Test III.

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