THE BEHAVIOURAL RESPONSES OF SITOPHILUS ORYZAE (L.) UNDER CONDITIONS MODIFYING THE EXPRESSION OF RESISTANCE IN STORED SORGHUM

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ABSTRACT

The behavioural responses of Sitophilus Oryzae (L.) under certain conditions modifying the expression of resistance to stored sorghum were investigated. The factors investigated include adult survival, feeding, oviposition and increase in moisture content of resistant and suceptible sorghum seeds. The results showed that S. Oryzae significantly fed more, laid more eggs and survived longer in the mixture treatment than it would normally do on resistant seeds in a no choice situation. Increase in moisture content of seed from 75 to 95% air R.H. increased egg production by x8 and x2.4 on the resistant and susceptible cultivars respectively. The results are discussed in relation to antibiosis and non-preference mechanisms in stored sorghum.

INTRODUCTION

ADETUNJI (1988) has shown that sorghum seeds vary in their susceptibility to attack by Sitophilus Oryzae (L.) and that non-preference and antibiosis (Painter, 1951) were the main mechanisms of resistance. This paper seeks to throw more light on the nature of non-preference and antibiosis in stored sorghum by a study of the behaviour of S. Oryzae when presented with situations modifying the expression of resistance. The factors which were investigated include adult survivorship, feeding, oviposition and equilibrium moisture content.

MATERIALS AND METHODS

Sorghum seeds and insect rearing

A susceptible cultivar, Ex-Mk and resistant cultivars TSC 212 and 303 (see Adetunji 1988) were used. The seeds were disinfested in the deep freeze (about -20°C) for at least 3 weeks and equilibrated in shallow layers in the experimental room maintained at 30°C and 70% R.H. for a minimum of 6 weeks before use. A culture of S. Oryzae was obtained from the Ministry of Agriculture, Fisheries and Food, Slough Laboratory and subsequent generations bred on a susceptible sorghum cultivar brought at a food shop in Southall, England.

Adult survivorship and Oviposition

Fifty seeds of each cultivar Ex-Mk (susceptible) and TSC 212 (resistant) were placed together in a 7,5 x2,5 cm glass tube and thoroughly mixed. Next, 100 seeds of the resistant and 100 seeds of the susceptible were placed in separate 7,5 x2,5 cm glass tubes. These were used as control. There were four replicates per treatment. The seed sizes used were similar in the three treatments having been standardized by using graded sieves. The standardization was necessary as Ewer (1945), Richards (1947), Reddy (1950), Segrove (1951) and Russels (1962) have shown that Sitophilus spp have preference for large seeds as compared to small seeds when presented with a choice situation.

Five pairs of rice weevils (less than 24 hours old) were placed in each replicate tube of each treatment and the tubes were covered by polytop with six pin head-size holes to allow aeration and placed in the experimental room. At weekly intervals, the tubes were inspected and the numbers of dead weevils and their sexes recorded. The surviving S. Oryzae were transferred to fresh seeds also on weekly basis until all the weevils had died. The old batch of seeds were stained by acid fuschin (Frankenfeld, 1948) to show up the egg plugs and the numbers of egg plugs recorded.

Feeding and oviposition

For the purpose of estimating the amounts of feeding, seeds of cultivar Ex-Mk, 212 and equal mixture of Ex-Mk and 212 used during the first week of the experiment described above were examined for feeding holes. The seeds having previously been stained by acid fuschin it was possible to distinguish between egg plugs and feeding holes using a binocular microscope. There was no further need to stain the feeding holes using iodine solution (e. g. Eden, 1952). The numbers of feeding holes and/ or egg plugs on each seed were recorded. Records were also taken of the numbers of seeds without feeding holes or egg plugs.

Increase in Equilibrium moisture content and Oviposition

The seeds of the susceptible cultivar Ex-Mk and the resistant 303 were conditioned at 70 % and 95 % air relative humidities and 30°C. The 30°C and the 70 % r.h. were the normal experimental conditions, while the 95 % r.h. was maintained in dessicators by appropriate solutions of caustic soda ("Analar" grade) (Madge, 1961).

One hundred seeds of each of the two test cultivars conditioned at the two test r.h. were separately placed in 7.5 X 2.5 cm glass tubes. There were 4 replicates per treatment. Ten paired S. oryzae were placed in each replicate tube for a 24 hours oviposition period at the normal experimental temperature and humidity (i.e. 30°C and 70 % r.h.). The tubes were sealed with polytop and ventilation provided as described above. The weevils were removed at the expiration of the 24 hours, seeds stained with acid fuchsin and the numbers of egg plugs determined.

The equilibrium moisture contents of the seeds at 70 and 95 % r.h.were determined by grinding each cultivar sample separately in a Moulinex grinder. Five grams of the ground sorghum were placed in a 5 X 2.5 cm unsealed glass tubes and dried in a ventilated oven at 130 C ± 3°C for 2 hours (ISO recommendation for cereal grains except maize) (Pixton, 1982). there were 5 replicates per cultivar treatment. The dried sorghum samples were cooled in a sealed dessicator over silica gel and weighed on a "Unimatic Stanton" balance.

TABLE 1 : Age specific oviposition and adult survivorship of S. oryzae on the susceptible cultivar (Ex-Mk) and the resistant cultivar TSC 212 and on an equal mixture of each cultivar.

Pivotal age	Survivorship (lx)			Mean numbers of eggs per female		
	Ex-Mk	Ex-Mk +212	212	Ex-Mk	Ex-Mk +212	212
0,5	0,96	1,00	0,81	4,95	3,45	0,95
1,5	0,93	1,00	0,54	10,6	7,85	1,3
2,5	0,91	0,99	0,30	10,8	8,4	0,2
3,5	0,88	0,98	0,10	10,1	8,1	0,25
4,5	0,83	0,95	0,03	6,6	4,65	0,0
5,5	0,75	0,86	0,0	4,5	4,0	0,0
6,5	0,61	0,59	0,0	2,7	1,25	0,0
7,5	0,39	0,21	0,0	0,5	0,0	0,0
8,5	0,15	0,05	0,0	0,1	0,0	0,0
9,5	0,03	0,05	0,0	0,0	0,0	0,0
10,5	0,0	0,03	0,0	0,0	0,0	0,0

RESULTS

Adult survivorship and oviposition

The survivorship of adult S. oryzae on the susceptible cultivar (Ex-Mk), the resistant (212) and the mixture of both is summarized in Table 1. Adult survivorship (lx) was the mean of the values at the beginning and at the end of the age interval and is expressed on the basis of 1.00 (Evans, 1982). The adult survivorship values were assumed to occur at mid-point of the age interval (Evans, 1982).

Adult survivorship on the mixture treatment was just as good as on the susceptible. Sitophilus oryzea lived for 10 and 11 weeks respectively. The presence of a resistant cultivar in the mixture did not in any way affect the survival of the adult S. oryzae. However, S. oryzae adults survived for only 5 weeks on the resistant cultivar (212). This is not unexpected.

TABLE 2 : Mean cumulative number of eggs per initial female S. oryzae during its reproductive period on the susceptible cultivar (Ex-Mk), resistant 212 and an equal mixture of Ex-Mk and 212.

Cultivar	Cumulative number of eggs ± S. E.
Ex-Mk (susceptible)	50,9 ± 5,96 a
Mixture*	37,7 ± 7,90 a
212 (resistant)	2,70 ± 0,88 b

Different superscripts indicate significant differences ($p < 0,05$) (LSD).

*Mixture composition : Ex-Mk 30,2 ± 7,51
212 7,50 ± 0,76
$t = 3,002$ - $0,02 < p < 0,05$

The mean cumulative numbers of eggs laid per initial S. oryzae female are shown in Table 2 for the different cultivar treatment. Overall 26 % fewer eggs were laid on the mixture as compared to the susceptible control treatment. The difference though not statistically significant (Table 2) was attributable to non-preference for the resistant seeds in the mixture. It is note worthy, however, that consistently fewer eggs were deposited weekly in the mixture (Table 1). As expected, significantly fewer eggs were laid on the resistant 212.

The mean cumulative numbers of eggs laid on the mixture treatment namely 37.7 eggs made up of 30.2 eggs (80 %) on the susceptible (Ex-Mk) and 7.5 eggs (20 %) on the resistant (212). The differences were significant (Table 2).

The ratio of eggs laid on the pure susceptible (Ex-Mk) treatment and the pure resistant (212) was 1.00 : 0.053 compared with 1.00 ; 0.25 when weevils were given a choice.

Feeding holes

The mean numbers of feeding holes per paired female for the week under investigation (see Materials and Methods) are summarized in Table 3. Significantly there were more feeding holes on the susceptible cultivar or on the mixture treatment than on the resistant cultivar ($F = 46.5$, $p < 0.001$). The numbers of feeding holes were not significantly different on both the susceptible and the mixture treatment. However, further analysis of the mixture treatment showed significantly more feeding holes on the susceptible cultivar than on the resistant, as might be expected.

TABLE 3 : Mean numbers of feeding holes and eggs plugs using cultivars Ex-Mk (susceptible), cultivar 212 (resistant) and an equal mixture of Ex-Mk and 212.

Cultivar	Number of feeding holes per paired female per week ± S. E.
Ex-Mk (susceptible)	10,7 ± 0,60 a
Mixture*	9,25 ± 0,65 a
212 (resistant)	3,30 ± 0,45 b

Different superscripts are significantly different - ($p<0{,}05$) (LSD)

*Mixture composition : Ex-Mk 6,80 ± 0,77
212 2,45 ± 0,21
$t = 4{,}60$ - $0{,}002 > p < 0{,}01$

Feeding and Oviposition

When a female S. oryzae comes into contact with a seed, it is capable of making one of the following decisions :

- (a) fedd only.
- (b) feed and oviposit.
- (c) oviposit only.
- (d) leave the seed untilized.

The percentage composition of seeds utilized for any of the above activities are shown in Table 4. The association between feeding ond oviposition was tested using chi-squared test. The χ^2 values were 47.7, 32.6 and 30.7 for the susceptible cultivar Ex-Mk, the resistant 212 and there mixture of both respectively. These differences were highly significant suggesting that feeding and egg laying on a seed are highly associated.

TABLE 4 : The percentage composition of seeds used for feeding, oviposition only, oviposition and feeding and seeds untilized for any activity by paired S. oryzae on the susceptible cultivar Ex-Mk (susceptible), 212 (resistant) and a mixture of Ex-Mk and 212.

Cultivar	seeds unutilized	seeds used for feeding only	seeds used for oviposition and feeding	seeds used for oviposition
Ex-Mk (susceptible)	62,5	21,25	11,25	5,0
Mixture	65	21,25	6,25	7,5
212 (resistant)	83,75	12,5	2,5	1,25

Increase in equilibrium moisture content and oviposition

the mean percentage moisture contents were similar on the resistant and on the susceptible seeds at a given R.H. However, the numbers of eggs laid on the resistant seeds were 8 times greater on seeds conditioned at 95% air relative humidity than at 70%. Similarly the numbers of eggs on the susceptible seeds were 2,4 times greater (Table 5).

DISCUSSION

The results on adult survival showed that adult S. Oryzae died quicker on the resistant seeds than on the susceptible suggesting differential levels of antibiosis were responsible for the differential adult survival. This is not unexpected (see Adetunji, 1988). However, in the choice treatment adult survival was just as good on the mixture as on the susceptible control (Table 1). Adult survival on the mixture treatment was unaffected no doubt because S. Oryzae was able to feed more on the susceptible cultivar (Table 3).

It was shown that in the presence of the susceptible cultivar (mixture treatment) S. Oryzae fed more,laid more eggs and survived longer than it would normally do on resistant seeds given a no choice situation (Table 1 and 2). These are behavioural responses of S. Oryzae indicative of non-preference and showing that preference for a seed cultivar can be modified.

Twenty-six percent fewer eggs were laid on the mixture compared with the susceptible control treatment (Table 2). Sitophilus Oryzae was unable to lay its full compliment of eggs on the mixture because the seeds of the resistant cultivar probably had some direct repellent effect even though in part S. Oryzae was "fooled" or confused by the presence of the susceptible. It could also be partly the result of the high degree of association between feeding and oviposition, so feeding on the susceptible stimulated oviposition on the resistant which was mixed with it. If in the presence of the susceptible cultivar, S. Oryzae fed more on the resistant seeds than it would normally do and since feeding and oviposition are highly associated, it is not surprising that in the presence of a susceptible cultivar more eggs were laid on the resistant seeds in the mixture than on the unmixed resistant seeds.

TABLE 5 : The Mean numbers of eggs per 10 paired S. oryzae per 24 hours at 70 and 95 % r.h. resulting from the susceptible cultivar Ex-Mk and the resistant 303, and their *equilibrium moisture contents.*

cultivar	Number of eggs		Equilibrium moisture content	
	70 % rh	95	70 % rh	95
303 (resistant)	2,0	16,0	11,98	15,26
Ex-Mk (susceptible)	11,5	28,0	12,04	15,29

The experiment on increase in equilibrium moisture content (Table 5) demonstrates that moisture content can also modify the expression of resistance of "resistant" and susceptible cultivars of stored sorghum. Sitophilus Oryzae's preference increased by x8 and by x2,4 for the resistant and susceptible seeds respectively. Reduced moisture content of resistant seeds would favour resistance and its increase will reduce the level of non-preference and antibiosis in resistant seeds. Rogers and Mills (1974) found that at 43% air relative humidity the resistant variety of sorghum caused 84% Sitophilus Zeamaïs.
Motschulsky to die as a result of antibiosis after 5 days oviposition period and that death of weevils did not occur at any other combination of variety and R.H. Birth (1945), Evans (1982) and Shazali and Smith (1985) have all shown that the moisture content of seed does have effect on egg laying and immature survival of S. Oryzae.

The work reported here demonstrate that non-preference (a behavioural response) and antibiosis (Painter 1951; Adetunji,1988) are operating together on stored sorghum cultivars and their expressions can be modified under certain conditions, although it becomes difficult to separate them at times. For example, part of the apparent non-preference for oviposition must be due to reduced feeding by adults on resistant seed which is therefore a secondary outcome of an antibiosis effect on the adult female, at any rate when only resistant seed is available.

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ETUDE DES REACTIONS COMPORTEMENTALES DE *SITOPHILUS ORYZAE* (L.) EN CONDITIONS MODIFIANT L'EXPRESSION DE LA RESISTANCE DES STOCKS DE SORGHO

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RESUME

Nous avons étudié les réponses comportementales de *Sitophilus oryzae* (L.) dans les conditions modifiant l'expression de la résistance. Les paramètres étudiés sont : la survie des adultes, l'alimentation, la ponte et l'augmentation du degré d'humidité dans les graines de sorgho résistantes et dans les graines de sorgho sensibles. Chez *Sitophilus oryzae* soumis à une alimentation mixte, les résultats ont montré un accroissement sensible de l'alimentation, de la ponte et de la durée de vie, augmentation supérieure à ce qui serait attendu d'une alimentation sur graines résistantes seules. L'augmentation du degré d'humidité des graines, le degré hygrométrique de l'air allant de 75 à 95 % (RH), a accru la ponte d'un facteur 8 sur cultures résistantes à 2,4 sur cultures sensibles, respectivement. L'examen des résultats tient compte des mécanismes d'antibiose et d'absence de choix dans le sorgho stocké.