FEEDING RESPONSE OF WESTERN CORN ROOTWORM ON SILKS OF FIFTEEN GENETIC SOURCES OF CORN

by 632

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B.S., Kansas State University, 1969

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

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INTRODUCTION

The western corn rootworm, Diabrotica virgifera Lec., is one of the most damaging corn pests in the midwest. Since its initial description on corn in Colorado, it has moved eastward and is now a serious pest in South Dakota, Nebraska, Kansas, Missouri, Iowa, Illinois, Ohio and Wisconsin.

Although the larval stage is considered to be the most destructive, adult beetles may feed on all parts of the corn plant above the ground. High populations of adults may consume enough corn silk to interfere with pollination of the ears.

Rootworms can be controlled by crop rotation, but intensive farming practices and the high value of corn compared to other crops often make this control method impractical. The first reliable chemical control of rootworm larvae was obtained with soil applications of the chlorinated hydrocarbons, aldrin and heptachlor. In the early 1960's resistance to these chemicals was reported in several states in the midwest. Recently, the western corn rootworm larvae has been effectively controlled by soil applications of organophosphate and carbamate insecticides. No resistance has been reported to these insecticides.

Some research has been directed towards selection of lines of corn with root systems resistant to the larvae of the rootworm, but the attempts have been largely unsuccessful. Very little research has been done on selection of lines of corn with silks resistant to the feeding of the adult western corn rootworm beetle. Granados (1967) screened 356 lines of corn for differential damage to the silks by the feeding of adult corn rootworm beetles, and classified several of the lines as resistant. The objective of this work was to study more closely in the field and laboratory the silk resistance of the lines of corn selected by Granados.

LITERATURE REVIEW

Biology

General life cycle. A good general description of the biology of the western corn rootworm can be found in Destructive and Useful Insects (Metcalf et al., 1962). There is one generation per year. In Nebraska, Ball (1957), found egg laying begins the second or third week in August, with most eggs being laid between August 20 and September 20. Most eggs go into a diapause lasting until June. After hatching, larvae move through the soil until they encounter corn roots (Tate and Bare, 1946). Larvae achieve full growth in July and pupate in the soil. Adults emerge from the soil in late July and August (Tate and Bare, 1946).

Egg stage. Eggs are deposited in the soil near the base of corn plants, with approximately 80 percent occurring in the upper six inches of soil (Ball, 1957). Patel and Apple (1967) found northern corn rootworm eggs were distributed in decreasing numbers at distances of four, eight, and ten inches from the base of corn plants with none in the middle of a 40-inch row.

Although most of the eggs require an exposure to cold temperatures before they hatch, a small percentage hatch without such exposure. In laboratory studies George and Ortman (1965) exposed eggs to a temperature of 4°C for varying periods of time and incubated them until hatching at 30°C. They found the period of egg incubation was reduced with longer exposures of up to 116 days. Most eggs hatch after surviving one winter, but Chiang (1965) demonstrated a small percentage of northern corn rootworm eggs (0.3%) hatched the second year after deposition.

Larval stage. After hatching, larvae are able to migrate up to 40 inches in search of corn roots (Suttle et al., 1967). The main food of larvae in the field is thought to be corn roots (Metcalf et al., 1962), but laboratory studies have shown larvae can develop to adults when feeding on 13 grass species (Branson and Ortman, 1970). George and Hintz (1966) showed that the larval period consists of 3 instars. In Kansas larval development may extend from the middle of June to the latter part of July (Bryson et al., 1953). Length of larval development is affected by the season, soil condition and environmental conditions.

Adult stage. In Nebraska, Ball (1957) observed that western corn rootworm beetles first appeared early in July. Adults emerge from soil around the bases of corn plants with the majority emerging at a distance of 4-10" (Short, 1970). After emergence beetles feed upon every part of the corn plant (Bryson et al., 1953). Feeding on the silks may interfere with pollination resulting in sparsely filled ears. Beetles may also consume tender end kernels on the ear (Bryson et al., 1953). Ball (1957) reported that mating occurred shortly after adult emergence. Egg laying began in August, with optimum evening temperatures for oviposition ranging between 60-65°F. The average number of eggs laid per female varied from 372 to 418.

Adult longevity ranged from 16 to 84 days. As the silk and tassel dry in a corn field, the beetles may move to nearby alfalfa, *Medicago sativa* (L.) and kochia, *Kochia scoparia* (L.) where they feed to a limited extent (Ball, 1957). Hixon et al. (1947) reported that in late August, the rootworm adults were abundant on various weeds such as the tumbling pigweed, Russian thistle, and kochia. They also noted that many beetles were present in alfalfa fields.

Cinerski and Chiang (1968) reported that adults of the northern corn rootworm would disperse to other, younger corn fields when silks and tassels of their original field were dried, and, during the oviposition period frequent feeding excursions occurred outside the field with trips back to the corn field for egg laying. Kaufman (1968) stated that after the peak beetle population, there was no evidence of beetle movement for either the western or northern species from early to late planted corn. He also observed small numbers of western corn rootworm beetles on sorghum plants.

Oviposition preference. In laboratory studies Kirk et al. (1968) observed that oviposition of the western corn rootworm female was influenced by the nature of the soil surface, with beetles preferring soil particles above 1 mm in diameter. They reported females laid eggs primarily in large soil cracks or aggregates and preferred moist areas. Cornstalks had no special attraction for the beetles except as ground cover. They suggested weeds or trashy areas would probably have more eggs than clean areas in the same field.

Varietal Resistance

Root resistance. Lonnquist and Kiesselbach (1946) tested several lines of corn in Nebraska for root resistance to larval feeding. There were differences observed in two heritable characteristics of resistance: (1) a tendency to grow new roots in replacement of those injured, and (2) the occurrence of a substance within the tissue which is repulsive to the rootworms.

Tate and Bare (1946) evaluated 29 hybrids and 1 open pollinated variety of field corn for root resistance to larvae of the western corn rootworm. They concluded that varietal differences appeared to be due to the inherent capacity of the plants to replace quickly and adequately a destroyed root system and that the roots of all strains were attacked and could be destroyed.

Field tests were carried out at Yankton and Canfield, South Dakota, to evaluate the performance of 160 corn inbred lines to rootworm infestation

(Ortman and Fitzgerald, 1964). The emerging adult population was approximately 95% western corn rootworm. These tests indicated that there was no evidence of antibiosis in any inbred line and superior performance was apparently due to tolerance which was manifested by capacity to regenerate a root system after damage, and capacity to survive and develop under rootworm attack.

Silk resistance. Three-hundred-fifty-six lines of corn were screened at Kansas State University for possible silk resistance to the feeding of adult western, northern and southern corn rootworms (Granados, 1965). Lines were evaluated both for silk and ear damage. Twelve lines were classified as resistant, 5 as susceptible, and 3 as highly susceptible. The mechanism involved in the resistance or susceptibility of these lines was thought to be preference or non-preference in which attractants, repellents, and continuous feeding factors could account for the high and low number of corn rootworm adults present on the different lines and also for the high or low damage to the silks. There were no tests made for the existence of antibiotic factors in the corn silks (Granados, 1967).

Ortman and Fitzgerald (1964) concluded that among 160 inbred corn lines there were differences in both silk and root damage, but there was no correlation between larval root feeding and adult silk feeding.

The presence of a feeding stimulant for western and northern corn rootworm adults was demonstrated in the kernel, pistillate branch, silks, leaves and roots of corn by Derr et al. (1964). Extracts were made by using water as a solvent. Stimulant concentration was highest in the corn kernel. Other plant part extracts ranking in decreasing activity were: pistillate branch, silks, leaves and roots.

MATERIALS AND METHODS

1969 Research

Material tested. The following sources of corn were used in silk tests conducted in 1969:

From Mexico:
Antigua 6D x C.B.C.*
Antigua 1D x C.B.C.*
Haiti x Manfredi
Veracruz x Manfredi
San Croix 2D x Manfredi
Lancaster x Zapalote Grande
Guadalupe x C.B.C.*

From Kansas:
Hybrid - K-1859

Inbred lines:
(released)
K-55
K-166
(unreleased)
KSU 63:1195-1
KSU 63:1192-1
KSU 63:1192-2
KSU 68:1195-2

South Dakota:
Inbred line: SD-10

With the exception of Antigua 1D x C.B.C., KSU 63:1192-2 and KSU 68:1195-2, which were substitutions for unavailable lines, all genetic sources in this work had been studied for field silk resistance to northern, western and southern corn rootworm adults by Granados in 1964 and 1965. They were evaluated for average damage to the ears, average number of adults per plant, average silk damage, average number of adults per plant, and given a general classification of resistant, susceptible, or highly susceptible.

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*The initials, C.B.C., refer to a corn belt composite which was made up of corn belt lines and used for crossing to Latin American lines.

Water extractions. Water extractions from the silks of each source were made. Silks were chosen for uniformity of age as much as possible. The extraction procedure was slightly modified from that used by Derr et al. (1964).

A 10-g sample of corn silk was boiled in 100-ml of distilled water for 5 min to denature the enzymes. The water was transferred to a 200-ml graduated cylinder, diluted to volume with distilled water, and added along with the solids to a Waring Blendor. This mixture was homogenized for 15 min, filtered through glass wool, and poured into 14-ml nalgene vials which were frozen and stored at -15°C.

Preparation of extractions. The extraction was prepared for feeding tests by incorporating it into 3 percent by weight plain agar disks. Disks were prepared by heating the extract with agar, pouring the hot solution into 16-mm I.D. glass tubing, allowing the solution to cool, and slicing off 6-mm-thick disks.

Two-choice feeding tests. All two-choice feeding tests were conducted in round, clear plastic dishes, 200-mm in diameter and 75-mm deep. Two agar disks, each containing a silk extract of a separate line, were placed on the bottom of the plastic dish equidistant from the center. During the tests, each dish was covered with a plastic lid with a cork stoppered hole in the center, through which test beetles were inserted. A source of moisture for the beetles was maintained in each dish by the presence of 2 small water-filled vials with cotton wicks. Thirty unsexed beetles were used in each test which lasted 24 hr.

In preliminary tests a plain agar disk without any silk extract was placed in the dishes as a control along with the other disks. Since the beetles did not feed on this disk, its use was discontinued for the regular tests.

Assay method. The assay of beetle feeding activity on the agar disk was the number of granules dropped by the mandibles on and around each disk (Derr et al., 1964). Granules were counted on top and around the base of each agar disk with the aid of a dissecting mocroscope. Granules on the side of the disk were ignored.

Preliminary testing. To obtain a preliminary ranking of feeding preferences, extracts of all 15 genetic sources were paired in a two-choice test with K-55. Each comparison was replicated twice. The inbred line K-55 was selected as a common tester because it had shown a high susceptibility to beetle feeding in the 1964 and 1965 tests by Granados. To obtain a ranking of the 15 lines, the number of agar granules produced from extracts of each line was subtracted from the number of granules from the K-55 extract. The lines with low numbers of agar granules were ranked lowest in feeding preference, and those with a higher number of granules were ranked highest in feeding preference.

Two-choice preference tests were conducted in the manner previously described, between lines ranked high and low in preliminary testing with K-55. Each test was replicated twice.

Multiple feeding tests. Multiple feeding tests were conducted in the same manner as the two-choice tests, but 5 lines were compared concurrently. Two types of multiple tests were made. In one, a source which appeared to be non-preferred in the preliminary two-choice tests was compared with 4 which were preferred. In another, a source which was highly preferred in the preliminary two-choice tests was compared with 4 non-preferred lines.

Caging of beetles on silks. Beetles were caged on silking ears of 14 of the corn lines to expose the silk to a high, uniform population of beetles so that the possibility of differential tolerance and antibiosis among lines could be investigated.

Cylindrical cages of fine wire screen, 85-mm in diameter and 140-mm long, were used. The top of the cage was a screen-filled quart jar lid soldered to the cage. The bottom of the cage was open with a cotton sleeve 130-mm long taped over the outside. The cage was placed over an ear of corn and secured by tightly wrapping string around the cloth sleeve on the bottom of the ear shoot. The top of the silk was approximately 25-mm below the top of the cage. The cage was braced by typing one end of a string around the top of the cage and the other end around the cornstalk (Plate I).

Five ears of corn were caged on each of the 14 lines of corn. Each ear was caged approximately 1 day after the silks had emerged. Fifteen beetles were placed in each cage through a hole in the screen top which was then closed with a cork. The beetles were left in the cages for 5 days. Then the cages were removed and the silks rated for damage. The silks were graded by an arbitrary rating scale adapted from Granados (1967) (Plate II). The scale was modified by adding ratings of .5, 1.5, and 2.5.

Ear damage rating. After harvest, ears used in silk ratings were rated for ear damage according to the number of kernels missing. The grading system was the same arbitrary scale used by Granados (1967) (Plate III).

1970 Research

Material used. The sources used in 1969 were tested in 1970. All corn seed planted in 1970 was obtained from self pollination of plants grown in 1969, except K-55 which was obtained from Kansas State University.

Silk comparisons. Silks of the 15 lines planted were assayed for beetle feeding in the laboratory in two-choice comparisons. Fifty

EXPLANATION OF PLATE I

Wire cage on corn ear used to confine western corn rootworm adults during silk feeding damage comparisons.

PLATE I

EXPLANATION OF PLATE II

Scale used to grade the damage done to corn silks by adult western corn rootworm caged on the silks.

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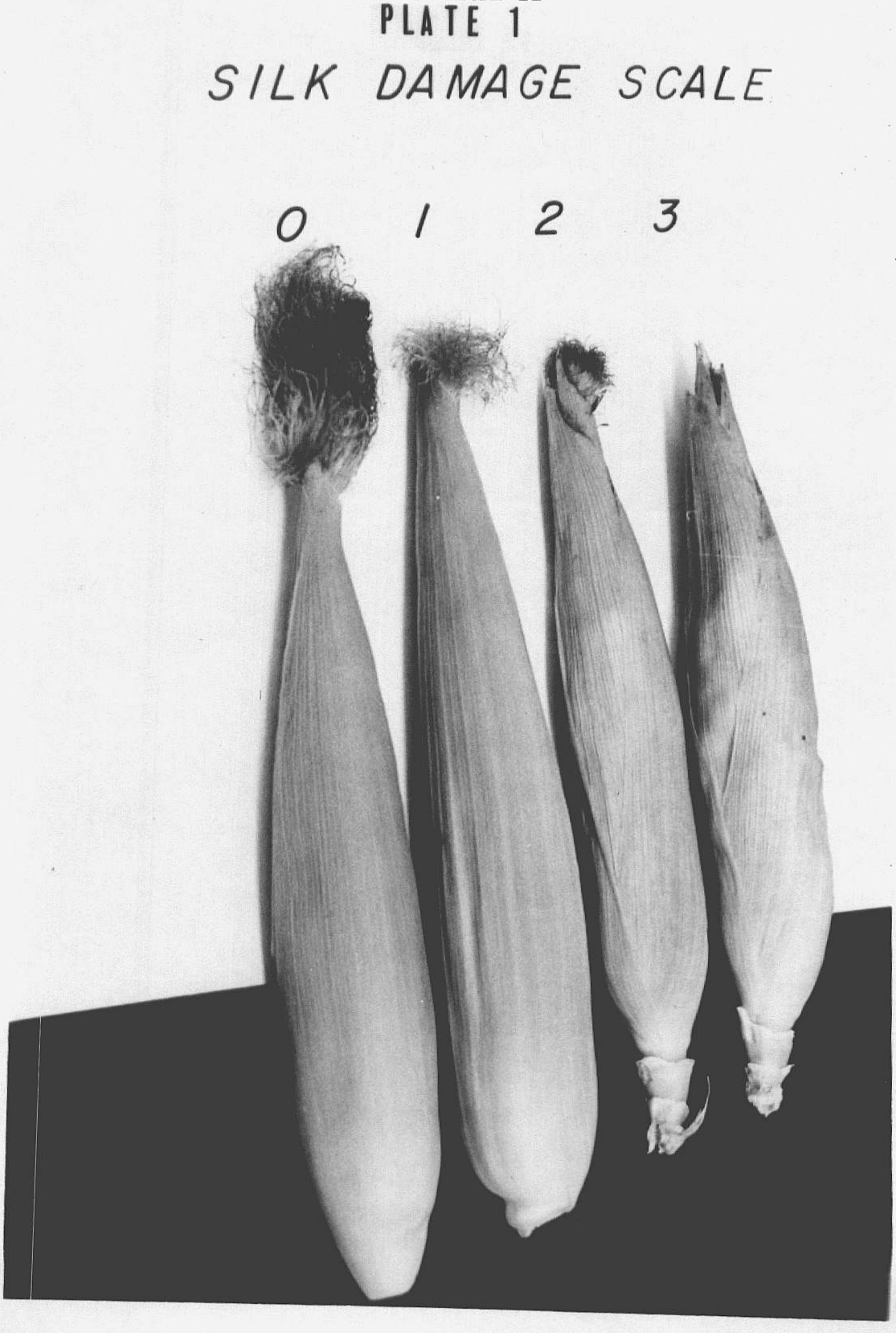
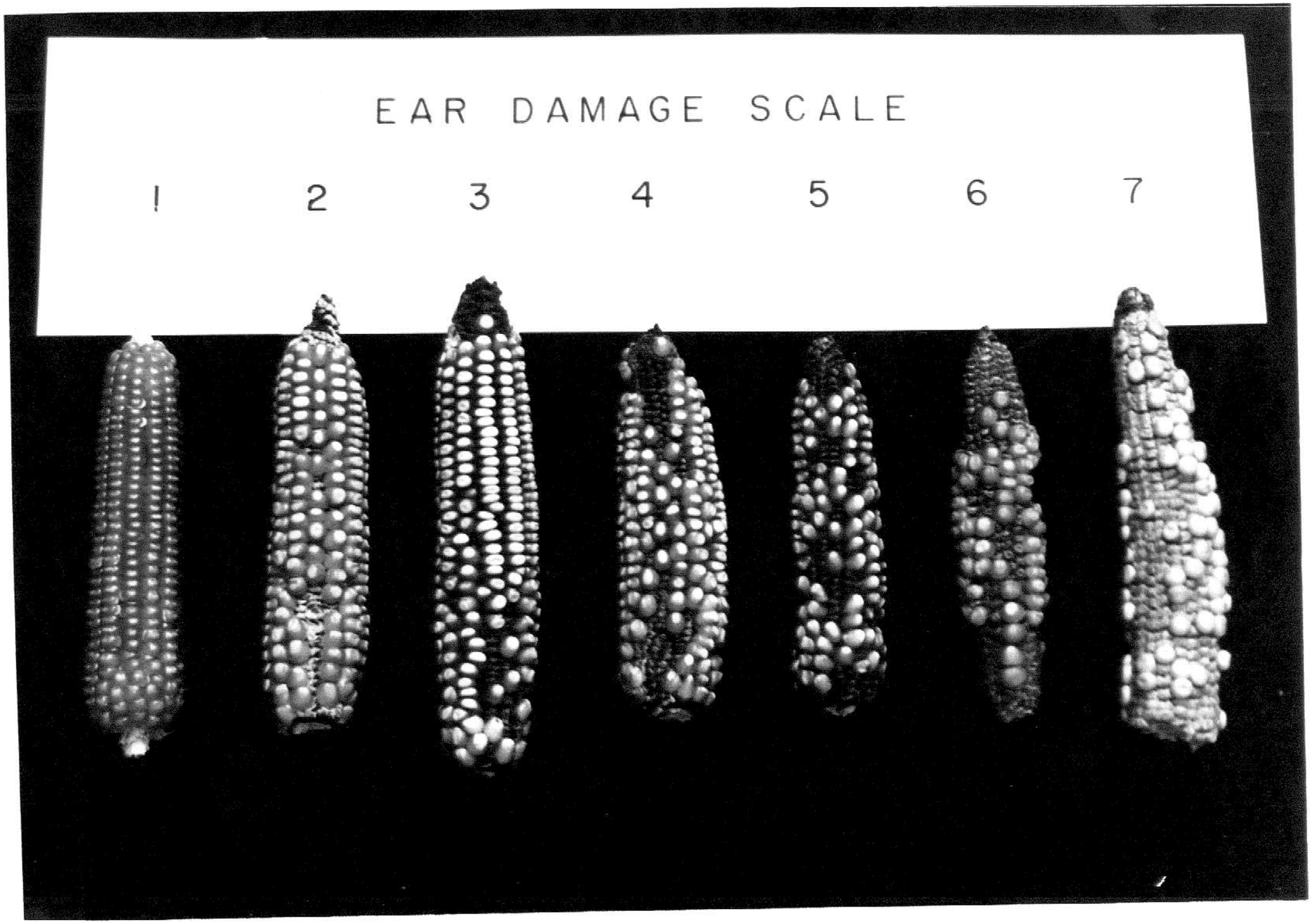
PLATE II



EXPLANATION OF PLATE III

Scale used to grade corn ears in relation to lack of kernels due to feeding on the silks by adults of western corn rootworm.

PLATE III



comparisons were made and all combinations were made at random.

Silks used in the comparisons were removed from the plant, sealed immediately in 20 x 38 mm plastic bags and quickly brought into the laboratory. The silks which were selected were uniform in age and ranged from 2-4 days old. All two-choice comparisons were replicated 3 times, and one complete silk of each line was used for each replication.

Silks were prepared in the laboratory by removing two small subsamples from each silk, weighing them, and putting them in metal salve boxes to dry in an oven. The remainder of each silk was inserted in a nalgene vial 27-mm in diameter and 9-mm high (Plate IV). Two small water-filled glass vials with a cotton wick were also placed in each dish to provide moisture for the beetles. During tests, each dish was covered with a plastic lid with a cork-stoppered hole in the middle through which beetles were introduced. Each comparison test used 24 unsexed beetles and lasted 24 hr. At the conclusion of each test, the remaining silk of each line was removed from the nalgene vial, placed in a previously weighed metal salve box, and put in an oven to dry.

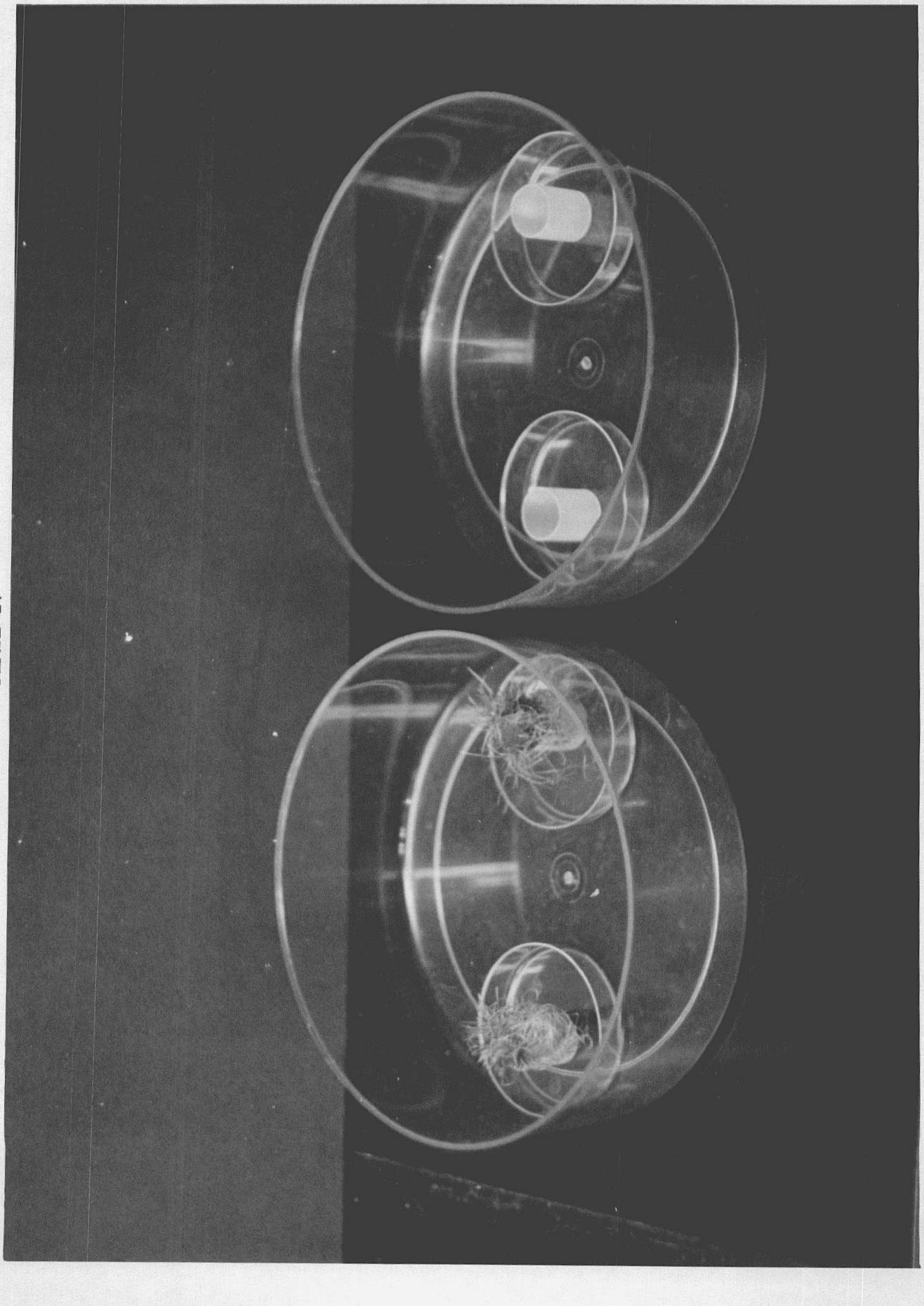
Drying procedures. Drying of silk samples was done in two sizes of metal salve boxes, 60 x 20 mm and 38 x 25 mm. The silks were dried for 6 hr at 66°C. During the drying period, the boxes were left uncovered. After drying, each box was covered with a tight-fitting lid before being removed from the oven.

Bioassay. Feeding tolerance was assayed by the percentage of dry material consumed during a 24 hr period. The weight of dry material in the silk before testing was estimated by multiplying the beginning fresh weight by the average percentage of dry weight in the two subsamples removed from it. The average percentage of dry weight in the subsamples was obtained by weighing them initially, drying them, weighing them again, and expressing the dry weight

EXPLANATION OF PLATE IV

Containers used to hold corn silk for 2-choice laboratory whole silk feeding tests.

PLATE IV



as a percentage. The dry weight remaining in the silks after the test was measured directly by drying the silks. The difference between the estimated beginning dry weight and the final dry weight obtained was expressed as a percentage of the original dry weight. Feeding preference was measured by estimating the amount of dry material consumed of each silk by subtracting the dry weight remaining from the estimated beginning dry weight.

Beetle sex ratio on corn leaves and silks. Beetles were collected separately from the leaves and silks of corn in 4 fields in Riley County, Kansas. The beetles were sexed by observing the ventral side of the abdomen (Mendoza and Peters, 1968).

The collections of beetles were made at 9:00 A.M. and 3:00 P.M. for approximately 45 minutes. Collection began on July 7 and continued intermittently until August 4. The 3:00 P.M. collection was discontinued on July 16. Beetles were collected from the corn plants by brushing them into a funnel attached to a glass jar. Since there was no specified number of beetles to be collected each day, numbers collected from each plant part fluctuated according to the field beetle population.

RESULTS

Water extracts. The ranking of extracts of the 14 lines after preliminary testing with K-55 is shown in Table 1. There was wide variation in the number of granules among the lines. Extracts of Veracruz x Manfredi, K-1859, and Antigua 6D x C.B.C. appeared least preferred by beetles, and extracts of SD-10, San Croix 2D x Manfredi and KSU 63:1195-1 most preferred.

The first two-choice feeding tests involving various sources are shown in Table 2. Beetles were nonstarved in the first 2 replications, and the same beetles were used for the second replications 24 hr later. In the first 2 replications, the average granule numbers for K-1859 were slightly higher than K-55. The average granule number of KSU 63:1192-2 was slightly higher than line Antigua 6D x C.B.C. Since these 2 lines were ranked adjacent to each other in the preliminary testing, one might expect that their average granule numbers would differ very slightly. In the other 3 pairs, the average granule number of Veracruz x Manfredi, Antigua 6D x C.B.C. and KSU 68:1195-2 were lower than the lines with which they were paired.

In the second test consisting of 2 replications using the same beetles 24 hr after the first tests, similar results were obtained except the granule number of K-55 was higher than K-1859. However, the differences between the averages of the pairs were much larger.

The results of another two-choice feeding test using the same 5 pairs but utilizing beetles which had been starved 24 hr prior to testing, are shown in Table 3. Again the average granule number of Veracruz x Manfredi, K-1859, Antigua 6D x C.B.C., and KSU 68:1195-2 was lower than the sources with which they were compaired.

Table 1. Preliminary feeding ratings on agar plugs of extracts of 14 lines compared with Kansas inbred K-55.

Line	Granule number relative to standard
Veracruz x Manfredi	-259
K-1859	-159
Antigua 6D x C.B.C.	-141
Lancaster x Zapalote Grande	-104
KSU 68:1195-2	-76
KSU 63:1192-2	-46
Antigua 1D x C.B.C.	-43
Haiti x Manfredi	-4
Guadalupe x C.B.C.	0
K-166	0
K-55 (standard)	standard
KSU 63:1192-1	+71
SD-10	+107
San Croix 2D x Manfredi	+116
KSU 63:1195-1	+132

Table 2. Paired comparisons of agar plugs containing extracts of various lines with unstarved beetles.

Lines	Average granule numbers/agar plug for 2 replications			
	Test 1		Test 2	
	Nonstarved beetles		Same beetles 24 hr. later	
		Mean difference		Mean difference
*Veracruz x Manfredi	50		67	
K-55	58	8	109	42
*K-1859	109		66	
K-55	103	6	122	66
*Antigua 6D x C.B.C.	68		102	
KSU 63:1192-1	139	71	178	76
*KSU 63:1192-2	53		54	
Antigua 1D x C.B.C.	44	9	51	3
*KSU 68:1195-2	37		56	
SD-10	95	68	236	180

*Indicates a higher ranking line in preliminary testing with K-55.

Table 3. Paired comparisons of agar plugs containing extracts of various lines. Beetles used in initial test were starved 24 hours before the test.

Lines	Starved beetles		Same beetles 24 hr. later	
	Average granule no.	Mean difference	Average granule no.	Mean difference
*Veracruz x Manfredi	154		144	
K-55	288	134	231	87
*K-1859	70		199	
K-55	195	125	244	45
*Antigua 6D x C.B.C.	92		85	
KSU 63:1192-1	189	97	219	134
*KSU 63:1192-2	64		78	
Antigua 1D x C.B.C.	42	22	132	54
*KSU 68:1195-2	140		65	
SD-10	229	89	182	117

*Indicates a hypothetical non-preferred line from preliminary testing with K-55.

Multiple feeding tests. The results of the multiple feeding test comparing 5 lines are shown in Table 4. The average granule numbers of San Croix 2D x Manfredi was the third largest in the first reading and largest in the second reading using the same beetles 24 hr later. The average granule number of KSU 68:1195-2 was the fourth largest in both readings. Veracruz x Manfredi and Antigua 1D x C.B.C. had the smallest average granule numbers in both readings.

The results of the multiple feeding test comparing 5 other sources are shown in Table 5. Antigua 1D x C.B.C. had the smallest average number of granules in the initial readings, and the second smallest average in the readings 24 hr later. The rankings of the 4 other lines fluctuated a great deal in the 2 readings.

In both of the multiple feeding tests, the average granule numbers produced by the beetles was higher when the same beetles were used 24 hr later.

Silk damage ratings. The averages of damage to silks of 14 lines subjected to caged beetles are shown in Table 6. An analysis of variance of .05 and .10 reveals that there are significant differences in silk damage among the lines. The averages range from 1.0 for SD-10 to 3.0 for KSU 63:1192-2 and KSU 68:1195-2.

Ear damage ratings. Damage ratings for the ears of the 14 lines upon which beetles were caged are shown in Table 7. The values range from 1.0 for 5 lines to 4.0 for Lancaster x Zapalote Grande. Although this range appears to be quite large, an analysis of variance at the .05 level reveals no significant differences among damage ratings for the 14 lines.

1970 laboratory silk comparisons. Table 8 presents comparisons between lines showing relative average percent of dry weight consumed in each pair. Table 9 is a summary of the comparisons in Table 8. In most comparisons,

Table 4. Multiple feeding tests involving four non-preferred lines and one preferred line.

Line	Average granule number
	Beetles starved 24 hours
Veracruz x Manfredi	68
Antigua 1D x C.B.C.	81
*San Croix 2D x Manfredi	95
KSU 68:1195-2	123
K-1859	133
	Same beetles 24 hours later
Antigua 1D x C.B.C.	101
Veracruz x Manfredi	110
K-1859	157
KSU 68:1195-2	205
*San Croix 2D x Manfredi	225

*Indicates a hypothetical preferred line from paired comparisons and preliminary testing with K-55.

Table 5. Multiple feeding tests comparing four preferred lines and one non-preferred line.

Line	Average granule number
	Beetles starved 24 hours
*Antigua 6D x C.B.C.	34
KSU 63:1192-1	91
K-55	110
SD-10	117
K-166	132
	Same beetles 24 hours later
KSU 63:1192-1	69
*Antigua 6D x C.B.C.	73
SD-10	117
K-166	170
K-55	184

*Indicates a hypothetical non-preferred line from paired comparisons and preliminary testing with K-55.

Table 6. Silk damage ratings from the caging of beetles on silks.

Line	Average silk damage
SD-10	1.0
Antigua 1D x C.B.C.	1.1
K-1859	1.4
Antigua 6D x C.B.C.	1.5
Guadalupe x C.B.C.	1.8
K-166	1.8
Veracruz x Manfredi	2.1
Lancaster x Zapalote Grande	2.5
KSU 63:1192-1	2.5
San Croix 2D x Manfredi	2.6
Haiti x Manfredi	2.6
K-55	2.8
KSU 63:1192-2	3.0
KSU 68:1195-2	3.0

Table 7. Ear damage ratings according to the number of kernels missing on each ear.

Line	Average ear rating
K-1859	1.0
SD-10	1.0
Guadalupe x C.B.C.	1.0
San Croix 2D x Manfredi	1.0
Veracruz x Manfredi	1.3
Antigua 1D x C.B.C.	1.3
Antigua 6D x C.B.C.	1.4
KSU 63:1192-2	1.8
KSU 68:1195-2	1.8
Haiti x Manfredi	2.0
K-166	2.0
KSU 63:1192-1	2.3
K-55	2.6
Lancaster x Zapalote Grande	4.0

Table 8. Two-choice comparisons of corn silks in the laboratory.

	Antigua 6D x C.B.C.	K-55	KSU 63:1195-1	San Croix 2D x Manfredi	Lancaster x Zapalote Grande	KSU 63:1192-1	K-1859	SD-10	K-166	KSU 63:1192-2	Guadalupe x C.B.C.	Antigua 1D x C.B.C.	KSU 68:1195-2	Veracruz x Manfredi	Haiti x Manfredi
Antigua 6D x C.B.C.		L	H	H		L	L	L	H			H		H	
K-55	H			H					H		H	H			H
KSU 63:1195-1	L						L	L			L	L	H		
San Croix 2D x Manfredi	L	L			H	L			H			L		L	L
Lancaster x Zapalote Grande				L							H	L	L	H	L
KSU 63:1192-1	H			H						H	H			H	
K-1859	H		H					H	L	H		H	H		
SD-10	H		H				L		H	H			H		
K-166	L	L		L			H	L				H			
KSU 63:1192-2						L	L	L			H	H		H	
Guadalupe x C.B.C.		L	H		L	L				L		L			H
Antigua 1D x C.B.C.	L	L	H	H	H		L		L	L	H			H	
KSU 68:1195-2			L		H		L	L							
Veracruz x Manfredi	L			H	L	L				L		L			H
Haiti x Manfredi		L		H	H						L			L	

L indicates that the line in the left hand margin of the table had a lower % consumed than the line to which it was compared.

H indicates that the line in the left hand margin of the table had a higher % consumed than the line with which it was compared.

Table 9. Total number of comparisons expressing relative high and low percentages of the silk dry weight consumed.

Line	Number times higher % consumed than other line	Number times lower % consumed than other line
KSU 63:1195-1	1	5
San Croix 2D x Manfredi	2	6
Veracruz x Manfredi	2	5
Guadalupe x C.B.C.	2	5
KSU 68:1195-2	1	3
K-166	2	4
KSU 63:1192-2	2	3
Lancaster x Zapalote Grande	2	4
Haiti x Manfredi	2	3
Antigua 1D x C.B.C.	5	5
Antigua 6D x C.B.C.	5	4
SD-10	5	1
K-1859	6	1
KSU 63:1192-1	5	0
K-55	6	0

there were no significant differences in the average percent of dry weight consumed of each line. When each pair was subjected to a paired T-test, 7 of the 49 paired comparisons were significantly different at the .05 level, and 14 pairs differed significantly at the .10 level. These pairs are listed in Table 10. Because of the low number of replications, and the large amount of variation, some of the 50 pairs failed to show any significance differences even though there were large differences between the average percentages of dry weight consumed. Six of these pairs in which the difference between the means was large, but not statistically significant, are also listed in Table 10.

The difference between the means of some of the pairs which were significant is less than the difference between the means of the nonsignificant pairs. This was due to differences in variation among the 3 replications of the tests. The most frequently occurring lines among the pairs in Table 10 with a low percentage consumed were Antigua 6D x C.B.C., Lancaster x Zapalote Grande, and Veracruz x Manfredi. The most frequently occurring sources among the pairs with a high percentage consumed were K-55 and KSU 63:1192-1.

The list of pairs showing significant differences between the mean dry weight consumed is shown in Table 11. The lines occurring most frequently in Table 11 with a higher mean dry weight consumed are KSU 63:1192-1 and K-55. The only line which occurs more than once with a significantly lower mean dry weight consumed is KSU 63:1195-1.

Table 12 lists pairs of silks which showed significant differences in both the average percent dry weight consumed and the average mean dry weight consumed. K-55 and KSU 63:1192-1 occur most frequently in Table 12 with a high percentage dry weight consumed and a high average dry weight consumed. No line occurred more than once with a low percentage of dry weight consumed and a low average dry weight consumed.

Table 10. List of pairs with large or significant differences in average percent of dry weight consumed.

Line	Avg. % dry weight consumed	Line	Avg. % dry weight consumed
*Antigua 6D x C.B.C.	7.9	K-1859	16.5
*Antigua 6D x C.B.C.	10.2	K-55	26.6
*KSU 63:1195-1	6.5	KSU 63:1192-1	16.8
*K-166	10.6	K-55	15.7
*Antigua 1D x C.B.C.	7.4	K-1859	12.2
*Haiti x Manfredi	21.3	K-55	31.6
*Guadalupe x C.B.C.	12.9	K-55	40.6
**Guadalupe x C.B.C.	10.3	K-55	45.1
**KSU 63:1195-1	10.7	K-55	32.7
**San Croix 2D x Manfredi	12.3	KSU 63:1192-1	21.0
**Lancaster x Zapalote Grande	8.8	Guadalupe x C.B.C.	13.3
**Veracruz x Manfredi	15.3	KSU 68:1195-2	13.7
**Veracruz x Manfredi	4.2	KSU 63:1192-1	24.9
**KSU 68:1195-2	1.0	Haiti x Manfredi	19.0
***Antigua 6D x C.B.C.	9.4	San Croix 2D x Manfredi	66.8
***KSU 68:1195-2	2.0	KSU 63:1192-1	25.6
***Lancaster x Zapalote Grande	6.7	Antigua 6D x C.B.C.	35.3
***Lancaster x Zapalote Grande	10.3	KSU 63:1192-2	34.6
***Veracruz x Manfredi	8.5	KSU 63:1195-1	13.9

* pairs which differ significantly in a paired T-test at .10

** pairs which differ significantly in a paried T-test at .05

*** pairs in which difference between means was large but not statistically significant

Table 11. List of pairs with significant differences in mean dry weight consumed of the silks.

Line	Avg. grams of dry wt. consumed	Line	Avg. grams of dry wt. consumed
*KSU 68:1195-2	.0187	K-1859	.0634
*K-166	.0498	K-55	.1159
*KSU 63:1195-1	.0403	Antigua 1D x C.B.C.	.1024
*KSU 63:1195-1	.0518	Guadalupe x C.B.C.	.0999
*Guadalupe x C.B.C.	.0603	K-55	.1048
*Lancaster x Zapalote Grande	.0731	K-55	.1317
**Haiti x Manfredi	.0163	KSU 63:1192-1	.1292
**San Croix 2D x C.B.C.	.0728	KSU 63:1192-1	.0930
**Veracruz x Manfredi	.0185	KSU 63:1192-1	.1268
**KSU 63:1192-2	.0674	San Croix 2D x C.B.C.	.1262

*significant at .10

**significant at .05

Table 12. List of pairs showing significant differences in both the average percent dry weight consumed and the average dry weight consumed of silks.

Line	Avg. % dry wt. consumed	Avg. dry weight consumed in grams	Line	Avg. % dry wt. consumed	Avg. dry weight consumed in grams
K-166	10.6	.0498	K-55	31.6	.1159
Haiti x Manfredi	21.3	.0163	K-55	45.1	.1048
Guadalupe x C.B.C.	10.3	.0603	K-55	32.7	.1317
KSU 63:1195-1	10.7	.0518	Guadalupe x C.B.C.	13.3	.0999
San Croix 2D x C.B.C.	12.3	.0728	KSU 63:1192-1	24.9	.1292
Lancaster x Zapalote Grande	10.3	.0731	San Croix 2D x C.B.C.	66.8	.1262
Veracruz x Manfredi	15.3	.0185	KSU 63:1192-1	25.6	.0930

Beetle sex ratio on leaves and silks. The results of sexing beetles collected separately from the leaves and silks of 4 different corn fields are shown in Table 13. For field Number 1, separate determinations for sex ratio were made for 2 different collection times, 9:00 A.M. and 3:00 P.M. The ratio of females to males on the leaves was 1:1 for the 9:00 A.M. collection period and 3:2 for the 3:00 P.M. collection. The ratio of females to males on the silks was 2:1 for both periods collectively. The lower ratio of females to males on the leaves at 9:00 A.M. may have been due to a lower temperature which reduced the agility of the males and made them more easy to capture. Usually, the males were more agile and more difficult to capture on the leaves than the females so that the sex ratios obtained in the field may have been somewhat biased in favor of the female beetle. This difference may also be a reflection of differences in preference between the sexes.

The general pattern of sex ratios shown in Table 11 indicated that the ratio of females to males on the silks increased from 2:1 to 3:1 later in the collection season. The ratio of females to males on the leaves increased from 1:1 to 6:1 at the end of the season. The sex ratios of the totals of all beetles collected revealed a slightly higher ratio of females to males on the silk than on the leaves, and the number of females collected outnumbered the males on both the silks and leaves of the corn plants in this study.

Table 13. Field collected beetle sex ratio on the leaf and silk of corn plants.

Collecting Dates	Field #	Leaves Total ♂	Leaves Total ♀	Leaves Sex ratio ♀ to ♂	Silks Total ♂	Silks Total ♀	Silks Sex ratio ♀ to ♂
July 7-16	1	219	235	1:1	81	155	2:1
July 21-22	2	26	50	2:1	26	48	2:1
July 23-28	3	76	73	1:1	26	38	1:1
July 29-August 4	4	43	224	6:1	15	139	10:1
				Totals from all fields			
July 7-August 4		364	582	3:2	148	380	3:1

DISCUSSION AND CONCLUSIONS

The differences in granule counts in agar plugs containing extracts of various lines of corn, indicate that there may be some water soluble substance or groups of substances absent or present in varying amounts in the silks of the 14 lines tested. The results of the extraction tests also indicated that it is difficult to make a precise ranking of feeding desirability because of the extreme variability which can occur in this type of test. This is supported by the results of the multiple tests which show that a hypothetical non-preferred line can be detected among preferred lines, but rankings within these lines may vary considerably. Preliminary comparisons were made between K-55 and other tested lines. Subsequent two-choice comparisons among lines indicated that feeding was less on the higher ranked lines. Tests using beetles starved for 24 hr had larger differences between the means. Possibly, beetles are better able to discriminate when hungry. Tests using beetles starved for longer periods of time showed no overall increase in differences between the means of lines tested. A 24 hr starvation period appears to be the most desirable for enhancing the beetles discriminatory power.

The damage rating from caged beetles on silks in the field indicated that there are damage differences among the 14 lines when they are exposed to uniform beetle populations. This phenomenon may be partially explained by the varying size of the silks produced by the lines tested. Lines which produced larger amounts of silk showed less relative damage than the lines with scanty amounts of silk because they had more silk for the beetles to consume. These differences may also have been due to different properties of the silks of the 14 lines which caused differential feeding.

The ear damage ratings were not as severe as might be expected from the feeding of 15 beetles for 5 days on the silks. Six of the lines had an average rating of 1.2 or less, which indicated that only a few kernels were missing from the ear. None of the lines had ear damage ratings of 5, 6, or 7 which were the most severe damage ratings on the scale. These results indicated that it would take an extremely large field population of beetles to cause enough silk damage to result in large amounts of missing kernels on ears due to lack of fertilization. These findings agree with Richter (1949) who found no significant increase in sterility of corn ears which had 25 beetles caged on them several days.

The calculated correlation coefficient, .0705, between ear and silk damage was nonsignificant. However, a comparison of the top and bottom 5 lines in Tables 6 and 7 shows that the lowest ratings in ear and silk damage have 3 lines in common and the bottom lines have 2 lines in common. This indicates a possible relationship. Reasons for lack of a significant correlation could have been that pollination and fertilization may have occurred before the beetle caging, some pollination may have been inhibited by high temperatures, and the cages may have interferred with pollination by prevention of free access of the pollen to the silks. Also, the beetle population in the cage may not have been high enough.

In laboratory tests of beetle feeding on complete silks in 1970, 50 of 105 possible comparisons were made. These were not sufficient to obtain a complete ranking of the lines. Since Antigua 6D x C.B.C., Lancaster x Zapalote Grande, Veracruz x Manfredi, Guadalupe x C.B.C., and KSU 68:1195-2 most frequently had a lower percent dry weight consumed than the lines with which they were compared, these were considered to show some tolerance to beetle feeding. Because K-55 and KSU 63:1192-1 often had a higher percentage of dry weight consumed, these were considered to show a low tolerance to beetle feeding.

Since K-55 and KSU 63:1192-1 also occurred most frequently in Table 11 with a high average dry weight consumed, these lines were considered to have been preferred by beetles. The lines occurring in Table 11 with a low average weight consumed may have been non-preferred by beetles.

Because the 7 pairs listed in Table 12 had significant differences both in average percentage and weight of dry material consumed, these pairs apparently showed differences in both tolerance and beetle preference.

The lines K-55 and KSU 63:1192-1 showed a low amount of tolerance and were preferred by beetles. Antiguq 6D x C.B.C., since it occurred in Table 10 three times with a lower percentage of dry weight consumed, exhibited tolerance to beetle feeding. However, there was no evidence of beetle non-preference since it was not listed among pairs with a significantly lower weight consumed. This may also have been true for any line listed several times in Table 10 with a low percentage consumed, but occurring only once in Table 11 or 12.

There are several possibilities which could have accounted for the differential feeding responses on the silk of the lines tested. Differences in tolerance could have been due to the amount of silk produced. Response differences could also have been due to other physical factors such as color, texture, or moisture percentage. The percentage of moisture was ruled out since moisture percentages of the silk compared generally did not differ more than one or two percent.

The silks of the 14 lines may have differed in chemical composition. The main theories of host plant selection by phytophagous insects due to differences in chemical composition fall into 2 categories. Thorsteinson (1958) believes that token stimuli provided by non-nutrients such as glucosides are important in feeding, but nutrients also play an important role.

Fraenkel (1959) believes that host plant selection is largely dependent on substances other than nutrients. Knapp (1966) analyzed the silks of 10 inbred lines of dent corn which were differentially susceptible to the corn earworm, and found no significant differences in amino acid content or concentration among the lines. The total percentage of reducing sugars was lower in the resistant lines. Differences in sugar concentrations among the 14 lines tested in this study were not measured. There also could have been other chemical differences since many other classes of compounds would be soluble in a water extraction.

The results of the collection and sexing of field collected beetles on the leaves and silks indicated that the ratio of females to males is slightly higher on the silks than on the leaves. Also, male emergence peaked before female emergence. The latter results compare with other unpublished studies (Wilde, personal communication*).

The higher ratio of females to males on the silks may have been due to differing nutritional requirements between the sexes. Females may require more carbohydrates and amino acids for oviposition than the males. The females may spend more time feeding on the silks than do the males to obtain these nutrients. Burkholder (1956) and George and Ortman (1965) reported that female western corn rootworms lay more eggs on a diet of corn silks than when fed any other substances they tested.

Differences in nutrient composition or concentration among the silk of preferred and non-preferred lines, may affect the fecundity of the female rootworms. This possibility should be investigated in the future. Beetles feeding on non-desirable silks may deposit fewer eggs in the field either

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because of a nutrient defficiency, or they may leave the field sooner to search for plants with more desirable silks.

ACKNOWLEDGMENTS

The author wishes to express deep appreciation to his major professor, Dr. Gerald E. Wilde, for his assistance and advice during the experimental work and preparation of the manuscript.

Sincere appreciation is also expressed to Dr. Robert B. Mills of the Department of Entomology and Dr. Clyde E. Wassom of the Department of Agronomy for their participation on the supervisory committee and help in the preparation of the manuscript.

Indebtedness is expressed to Dr. Arthur D. Dayton of the Department of Statistics and Computer Science for his advice in the statistical analysis of the data presented.

The writer also wishes to express gratitude to the National Science Foundation for providing him with financial assistance in the form of a traineeship.

A special note of gratitude is expressed to the writer's wife, Rita, for her encouragement, understanding, and assistance throughout the period of study.

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FEEDING RESPONSE OF WESTERN CORN ROOTWORM ON SILKS
OF FIFTEEN GENETIC SOURCES OF CORN

by

WILLIAM HARVEY REISSIG

B.S., Kansas State University, 1969

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

The objective of this work was to study more closely in the field and in the laboratory the silk resistance of various lines of corn.

Fifteen genetic sources of corn were investigated in this study. Seven sources were from Mexico, 1 hybrid line from Kansas, 2 unreleased inbred lines from Kansas, 4 unreleased lines from Kansas, and 1 inbred line from South Dakota.

In 1969, feeding tests were conducted on water extractions of silks from each genetic source. Preliminary tests were conducted to compare all of the lines using K-55 as a standard. Extracts of Veracruz x Manfredi, K-1859, and Antigua 6D x C.B.C. appeared the least preferred by beetles, and extracts of SD-10, San Croix 2D x Manfredi, and KSU 63:1195-1 appeared to be the most desirable.

Two-choice feeding tests were conducted on extracts of lines ranked high and low in preliminary testing. Multiple-choice feeding tests were conducted on extracts of 4 high ranking lines and 1 low ranking from preliminary testing and on extracts of 4 low ranking and 1 high ranking line from preliminary testing. It was difficult to make a precise ranking of feeding desirability because of the extreme variability which occurred in the tests. In the multiple feeding tests a hypothetical non-preferred line could be detected among preferred lines, but rankings within these tests varied considerably. Usually, extracts of the higher ranked lines showed less feeding than lines ranked low in preliminary testing.

Beetles were caged on 5 silking ears of each of the 14 lines in the field and subsequent silk and ear damage was rated. Significant differences in silk damage occurred among the lines, but the subsequent differences in ear damage were nonsignificant. The correlation coefficient between ear damage and silk damage was nonsignificant.

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In 1970, two-choice comparisons of whole silk beetle feeding were carried out in the laboratory. The average percentage of dry material consumed was used to measure tolerance of the line and the average weight of dry material consumed was used to measure beetle preference. Nineteen of the pairs had large or significant differences between the average percentage of dry weight consumed. Ten of the pairs had significant differences is the average weight of dry material consumed. Seven of the pairs had significant differences in both the average percentage and weight of dry material consumed. Antiqua 6D x C.B.C., Lancaster x Zapalote Grande, and Veracruz x Manfredi showed some tolerance to beetle feeding, and K-55 and KSU 63:1192-1 exhibited low tolerance and were preferred by beetles.

Differences in tolerance may have been due to the amount of silk produced by each line. Feeding response differences could have been due to physical factors, such as color, texture, or moisture percentage, or silks of the lines may have differed in chemical composition.

Beetles were collected separately from the leaves and silks of 4 fields in Riley County, Kansas. The ratio of females to males on the silks increased from 2:1 to 3:1 later in the season, and the ratio of females to males on the leaves increased from 1:1 to 6:1 at the end of the season. The sex ratio of the total of all beetles collected revealed a slightly higher ratio of females to males on the silks than on the leaves. This higher ratio of females to males on the silks may have been due to differing nutritional requirements between the sexes.

Differences in nutrient composition or concentration among the silk of preferred and non-preferred lines may affect the fecundity of the female rootworms. Beetles feeding on non-desirable silks may deposit fewer eggs in the field either because of a nutrient deficiency, or they may leave the field sooner to search for plants with more desirable silks.