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REACTION OF TWENTY MAIZE CULTIVARS TO *Sesamia cretica* AND EFFECTS ON THE INSECT REPRODUCTION

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ABSTRACT

The objectives of the present study were: (1) to evaluate 20 Egyptian maize cultivars including the Bt corn (SC Ageeb) for resistance to the pink stem borer S. cretica under natural and artificial infestation in two planting dates and (2) to study the effect of rearing this insect on maize plants of different resistance to S. cretica. Two field and one laboratory experiments were conducted during 2005, 2006 and 2007 seasons. Results of the field experiment under natural infestation recommended the use of the 1st planting date (20th of April) for more accurate evaluation, because of the more existence of this insect in large populations shortly after mergence of plants at this time as compared with the 2nd planting date (15 May). Results from the 1st planting date of this experiment indicated that the three cultivars SC Ageeb (Bt corn), TWC 351 and TWC 352 were considered resistant to S. cretica by using percentage of avoidance (AV) and leaf feeding plants (LF), however, percentage of dead hearts (DH_n) considered only SC Ageeb and TWC 351 as resistant. Result of the 2nd field experiment under artificial infestation assured the resistance of the Bt cultivar SC Ageeb by using the three studied resistance traits; susceptible plants % (SP), dead hearts % (DH) and intensity of damage % (ID), the cultivar TWC 351 by using two traits (DH and ID) and the cultivar TWC 352 by using only one trait (DH). Chlorophyll content in maize leaves was significantly correlated with dead hearts (0.581**); leaf feeding (0.517*) and avoidance (- 0.586**); leaf angle showed also significant and positive correlation (0.518*) with dead hearts. Chlorophyll content and leaf angle could therefore be used as selection criteria in maize for S. cretica resistance. Laboratory experiment indicated that artificial rearing of S. cretica on plants of the most resistant (genetically modified) maize cultivar (SC Ageeb) resulted in 100 % larval mortality (LM), and rearing on the 2nd resistant cultivar (TWC 351) caused the 2nd highest percentage of LM (80 %), highest deformed pupa (DP) (10.37 %), the shortest pupal duration(PD) (8.6 days), less fecundity (Fec) (92.6 eggs / female), less hatchability (Hat) (56.9 %) and a significant change in sex ratio of pupa. On the contrary, rearing S. cretica on the susceptible cultivar TWC 321 resulted in the lowest LM (32.2 %), the longest larval duration (44.7 days), the 2nd longest PD (10.2 days), the lowest DP (3.33 %), the highest Fec (241.2 eggs / female) and the highest Hat (94.4 %). Such differences were attributed to preference of susceptible plants (TWC 321) and non-preference of resistant plants (SC Ageeb and TWC 351) to S. cretica.

Key words: ***Maize, Resistance, Sesamia cretica, Bt corn, Infestation, Dead hearts, Fecundity, Larval mortality, Hatchability*.**

INTRODUCTION

The pink stem borer *Sesamia cretica* is a serious corn borer in Egypt that attacks young maize plants shortly after emergence, devours the whorl leaves and may kill the growing points, causing dead hearts. It is also capable of damaging older plants and excavating tunnels into the stems, ears and/or cobs (Soliman 1994).

One of the most important methods for controlling insect pests in the context of integrated pest control is to grow insect-resistant cultivars (Pathak 1991), which neither increase cost nor cause environmental pollution. In addition, the efficiency of this method is compatible with other chemical and biological control methods (Scott *et al* 1977). Mihm (1985) stated that the components necessary for implementing a breeding program for resistance to stem borers in maize include a colony of the pest, an array of the available maize germplasm, rating scales and evaluation methods that permit identification of genetic resistance and knowledge of screening and breeding methods. Considerable efforts have been devoted to identifying and developing maize germplasm with resistance to damage by the pink stem borer *S. cretica* (Al-Naggar *et al* 2000b, Saafan 2003 and El-Kheshin 2006).

Several researches were carried out to study genotypic differences in maize resistance to the pink stem borer, some of them were conducted in Egypt either under natural (El-Sherif *et al* 1986) or artificial infestation (Al-Naggar *et al* 2000b, Saafan 2003 and El-Kheshin 2006). Some sources of resistance were identified.

The Bt (genetically modified, GM) corn introduced in the last decade into the world agriculture contributed markedly to insect resistance of maize. Of the 148 million hectares of maize planted on a global basis in 2007, 24 % (35.2 million hectares) were planted to biotech maize; 20.6 % (29.7 million hectares) were planted to Bt corn (James 2007). The effect of feeding of corn borers on maize plants of different genetic backgrounds with regard of resistance / susceptibility to the insect was studied by many investigators; most of them were working on *Ostrinia nubilalis*. Feeding of *O. nubilalis* on maize resistant plants caused retardation of the development (Everrett *et al* 1958, Reed *et al* 1972 and Guthrie *et al* 1980), higher larval mortality, lower survival (Guthrie *et al* 1960, 1970 and Metwally 1995), less pupae weight (Reed *et al* 1972, Ward *et al* 1975, Eghlidie *et al* 1977, Guthrie *et al* 1980 and Metwally 1995), fewer egg masses, less larval development and duration (Reed *et al* 1972 and Robinson *et al* 1978), less larva number and activity (Reed *et al* 1972 and Barry and Darrah 1991) than feeding on susceptible plants. This was attributed to antibiosis (Guthrie *et al* 1980) and high DIMBOA content (Ward *et al* 1975). Larvae death on

Bt corn was also reported by many investigators on corn insects other than *S. cretica* (Pilcher et al 1997 and Lozzia *et al* 1997). However, for *S. cretica* a few number of reports had been published in this respect; they were mainly performed in Egypt. Laboratorial work on *S. cretica* stages fed on different maize genotypes of different resistance backgrounds revealed differential response in the rate of development and mortality, larval and pupal stage duration, number of egg masses, hatching % and adult longevity period (Ali *et al* 1987 and El-Naggar 1997). Resistant maize plants to *S. cretica* caused retardation of larval development, increase in rate of larval mortality and elongation of pupal stage (Ali *et al* 1987) and decrease in the number of egg masses laid by the female moth, hatched larva and hatching % and elongation of adult longevity (El-Naggar 1997) than the susceptible ones. To the best of our knowledge, no available report in the literature had been published on the effect of feeding *S. cretica* on Bt corn plants.

The main objectives of the present investigation were to (1) evaluate twenty Egyptian maize cultivars for resistance to the pink stem borer *S. cretica* under natural and artificial infestation conditions, (2) study the effect of environmental conditions (planting dates and seasons) on maize reaction to the pink stem borer and (3) determine the effect of feeding on maize plants of different genetic backgrounds with respect of *S. cretica* resistance on the reproduction of this insect.

MATERIALS AND METHODS

The experimental field work of this investigation was carried out at Sakha Agricultural Research Station of the Agricultural Research Center (ARC) and the Agricultural Experiment Station Faculty of Agriculture, Cairo University, Giza during 2005, 2006 and 2007 seasons.

Materials

Twenty genotypes of maize (*Zea mays* L) were used in this study (Table 1). Nineteen of them (nine single cross and ten three-way cross hybrids) were obtained from Maize Research Section , Field Crops Research Institute (FCRI), ARC , Egypt as the most popular commercial local maize cultivars and one single cross (recently registered) cultivar (Ageeb) was provided by Fine Seeds Company, Egypt as insect resistant genetically engineered cultivar (Bt corn) developed by Monsanto Co., USA

Field evaluation experiments

Two field evaluation experiments were carried out. One of them was under natural infestation of the pink stem borer *S. cretica* in t two seasons (2005 and 2006) and the other one was carried out under artificial infestation conditions of the same borer in one season (2007).

Table 1. Designation, pedigree and grain colour of twenty maize genotypes used in this study.

Designation	Pedigree	Grain colour
SC 10	Sd 7 x Sd 63	White
SC 13	Gm 4 x Gm 30	White
SC 14	Sd 7 x Gm 30	White
SC 15	Sd 63 x Gm 30	White
SC 122	Gz 628 x Gz 603	White
SC 123	Gz 628 x Gz 602	White
SC 124	Gz 629 x Gz 603	White
SC 129	Gz 612 x Gz 628	White
SC 155	Gm 1002 x Gm 1021	Yellow
TWC 310	SC 10 x Sd 34	White
TWC 311	SC 21 x Sd 34	White
TWC 314	SC 24 x Sd 34	White
TWC 321	SC 21 x Sd 7	White
TWC 322	SC 22 x Sd 7	White
TWC 324	SC 24 x Sd 7	White
TWC 325	SC 25 x Sd 7	White
TWC 327	SC 27 x Sd 7	White
TWC 351	SC 51 x Gm 1021	Yellow
TWC 352	SC 52 x Gm 1021	Yellow
SC Ageeb	developed by Monsanto Co. ,USA as Bt corn	White

SC = Single cross, TWC = Three-way cross
Sd = Sids, Gm = Gimmeza Gz = Giza

Experiment one (under natural infestation)

The 20 maize genotypes were tested for resistance to *S. cretica* under natural infestation conditions at two seasons (2005 and 2006). Two sowing dates were used each year. The first sowing date (April. 20) was chosen so that the plants reach the growth stage of 30-45 cm in height most preferred by *S. cretica*, when the moth emergence from hypernating larvae reached its maximum (its peak time) to assure maximum natural infestation. The second sowing date (May, 15) was chosen, so that minimum moth emergence from hypernating larvae to be matched with maize plants of 30-45 cm height. The randomized complete blocks design (RCBD) with three replicates was used. The experimental plot was one row of 6 m length and 80 cm width. No pest control was carried out in both years.

Experiment two (under artificial infestation)

In 2007 season, a field experiment was carried out under artificial infestation at the Experimental Farm of Faculty of Agriculture, Cairo

University, Giza., Egypt. Sowing date was May, 20. The experiment included the same 20 maize genotypes. The RCBD with three replications was used. The experimental plot was represented by one row, with a plot size of 4.8 m. All pest control practices were entirely avoided.

Rearing technique

The successful rearing technique of the pink stem borer *S. cretica* on the artificial diet (Diet A) developed by Al-Naggar *et al* (2000a) and used by the Corn Borer Research Lab (CBRL),Maize Research section, ARC, Giza , Egypt was used in this experiment. According to this rearing technique a total of 4800 egg masses of the pink stem borer were obtained from the CBRL laboratory, mixed with wheat "Senn" flour and transferred to big glass gars of one gallon size, such that each jar contained 300-350 egg masses. A mist of water was sprayed inside the jars. Then the egg-masses were gently scattered and adhered on the walls of the jars. The jars were then covered with their lids. These egg-masses were incubated at 27-28°C and 75-80 % relative humidity for 2 - 4 days. The newly hatched larvae were collected from the jars by adding a small amount of corn cob grits (grade $10^{-14)}$ to each jar and mixing them together by gently rotating the jar. The mixture from several hatching jars was accumulated in a large size container, depending on the number of larvae and plants to be infested.

Infestation technique

In the field, ten plants from each plot were artificially infested with newly hatched larvae of the pink stem borer. Each plant received 8 neonate larvae of the pink stem borer which were mixed with maize cob grits and placed into the whorl of the plant with age of 15 days from planting using the Bazooka as mechanical dispenser. Two shots of Bazooka were used on each plant; each shot delivered 4 larvae. It was necessary that the newly hatched larvae should be immediately applied to the plant because the capability of the larvae to attack corn plants could be reduced if kept unused for a period of time.

Experiment three (artificial infestation in the laboratory)

A laboratory experiment was carried out in 2007 season at the Entomology Laboratory of the Dept. of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt. Five maize genotypes (three single crosses, i.e. SC 10, SC 155, and SC Ageeb and two 3- way crosses i.e. TWC 321, TWC 351) chosen to represent different genetic backgrounds with respect of resistance to *S. cretica* were used in this experiment as a source of small plants for feeding (natural rearing) of the insect Newly hatched larvae obtained from CBRL were introduced into rearing vials containing fresh maize plant parts, using a soft brush to place 10 larvae in each vial and kept at a constant temperature of 27°C.The completely randomized design with 10 replicates was used; each vial containing 10 larvae represented one replicate. Young instars larvae of *S. cretica* were supplied with small fresh pieces of tender rolled leaves surrounding the growing point of young maize plants. Older larvae were fed on small tender cuttings of older maize stem. Larvae were transferred to clean vials provided with fresh pieces of maize plants every other day until pupation. The pupae were separated into males and females, then weighed and kept individually in glass tubes with moistened cotton till moth emergence. Each three adult moths (2 males + 1 female) were kept in a glass jar containing three seedlings of the studied maize genotypes (about 20-25cm length) for meeting and oviposition. The glass jar was provided with a piece of cotton socked in 10 % sugar solution as a source of moth food and covered with muslin fixed with a rubber band.

Data recorded

Data were recorded in field under natural infestation (after 30 days from planting date) as avoidance plants (AV %), leaf feeding (LF %), dead hearts (DH_n %), leaf angle (LA) and leaf chlorophyll (Chl). In the field under artificial infestation, data were recorded on susceptible plants (SP %), dead heart (DH_a %) and intensity of damage (ID). In laboratory under artificial infestation data were on fecundity (Fec) as number of eggs laid by each female moth, hatchability (Hat %) as No. of eggs hatched / No. of eggs laid per female) X 100, larval duration (LD) in days measured as the longevity of larval stage from hatching to pupation., larval mortality (LM %) No. of dead larvae / total No. of larvae) X 100, pupal weight (PW) in mg measured immediately after pupa formation., pupal duration (PD) in days measured as the longevity of pupal stage from pupation to adult (moth) emergence, sex ratio (SR)) measured as the ratio of males : females determined immediately after pupation, deformed pupa (DF %) and adult emergence (AE %).

Genotypes were classified according to their mean percentage of AV into: resistant (70 % AV or above), moderately resistant (35 to less than 70 % AV) and susceptible (less than 35 % AV), LF into: resistant (less than 35 % LF), moderately resistance (35 % to less than 70 % LF) and susceptible (70 % or above LF), DH_n into: resistant (0 % to less than 7 % DH_n), intermediate (7 % to less than 15 % DH_n) and susceptible (15 % DH_n or more), SP into resistant (0 to less than 35 % SP), moderately resistant (35 % to less than 70 % SP) and susceptible (70 % SP and more), DH_a into resistant (0 % to less than 7 % DH_a), intermediate (7 % to less than 15 % DH_a) and susceptible (15 % DH_a and more) and ID into: resistant (0 % to less than 1.7 ID) moderately resistant (1.7 to less than 2.7 ID) and susceptible (2.7 ID or above).This classification was according to Al-Naggar *et al.* (2000 b)

Biometrical analysis

The collected data were subjected to the normal analysis of variance of the RCBD in experiments one and two and completely randomized design in experiment three. Combined analysis of variance across years was performed in experiment one if the homogeneity test was not significant. LSD values were calculated to compare means. Data collected in percentages were subjected to arcsine transformation for the purpose of statistical analysis. However, presentation of such data hereafter will be in original percentages. Rank correlation coefficient was computed between traits in each experiment and Chi square test was performed for the sex ratio. All biometrical analyses mentioned previously were performed according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Experimental one

Field evaluation of maize resistance to *S. cretica* under natural infestation
Analysis of variance

Analysis of variance of the three studied resistance traits to the pink stem borer *S. cretica i.e.* percentage of avoidance plants (AV %), percentage of dead hearts (DH_n %) and percentage of leaf feeding plants (LF %) for 20 maize genotypes grown in the field under natural infestation across two planting dates and two years was performed (data not presented). Mean squares due to years were insignificant for all studied traits, indicating that climatic conditions of the two years (2005 and 2006) were not significantly different. Mean squares due to planting date were highly significant for all studied resistance / susceptibility traits, suggesting a significant role for planting date in the maize resistance to *S. cretica*. These results agreed with those reported by previous investigations (Shahoudah 1964, Isa and Awadallah 1975, El-Naggar 1991, and Soliman 1997). Moreover, mean squares due to maize genotypes were highly significant for the three studied traits, indicating the important role of maize genotype in the resistance of *S. cretica*. Ali *et al* (1987) and El-Naggar (1997) also reported genotypic differences in maize resistance to the pink stem borer. Mean squares due to all possible interactions between genotypes, years and planting dates were not significant, except for those due to genotype X planting date interaction which were highly significant for all studied traits, suggesting that maize genotypes performed differently under different planting dates for the three studied traits of *S. cretica* resistance. These results were in agreement with those reported by Isa and Awadallah (1975), El-Naggar (1991), Metawi (1996) and Soliman (1997). Mean squares due to the maize genotype for the five studied traits were highly significant only in the first planting date. Non significance of differences among genotypes in their resistance/

susceptibility to *S. cretica* in the 2nd planting date indicated the importance of the three factors required for accurate evaluation of insect resistance, *i.e.* presence of the host, presence of the insect of interest and the suitable environmental conditions for occurrence of infestation.

Means of traits expressing resistance to *S. cretica*

Table (2). shows that differences between the two years for studied traits were very small, supporting results of analysis of variance where years did not differ significantly for all studied traits. This assured that climatic conditions and populations of *S. cretica* insect were similar in both years at each planting date. In general, means for the 1st planting date were higher than those for the 2nd planting date for dead hearts (DH_n %) and leaf feeding (LF %) and the opposite was true for avoidance (AV %) trait. This indicated that the population of *S. cretica* insect from the first planting date was completely sufficient to cause natural infestation as compared with that following the 2nd planting date, so genotypic differences in *S. cretica* resistance among maize cultivars would be more obvious in the 1st than in the 2nd planting date.

Table 2. Summary of means and ranges for avoidance (AV), dead hearts (DH), and leaf feeding (LF) traits combined across 20 maize genotypes evaluated under natural infestation in 2005 and 2006 as well as their combined estimates across years.

Year / planting date		AV%			DH%			LF%		
		Mean	Range Low	Range High	Mean	Range Low	Range High	Mean	Range Low	Range High
2005	1st	51.3	30.0	98.3	27.8	0.0	61.7	48.7	1.7	70.0
	2nd	95.9	90.0	98.3	0.5	0.0	3.0	4.1	1.7	10.0
2006	1st	49.7	18.3	98.3	30.2	0.0	71.7	50.3	1.7	81.7
	2nd	97.3	81.7	100.0	0.1	1.7	0.0	2.3	0.0	11.7
Combined	1st	50.5	25.8	98.3	29.0	0.0	60.0	49.5	1.7	74.2
	2nd	96.6	87.5	99.2	0.3	0.0	1.7	3.2	0.8	9.2

1st = first planting date (most suitable for natural infestation).
2nd = second planting date (less suitable).

Results in Table (2) indicated very clearly that the range of all studied traits in the 1st planting date was much wider than that in the 2nd planting date. Therefore, the 1st planting date would be expected to show the differences among maize genotypes for resistance to *S. cretica* in a way much better than the 2nd planting date. This study therefore recommended the use of the 1st planting date (20th of April) in field evaluation of the *S. cretica* resistance among maize genotypes under natural infestation conditions in Egypt. Since analysis of variance indicated that mean squares due to planting dates were highly significant, so means of the 3 studied resistance traits will be presented and discussed for each planting date, separately. Moreover, since mean squares due to years and their interaction with genotypes (years X genotypes) and with genotypes and planting dates (years X planting dates X genotypes) were not significant, indicating non-existence of climatic variation between years and that genotypes performed similarly in the two years, so means of the studied traits will be presented and discussed as combined data across the two years for each planting date.

First planting date

Percentage of plants showing avoidance (AV) to *S. cretica*, i.e. plants showing no symptoms of leaf feeding and/ or dead hearts (Table 3) ranged from 25.8 % (for SC 129) to 98.3 (for SC Ageeb). The three genotypes SC Ageeb, TWC 351 and TWC 352 were considered as resistant to *S. cretica* across the two years. The two genotypes SC 129 and SC 124 were found susceptible, while the rest of genotypes were considered moderately resistant (intermediate). Under natural infestation, mean percentage of plants showing leaf feeding on the stem and/or on the whorl for each studied genotype (LF) ranged from 1.7 % for SC Ageeb to 74.2 % for SC 129(Table 3). LF trait classified the studied maize genotypes in the same manner like AV trait i.e. into three resistant (SC Ageeb, TWC 351 and TWC 352), two susceptible (SC 124 and SC 129) and fifteen moderately resistant genotypes (the rest of genotypes). Similarity of genotypes classification with respect of reaction to *S. cretica* among AV and LF traits could be attributed to that each of these two traits complement the other one in the way of estimation. DH_n average across the two years ranged from zero % for SC Ageeb to 60.0 % for SC 310 (Table 3). Combined data showed that two genotypes (SC Ageeb and TWC 351) were considered as resistant to *S. cretica*, five were moderately resistant (TWC 325, SC 352, TWC 324, SC 14 and SC 10) and thirteen were susceptible genotypes (the rest genotypes).

Rank correlation coefficients (Table 4) among the five studied traits across all genotypes evaluated in the first planting date across two years indicated a very strong negative association between AV and LF traits (r = –0.987**). Correlation coefficients were significant and positive for DH_n vs. LF (r = 0.518*), and highly significant and negative for DH_n vs. AV (r = –0.721**). Thus, under natural infestation conditions, beside dead hearts trait it could be recommended that only one of the other two studied traits, i.e. AV or LF could be used for screening maize genotypes for their resistance to S. cretica. The two latter traits are characterizing the same reaction to S. cretica but with opposite directions.

Table 3. Mean percentage of avoidance, leaf feeding and dead hearts for twenty maize genotypes evaluated under natural infestation with *S. cretica* at Sakha (data are combined across years) for the 1st planting date.

Genotypes	AV		LF		DH	
	%	Reaction	%	Reaction	%	Reaction
SC 10	45.8	I	54.2	I	14.8	I
SC 13	55.8	I	44.2	I	33.3	S
SC 14	51.7	I	48.3	I	14.6	I
SC 15	50.0	I	50.0	I	20.5	S
SC 122	40.0	I	60.0	I	37.5	S
SC 123	51.7	I	48.3	I	34.2	S
SC 124	29.2	S	70.8	S	30.9	S
SC 129	25.8	S	74.2	S	57.5	S
SC 155	47.5	I	52.5	I	20.7	S
TWC 310	35.8	I	64.2	I	60.0	S
TWC 311	40.0	I	60.0	I	31.7	S
TWC 314	39.2	I	60.9	I	53.3	S
TWC 321	46.7	I	53.3	I	50.8	S
TWC 322	50.0	I	50.0	I	30.0	S
TWC 324	53.3	I	46.7	I	14.4	I
TWC 325	61.7	I	38.3	I	13.3	I
TWC 327	40.0	I	60.0	I	45.0	S
TWC 351	77.5	R	22.5	R	3.4	R
TWC 352	70.3	R	29.7	R	13.7	I
SC Ageeb	98.3	R	1.7	R	0.0	R
LSD 0.05	20.2		20.3		19.3	

R= resistant , S= susceptible and I= intermediate

Table 4. Rank correlation coefficients (r) between pairs of all studied traits under natural infestation (data are combined a cross years) in the 1st planting date.

Trait	Avoidance %	Leaf angle (°)	Leaf chlorophyll mg/ m2	Leaf feeding %
Dead hearts%	– 0.721**	0.518*	0.581**	0.697**
Leaf feeding%	– 0.987**	0.204	0.517*	
Chlorophyll content	– 0.586**	0.453*		
Leaf angle	– 0.250			

*, ** Indicate significance at 0.05 and 0.01 levels of probability, respectively.

Chlorophyll content in maize leaves was significantly correlated but in low magnitude with all resistance traits, i.e. with dead hearts (r = 0.581**), leaf feeding (r = 0.517*) and avoidance (r = -- 0.586**). Therefore, chlorophyll content could be taken into consideration as a selection criterion to S. cretica resistance. The green colour of maize leaves could be considered as an attraction factor of the S. cretica moths to lay their egg masses on the leaves. The results of the present investigation indicated that plants of higher chlorophyll content may cause higher percentage of dead hearts and leaf feeding and lower percentage of avoidance plants, i.e. more preference and attractiveness of moths of S. cretica. These results, under natural infestation were in agreement with those reported by Soliman (1997). However, Metawi (1996) reported that chlorophyll A and B did not exhibit any association with infestation percentage caused by S. cretica. Differences in results reported by different investigators for the role of chlorophyll content in maize plant reaction to S. cretica could be attributed to the genetic background of maize genotypes used in different investigations.

Leaf angle trait showed significant and positive correlation, but in low magnitude with DH_n (r = 0.518*) and chlorophyll content (r = 0.453*). The positive correlation between leaf angle and dead hearts in this study agreed with Soliman (1997) who reported a positive correlation coefficient between leaf angle and percentage of infested plants under natural infestation, indicating that selection for narrower leaf angle helps in preventing moths of *S. cretica* to lay their eggs beneath the leaf sheath. Further experiments should be conducted on the role of maize leaf angle in attraction of *S. cretica* insect to lay their egg masses, by using maize genotypes of different genetic backgrounds with regard of leaf angle and chlorophyll content.

Second planting date

Results (not presented) indicated absence of significant differences among all studied genotypes with regard to their resistance/susceptibility to *S. cretica* expressed by all three studied traits (AV, LF and DH_n). The ranges were very narrow to the extent that all maize genotypes showed only resistance reaction to *S. cretica*. The reason was due to that this planting date (2^{nd} one) was unsuitable to natural infestation with *S. cretica*. Non-existence or existence with small populations of *S. cretica* at the time of evaluation in this 2^{nd} planting date could be the main reason for the resistance reaction shown by all studied maize genotypes. These results are in agreement with those reported by Shahoudah (1964), Isa and Awadallah (1975) and El-Naggar (1991).

Shahoudah (1964) concluded that *S. cretica* has two egg-mass populations with two distinct peaks of abundance, one in May and the other in September. He added that egg-mass population is high from late March to late May, while in June and July, it is very low. Isa and Awadallah (1975) found that maize field planted in Nile Delta as early as late of March or during April is subjected to have infestation by *S. cretica* during May. They added that occurrence of this insect is rare during June and July when maize is planted during May and June. El-Naggar (1991) found that the mid-April and first of May cultivation of maize had the most infestation. His results also clarified that planting at the beginning of June or July resulted in the least infestation with *S. cretica*.

Experiment two

Field evaluation of maize resistance to *S. cretica* under artificial infestation

Analysis of variance

Analysis of variance (not presented) of the three studied resistance traits to *S. cretica*, in this field evaluation of 20 maize genotypes under artificial infestation in 2007 season indicated that mean squares due to maize genotypes were highly significant for the three studied traits, i.e. susceptible plants %, dead hearts % and intensity of damage. This indicated the existence of significant differences among studied maize genotypes in their resistance/susceptibility to the pink stem borer *S. cretica* under artificial infestation conditions. These results were in agreement with those reported by some investigators under artificial infestation with *S. cretica* (Al–Naggar *et al* 2000 b, Saafan 2003 and El-Kheshin 2006)

Means of traits expressing resistance to *S. cretica*

Means of the three studied resistance traits under artificial infestation and the resistance/susceptibility reaction of each maize genotype are presented in Table (5). A wide variation was observed between studied maize genotypes in their resistance/susceptibility to *S. cretica*. Under artificial infestation with *S. cretica*, mean percentage of susceptible plants (SP %) as the first criterion of resistance to *S. cretica* in this experiment (Table 5), ranged from 6.7 % (for SC Ageeb) to 80 % (for SC 155, TWC 311, TWC 314 and TWC 325). Based on SP % trait maize genotypes could be classified into three groups; the 1st group represented the resistant cultivars and included only SC Ageeb, the 2nd represented the intermediate resistant and included six genotypes (TWC 351, SC 15, TWC 310, SC 122, TWC 322 and TWC 352) and the 3rd group represented the susceptible genotypes which included 13 maize cultivars. Means of dead hearts (DH_a) ranged from 0.0 % for SC Ageeb and TWC 351 to 50 % for TWC 321. Based on DH_a maize genotypes could be classified for their resistance to *S. cretica* other than the two studied traits. DH_a discriminated maize genotypes

Table 5. Mean percentage of susceptible plants, dead hearts and intensity of damage for twenty maize genotypes evaluated under artificial infestation with *S. cretica* at Giza in 2007season.

Genotypes	Susceptible plants%	Reaction	Dead hearts%	Reaction	Intensity of damage	Reaction
SC 10	70.0	S	13.3	I	2.5	I
SC 13	70.0	S	16.7	S	3.3	S
SC 14	70.0	S	7.6	I	3.4	S
SC 15	53.3	I	7.6	I	2.5	I
SC 122	63.3	I	23.3	S	3.7	S
SC 123	70.0	S	30.0	S	4.4	S
SC 124	76.7	S	10.0	I	3.5	S
SC 129	70.0	S	20.0	S	3.4	S
SC 155	80.0	S	13.3	I	4.6	S
TWC 310	56.7	I	40.0	S	4.7	S
TWC 311	80.0	S	13.3	I	4.2	S
TWC 314	80.0	S	20.0	S	4.4	S
TWC 321	70.0	S	50.0	S	5.4	S
TWC 322	63.3	I	10.0	I	3.4	S
TWC 324	76.7	S	10.0	I	2.6	I
TWC 325	80.0	S	10.0	I	3.4	S
TWC 327	70.0	S	23.3	S	4.8	S
TWC 351	36.7	I	0.0	R	2.1	I
TWC 352	66.7	I	13.3	I	2.9	I
SC Ageeb	6.7	R	0.0	R	1.0	R
LSD 0.05	2.34		3.82		1.32	

R= resistant , S= susceptible and I= intermediate

into three groups; the 1st represented the resistant genotypes that included SC Ageeb and TWC 351, the 2nd represented the intermediate genotypes which included ten cultivars (SC 14, SC 15, SC 124, TWC 322, TWC 324, TWC 325, SC 10, SC 155, TWC 311 and TWC 352) and the 3rd represented the susceptible genotypes which included eight cultivars (SC 13, TWC 314, SC 129, TWC 327, SC 122, SC 123, TWC 310 and TWC 321). With respect to intensity of damage (ID) trait under artificial infestation, means (Table 5) ranged between 1.0 (resistant) for the single cross SC Ageeb to 5.4 (susceptible) for TWC 321. Based on ID maize genotypes could be classified into three groups; the 1st included one resistant cultivar, i.e. SC Ageeb (1.0), the 2nd included five moderately resistant genotypes (TWC 351, SC 10, SC 15, TWC 324 and TWC 352) and the 3rd group included 14 susceptible to *S. cretica* genotypes.

Rank correlation coefficients computed among resistance traits recorded under artificial infestation in one season and those recorded under natural infestation at the 1st planting date combined across two season are presented in Table (6). Dead hearts (DH_a) and intensity of damage (ID) under artificial infestation had positive and highly significant correlation coefficients with each of avoidance %, dead hearts (DH_n) and leaf feeding percentage under natural infestation. The strongest correlation were recorded among dead hearts under natural infestation DH_n and dead hearts under artificial infestation DH_a (r = 0.870**). This indicated the similarity of results of dead hearts under both artificial and natural infestation conditions (in the 1st planting date).

Table 6. Rank correlation coefficients among resistance traits recorded under artificial infestation and those recorded under natural infestation in the 1st planting date.

Trait	Avoidance	Dead hearts (natural)	Leaf feeding
Intensity of damage (ID)	0.584**	0.732**	0.582**
Dead hearts% (DH_a)	0.599**	0.870**	0.603**
Susceptible plants% (SP)	0.305	0.195	0.295

** Indicates significance at 0.01 probability level

It could be concluded that natural infestation with *S. cretica* matched with the 1st plating date could be considered an efficient technique in screening a large number of maize genotypes for their resistance to *S. cretica* and the artificial infestation could be utilized for detailed screening of small number of maize genotypes. The most reliable resistant trait recommended by results of this study was percentage of dead hearts. The percentage of leaf feeding (or avoidance) under natural infestation in the 1st planting date could also be used.

Experiment three (Laboratorial experiment)

Reproduction of *S. cretica* reared on different maize genotypes

Analysis of variance

Analysis of variance (not presented) indicated that mean squares due to maize genotypes were highly significant for larval mortality (LM), larval duration (LD), fecundity (Fec), hatchability (Hat) and deformed pupa (DP). This suggests that the *S. cretica* insect performed differently, during its reproduction when it was fed (reared) on maize plants of different resistance / susceptibility reaction. Thus, a significant role of maize genotype existed in the reproduction process of the pink stem borer *S. cretica*. On the contrary, genotypes of maize did not differ significantly in three attributes of *S. cretica* reproduction, i.e. pupal weight, pupal duration and adult emergence, where mean squares due to genotypes for these characteristics were not significant. Female of pupa differed significantly from males in their performance with regard of pupal weight and pupal duration characteristics, where mean squares due to sex for these two characteristics were highly significant. Mean squares due to genotype X sex interaction were highly significant for larval duration, indicating that the duration of *S. cretica* larva was different when fed on plants of different maize genotypes.

Means of *S. cretica* reproduction attributes

Mean percentage of larval mortality (LM) (Table 7) differed widely when larvae of *S. cretica* were fed on plants of different maize cultivars. Larval mortality reached 100 % when larvae were fed on the Bt single cross of corn SC Ageeb. The death of all larvae of *S. cretica* after feeding them on small plant parts of SC Ageeb, could be attributed to the presence of a toxic protein produced in plants as a result of expression of the Bt gene introduced *via* genetic engineering of this maize cultivar. Larvae death on Bt corn was also reported by many investigators on many corn borers other than *S. cretica* (Pilcher *et al.*, 1997 and Lozzia *et al.*, 1997). To the best of our knowledge, there was no any report in the literature presenting laboratorial results on the mortality of larvae of *S. cretica* due to feeding on Bt corn.

Table 7. Means of larval mortality (LM) and larval duration (LD) of *S. cretica* fed on plants of different maize genotypes.

Genotypes	LM%	LD (days) ♀	LD (days) ♂
SC10	66.7	33.6	32.8
SC155	65.6	29.7	32.6
TWC321	32.2	45.0	44.4
TWC 351	80.0	42.5	35.0
SC Ageeb	100.0	–	–
L.S.D. 0.05	3.95	G = 2.31, S= 1.63, G x S =3.27	
L.S.D. 0.01	5.75	G = 3.07, S = 2.17, G x S = 4.34	

– indicates complete death of larvae in the 2nd instars,
G = genotypes and S = sex.

In the 2nd rank after SC Ageeb, came the maize cultivar TWC 351 which exhibited a larval mortality percentage of 80 % (Table 7). This cultivar proved, in this laboratorial experiment very high percentage of LM; that was close to LM of SC Ageeb. This three-way cross is not genetically engineered for insect resistance, which assured that *S. cretica* resistance could also be found in the available maize germplasm without introducing genetically modified plants. High larval mortality on this cultivar (TWC 351) could be attributed to non- preference for the *S. cretica* insect which is a form of resistance in maize plants. Further chemical analysis of plants of TWC 351 cultivar should be performed to determine the presence of any substance such as DIMBOA in the leaves and stems, which may cause death of *S. cretica* larvae.

On the contrary, larval mortality percentage reached to a minimum of 32.2% when *S. cretica* was fed on the three-way cross TWC 321. This cultivar was therefore considered as sensitive to feeding by *S. cretica* larvae. The other two varieties of maize, i.e. SC 10 and SC 155 showed average percentage of LM (66.7 and 65.6 %, respectively). The present results are in general harmony with those of Eghlidie *et al.* (1977) and Barry and Darrah (1991) on *Ostrinia nubilalis* and EL-Naggar (1997) on *S. cretica*. It is worth noting that the results of this investigation on LM in the laboratory assured the results recorded in the field on DH and SP traits, where the two cultivars SC Ageeb and TWC 351 were resistant to infestation (both natural and artificial) with *S. cretica*. Mean larval duration (Table 7) differed among maize cultivars. It ranged from 29.7 to 45.0 days for females and from 32.6 to 44.4 days for males on. SC 155 and TWC 321, respectively. It is worthy to note that the maize cultivar TWC 321 which caused the minimum mortality to larvae caused also the longest larval duration to both females and males of larvae of *S. cretica* (Table 7). Means of the rest of reproduction attributes of *S. cretica* did not include those on the cultivar SC Ageeb, since all larvae fed on this cultivar died, so all the successive stages of insect after larva were absent. In general, LD was for females 1.5 days longer than for males. The males of larvae on TWC 321 showed shorter LD (35.0 days) than their females (42.0 days). On the contrary, on the cultivar SC 155, the LD of larval male was longer (32.6 days) than that of larval female (29.7 days). This assured the role of insect sex X maize cultivar interaction on the larval duration. The results of this study are in agreement with those reported by Reed *et al* (1972), Robinson *et al* (1978) and Barry and Darrah (1991).

Mean pupal weight (PW) (Table 8) did not differ significantly when the insect was reared on different maize cultivars. There was a significant difference between females and males of *S. cretica* pupa. the heaviest pupa was 167.9 mg for female and 112.3 mg for male on SC 10 and TWC 351, respectively, while the highest pupa was 147.0 mg for female and 109.9 mg for male on SC 155 and SC 10, respectively. Differences in PW of *S. cretica* were previously reported by other investigators between males and females (Soliman 1997 and El-Metwally 1997) and when the insect was reared on different maize genotypes (Reed *et al* 1972, Ward *et al* 1975 and Eghlidie *et al* 1977).

Table 8. Means of pupal weight (PW), pupal duration (PD) and deformed pupae (DP) of *S cretica* fed on plants of different maize genotypes.

Genotypes	PW (mg)		PD (days)		DP %
	♀	♂	♀	♂	
SC10	157.9	109.9	11.3	8.6	3.3
SC155	147.0	111.6	11.2	9.7	9.7
TWC321	163.6	110.5	10.6	9.8	3.3
TWC 351	164.9	112.3	10.5	8.6	10.4
L.S.D. 0.05	G = ns	Sex = 0.01	G = ns	Sex = 0.65	7.4
L.S.D. 0.01	G = ns	Sex = 0.02	G = ns	Sex = 0.86	10.9

G = genotypes, ns = nonsignficant.

Means of pupal duration (PD) in days of both males and females of S. *cretica* pupa reared on plants of different maize genotypes are presented in Table (8). In general, pupa of *S. cretica* reared on TWC 351, the most resistant genotype (after SC Ageeb) in this study showed the shortest PD for both females and males. The longest PD was recorded by pupa females on SC 10 and SC 155, and by pupa males on TWC 321 and SC 155. As an average of males and females the pupa reared on SC 155 was the heaviest. PD was longer for females than that for males, which was matching with the results of pupal weight. The longest PD of females than males could interpret the reason of the heavier PW of females than males.

The results of the present study are in agreement with those reported by Ali *et al.* (1987) and El-Naggar (1997) on the differences in mean PD of ECB reared on different maize genotypes. Mean percentages of deformed pupa (DP) of *S. cretica* reared in the laboratory on maize plants are presented in Table (8). The highest percentage of DP was shown by the resistant cultivar TWC 351 (10.37 %), while the lowest percentage (3.33 %) was exhibited by the maize cultivars SC 10 and TWC 321. Based on this insect attribute (DP), TWC 351 could be assured to be resistant and TWC 321 and SC 10 could be considered as susceptible to infestation with *S. cretica*. The results of the present study are in agreement with those reported by Everrett *et al* (1958) and Reed *et al* (1972) on DP of *O. nubilalis*.

Results of sex ratio of pupae of *S. cretica* reared in the laboratory on different maize cultivars are presented in Table (9). All chi square values computed on observed and expected estimates of sex ratio were highly significant, which means that the observed ratio in different significantly from the expected one; the most close observed to expected sex ratio (1 : 0.9) was recorded when the insect was reared on one of the susceptible maize genotypes (SC 155) which was preferred to the insect, while the observed sex ratio was faraway of expected ratio, when the insect was reared on the other three studied cultivars, including the most resistant one

Table 9. Sex ratio of pupae of *S. cretica* reared in the laboratory on plants of different maize cultivars.

Genotypes	Sex ratio Female : Male	X^2
SC 10	18 :11 (1 : 0.6)	0.099**
SC 155	13 : 10 (1 : 0.9)	0.343**
TWC 321	20 : 29 (1 : 1.5)	0.044**
TWC 351	12 : 7 (1 : 0.6)	0.149**

** Indicates significance at 0.01 probability level. X^2 = Chi-square.

TWC 351 (the least preferable by the insect). The results of the present study were in agreement with those reported by Guthrie *et al* (1980) and Soliman (1997).

Mean percentage of adult (moth) emergence (AE) of *S. cretica* reared on four maize cultivars is presented in Table (10). Results showed no significant differences among maize genotypes for AE of *S. cretica* reared on them. All adults (100 %) were emerged on three maize cultivars SC 155, TWC 321 and TWC 351, while on the cultivars SC 10, AE percentage was 96.7 % with no significant difference among this and other cultivars. The results of the present study were in agreement with those reported by Ali *et al.* (1987) and El-Naggar (1997).

Table 10. Means of adult emergence (AE), fecundity (Fec) and hatchability (Hat) of *S. cretica* fed on plants of different maize cultivars at the laboratory.

Genotypes	AE%	Fec	Hat %
SC10	96.7	59.0	37.3
SC155	100.0	117.4	72.6
TWC321	100.0	241.2	94.4
TWC 351	100.0	92.6	56.9
L.S.D. 0.05	ns	51.11	22.52
L.S.D. 0.01	ns	69.03	30.41

AE = adult emergence, Fec = fecundity and Hat = hatchability
ns = Non significance

Mean number of eggs laid by each female moth (fecundity,Fec) of *S. cretica* differed widely among maize genotypes; it ranged from 59.0 eggs/ female when rearing was on SC10 to 241.2 eggs / female when rearing was on TWC 321 cultivar (Table 10). Based on fecundity results, the cultivar TWC 321 could be considered the most susceptible (the most preferable to egg deposition), while SC 10 and TWC 351 cultivars could be regarded as the most resistant (the least preferable to egg depositing). Our results are in agreement with those reported by El-Naggar (1997).

Mean percentage of hatchability (Hat) of *S. cretica* (Table 10) was maximum (94.4%) on the most susceptible cultivar in this study (TWC 321), while it was minimum on SC 10 (37.3 %) followed by the most resistant cultivar TWC 351 (56.9 %). Results of fecundity and hatchability of *S. cretica* ranked the studied maize cultivars for resistance (non-preference) or susceptibility (preference) in the same manner. Differences among maize genotypes for hatchability of *S. cretica* reported in this study were in harmony with those reported by previous investigators (Reed *et al* 1972, Ali *et al* 1987, El-Naggar 1997 and Robinson *et al* 1978).

Rank correlation coefficients computed among pairs of reproduction traits of *S. cretica* reared in the laboratory on maize plants of different maize cultivars are presented in Table (11). Fecundity had a strong positive and significant or highly significant correlation coefficient with each of larval mortality percentage (r = 0.80**), larval duration (r = 0.70**) and percentage of deformed pupa (r = 0.65*), and a strong negative and highly significant correlation coefficient with pupal weight (r = – 0.80**).

Table 11. Rank correlation coefficients between pairs of reproduction characters of *S. cretica* reared on plants of different maize genotypes in the laboratory.

	Hat	Fec	AE	DP	PD	PW	LD
LM	0.31	0.80**	- 0.10	0.49	0.31	- 0.06	0.09
LD	0.45**	0.70**	0.34	0.01	- 0.05	0.11	
PW	- 0.23	- 0.80**	- 0.05	- 0.28	- 0.14		
PD	0.29	- 0.03	0.47	- 0.32			
DP	0.52	0.65*	- 0.21				
AE	0.42	0.28					
Fec	0.80**						

***, ** Indicate significance at 0.05 and 0.01 levels of probability, respectively.**

Moreover, hatchability showed a highly significant and positive correlation coefficient (r = 0.45**) with larval duration. The results indicated that the longer larval duration period, the higher fecundity and hatchability percentage, which is logic. Moreover, fecundity and hatchability traits of *S. cretica* are strongly associated

In this study a high percentage of fecundity was associated with high percentage of mortality and deformed pupa and low weight of pupa. Further experiments should be conducted in the future to test these correlations by feeding the stem borer *S. cretica* on more maize genotypes of different resistance to this insect.

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مقاومة عشرون صنفا من الذرة الشامية المصرية لدودة القصب الكبيرة وتأثيرها على تكاثر الحشرة

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تعتبر ثاقبة الساق القرمزية *Sesamia cretica* (دودة القصب الكبيرة) اخطر الثاقبات التي تهاجم الذرة الشامية فى مصر كانت أهداف هذه الدراسة هى (١) تقييم عشرون صنفا من الذرة الشامية المنزرعة فى مصر (بما فيها الصنف الجديد الهجين الفردى عجيب المهندس وراثيا بجين ال Bt) من حيث مقاومتها لدودة القصب الكبيرة تحت ظروف العدوى الطبيعية والصناعية فى ميعادين مختلفين للزراعة و (٢) دراسة تأثير تغذية الحشرة فى المعمل على نباتات من الذرة تابعة لأصناف مختلفة فى درجة مقاومتها للحشرة.

تم أجراء تجربتين حقليتين وتجربة معملية خلال المواسم ٢٠٠٥، ٢٠٠٦، ٢٠٠٧ أوصفت نتائج التجربة الحقلية تحت ظروف العدوى الطبيعية باستخدام ميعاد الزراعة الأول (٢٠ ابريل) للحصول على نتائج أكثر دقة عن مقاومة الذرة لهذة الثاقبة وذلك بسبب توافد أعداد كبيرة من هذه الحشرة فى الطبيعة عندما تكون النباتات الصغيرة للذرة منزرعة هذا الميعاد، مقارنة بالميعاد الثانى (١٥ مايو). أشارت نتائج الميعاد الأول لهذه التجربة إلى أن الأصناف الثلاثة الهجين الفردى (عجيب) المهندس وراثيا، والهجينين الثلاثين ٣٥١ ، ٣٥٢ كانت مقاومة لهذه الثاقبة باستخدام الصفتين نسبة النباتات التى تجنبت الإصابة والتى حدث بأورلقها تغذية الحشرة ، بينما اعتبرت صفة القلب الميت إن الصنفين عجيب والثلاثى ٣٥١ هما المقاومين فقط . وأكدت نتائج التجربة الحقلية الثانية تحت ظروف العدوى الصناعية مقاومة للصنف (عجيب) المهندس وراثيا باستخدام الثلاثة صفات للتعبير عن المقاومة (نسبة [illegible] القلوب الميتة وشدة الإصابة) ومقاومة الهجين الثلاثى ٣٥١ باستخدام صفتى

(القلب الميت و شدة الإصابة) ومقاومة الهجين الثلاثى ٣٥٢ باستخدام صفة واحدة فقط (القلب الميت). ارتبط محتوى كلوروفيل الأوراق فى الذرة ارتباطا عالى المعنوية مع صفات القلب الميت (٠,٥٨١**)و نسبة النباتات التى تم التغذية على أوراقها (٠,٤٩٣**) والنباتات التى تجنبت الإصابة (٠,٥٢٤**) ، مما يقترح أن محتوى الكلوروفيل يمكن أن يؤخذ فى الاعتبار كمعيار انتخابى فى الذرة للمقاومة لهـذه الحشرة . أشارت نتائج التجربة المعملية الى أن التغذية الصناعية لهـذه الحـشرة علـى نباتـات ذرة المهندسة وراثيا للصنف عجيب أدت الى ١٠٠ % موت لليرقات والتغذية على نباتات مقاومة للهجـين الثلاثى ٣٥١ أدت إلى ثانى أعلى نسبة موت اليرقات (٨٠ %) وأعلى نسبة عذارى مشوهة (١٠,٣٧ %) واقصر فترة حياة عذراء (٨,٦ يوم) وعدد قليل من البيض الموضوع لكل أنثـى (٩٢,٦) ونـسبة فقس منخفضة (٥٦,٩ %) وتغير معنوى غير مرغوب فى النسبة الجنسية للعـذارى المتوقعـة عـن الحقيقية. وعلى العكس فان تربية الحشرة على الصنف الحساس هجين ثلاثى ٣٢١ أدى الى اقل نسبة موت لليرقات (٣٢,٢ %)، وأطول فترة يرقية (٤٤,٧ يوم)، ثانى أطول فترة عـذراء (١٠,٢ يـوم)، وأعلى عدد بيض لكل أنثى (٢٤١,٢) وأعلى نسبة فقس (٩٤,٤ %). أعزيت هذه الفـروق لتفـضيل الحشرة لنباتات الصنف الحساس (ثلاثى ٣٢١) وعدم التفضيل لنباتات الصنف المقاوم (الفردى عجيـب والثلاثى ٣٥١).