

Article

Identification of Resistance to the Corn Weevil (*Sitophilus zeamais* M.) in Mexican Maize Races (*Zea mays* L.)

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Abstract: *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) has a global distribution and is one of the most important post-harvest pests of maize (*Zea mays* L.). Some Mexican maize races could be a novel source of resistance against *S. zeamais* to improve commercial maize, generating lines, varieties, and hybrids with resistance to the pest. These are original races from the center of origin of maize and have been exposed to selection pressure from *S. zeamais* and other pests for thousands of years, which probably resulted in some type of resistance. We studied the resistance of Mexican maize races to *S. zeamais* focusing on antixenosis, antibiosis, and tolerance. Antibiosis reduces the survival and reproduction of the insect or prolongs the time between generations, whereas antixenosis reduces the rate of initial and successive accumulation of the insect population. Obligate-antibiosis and free-choice bioassays were performed, and in those experiments, resistance traits and the consumption by *S. zeamais* were measured. The most resistant accession showed high adult mortality and fewer adults in the 1st generation, where the number of emerged adults is an ideal trait to evaluate the level of maize resistance to the pest. Net consumption (in grams) is the most economically important trait because it indicates the gross amount lost in a store, while yield losses (percentage of consumption) allow for studying tolerance. The type of endosperm did have an influence on the resistance/tolerance to *S. zeamais*. The flint varieties showed significantly higher mortality of adults before entering the seed, a lower quantity of emerged adults of the first generation in the obligate-antibiosis bioassay, and lower net consumption and percentage in both bioassays. In general, the varieties of the races Cristalino de Chihuahua, Apachito, and Azul showed high levels of resistance, with the Cristalino-079 (flint) and Azul (floury) accessions also being tolerant, and low levels of consumption by *S. zeamais*. Both could be used in breeding programs to reduce losses caused by *S. zeamais*.

Keywords: *Zea mays*; *Sitophilus zeamais*; insect resistance; grain yield losses; Mexican maize races



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1. Introduction

Across the world, the area within which maize was sown in 2020 exceeded 243.2 million hectares, with a total production of 1423.2 million tons and a yield of 5.85 tons ha⁻¹ [1]. Post-harvest losses due to storage pests are generally estimated to range from 20 to 30% [2].

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) is one of the most important and destructive pests of stored grains, particularly in maize [3]. In addition, *S. zeamais* has a worldwide distribution [4]. Particularly for *S. zeamais*, losses ranged from 10 to 20%, which means a loss from 142.3 to 284.6 million tonnes per year [2]. Even in a short storage

time of three months, losses can be as high as 6.9%, which means a loss of 112.4 million tons per year [5]. *S. zeamais* infests ears in the maize field during grain drying and before harvest or when the grain is stored. The greatest damage to the grain is caused by adults and larvae. Adults pierce the grain to oviposit, and the larvae feed on the endosperm of the grain until they transform into pupae. When pupae become adults, they pierce the grain and go out into the environment. Females can lay up to 250 eggs in their reproductive life. Its life cycle depends on temperature and varies between 30 and 113 days. In temperate zones, there are two to three generations per year [4].

Fumigation with aluminum phosphide (phosphine) is recommended for controlling *S. zeamais* [4]. However, the use of these insecticides leads to environmental contamination, human health problems, pest resistance, ozone layer depletion, and toxicity to beneficial insects [6,7]. Controls based on chemical insecticides need to be repeated periodically, and in general, are more expensive than using crops that are genetically improved [8]. The development of resistant varieties to *S. zeamais* and other warehouse pests seems to be a very good strategy and has a number of comparative advantages, particularly the decreased use of chemical insecticides [4,9].

Many resistant genotypes to *S. zeamais* have been reported such as Naltel (Yucatan-7), Chapalote (Sinaloa-35), and Palomero o Bolita (Oaxaca-130), which presented low oviposition, a high mortality percentage on oviposition, low progeny, high mortality on emergence replicates, the lowest Dobie index, and low weight loss [10]. The variety 'BHQP-542' was reported with the lowest index of susceptibility and was regarded as resistant [11]. Maize accessions 2000SYNEE-WSTR and TZBRELD3C5 have been reported as being resistant to *S. zeamais* by Serratos et al. [12] because they had more crude fiber, phenolic acid, and trypsin inhibitor contents in the grain, which significantly increased the mortality and reduced the survival rate of *S. zeamais* adults and the percentage of grain damage, percentage of weight loss, the weight of grain flour, and oviposition. Other researchers have reported resistant accessions to *S. zeamais* as Ikenne 83-TZSR-W-1 (open-pollinated) and 8329-15 (hybrid) [13], and the ZM421 and ZM521 varieties showed potential for *S. zeamais* progeny suppression [14]. Furthermore, CKPH08004 accession with yield from 6.7 to 8.6 t·ha⁻¹ and the best response to *S. zeamais* could be a good prospect for maize production and *S. zeamais* control [15]. Out of a total of 175 genotypes, including hybrids, landraces, open-pollinated varieties, and controls, the most resistant genotypes were CKPH08003 and BRAZ 2451 [16]. The conventional inbred line KU1414SR/SR, out of five maize varieties studied, showed the best level of resistance to *S. zeamais* [17]. KH631Q was the most resistant hybrid to *S. zeamais* out of 22 hybrids and 2 controls [18]. The variables that have been mainly used for characterizing resistance were the mortality of adults or parental mortality [19], grain damage, grain weight loss, kernel consumption, days to adult emergence, number of eggs, adult weight, susceptibility index [10–12,15,19–21], percentage of damaged kernels [13], and weight of powder produced [16,22]. Few studies of resistance to *S. zeamais* have measured adult mortality in the infestation (mortality of adults before entering the seed). This trait is highly correlated with first generation adults and consumption.

The novelty of this manuscript is that we focus on almost all stages of the biological cycle of *S. zeamais* and explore the different resistance mechanisms (antixenosis, antibiosis, and tolerance) to gain a more detailed picture of the performance of maize varieties against *S. zeamais* attack. Antibiosis reduces the survival and reproduction of the insect or prolongs the time between generations; antixenosis reduces the rate of initial and successive accumulation of the insect population; and tolerance is when plants have the ability to grow and produce a good grain or forage yield even when attacked by the pest [23,24]. The traits studied sometimes encompass more than one category of resistance to *S. zeamais*; therefore, we studied all resistance traits reported in the literature for obligate- and free-choice antibiosis trials. Therefore, the objective was to look for new sources of resistance to *S. zeamais* in Mexican maize races and the stages where the diverse types of resistance are highest.

2. Materials and Methods

2.1. Plant Material

Thirteen accessions from seven Mexican maize races were used to study resistance to *S. zeamais*. The seed of the Mexican maize races used in the present research was recollected at Chihuahua State, Mexico and reproduced in 2019 at INIFAP Experimental Station at Bachiniva, Chihuahua, Mexico.

2.2. Bioassays for Resistance Studies

2.2.1. Obligate-Antibiosis Bioassays

Two bioassays in obligate antibiosis, initiated on 2 September 2020 and finished on 24 June 2021, were conducted under laboratory conditions at 17.9 °C following a completely randomized design with three repetitions. The *S. zeamais* used in the experiments were recollected from a warehouse of a maize farmer from Durango, Mexico, and reared in Cuauhtémoc, Chihuahua. We evaluated 13 maize accessions in bottles, 5 cm in diameter and 5 cm in height, using 10 seeds per bottle and 3 repetitions (bottles) per bioassay; the total weight of seeds introduced in each bottle was recorded (initial weight). The insect adults used emerged from 30 August to 1 September 2020. The following variables were measured on those adults: (1) adult antixenosis, measured as the percent of 10 adults that were away from the seed, calculated with the following formula: $\text{antixenosis} = \frac{\text{number of adults away the seed}}{10} \times 100$; antixenosis was accounted for each day from 3 to 8 September 2020; and (2) adult mortality, measured as the number of initial adults that were found dead outside the seed. The adults of the infestation were removed on 20 October 2020, and the next variables were measured in the 1st generation: (3) the number of first-generation adults emerged and (4) the seed weight (g) after the first generation attack measured from 10 seeds with a precision balance in grams, which was used to calculate: (5) net consumption by the first generation (g) = initial weight–final weight and (6) percentage of consumption by the first generation = $\frac{\text{net consumption}}{\text{initial weight}} \times 100$. Finally, (7) the first generation duration (days), was estimated from the date of the beginning of experiments until the first-generation adults emerged.

2.2.2. Free-Choice Bioassays

In addition, two experiments using free-choice bioassays were initiated on 26 June 2021 and finished on 1 February 2022 under warehouse conditions at 18.5 °C. The free-choice experiment followed a completely randomized design with three repetitions. We used the seed that resulted from the obligate bioassays; therefore, seed final weight from the obligate bioassays was used as the seed initial weight in the free-choice bioassays. After the obligate antibiosis experiments, an attempt was made to imitate what happens in a warehouse highly infested with *S. zeamais*, since after one generation of adults comes the next, and the seeds present different degrees of damage. We evaluated 13 maize accessions on the top of the bottles used in obligate antibiosis because the adults cannot climb the bottles (Figure 1). We placed ten seeds per repetition and three repetitions per bioassay. We measured: (1) net consumption (g), (2) percentage of consumption, and (3) adult preference (n), as the number of adults per repetition at the end of free-choice bioassays which was considered a similar trait to the emerged adults in the obligate-antibiosis bioassay.



Figure 1. Most resistant accessions (Cristalino-079 and Azul) and some of the most susceptible (Bofo, E-Zapata-T and Gordo) after consecutive bioassays, obligate-antibiosis and free-choice bioassays. Experiment 1 (A) and experiment 2 (B) after the free-choice bioassays.

2.3. Statistical Analysis

For each type of experiment (obligate-antibiosis and free-choice), analyses were carried out to determine if there were significant differences between the two bioassays carried out. No differences were found between the bioassays or in the bioassay \times accession interaction, so they were considered as a single bioassay with six replicates. A combined and individual generalized linear mixed model analysis for each experiment was carried out using the GLIMMIX procedure of SAS 9.4 [25]. To analyze adult mortality before entering the seed, a binomial distribution was used, while for the first generation of adults that emerged, a negative binomial distribution was chosen. For antixenosis and percentage of consumption, we used a beta distribution, while net consumption and biological cycle were considered normally distributed (Gaussian) data. For mean comparisons, we used the LSmeans obtained with the GLIMMIX procedure. Pearson correlation coefficients between traits were calculated with the procedure CORR of SAS for each type of bioassay separately.

The maize accessions were classified into types of endosperms according to grain color, and a comparison was made between groups. We found seven accessions with white coloration, three with yellow, and three colored (one black, one purple, and one pink) (Table 1).

Table 1. Characteristics of seeds of thirteen maize races (*Zea mays*) originating from INIFAP evaluated for antibiosis, antixenosis, and tolerance to *S. zeamais*.

Accession	Maize Race	Color	Endosperm Type ^a
8-Carreras-PP	8-Carreras	White	Dent
8-Carreras-RP	8-Carreras	White	Dent
Azul	Azul	Black	Floury
Cristalino-061	Cristalino de Chihuahua	White	Flint
Bofo	Bofo	Purple	Floury
E-Zapata-T	–	White	Dent
Cristalino-279	Cristalino de Chihuahua	White	Flint
Cristalino-282	Cristalino de Chihuahua	Yellow	Flint
Apachito-r	Apachito	Pink	Flint
Cristalino-079	Cristalino de Chihuahua	Yellow	Flint
Apachito-b	Apachito	White	Flint
Gordo	Gordo	White	Floury
Palomero	Palomero de Chihuahua	Yellow	Popcorn

^a The races are distributed among classical maize groupings based on relative endosperm hardness: floury, dent, semi-flint, flint, and popcorn. The latter is comparable with flint in its hardness.

In order to study the tolerance of the different accessions, a regression was performed using grain yield losses due to the first and second generations as a dependent variable and the sum of adults of the first and second generations as the independent variable [26].

3. Results

In the combined analysis of the two types of experiments (obligate-antibiosis and free-choice) significant differences were found between both experiments, and the accession × experiment interaction was significant for those traits that were used in both experiments: number of emerged adults, net consumption, and percentage of consumption. Therefore, the obligate-antibiosis and free-choice bioassays have been analyzed separately.

Three traits were used exclusively in the obligate-antibiosis bioassay: antixenosis, adult mortality before entering the seed, and biological cycle. Significant differences were found for adult mortality before entering the seed between the maize accessions. The accessions with more mortality of initial adults were Apachito-b (93% of the infestation generation) and Cristalino-079 (90% of the infestation generation). In addition, the maize accessions with lower mortality were 8-Carreras-RP, E-Zapata-T, Bofo, and 8-Carreras-PP (Figure 2). Significant differences were found for the first generation duration between maize accessions. Almost all accessions had a similar biological cycle ranging from 47.6 to 48 days, except Gordo with 46 days (Figure 3). After 48 days from the start of the bioassay, we could not see the first-generation adults in Cristalino-079, Cristalino-282, and Cristalino-061.

In both bioassays, there were significant differences in the number of emerged adults in obligate-antibiosis and adult preference in the free-choice bioassays between maize accessions. Cristalino-079, Cristalino-279, and Apachito-b formed the group with the lowest number of emerged adults. Cristalino-282, Palomero, Apachito-b, Apachito-r, Azul, and Cristalino-279 formed the group with the lowest number of adult preferences. In contrast, the accessions: E-Zapata-T, Bofo, and 8-Carreras-RP showed a significantly higher number of emerged adults in obligate-antibiosis. In addition, 8-carreras-PP, Cristalino-061, and Gordo showed a significantly higher number of adult preferences in the free-choice experiment (Figure 4).

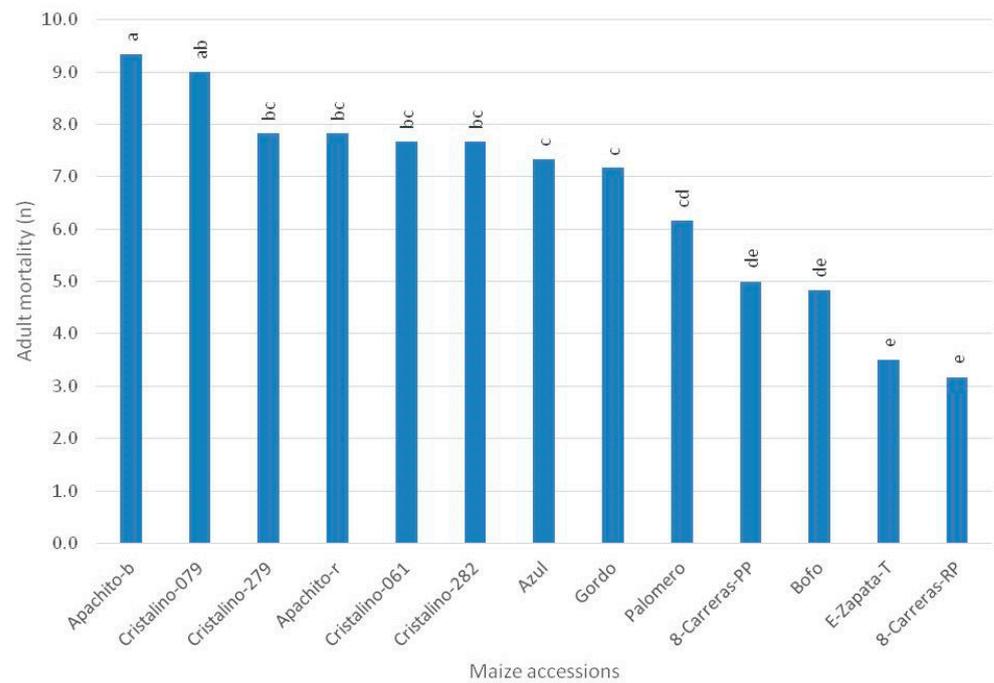


Figure 2. Adult mortality before entering the seed of thirteen maize accessions. Lsmeans followed by the same letter were not significantly different ($p < 0.05$).

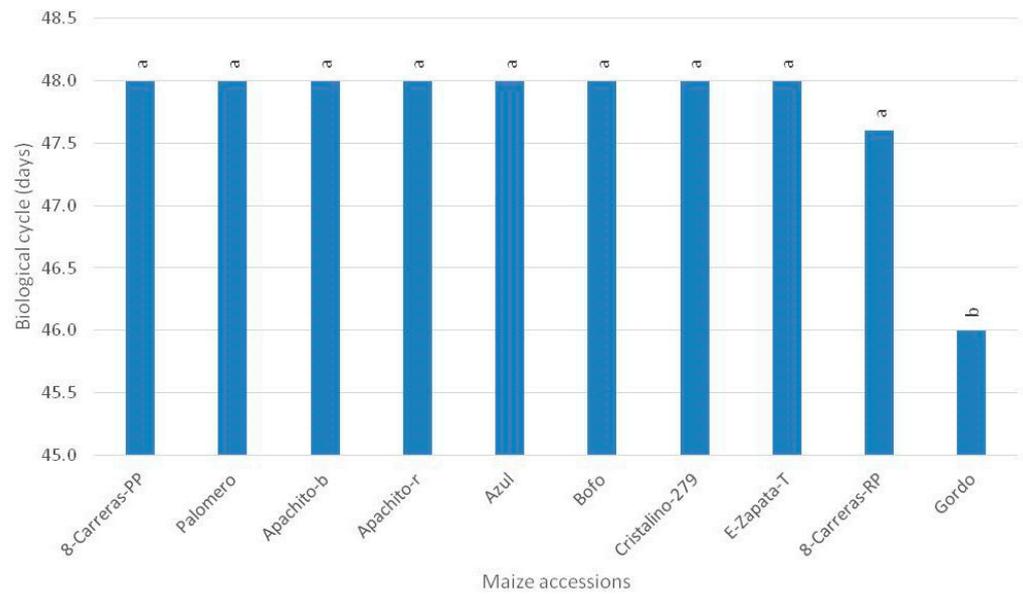


Figure 3. Biological cycle for thirteen maize accessions. Lsmeans followed by the same letter are not significantly different ($p < 0.05$).

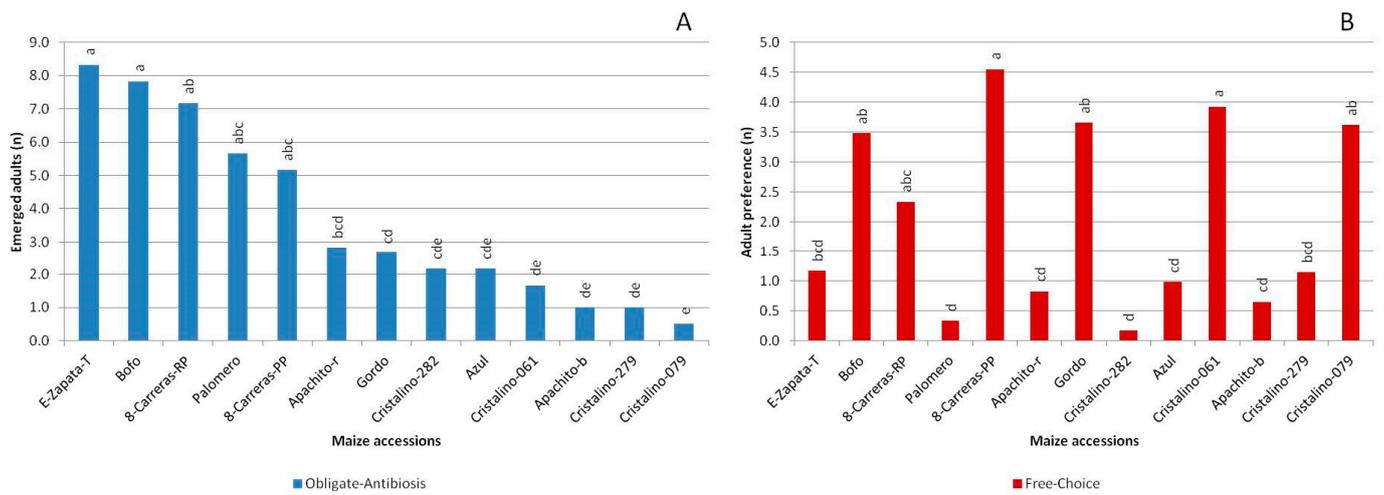


Figure 4. First-generation adults emerged from thirteen maize accessions in the obligate-antibiosis bioassays (A) and adult preference (B) in the free-choice experiment. Lsmeans followed by the same letter within each experiment are not significantly different ($p < 0.05$).

The accessions presented similar net consumptions in the bioassays of obligate antibiosis and in those of free choice, except Gordo, including the accessions from Cristalino de Chihuahua, Apachito, and Azul races, which were less damaged by *S. zeamais* and presented less net consumption (Figure 5).

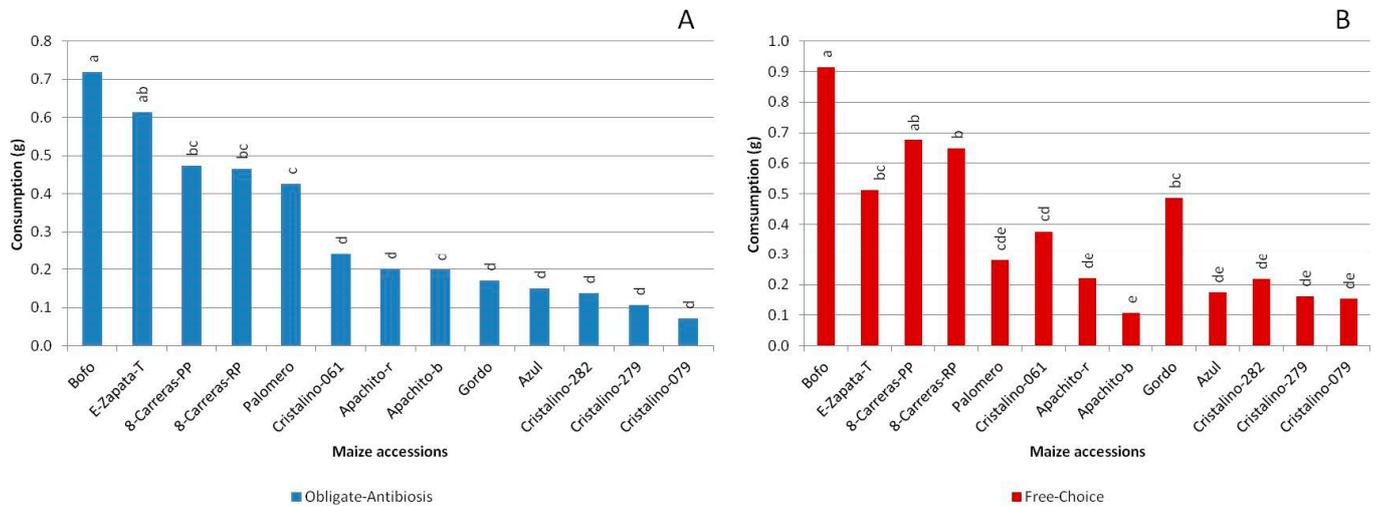


Figure 5. Net consumption (g) by *S. zeamais* on kernels from thirteen maize accessions in the obligate-antibiosis (A) and free-choice (B) experiments. Lsmeans followed by the same letter within each experiment were not significantly different ($p < 0.05$).

The varieties that showed a low net consumption also showed a low percentage of consumption. In some accessions such as Bofa, *S. zeamais* consumed a large proportion of the grain, specifically 25% in the obligate-antibiosis bioassay and 43% in the free-choice bioassay. Grain damage was larger in the free-choice than in the obligate-antibiosis bioassays (Figure 6).

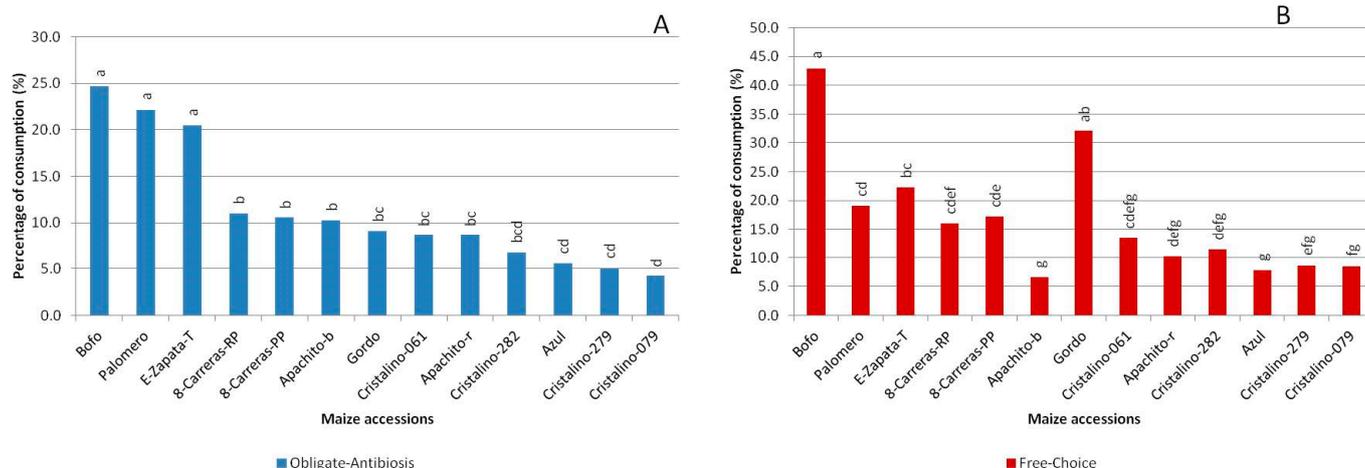


Figure 6. Percentage of kernel consumption for thirteen maize accessions in the obligate-antibiosis (A) and free-choice (B) experiments. Lsmeans followed by the same letter within each experiment were not significantly different ($p < 0.05$).

Grain color did not show significant differences for most of the traits in both bioassays. However, it appears that the yellow varieties showed a significantly higher mortality of adults before entering the seeds than the white varieties and also showed a significantly lower net consumption than the rest of the varieties in the free-choice bioassay. However, the type of endosperm did have an influence on the resistance/tolerance to *S. zeamais*. The flint varieties showed significantly higher mortality of adults before entering the seed, fewer emerged adults of the first generation in the obligate-antibiosis test, and lower net consumption and percentage in both bioassays (Table 2).

Table 2. Lsmeans of endosperm types for those traits that differed significantly.

Trait. Type of Bioassay	Endosperm Type		
	Dent	Flint	Floury
Adult’s mortality. Obligate-antibiosis	0.389 c	0.793 a	0.644 b
Emerged Adults. Obligate-antibiosis	6.9 a	2.1 b	4.2 a
Net consumption (g). Free-choice	0.612 a	0.216 b	0.525 a
Net consumption (g). Obligate-antibiosis	0.517 a	0.198 c	0.346 b
Percentage of consumption (%). Free-choice	19.9 a	12.3 b	24 a
Percentage of consumption (%). Obligate-antibiosis	14.7 a	9.5 b	12.1 ab

Lsmeans followed by the same letter within each row were not significantly different ($p < 0.05$).

As a summary, grain yield losses (measured as the percentage of consumption) were represented against *S. zeamais* antibiosis and/or antixenosis (measured as the number of emerged adults of the first and second generations). The line of regression for the percentage of consumption (dependent variable) on emerged adults (independent variable) divided the accessions into tolerant, when they had lower yield losses than expected based on the value of the independent variable, and sensitive, when the losses exceed the values marked by the line of regression. In addition, a vertical line drawn on the average of emerged adults divided the accessions into resistant, when the number of emerged adults was fewer than the average of all varieties, and susceptible, when they exceed the average (Figure 7).

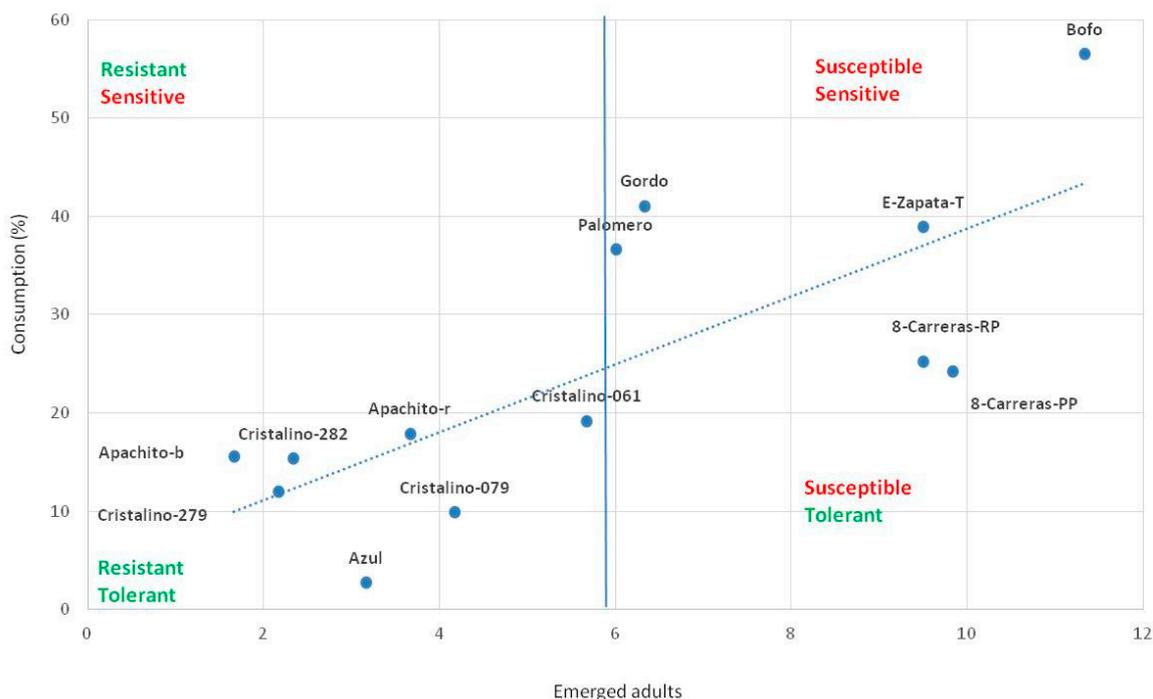


Figure 7. Relationship between percentage of consumption and emerged adults in 13 maize accessions.

A negative correlation was found between dead adults before entering the seed and 1st-generation emerged adults (-0.94) in the obligate-antibiosis bioassay (Table 3). Resistant accessions had more dead adults outside the seed and fewer adults of the 1st generation emerging from the seed. We found a negative correlation between dead adults and net consumption of -0.85 , and between dead adults and percentage of consumption of -0.61 . This means that resistant races had more dead adults outside the seed and, therefore, lower net consumption and percentage of consumption. On the other hand, a positive correlation was shown between emerged adults and consumption and percentage of consumption in the obligate-antibiosis bioassays. This means that the resistant accessions had fewer numbers of adults and lower net consumption and consumption percentage than the susceptible accessions.

Table 3. Pearson’s correlations between seed and resistance traits measured in the *S. zeamais* individuals and the seeds from Mexican maize races on which insects were fed. Above the diagonal for the free-choice bioassay and below the diagonal for obligate-antibiosis.

	Initial Weight (g)	Adult Mortality (n)	Biological Cycle (days)	Emerged Adult (n)	Net Consumption (g)	Percentage of Consumption (%)
Initial weight (g)				0.38	0.42	-0.16
Adult mortality (n)	-0.67 *					
First generation duration (days)	0.29	-0.01				
Emerged adults (n)	0.50	-0.94 *	0.15		0.58	0.40
Net consumption (g)	0.50	-0.85 *	0.26	0.95 *		0.81 *
Percentage of consumption (%)	0.02	-0.61 *	0.09	0.82 *	0.86 *	

* Significant correlations $p < 0.05$.

4. Discussion

Some efforts have been made to study differences among maize genotypes for resistance to *S. zeamais* [10,11,19]. Some genotypes were found to be highly resistant to *S. zeamais*, but the resistance of Mexican races has been scarcely explored. These maize varieties would be promising materials according to the classic theory proposed by Ehrlich and Raven [27], as maize originated from México [10]. Ehrlich and Raven [27] claimed that: co-evolution

is a dynamic process whereby plant and insect species exhibit reciprocal selective pressure. Along this evolutionary process, insects diversify their feeding habits and behaviors, whereas plants develop defense strategies against insect herbivores [27–31]. The strategies of plant defense are based on physical barriers, constitutive chemical defenses, and indirect inducible defenses including volatiles [27,28].

We have found genetic variability for resistance to *S. zeamais* among Mexican maize races considering the number of emerged adults as a proxy for resistance. However, we also see variability among maize races for tolerance. When considering a tolerant variety as one with grain yield losses (measured as the percentage of consumption) that are lower than those expected based on its resistance level [26], Cristalino-279, Cristalino-282, Apachito-b, and Azul can be considered resistant varieties. These had good performance both in the obligate-antibiosis and free-choice bioassays. On the other hand, the 8 Carreras RP, 8 Carreras PP, and Bofo varieties are susceptible to attack by *S. zeamais*. The rest of the varieties either have different behavior depending on the type of bioassay, or they showed intermediate values. Cristalino-079, Cristalino-279, Cristalino-282, and Azul are also tolerant varieties, having a low percentage of consumption and low net consumption. This occurs considering the two types of bioassays separately and when they are analyzed together. In the latter case, the varieties Azul and Cristalino-279 stand out. In present study, the percentage of consumption ranged from 5.1 in Cristalino-079 to 33.7 in Bofo. Furthermore, the consumption is higher than that observed in previous reports by Arnason et al. [10], which ranged from 0.0 to 9.6, and by Abebe et al. [11], which ranged from 4.4 to 8.3. These differences could be due to the duration of the trials, the maize varieties, and perhaps the *S. zeamais* colony used. In addition, the four accessions of the Cristalino de Chihuahua race showed high levels of resistance to *S. zeamais* and a low net consumption and consumption percentage. Therefore, this race can be used as a new source of resistance to improve varieties against the attack of *S. zeamais*, especially for Chihuahua State. In addition, this race is very productive in the area due to its adaptation. It is the most productive in Chihuahua due to its plant height, cob length, and grain yield. Furthermore, it is a good product for forage and a grain alternative in the North of Mexico [32].

It appears that the yellow varieties showed significantly higher mortality of adults before entering the seeds than the white varieties, and these varieties also showed a significantly lower net consumption than the rest of the varieties in the free-choice bioassay. The type of endosperm did have an influence on the resistance/tolerance to *S. zeamais*. The flint varieties showed significantly higher mortality of adults before entering the seed, fewer emerged adults of the first generation in the obligate-antibiosis test, and lower net consumption and percentage in both bioassays. Apparently, the flint varieties could limit the penetration of adults compared to the dent varieties. This is indirectly explained by the fewer adults of the first generation from flint varieties and the lower grain consumption. It is also important to mention that the groups are unbalanced and the genetic relationships within some groups have not been taken into account. Similar results were found by Suleiman et al. [33], who suggested that flint accessions are more tolerant than dent accessions to *S. zeamais*. Grain color does not seem to be important for resistance to *S. zeamais*. However, yellow varieties could limit the entry of adults. Accordingly, Serratos et al. [12] found that ferulic acid in grain sections is located in the pericarp and aleurone layer and is especially intense in the most resistant grain variety “Local yellow”.

High resistance in cereal grains to storage insects has been due to low adult progeny and a prolonged developmental period [9,26,34,35]. Abebe et al. [11] found that *S. zeamais* fed with the resistant variety produced low numbers of F₁ progeny, and a low percentage of seed damage and seed weight loss. Muzemu et al. [14] found that varieties with resistance to *S. zeamais* had high parent *S. zeamais* mortality, low *S. zeamais* emergence, lower grain weight loss, and low grain damage. Nhamucho et al. [36] found that parental mortality showed a significant negative correlation with the emergence of the 1st adult generation of *S. zeamais*, seed damage, and grain weight loss. In agreement with the previous studies mentioned, in the present study, we found a negative correlation between adult mortality

before entering the seed and the 1st adult generation and consumption. Both results could be explained because when adult mortality is high, the number of eggs during the oviposition period can be low and the numbers in the 1st adult generation are low too. Few eggs result in few emerged insects; therefore, the grain damage is low. The most interesting negative correlations are between the mortality of adults before entering the seed and the 1st adult generation and consumption; this means higher adult mortality outside the grain, lower 1st adult generation, and a lower level of consumption. The mortality of adults before entering the grain could be due to the pericarp of the resistant accessions, which makes it interesting to study the chemical elements, proteins, or genes expressed in that component of the maize kernel. On the other hand, a positive correlation is shown between the 1st adult generation and the net consumption and percentage. This means that the resistant accessions had fewer adults from the 1st generation and a lower net consumption and consumption percentage.

Chapalote, Naltel, Bolita, and Palomero have been reported with a high level of resistance to *S. zeamais*. On the contrary Bofo, Gordo, Apachito, and Azul have been reported as susceptible [10]. Our study agrees with previous researchers because we detected Bofo and Gordo as susceptible races. However, we disagree on the classification of Palomero and especially of Azul, which have behaved as one of the most promising accessions together with the accessions of the Cristalino Chihuahua race. However, this may be because the other studies have used other varieties than the Azul race or even *S. zeamais* from another region. In the present study, we used only one population of *S. zeamais*. The resistance of maize to storage pests is influenced by biophysical, biochemical, and genetic factors including kernel hardness [37,38], pericarp thickness [17], phenolic compounds, enzymes, and structural components of the kernel [37,39–43]. However, all these characteristics vary with the environment, making it important to evaluate and improve varieties for specific environments.

Arnason et al. [10], Abebe et al. [11], and Serratos et al. [12] have identified some maize accessions with a high level of tolerance to *S. zeamais*. We have detected varieties with resistance to two storage pests, *Sitotroga cerealella* and *S. zeamais*, and Cristalino-079, in a previous study, had only 4.5% weight loss caused by *S. cerealella* [44]. Meanwhile, in the current study, we observed 4.2% weight loss in a nine-month period of obligate feeding and 8.5% weight loss in an eight-month period under free-choice conditions. The differences in the percentage of weight loss could be due to pest diversity, exposure time, temperature, etc. Therefore, we trust that Cristalino-079 could be used in breeding programs for resistance to *S. cerealella* and *S. zeamais* [44]. In addition, the Azul race can be used for maize genetic improvement since it presents a good level of resistance to *S. zeamais*. The highly resistant maize accessions should be included in the maize breeding programs for pest resistance [19]. In fact, we are preparing a multi-parental population of eight parents (Cacahuacintle x Bofo x Cristalino-079 x Cristalino-279 x Azul x Cristalino-282 x Apachito-r x Apachito-b), including among them the resistant varieties to study *S. zeamais* resistance at the genomic level and to carry out genetic improvement in maize. Overall, we agree with López-Castillo et al. [45] and Derera et al. [46] because they have considered that the development of insect-resistant genotypes would be a sustainable alternative for pest control, especially in developing countries.

We would be interested in tolerant varieties because they have low yield loss and, at the same time, are resistant. In this way, when few adults emerge, the attack of the new generations of *S. zeamais* would be reduced. Thus, Azul and Cristalino-079 would be the best varieties.

5. Conclusions

The most resistant accession showed high adult mortality and fewer 1st-generation adults. The 1st-generation adults are an optimum trait to evaluate resistance to *S. zeamais* in corn. Net consumption is the most economically important trait because it indicates the gross amount lost in a store, while yield losses (percentage of consumption) allow studying

tolerance. The type of endosperm did have an influence on the resistance/tolerance to the *S. zeamais*. Apparently, the flint varieties showed significantly higher mortality of adults before entering the seed, fewer emerged adults of the first generation in the obligate-antibiosis test, and lower net consumption and percentage in both bioassays.

In general, the varieties of the races Cristalino Chihuahua, Apachito, and Azul showed high levels of resistance, with the Cristalino-079 (flint) and Azul (floury) accessions being the most interesting because they were also tolerant, showing low levels of consumption by *S. zeamais*. Both could be used in breeding programs to reduce losses caused by *S. zeamais*.

Author Contributions: J.C.J.-G. and G.C.-P. carried out bioassays in the laboratory. J.C.J.-G. and R.A.M. realized the statistical analysis of data. J.C.J.-G., R.A.M., A.O.-O., A.C.-R., G.C.-P., F.O.-G., D.P.-C., A.B., and P.R. realized the draft of initial manuscript. All authors have read and agreed to the published version of the manuscript.

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