RESISTANCE OF SORGHUM VARIETIES TO THE RICE WEEVIL SITOPHILUS ORYZAE (L.) AND TO THE ANGOUMOIS GRAIN MOTH SITOTROGA CEREALELLA (OLIVIER)

by

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INTRODUCTION

Sorghum is one of the major food grains in the world, ranking close to wheat, rice and maize (Rano and House 1970). Many people in Africa and Asia depend on it, eg. in the Sudan the grain is a major food for man, and the vegetative growth of the crop is fed to domestic animals.

In the United States sorghum production was 24.2 million tons during 1971, according to the Economic Research Service in the U.S. Department of Agriculture. Apart from its use as food and feed, it is used industrially for various purposes.

With increasing human population and their needs for food and animal protein, concerend efforts must be made to develop high yielding varieties of sorghum, and to minimize the storage losses due to insects and other pests. In developing countries, especially the tropical and subtropical ones like Sudan, storing sorghum and other grains is a major problem because the environment there is suitable for insect development. Losses caused by stored-product insects was estimated to be 5-10% of the world production (Wilbur and Mills 1978). In the United States where the environment is less favorable for insect infestation, losses were reported to be as high as 300-600 million dollars annually (Wilbur and Mills 1978). By reducing post-harvest losses to insects, rodents and other stored-grain pests, the availability of sorghum grains to man and animals may be increased. To control stored-grain insects we can use fumigation, air tight storages or conditioned storages; such controls are not available in developing countries. On the other hand, chemical control has serious limitations, e.g. insecticide residues, and some insects have developed resistance to some insecticides (Georghious and Taylor 1977). One alternative which is for controlling insects in agricultural production is host resistance to insects.

Plants or varieties that are inherently less injured or infested by insects than other varieties of the same species under comparable environmental conditions are considered resistant (Painter 1951). Utilization of such resistant varieties in developing countries like Sudan, where storage facilities are not adequate, is important.

LITERATURE REVIEW

Many factors are related to insect resistance in grains and several have been identified:

Factors that have been mentioned to cause resistance in grains are: 1) hardness or thickness of the pericarp, 2) seed size, 3) chemical composition, 4) smoothness. Rout (1973) using 10 sorghum cultivars ranked for resistance to Sitotroga cerealella, drilled small holes and then infested the samples with eggs. He found that the newly-hatched larvae readily entered the holes and developed in all selections; Thus the resistance in the pericarp was destroyed in all cultivars. However, in another experiment with Tribolium castaneum he found an exception in one cultivar where larval development was slowed even though the sorghum was offered as flour, thus suggesting resistance factors other than the intact pericarp. (White 1975) found that susceptible cultivars had thicker pericarps with conspicuous quantities of starch granules present in the mesocarp layers while the resistant varieties generally had thinner pericarps and seed coats. Schoonhoven et al. (1976) using maize weevils and maize found that damaging the pericarps in various ways made the corn more susceptible to weevils. Nwanze et al. (1975) found that cowpeas with smooth seed coats were more susceptible to cowpea weevils than rough varieties.

Hardness has been frequently suggested as a factor in resistance of cereal grains to stored product insects. Relative hardness was measured by Russell (1966) using a Strong Scott Barley Pealer Model 38. He found that the harder the grain, the fewer the eggs deposited by the rice weevil.

Maneechoti (1974) measured relative hardness by the penetrating point method; he used the impression made by a diamond point under 1 kg weight to measure hardness or sorghum cultivars.

Chemical composition of grains is also a major factor in resistance to insect. Eickmeier (1965) showed that high amylose content in corn resulted in resistance to the Angoumois grain moth. He also showed that high amylose content was a factor in hardness of the seed coat of corn.

Su et al. (1972) demonstrated that soybean saponin and its calcium salts were highly toxic to the rice weevil. Infestation of 39 sorghums, including Brazillian varieties and hybrids, under laboratory conditions revealed that host resistance, grain tannin content and grain hardness were not correlated but that weevil weight was significantly and positively correlated with grain volume, and number of emerging weevils (Ramalho 1977).

Condensed tannins are found in many plant species but among cereal grains have been reported only in sorghum and barley. Tannins are responsible for most of the bird-resistant grain sorghum (Harris 1969, Tipton et al. 1970, McMillian et al. 1972). High tannin content has also been related to reduction in germination in the head and pre-harvest molding (Davis 1976). Tannin in grain sorghum is associated with the testa layer (Armstrong 1974, Featherston and Rogers 1975).

The presence of the testa layer in sorghum is controlled by two complimentary dominant genes (Kofoid et al.). Thus, genetically eliminating the testa and tannin from sorghum lines and hybrids is not difficult and it might be desirable because many think high tannin content has resulted in problems in the utilization of grain sorghum. However, the complete elimination of tannin from all grain sorghum likely would eliminate sorghum production in certain areas, where bird damage is a problem.

Resistance of some sorghum varieties to the rice weevil has been studied by Chatterji (1953). Russell (1962) found that the harder the sorghum grain the less the attack by the rice weevil. When the varieties were mixed, the size of the grain influenced the intensity of infestation. Shallu MP-10 was reported to be the most resistant to rice weevils among 92 cultivars of sorghum (Maneechoti 1974). Teetes et al. (1981), searching for resistance in exotic sorghum kernels, found that the lines SC0226, SC0309, SC0311 and SC0331 has a promising source of resistance to maize weevil.

Cohen and Russell (1970) found different levels of susceptibility in rice varieties to S. oryzae and Sitotroga cerealella, which they attributed to the tightness of the husk. Cogburn (1974) suggested that the tightness of the husk was not the sole factor and that chemicals in the husk of the grain may attract or repel insects. Russell (1976) maintained that resistant factors were working against the first instar of S. cerealella before they penetrated the rice grain rather than affecting development and mortality of later stages so hardness is the resistant factor. Preliminary evidence of resistance of high amylose corn to attack by the Angoumois grain moth was obtained by Peters et al. (1960). Rhine and Staples (1968) showed that high amylose corn adversely affected larval nutrition of the rice weevils and the Angoumois grain moth. Rogers and Mills (1974) found that varieties with tight glumes were almost immune to S. zeamais.

Taxonomy of the Rice Weevil

Linnaeus (1763) first described and named the rice weevil, Curculio oryza L.; Motschulsky, in 1855 described a large weevil, Sitophilus zea-mais Motsch. from corn. Sasaki (1899) studied a small weevil and named it Calandra oryzae var. minor. In later studies Takahashi (1928) redescribed and raised Sasaki's Calandra oryza (L.) minor (Sasaki) to Calandra sasaki (Tak). A review by Floyd and Newsom (1959) showed morphological as well as biological differences between the two populations of the rice weevils. They referred to the large strain as Sitophilus oryzae (L.) and to the small weevil as S. sasakii (Tak) and recognized each as an isolated species. (Stevens 1966). Later studies separated the two species on the basis of the male genitalia and found the small rice weevil to be Sitophilus oryzae (L.) and the large Sitophilus zeamais Motsch. (Kuschel 1961).

Biology of the Rice Weevil

The adult weevil is some 2-4mm in length with the typical snout of weevil and mandibles. It is usually brown in color but may be almost black and there are 4-light reddish or yellowish spots on the elytra. The adult can fly strongly. Eggs may be laid in grains in the field or in the storage. The female chews a hole in the grain, almost never attacking it through the embryo, and places an egg in the hole which is then plugged with mucilaginous material. The egg is white, measuring 0.7mmx0.3mm and each female lays 250 or more. These may hatch in four to nine days, depending on the temperature and humidity. The grub, which is white with a brown head and strong mandibles, feeds within the grain, mostly in the endosperm, but ignores the embryo. The larval stage lasts for 15-40 days, depending on conditions. The pupa forms within the grain and lasts 3-6 days. The adults usually remain in the grain a few days before emerging and mating.

The rice weevil cannot breed at temperatures consistently above 30-32°C and the life cycle takes a minimum of 26 days at the higher temperature but is prolonged at lower temperatures (Doggett, Hugh 1970).

Taxonomy of the Angoumois Grain Moth

Oliver named the moth *Alucita cerealella* in 1789. Simmons and Ellington (1933) stated that the Angoumois grain moth has been assigned by various authors to different genera including *Alucita*, *Oecophora*, *Tinea*, *Phalena*, *Ypsolophus*, *Anacampsis*, *Gelechia*, and *Sitotroga*. The Angoumois grain moth is *Sitotroga cerealella*, (Oliv.) in the family Gelechiidae (Rout 1973).

General Biology of the Angoumois Grain Moth

Natural food of the larvae include kernels or seeds of corn, wheat, sorghum and many other cereals (Bell 1971). Crombie (1943) showed that newly hatched larvae could attain the adult stage in wheat flour although mortality was high. Rachesky (1966) demonstrated that the insect could be reared in pellets made of ground wheat mill fractions. Chippendale (1970) demonstrated that the Angoumois grain moth could be reared in diets of ground wheat or corn.

Many workers have reported on the number of eggs laid by the female. According to Pederson et al. 1980 each female may lay 40-389 eggs. Crombie (1944) reported that 86% of a group of moths reared in wheat under the same conditions had larval period from 29-36 days. Mills (1964) who reared the Angoumois grain moth in wheat, sorghum and corn, obtained mean larval periods of 37.9 days, 33.0 and 35.3 days respectively. Mills (1965b) demonstrated the importance of early germ feeding by young larvae inside the wheat kernels. Mills and Wilbur (1967) reported a pupal period averaging about 9.6 days with a range from 8 to 11 days and individuals having a longer larval period also tended to have a longer pupal period.

Simmons and Ellington (1933), who made extensive studies of its biology and life cycle, found in 1933 that females generally lived longer than males. Mills (1964) found that for mated individuals reared in wheat without water or food, 15 males lived an average of 8.4 days and 15 females 10.9 days. When given a sucrose solution, the longevity was greatly enhanced; males averaged 14.9 days and females, 17.9 days.

Structure of the sorghum kernel

The mature sorghum seed consists of the embryo, or germ, and the endosperm, both surrounded by a seed coat. The seed is enclosed in the pericarp. The whole structure is called the caryopsis or kernel. In certain sorghums a pigmented layer, called the testa, is present beneath the pericarp.

In sorghum grain, polyphenols are located primarily in the pericarp and the testa. Bird-resistant sorghums have a pigmented testa containing condensed tannins that remain in the kernel at maturity, whereas in sorghums susceptible to birds the testa is resorbed during maturation. The testa sometimes consists of two overlapping layers of distinct blocky cells. The thickness of the testa varies around the kernel (Hulse 1979). See diagram of a sorghum caryopsis in Fig. 1.

EXPLANATION OF FIG. 1

Diagram of the major anatomical parts of a mature sorghum caryopsis.

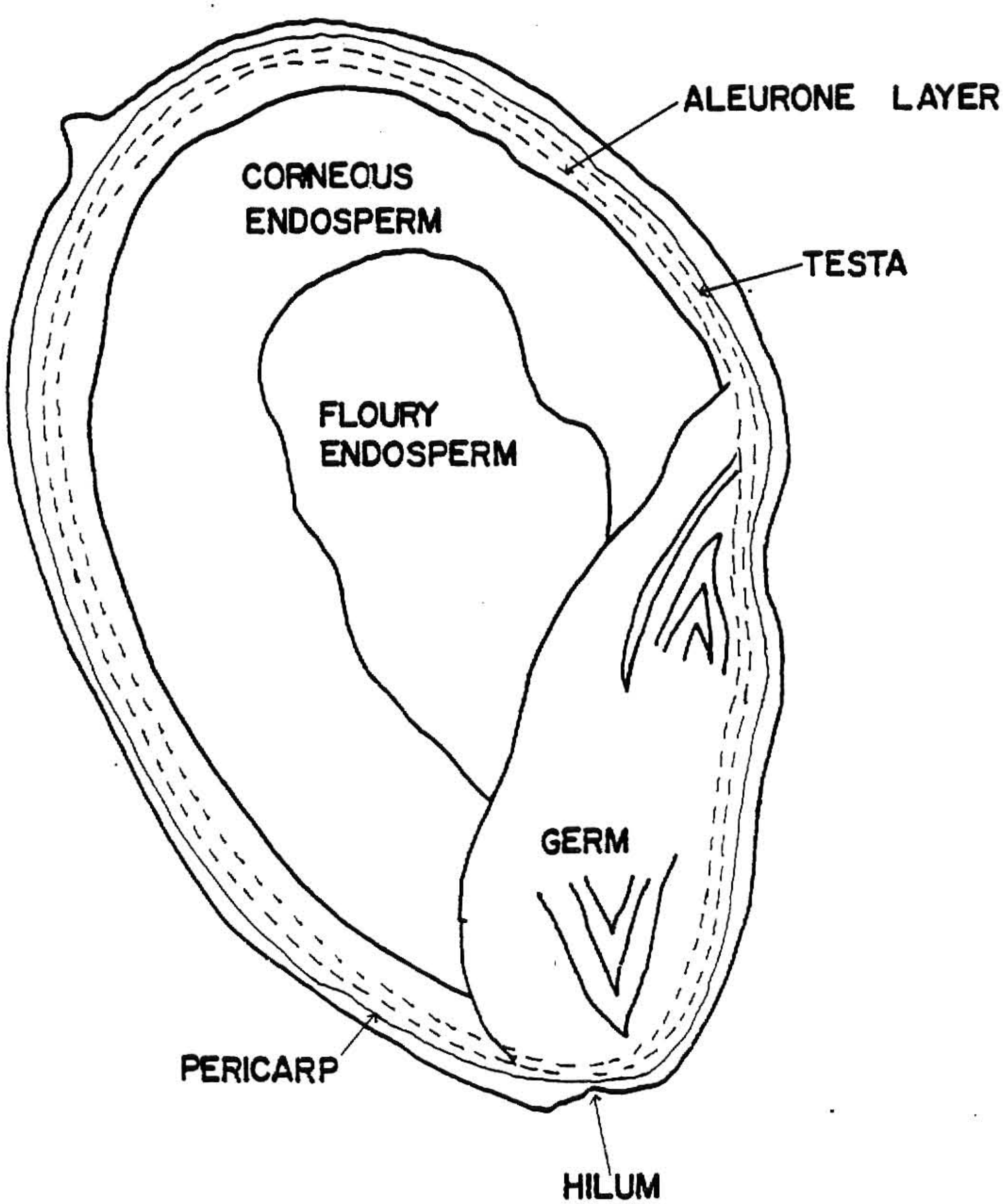


Fig.1. DIAGRAM OF A SORGHUM CARYOPSIS (Rooney et al)

Laboratory Studies of Resistance

Resistant plants or varieties are those which are markedly less damaged or less infested than neighboring plants. There are three components of resistance: tolerance, antibiosis and nonpreference (Painter 1951). Antibiosis is exhibited when the plant contains material injurious to the insects that feed on it or the part fed on may lack some necessary nutrient or vitamin. Insects die when young, produce fewer progeny, or grow more slowly (Painter 1951).

Antibiosis type of resistance can be detected and studied using suitable techniques. When the cause of resistance is expected to be chemical, the physical causes of resistance, e.g. hardness or smoothness, has to be excluded. Pelleting techniques is a convenient way to study antibiosis against stored-grain insects.

In these laboratory studies, we are trying to find an explanation for possible causes of resistance of some Sudanese and American sorghum varieties to the rice weevil and to the Angoumois grain moth.

MATERIALS AND METHODS

Sources of Sorghum Grains

Samples of seven sorghum varieties were collected from 1982 harvest from farms near El Obeid area in Sudan. Five varieties were received from the Agronomy Department, Kansas State University.

Table 1.

The vernacular name of the variety (Short version used in tables and graphs)		Origin	Color
1. Gadum el Tatel	(Tatel)	Sudan	Orange
2. Kulum Safra	(Safra)	"	Yellow
3. Zinary Sarehi	(Zinary)	"	White
4. Gasabi		"	Light Yellow
5. Feterita Kharmhk	(F. Kharmhk)	"	White
6. Wad Umm Sadari	(Sadari)	"	White
7. Feterita Wad Yabis	(F. Yabis)	"	White
8. Double Dwarf Early Shallu	(Shallu)	U.S.A.	White
9. Early Kalo	(Kalo)	"	Light Red
10. KS1		"	Light Red
11. KS3			Light Red
12. Sagrain		"	Light Red

Source of Insects

Insects used in the experiments were obtained from a stock culture maintained in the Department of Entomology Stored-Product Insects Laboratory. The colony of the Angoumois grain moth was originally acquired in 1960 from infested corn in Anderson County, Kansas, and the original stock of the rice weevil was obtained from the U.S.D.A Stored-product Insects Research and Development laboratory, Savannah, Georgia.

Maintenance of the Rice Weevil Stock Culture

Stock cultures were maintained in two widemouth quart jars with 40-mesh screen in the caps. Each jar was half filled with insect free sorghum, to which we introduced approximately 200 unsexed adults. After a one-week oviposition period, adults were removed by screening with a No. 10 sieve. The cultures were kept in a rearing room with relative humidity controlled at 67±3% and temperature at 27°±1°C. New cultures were started every two weeks.

The rearing procedure for the Angoumois grain moth were similar. except new cultures were set up every week and the adults were not removed.

Collection of Angoumois Grain Moth Eggs

The technique and equipment for collecting the eggs were similar to an adaptation of Ellington's (1930), used by Mills (1965). Pint jars with four double black paper oviposition strips inserted through slots in the metal lids were used. Twenty-five newly emerged, unsexed moths were put in each jar. Eggs were placed by the female between the strips of paper. The oviposition strips were removed every 24 hours and new strips inserted.

RESISTANCE OF SORGHUM TO THE RICE WEEVILS

Free Choice Test

In this experiment, the sample size was 100 whole kernels chosen at random from a sample of each variety in 48 x 48 x 18 mm plastic test boxes without lids. There were three replicates per each variety. The thirty six replicates of the twelve varieties were placed in a circle in an oviposition wooden chamber. The chamber was round, had a diameter of 32.5 cm and a depth of 7.5 cm. Three hundred and sixty rice weevils, 1-2 weeks old, were placed in the center of the chamber so that the insects had an equal chance to choose any variety. The oviposition chamber was covered with a circular piece of masonite and sealed with tape, then left in the rearing room for 5 days for free choice oviposition. The adults were removed and the plastic boxes were covered and returned to the rearing room. They were left for six weeks for progeny emergence. Then the progeny were counted and the frass was weighed on a SARTORIUS (1219) electronic balance. The number of progeny emerging, and the frass weight from each variety, were used as a measure for the relative resistance of the different varieties.

Results of the Free choice Test

Table 2 and Figure 2 shows that the highest number of adults emerged from Zinary variety, averaging 22.6 per replicate, followed by Safra which averaged 21.6 per replicate. The lowest number emerged from Shallu, 2.7, followed by Sagrain and Tatel. The statistical analysis (Duncan's Multiple Range Test) of the data revealed that differences in the number of adults emerged from Zinary, Safra and those emerged from Shallu, Sagrain and Tatel were significant.

The highest amount of frass was collected from Safra (Table 3 and Figure 2), averaging 54 mg. per replicate, follwed by Sadari averaging 33.7 per replicate. The lowest amount was collected from Shallu, averaging 4 mg. per replicate. The statistical analysis (Duncan's Multiple Range Test) revealed that there is a significant difference in the amount of frass collected from Shallu, Sagrain, and the amount collected from Safra and Zinary under the 5% level.

Table 2. Summary of Duncan's Multiple Range Test with .05 protection level of number of progeny emerged from each variety in 6 weeks to show the relative resistance or susceptibility of the variety in the free choice tests.

Sorghum Variety	No. of adults/rep. A	B	C	Mean no.* of adult
Zinary	21	25	22	22.6 a
Safra	19	25	21	21.66 ab
Gasabi	12	18	19	16.33 abc
F. Yabis	14	12	21	15.66 abc
F. Kharmhk	10	19	15	14.66 bc
KS3	13	10	17	13.33 cd
Sadari	14	13	13	13.33 cd
Kalo	13	4	19	12.00 cd
KS1	19	8	9	12.00 cd
Tatel	16	7	12	11.66 cd
Sagrain	10	3	7	6.66 de
Shallu	3	2	3	2.66 de

* Mean with the same letter are not significantly different.

Table 3. Summary of Ducan's Multiple Range Test with .05 protection level for the data of the Frass weight in the free choice test.

Variety	Frass Weight (mg) Reps. 1	2	3	Mean*
Safra	49	60	53	54.00 a
Sadari	33	41	27	33.70 b
Zinary	34	27	34	31.70 b
Gasabi	31	25	29	28.30 b
F. Kharmhk	26	30	25	27.00 bc
F. Yabis	25	17	35	25.70 bc
Kalo	20	18	36	24.70 bc
Tatel	34	14	23	23.70 bcd
KS1	21	13	14	16.00 cde
KS3	11	16	13	13.30 def
Sagrain	16	4	11	10.30 ef
Shallu	4	3	5	4.00 f

* Means with the same letter are not significantly different.

EXPLANATION OF FIG. 2

Mean number of progeny emerged and mean frass weight from 12 sorghum varieties in the free choice test.

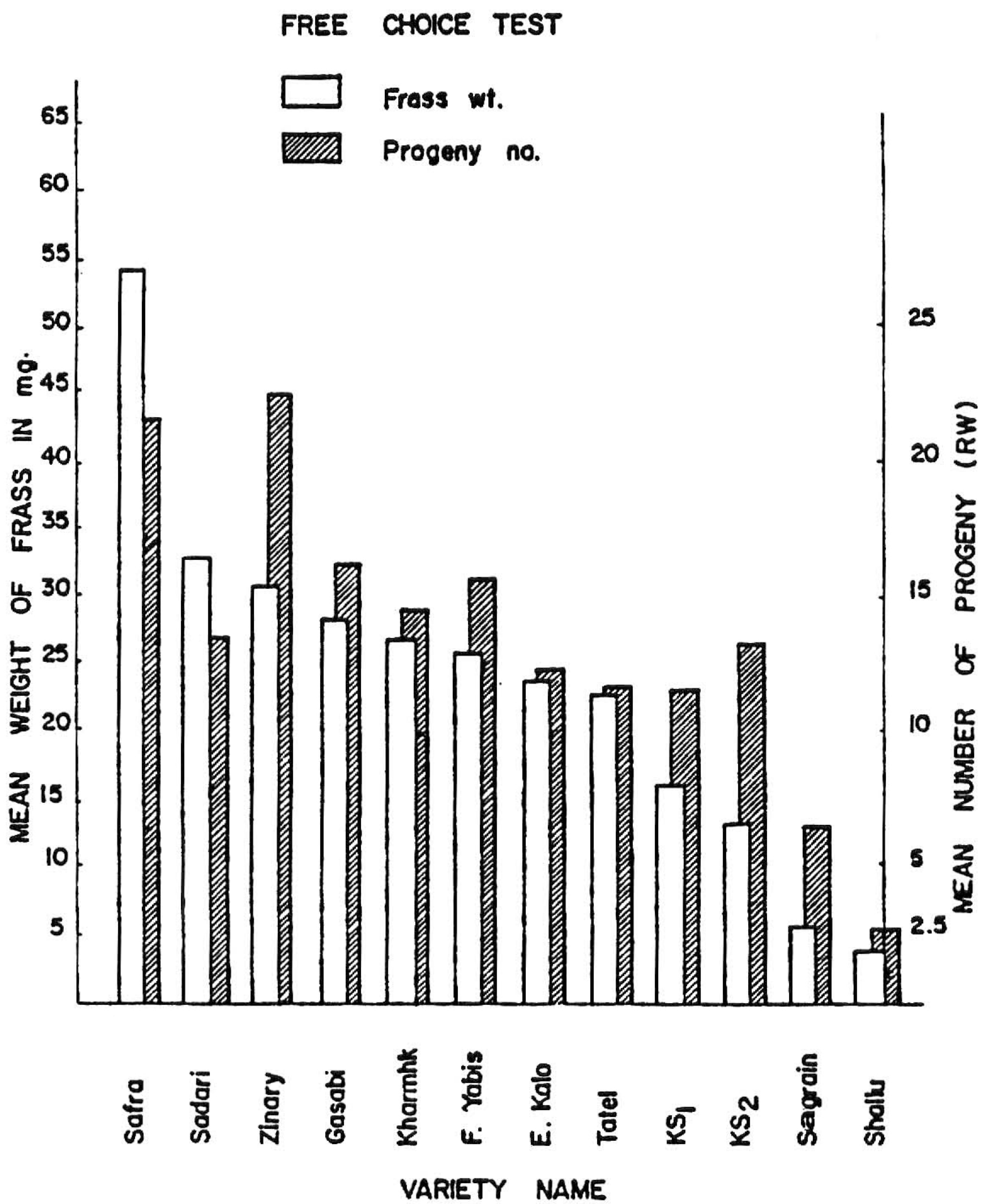

FREE CHOICE TEST
Frass wt.
Progeny no.
MEAN WEIGHT OF FRASS IN mg.
5
10
15
20
25
30
35
40
45
50
55
60
65
MEAN NUMBER OF PROGENY (RW)
2.5
5
10
15
20
25
Safra
Sadari
Zinary
Gasabi
Kharmhk
F. Yabis
E. Kalo
Tatel
KS1
KS2
Sagrain
Shallu
VARIETY NAME

Fig. 2

No Choice Test

Techniques for this test were similar to those in the free choice test, except that 10 rice weevils were taken at random and used to infest a sample variety in a plastic test box, allowed an oviposition period of 5 days and then removed. The insects had no choice in selecting a variety.

In both tests, after six weeks, the infested varieties were removed from the rearing room, put in the freezer for 48 hours, and the number of progeny counted and the frass from each variety was collected and weighed on a SARTORIUS (1219) electronic balance.

Results of the No Choice Test

Table 4 and Figure 3 shows that the highest number of adults emerged from KS1 averaging 33.5 per replicate, follwed by Zinary and Safra which averaged 32.5, 32.00 respectively. The lowest number emerged from Tatel averaging 9.5 per replicate and Shallu averaging 10.5 per replicate. The statistical analysis (Duncan's Multiple Range Test) revealed that the differences in the number of adults emerged from KS1, Zinary, Safra and those emerged from Shallu and Tatel were significant under the 5% level.

Table 5 and Figure 3 shows that the highest amount of frass was collected from Safra, averaging 118.5 mg per replicate follwed by Sadari averaging 64 mg per replicate. The lowest amount of frass collected was from Shallu, averaging 11 mg per replicate and Tatel averaging 26 mg per replicate. The statistical analysis (Duncan's Multiple Range Test) revealed that there is a significant difference in the amount of frass collected from Safra, Sadari and those collected from Tatel and Shallu under the 5% level.

The results of the free choice test and the no choice test indicated that Shallu was the most resistant variety and Safra, Zinnary were the most susceptible varieties.

Table 4. Summary of Duncan's Multiple Range Test (protected with .05 level) for number of progeny emerged from each variety in 6 weeks to show the relative resistance or susceptibility of the variety in the no-choice tests.

Sorghum Variety	No. of adults/rep.		Mean No.* of adults
	A	B	
KS1	26.00	41.00	33.5 a
Zinary	33.00	32.00	32.5 ab
Safra	30.00	34.00	32.00 ab
F. Yabis	34.00	28.00	31.00 ab
KS3	34.00	24.00	29.00 ab
Kalo	27.00	20.00	23.5 abc
Sagrain	17.00	29.00	23.00 abc
Gasabi	25.00	19.00	22.00 abc
Sadari	16.00	27.00	21.5 abc
F. Kharmhk	23.00	10.00	16.5 abc
Shallu	7.00	14.00	10.5 c
Tatel	9.00	10.00	9.5 c

* Means with the same letter are not significantly different.

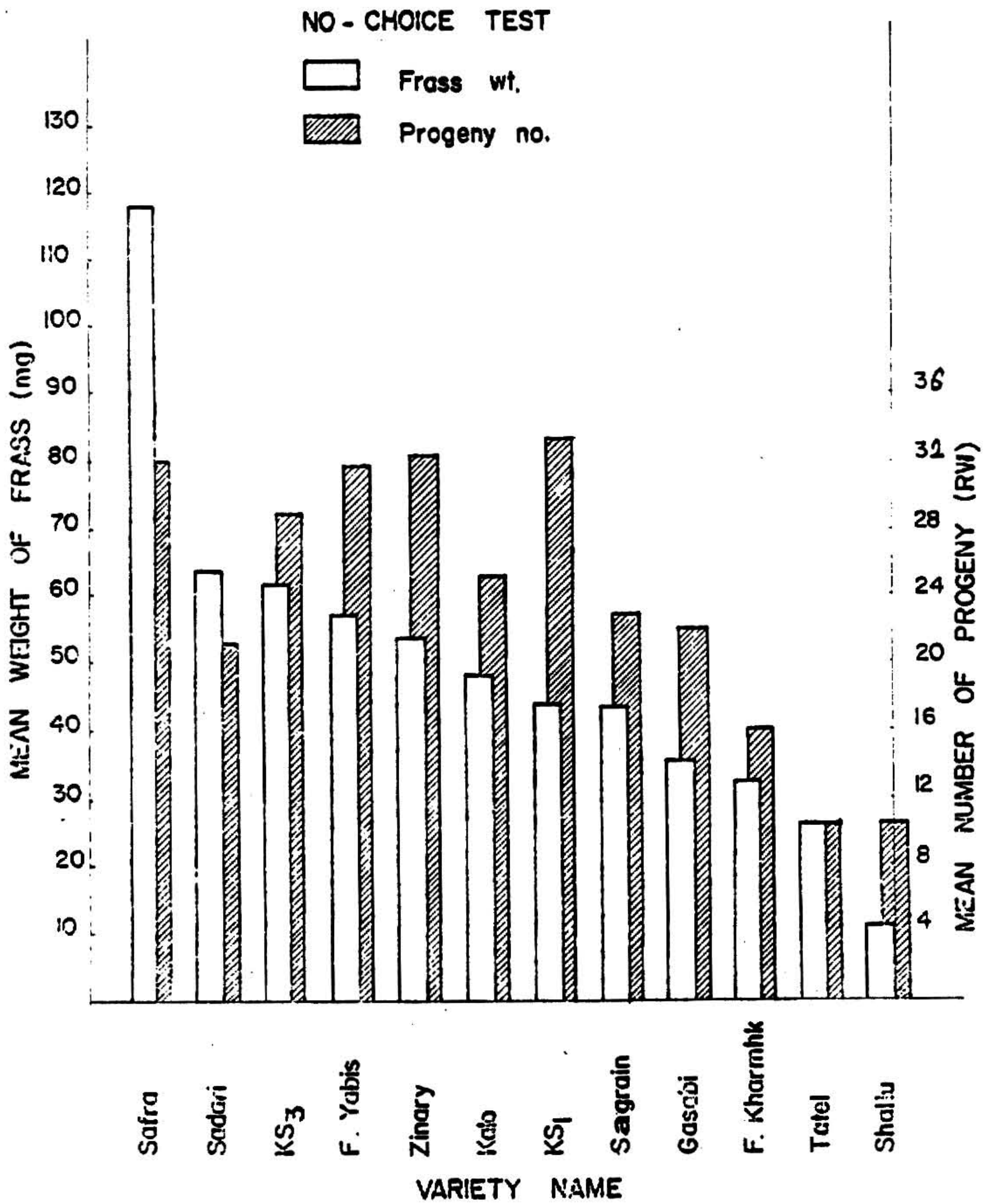
Table 5. Summary of Duncan's Multiple Range Test with 0.50 protection level for the frass weight data in the no-choice test.

Variety	Frass weight (mg)/rep. 1	2	Mean
Safra	117	120	118.5 a
Sadari	47	81	64.00 b
KS3	60	64	62.00 bc
F. Yabis	53	62	57.5 bc
Zinary	61	47	54.00 bcd
Kalo	48	48	48.00 bcd
KS1	33	55	44.00 bcd
Sagrain	31	56	43.5 bcd
Gasabi	45	26	35.5 bcde
F. Kharmhk	40	25	32.5 cde
Tatel	13	39	26.00 de
Shallu	8	14	11.00 e

* Means with the same letters are not significantly different.

EXPLANATION OF FIG. 3

Mean number of progeny emerged and mean frass weight from 12 sorghum varieties in no-choice test.



NO - CHOICE TEST
Frass wt.
Progeny no.
MEAN WEIGHT OF FRASS (mg)
130
120
110
100
90
80
70
60
50
40
30
20
10
MEAN NUMBER OF PROGENY (RW)
36
32
28
24
20
16
12
8
4
Safra
Sadari
KS_3
F. Yabis
Zinary
Kalo
KS_1
Sagrain
Gasabi
F. Kharmhk
Tatel
Shallu
VARIETY NAME

Fig. 3

CORRELATION BETWEEN TANNIN CONTENTS IN SORGHUM VARIETIES AND THE RELATIVE RESISTANCE TO THE RICE WEEVIL

Chemical Method for Estimation of Tannin in Grain Sorghum

Reagents: The reagent was prepared by combining equal volumes of 8% concentrated hydrochloric acid in methanol and 4% vanillin in methanol. These reagents were prepared daily. One gram of seed of each variety, ground through a 40 mesch screen, was put in a 125-ml beaker, 50 ml methanol was added and each beaker was covered and swirled. The beakers were swirled occasionally to mix the contents. After 26 hours, the beakers were swirled and the sediment settled. One ml of the supernatant of each beaker was pipetted into each of the two tubes, then 5 ml of vanillin-HCL reagent was added to each tube, the absorbance was read on a Beckman-DB ultraviolet spectrophotometer at 500 nm wave length, after a uniform length of time (10 minutes) (Burns 1970).

Standard Curve

The standard curve was prepared by adding 100 mg catechin to 50 ml methanol. This solution was used at various dilutions from full strength to 1:10 to construct the standard curve. One ml of each dilution was pipetted into each of two separate tubes. After the 10 dilutions had been prepared, 5 ml of vanillin-HCL reagent were added to each tube. After a uniform length of time, the absorbance was read on the spectrophotometer at 500 nm wave length. The vanillin-HCL was used for the 100% absorbance blank. Then the absorbance was plotted against the catechin concentration.

Table 6. Catechin concentration and the absorbance at 500 nm.

Catechin concentration (mg)	Absorbance at 500 nm
1	.025
2	.035
3	.043
4	.051
5	.059
6	.069
7	.075
8	.083
9	.092
10	.131
20	.213
30	.295
40	.377
50	.460
60	.542
70	.624
80	.706
90	.788
100	.871

Table 7. Estimation of Tannin in Sorghum Varieties.

Sorghum Variety	Absorbance at 500 nm	% Tannin (in 1g-sample)
Sagrain	.697	7.7
F. Yabis	.060	.67
Kalo	.052	.58
KS3	.051	.57
Safra	.050	.56
KS1	.049	.54
Tatel	.041	.45
F. Kharmhk	.017	.18
Sadari	.015	.17
Gasabi	.013	.14
Zinary	.009	.10
Shallu	.005	.06

Results of the Estimation of Tannin

The percent tannin was calculated using the absorbency. The highest amount of tannin was from Sagrain 7.7% (susceptible). The lowest amount was from Shallu .06% (resistant) and Zinary .1% (susceptible). The remaining varieties ranged between .67 and .14%.

The values obtained from this test are based on Catechin equivalents, not actual tannin content. On the lower range of the test, other factors appeared to affect the reaction.

SCANNING ELECTRONE PHOTOGRAPHS OF THE DIFFERENT VARIETIES

We have examined a cross-section of each variety under the SEM to determine the presence or absence of the testa layer which is associated with the presence of polyphenols (tannin).

All the Sudanese varieties showed presence of testa with the exception of Zinary. The SEM of F. Kharmhk (Fig. 4) shows presence of a distinct testa layer. F. Kharmhk is a susceptible variety. Zinary, which is also a susceptible variety, shows absence of the testa layer, (Fig. 5). Shallu, which is a resistant variety, also shows absence of the testa. Fig. 6, however, Tatel, shows presence of a partial testa which is not continuous and disappears in some parts (Fig. 7a and 7b).

The presence or absence of the testa layer is consistent with the percent tannin (the chemical test) in the different varieties with the exception of KS1 and Kalo which showed absence of the testa layer in the SEM but has a sustantial percent tannin in the chemical test .54% and .58% respectively.

Sagrain, which has the highest percent tannin in 1g sample, showed a very wide testa layer (Fig. 8).

EXPLANATION OF FIG. 4

SEM of a cross-section of a sorghum kernel, variety Kharmhk (a susceptible variety) which shows the presence of the testa layer at 700X.

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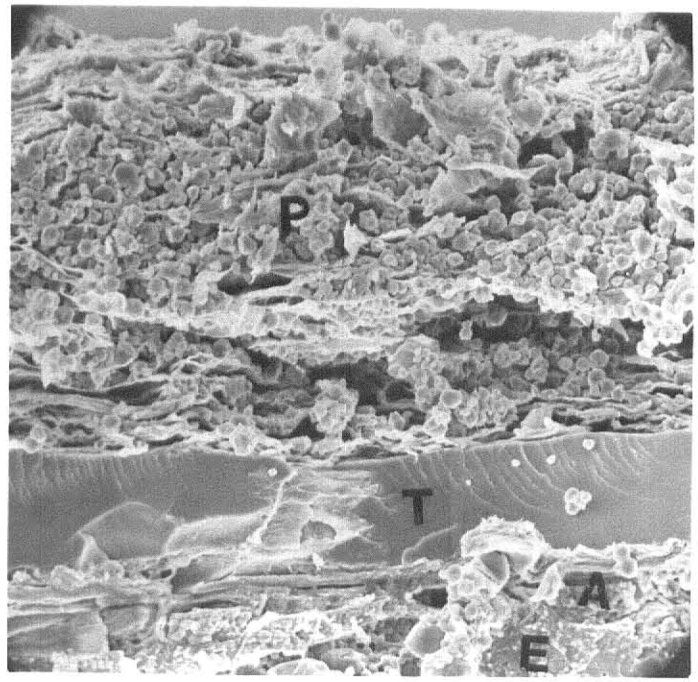


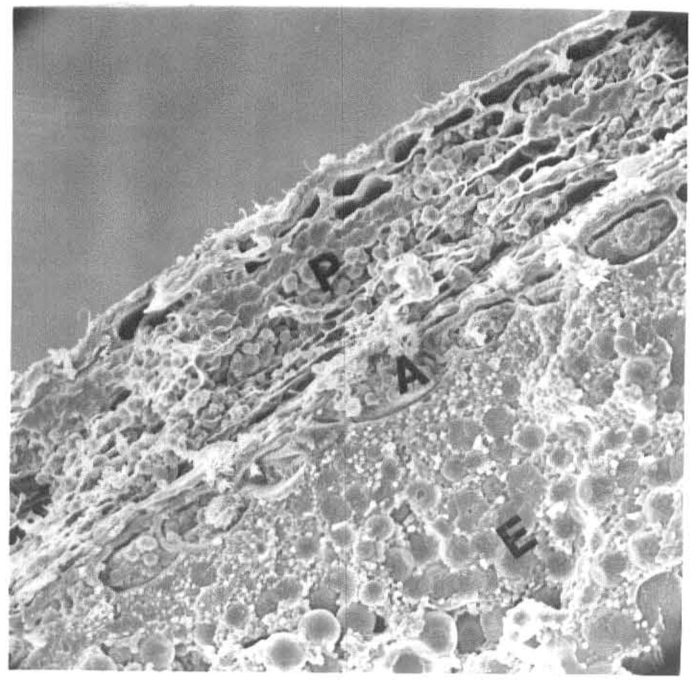
F. Kharmhk

FIG. 4

P = Pericarp layer
A = Aleurone layer
T = Testa layer
E = Endosperm

EXPLANATION OF FIG. 5

SEM of a cross-section of a sorghum kernel, variety Zinary (susceptible), which shows the absence of the testa layer at 700X.



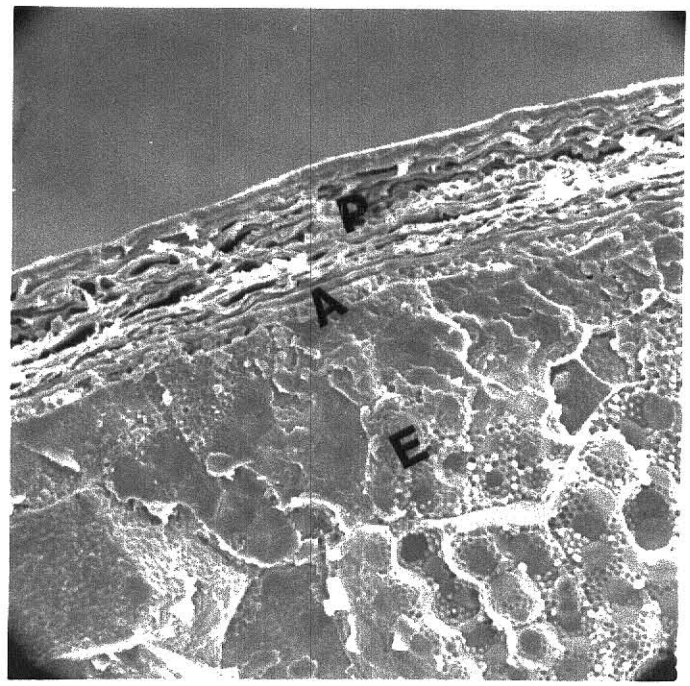
Zinary

FIG. 5

P = Pericarp layer
A = Aleurone layer
E = Endosperm

EXPLANATION OF FIG. 6

SEM of a cross-section of a sorghum kernel, variety Shallu (resistant), to show absence of the testa layer.



Shallu

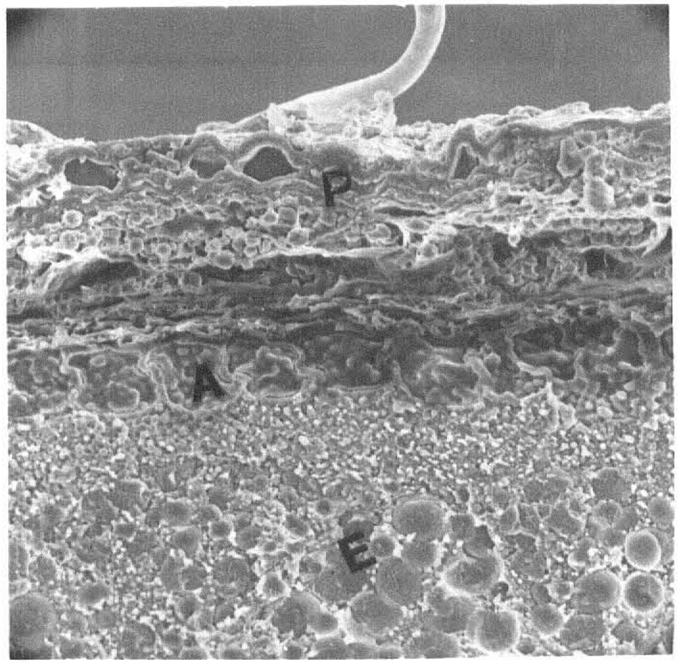
FIG. 6

P = Pericarp layer
A = Aleurone layer
E = Endosperm

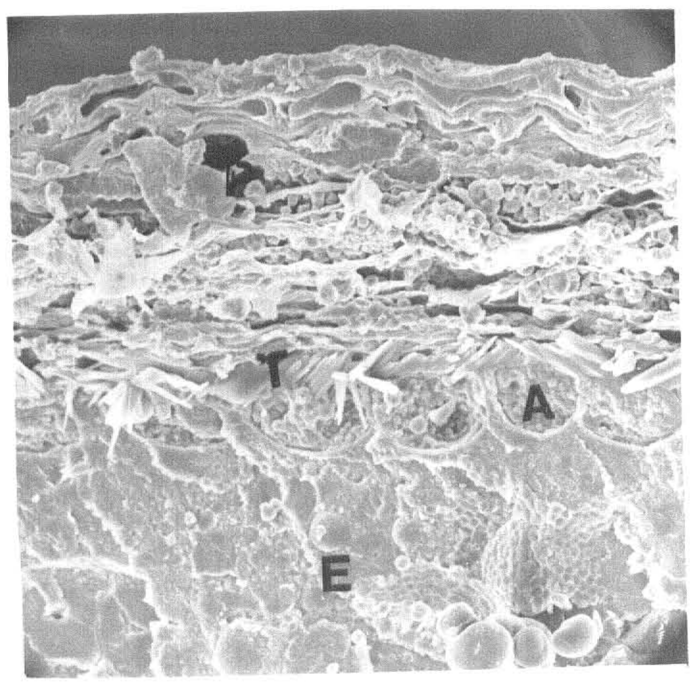
EXPLANATION OF FIG. 7

Fig. 7a SEM of a cross-section of a sorghum kernel, variety Tatel (resistant), which shows the absence of the testa at 700X.

Fig. 7b SEM of a cross-section of a sorghum kernel, variety Tatel (resistant), which shows presence of the testa layer



Tatel FIG. 7a

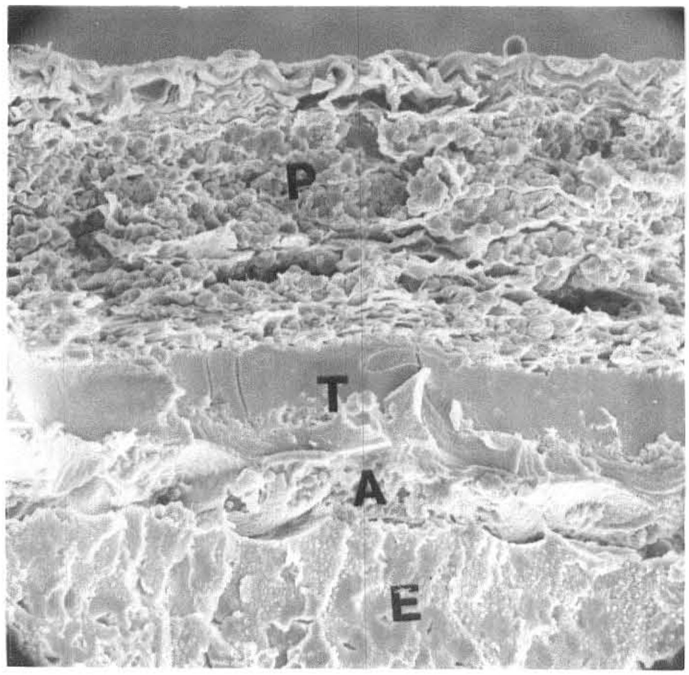


Tatel FIG. 7b

P = Pericarp layer
A = Aleurone layer
E = Endosperm
T - Testa layer

EXPLANATION OF FIG. 8

SEM of a cross-section of a sorghum kernel, variety Sagrain, which shows presence of the testa layer.



Sagrain

FIG. 8

P = Pericarp layer
A = Aleurone layer
T = Testa layer
E = Endosperm

CORRELATION BETWEEN HARDNESS OF SORGHUM KERNEL AND RESISTANCE

Procedure

The apparatus used for the hardness test was a diamond-crystal apparatus, which is composed of 2 pieces of plywood with a hinge on one end. The smaller piece could be moved up and down. A steel cylinder was inserted in the smaller piece, with a diamond crystal cemented at the free end (Maneechoti 1974). A weight of 4 lbs. was placed directly above the crystal and the point of the crystal rested on the kernel for one minute. The crystal made a diamond-shaped impression in each kernel. The softer the grain, the longer the diagonals of the impression. Ten kernels of each sorghum variety, which had been equilibrated in the rearing room for 2 weeks, were selected randomly and glued in a row on a small piece of fiberglass board. Each kernel was set under the diamond crystal. A 4 lb. weight was placed above the cylinder holding the diamond crystal carefully against the kernel where it remained for 60 seconds. The diagonal was measured using an ocular micrometer in a binocular microscope. The reading was converted to millimeters by multiplying the ocular units with a conversion factor .00333.

Results of the Hardness Test

Table 8 shows the shortest average diagonal length of the diamond impression on the surface of the kernel was .426 mm in Shallu, follwed by Tatel .500 which indicates that the kernels were relatively harder in those varieties. The longest diagonal was .780 mm in F. Kharmhk, which indicates that the grain was relatively softer. Safra and Zinary, the most susceptible varieties, got a relatively large diagonal impression, .776 and .779 respectively which indicates that those varieties were relatively softer. The length of the diagonal impression in the remaining varieties ranged between .699 and .619.

The statistical analysis (Duncan's Multiple Range Test) revealed a significant difference between Shallu, Tatel and Safra, Zinary in the length of the diagonal impression, under 5% level.

Feeding of the rice weevil on the softer varieties was usually all over the kernel (Fig. 9) like Safra, while in Shallu and Tatel the harder varieties,the feeding was mostly on the sides of the kernel (Fig. 10 and 11).

Table 8. Summary of Duncan's Multiple Range Test with .05 protection level for data of hardness of the sorghum varieties as measured by the diamond impression.

Weight = 4 lbs.
Time = 1 min.

Variety	Length of the diagonal of the diamond impression (mm)					Mean*
	1	2	3	4	5	
F. Kharmhk	.866	.833	.733	.733	.733	.780 a
Zinary	.833	.699	.699	.799	.866	.799 a
Safra	.833	.733	.666	.699	.899	.766 a
F. Yabis	.666	.666	.733	.733	.699	.699 b
KS3	.666	.633	.599	.666	.666	.646 bc
Kalo	.699	.666	.666	.599	.599	.646 bc
Gassabi	.666	.666	.666	.599	.599	.639 bc
KS1	.599	.666	.599	.666	.633	.633 bc
Sagrain	.633	.633	.633	.633	.599	.626 c
Sadari	.599	.599	.666	.633	.599	.619 c
Tatel	.500	.500	.500	.500	.500	.500 d
Shallu	.533	.433	.433	.433	.400	.426 e

*Means with the same letter are not significantly different.

EXPLANATION OF FIG. 9

Feeding pattern of the rice weevil
in Safra (soft, susceptible) variety.

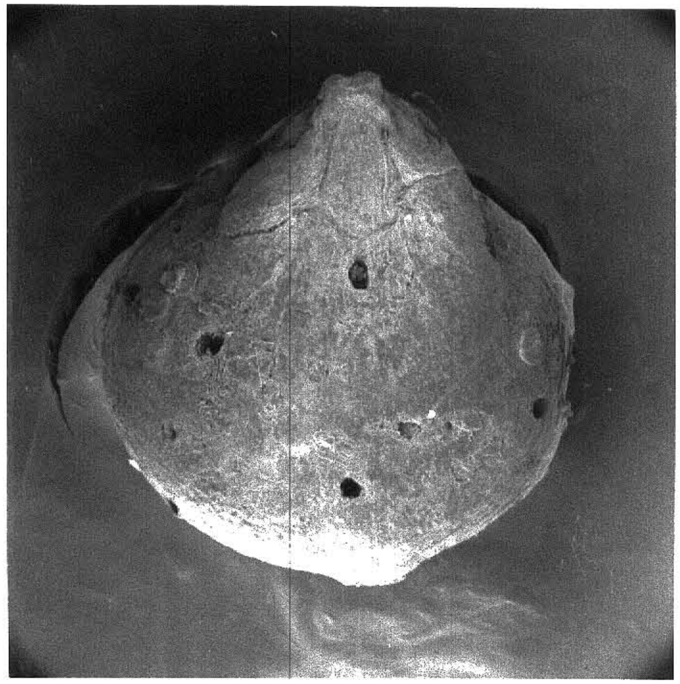


FIG. 9

EXPLANATION OF FIG. 10

Feeding pattern of the rice weevil
on Shallu variety (hard resistant)

FIG. 10

EXPLANATION OF FIG. 11

Feeding pattern of the rice weevil on Tatel variety (hard resistant).

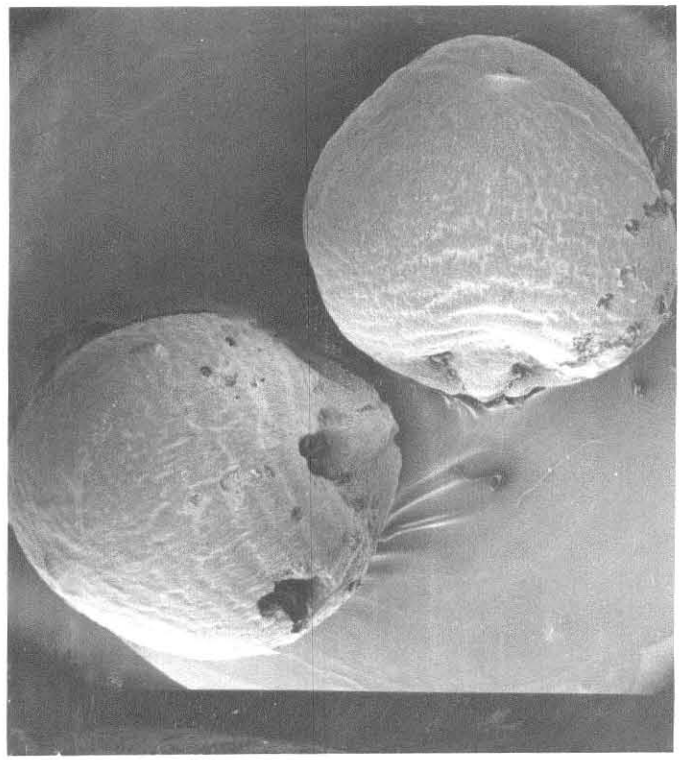


FIG. 11

THE CORRELATION BETWEEN THE PROGENY NUMBER, FRASS WEIGHT AND THE PERCENT TANNIN AND THE LENGTH OF THE DIAGONAL IMPRESSION.

Table 9 is a summary of the data of the free choice and no choice test, _____ the percent tannin and the hardness test. The statistical analysis (Table 10) revealed a .83 positve correlation between the progeny numbers and the frass weight (free choice test) at the .0008 probability level. Also the statistical analysis indicated that there is a positve .81 correlation between progeny number (free choice test) and the length of the diagonal impression at the .001 probability level. Thus, the harder the grain and the less the frass, the more resistant the variety. However, the percent tannin showed a negative correlation with the progeny number - .373 at the .23 level and also a negative correlation with the frass weight and the length of the diagonal impression. Thus the tannin content of the grain has no effect on the resistance of the variety.

Similar correlations existed in the no choice test (Table 10).

Table 9. Summary of Duncan's Multiple Range Test with .05 protection level for data of progeny number, frass weight (in the free choice and no choice test), percent tannin and the hardness test.

Sorghum Variety	Free choice test: Mean number of progeny	Free choice test: Mean Frass weight (mg)	No choice test: Mean numbers of progeny	No choice test: Mean Frass weight (mg)	% Tannin	Mean length of the diagonals at diamond impression (mm)
Zinary	22.7 a	31.7 b	32.5 ab	54 bcd	.1	.779 a
Safra	21.7 ab	54.0 a	32.0 ab	118.5 a	.56	.776 a
Gasabi	16.3 abc	28.3 b	22.0 abc	35.5 bcde	.17	.639 bc
F. Yabis	15.7 abc	25.2 bc	31.0 ab	57.5 bc	.67	.699 b
F. Kharmhk	14.7 bc	27.0 bc	16.5 abc	32.5 cde	.18	.780 a
KS3	13.3 cd	13.3 def	29.0 ab	62.0 bc	.57	.646 bc
Sadari	13.3 cd	33.7 b	21.5 abc	64.0 b	.17	.619 c
Kalo	12.0 cd	24.7 bc	23.5 abc	48.0 bcd	.58	.646 bc
KS1	12.0 cd	16.0 cde	33.5 a	44.0 bcd	.54	.633 bc
Sagrain	11.7 cd	10.3 ef	23 abc	43.5 bcd	7.7	.626 c
Tatel	6.7 de	23.7 bcd	9.5 c	26.0 de	.45	.500 d
Shallu	2.7 de	4.0 f	10.5 c	11.0 e	.06	.426 e

*Means with the same letter are not significantly different.

Table 10. Correlation between progeny, frass weight, hardness and tannin content in the free-choice and no-choice tests.

	Progeny number	Frass weight	Length of diagonal impression	Tannin content
A. <u>Free Choice Test</u>				
Progeny	-	*.8324	.8151	-.3737
	-	.0008	.0012	.2314
Frass Weight	-	-	.6371	-.3204
			.0259	.3099
Impression	-	-	-	-.0448
				.8898
B. <u>No-Choice Test</u>				
Progeny	-	.6712	.6726	.02612
		.016	.016	.935
Frass weight	-	-	.605	-.015
			.037	.963
Impression	-	-	-	.04489
				.8898

* Probability level

THE PERICARP AS A RESISTANT FACTOR IN SORGHUM VARIETIES

Procedure

The removal of large portions of the pericarp were done by using a Strong Scott Barley Pearler, Model-38. This is a technique adapted by McCluggage (1943) and further modified by Russell in 1962. Each sample was cleaned and foreign materials and broken kernels were removed. The samples were equilibrated in the rearing rooms for moisture content for 2 weeks prior to pearling. Twenty gm. of each variety were pearled for 30 seconds, then the broken kernels were removed and the sound ones were used for the experiments.

As in the whole kernels experiment, 12 varieties were used, 50 kernels per each replicate and 3 replicates/variety.

Kernels were put in 48x48x18 mm plastic boxes and 10 weevils were added to infest each sample.

Results After the Removal of the Pericarp

After the removal of the pericarp, the formerly resistant varieties Shallu and Tatel got the highest average number of emerged progeny which was 36.3 and 35.66 respectively, in this no-choice test (Table 11). The progeny number for the other 10 varieties ranged from 35.6 to 22.6

The statistical analysis revealed that there were no significant differences between the different varieties at the .05 level except sagrain. These results show that the resistance factors in these sorghum grain is in the pericarp.

Table 11. Summary of Duncan's Multiple Range Test with 0.05 protection level for number of progeny emerged from each variety after removal of the pericarp in no-choice test.

Variety	Number of progeny/rep.			Mean number*
	A	B	C	
Tatel	39	35	35	36.3 a
Shallu	38	35	34	35.6 a
Zinary	36	35	36	35.5 a
Gasabi	29	35	35	33.0 ab
Kalo	30	33	34	32.3 ab
KS3	34	23	34	30.6
F. Yabis	30	32	30	30.6 ab
F. Kharmhk	34	37	20	0.3 ab
Safra	30	30	30	30.0 ab
KS1	31	37	20	29.3 ab
Sadari	17	35	35	29.0 ab
Sagrain	23	21	24	22.6 b

* Means with the same letter are not significantly different.

OVIPOSITION PREFERENCE BY S. ORYZAE AND RATE OF DEVELOPMENT IN EACH VARIETY

Procedure

Four varieties were selected for this test: two varieties, Tatel and Shallu, the most resistant ones, Sagrain, a less resistant one than the former two, and Safra, a very susceptible one.

50 kernels were chosen at random from a sample of each variety and placed in 48x48x18 mm plastic boxes, 5 replicates for each variety. Ten weevils were introduced into each box, and allowed to oviposit for 5 days, after which the weevils were removed. Individual grains in each replicate of each variety were examined under a binocular microscope and the total number of egg plugs in each replicate was recorded. The samples were then returned to the rearing room for the progeny emergence. Starting from the 30th day, when the emergence was expected, the progeny were removed daily and recorded to determine the rate of development in each variety.

Results of the Oviposition Preference and the Mortality

The oviposition rate in each variety and the mean percent mortality are shown in Table 12 and Figure 12. The statistical analysis (Duncan's Multiple Range Test) revealed a significant difference in the oviposition preference between Safra, Tatel and Shallu. Safra and Sagrain were more prefered by the female than Tatel and Shallu. The mortality was high in Safra which is the most susceptible one but still it was high in Shallu which is the most resistant variety. The statistical analysis revealed there was no significant difference between Safra, Shallu and Sagrain in the mortality but there is significant difference between Tatel and Safra.

Table 11. Duncan's Multiple Range Test for oviposition preference by the rice weevil and mortality rate at .05 protection level.

Variety	*Mean no. of eggs deposited	% Mortality
Safra	55.8 a	65.5 a
Sagrain	46.4 a	47.0 ab
Tatel	20.8 b	23.7 b
Shallu	16.8 b	48.7 ab

* Means with the same letter are not significantly different.

EXPLANATION OF FIG. 12

Mean number of weevil progeny from 4 sorghum varieties emerging on indicated days.

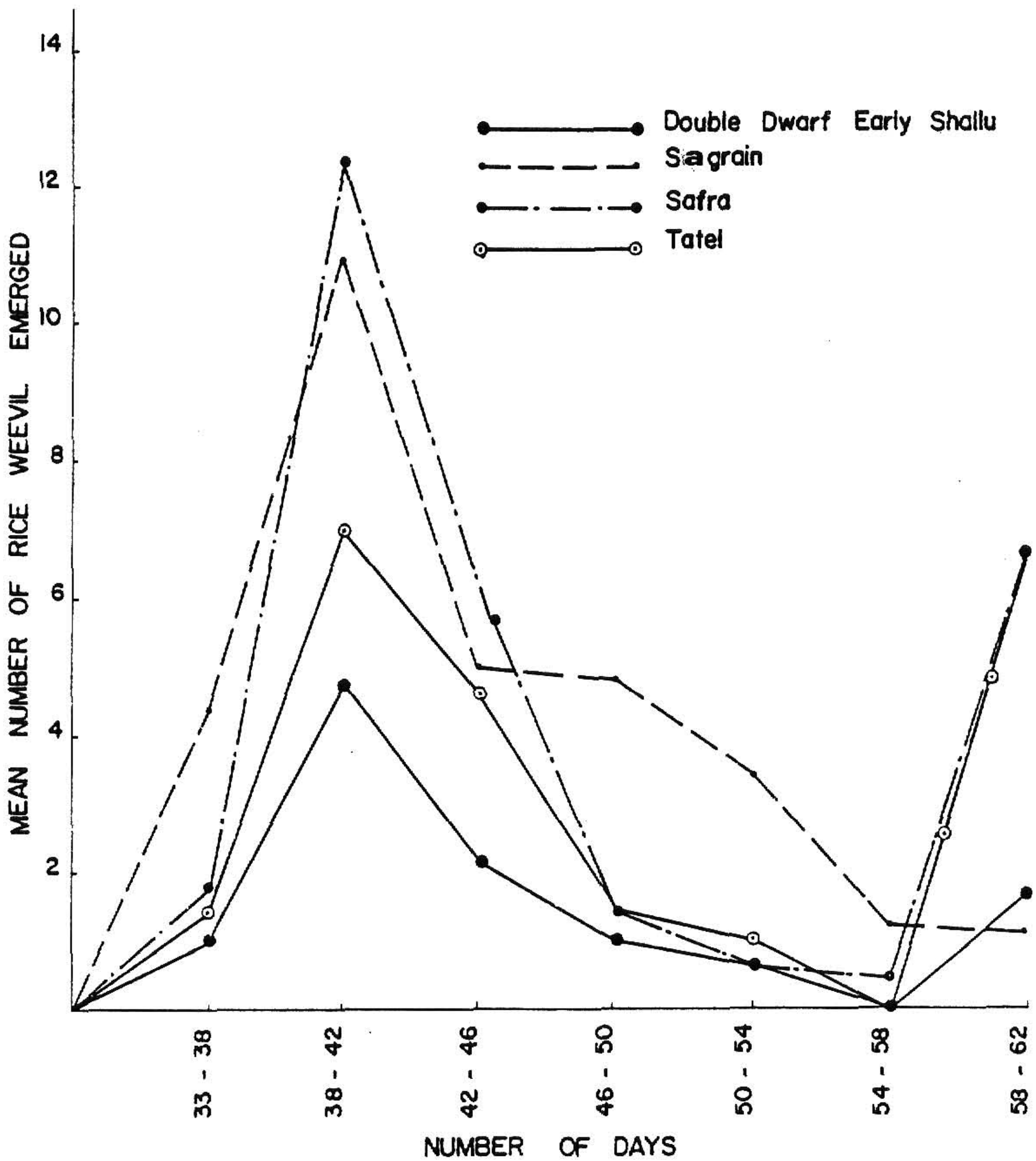

Double Dwarf Early Shallu
Sagrain
Safra
Tatel
MEAN NUMBER OF RICE WEEVIL EMERGED
14
12
10
8
6
4
2
33 - 38
38 - 42
42 - 46
46 - 50
50 - 54
54 - 58
58 - 62
NUMBER OF DAYS

Fig. 12

Results

Figure 12 shows the rate of development in Shallu, Tatel, Sagrain and Safra. In Shallu the peak of the progeny emergence was in 38-42 days. This is also similar to the other three varieties, although Shallu and Tatel were resistant and Safra and Sagrain were susceptible. However, Tatel and Safra had another peak in 58-62 days.

RESISTANCE OF SORGHUM VARIETIES TO THE ANGOUMOIS GRAIN MOTH

The sorghum varieties used in the experiment were of the same origin as those used in the rice weevil experiment (Sudanese and American). Eight sorghum varieties were used in these tests.

Procedure

One hundred grains of each variety were selected at random and placed in small glass jars (159 cc). There were three replicates of each variety. The grain was equilibrated in the rearing room for moisture content and temperature. Each replicate was infested with twenty-five 3 days old fertilized eggs on small pieces of black paper oviposition strips. After 10 days the oviposition strips were checked under a binocular microscope and the unhatched eggs in each replicate were recorded; unhatched eggs appeared white and opaque, while the hatched ones were silvery, transparent with an opening at one end. After infestation, samples were returned to the rearing room and kept for 6 weeks for progeny emergence. The emerging adults were removed daily from the jars to avoid progeny oviposition.

The number of adults emerged, those that had entered the kernel as larva, were used to determine the relative resistance of the different varieties.

Results of the Whole Kernel Test

There was greater survival in Sagrain 82.7%. Safra, Kalo and Shallu sustained the lowest infestation 34.7, 58.6, 61.3 percent respectively (Table 13). In the other varieties, the percent emergence ranged between 72.0 - 80%. The statistical analysis (Duncan's Multiple Range Test) revealed that no significant difference between Safra, Shallu and Kalo and the remaining varieties under the .05 significant level.

Table 13. Percentage of hatched AGM larvae which emerged as adults from eight sorghum varieties given a choice for entry (25 eggs/100 whole kernels).

Variety	% moth emergence in each replicate			Mean**
	A	B	C	
Shallu	80	64	40	61.3 ab
	*(20)	(16)	(10)	(15)
Sagrain	56	89	96	82.7 a
	(14)	(24)	(24)	(20.7)
Kalo	48	80	48	58.6 ab
	(12)	(20)	(12)	(14.7)
KS1	72	72	96	80.0 a
	(18)	(18)	(24)	(20)
KS3	96	56	88	80.0 a
	(24)	(14)	(22)	(20)
Safra	48	32	24	34.7 ab
	(12)	(8)	(6)	(8.7)
Tatel	88	80	72	80.0 a
	(22)	(20)	(18)	(20)
Sadari	80	56	80	72.0 a
	(20)	(14)	(20)	(18)

* Number in parenthesis is the number of adults emerged.
** Mean with the same letter are not significantly different.

EXPERIMENT WITH AGM AFTER REMOVAL OF THE PERICARP

Procedure

Removal of the pericarp was done as in the rice weevil experiment using Barley Pearler, Model 38-8.

One hundred kernels were chosen at random from a sample of each variety with four replicates for each variety. The rest of the experiment was done as in the whole kernel experiment.

Results

The lowest number of adults emerged from Safra 58.2% and the highest from KS1 93.3%, the remaining varieties ranged between 63.3 and 77.3 (Table 14).

The statistical analysis (Duncan's Multiple Range) revealed that there is no significant difference between the different varieties under the .05% significant level, indicating that removal of the pericarp has no effect on the resistance. Safra still has the lowest number of emerged progeny (58.7%).

Table 14. Percentage of hatched AGM larvae which emerged as adults from sorghum varieties after removal of the pericarp, given a choice for entry 25 eggs/100 kernels.

Variety	% Moth Emerged in each Replicate A	B	C	D	Mean
Shallu	88 *(22)	40 (10)	8 (2)	88 (22)	74.7 a (14)
Sagrain	72 (18)	40 (10)	40 (10)	80 (20)	73.3 a (14.5)
Kalo	56 (14)	64 (16)	64 (16)	48 (12)	77.3 a (13.0)
KS1	48 (12)	72 (18)	64 (16)	96 (24)	93.3 a (17.5)
Safra	48 (12)	40 (10)	48 (12)	40 (10)	58.7 a (11.0)
Tatel	48 (12)	48 (12)	56 (14)	56 (14)	69.3 a (13.0)
Sadari	88 (22)	32 (8)	64 (16)	64 (16)	63.3 a (15.0)

* The number in parenthesis is the number of adults emerged.

The correlation between number of progeny from the whole kernels and those without pericarp is positve .413 (Table 15), but the probability level is .3 which is more than .05 so there is no correlation between progeny emerged from those kernels with pericarp and those without pericarp The length of the diagonal impression and the progeny number (whole kernels) has no correlation under the 5% level. Also, in the percent tannin and the number of progeny has no correlation under the 5% level.

Table 15. Correlation between progeny, hardness and tannin content for the AGM data whole kernels and those without pericarp.

	Progeny number in whole kernel	Progeny number in pericarpless	Length of diagonal impression	Tannin content
Progeny number in whole kernels	-	.413 *.35	.317 .48	.432 .333
Progeny number in pericarpless	-	-	.413 .35	.637 .123
Length of Diagonal impression	-	-	-	.286 .53

*Probability level

SUMMARY AND DISCUSSION

This study was to compare resistance in 12 sorghum varieties, some of which were of Sudanese, others of American origin. These varieties were tested against the rice weevil (S. oryzae) and the Angoumois grain moth (S. cerealella) to evaluate various factors which may influence resistance, especially tannin content, hardness, and pericarp. Experiments were conducted in the Stored Product Insects Laboratory, Department of Entomology, Kansas State University.

Ten rice weevils were used per 100 kernels in both the no-choice test (with 3 replicates) and the free choice test (360 for the 12 varieties). The sorghum samples were kept in the rearing room for conditioning for two weeks before used in the experiments. Plastic test boxes 48x48x18 mm were used for the tests. The adult rice weevils were left for 5 days for oviposition then removed and the samples returned to the rearing room, and kept for six weeks for progeny emergence. The progeny emerging from each variety and the frass weight were used as a measure of the relative resistance.

The smallest number of progeny of rice weevil emerged from varieties Tatel and Shallu, 9.5 and 10.5 respectively, in the no-choice test, and from Shallu, Sagrain and Tatel in the free-choice test, 2.66, 6.66, 11.6 respectively.

Tannin content of the varieties was determined (in one gram of ground sample) to test the correlation between resistance and tannin content in the grain. Sagrain was found to have the highest tannin content, 7.7%, Shallu and Zinary had the lowest content, .06% and .1% respectively. The correlation coefficient between tannin content and the number of progeny emerged was negative in the free choice test -.373 and only .026 in the no-choice test.

Grain hardness was determined for the 12 varieties using the diamond crystal impression method. Shallu was found to be the hardest and the statistical analysis revealed that there is a high correlation between hardness of the variety and the number of progeny emerged. Removal of the pericarp (using a barley pearler) caused the resistant variety to be more susceptible indicating that the resistance factor is located in the pericarp.

For the Angoumois grain moth experiment, twenty-five 3 days old AGM eggs in black paper strips were used per 100 kernels in 159 cc jars, with 3 replicates. Success of larval entry and development to adults were used to determine relative resistance of the varieties. The lowest survival was observed in Safra 34.7%; Kalo 58.6% and Shallu 61.3%, the maximum was from Sagrain 82.7%. When survival of AGM from larvae to adults in whole kernel was compared with that in the kernels without pericarp, a very weak correlation was found, but still Safra yielded the lowest percentage 58.7%.

Data from those tests indicated that as previous investigators have found hardness of sorghum grains correlated with resistance whereas tannin content did not correlate with resistance in the rice weevil test and there also was no correlation in the Angoumois grain moth test.

The varieties found to be resistant to the rice weevil, Shallu and Tatel, are not identical to those resistant to the AGM except for Shallu which is resistant to both. However, in both cases, removal of the pericarp causes the varieties to be more susceptible. Therefore, resistance is located in the pericarp.

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RESISTANCE OF SORGHUM VARIETIES TO THE RICE WEEVIL *SITOPHILLUS ORYZAE* (L.) AND TO THE ANGOUMOIS GRAIN MOTH *SITOTROGA CEREALELLA* (OLIVIER)

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In this study, twelve sorghum varieties, some of which were Sudanese, and others of American origin, were evaluated for resistance to the rice weevil *Sitophilus oryzae* and the Angoumois grain moth *Sitotroga cerealella* (Olivier).

Experiments were conducted in the Stored Product Insects Laboratory, Department of Entomology, Kansas State University. Ten rice weevils were used per 100 kernels in both the no-choice and the free-choice test (360 RW for the 12 varieties). The sorghum samples were kept in the rearing room for conditioning for two weeks before used in the experiments. Plastic boxes 48x48x13 mm were used for the tests. The adult rice weevils were left for 5 days for oviposition, then removed and the samples returned to the rearing room, and kept for six weeks for progeny emergence. The progeny emerging from each variety was used as a measure of the relative resistance.

In the Angoumois grain moth experiment, twenty-five 3 day-old AGM eggs in black paper strips were used per 100 kernels in 159 cc jars with 3 replicates. Success of larval entry and development to adults were used to determine relative resistance of the varieties. Tannin content of the different varieties was determined using a modification of the Vanillin test, absorbancy was read on a Beckman ultraviolet spectrophotometer at 500 wave length. Grain hardness was determined using a diamond crystal impression method. Removal of the pericarp was done by using a barley pearler for 30 seconds.

The smallest number of progeny of rice weevils emerged from varieties Tatel (9.5), Shallu (10.5) in the no-choice test and from Shallu, Sagrain and Tatel in the free-choice test.

In the test for tannin content, Sagrain was found to have the highest with 7.7 in 1g sample, Shallu and Zinary the lowest, with .06% and .1% respectively. The correlation coefficient between tannin content and the number of progeny emerged was negative in the free-choice test -.373 and only .026 in the no-choice test.

Shallu was found to have the hardest grain and the statistical analysis revealed that there was a high correlation between hardness and the number of progeny emerged in the rice weevil experiment, however the correlation was very weak for the AGM, only .317.

Removal of the pericarp rendered most of the varieties more susceptible to the rice weevil and the AGM.