

Susceptibility of native maize populations and preference of the weevil in Yucatán, México

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Abstract

One of the problems with the storage of seeds in corn is the presence of pests that reduce quality. To deal with this problem, materials with little or no susceptibility are used. The native varieties of maize in Yucatan are reservoirs of genes for resistance to storage pests. The objective of this study was to evaluate the attack of weevils (*Sitophilus zeamais* Motschulsky) in eight populations of corn and two control varieties. To determine the susceptibility, 100 seeds of each population were exposed to the presence of 100 weevils for 15 days, with 10 repetitions, the variables were number of live weevils, weight loss and percentage of damaged grain. Preference was determined by placing 100 seeds from each population and 1 000 weevils in the same container, they were stored for 30 days and repeated five times. The percentage of grain damaged by color was recorded. The highest susceptibility was presented in the populations Chichen Itza, Sac beh and Nal t'eel white, the first two registered the highest percentage of damaged grain, with 15% higher than the other populations evaluated, while the Nal t'eel White population registered 6.1% above the lower loss. The insect's preference was for white grains. The pericarp (0.495) and scutellum (0.418) showed a relationship with the presence of damaged grains. There are differences in the susceptibility and preference of the evaluated populations, which indicates variability that can be used in genetic improvement programs.

Keywords: endosperm, color preference, Nal t'eel, perforated seed, purple corn, red corn.

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Introduction

Corn (*Zea mays* L.), is one of the oldest known food grains and due to its multiple uses, it has become the most important crop among cereals in the world for its production, they are commercialized in the market over 90 million tons of corn (USDA, 2018). In Mexico, corn is the main crop, in 2017 7 230 million hectare were cultivated, of which 21.5% were irrigated and 78.5 were rainfed, with an average yield of 8.76 t ha⁻¹ and 1.87 t ha⁻¹ respectively (SIAP 2018).

In the state of Yucatán, corn is part of the daily diet, more than 80% is grown under rainstorm conditions in sowings of less than 1 ha, in slash, slash and burn systems, the harvest is mostly destined for self-consumption and to select the seeds that will be used in the next crop cycle, so it is relevant to conserve them and keep them in an optimal state of germination and free of pests (Palafox-Caballero *et al.*, 2008).

One of the main problems faced by the producer when storing the grain is the loss caused by pests and diseases, mainly the corn weevil (*Sitophilus zeamais* Motsch.), which causes losses of up to 80% (García-Lara *et al.*, 2003) and it is in the tropic regions where the greatest damage is observed, due to the environmental conditions that favor the development of the insect (Palafox-Caballero *et al.*, 2008).

At the time of harvest, it is estimated that 10% of the grains show signs of infestation and if the contamination is not controlled, the losses in the warehouse amount to 70% after six months (Cerna *et al.*, 2010). Rodríguez and Herrera (2003) report 38 species of insects that attack stored grains of corn and beans, *Sitophilus zeamais* Motschulsky being one of the most destructive.

There are economic and sustainable alternatives for the control of this pest, among the options is genetic improvement, since evaluations show that there are native materials with tolerance to the attack of the weevil (García-Lara and Bergvison, 2013), which involve various biochemical mechanisms, such as the presence of soluble peroxidases (García-Lara *et al.*, 2007) or physicochemical characteristics such as the hardness of the grain or the type and amount of starch present in the grain (García-Lara *et al.*, 2003).

The native populations of maize in Yucatan are important gene reservoirs, since farmers carry out conservation and selection practices due to their adaptive advantages to diverse environmental conditions (Antonio *et al.*, 2004); however, the degree of susceptibility of the grains to the warehouse pest caused by *Sitophilus zeamais* Motschulsky is unknown. In the Yucatan peninsula, there are native populations of maize of the 'Nal Tel' breed that show a certain tolerance to the attack of weevils; however, there is no information on the mechanisms involved.

In this context, the objective of the present study was to evaluate grains from ten maize populations of the state of Yucatán, in order to know the degree of susceptibility of the populations and preference of the weevil (*Sitophilus zeamais* Motschulsky).

Materials and methods

Vegetal material

Seeds from ten populations of corn were used, eight were collections of native materials obtained from local producers in Yucatán identified by the local name: Nal t'eel white; Nal t'eel yellow (two accessions, one of them identified as gallito yellow), Chak choc (two accessions red seed one of them identified as Chak); Nal xoy (yellow seed); Sak nal (white seed) and X ej'ub (purple seed) and two control varieties of free pollination Sac beh (white seed) and Chichen Itza (yellow seed) developed at the National Institute of Forestry, Agricultural and Livestock Research (INIFAP).

The collections increased during the spring-summer (SS) 2018 cycle at the facilities of the Uxmal Experimental Site in the municipality of Muna, Yucatán. The harvest was carried out manually, when the seed presented 14% humidity and ears were selected that did not present damage by insects or rot.

Grain handling

The seeds of each population were inspected to avoid perforated seeds, with the presence of eggs or with some stage of development of *Sitophilus zeamais* or another insect. The seeds were placed in plastic jars and stored under laboratory conditions (25 °C \pm 1 and 45% RH) until the establishment of the susceptibility and preference tests for seed color.

Sitophilus zeamais Motschulsky collection

The insect collections were made in local warehouses in the region. The insects were transported to the laboratory and about 1 000 insects were placed in plastic jars containing 500 g of corn for feeding and reproduction of the insects, the lid of the jar was perforated and covered with steel mesh to allow ventilation.

Germination and moisture of seeds of maize populations

To evaluate germination, 25 seeds from each population distributed in four repetitions were used, under a completely random design. The seeds were previously disinfected with 1% sodium hypochlorite and placed between paper towels moistened with distilled water and finally in a germination chamber at 25 °C \pm 1. Germination evaluations were carried out according to ISTA standards (2015), with a first count on the fourth day and the last on the seventh day.

The results were reported in percentage of germination of normal seedlings. Moisture was measured in 20 g of seeds from a moisture tester (Moisture check PLUSTM, Johnn Deere brand), in four replications of each population and the result obtained was reported in grams of water per kilogram of dry weight.

Seed susceptibility

100 seeds, previously weighed, plus 100 weevils for 15 days were placed in Petri dishes of three centimeters in diameter and 1.2 cm high, with perforations covered with steel mesh for ventilation. A completely randomized experimental design with 10 repetitions was used. At the end of the infestation period, the live insects were counted and the number of grains perforated or with galleries was recorded visually. The results were expressed in percentage of live weevils and percentage of damaged seed, finally, the seeds of each repetition were weighed and the percentage of weight loss was obtained by difference.

Seed color preference

A sample of 100 seeds was obtained from each maize population, which were placed in a 2 L plastic bottle, with perforated lid and covered with steel mesh to allow ventilation of the insects, while 1 000 weevils were added, closed and stored for one month.

The experiment was set up in a completely randomized design with five replications. At the end of the test, the seeds were separated by color and the damaged seeds, with perforations or galleries, were counted. The result was expressed as a percentage of damage.

Structural composition of the seeds

10 seeds were taken from each corn population, weighed and placed in beakers with distilled water at 75 °C for 15 min, after this time the seeds were removed from the water and excess water was removed. With the help of a scalpel, the pedicel, pericarp and embryo were detached together with the scutellum, leaving only the part that corresponds to the endosperm.

With the help of a drill (type Dremel model 300) the crystalline and mealy endosperm of each seed was separated. All the structures obtained from the seeds were dried in an oven at 80 °C for 48 h and subsequently weighed and the result was expressed as a percentage. This phase of the study was carried out in two replications.

Statistical analysis

The results expressed as a percentage were transformed with the arcsine function and later the analysis of variance and comparison of Tukey means ($p \leq 0.05$) were used using the SAS statistical package version 9.4.

Results and discussion

The seeds of all the corn populations maintained the germination in general average of 82%, with the Sak nal population standing out and the one with the lowest percentage was Chak with a difference of 14%, with respect to humidity the populations registered differences having Chichen Itza and Sac beh with the highest humidity, while the gallito yellow registered 11.9% (Table 1).

Table 1. Comparison of means of the germination response and moisture content of ten corn populations.

| Population | Response variable | | | |
|-----------------|-------------------|---|--------------|-----|
| | Germination (%) | | Humidity (%) | |
| Gallito yellow | 80 | a | 11.9 | d |
| Nal t'eel white | 79 | a | 12.08 | cd |
| Chak choc | 83 | a | 12 | cd |
| X'ejub | 80 | a | 12.2 | cd |
| Chak | 75 | a | 11.98 | cd |
| Nal xoy | 86 | a | 12.6 | bc |
| Nal teel yellow | 84 | a | 12.9 | b |
| Chichen Itza | 87 | a | 13.93 | a |
| Sac beh | 79 | a | 13.73 | a |
| Sak nal | 89 | a | 12.3 | bcd |
| HMD | 25.6 | | 0.68 | |

HMD= honest minimum difference. Means with the same letter in each variable in the columns are not statistically different (Tukey, $p < 0.05$).

Although there is no evidence on the preference of the weevil for seeds with the live germ, Lara *et al.* (2018), reveal that some structures of the maize seed influence the behavior and interaction of the insect with the seed, in their study they detected the presence of chemical components in the epicuticle that influenced the recognition and attraction as a source of food and reproduction, the presence of feluric and coumaric acid substances among others that influence attack tolerance, many of these systems work more effectively in living organisms.

On the other hand, it has been reported that seeds stored at low moisture content reduce the emission of CO₂ and O₂, which is unfavorable for the survival of insects (García-Leños *et al.*, 2007). Seed moisture is an important variable for storage, according to Cespon *et al.* (2015) the optimal conditions to maintain the integrity of warehouse corn is 12% humidity (120 gH₂O kg⁻¹ps) with relative humidity less than 70%.

When the seed is stored with higher humidity there is a risk of the presence of fungi and yeasts; however, the storage temperature is also essential for the control of pest insect populations. It is reported that even with seed humidities lower than optimum, insects can appear when storage temperatures exceed 20 °C, between 25 and 35 °C the growth rates of these populations soar (Mansoor-ul *et al.*, 2017).

These drawbacks directly affect the physiological quality of the seed, considerably reducing germination and vigor due to the fact that there may be a high presence of insects that compromise vital structures such as scutellum and embryo (Tefera *et al.*, 2011).

Likhayo *et al.* (2018) they observed that high temperature and humidity of the seed during storage contribute to an increase in weevil populations, finding up to 1 273 adults per kilogram of seed, so this is an important reason to carry out storage in adequate containers and with the correct humidity and temperature conditions, which reduce or minimize the growth of these populations.

Seed susceptibility

Differences were detected in the susceptibility of the seeds to the attack of the weevil ($p \leq 0.05$), the populations with the greatest affectation, in terms of percentage of damaged seed were Chichen Itza and Sac beh which presented 15.4 percentage points above the less affected material (Nal t'eel yellow) (Table 2)

Regarding the percentage of live weevils, at the end of the evaluation, there were no differences and most populations conserved more than 50% of live insects; however, in terms of weight loss, the Nal t'eel white sample registered the highest percentage with 6.1% higher than the Sak nal population, which only had a 2.9% reduction.

Table 2. Averages of the variables for susceptibility in each evaluated maize population.

| Population | Damaged grain (%) | Live weevils (%) | Weight loss (%) |
|------------------|-------------------|------------------|-----------------|
| Sak nal | 24.3 bc | 70 a | 2.9 c |
| X'ejub | 25.1 bc | 64.8 a | 3.4 bc |
| Gallito Yellow | 20.1 c | 57.2a | 4 bc |
| Nal xoy | 26 bc | 67.3 a | 5.6 abc |
| Chak choc | 30.4 ab | 69.8 a | 4.8 bc |
| Nal t'eel white | 31.3 ab | 68.2 a | 9 a |
| Nal t'eel yellow | 32.6 ab | 70.4 a | 6.2 ab |
| Chak | 32.3 ab | 65.3 a | 4.8 bc |
| Chichen Itza | 35.5 a | 85.7 a | 6.2 abc |
| Sac beh | 35.4 a | 68.2 a | 6.62 ab |
| DSH | 9.1 | 24.7 | 3.5 |

HSD= honest significant difference. Means with the same letter in each variable in the columns are not statistically different (Tukey, $p < 0.05$).

The results obtained agree with that reported by Caneppele *et al.* (2003) who report weight loss in maize infested by weevil at an average of 0.36% per day; Derera *et al.* (2014) record weight losses that can range from 19 to 56% after five months of infestation. Some authors indicate that these losses are related to the type of material, the amount and type of endosperm that confer particular characteristics such as hardness (Figuroa *et al.*, 2013; García-Lara and Bergvinson, 2007).

Seed color preference

The populations were separated into four groups, of which they presented significant differences ($p \leq 0.05$), the white color presented the highest amount of damaged seeds, surpassing the rest of the colors. The least preferred color was purple seed, with a difference of 14.6% with respect to white; yellow and red maize registered 9.6 and 13.6% difference with respect to the one with the greatest affectation (Figure 1).

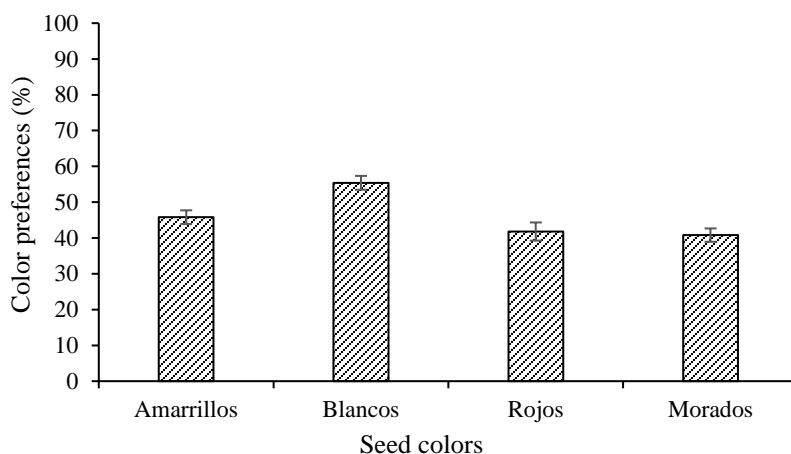


Figure 1. Percentage of damaged grain according to the preference of *Sitophilus zeamais* by grain color.

According to Groote *et al.* (2017) *Sitophilus* shows a preference for colored and small seeds (approximately 8 mm); however, the answer in the present study was the opposite, which may be due to the fact that these authors used artificial colorants.

On the other hand, Nwosu (2016) observed that the behavior of *Sitophilus zeamais* is conditioned by the susceptibility of the material, the preference of oviposition is in highly susceptible materials, with high levels of protein and starch. This same behavior was observed by Abebe *et al.* (2009) who related the susceptibility to the hardness of the seed, in hard seeds the insects only fed, but there was no oviposition, therefore the insect population was considerably reduced.

No more reports have been found that indicate the preference of the insect for a color, however, the color of the seeds is related to the presence of compounds that affect the preference of the insect. In the individual evaluation of insect damage (Table 2), it is observed that within the materials that recorded the highest percentages of damaged seed are red (Chak choc and Chak) and yellow (Nalt'eel and Chichen Itza), for what is necessary to perform the analysis of the components of these populations to determine what causes their preference.

On the other hand, it is pertinent to highlight that one of the populations evaluated Nal t'eel yellow registered the lowest percentage of damaged seed (Table 2), this coincides with that reported by Arnason *et al.* (1994); García-Lara and Bergvinson (2013), who reported that populations of Nal t'eel (Gallito) registered resistance to the attack of weevils; however, it is clear that within the Nal t'eel population there is variability with respect to resistance or tolerance to *Sitophilus*.

Structural composition of the seeds

The endosperm and the embryonic axis are one of the structures that *Sitophilus zeamais* prefers and correspond where the majority of proteins and starches are concentrated, therefore they directly influence the susceptibility of populations (Nwosu, 2016). Differences ($p \leq 0.05$) were registered in all the evaluated structures.

The populations with the highest seed weight were Sak nal and Nal xoy, while those with the lowest weight were gallito yellow and Nal t'eel white, the latter stand out in pedicel percentage. Regarding the percentage of pericarp, the Sac beh and Chak choc materials stood out from the rest, which indicates that the covering of these materials is thicker.

The largest scutellum was recorded by Chichen Itza followed by Chac and Sac beh. Nal t'eel yellow yielded the highest percentage of crystalline endosperm, while X'ejub presented mealier endosperm followed by white Nal t'eel, Sac beh and Chiche Itza (Table 3).

Table 3. Means of the structural composition of the seed of 10 varieties of corn.

| Population | Grain weight (g) | Pedicel (%) | Pericarp (%) | Scutellum (%) | Crystalline endosperm (%) | Floury endosperm (%) |
|------------------|------------------|-------------|--------------|---------------|---------------------------|----------------------|
| Sak nal | 3.2 a | 0.53 ef | 5.68 bc | 9.7 bcd | 46.95 abc | 36.05 abc |
| X'ejub | 2.35 de | 0.4 f | 5.3 bc | 8.05 d | 44.8 bc | 40.35 a |
| Gallito yellow | 1.6 f | 1.29 a | 5.16 bc | 10.65 abc | 48.5 abc | 32.05 bc |
| Nal xoy | 3.25 a | 0.99 b | 5.4 bc | 10.9 abc | 51.3 ab | 29.75 c |
| Chak choc | 2.15 e | 0.8 cb | 8.2 ab | 9.25 cd | 49.45 ab | 30.65 c |
| Nal t'eel white | 1.2 g | 1.2 a | 6.56 abc | 9.92 abcd | 41.2 c | 38.8 ab |
| Nal t'eel yellow | 2.7 cb | 0.76 cd | 6.2 bc | 8.95 cd | 53.05 a | 30.85 c |
| Chak | 2.65 cb | 0.58 def | 6.15 bc | 11.6 ab | 47.9 abc | 32.6 bc |
| Chichen Itza | 2.6 cd | 0.58 def | 6.2 bc | 11.9 a | 46.2 abc | 33.8 abc |
| Sac beh | 2.9 b | 0.66 cde | 8.95 ab | 11.4 ab | 41.35 c | 36.25 abc |
| DSH | 0.25 | 0.2 | 2.11 | 2.06 | 7.88 | 7.81 |

DSH= honest significant difference. Means with the same letter in each variable in the columns are not statistically different (Tukey, $p < 0.05$).

When observing the results of the percentage of damaged seed (Table 2), it is evident that X'ejub, despite excelling in the percentage of mealy endosperm, did not highlight the percentage of damaged seed, on the other hand, the populations that coincide with large scutellum (greater than 11%) and percentages of mealy endosperm greater than 30%, with the exception of X'ejub, registered percentages of damaged seed greater than 30%.

According to the results of the correlation, the association index of the mealy endosperm with the percentage of damage was -0.049, while for the crystalline endosperm it was obtained -0.167, these results coincide with that obtained by Bourne-Murrieta *et al.* (2014). They found low correlation between seed hardness and weight loss ($r = 0.15$) and with the percentage of damaged grain ($r = 0.2$), which concludes that grain hardness is not a determining factor in preference, but the

relationship obtained in this study, between the embryo and pericarp structures for damaged seed, the indices were 0.418 and 0.495 respectively, which indicates that these structures influence the preference of the weevil.

According to García-Lara *et al.* (2004) mention the importance of phenols in tolerance to warehouse pests and these compounds have been located more frequently in the pericarp and scutellum. Cabrera-Soto *et al.* (2009) correlate the amount of phenols, grain color and hardness, while Bourne-Murrieta *et al.* (2014) reported that the more opaque they are, the less damaged they are.

These results suggest that there are different physical, physiological and biochemical mechanisms that interact and contribute to generating susceptibility of the seed or increasing the preference of the insect, either for reproduction or feeding. Cázares-Sánchez *et al.* (2015) analyzed 41 native populations of Yucatan maize and recorded high protein contents, particularly the populations called Nal t'eel, while the populations of Xnuuk nal presented high fiber contents, elements that have been reported as attractive to *Sitophilus*, which coincides with what was observed in the present study, where the materials with the greatest affectation belong to these groups.

According to Khakata *et al.* (2018) there is the possibility of selecting materials for genetic improvement based on their response to weight loss caused by the *Sitophilus* attack. On the other hand, it is reported that native materials are the best sources for genetic improvement for resistance to *Sitophilus*, since the losses that are registered are below the records of hybrid materials (Maggioni *et al.*, 2016).

This information coincides with what was found in this study, the control varieties (Chichen Itza and Sac beh) registered the highest amount of damaged grains, around 15.4% above the gallito yellow. Regarding the weight loss, this was 3.7% percentage, higher than the material with the lowest loss (Sak nal), surpassed only by Nal t'eel white (Table 2).

Conclusions

The white materials (Sac beh and Gallito white) presented susceptibility to the weevil (*Sitophilus zeamais* Motschulsky) as they registered greater weight loss and damaged seed. The X 'ejub (purple) population and the red populations, despite having a high percentage of mealy endosperm, did not have a preference for feeding and reproduction insects.

A different response was observed in the Nal t'eel materials (white and yellow) which indicates variability in the response to tolerance to weevil. The type of endosperm was not determining in the susceptibility or preference of the insect; however, the scutellum and pericarp influence preference.

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