

INTERACTIONS OF THE SQUASH BUG, ANASA TRISTIS (DEGEER)
(COREIDAE, HEMIPTERA), AND SIX VARIETIES OF SQUASH (CUCURBITA SPP.)

by

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INTRODUCTION

Man's struggle to combat his leading competitors, the insects, has led to great progress in the search for various methods of insect control. The great diversity of insects' habits and behavior, their unusual capacity to cope with changes in their environment, and their enormous rate of reproduction need to be balanced by a continuous effort to find effective methods of control, either with the use of chemicals, insects' natural enemies, or by developing natural defense within the plants, or else crop production might be a failure.

The economic importance of the members of the family Cucurbitaceae, which includes squash, cucumber, watermelon, muskmelon, pumpkin and gourd, is well known, both for their uses as food and for ornamental purposes. Their production, however, is often limited to certain parts of the world because of various insect pests which sometimes destroy the entire planting. The great losses in cucurbit crops, especially squash, due to severe damages by the common squash bug, Anasa tristis (DeGeer), justify a thorough consideration of the control of this pest.

The unusual resistance of the squash bug, particularly the adults, to insecticides makes chemical control less dependable (Cummings 1942 and Thompson et al. 1955). Nymphs are less resistant but their tendency to congregate underneath the leaves, vines and fruits of squash, protects them from being hit by the spray material (Compton 1932 and Watson 1942). Two to three sprayings per week are needed to attain a better killing of the bugs, amounting to a great expense scarcely compensated by the profit derived from the crop saved (Eichmann 1943). The laborious mechanical methods such as daily hand-picking of egg masses, nymphs and adult bugs is therefore often

used (Jarvis 1909, Haseman 1937). This is impractical on commercial scale squash production. Gould (1951) commented that the squash bug is the one major pest of vegetable crops for which there is no satisfactory control.

The use of resistant varieties of squash is a method of control with great possibilities for controlling squash bugs, and would be economically fitted both for home garden and for large scale planting. Screening of some Cucurbita plants for resistance to this insect and to some other pests of cucurbits in Manhattan, Kansas, was started by Hall and Painter in 1956. In the field, they observed some varieties to be resistant, some moderately resistant and the others susceptible to squash bug. However, the mechanisms involved in the various levels of resistance observed are still unknown.

The present work aims to determine the components of resistance or susceptibility in the greenhouse of the six varieties of squash whose performances had already been observed in the field and their degree of resistance evaluated. These are Cucurbita moschata Duch. -- varieties Sweet Cheese and Butternut (both resistant), C. pepo L. -- varieties Royal Acorn (resistant) and Black Zucchini (susceptible), C. maxima Duch. -- varieties Green Striped Cushaw and Pink Banana (both moderately resistant).

LITERATURE REVIEW

Squash bug has a wide geographical distribution. Uhler (1876) recorded its presence in the West Indian Islands, Central America and Mexico. Hoerner (1938) reported these bugs in Colorado doing serious damage especially to winter squash. Weed (1902) noted that these bugs were the most vexatious insects which the gardeners in New Hampshire had to contend with in 1901.

In Ontario, they are abundant and injurious throughout all the southwestern counties (Bethune 1917 and Gibson 1921). Gould (1944) reported an estimate of insect damage to cucurbit crops in Indiana to the extent of \$525,000 annually, most of which was caused by squash bug, cucumber beetle and other minor pests. Davis (1955) reported the same great loss in commercial plantings of squash and pumpkin, particularly in Jackson county, caused by the very high squash bug population that built up by late August or early September throughout the state. Hutchins (1941) observed gourds in Minnesota being attacked by squash bugs. Eichmann (1943, 1945) stated that the same pest had been invading eastern and central Washington in outbreak proportions since 1937 when it appeared in Kennewick county. The production of home garden squash ceased throughout the region and the invasion of the Yakima Valley practically ended the commercial production of about 1,000 acres of Marble head squash, a winter variety. Vorhies (1946) observed the same pest in Arizona, Parsley (1914) found them in New England and Smith (1939) reported the squash bug outbreak in 1938 killing the squash, melon and pumpkin in the Kansas River Valley, this insect being one of the major truck pests of the state.

The nature of the feeding injury of squash bugs to the plants has been investigated and described by various workers. Slingerland (1895), Quaintance (1899), Weed (1902), Pettit (1905), Worthley (1923), Pack (1930) and Balduf (1950) attributed wilting of the leaves followed by the death of the plant when attacked in the seedling stage, not only to the enormous amount of plant material removed by the bugs, but also to the toxic substance injected into the plant prior to, or during the feeding process. In addition, Richards (1930) reported that squash wilt disease is induced by the feeding of the

bugs. Selby (1896) and Baldwin (1912) stated that the bugs transmitted bacteria blight organism from plant to plant. In contrast, Rand (1915, 1916) reported that in his experiments, squash bugs failed to transmit the disease. Robinson and Richards (1931), Knowlton (1935) and Hoerner (1938) termed the injury caused by squash bugs as "Anasa wilt of cucurbits."

Evidences of resistance of some varieties of squash to squash bug have been reported. Chamberlin (1916) noted that in western New York, the pest would seldom attack summer squash plants when winter varieties were available. Hoerner (1938) and Knowlton (1952) made a similar report that winter squash were preferred to summer varieties and that the insects seemed to move to them only when the preferred hosts were dead. Eichmann (1945) reported that Clore, horticulturist at Prosser Station, Washington, found that Kentucky pumpkin survived squash bug attack best and Marblehead succumbed most easily. Elliot (1935) stated that some varieties of squash in Connecticut were seriously injured while others were not when they were planted together in the field.

Painter (1951) compiled and summarized the studies on insect resistance, dividing the components involved into three main groups, namely, tolerance, non-preference and antibiosis. The above reported evidence of resistance in squash might, therefore, have been due to any one of the three, a combination of any two, or of all components.

MATERIALS AND METHODS

Sources of Test Insects and Plant Materials

Adult and nymph squash bugs were collected from the field in Ashland Horticulture Farm, Manhattan, Kansas, in the fall of 1958 when the insects were still abundant; overwintering adults were collected in December from

the same field.

Fruits of the six varieties of squash to be studied were also collected from the same field. Seeds were obtained from Ferry-Morse Seed Co., Burrels Seed Co. and Lawrence Robinson and Sons, Inc.

The experiments were mostly conducted in the Horticulture and Entomology greenhouses with some in the laboratory. Maximum temperature in the greenhouse varied with the seasonal changes and daily period of sunlight. The minimum night temperature was maintained at approximately 60° F. during winter. The temperature in the laboratory ranges between 65° and 85° F.

Multiplication of the Test Insects in Stock Cultures

Nymph and adult bugs collected from the field were reared separately. In the laboratory, the rearing cage was six-sided, five sides of which were made of 1 mm. mesh wire screen supported by a wooden framework forming a 12" x 12" x 12" box. The front side was a removable glass which facilitated handling of the insects. The inside of the box was lined with cheesecloth on which the bugs could oviposit. Pink Banana squash fruit was used as food. This method of rearing was similar to that used by Dietrich and Bosch (1957). The nymphs were reared in separate cages.

Beard (1940) reported that squash bug has only a few days pre-oviposition period, has one generation a year, passing the winter in the adult stage. In the studies here reported, when after two months rearing eggs were not yet produced, some adult bugs about a month old were subjected to cold treatment to try to break the diapause in which the insects were suspected to be at that time of the year. The squash bugs were divided into six groups of five males and five females each. Each group was placed in a box filled with straw

and the boxes were placed in a refrigerator with a temperature of 38° F. at 5°. Three groups were subjected to alternate cold and laboratory temperature by taking them out of the cold chamber every other day. The first group was given a week of this alternate cold and laboratory room temperature, the second two weeks and the third, a month. The other three groups were given continuous cold treatment for the same lengths of time, a week, two weeks and a month. After the treatments the different groups were kept separately in the rearing cages and mature Pink Banana fruits were used as food.

Some adults were kept in the greenhouse on young leaves of mature Black Zucchini and Pink Banana plants. The rearing cages were made of three mm. mesh plastic material. The two sides of the 18" x 14" piece of material were glued together using Duco cement, forming a cylinder. The top end was then glued leaving only the bottom end open. Adults were placed inside the cage which was then placed over one or two young leaves of the plants grown on greenhouse beds. Cotton pads were placed around the petioles, and with these petioles held between the edges of the cage, the open end was closed by means of paper clips. Five males and five female bugs were kept in each cage, moving them to other leaves as often as necessary.

Methods of Studying the Effects of Host on Fecundity and Gain in Body Weight of Adult Bugs

This experiment was conducted in the laboratory and designed to compare the number of eggs produced by bugs feeding on the fruit of a resistant variety with eggs produced by those feeding on a susceptible one, as well as their weekly changes in body weight.

Ten pairs of day-old adult bugs were reared separately on Pink Banana fruit and another ten pairs on Sweet Cheese, using the rearing cages described above. Each bug was marked with colored paint on the wing and weighed, using a Roller Smith precision balance, before confining them in the cages. The cages were lined with cheesecloth inside where the bugs could oviposit. Observations were made daily to record oviposition and mortality of the two groups. The bugs were weighed weekly but weighing was stopped after a month as the insects in both cages began to show abnormal behavior.

Methods of Studying the Amount of Plant Material Drawn by the Bugs from the Resistant and Susceptible Plants

Comparisons of the amount of plant material drawn by both nymphs and adults from the mature plants as well as from the seedlings were made. The number of hours of starvation and the length of feeding opportunity given to the bugs were based on the preliminary experiments done before these tests. Results of the preliminary experiments not here recorded indicated that 48 hours of starvation for the adults, 24 hours for the nymphs and three hours opportunity to feed for either, were the most satisfactory periods of time. Starvation should be long enough to make the bugs feed readily once they had a chance, but not so long as to cause detrimental effects to the bugs. The opportunity to feed must be sufficiently long to enable them to get as much plant material as they could, but the determination of the amount drawn should be done before digestion had proceeded very far.

In determining the amount of plant material drawn by adults and third instar nymphs from seedlings and mature plants of the six squash varieties, a general procedure was used making some slight modifications when needed. Individually marked adult males were starved for 48 hours; marked third

instar nymphs, 24 hours; each individual then was weighed separately. Marking was necessary in order to distinguish one bug from the other during the weighing process. Males were used instead of females because in the latter, the development of eggs would mask the weight differences due to varietal effects on food intake. Three hours of feeding opportunity was given to both the nymphs and the adults, after which time they were removed from the plants and weighed individually again.

The third from the youngest leaves of the mature plants of the six squash varieties were used in all cases. Using the 3 mm. mesh plastic cages which have been previously described, two adult bugs were confined on each undetached leaf. With the nymphs, 0.5 mm. mesh plastic cages were used for confining two individuals to a leaf. In both cases, a cotton pad was placed around the petiole of the leaf to prevent the cage from crushing it and to hold the stem between the unglued edges of the cages. The open end was closed by means of paper clips.

Cellulose-nitrate cages were used to confine the adults and also the nymphs on the seedlings. Two individuals were placed in each cage. The cages were 12" high and $3\frac{1}{2}$ " in diameter, with tops covered with nylon-orlon cloth and each provided with two nylon-orlon covered windows for ventilation.

Two replications were made in each test, each time using 10 insects per variety and another 10 for control to which no opportunity was given for feeding.

Preference Tests

Separate preference tests were made for the nymphs and the adults. Seedlings of the six varieties were paired, giving 15 possible combinations.

In these tests, it was desirable to have uniform size, age and condition of plant growth. However, with six varieties uniformity in size was not always possible if similarity in age was to be maintained, because seedlings of some varieties were inherently small compared with others of the same age. In these tests, therefore, uniformity in age and condition of plant growth were maintained and the differences in size kept to a minimum. Approximate leaf size measurements were recorded.

The seeds of the six varieties were germinated in ordinary garden soil placed in small paper boxes. The paper boxes were helpful both in keeping the sowing depth uniform and in transplanting the seedlings without damaging the root system. The dates of sowing the seeds of the six varieties were varied because some required a longer period for germination than others. Seedlings used were those with the first true leaf formed, ten days old, big enough to last unwithered until the end of the experiment.

Large glass cylinders (8" diameter x 12" height) wrapped with brown paper were used to confine the bugs with the plants. As it was previously observed that the bugs tend to congregate in shaded areas, wrapping the cylinder tended to reduce the possibility of bugs going to the plant as a negative response to light.

Three females and two males, marked and starved for 48 hours, were confined in each cylinder. The top of the cylinder was covered with a 1 mm. mesh wire screen cover, and above this a thick cardboard was placed to provide complete shade inside the cylinder. Five replications were used. Records of the bugs found on each of the paired plants were taken at 30 minute intervals for three hours, followed by daily observations for three days.

Preference tests with nymphs were done in almost the same manner as that with the adults. Third instar nymphs properly marked and starved for 24 hours

were used, also confining five nymphs per cage (Plate I). Since the nymphs were small, cellulose-nitrate cages were used. The seedlings used were three-day old, small enough to be accommodated inside the cage without folding the leaves, crushing them or allowing them to touch each other. No shading was provided as all the nymphs could be accommodated underneath the leaves without crowding. Five replications were used and observations similar to those taken with the adults were recorded.

An attempt was made to trace the pattern of movement of the adult bugs in finding the host plant, whether by random movement or by following a definite pattern as a result of a stimulus or stimuli from the host. Six seedlings, one of each variety, were planted in a circular form keeping the distances between the plants the same in a flat wooden seedbox as in Plate II, except that seedlings were used instead of the sticks.

A 48-hour starved bug was released at the center of the graph paper and the path it traveled till it found the host was reproduced on the duplicate graph paper during the experiment. Some squares on the paper were numbered to serve as reference points in following up the bug's movement. Every time the bug got off the paper, it was placed back at the center and the path it had traveled was discarded. The length of time it took the bug to find the plant was recorded.

After the first insect had settled on a seedling, it was removed, the graph paper was changed and the seed box was rotated in a clockwise direction such that a seedling occupied the direction previously occupied by its adjacent seedling. The second bug was then released at the center of the graph paper for observations similar to those made with the first bug. Twenty trials were made using 10 males and 10 female bugs.

In another experiment, sticks painted green were used in place of the three seedlings as shown in Plate II. The purpose was to find out if the bugs were attracted equally to both plants and sticks which might be the case if host-finding is by chance through random movements, or if they have the tendency to climb vertical objects as some insects do. The same procedure outlined above was followed making similar observations.

All the above tests were done in a shaded place to minimize the possible effects of sunlight on the pattern of movement of the bugs.

Tolerance Tests

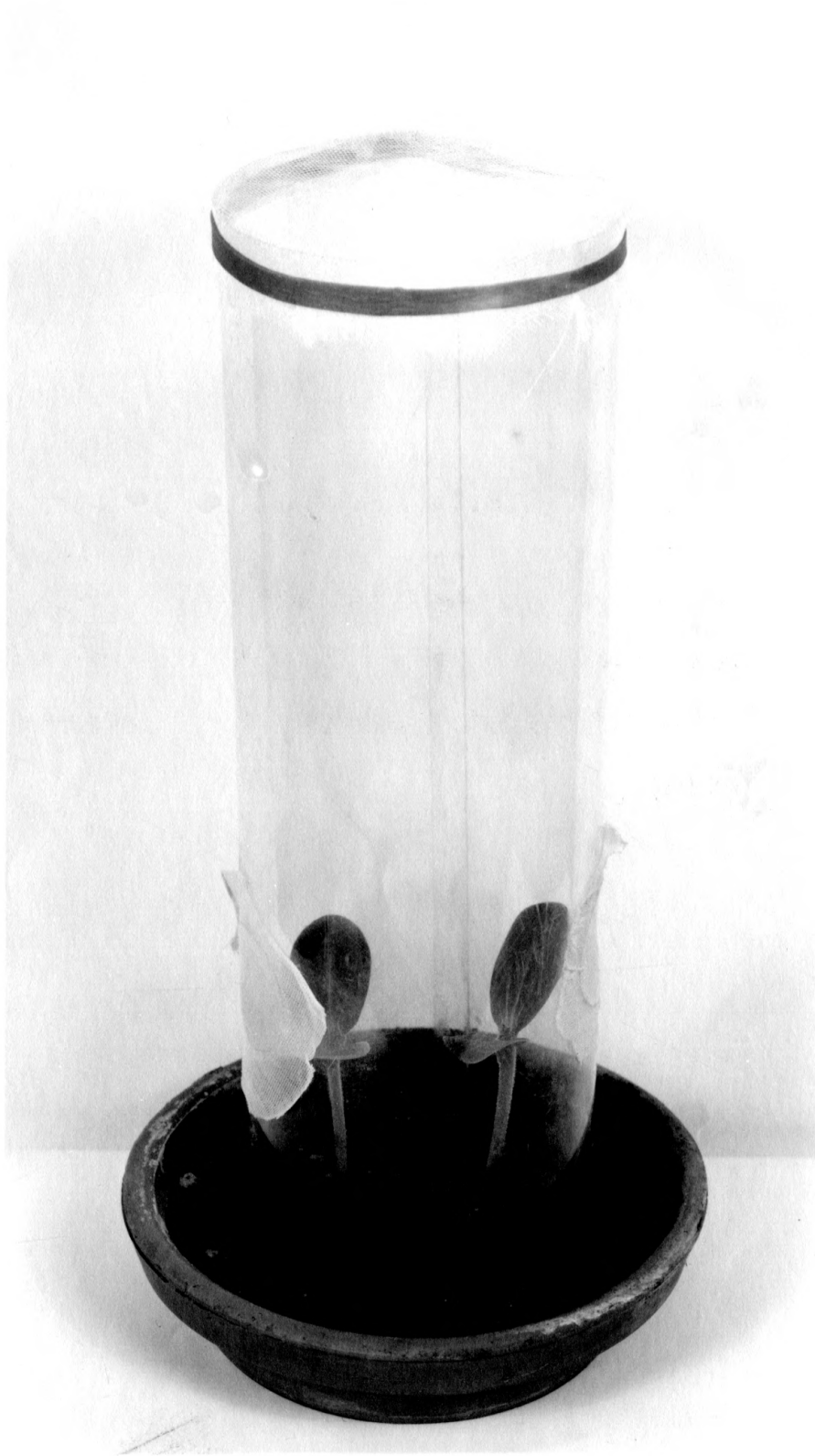
A plant is said to be tolerant when it shows an ability to grow and reproduce itself or to repair itself to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host (Painter 1951). In this study, tolerance was measured by the number of hours that the seedlings of each of the six varieties of squash survived, each plant supporting the same number of adult squash bugs.

Prior to these experiments, tests were conducted to determine the most satisfactory stage of the seedlings for the study, and the minimum number of adult bugs that should be allowed to feed on each seedling to bring about the most marked feeding injury in a reasonable length of time. Thus the seedling passed through various stages of injury more slowly, rather than being killed quickly. It was found that three-day old seedlings with cotyledon leaves about three-fourths grown were best, that five bugs per plant were the ideal number, and that three hour intervals between observations were needed to study the successive stages of the injury.

EXPLANATION OF PLATE I

Cellulose-nitrate cage, 12" high and $3\frac{1}{2}$ " in diameter, used to confine the third instar squash bug nymphs with the paired seedlings for the preference tests.

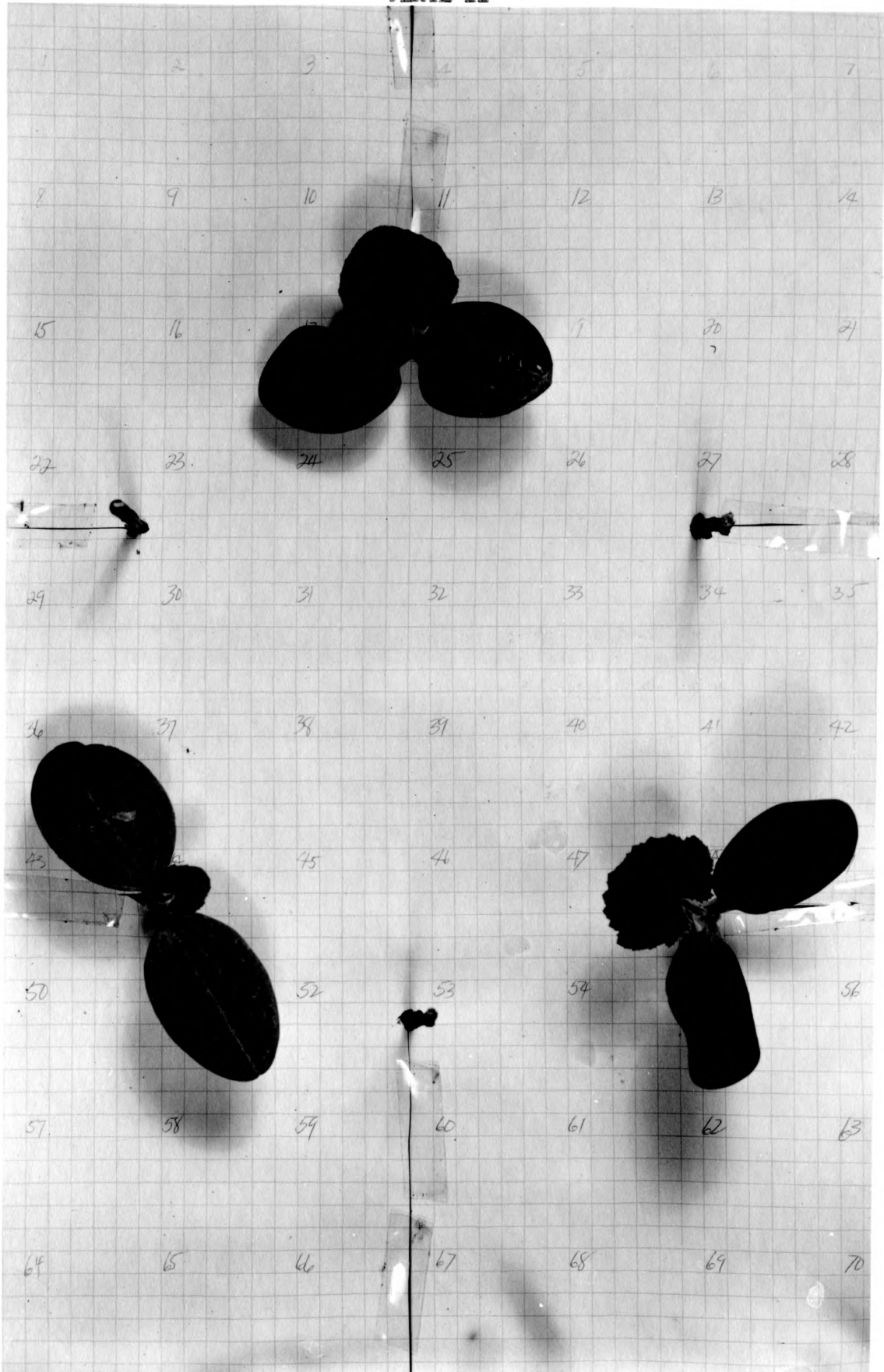
PLATE I



EXPLANATION OF PLATE II

Individually marked 24-hour-starved adult squash bugs were released, one at a time, at the center of the graph paper and the path that each bug traveled till it climbed either the seedling or the stick was reproduced on a duplicate sheet.

PLATE II



As in the preference tests, uniformity of the seedlings with respect to age, condition of plant growth and size was maintained as nearly as possible. "Germination in paper box" technique was employed for the purposes already mentioned. Three-day old seedlings of the six varieties, with the cotyledon leaves about three-fourths grown, were used. They had been randomly planted in wooden flats, using two plants of each variety per replication. The experimental arrangement is shown in Plate III.

Five properly marked female bugs, starved for 48 hours and weighed, were confined with each seedling using the cellulose-nitrate cages. After three hours opportunity of feeding, the bugs were removed from the plant, weighed individually and then returned to the same plant. The purpose of weighing was to be sure that all the bugs were feeding. The plants were watered two to three times a day, depending on the weather, to eliminate the possibility of wilting due to lack of moisture in the soil rather than as a result of the feeding of the bugs. Observations were made at three-hour intervals, except at night, each time noting the condition of the plants. The experiment was begun in such a way that the probable time when the first symptom of the injury would show occurred in day time. In noting the condition of the plants the following ratings were used in recording results:

- 1 = no injury
- 2 = leaves beginning to wilt
- 3 = both leaves wilted
- 4 = whole plant wilted
- 5 = plant dead

The bugs were removed after the death of the seedlings. Five replications were used (conducted at different times) each time recording the range of temperature during the experiment.

EXPLANATION OF PLATE III

One replication of the tolerance tests. Cellulose-nitrate cages were used to confine five adult squash bugs with each seedling.

PLATE III



Antibiosis Studies

The antibiosis of a particular host plant was studied by rearing newly hatched nymphs on the seedlings. The seedlings used were a week old at the start of the experiment, randomly planted on flat wooden seed boxes, and were replaced every 6 weeks. The experimental situation is illustrated in Plate IV.

The cages were made of 0.5 mm. mesh plastic material. The two edges of a 14" x 12" piece of material were glued together forming a cylinder and then the top edges were glued leaving only the bottom open. A newly hatched nymph was placed in each cage, a leaf was introduced through the open end, and a piece of cotton was placed around the petiole to protect it from being injured or crushed by the edges. Paper clips again were used to close the cage and to hold the petiole between the edges. A culture number, indicating the host, was assigned to each nymph. The nymphs were transferred to new leaves of the same or of the new seedlings every six days. A total of 25 nymphs was used on each variety. Daily observations were made to record mortality, date of molting, size and weight of newly emerged adults, and other pertinent information.

RESULTS

Multiplication of the Test Insects

Exposure of the adult squash bugs to a continuous cold treatment, or to alternate cold and laboratory room temperature, for a week to a month did not initiate significantly earlier oviposition, although oviposition did occur earlier than that by the overwintered adults in the field. One

EXPLANATION OF PLATE IV

Plastic rearing cages used in the antibiosis studies. Host plant and culture number of each squash bug nymph were written on the piece of paper clipped with the cage.

PLATE IV



or two female bugs in each of the groups that were subjected to experimental cold treatment began to lay eggs after four to five months, as did also the overwintering bugs collected directly from hibernation in the field in December, 1958. Some bugs in the stock cultures kept in the laboratory and not subjected to any cold treatment began to lay eggs on the cheesecloth lining of the rearing cage after three months on Pink Banana fruit; the remainder, as well as those in the greenhouse which were fed on young leaves of mature plants of Pink Banana and Black Zucchini, laid eggs about two months later.

The above results are in agreement with Beard's (1940) observations that when adult squash bugs were kept in the laboratory under fairly high temperature and humidity, mating and deposition of fertile eggs might be induced. However, this response was not uniform and many individuals withstood all attempts to break the resting condition of their reproductive organs.

Effects of Host on Fecundity and Gain in Body Weight of Adult Bugs

Egg masses were found in the rearing cage with Pink Banana squash fruit used as food, after 96 days. The eight live female bugs laid a total of 439 eggs of which 70.61 per cent hatched. The bugs reared on Sweet Cheese fruit began laying eggs after 121 days rearing. The seven live females produced a total of 243 eggs and only 50.61 per cent hatched.

As shown in Table 1, there was no significant difference between the number of days the squash bugs reared on Sweet Cheese and on Pink Banana fruits survived. The females lived longer, though not significantly, than the males in both cases. However, a significant difference was found between the gains in body weight, after a week, of the adult bugs kept on each fruit

Table 1. Number of days adult squash bugs survived when reared on fruits of susceptible and resistant varieties of squash. The bugs were confined with the fruits a day after molting to adult. Laboratory, Manhattan, Kansas, 1958.

Bug No.	Pink Banana Fruit		Sweet Cheese Fruit	
	Male	Female	Male	Female
	Days		Days	
1	31	183	27	101
2	146	162	188	188
3	96	42	89	36
4	90	179	32	173
5	29	18	144	180
6	187	176	175	45
7	183	201	170	170
8	98	213	95	191
9	167	189	90	199
10	89	198	96	207
Average	111.60	156.10	110.6	149.0

t = non-significant (between varieties for both males and females)

of the two varieties. Data for the adult males are shown in Table 2. The loss of body weight of the bugs which were given no food was greater than that of those kept on Sweet Cheese fruit.

Amount of Plant Material Drawn by the Bugs
from the Resistant and Susceptible Plants

During the experiment it was observed that both the adults and the nymphs of squash bug began to move on to the plant from the cage about 30 minutes to one hour after their confinement with the host. Many preliminary attempts at feeding by the bugs were indicated by the fairly short time of insertion of the stylets into the plant tissue. The bugs finally settled on definite spots and started feeding with their stylets about one-half to one-third inserted. Here the feeding process lasted for about an hour or more. The preferred feeding areas were on the veins of the leaves of mature plants and on veins, petioles and stems of seedlings. No local or systemic feeding injury to the plant was observed after the three hours feeding period of the bugs.

Results of the tests for measuring the amount of plant material taken in by the adult squash bugs from the resistant and susceptible seedlings and leaves of mature plants are shown in Tables 3 and 4.

On seedlings, the largest amount of plant material drawn was from Pink Banana and the least was from Green Striped Cushaw and Butternut. The amounts taken from Pink Banana and Black Zucchini were significantly higher than those from Royal Acorn, Butternut and Green Striped Cushaw but not from Sweet Cheese. The amount taken from Sweet Cheese was significantly higher than the amount of material from Butternut and from Green Striped Cushaw but not from Royal Acorn.

Table 2. Per cent gain or loss in weight (and their corresponding transformed angles) of ten adult male bugs after a week of confinement with the squash fruits. The one-day old marked adult bugs were weighed before and after confining them with the fruits. Laboratory, Manhattan, Kansas. September, 1958.

Varieties	Bug Number																							
	1		2		3		4		5		6		7		8		9		10		Average			
	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle	:Per	:Angle		
<u>C. moschata</u>																								
Sweet Cheese	-15.23	-22.95	19.77	26.42	-9.90	-18.34	-5.64	-13.69	15.43	23.11	16.45	23.97	-11.14	-19.46	12.53	20.70	1.29	6.55	-7.29	-15.68	1.63	7.27		
<u>C. maxima</u>																								
Pink Banana	86.91	68.78	41.38	40.05	95.11	77.21	23.28	28.86	16.71	24.12	15.50	23.19	24.10	29.40	36.36	37.11	9.41	17.85	-7.80	-16.22	34.10	35.73		
Control (no food)	-20.10	-26.64	-11.16	-19.55	-15.12	-22.87	x	x	-20.11	-26.64	x	x	-16.21	-23.73	-18.12	-25.18	-18.22	-25.25	x	x	-17.10	-24.35		

x = died before one week.

t = 3.17** (between Sweet Cheese and Pink Banana).

t = 2.67* (between Sweet Cheese and Control).

Table 3. Average per cent gain or loss in weight (and their corresponding transformed angles) of ten adult male squash bugs after three hours feeding on the seedlings and on the leaves of mature plants of each of six varieties of squash. The bugs were starved for 48 hours prior to feeding (two replications). Greenhouse, Manhattan, Kansas. April, 1959.

Species	<u>Cucurbita pepo</u>				<u>C. moschata</u>				<u>C. maxima</u>					
:	:	:	:	:	:	:	:	:	:	:	:	:	:	
Variety	<u>Royal Acorn</u>	<u>Zucchini</u>	<u>Butternut</u>	<u>Sweet Cheese</u>	<u>Cushaw</u>	<u>Pink Banana</u>	<u>Control</u>	<u>(no food)</u>						
:	Per	Per	Per	Per	Per	Per	Per	Per	Per	Per	Per	Per	Per	
:	cent	Angle	cent	Angle	cent	Angle	cent	Angle	cent	Angle	cent	Angle	cent	Angle
Seedlings	22.78	28.52	34.27	35.85	19.89	26.49	29.30	32.77	19.79	26.42	37.38	37.70	-1.53	-7.04
Mature plants	19.22	25.99	30.08	33.27	17.39	24.65	25.81	30.53	21.83	27.83	24.08	29.40	-1.60	-7.27

Analysis of Variance¹

Sources of Variation		D/F	S _B	M _B	F
Seedlings	Varieties	5	1158.41	231.68	4.76**
	Individuals same variety	54	2627.93	48.66	
Mature plants	Varieties	5	476.83	95.36	2.73*
	Individuals same variety	54	1886.22	34.93	

¹ Analysis based on transformed angles of the percentages.

* Significant at 5% level.

** Significant at 1% level.

Table 4. Mean difference, and its significance, (transformed angles) between the gain or loss in weight of adult squash bugs after 3 hours opportunity to feed on seedlings and on leaves of mature plants of the six varieties of squash (see Table 3). Greenhouse, Manhattan, Kansas. March, 1959.

Species	<u>Cucurbita pepo</u>		<u>C. moschata</u>				<u>C. maxima</u>			
Variety	<u>Black Zucchini(b)</u>		<u>Butternut(c)</u>		<u>Sweet Cheese(d)</u>		<u>Green Striped Cushaw (e)</u>		<u>Pink Banana(f)</u>	
	S ¹	M ²	S	M	S	M	S	M	S	M
a) Royal Acorn <u>C. pepo</u>	b > c 7.33*	b > a 7.28*	2.03	1.34	4.25	4.54	2.10	1.84	f > a 9.18**	3.41
b) Black Zucchini			b > c 9.36**	b > e 8.62*	3.08	2.74	b > e 9.43**	b > e 5.44*	1.85	3.87
c) Butternut					d > c 6.28*	d > e 5.88*	0.07	3.18	f > c 11.21**	4.75
d) Sweet Cheese							d > e 6.35*	2.70	4.93	1.13
e) Green Striped Cushaw									f > e 11.28**	1.57

lsd	1 Seedlings	2 Mature plants
*	6.27	5.31
**	8.33	ns

On the leaves of mature plants, the amount taken from Black Zucchini, as in the seedlings, was significantly higher than that from Royal Acorn and Butternut. The amount drawn from Pink Banana was neither significantly greater nor significantly less than that from any other varieties.

Tables 5 and 6 show the amounts of plant material drawn by the third instar nymphs from the seedlings as well as from the mature plants of the six varieties. With the seedlings, significant differences were found only when the amounts drawn from Pink Banana and Black Zucchini were compared with those from Butternut and Royal Acorn; other seedling comparisons gave non-significant differences. With the leaves of mature plants, the amount drawn from Black Zucchini was significantly higher than from Royal Acorn, Sweet Cheese and Butternut; the amounts from Pink Banana and from Green Striped Cushaw were significantly higher than from Butternut.

When the six varieties were arranged in descending order of the amount of plant material drawn in by the bugs in each of the four tests (i.e., adults on seedlings, adults on mature plants, nymphs on seedlings and nymphs on mature plants) the rankings or relative positions of Black Zucchini and Pink Banana (either one or two), Royal Acorn and Butternut (either five or six) were nearly the same; that of Sweet Cheese and Green Striped Cushaw frequently changed. The rank of Sweet Cheese was four, five, three and two, that of Green Striped Cushaw was three, three, six and four, respectively, for the four tests.

Preference Tests

Tables 7 and 8 present the data on the preference tests on seedlings using the adult squash bugs. Statistical analysis of the number of bugs observed on each variety, 30 minutes after their being confined with the

Table 5. Average per cent gain or loss in weight (and their corresponding transformed angles) of ten third instar squash bug nymphs after three hours feeding period on each of the six varieties of squash. The nymphs were starved for 24 hours prior to feeding (two replications). Greenhouse, Manhattan, Kansas. March, 1959.

Species	<u>C. pepo</u>		:		<u>C. moschata</u>		:		<u>C. maxima</u>		:			
	:	Black	:	:	:	:	:	Green	:	:	:	:	Control	:
Variety	<u>Royal Acorn</u>	<u>Zucchini</u>	:	<u>Buttermut</u>	:	<u>Sweet Cheese</u>	:	<u>Cushaw</u>	:	<u>Pink Banana</u>	:	<u>(no food)</u>	:	
	Per	Per	:	Per	:	Per	:	Per	:	Per	:	Per	:	
	cent	Angle	:	cent	Angle	:	cent	Angle	:	cent	Angle	:	cent	Angle
Seedlings	27.75	31.76	55.06	47.93	28.92	32.52	35.38	36.51	48.96	44.43	55.68	48.27	-2.12	-8.33
Mature plants	22.58	28.38	34.80	36.15	13.71	21.72	22.47	28.32	28.15	32.01	29.56	32.96	-2.23	-8.53

Analysis of Variance¹

Sources of Variation		:	D/F	:	S _g	:	M _g	:	F
Seedlings	Varieties		5		3859.72		771.94		3.17**
	Individuals same variety		54		13147.92		243.48		
Mature plants	Varieties		5		1241.34		248.27		3.88**
	Individuals same variety		54		3451.62		63.91		

¹ Analysis based on transformed angles of the percentages.

** Significant at 1% level.

Table 6. Mean difference, and its significance, (transformed angles) between the gain or loss in weight of squash bug nymphs after 3 hours opportunity to feed on seedlings and on leaves of mature plants of the six varieties of squash (see Table 5). Greenhouse, Manhattan, Kansas. March, 1959.

Species	<u>Cucurbita pepo</u>		<u>C. moschata</u>				<u>C. maxima</u>			
Variety	<u>Black Zucchini(b)</u>		<u>Butternut(c)</u>		<u>Sweet Cheese(d)</u>		<u>Green Striped Cushaw(e)</u>		<u>Pink Banana(f)</u>	
	S ¹	M ²	S	M	S	M	S	M	S	M
a) Royal Acorn <u>C. pepo</u>	b>a 16.17*	b>a 7.77*	0.76	6.66	4.75	0.06	12.67	3.63	f>a 16.51*	4.58
b) Black Zucchini			b>c 15.41*	b>c 14.43**	11.41	b>c 7.83*	3.50	4.14	0.34	3.19
c) Butternut					3.99	6.60	11.91	e>c 10.29**	f>c 15.75*	f>c 11.24**
d) Sweet Cheese							7.92	3.99	11.76	4.64
e) Green Striped Cushaw									3.84	0.95
	lsd		¹ Seedlings				² Mature plants			
	*		13.87				7.20			
	**		18.42				9.56			

plants (not presented separately in the table) gave no significant differences. However, highly significant differences, as shown in Table 7, were obtained upon analysis of the total number of bugs counted on the different squash varieties observed for three hours at 30 minute intervals and on those observed for three days at one-day intervals.

Table 8 shows the difference among the mean total numbers of adult squash bugs observed on the six varieties of squash in three hours and in three days as well as the level of significance of their differences for all the possible paired varietal comparisons. Comparing the mean total number for Royal Acorn in the three-hour data as well as in three-day, with that of any of the other five varieties, significant differences were obtained, Royal Acorn being the non-preferred host in all cases. The level of significance for the three-hour and for the three-day data were not always the same.

In the three-hour data, the Black Zucchini mean total number was also significantly higher than those for either Sweet Cheese, Butternut or Green Striped Cushaw; that for Pink Banana was significantly higher than for Sweet Cheese.

Aside from the comparisons of the mean total number of adults observed on Royal Acorn in three days with any of the other five varieties, no significant differences were obtained.

Data for the preference tests on seedlings using the nymphs are shown in Tables 9 and 10. Statistical analysis of the data for three-hour and for three-day observations gave significant F values.

The differences among the mean total numbers of nymphs observed on the six varieties for all the possible comparisons are shown in Table 10. As in the tests using adult squash bugs, significant differences were obtained when the mean total for Royal Acorn was compared with those for either of the other

Table 7. Mean total number of adult squash bugs observed on each of the paired seedlings of the six varieties. Five 48-hour-starved, marked bugs were confined to paired seedlings and counts of bugs on each plant were made at 30 minute intervals for three hours and then daily for three days (five replications). Greenhouse, Manhattan, Kansas. February, 1959.

Species	<u>Cucurbita pepo</u>		<u>C. moschata</u>		<u>C. maxima</u>	
Variety	Royal Acorn	Black Zucchini	Butternut	Sweet Cheese	Green Striped Cushaw	Pink Banana
Within 3 hours	5.88	13.92	10.84	9.88	10.28	12.96
Within 3 days	2.84	8.32	6.72	7.08	7.11	7.36

Analysis of Variance
(Balanced Incomplete Block Design with $r = 5$)

Sources of Variation		D/F	S_s	M_s	F
3 hours	Replications	24	314.43	13.10	n. s.
	Varieties (unadjusted)	5	988.61	197.72	7.79***
	Blocks same replication (adjusted)	50	859.22	17.18(E_b)	
	Intra-block error	70	1776.83	25.38(E_e)	
3 days	Replications	24	91.29	3.80	n. s.
	Varieties (unadjusted)	5	455.55	91.11	9.81***
	Blocks same replication (adjusted)	50	347.12	6.94(E_b)	
	Intra-block error	70	649.67	9.28(E_e)	

Note: Since E_b , E_e no adjustment of variety mean needed.
n. s. = non-significant.
*** = significant at 0.1% level.

Table 8. Mean difference, and its significance, among the numbers of adult bugs observed on the six varieties (see Table 7).

Species	<u>Cucurbita pepo</u> :		<u>C. moschata</u> :		<u>C. maxima</u> :		Green Striped :		Pink Banana(f)	
Variety	<u>Black Zucchini(b)</u> :		<u>Butternut(c)</u> :		<u>Sweet Cheese(d)</u> :		<u>Cushaw(e)</u> :		<u>Pink Banana(f)</u>	
	3 hrs. :	3 days :	3 hrs. :	3 days :	3 hrs. :	3 days :	3 hrs. :	3 days :	3 hrs. :	3 days :
a) Royal Acorn <u>C. pepo</u>	b>a 8.04***	b>a 5.48***	c>a 4.96***	c>a 3.88***	d>a 4.00**	d>a 4.24***	e>a 4.40**	e>a 4.27**	f>a 7.08***	f>a 4.52***
b) Black Zucchini			b>c 3.08*	1.60	b>d 4.04**	1.24	b>e 3.64**	1.21	0.96	0.96
c) Butternut					0.96	0.36	0.56	0.39	2.12	0.64
d) Sweet Cheese							0.40	0.03	f>d 3.08*	0.28
e) Green Striped Cushaw									2.69	0.25

lsd	3 hours	3 days
*	2.85	1.72
**	3.76	2.28
***	4.92	2.96

Table 9. Mean total number of third instar squash bug nymphs observed on each of the paired seedlings of the six varieties. Five 24-hour-starved, marked nymphs were confined to a paired seedling and counts of nymphs on each seedling made at 30 minute intervals for three hours and then daily for three days (five replications). Greenhouse, Manhattan, Kansas. April, 1959.

Species	<u>Cucurbita pepo</u>		<u>C. moschata</u>		<u>C. maxima</u>		
Variety	Royal Acorn	Black Zucchini	Butternut	Sweet Cheese	Green Striped	Cushaw	Pink Banana
Within 3 hours	5.56	11.32	9.48	9.60	8.96	12.28	
Within 3 days	3.04	7.60	4.36	6.36	7.12	7.04	

Analysis of Variance
(Balanced Incomplete Block Design with $r=5$)

Sources of Variation		D/F	S_s	M_s	F
3 hours	Replications	24	339.00	14.12	n. s.
	Varieties (unadjusted)	5	671.50	134.30	6.61***
	Blocks same replication (adjusted)	50	412.65	8.25	
	Intra-block error	70	1423.15	20.33	
3 days	Replications	24	129.41	5.39	n. s.
	Varieties (unadjusted)	5	470.00	94.00	7.44***
	Blocks same replication (unadjusted)	50	284.93	5.70	
	Intra-block error	70	884.34	12.63	

n. s. = non-significant.

*** = significant at 0.1% level.

Table 10. Mean difference, and its significance, among the numbers of squash bug nymphs observed on the six squash varieties (see Table 9).

Variety	<u>Cucurbita pepo</u>		<u>C. moschata</u>		<u>C. maxima</u>		Green Striped		Cushaw		Pink Banana(f)	
	3 hrs.	3 days	3 hrs.	3 days	3 hrs.	3 days	3 hrs.	3 days	3 hrs.	3 days	3 hrs.	3 days
a) Royal Acorn <u>C. pepo</u>	b>a 5.76***	b>a 4.56***	c>a 3.92**	1.32	d>a 4.04**	d>a 3.32**	e>a 3.40*	e>a 4.08***	f>a 6.72**	f>a 4.00***		
b) Black Zucchini			1.84	b>c 3.24**	1.72	1.24	2.36	0.48	0.96	0.56		
c) Butternut					0.12	d>c 2.00*	0.52	e>c 2.76**	f>c 2.80*	2.68**		
d) Sweet Cheese							0.64	0.76	f>d 2.68*	0.68		
e) Green Striped Cushaw									f>e* 3.32	0.08		

lsd	3 hours	3 days
*	2.54	2.00
**	3.37	2.65
***	4.37	3.44

five varieties for three-hour data and also for three-day, except for Butternut in the latter case. Again, the levels of significance for the two sets of data were not always the same. The mean total for Butternut was significantly lower than that for either Black Zucchini, Green Striped Cushaw or Sweet Cheese for the three-day data. On the other hand, the mean total for Pink Banana was significantly higher than those for Sweet Cheese, Green Striped Cushaw and Butternut for the three-hour data.

There were differences between the preference responses of the nymphs and the adults to the six varieties of squash particularly when responses to Royal Acorn and Butternut were compared and in considering the three day data. The mean total number of adult squash bugs for Butternut was significantly higher than that for Royal Acorn, while there was no significant difference between the mean totals of nymphs for the two varieties. On the contrary, Butternut's mean total number of nymphs, but not adults, was significantly lower than those of Black Zucchini, Green Striped Cushaw, Pink Banana and Sweet Cheese. With the other variety comparisons, preference responses of adults and nymphs were almost the same.

As will be discussed later, results of the above preference tests indicated that Royal Acorn was least preferred by both the nymphs and the adults, followed by Butternut. The most preferred appeared to be Black Zucchini with Pink Banana ranking next. With this information, it was made possible to pick out from the preference test data on hand, those variety pairs which would furnish information on the movement of the bugs between the seedlings of the following pairs, namely, least and most preferred, both non-preferred, and both preferred. The pairs selected were Royal Acorn-Black Zucchini, Royal Acorn-Butternut, and Black Zucchini-Pink Banana (Table 11). The number of bugs indicated in the table as shifting between the paired

varieties included only those which were found on a seedling during an observation which were later found to have transferred to the other seedling of the pair in the succeeding observation. Those bugs which, after having been on a plant during one observation were found to be on the cage or on the soil during the next, and then on the other seedling during the third observation, were not included. Similarly, the number of bugs indicated as having remained on either of the two seedlings during the two successive observations did not include these bugs which were found to have left the plant in the second, coming back to the same plant in the third observation. In all cases, the bugs seemed to stay longer on the seedling where they first came to rest. The data show that when most and least preferred varieties were paired as Black Zucchini and Royal Acorn, the number of bugs that remained on the preferred seedling increased with time, with the reverse true of the non-preferred member of the pair. When the paired varieties were about equally preferred, as were Pink Banana and Black Zucchini, the number of bugs observed remaining on the two seedlings were almost the same during the different times of observation.

Results of the experiments designed to trace the pattern of movement of the adult squash bugs in finding the host plants are shown in Table 12. Statistical analysis of the number of bugs that climbed each seedling, the total time they spent and the total distance they traveled in finding the plants, all gave non-significant differences between the varieties. No effects of the relative position of the plants on their being found by the bugs were shown by the data gathered which were not presented in the table.

When three seedlings used in the above tests were replaced with sticks painted green, the bugs climbed the sticks just as they did the seedlings as shown in Table 13. The total number of bugs that climbed the plants compared

Table 11. Total number of individually marked bugs that moved from one member of paired varieties to the other, observed at 30-minute intervals for 3 hours and at one-day intervals for 3 days.¹

Paired Varieties	Adults								Nymphs							
	: 1	: 1.5	: 2	: 2.5	: 3	: 1	: 2	: 3	1	: 1.5	: 2	: 2.5	: 3	: 1	: 2	: 3
	: hr.:	hrs.:	hrs.:	hrs.:	hrs.:	day:	days:	days:	hr.:	hrs.:	hrs.:	hrs.:	hrs.:	day:	days:	days:
1) Royal Acorn & Black Zucchini																
(a) R.A. → B.Z. ²	0	1	3	1	0	0	2	0	1	0	0	3	4	0	1	1
(b) R.A. → R.A.	6	4	5	5	4	5	3	3	7	7	1	2	0	1	1	0
(c) B.Z. → R.A.	0	0	0	0	1	0	0	1	0	2	0	0	0	0	1	0
(d) B.Z. → B.Z.	7	8	11	10	9	8	10	10	3	3	5	10	12	7	10	14
2) Royal Acorn & Butternut																
(a) R.A. → But.	0	2	0	2	1	3	2	0	0	0	2	3	2	0	1	0
(b) R.A. → R.A.	3	2	3	5	4	4	6	3	3	3	2	2	2	2	1	2
(c) But. → R.A.	1	0	1	1	0	2	0	0	0	1	1	2	0	0	1	0
(d) But. → But.	5	3	6	5	6	7	8	9	4	5	5	7	6	5	7	8
3) Pink Banana & B. Zucchini																
(a) P.B. → B.Z.	0	2	1	0	0	2	0	1	0	0	0	2	1	0	0	1
(b) P.B. → P.B.	3	9	9	9	10	9	10	10	6	5	11	8	8	9	12	10
(c) B.Z. → P.B.	2	0	0	1	0	2	3	2	1	0	0	2	2	1	0	2
(d) B.Z. → B.Z.	5	8	10	10	11	11	11	12	5	7	12	10	10	10	12	11

¹ Totals of 5 replications with 5 bugs per replication.

² Arrow indicates direction of movement of the bugs.

Table 12. Pattern of movement of the adult squash bugs in finding the host plant. The 48-hour-starved bugs were released one at a time at the center of the graph paper¹ and the pattern of movement of each bug until it reached the plant was reproduced on a duplicate graph paper. Greenhouse, Manhattan, Kansas. March and May, 1959.

Species	<u>Cucurbita pepo</u>				:				<u>C. moschata</u>				:				<u>C. maxima</u>																			
Variety	Royal Acorn				:				Black				:				Green Striped				:				Pink											
	Zucchini				:				Butternut				:				Sweet Cheese				:				Cushaw				:				Banana			
Bug Number	8	12	2	13	7	3	18	17	10	19	6	15	5	9	14	16	20	1	10	4																
Total time (minutes)	25	20	10	3	11	20	18	3	35	8	2	29	17	28	19	21	23	1	20	25																
Total distance (no. squares crossed) ²	82	41	55	19	42	50	62	15	163	28	27	126	50	106	96	73	83	20	101	47																
Pathway: Direct or Indirect	I	I	I	D	I	I	I	D	I	D	D	I	I	I	I	I	I	D	I	I																
Average: Total time	14.50				18.33				15.33				16.00				22.75				15.33															
Average: Total distance	49.25				51.33				68.66				67.66				89.50				56.00															

¹ The experimental situation was similar to that in Plate II except that plants were used instead of sticks.

² 1 square = 8 mm. sq.

Table 13. Attraction of the adult squash bugs to both the squash seedlings and sticks. The 48-hour-starved bugs were released at the center of the graph paper¹ and the pattern of movement of each bug until it climbed the object was reproduced on a duplicate graph. Greenhouse, Manhattan, Kansas. March and May, 1959.

Variety	<u>Cucurbita pepo:</u>								<u>C. moschata</u>				<u>C. maxima:</u>											
	Black				Zucchini				Sweet Cheese				Pink											
	Stick No. 1								Stick No. 2				Stick No. 3											
Bug Number	8	5	9	11	2	14	20	3	1	12	13	7	10	16	18	4	17	6	15	19				
Total time (minutes)	19	38	43	16	25	39	23	36	39	10	10	2	38	29	23	28	30	15	28	26				
Total distance (no. squares crossed) ²	52	75	130	31	42	176	207	79	62	33	179	24	157	94	130	92	102	35	109	98				
Pathway: Direct or Indirect	I	I	I	D	I	I	I	I	I	D	I	D	I	I	I	I	I	D	I	I				
Average: Total time	33.33				25.75				23.75				23.00				29.00				23.00			
Average: Total distance	85.66				139.00				88.25				101.25				96.00				80.66			

¹ See Plate II for illustration.

² 1 square = 8 mm. square.

to those that climbed the sticks, the time spent and the distance traveled by the bugs in finding either the sticks or the plants were not significantly different. The position of the plants and the sticks in relation to the sun, which was omitted in the table, also seemed to have no effect on their being found by the bugs. At one instance, when the bugs were kept with the seedlings and the sticks for some time with the use of a large glass cylinder, a bug was observed trying to insert its stylets into the stick.

During the experiments using plants only and those using three plants and three sticks, it was observed that some of the adult squash bugs, which had been individually released at the center of the graph paper, moved quickly upon their release as if no definite pattern of movement was being followed. The others remained in place for a time, wiggling the antennae, stretching the legs and often rubbing together the tips of the tarsi of the forelegs before starting their movement. When the fast moving bugs happened to encounter either a plant or a stick, the majority of them climbed the object but a few continued to move around.

Tolerance Tests

Results of the tests to determine whether or not there were significant differences between the lengths of time the seedlings of the six varieties of squash could support the same insect population are shown in Table 14. In these tests, there are two variable factors, namely, the maximum temperature during the experiment and the size of the seedlings evaluated in terms of leaf area, which might have had some effect upon the length of time that the seedlings survived. No significant correlation (by ranking¹) was found

¹ Suggested by Dr. H. C. Fryer, Head of Statistics Department.

Table 14. Number of hours the seedling of the six varieties of squash survived with two males and three female bugs feeding on each seedling. Three-day old seedlings with the cotyledon leaves about three-fourths grown were used and condition of plants noted at three-hour intervals. Greenhouse, Manhattan, Kansas. 1959.

Repl- cation	Maximum temp. during experiment (F°)	Species Variety Plant No.	<i>Cucurbita pepo</i>				<i>C. moschata</i>				<i>C. maxima</i>			
			Royal Acorn		Black Zucchini		Butternut		Sweet Cheese		Green Striped Cushaw		Pink Banana	
			leaf area ¹ sq.mm.	Hrs. the plant survived	leaf area sq.mm.	Hrs. the plant survived	leaf area sq.mm.	Hrs. the plant survived	leaf area sq.mm.	Hrs. the plant survived	leaf area sq.mm.	Hrs. the plant survived	leaf area sq.mm.	Hrs. the plant survived
I	85	1	875	(120) ²	960	126	400	(78) ²	535	123	450	177	910	171
		2	600	120	777	72	360	78	672	(123) ²	567	123	700	171
II	96	3	660	96	1200	144	432	96	480	102	700	147	900	84
		4	562	99	981	120	362	99	683	129	590	120	689	81
III	97	5	660	84	810	72	570	75	825	81	816	78	1085	81
		6	700	81	740	78	500	78	689	78	864	78	1360	78
IV	101	7	660	72	640	96	450	75	480	60	569	60	600	72
		8	475	75	448	60	630	78	600	96	701	75	840	75
V	104	9	650	72	742	72	315	54	526	57	673	57	571	57
		10	563	75	669	75	540	57	672	60	721	72	672	57
		Average	640.5	89.4	796.7	91.5	455.9	76.8	616.2	90.9	665.1	98.7	832.7	92.7

¹ No correlation was found between the leaf area and the number of hours that the plant survived for any of the six varieties.
² Plants still alive two weeks after the death of the other seedlings. Values were assigned to facilitate analysis of the data.

Analysis of Variance

Sources of Variation	D/F	S _s	M _s	F
Variety	5	2,506.40	501.28	2.49 n.s.
Temperature	4	30,886.00	7,722.15	38.46***
Variety-Temperature Interaction	20	14,053.10	702.65	3.50**
Plants same variety and temperature	27	5,420.5	200.76	

between the leaf area of the seedling and the number of hours it survived for any of the six varieties. Comparing the number of hours that the seedlings of the six varieties survived, regardless of their leaf areas, no significant differences were obtained. The time interval between the appearance of the first symptom of the feeding injury and the death of the seedlings of the six squash varieties were compared and again the differences were found to be non-significant.

Temperature affected the length of survival of the seedlings as indicated by the highly significant F value shown in Table 14. The higher the temperature the shorter the time of seedling survival.

In the first replication conducted with the maximum temperature of 85°F. during the experiment, three plants (one each of Royal Acorn, Butternut, and Sweet Cheese) survived and were vigorously growing when all the others were dead.¹ Two weeks after all the others had died, the bugs were removed from the three living plants and the experiment was discontinued as it appeared probable that the plants would continue to grow in spite of the squash bugs' feeding on them. No similar case of survival occurred in the other four replications carried on at higher maximum temperature.

Antibiosis Studies

High mortality (76-85%) of the nymphs reared on the seedlings of any of the six varieties of squash was observed and no significant differences were found among such high mortality of the groups of nymphs on the six squash varieties.

¹ It may be significant that these three plants were similar to those classified as resistant in the field. Repeated experiments at lower temperature might show consistent differences. See Discussion.

Out of a total of 300 nymphs used on the six squash varieties, only five were able to reach the adult stage. Of these five, one (male) was reared on Royal Acorn, two (both males) on Black Zucchini and two (one male and one female) on Pink Banana. The other surviving bugs were still in the fourth and fifth instars after four months, at which time the observation was discontinued. Beard (1940) stated that the total developmental time required (from egg to adult) was 45 to 66 days in Kansas.

Due to the insufficient number of bugs that developed to adults, comparisons of the antibiotic effects of the host plants on the squash bugs as measured in terms of length of life cycle, fecundity and hatching percentage, size of newly emerged adult bugs, and longevity, were inconclusive. Antibiotic effects of the six squash varieties were, therefore, based on the nymphal instar attained and the number of days lived by these nymphs that failed to reach maturity as shown in Tables 15 and 16.

Nymphs reared on Black Zucchini and Pink Banana reached a significantly later instar than did those reared on Royal Acorn, Sweet Cheese, Green Striped Cushaw and Butternut. Those reared on Pink Banana lived significantly longer than those reared on Green Striped Cushaw and Butternut. The instar reached and the number of days lived by the nymphs reared on Royal Acorn, Sweet Cheese, Green Striped Cushaw, and Butternut were not significantly different from one another.

Most of the nymphs died in the second and third instars. Among those reared on Royal Acorn, Green Striped Cushaw, Sweet Cheese and Butternut, death occurred mostly just before, during, or immediately after molting. Death of the nymphs on Black Zucchini and on Pink Banana occurred at various times.

Table 15. Average instar reached and length of life (days) of 15 squash bug nymphs that died out of the 25 newly hatched nymphs reared on the seedlings of each of the six squash varieties.¹ Greenhouse, Manhattan, Kansas. 1959.

Species	<u>Cucurbita pepo</u>		<u>C. moschata</u>		<u>C. maxima</u>	
Variety	Royal	Black	Sweet	Green Striped	Pink	
	Acorn	Zucchini	Butternut	Cheese	Cushaw	Banana
Instar reached	2.87	3.93	2.47	2.67	2.73	3.86
No. days lived	26.73	38.93	20.67	25.13	21.93	33.60

¹ These tests were conducted from February 10 to June 15.

Analysis of Variance

Sources of Variation		D/F	S _s	M _s	F
Instar	Between varieties	5	30.85	6.17	4.97**
	Individuals same variety	84	104.40	1.24	
No. days lived	Between varieties	5	3,767.03	753.40	3.18*
	Individuals same variety	84	19,875.47	236.61	

* = significant at 5% level.

** = significant at 1% level.

Table 16. Mean difference, and its significance, between the instar reached and the number of days lived by nymphs reared on seedlings of the six squash varieties (see Table 15).

Variety	<u>Cucurbita pepo</u> :		<u>C. moschata</u> :		<u>C. maxima</u> :					
	<u>Black Zucchini(b)</u>	<u>Butternut(c)</u>	<u>Sweet Cheese(d)</u>	<u>Green Striped</u>	<u>Cushaw(e)</u>	<u>Pink Banana(f)</u>				
	Instar	Days	Instar	Days	Instar	Days	Instar	Days		
a) Royal Acorn	b > a 1.06**	b > a 12.20*	0.40	6.06	0.20	1.60	.14	4.80	f > a 0.99*	6.87
b) Black Zucchini			b > c 1.46**	b > c 18.26*	b > d 1.26**	b > d 13.80*	b > e 1.20**	b > e 17.00*	0.07	5.33
c) Butternut					0.20	4.46	0.26	1.26	f > c 1.39**	f > c 12.93*
d) Sweet Cheese							0.06	3.20	f > d 1.19**	8.47
e) Green Striped Cushaw									f > e 1.13**	f > e 11.67*

lsd	Instar	Days
*	0.80	11.03
**	1.06	n. s.

DISCUSSION

Why a certain plant is resistant should, if possible, be explored because such information might be of vital importance in breeding plants for insect resistance. In this greenhouse study, several criteria were used in measuring and separating the components of resistance of certain varieties of squash to the squash bug; these varieties had exhibited differing degrees of resistance in the field.

Eichmann (1945) working in Washington, stated that the overwintered adult squash bugs emerging from hibernation and seeking squash seedlings at ground level during late May to early June, were responsible for most of the destruction of seedlings in the field, while Davis (1955) reported that the surprisingly large population of squash bugs, consisting mostly of nymphs, that built up by August or September, had been responsible for heavy losses in commercial plantings of squash and pumpkin in Indiana. Because the nymphs and the adult squash bugs might have differences in their interactions with a particular variety of squash, partly because of some differences in their nutritional requirements, separate tests were made for the nymphs and the adults. When possible and applicable, seedlings and mature plants of each squash variety were tested because a plant may develop or lose resistance as it matures. To facilitate discussion, Table 17 is presented as a summary of all the tests conducted in this study.

Results of the preference tests indicated that non-preference was one of the components of resistance of Royal Acorn when compared with any of the other five varieties, of Butternut compared with the four varieties excluding Royal Acorn, and of Sweet Cheese and Green Striped Cushaw with Black Zucchini and Pink Banana. Considering Royal Acorn, it can be noted in Tables 8 and 10,

Table 17. Ranking of the squash varieties according to their resistance¹ to squash bugs as observed in the greenhouse and a comparison of these results with field observations.²

Resistance measured by	Variety Rank (based on means)						Significant comparisons among means ³
	Cucurbita pepo	C. moschata	C. maxima	Green	Royal	Black	
	Acorn	Zucchini	nut	Cheese	Cushaw	Banana	

Preference:

Adult bugs attracted in 3 hrs.	1	6	4	2	3	5	6 > 1,2,3,4; 5 > 1,2; 2,3,4 > 1
Adult bugs attracted in 3 days	1	6	2	3	4	5	2,3,4,5,6 > 1
Nymphs attracted in 3 hrs.	1	5	3	4	2	6	6 > 1,2,3,4; 5 > 1,2; 2,3 > 1
Nymphs attracted in 3 days	1	6	2	3	5	4	3,4,5,6 > 1,2
Amount of plant material drawn:							
Adults from seedlings	3	5	2	4	1	6	5,6 > 1,2,3; 4 > 1,2
Adults from mature plants	2	6	1	5	3	4	6 > 1,2,3; 5 > 1,2
Nymphs from seedlings	1	5	2	3	4	6	5,6 > 1,2
Nymphs from mature plants	3	6	1	2	4	5	6 > 1,2,3; 5,4 > 1

Table 17. (concl.)

Resistance measured by	Variety Rank (based on means)							Significant comparisons among means ³
	<u>Cucurbita pepo</u>	<u>C. moschata</u>	<u>C. maxima</u>	Green	Royal	Black	Butter-	
	Acorn	Zucchini	nut	Cheese	Cushaw	Banana		
Antibiosis:								
Instar attained by nymphs	4	6	1	2	3	5	5,6 > 1,2,3,4	
No. days nymphs survived	4	6	1	3	2	5	6 > 1,2,3,4; 5 > 1,2	
Tolerance:								
No. hours plant survived	5	3	6	4	1	2	All comparisons are non-significant	
Average rank ⁴	2.1	5.7	1.9	3.1	3.1	5.1		
Field Classification ²	2-3	6	1-2	1	3	3		

¹ Resistant = 1; Susceptible = 6.

² Information furnished by Prof. Hall of the Horticulture Department. Ranking based both on percentage survival of seedlings and counts of squash bugs per plant.

³ Interpretation: (Example: Adult bugs attracted in 3 hours indicated by ranking as variety ranking No. 6. 6 > (is significantly greater than) 1,2,3,4). The bugs attracted to Black Zucchini were significantly more than those on Royal Acorn, Sweet Cheese, Green Striped Cushaw or Butternut.

⁴ Ranking for tolerance (which gave no significant differences) not included.

that there were significantly fewer squash bugs attracted to this plant in all the four tests made, as compared with the number of bugs observed on any of the other five varieties. The greatest number of bugs was observed on Black Zucchini and Pink Banana, indicating that these were the most preferred varieties.

The differences between the preference responses of the squash bug nymphs and adults which is evident in most of the experiments, could have been brought about by certain differences in their bodily needs. Insects use a particular plant for food, for oviposition, for shelter or for combinations of the three (Painter 1951). It was obvious that the attraction of the nymphs to the plant, unlike the adults, was not governed by the oviposition needs but by either or both the needs for food and for shelter. Much more, the adults' requirement for food was for body maintenance and for egg production (in females) while that of the nymphs was primarily for growth and development.

Considering the three-day preference test data for the Royal Acorn-Butternut pair, the significantly greater number of adult squash bugs found on Butternut suggested that the adult bugs found this plant to be a better host than Royal Acorn. Similarly, the non-significant difference between the number of nymphs observed as attracted to the two varieties implied that probably these two varieties were equally unsuited as host plants for the nymphs. The above reasons could also be given for the difference in the preference response of the nymphs and the adults between other variety pairings.

Taking the four measures of squash bug preference as one (Table 17), Royal Acorn appeared to be the least preferred variety, Black Zucchini and

Pink Banana were the most preferred, and Butternut, Sweet Cheese and Green Striped Cushaw were intermediate.

Painter (1951) stated that generally host-finding by the insects is either directed by a stimulus or stimuli originating from the host, or by mere chance, through random movement of the insects. The second possibility of host-finding by the squash bug was strongly implied by the results of the preference tests conducted.

Comparing the variety rankings based on the three-hour data, with that based on the three-day data for both the nymphs and the adult squash bugs, differences in the ranking of Butternut, Sweet Cheese and Green Striped Cushaw in the four tests, were evident. Butternut, for example, which was ranked four and three (for the adults and nymphs, respectively) using the three-hour data, was ranked number two (for both nymphs and adults) when the three-day data were used. The reverse was true of Green Striped Cushaw. It was ranked number three and two (adults and nymphs) based on the three-hour data, but the ranks were lowered to four and five, respectively, when the three-day data were considered. These results were evidence that it took the squash bugs some time to detect the preferred host.

Location of a suitable host through random movements of the bug was indicated by the non-significant differences among the numbers of bugs observed on the six varieties 30 minutes after the insects were introduced; by the non-significant differences among the numbers of bugs that climbed the plants of the six varieties, the distance traveled and the time spent by the insects in finding the plants; by the fairly equal attraction of the insects to both the plants and the sticks; and by the observation that one bug was attempting to feed on a stick. It appears, also, that final choice of one host plant among several possibilities took considerable time and that

it possibly was accomplished by "tasting" or "sampling" each plant onto which the bug climbed. This would account for the increase, with time, in number of bugs that remained on susceptible plant of the paired seedlings, and the decrease in number of bugs on the resistant plant of the pair.

From the results of the preference tests, it was evident that the time needed by the bugs to detect which was the "better" of the two hosts offered depended greatly on the degree of preference for the plants used. When the seedlings paired were the most and the least preferred, the bugs were able to respond to the difference in a shorter time (possibly in three hours or less) than when either highly or least preferred was paired with a moderately preferred plant.

The ability of the squash bugs to select between most and least preferred plants in a relatively short time apparently was due to a wide differential in plant characteristics responsible for the non-attractiveness or repellence of the least preferred as opposed to the attractiveness of the most preferred. On the other hand, the longer time required for the bugs to select between moderately and least (or most) preferred was possibly due to a less pronounced differential in the plant characteristics mentioned above. This, therefore, would explain the uniformity of the preference ratings of Royal Acorn, Black Zucchini, and Pink Banana in both the three-hour and the three-day observations.

Another measure of the components of resistance used in this study was the differential amount of plant material that the squash bugs extracted from the six varieties of squash. An observation during these feeding tests that many attempts on the various spots on the leaves or seedlings were made by the bugs also suggested a "tasting" of the plants first before the actual feeding

was started. Based on the amount of plant material extracted from the plants, results of the four tests indicated that Butternut furnished the smallest amount of material extracted and therefore had the highest level of resistance, followed by Royal Acorn. Black Zucchini was the most susceptible with Pink Banana ranking next. Fairly uniform ranking of the four varieties just mentioned was obtained in the four tests, but again there were variations in the ranking of Sweet Cheese and Green Striped Cushaw. The great variability was related to the two stages of the insect used (nymphs and adults) and not to the two stages of the plants (seedling and mature plants). Sweet Cheese ranked four and five (seedlings and mature plants) when adult bugs were used, three and two when nymphs were used, while Green Striped Cushaw was ranked one and three, using adult squash bugs and four, using the nymphs.

The difference between the amount of plant material drawn by the adults and by the nymphs from the same variety of squash as mentioned above, could have been due to some morphological characteristics of the plant. Considering Sweet Cheese and Green Striped Cushaw, such plant characteristics of the former might have had interfered with, or had acted as "mechanical barriers" against the feeding of the nymphs but not the adults, they being in two different stages of insect development. The reverse should have been true with the latter variety.

Significant differences were obtained when the amounts of plant material withdrawn by the nymphs and adult squash bugs from the resistant and from the susceptible varieties of squash were compared. Such differences could be attributed to at least two causes or possibilities, namely, the impeding external or cellular structure of the resistant plants to the feeding process

of the insect, or the "dislike" of the insect for the plants, though they were inclined to feed on these plants just the same, since they were starved before being confined with the plants. The reasons for the possible "dislike" of the insect for feeding on a particular variety manifested by the small amount of plant material drawn, could be the absence of feeding stimulants in the plant or the presence of some antibiotic or repellent effects of the plant material to the insect.

Another plant characteristic useful against insect attack that a resistant plant might have is a single, or a group of its adverse effects on the life history of the insect. Such effects are reduced fecundity, decreased size, abnormal length of life cycle and increased mortality (Painter 1951, 1958).

As has been mentioned in the Results, because of the insufficient number of nymphs that reached the adult stage, the determination of the antibiotic effects of the resistant varieties was based on the instar reached and the number of days lived by those nymphs that died.

The high mortality of the nymphs reared on the seedlings of each of the six squash varieties could be attributed to the adverse effects of the environmental conditions, rather than to the effects of the host on which they were being reared, as no significant differences were found among the mortalities of the six groups of nymphs.

As had been reported by various workers, the squash bug has only one generation a year and reports of individuals that were raised from egg to adult under laboratory conditions, during winter, were extremely rare in the literature. However, several generations a year were reported in the southern states and in California, where no extreme cold weather conditions were present. This information suggested the sensitiveness of this insect to

adverse weather conditions such as low temperature and humidity.

It was true that temperature and humidity in the greenhouse during the time of rearing of the nymphs, were not fluctuating as much as the outdoor conditions did, but probably 60° F. (± a few degrees) was not high enough for the normal development of the nymphs.

Antibiotic effects exerted by the four more resistant squash varieties (Butternut, Royal Acorn, Sweet Cheese and Green Striped Cushaw) on the nymphs were suggested by the significantly later instar attained by the nymphs reared on Pink Banana and on Black Zucchini compared to that attained by those on the four varieties just mentioned; by the significantly more days lived by the nymphs reared on Black Zucchini compared to that lived by those on these four varieties; by those on Pink Banana compared to that on Butternut and Green Striped Cushaw.

It appears probable that the above significant differences among the instars attained and the numbers of days lived by the six groups of nymphs were manifestations of some antibiotic effects of the resistant squash varieties on the insects, as had been pointed out, rather than the effects of the environmental conditions during the experiment. However, there was a possibility that the unfavorable weather conditions might have aided in accentuating such varietal effects on the nymphs.

Death of the nymphs reared on Butternut, Sweet Cheese, Royal Acorn and Green Striped Cushaw shortly before, during, or immediately after molting, seemed to suggest the absence of, or the presence of inadequate amount of, certain plant materials needed by the nymphs for the molting process.

Other indications, though not so conclusive, of the antibiotic effects of the resistant variety Sweet Cheese, were the fewer number of eggs laid (and lower hatching percentage) by, and the significantly lower increase in

body weight of, the adult bugs reared on Sweet Cheese fruit compared to those of the bugs on Pink Banana fruit. However, such difference between the increase in body weight of these two groups might have been a matter of "dislike" for Sweet Cheese, a response belonging to "non-preference". The fact that the control bugs, which were given no food, suffered a greater decrease in body weight than those kept on Sweet Cheese fruit, was an indication that the bugs fed at least to a limited extent on the Sweet Cheese fruit, since they were given no choice of food.

Since the above tests were not done on the other four varieties because of lack of fruits, they were not included in the summary table (Table 17).

The non-significant differences among the lengths of survival of the seedlings of the six squash varieties, supporting the same number of adult bugs, seemed to suggest that tolerance was not one of the mechanisms involved in squash resistance to squash bugs. The variety ranks for these tests were markedly different from those based on other tests. However, this result did not necessarily mean that there was no field tolerance in the resistant varieties studied. Tolerance in the field might have been in the form of re-growth or repair of the injury to the plants by the bugs, a factor which would be quite difficult to determine in the greenhouse. Besides, tolerance is the component of resistance which is most easily affected by the conditions under which the plants grow (Painter 1951, 1958). In this study, such an effect was supported by the significantly great effect of temperature on the length of survival of the seedlings.

The existence of the three plants (one each of Royal Acorn, Sweet Cheese, Butternut) in the first replication of the tolerance studies, which survived after all the other seedlings were dead, was a good indication of tolerance

of these plants. Painter and Hall (unpublished, 1956) observed high degrees of tolerance in the field particularly with Sweet Cheese. Aside from the possibility of tolerance in the form of regrowth or repair of injury mentioned above, it could be that those three plants that survived (in the greenhouse) were tolerant strains of those three squash varieties. Had they been raised for multiplication, they might, in the future, prove to be good sources of resistant squash plants. There was a possibility that the surviving plants of Royal Acorn, Butternut and Sweet Cheese observed in the field and those three in the greenhouse, were of similar resistant strains of those varieties.

As shown in Table 17, field and mean greenhouse rankings of the six varieties (resistant (1) to susceptible (6)) were nearly the same except for Sweet Cheese and Pink Banana. The former was classified as the most resistant and the latter, moderately resistant. For Sweet Cheese, such difference could have been the result of its failure to manifest, in the greenhouse, the observed high degree of tolerance in the field. Professor Charles V. Hall (through personal communication) stated that the rank assigned to Pink Banana was based on only one year of field observation and was therefore less reliable than the ranks of the other five varieties which were based on two years of field observation. If additional field observations on Pink Banana were to still indicate that this variety was moderately resistant, then the difference between its performance in the greenhouse and in the field could have been due, as might be true also for Sweet Cheese, to the effects of the environmental conditions under which the plants were grown.

Comparisons could be made among the ranges of resistance (resistant to susceptible) to the squash bug found within each of the three species studied, namely, C. pepo, C. moschata and C. maxima, using both the greenhouse and

field classifications of the six representative squash varieties tested. The maximum range was found within the first species represented by the resistant Royal Acorn and the highly susceptible Black Zucchini; those within the latter two species were fairly narrow and were nearly the same. However, had other varieties been used instead, results might have been different from the above.

SUMMARY AND CONCLUSIONS

The present work mainly involves determination, in the greenhouse of the components of resistance or susceptibility observed in the field, of Cucurbita pepo L. varieties Royal Acorn (resistant) and Black Zucchini (susceptible); C. moschata Duch. varieties Butternut and Sweet Cheese (both resistant to differing extents), and C. maxima Duch. varieties Green Striped Cushaw and Pink Banana.

Several criteria were used in measuring and separating the mechanisms of resistance involved. Nymph and adult squash bugs were used separately in each experiment. When possible and applicable, seedlings and mature plants of each variety were tested.

Preference tests showed that Royal Acorn was the least preferred variety, with Butternut ranking next, when either was paired with any of the other four squash varieties and the bugs were given a chance to select from between the two seedlings. Black Zucchini and Pink Banana were the most preferred, Sweet Cheese and Green Striped Cushaw were moderately preferred.

There were differences between the preference responses of the nymph and the adult squash bugs to the same squash variety. Such could have been the result of some differences in the nutritional requirements of these two life history stages.

The squash bugs found the plants by chance, through random movement aided in part by a tendency to react toward upright objects. The time needed to detect the more preferable of the two varieties depended greatly on the degree of preference for the plants tested. When the paired seedlings were the most and the least preferred, the bugs were able to respond to the differences more readily than when paired with a moderately preferred plant.

The amounts of plant material taken in by nymphs and adults from the seedlings and from the leaves of mature plants of the six varieties were determined. Results showed that Butternut, from which the smallest amount was extracted, was the most resistant, followed by Royal Acorn. Black Zucchini and Pink Banana were the most susceptible. The other two varieties were intermediate.

The significantly smaller amount of plant material withdrawn from the resistant than from the susceptible varieties, was attributed to the impeding external or cellular structure of the plants to the feeding process of the insect, or the insects' "dislike" for the plants either because of the absence of feeding stimuli or the presence of some antibiotic or repellent effects of the plant material to the insect.

The high mortality of the nymphs reared on the seedlings of the six squash varieties was the result of adverse effects of the unfavorable environmental conditions, such as low temperature and humidity which was prevalent during the rearing period.

Due to an insufficient number of individuals that developed to adults, antibiotic effects of each squash variety were based on the instar attained and the number of days lived by nymphs which failed to reach maturity. Nymphs reared on Black Zucchini and Pink Banana attained a significantly later instar and lived significantly longer than did those on any of the other four

squash varieties. It is concluded, therefore, that these four resistant varieties have some antibiotic effects on the nymphs.

Resistant seedlings did not live significantly longer than the susceptible ones when each plant supported the same number of bugs. This indicated that tolerance was not present in the stage of the resistant plants used. However, this component could also be present but in the form of regrowth or repair of the injury, a factor quite difficult to determine in the greenhouse. Besides, it is the component most easily affected by the conditions under which the plants were grown (Painter 1951, 1958).

Three plants, one each of Royal Acorn, Butternut and Sweet Cheese, in the first replication of the tolerance tests, survived when all the other seedlings had died. These, as well as those surviving plants observed in the field, could have been resistant strains of these three varieties.

Results of all the greenhouse experiments indicated that Butternut was the most resistant among the six squash varieties studied, due to its high degree of antibiosis, non-preference and the inability of the bugs to get from this host as much plant material as they were able to take from any of the other five varieties.

Royal Acorn ranked next to Butternut in resistance. The components involved were a high degree of non-preference over any of the other five varieties, a slight degree of antibiosis, and the presence of some plant characteristics that somewhat limited the amount of sap that the bugs were able to extract from the plant.

Sweet Cheese and Green Striped Cushaw were classified as moderately resistant. Both had some antibiotic effects on the nymphs, were slightly non-preferred, and possessed some plant characteristics that limited to a slight degree the amount of plant material that the bugs were able to

extract from the plant.

Black Zucchini was the most susceptible and Pink Banana ranked next. Both were highly preferred by the bugs, had the least antibiotic effects on the nymphs, and the insects were able to extract the largest amount of plant material from them.

Greenhouse and field classifications of the six squash varieties according to their resistance to squash bug, were similar except for Sweet Cheese and Pink Banana. The recorded differences in the performance of these two varieties in the field and the greenhouse could have been due to the effects of the environmental conditions under which the plants were grown.

Resistance was found in all the three Cucurbita species studied. Based on the two representative varieties of each species used, the maximum range of resistance was found in C. pepo; those of C. moschata and C. maxima were fairly narrow and the two were nearly the same.

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INTERACTIONS OF THE SQUASH BUG, ANASA TRISTIS (DEGEER)
(COREIDAE, HEMIPTERA), AND SIX VARIETIES OF SQUASH (CUCURBITA SPP.)

by

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The use of resistant squash plants might prove effective in controlling the squash bugs, Anasa tristis (DeGeer), which are unusually difficult to control with insecticides.

Nymph and adult squash bugs were tested on seedlings and mature plants of six squash varieties.

Preference tests were conducted by pairing seedlings of the six varieties to form 15 possible combinations. Five starved bugs were confined with each pair, and the bugs attracted to each seedling were observed at regular time intervals; this showed that Royal Acorn and Butternut were least preferred, Sweet Cheese and Green Striped Cushaw were moderately preferred, Black Zucchini and Pink Banana were most preferred.

Differences between the preference responses of nymphs and adults to the same squash variety were observed. The bugs found the plants by chance, through random movement aided in part by a tendency to react to upward objects. The time needed to detect the more preferable of the two varieties depended greatly on the degree of preference for the plants tested. When the paired seedlings were the most and the least preferred, the bugs were able to respond to the difference more readily than when paired with a moderately preferred plant.

Starved bugs were given three hours feeding opportunity on resistant and on susceptible plants. Their gains in body weights, corresponding to the amount of sap extracted from the plants, were determined. The smallest amount was taken from Butternut, then Royal Acorn, and the most from Black Zucchini and Pink Banana.

In tolerance tests, five starved bugs were confined on each seedling of the six varieties. The condition of each plant was noted at three-hour intervals until the plant died. Three plants in the first replication

(on each of Royal Acorn, Butternut and Sweet Cheese), possibly resistant strains, survived after all other seedlings had been killed. No significant differences were found among the lengths of survival of the other seedlings. The seedlings lived significantly longer at lower than at higher temperatures.

Low temperature and low humidity caused high mortality of the nymphs. Antibiosis was therefore based on the instar attained and the number of days lived by nymphs that failed to reach maturity. Those reared on Black Zucchini and Pink Banana lived significantly longer and attained significantly later instars than those on any of the other four varieties, indicating antibiotic effects of the latter four.

Greenhouse tests indicated that: Butternut (Cucurbita moschata) was most resistant, then Royal Acorn (C. pepo), the former, due to the high level of antibiosis, moderate non-preference, and great reduction in intake of food by the bugs, the latter, due to high level of non-preference and moderate degrees of antibiosis and reduction in food intake; Sweet Cheese (C. moschata) and Green Striped Cushaw (C. maxima) were moderately resistant, possessing a moderate level of antibiosis, slight non-preference and some reduction in food intake; Black Zucchini (C. pepo) and Pink Banana (C. maxima) were susceptible, being highly preferred, having little evident antibiotic effects on the nymphs, and the bugs were able to extract large amounts of sap from them.

Differences between greenhouse and field classifications were found only in Sweet Cheese and Pink Banana, classified as resistant and moderately resistant, respectively, in the field. These differences could have been the results of different environmental conditions.