Screening for Resistance against the Maize Weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) in Peruvian Maize Accessions[1]

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ABSTRACT

Maize (*Zea mays* L.) accessions (114) from the Peruvian maize germplasm bank were exposed to laboratory infestations of the maize weevil *Sitophilus zeamais* Motsch. Susceptibility, as assessed by the Dobie Index, ranged from 0.0 to 9.90 in the freechoice test and from 6.35 to 11.30 in the no-choice test. Floury endosperm types were significantly more susceptible than flint endosperm types in both tests. Weevils emerging from flint accessions had a longer developmental period (39.9 days) and were heavier (1.54 mg) than those emerging from floury accessions (38.1 days, 1.48 mg, respectively). Maize cultivated in the lowland tropical rain forest was significantly more resistant than maize cultivated in the inter-Andean valleys. Maize from the lowland coastal region reacted more like highland types. The Rabo de Zorro race was the most susceptible, followed by Ancashino and Huayleno. The race Huancavelicano and undetermined flints were the most resistant.

COMPENDIO

Más de un centenar de accesiones de maíz (*Zea mays* L.), obtenidas del Banco de Germoplasma de la Universidad Nacional Agraria La Molina, Lima, Perú, fueron expúestas a infestaciones del gorgojo del maíz *Sitophilus zeamais* Motsch. La susceptibilidad fue evaluada por medio del Indice Dobie y fluctuó de 0.00 a 9.90, en un ensayo de escogencia libre y de 6.35 a 11.30, en un ensayo de no-escogencia. Los tipos de maíz de endospermo harinoso fueron significativamente más susceptibles que los tipos de endospermo duro, en ambos ensayos. Los gorgojos que emergieron de accesiones de endospermo duro tuvieron un período de desarrollo más prolongado (39.9 días) y fueron más pesados (1.54 mg) que aquéllos que emergieron de las accesiones de endospermo harinoso (38.1 días y 1.48 mg, respectivamente). Los tipos de maíz de endospermo harinoso fueron significativamente más tivamente más resistentes que los obtenidos de los valles interandinos. Maíces obtenidos de la región costera reaccionaron en forma similar a aquéllos de los valles interandinos. La raza denominada Rabo de Zorro fue la más susceptible, seguida, por las razas Ancashino y Huayleño. La raza Huancavelicano y los maíces duros no determinados de la región de la Selva, fueron los más resistentes.

INTRODUCTION

When screening a large number of plant accessions, a preliminary free- or no-choice test is advisable to pinpoint those accessions obviously susceptible or resistant. This reduction of the original material to a manageable number for further study is practical in field breeding programs (11). Earlier scientists tended to use large amounts of grain in screening tests involving only a few varieties (10, 26). This was not feasible for material requested from germplasm banks, which normally supply a limited number of seeds per accession. Therefore, techniques involving fewer kernels were developed (2, 17, 23).

The number of maize weevil progeny was a better parameter for rating resistance in corn varieties than percent damaged kernels, percent weevil mortality, progeny weight, or kernel weight loss (27). Other investigators confirmed that mortality of the maize weevil was an unreliable parameter in rating maize resistance (9). The latter authors indicated that 40 kernels per sample was adequate, and that a minimum three-week oviposition period should be considered

Stevens and Mills (20) compared the attributes of free- and no-choice screening tests and concluded that both could be used sequentially. The first test should be free-choice, in which there is no need to sex the weevils, followed by the no-choice test, in which sexed weevils are used, but stronger selection pressure is exerted, so that escapes from the choice test can be detected. Several laboratory screenings for resistance against stored-grain pests have used these or slightly modified techniques in rice (1), in maize (14, 16), and in sorghum (15, 22).

Resistance of grains to insects involves two components: non-preference for oviposition or feeding, and antibiosis, as reflected by an adverse effect on the

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biology of the insect. Tolerance is unlikely because grains do not have the ability to compensate for damage done by insects (5, 22). The two screening techniques mentioned above have proven effective in discriminating between susceptible or resistant genotypes because both permit evaluations of non-preference and antibiosis.

When laboratory screenings are considered as part of a population improvement program, their effectiveness as evaluating tools becomes apparent. In breeding programs, advance is measured by genetic gain per cycle of selection. The least time spent, the higher the genetic progress, provided the best breeding plan was chosen.

With this in mind, a preliminary screening for resistance of Peruvian maize (*Zea mays* L.) accessions against the maize weevil (*Sitophilus zeamais* Motsch.) was undertaken.

MATERIALS AND METHODS

The Peruvian maize germplasm bank of the Universidad Nacional Agraria La Molina, at Lima, provided 114 accessions of maize from three regions within Peru.

a. Free-choice Test

Forty kernels per accession, as well as the hybrid maize used to rear the maize weevil at this laboratory (susceptible check), were placed in the bottoms of 48 x 48 x 5 cm plastic boxes and equilibrated in an environmental room at 27 +/−1°C, 67 +/− 3% RH, and a 16:8 light: dark photoperiod for 14 days. The moisture content of the samples was not determined, but it was expected to be 12.5 to 13.5%, the normal rante of moisture equilibration at this temperature and relative humidity (19).

Unsexed weevils (1-3 weeks old) were used to infest 17 samples (10 weevils/sample). The samples were randomly distributed around the interior perimeter of a wooden, circular chamber (45 cm diameter and 5 cm height) (28). To accommodate all accessions, seven chambers were used, and to facilitate contact of weevils with the kernels, the weevils were given a paper ramp extending from the chamber floor to the rim of each plastic box. The weevils were released in the center of the chamber through an orifice in the center of the lid, which was then closed with a rubber stopper.

Strips of masking tape 3 cm wide were used to seal each chamber to prevent weevils from escaping. The chambers were kept in the environmental room, and opened after seven days. The plastic boxes were immediately closed to trap the seven weevils in each sample. Few weevils were observed wandering on the floor and wall of the chambers and mortality was negligible (< 1%). After the weevils were counted and removed, the grain samples were incubated in the environmental room for progeny development. Starting on the 27th day after infestation, the boxes were checked daily for weevil progeny until no more emerged (day 67). Progenies from each sample were placed in small glass vials and stored in a freezer at −20°C. Dry weight was determined by placing the weevils in aluminium tins (15 mm diameter and 10 mm height) which were held in an oven for six days at 60°C (18). At the end of this period, the tins were placed in a desiccator with calcium sulfate (DrieriteR) for 24 hours. The weevils were weighed on a Mettler Electrobalance Model H16 and returned to the dessicator. A second weighing 24 hours later agreed with the first. Therefore, all subsequent dry weight determinations were performed after one 24-hour period in the desiccator.

The parameters evaluated in this test were: a) numbers of parent weevils attracted to each sample; b) developmental period; c) numbers of progeny emerged; and d) dry weight. The rating scale was based on the susceptibility index (SI) published by Dobie (3).

b. No-choice Test

Accessions were selected according to the value calculated for the susceptibility index as observed in the free-choice test and other criteria indicated above. Of the 35 chosen, 20 were regarded as susceptible (9.90-8.33 SI) and 15 were regarded as resistant (5.33-0.0 SI). The definition of the limits of resistance and susceptibility was arbitrary, but intended to eliminate the majority of intermediate ranking accessions.

For each accession, 120 kernels were divided into three subsamples of 40 in order to conform to a three-replicate experiment in a completely randomized design. Each subsample was placed in a 48 x 48 x 5 mm plastic box, covered with a screened lid, and kept in the environmental room for moisture equilibration under the same conditions observed for the free-choice test.

Infestation was accomplished by putting six females and three males, 1-3 weeks old, into each sample for seven days, then removing them with an aspirator. The boxes were returned to the environmental room for progeny development and the removed weevils were discarded.

Weevils were sexed by snout characteristics (12) under a stereo microscope. To facilitate snout observation, the weevils were held from the dorsum with a Schuco-Vac[R] aspirator.

Data obtained from this test were subjected to analysis of variance in order to determine accession differences based on the index of susceptibility, days to first and last emergence, and dry weight. Duncan's Multiple Range Test (DMRT) was used to test for statistical differences between individual means at the 0.05 probability level.

RESULTS AND DISCUSSION

Free-choice test

A comparison of the values of the susceptibility index among all accessions suggested that floury accessions were more susceptible than flinty ones (Table 1). To test this, the 59 floury accessions were arranged into 10 random groups of 17 each to compare to the 17 flint accessions used. As suspected, floury samples were significantly more susceptible than flints ($P < 0.01$) (Table 2). This indicated that the flinty character was associated with resistance and the floury character with susceptibility. Comparable results were reported by Dobie (3) in which flint varieties from Malawi and Colombia were more resistant to the maize weevil than medium or dent varieties from Rhodesia and Tanzania.

On the average, the weights of weevils that emerged from flint accessions were significantly higher ($P < 0.01$) than of those of weevils that emerged from floury accessions (1.54 vs 1.42 mg). However, some floury accessions also produced heavier weevils (e.g., CUZ-354, 1.97 mg; ANC-049, 1.76 mg; LIM-019, 1.70 mg). When the floury accessions were rearranged into smaller random groups of 17 each, this level of difference remained only in one random group (group 1). Although these 10 groups were a small and arbitrary fraction of all random groups of 17 that could be formed with 59 floury accessions, they did show how the contribution of individual accessions affects the overall pattern of susceptibility.

With regard to dry weight, it has been indicated that it could be the most important parameter for rating resistance when an antibiotic factor is suspected (16). In this investigation, the dry weight of the weevils was more affected by the flinty character

Table 1. Results of a free-choice test for susceptibility of Peruvian maize accessions to the maize weevil, *S. zeamais*[1, 2]

Accession code	Parent weevils/sample	Progeny/sample	Avg. develop. period (days)	SI[3]	Avg. DW/adult (mg)[3]
JUN-159	33	39	37	9.90	1.36
TAC-042	19	29	35	9.62	1.39
LIM-104	34	33	37	9.45	1.39
CONTROL[4]	24	36	39	9.13	1.53
AREQ-077	33	26	36	9.05	1.47
AREQ-074	24	51	44	8.94	1.31
LIB-202	9	27	39	8.45	1.59
ANC-522	8	14	38	6.94	1.44
CUZ-370	9	12	37	6.72	1.28
SMTI-061	6	12	40	6.21	1.68
HVCA-175	7	8	37	5.62	1.55
ANC-585	2	6	36	4.98	1.40
SMTI-057	7	8	42	4.95	1.73
CAJ-208	7	6	40	4.48	1.43
JUN-162	4	5	41	3.93	1.10
LBQU-141	3	4	37	3.75	1.58
SMTI-058	3	4	38	3.65	1.33

1 Test conducted with no replication.

2 The accessions included in this table represent a selected sample of those tested (114). The full table can be seen in (21)

3 SI = Susceptibility Index, DW = Dry Weight.

4 Locally grown hybrid maize

Table 2. Comparison of flint and floury characters in Peruvian maize accessions with regard to their susceptibility to and their influence on the developmental period and dry weight of the maize weevil[1].

Types of accessions	SI[2]	Avg. Dry Wt/adult (mg)	Avg. Develop. period (days)
Avg. flint	4 74	1.54	39 18
Avg. floury[3]			
A	7 58 **	1.39 **	38 88 ns
B	6 86 **	1 47 ns	38.17 ns
C	6.87 **	1 40 *	38 88 ns
D	7.17 **	1 40 *	37.41 *
E	6.16 *	1.47 ns	38.53 ns
F	6 84 **	1 44 ns	38.35 ns
G	6 99 **	1.43 ns	37 53 *
H	7 05 **	1 43 ns	37 70 ns
I	6 38 *	1.41 *	38 18 ns
J	7 44 **	1 44 ns	38 35 ns
ALL	6 70 **	1.42 **	38 10 ns

1 Means followed by * or ** are significantly different from the flints at the 0.05 and 0.01 percent probability t-test respectively.

2 SI = Susceptibility Index

3 Random groups of 17 accessions each.

than the developmental period from oviposition to emergence. Significant differences ($P < 0.05$) were observed only in two of ten comparisons (flint vs. random floury groups four and seven) It has been reported (14, 16) that, on susceptible maize lines, maize weevil progeny emerged on the average four days earlier and weighed slightly less than in resistant lines. These observations agree with those recorded here.

The fact that the accessions were grown in different geographic and altitudinal regions of Peru prompted an analysis of variance to compare the mean value of the susceptibility index by region (Table 3). Three altitudinal regions were considered: low altitude-Costa (010-300 m), low altitude-Selva (314-1 000 m), and high altitude-Sierra (1 400-3 450 m). There were significant differences ($P < 0.05$) in susceptibility to the maize weevil by region. The mean value for the susceptibility index was significantly higher for those accessions growing in the high altitude-Sierra than for those in the low altitude-Selva. Similar observations were reported by Diaz (2), Fortier *et al* (6), and Van der Schaaf *et al* (23). Accessions obtained from the low altitude-Costa occupied an intermediate position between the other two.

The analysis of variance showed a high degree of variability, of which 22 percent was explained by the ANOVA model. This indicates that the variability within each group, although high, was not enough to override the individuality of the altitudinal groups. The variability within groups was more likely to be due to different racial origins of the accessions (Table 4).

It has been indicated by Wellhausen *et al.* (24) that factors of resistance to insects were likely in the lowland races because insect pressure is higher there. The moderately high temperatures and relative humidities of this region are regarded as the optimum conditions for development and reproduction of the maize

Table 3. Susceptibility of Peruvian maize accessions to the maize weevil, according to altitudinal regions. Means of 13 accessions per region.

Region	Susceptibility Index[1]
High altitude-Sierra (1 400-3 450 m[2]	7.71 a
Low altitude-Costa (010-300 m)	6.58 ab
Low altitude-Selva (314-1 000 m)	5 35 bc

C.V. = 27.38

1 Means followed by the same letter are not significantly different ($P < 0.05$), DMRT.

2 Meters above sea level.

Table 4. Racial differences in susceptibility index of Peruvian maize accessions against the maize weevil *S. zeamais* and their effect on weight gain.[1, 2]

Races	Susceptibility Index	Dry weight (mg) of adults
Rabo de Zorro	8 95 a	1 51 ab
Ancashino	7.48 ab	1.43 ab
Huayleno	6.96 abc	1.45 ab
San Jeronimo	6.15 bcd	1.40 ab
Cuzco Cristalino	5.91 bcde	1.35 b
Paro	5 87 bcde	1.33 b
Undetermined flints[3]	4 74 cde	1.54 a
Huancavelicano	3.41 e	1 48 ab

1 Data collected under a free-choice test.

2 Means followed by a common letter are not significantly different ($P < 0.05$), according to Fisher Projected Least Significant Differences.

3 Includes accessions collected in the Selva region.

weevil. In connection to this, Fortier *et al.* (6) stated that local maize populations play an important role in present day, small-farm agriculture. Some of them, especially flint types, afford the best protection against primary insect pests.

It is interesting to notice that the accession JUN-159 derived from the race Cuzco Gigante was the most susceptible (SI 9.90 in free-choice and 10.28 in no-choice tests). According to Grobman *et al.* (7), the development of this race parallelled that of the Andean Inca Empire, suggesting that this maize formed the basis of the diet for ancient people of the Cuzco region. There is no reason to believe that this maize was resistant to weevil attack then. It is more likely that damage by weevils or other stored-product insects was prevented by low temperature. Low temperatures occurring at high altitudes in Mexico were observed by Wilbur *et al.* (25) to have a depressing effect on rice and granary weevil productivity and development.

Table 5. Analysis of variance of susceptibility index (SI), days to first emergence (DFE), days to last emergence (DLE) and dry weight (DW) of newly emerged adult maize weevil *S. zeamais* in a no-choice test.

Source	d.f.	SS	MS	F	Pr > F
SI					
Accessions	34	163 98	4.82	9 70	0.001
Error	69		34.30	0.50	
Total	103		198 27		
DFE					
Accessions	34	197 58	5.81	1.76	0.023
Error	70		230.66	5.30	
Total	104		428 25		
DLE					
Accessions	34	443.43	42.25	2.64	0.0003
Error		70	124 00	16.06	
Total		104	567 43		
DW					
Accessions	34	1 180	0.034	3 12	0.0001
Error		70	0 778	0 011	
Total		104	1.958		

C.V. (SI) = 7.35
C.V. (DFE) = 5.92
C.V. (DLE) = 8.66
C.V. (DW) = 7.48

The appearance of the semicrystalline or semiflint character appears to have afforded some degree of resistance to the maize weevil in some races (Confite Punteagudo, Paro, Cuzco Cristalino), but not in others (Rabo de Zorro)

The ecological significance of these life history strategies can only be understood by considering the association of maize and the maize weevil as a long coevolution, in which the diverse genetic backgrounds were tested against each other Lowland farmers undoubtedly preferred flint types, whereas highland farmers selected for floury types (7) In both cases, insect attack was diminished either by resistant traits of ears and kernels or by low temperature and high altitude, respectively. Compatibility and agressiveness of the maize weevil were forced to develop under conditions that modified the expression of resistance of these genotypes, for example, high moisture during storage for flint types, and removal of floury types from the highlands to the coastal or lowland tropical areas during trade

No-choice test

The no-choice test is a more sensitive screening procedure than the free-choice test. Teetes *et al* (22), in their search for resistance against *S. zeamais* among converted exotic sorghum varieties, found that the F values for variety differences were much higher in the no-choice test than in the free-choice test Effectiveness increased because the test insects were confined to a certain number of kernels of each variety for a number of days. The females must oviposit on the kernels in order for the population to survive Because of the higher "selective pressure" exerted by the weevils, this test is useful to detect varieties that may have "escaped" infestation in the free-choice test (20)

The susceptibility indices obtained in this test were significantly larger ($P > t = 0.0001$) than those in the free-choice test. This confirms the higher selection pressure exerted by the weevils in the no-choice test

An analysis of variance of susceptibility indices gave significant differences among accessions (Table 5) In general, there was agreement with the results obtained in the free-choice test. Most accessions that were susceptible in the free-choice test were also susceptible in the no-choice test Resistant accessions also performed consistently.

The degree of association between the number of parent weevils attracted to each sample in the free-choice test with the number of F_1 progeny was not determined because of no replications in the free-choice test. In corresponding correlation analysis of the same variables, significant correlation coefficients were found by McCain *et al.* (10) and Stevens and

Table 6. Duncan's Multiple Range Tests for susceptibility index (SI), days to first emergence (DFE), days to last emergence, and dry weight (DW) of newly emerged adults of the maize weevil *S. zeamais* in a no-choice test.[1]

Accession Code	Means[2]			
	SIE	DFE	DLE	DW
AREQ-077	11.30 a	29.33 bd	43.33 dg	1.38 di
APUC-242	11.07 ab	29.00 cd	44.33 bg	1.43 ci
TAC-042	10.96 ab	29.33 bd	44.68 bg	1.31 hi
JUN-159	10.55 ad	29.67 bd	41.33 fg	1.23 i
ANC-585	10.64 ad	29.00 cd	46.00 bg	1.34 fi
CUZ-292	10.41 ae	30.67 bd	55.68 a	1.46 bh
CONTROL	10.06 ae	30.33 bd	49.00 af	1.65 ab
UCAY-032	9.97 af	29.33 bd	45.68 bg	1.39 di
LBQU-141	8.75 fj	30.00 bd	52.33 ab	1.33 gi
ANC-212	8.60 gj	31.33 ad	42.33 fg	1.31 hi
LIB-242	8.40 hj	31.67 ad	47.33 bf	1.67 a
CAJ-208	7.53 jk	32.67ab	45.33 bg	1.45 bh
SMII-031	7.43 jk	32.33 ac	49.00 af	1.58 ad
SMTI-057	6.35 k	32.33 ac	51.33 ad	1.55 af

1 Results presented in this table are selected examples of the larger experiment Full results are included in (21).

2 Means followed by a common letter are not significantly different at 0.05, DMRT.

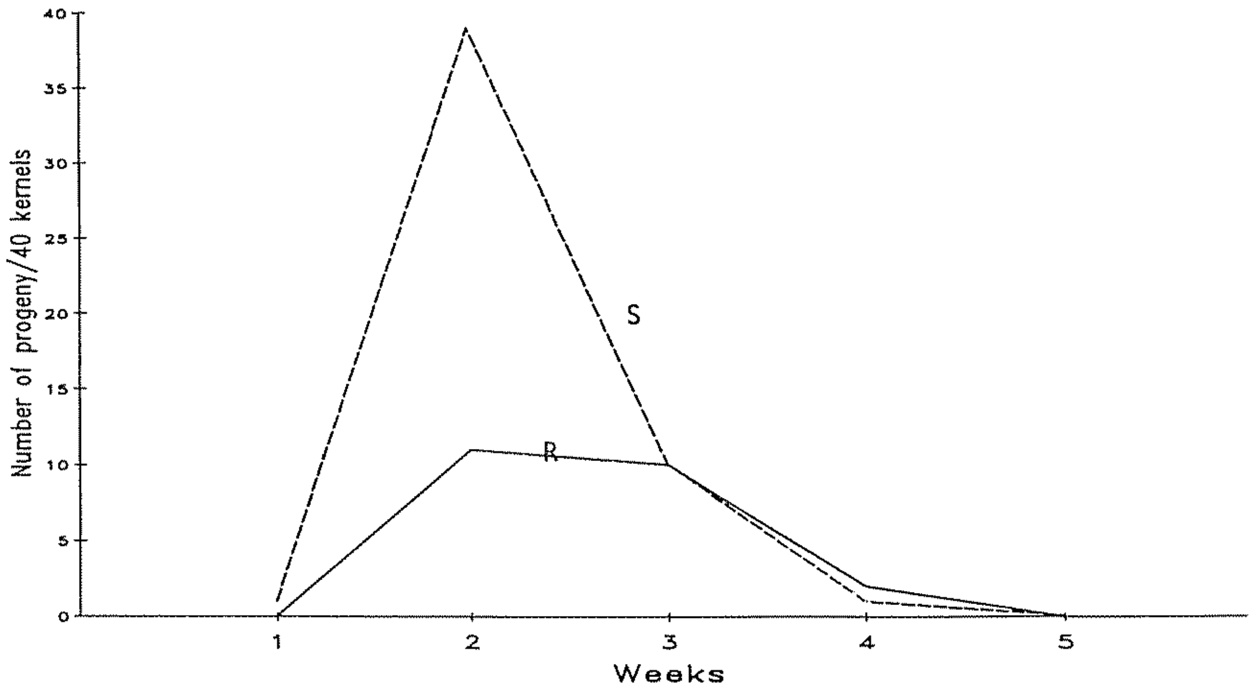

Fig 1. Emergence pattern of *Sitophilus zeamais* Motsch on resistant (R) and susceptible (S) maize.

Mills (20), meaning that both tests were effective in discriminating maize cultivars according to their susceptibility to the rice weevil. However, little correlation was found by Teetes *et al.* (22) between screening tests with sorghum varieties against the maize weevil.

The mean separation of accessions by susceptibility index (Table 6) showed a wide array of groupings, indicating that diverse genetic backgrounds were responsible for susceptibility or resistance. The majority of the floury accessions grouped as the susceptible "a," which also contained the susceptible check. The most resistant group "k" was composed of flint accessions. The SI increased as the F, progeny increased and the developmental period decreased.

In Figs. 1 and 2, the cumulative and non-cumulative frequency distribution of F_1 progeny emergence were grouped by weekly count. The weekly emergence in the floury-susceptible (JUN- 159) peaked at the second week. In the flint-resistant (SM-058), the peak was spread between weeks two and three. These figures are comparable to those of the granary weevil reported by Howe and Hole (8) and the maize weevil by Dobie (4).

Since the full developmental period was excluded from the analysis of SI, which takes into account only the initial 50 percent emergence, in order to study developmental variability in the population, further analyses of variance of days elapsed to first (DFE) and to last (DLE) were performed. These analyses showed significant differences. According to DMRT for DFE, three groups were formed. Floury accessions induced some weevils to emerge earlier than did flint accessions. In this connection, developmental period in the granary weevil has been found to be a genetically controlled trait, but influenced by environmental factors (13). These results suggested that genetic differences among F_1 offspring were narrow for DFE; as a result, early-developing individuals emerged within a short minimum time period. It appears, therefore, that despite the relative lack of genetic plasticity in the weevils, the kernel characteristics of the different maize accessions seem to have forced the genetic or physiological factors controlling early emergence to be expressed differently.

By contrast, the groupings of days to last emergence was wider and more diverse. In general, flint accessions induced some individuals to delay emergence. In this case, the variability among late emerging individuals was more evident than for DFE.

Of significant value to modern breeding programs would be a phenotype that decreases progeny number, increases developmental period, and decreases the weight of the weevils. As indicated earlier, floury accessions yielded more progeny, a shorter developmental period, and smaller insects, whereas flint

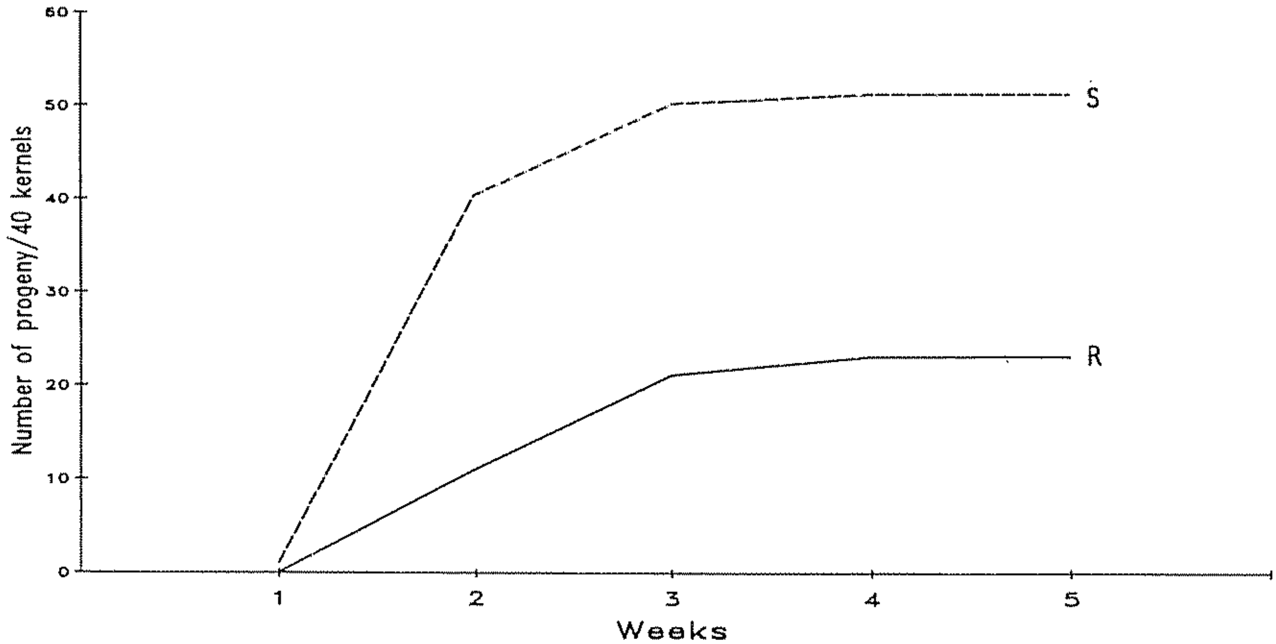

Fig. 2. Cumulative frequency distribution of emergence of *Sitophilus zeamais* Motsch. in resistant (R) and susceptible (S) maize.

accessions yielded fewer progeny, a longer developmental period, and larger weevils We found that the accessions in the no-choice test included some which constituted exceptions to the general trend, that is, susceptibles which prolonged the developmental period (CUZ-292, 55.68 days), and resistants which decreased weight (LBQU-141, 1.33 mg) (Table 6).

Decreased weight and prolonged developmental period of the weevils are factors that favor the permanence of resistance in maize genotypes (3), Thus, it would be desirable to make crosses between resistant and susceptible accessions and select those progenies that segregate jointly for these two characters

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