STUDIES OF RESISTANCE OF 92 SORGHUM AND 38 MAIZE CULTIVARS TO 4 SPECIES OF STORED-PRODUCT INSECTS

by

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INTRODUCTION

Sorghum and maize are grown extensively in many countries of the world. They are used as food by man, and as in the United States, as feed for livestock.

Both sorghum and maize are attacked by stored-grain insects in farm and commercial granaries. Total losses of the world production of cereals due to storage insects have been estimated at about 10% (Munro, 1966).

The rapid human population increase as well as the current emphasis on production, storage, and marketing of grain free from insect damage and contamination make it more important that insect infestations in grain be prevented and controlled. Control is often accomplished by chemical treatment which is costly and may result in undesirable residues.

Stored-product insect control by using resistant crop varieties is being explored to reduce the possible undesirable effects of insecticides. If crop varieties resistant to stored-product insects were available to growers, control could be greatly enhanced with reduced cost and reduced usage of chemicals.

Painter (1951) stated that the first potential sources of resistance should be the common varieties grown and adapted in the area where the experiments are being conducted. If resistance can be found among such varieties, breeding for a satisfactory variety is simplified.

The primary purpose of this research was to evaluate resistance to 4 important stored-product insects of cultivars of sorghum and maize, most harvested from field trials in Kansas, and to study factors which may cause the resistance. The insect species used were the rice weevil, Sitophilus oryzae (L.), maize weevil, Sitophilus zeamaize (Motsch.), lesser grain borer, Rhyzopertha dominica (F.), and the red flour beetle, Tribolium castaneum (Hbst.)

The female weevil eats a hole in the kernel, lays an egg in it, then plugs the hole with a gelatinous material. The larva remains inside the kernel where it develops through 4 larval instars and the pupal stage before emerging as an adult.

The female lesser grain borer lays eggs outside the kernels. After hatching, the small larva chews its way into the kernel where it develops through 4 larval stages and the pupal stage before emerging as an adult.

Red flour beetles, unlike weevils and lesser grain borers, are external feeders in the larval and adult stages, and usually start feeding on the germ, then attack the endosperm. The larva commonly develops through 7-8 instars.

REVIEW OF LITERATURE

Host Resistance in Sorghum Grain

Ali (1950) studied 15 varieties of sorghum and found that only Martin and Cody were suitable for reproduction of the rice weevil. However, a low moisture content (9.6%) in each of the sorghums may explain the high level of resistance he obtained.

In India, Samuel and Chatterji (1953) studied the resistance of varieties of jowar (sorghum) to 6 species of stored grain insects, including the rice weevil, lesser grain borer, and red flour beetle.

By using weight loss and percentage of damaged grain, they found Js 20, a non-huskable variety, almost fully resistant to all of the insect species except the lesser grain borer. No variety was immune and the degree of resistance or susceptibility of the different varieties seemed to depend upon a number of factors such as hardness, texture, husk cover, and moisture content of the grain.

Victoria Lieu, in unpublished work done at Kansas State University in the 1950's, noted that the rice weevil, granary weevil, and lesser grain borer did not reproduce or survive in two non-waxy sorghum varieties, Double Dwarf Yellow Sooner and Double Dwarf White Sooner, of 12 per cent moisture content.

Doggett (1957) described a no-choice test method of estimating weevil damage to 17 different sorghum varieties. He found a positive relationship between the low level of damage to sorghum grains by weevils and thickness of the corneous endosperm shell. He also observed that small grains appeared less damaged than larger ones. Doggett (1958) stated that a thick corneous endosperm shell in grain had been successfully incorporated in a breeding program in Tanganyika for weevil resistance.

Morrison (1964) found that whole kernels of Atlas sorghum yielded more maize weevil adult progeny than halved or coarsely ground kernels. This tends to support Doggett's finding regarding kernel size.

Davey (1965) studied the factors such as moisture content and hardness of the endosperm, that affected the susceptibility of sorghum kernels to the attack of rice weevils. She devised a method to measure the relative amounts of vitreous corneous and mealy endosperm in a seed and found that the greater the percentage of vitreous endosperm, the higher the degree of resistance to the rice weevil.

Davey (1964) stated that eggplug counts and X-rays were unreliable methods for counting eggs and young larvae of the rice weevil because they are small and difficult to see. She concluded that counts of emerging adults were adequate for comparing damage by weevils to different varieties of sorghum.

Russell (1962) found that harder-grained varieties, with the exception of Texioca-54, were least attractive to the maize weevil for oviposition. Hardness was measured by average per cent weight lost by pearling for a given period. Per cent mortality of immatures was not significantly different among the varieties, thus, first generation emergence paralleled the oviposition findings. Oviposition preference was greatest for the larger seeds.

Russell and Rink (1965) studied the effects of sorghum varieties on the development of maize weevils and concluded that their reactions were similar to those of rice weevils. They tested 4 varieties using length of developmental period and number of first generation progeny as indicators of resistance. Resistance was correlated to hardness, i.e., the softer the variety the shorter was the developmental period.

Russell (1966) found that harder varieties of sorghum reduced rice weevil adult longevity and oviposition rate, which results in reduced grain damage.

By using flour of several varieties of sorghum, Dang and Pant (1965) observed a difference in larval survival of red flour beetle and stated that chemical factors in the sorghum may have been responsible for the differences.

Rogers (1970) screened 1511 cultivars of sorghum received from the International Germ Plasm Seed Bank, Chapingo, Mexico, for resistance to the maize weevil. He found that 161 cultivars produced as few as or fewer progeny than his resistant check (Double Dwarf Early Shallu). He also found that an increase in relative humidity gave an increase in the number of progeny produced by 1 resistant and 2 susceptible varieties but the increase was much smaller for the resistant variety than for the 2 susceptible varieties.

Hunkapiller (1970) screened 269 cultivars of sorghum to determine resistance to the maize weevil and lesser grain borer. Only 13 of the cultivars exhibited some degree of resistance to maize weevil when compared to the susceptible check. Double Dwarf Early Shallu was the most resistant and Shambul from Nigeria the most susceptible cultivar tested. Only 49 of the cultivars exhibited some degree of resistance to lesser grain borer. Martin X Norg-mid 7319-1 was the most resistant and 60M 1459 the most susceptible cultivar tested. Size of kernels did not appear to affect resistance or susceptibility. The yellow cultivars tested were the most susceptible to both insects.

Rout (1973) compared red flour beetle resistance of 21 sorghum cultivars of world-wide origin, which Rogers (1970) found to be most resistant or most susceptible to maize weevils. Rout compared samples of these, grown in Kansas in 1970, to red flour beetles using sound kernels, 90% sound: 10% broken kernels, and flour, infested with 25 0-24-hr-old larvae. Some degree of resistance in the sorghum cultivars was observed. No progeny emerged in the sound kernels of cultivar 173 while the percentage of larvae which developed to adults in sound kernels of other cultivars ranged from 21.33 to 89.33. Percentage survival to adult was highest to lowest in flour, sound:broken kernels, and sound kernels, respectively. The larval-pupal periods were shorter in flour than in sound:broken or sound kernels.

Using 26 sorghum cultivars, which Rogers (1970) found to be most resistant to the maize weevils and grown in Kansas in 1970, Lange (1973) found that maize weevil oviposition and kernel hardness were negatively correlated and that there was less weevil emergence from the resistant cultivars. Soil nitrogen fertilization had little effect on resistance to maize weevils but grain maturity at harvest did; mature grain was more resistant to insect attack.

Stevens and Mills (1973) compared the suitability of 2 free-choice tests (random-distribution and uniform-distribution) with a no-choice technique to determine relative resistance of 36 varieties of sorghum to rice weevils and found that the 3 types of tests were nearly equal for ranking varieties of sorghum as to rice weevil resistance; however, more progeny were produced in the no-choice tests.

Host Resistance in Maize Grain

The value of husk cover in preventing rice weevil injury to ear corn has been discussed by Smith (1909), Wilson (1912), Hinds (1914), Kyle (1918), Back (1919), Cartwright (1930), Eden (1952), Floyd and Powell (1958), and Floyd, Oliver, and Powell (1959).

Warren (1954) reported that the rice weevil was capable of surviving in hulled teosinte, a primitive type of corn.

Singh and McCain (1963) reported a highly significant positive correlation between sugar content of corn kernels and extent of field infestation by rice weevils, and a negative correlation between kernel hardness and rice weevil infestation.

Pant, Kapoor, and Pant (1964) studied the relative resistance of 11 varieties of maize to the rice weevil and noted that the flint type of maize varieties tended to fall in the resistant groups and the dent type in the susceptible groups.

Schoonhoven (1972) stated that selection for maize weevil resistance in corn kernels was successful in dent lines, mainly derived from an open-pollinate variety, but was not successful in flint lines. He measured hardness of kernels with opaque (high lyzine) and normal endosperm by applying 4 kg pressure on a diamond crystal placed against the back of kernel and found no correlation between resistance and hardness. He also stated that damage to the pericarp such as hot water treatment, scratching or rubbing between sandpaper made the kernel susceptible. Kernel size, moisture equilibration of the sample in screen-lidded cages prior to testing, or extended storage periods did not influence progeny number but temperature did.

McCain, Eden, and Singh (1964) described a laboratory technique for selecting rice weevil resistance in corn varieties. A promising test was designed that offered weevils free-choice of several varieties. Weevils readily selected the most susceptible hybrids.

Diaz (1967) suggested that, in 139 Mexican maize collections he screened, the resistance to the maize weevil came from lowlands in Tepalcingo, Morelos, Mexico, or primitive corn from other areas. He also stated that the best measure of resistance in free-choice and no-choice tests was in the number of emerged weevils.

VanDerSchaaf, Wilbur, and Painter (1969) screened 337 corn strains using the maize weevil in a no-choice and free-choice test. They found 20 strains, which had their origin in lowland tropical regions, with some degree of resistance. This agrees with Diaz (1967).

Kirk and Manwiller (1964) developed a method of supplementing low field populations of weevils for resistance ratings of breeding material and new hybrids. They broadcast collected weevils (30,000-70,000 insects/acre) through yield test fields. Resistance or susceptibility of the corn to the weevils was evaluated by using per cent ears infested.

Rhine and Staples (1968) found that high amylose content in maize varieties adversely affected larval nutrition of rice weevils and granary weevils, but did not affect either nutrition or larval survival of lesser grain borer or red flour beetle. It was suggested that larval survival of some stored-product insects may have been influenced by other resistant factors since the high amylose and normal amylose maize were grown under different breeding programs.

Hopkins (1970) screened 314 genetic sources of corn from the International Germ Plasm Seed Bank, Chapingo, Mexico for resistance to the lesser grain borer. He stated that corn sources which had large amounts of hard endosperm and small amounts of soft endosperm were more resistant to the lesser grain borer.

Host Resistance in Wheat, Rice, and Other Stored Grain

Ewer (1945) noted that larger grains of wheat were preferred for oviposition by granary weevils.

Singh, Kundu, and Gupta (1968) tested 29 varieties of wheat and suggested that hardness could be a component of resistance to the rice weevil.

Breese (1960) reported that sound, mature rough rice with intact husks appeared to be almost immune to infestation by rice weevils. Infestation developed in grains with lemma and palea separated, but the developing adults were often unable to emerge. Rossetto (1966) screened 1700 varieties of rough rice for resistance to maize weevils and reported the same relationship.

Russell (1968) tested 6 American varieties of rice for resistance to rice and maize weevils. He found that grains with gaps between the palea and lemma were more susceptible to weevil oviposition.

Sinha (1969) determined the reproduction of 5 cosmopolitan stored-grain insects on 39 varieties of cereals grown in Canada and reported the low resistance of the commonly-grown barley varieties to red flour beetles. Hulls of oats prohibited reproduction of the granary weevil and lesser grain borer but none of the oat varieties were particularly resistant to red flour beetle.

GENERAL MATERIALS AND METHODS

Sorghum Samples

All sorghums used in the studies were grown in the field in 1972. Eighty-two cultivars were from field trials grown in Brown County, Kansas, by the Kansas Agricultural Experiment Station, 8 cultivars (C42Y, C42Y-1, F65A-1, F65a, BR54, BR54-1, E57-1, and C42C-1) were obtained from Sorghum Research, DeKalb AgResearch, Inc., Lubbock, Texas, and 2 cultivars (MP10 Sh and DDES) were obtained from Fort Hays Kansas Agricultural Experiment Station, Hays, Kansas.

Maize Samples

All 38 maize cultivars used were from field trials grown in Republic County, Kansas, by the Kansas Agricultural Experiment Station in 1972.

Storing Grain Samples

Upon receipt, the grain samples were placed in a freezer for at least 2 weeks at approximately $-16^{o}C$ to destroy any possible insect infestations and then stored in a cold room at $4^{o}C$.

Rearing Room

All insect cultures and tests were kept in a rearing room with constant $67 \pm 3\%$ relative humidity (RH) and a temperature of $27 \pm 1^{o}C$. An automatic mist-type humidifier was used to maintain the relative humidity and thermostatically controlled electric heating and cooling units maintained the temperature. The culture room was maintained in a 12:12 light, dark photoperiod.

Sources of Insects

Insects used in the studies were obtained from stock cultures maintained in the Department of Entomology Stored-Product Insects

Laboratory. The rice weevils, lesser grain borers, and red flour beetles originated from field collections in Kansas and have been maintained in the laboratory for several years. The maize weevil culture was obtained from Stuttgart, Arkansas in 1955 and since maintained in the laboratory.

Maintenance of the Stock Insect Cultures

Insect cultures were kept in wide-mouth quart jars having caps fitted with both 60-mesh brass screens and 9 cm kelthane-treated filter papers for mite control. About 25 g of hard red winter wheat at 12.5 to 13.5% moisture content were placed in each jar for weevils and lesser grain borers, and about 200 g of a mixture of 60 parts of whole wheat flour, 40 parts of cornmeal, and 5 parts of dry yeast was used as a rearing medium for red flour beetles. About 200-300 unsexed adult weevils, 300-400 unsexed adult lesser grain borers, and 300-400 unsexed adult red flour beetles were allowed to oviposit in each appropriate medium for 7 days and then removed so that the age of the progeny insects was fairly uniform.

Testing Chambers

Five circular, wooden chambers were used in a preference test to determine the relative resistance of the samples (Plate I). Each chamber had a diameter of 42 cm and a depth of 8.5 cm. Twenty 48 x 48 x 6 mm plastic box lids which held grain samples during oviposition could be arranged in a circle near the chamber wall. The chamber was closed with a circular piece of 3/16" masonite and sealed with masking tape to

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EXPLANATION OF PLATE I

Fig. 1. Test chamber and lid in which 19 plastic box lids of maize samples were arranged.

Fig. 2. Twenty box lids containing sorghum samples arranged in the test chamber, and pieces of paper leaned against the lids to serve as bridges for testing with red flour beetles.

PLATE I

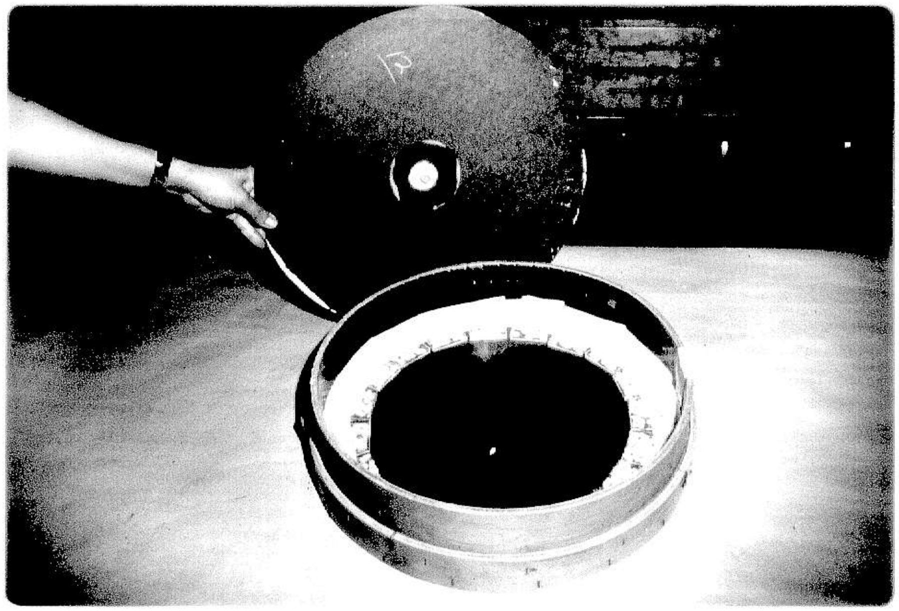

Fig. 1

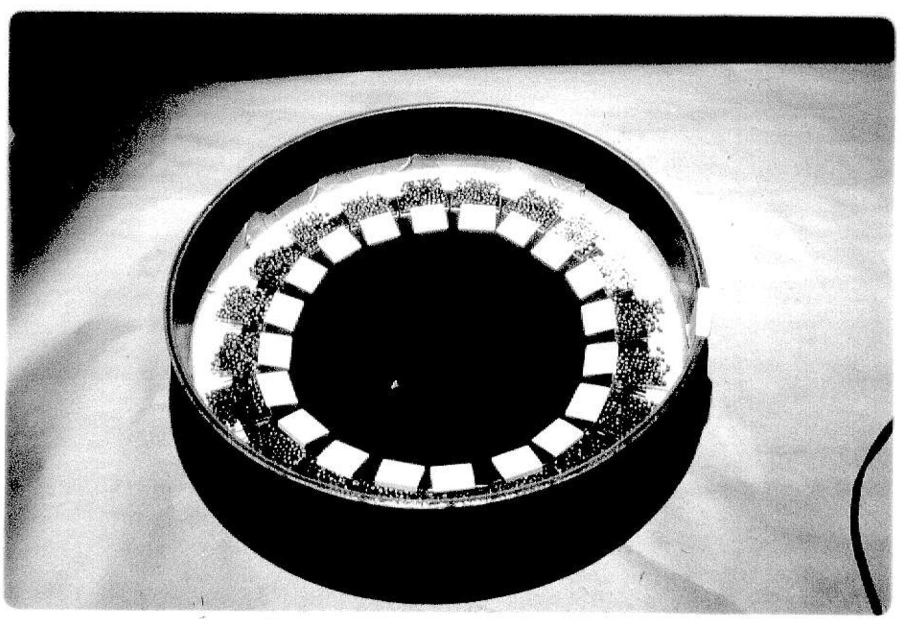

Fig. 2

prevent escape of insects. In the center of the lid was a circular opening (9 cm diam) closed with 60-mesh brass screen. A small hole in the center of the screen through which insects could be introduced was closed with a rubber stopper.

Grain Equilibration

Measurement of the moisture content of each sample was impractical because of the small amount of grain, but all samples (100 kernels of each sorghum and 20 maize kernels) were placed in 48 x 48 x 18 mm plastic boxes with lids having 60-mesh screen in the rearing room (15 days for sorghum and 21 days for maize) so moisture content could equilibrate with the 67% relative humidity.

RESISTANCE OF SORGHUM CULTIVARS TO RICE WEEVILS

Materials and Methods

Three replicates of 100 kernels each for each cultivar, which had been equilibrated in the rearing room, were selected randomly and placed in 48 x 48 x 6 mm plastic box lids. Twenty lids were arranged in each testing chamber (Plate I, Fig. 2). The chamber was covered with a lid and sealed with masking tape before dropping 200 7 to 14-day-old adult rice weevils through a central hole. The chamber was then placed in the rearing room. The rice weevils were allowed free-choice for ovi-posting among all the cultivars in the chamber for 5 days and then removed. The sorghum samples were transferred to 48 x 48 x 18 mm plastic boxes and covered with screened lids, put in cardboard trays and returned to the rearing room. Beginning 25 days after the parent weevils were removed the numbers of emerged adult progeny were counted and recorded daily until no progeny emerged from the cultivar for 7 days.

Results and Discussion

The smallest average number of rice weevil progeny emerged from cultivar MP10 Sh (8/replicate) and the largest average number (71.7/replicate) from cultivar X101 (Table 1). The average number that emerged from the remaining 90 cultivars ranged from 26.0 to 69.3/replicate. Statistical analysis (Table 2) revealed significant differences in the numbers that emerged from different cultivars. Based on a least significant difference (5% level) of 11.53, the sorghum cultivars could be placed in 4 groups according to the degree of resistance: (1) the most resistant cultivar, MP10 Sh, (2) the 11 resistant cultivars from which the average numbers of emerged insects ranged from 26.0 to 36.3/replicate, (3) intermediates, and (4) the 5 most susceptible cultivars from which the average numbers of emerged insects ranged from 61.0 to 71.7/replicate.

Plate II shows the contrast in damage and numbers of emerged insects between the most resistant and the most susceptible replicates.

EXPLANATION OF PLATE II

MP10 Sh and X101, the most resistant and the most susceptible sorghum cultivars, respectively, to rice weevils. The progeny that emerged from each sample are shown.

PLATE II

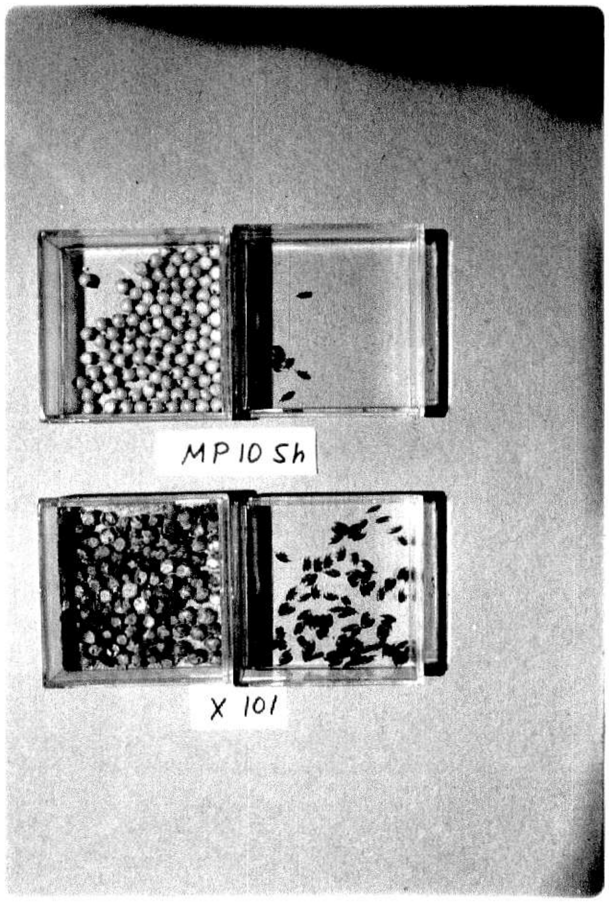

Table 1. Numbers of progeny and the developmental period of rice weevils in 92 sorghum cultivars (100 kernels/sample; 3 replicates) when 10 unsexed adults 7-14 days old per sample (200 total) had free-choice for 5 days oviposition among 20 samples in each test chamber.

Cultivar	Developmental period* (No. days + 2)		Number of emerged adults			
			Replicate			All replicates
	Range	Avg.**	1	2	3	Avg.
MP10 Sh	29-46	37.5	9	8	7	8.0
65MH 340	29-49	39.0	24	20	34	26.0
C42Y-1	29-48	38.5	26	30	26	27.3
E57	29-43	36.0	31	30	23	28.0
Jumbo L	28-46	37.0	23	37	34	31.3
F65A-1	30-48	39.0	31	28	40	33.0
G814	28-48	38.0	34	33	33	33.3
E57-1	28-51	39.5	24	37	41	34.0
DDES	29-42	35.5	38	33	34	35.0
521	28-47	37.5	33	30	42	35.0
BR54-1	30-52	41.0	43	33	30	35.3
233	29-52	40.5	35	44	30	36.3
880	29-51	40.0	42	42	31	38.3
BR54	30-45	37.5	42	36	38	38.7
F65 a	28-44	36.0	33	46	38	39.0
820	29-48	38.5	37	36	45	39.3
R1019	28-48	38.0	43	44	31	39.3
C42C-1	28-43	35.5	37	44	38	39.7
77A	29-47	38.0	43	33	43	39.7
RS671	29-47	38.0	39	46	38	41.0
ES702	28-46	37.0	40	46	37	41.0
842	29-54	41.5	34	48	43	41.7
7131	28-46	37.0	33	49	44	42.0
760	29-50	39.5	49	38	40	42.3
W851	28-51	39.5	37	45	46	42.7
8681	30-49	39.5	46	48	34	42.7

Table 1 (cont'd).

Cultivar	Developmental period * (No. days + 2)		Number of emerged adults			
			Replicate			All replicates
	Range	Avg.**	1	2	3	Avg.
8375	29-54	41.5	38	50	41	43.0
180	29-50	39.5	54	42	34	43.3
96	28-43	35.5	42	37	51	43.3
70X	28-48	38.0	35	44	52	43.7
634	29-52	40.5	40	45	46	43.7
F61	28-49	38.5	41	38	53	44.0
E57 a	27-47	37.0	35	46	52	44.3
Dorado M	28-49	38.5	43	46	44	44.3
808	29-46	37.5	41	45	49	45.0
66X	28-51	39.5	45	52	38	45.0
Super 400A	29-51	40.0	42	52	42	45.3
2529	29-47	38.0	38	34	65	45.7
833	30-49	39.5	44	39	54	45.7
Double TX	29-50	39.5	50	35	52	45.7
G522	28-52	40.0	44	47	49	46.7
8417	28-47	37.5	43	45	54	47.3
R1090	28-50	39.0	46	42	55	47.7
Total	28-46	37.0	37	54	53	48.0
511	28-50	39.0	45	52	47	48.0
E59	28-50	39.0	46	45	54	48.3
RS690	28-47	37.5	54	34	57	48.3
GX701	28-51	39.5	55	45	45	48.3
G490	29-51	40.0	53	38	54	48.3
91	28-52	40.0	55	47	43	48.3
C42Y	27-50	38.5	42	45	60	49.0
C42 c	27-46	36.5	46	51	51	49.3
635	29-45	37.0	48	51	49	49.3
729	28-48	38.0	42	51	55	49.3
Dorado E	28-51	39.5	58	42	49	49.7
735	28-51	39.5	55	51	43	49.7
R1029	28-48	38.0	38	57	55	50.0

Table 1 (cont'd).

Cultivar	Developmental period * (No. days ± 2)		Number of emerged adults			
			Replicate			All replicates
	Range	Avg.**	1	2	3	Avg.
412	28-51	39.5	60	45	45	50.0
SG41	28-50	39.0	55	40	56	50.3
516	29-51	40.0	54	41	56	50.3
Y101	28-51	39.5	47	56	50	51.0
691	27-52	39.5	52	50	51	51.0
811A	28-50	39.0	53	54	47	51.3
G820	28-48	38.0	57	45	52	51.3
650	28-52	40.0	53	54	47	51.3
270A	30-51	40.5	63	43	49	51.7
W839	28-45	36.5	52	41	62	51.7
SG40	27-52	39.5	61	46	49	52.0
80	29-51	40.0	51	53	52	52.0
95	28-51	39.5	51	58	47	52.0
Dorado	28-53	40.5	49	53	55	52.3
45	29-50	39.5	49	55	55	53.0
Grain MasterA	28-50	39.0	50	57	53	53.3
Oro	28-46	37.0	63	49	48	53.3
RS628	28-50	39.0	48	58	55	53.7
C42 a	28-47	34.5	61	47	53	53.7
Early Oro	29-48	38.5	56	54	52	54.0
733	28-53	40.5	66	54	44	54.7
R109	28-47	37.5	56	46	64	55.3
RS610	30-47	38.5	52	48	66	55.3
707A	28-50	39.0	64	53	50	55.7
W869	28-49	38.5	50	57	60	55.7
634A	28-50	39.0	62	58	48	56.0
W85	30-46	38.0	55	63	53	57.0
H7043	28-47	37.5	49	58	69	58.7

Table 1 (concluded).

Cultivar	Developmental period * (No. days ± 2)		Number of emerged adults			
			Replicate			All replicates
	Range	Avg.**	1	2	3	Avg.
846	28-50	39.0	62	61	54	59.0
402	29-49	39.0	64	61	55	60.0
RS700	30-53	41.5	63	57	63	61.0
8674	28-51	39.5	72	44	68	61.3
G766 W	28-46	37.0	57	71	64	64.0
GX266	30-50	40.0	66	71	71	69.3
X101	27-54	40.5	67	55	93	71.7

* Calculated from third day of oviposition.

** Average of the minimum and maximum developmental period.

Table 2. Analysis of variance of the emerged progeny rice weevils in 92 sorghum cultivars.

Source of variation	d.f.	Sum of squares	Mean square	F.
Sorghum cultivars	91	24352.324	267.607	5.156**
Experimental error	184	9549.714	51.900	
Total	275	33902.039		

** Significant at 0.05 level of probability.

LSD = 11.5291.

RESISTANCE OF SORGHUM CULTIVARS TO MAIZE WEEVILS

Materials and Methods

Materials and methods were the same as described in the previous rice weevil test except numbers of emerged adult progeny were counted and recorded 3 times a week.

Results and Discussion

The smallest average number of maize weevil progeny emerged from cultivar MP10 Sh (7/replicate) and the largest (72.3/replicate) from cultivar GX266 (Table 3). The average numbers of emerged weevils from the remaining 90 cultivars ranged from 28.3 to 67.0/replicate. Statistical analysis (Table 4) revealed significant differences in the numbers that emerged from different cultivars. Based on a least significant difference (5% level) of 14.59, the sorghum cultivars could be placed in 4 groups according to the degree of resistance: (1) the most resistant cultivar, MP10 Sh, (2) the 30 resistant cultivars from which average numbers of emerged insects ranged from 28.3 to 42.7/replicate, (3) intermediates, and (4) the 9 most susceptible cultivars from which the average numbers of emerged insects ranged from 58.0 to 72.3/replicate.

Plate III shows the contrast in damage and numbers of emerged insects between the most resistant and the most susceptible replicates.

EXPLANATION OF PLATE III

MP10 Sh and GX266, the most resistant and the most susceptible sorghum cultivars, respectively, to maize weevils. The progeny that emerged from each sample are shown.