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Protective Effects of Resistant Beans on Maize Damage by *Mythimna unipuncta* and *Sitotroga cerealella*

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Abstract: *Sitotroga cerealella* is the main pest of maize grains and *Mythimna unipuncta* is a generalist defoliating pest that often attacks maize. Two Tepary bean (*Phaseolus acutifolius*) genotypes, Tepary pinto yellow (Yellow T) and Tepary pinto negro (Black T), repel pest attacks on beans and, since beans and maize have co-evolved in America, we hypothesized that they could also protect maize against the attack of similar pests. Yellow T showed antixenosis against larvae in young maize plants. Pinto Saltillo (P. Saltillo) (*P. vulgaris*) and Yellow T controlled the consumption of maize leaves. No significant differences were found between these two genotypes for *Mythimna unipuncta* growth in bioassays with artificial diets. We found significant differences for number of holes caused by *Sitotroga cerealella* attack in maize grains being lowest for Yellow T (43.3) and highest for PS-AZH-15 (*P. vulgaris*) (53.6). Number of adults was lowest for Yellow T and PS-AZH-15 and highest for Black T. Yellow T showed antixenoxis against *Mythimna unipuncta* and *Sitotroga cerealella* and can be used for partially controlling these maize pests. Furthermore, Yellow T was consistently superior to the control for both maize pests and could be used for future studies of maize protection; suggesting that there is a clear genetic regulation of this antixenotic effect. The protective mechanism has not insecticide properties; conversely, we believe that there could be substances that increase the hardness or reduce the palatability of tissues.

Keywords: Phaseolus Vulgaris, Phaseolus Acutifolius, Maize, Zea Mays, Mythimna Unipuncta, Sitotroga Cerealella, Antixenosis

1. Introduction

Maize (*Zea mays* L.) is the crop with greatest production in the world. Mythimna unipuncta Haworth (Lepidoptera: Noctuidae) cause devastating damages to several crops, including maize [1], and has developed alleles for resistance to *Bt* corn [1]. The angoumois grain moth, *Sitotroga cerealella* (Lepidoptera: Gelechiidae) is one of the main insect pests of stored grains around the world [2]. Was reported an overall mean loss in weight per grain of 18.3% and 2.0 insects emerging of *Sitophilus zeamais* per seed on average [3]. In tropical countries, the losses can reach 50% [4]. Furthermore, the often inadequate storage facilities can cause enormous losses on stored grains.

There are different mechanisms of defense against the attack of insects: antixenosis, antibiosis and tolerance [5]. Antioxenosis reduces the probability of contact between pests and plants [6]. Chemical control based on the use of synthetic pesticides has become the most widespread pest management strategy [7]. But pesticides can cause harmful effects on health and environment. The development of effective pest management strategies involving ecologically safe methods would benefit local populations at competitive costs [7]. Screening crop diversity for insect-resistance can be a valuable strategy, especially in developing countries where small farmers do not have access to commercial pesticides.

Co-evolution between crops and pests often leads to the development of plant resistance characters that limit the survival or the damages caused by their pests [8]. Maize and beans have the same center of origin and together have co-evolved with common defoliant insects and pests of grains. Two *P. acutifolius* genotypes, Yellow T and Black T, have shown resistance to *Acanthoscelides otectus* [9], and previous observation indicate that Yellow T has also antixenosis and antibiosis effects against *Brachystola magna*, one of the most important defoliating pest of beans in Mexico North. We hypothesize that bean genotypes could have developed antixenosis against these pests and that could have a protective effect on maize. Our objective was to study if the antixenosis effects detected in Yellow T and Black T could also protect maize against the attack of *Mythimna unipuncta* and *Sitotroga cerealella.*

2. Materials and Methods

We carried out one experiment with *Mythimna unipuncta* in a greenhouse and one bioassay in a laboratory, while with *Sitrotoga cerealella* we made one bioassay in a laboratory. For the experiment in greenhouse with *M. unipuncta* we used Yellow T and Black T, as resistant genotypes [9], and P. Saltillo as susceptible control [10, 11]. In the bioassay with *M. unipuncta* we used as resistant genotypes Yellow T and Black T, and maize polenta as control. And finally for the bioassais with *S. cerealella* we used Yellow T and Black T as resistant genotypes, PS-AZH-15 as susceptible control [12], and treatment control with maize without beans (Table 1).

Table 1. *Characteristics of four bean genotypes seed origin from INIFAP evaluated for antixenosis, and antibiosis to M. unipuncta and S. cerealella.*

Genotype	Race	Species	Growth habit	Seed size
Tepary pinto yellow (Yellow T)	Cultivated tepary	*P. acutifolius*	III	Small
Tepary pinto black (Black T)	Cultivated tepary	*P. acutifolius*	III	Small
Pinto Saltillo (P. Saltillo)	Durango	*P. vulgaris*	III	Medium
PS-AZH-15	N. Granada	*P. vulgaris*	II	Large

We evaluated the effects of aqueous extracts from leaves of Yellow T, Black T and P. Saltillo as protective treatments against *M. unipuncta* attack in maize young plants in a greenhouse experiment carried out in September 2016. The maize inbred line A662 was chosen as previous observations have shown its susceptibility to *M. unipuncta*. Aqueous extracts of the two *P. acutifolius* and the *P. vulgaris* genotypes were prepared in 2 L Erlenmeyer flasks by immersing 10 g of previously air-dried and slashed leaves in 500 mL of distilled water (1:50 w/v). Flasks were left in the dark at room temperature for 24 h, and gently soaked every 8 h. The aqueous extract was filtered through muslin cloth bags until no visible plant material remained. The three aqueous extracts obtained were immediately used for assay.

Pots of 13 cm diameter were filled with peat, and three seeds of *Z. mays* A662 were sown in each pot and thinned to 1 plant per pot after emergence. Three aqueous extracts of bean leaves (Yellow T, Black T and P. Saltillo) plus a control treatment with distilled water were established in four mesh boxes, each of them containing a different treatment, and nine pots were placed in each box. The assay started when maize reached the four-leaf stage (the 9th of September, 2016). Medium-size larvae of *M. unipuncta* were collected in the field the same morning, and immediately each maize plant was infested with seven larvae placed between the leaves. A first treatment consisting of spraying the maize plants with 100 mL of each aqueous extract per box was immediately applied. Two subsequent applications were made at 3-day intervals (the 12th and the 15th of September). Eleven days after infestation, we recorded leaf damage using a subjective visual scale from 1 to 9 (in which 1 indicated complete damaged and 9 indicated no damage) for each maize plant. The proportion of remaining (not eaten) leaf tissue (leaf ratio), the individual leaf weight (g), the number of live larvae, and the number of pupae at the end of the experiment were also recorded.

A bioassay consisting on rearing *M. unipuncta* larvae with artificial diets containing dried and powdered leaves of Yellow T and Black T was performed in a laboratory at 22°C. Diets were prepared according to the following recipe: water (500 mL), benzoic acid (1.25 g), agar (13 g), wheat germ (20 g), beer yeast (21.5 g), maize polenta (73 g), ascorbic acid (3 g), nipagin (0.5 g), mixture of salts (0.775 g), and powdered leaves of bean (Yellow T or Black T) (7 g). A control diet was prepared by replacing powdered leaves of bean with maize polenta. Thirty-one larvae were fed with each type of diet in 13 cm diameter Petri dishes. The bioassay was performed from September 27, 2016 to October 10, 2016 and two times per week, larvae weight and larvae survivals in days were recorded.

The effects of mixing grains of *P. acutifolius* and maize on *S. cerealella* were tested in plastic boxes of 0.5 litters at 22°C in laboratory. The experiment began on September 30, 2016 and ended on December 5, 2016. Seeds of two *P. acutifolius* genotypes Yellow T, Black T and one *P. vulgaris* as susceptible control (PS-AZH-15), were mixed with two lines of maize (EP66, European flint with white seeds, and Oh545, Corn Belt Dent with yellow seeds). Treatments consisted of plastic boxes of 0.5 L where 50 seeds of EP66, and 50 seeds of OH545 were placed. For the *P. acutifolius* treatments, 20 seeds of each genotype were placed in different boxes, plus 30 seeds of maize infected with *S. cerealella* acting as inoculum. Control treatment consisted on boxes containing maize grains without beans. Each treatment was replicated four times. After one week, the numbers of *S. cerealella* adults were counted and were removed to wait for the new generation of adults. The variables measured, at the end of bioassay on December 5, 2016, were number of holes in the seeds of each maize inbred line and number of emerged adults.

For each experiment, analyses of variance were performed with treatments and genotypes as fixed effects and repetitions and its interactions as random effects. Means were compared by using the Fisher's protected Least Significant Difference.

Repeated measures analysis was used to analyze the weekly weight measurements of larvae. A growth curve of weight on time was estimated for each treatment, and homogeneity of linear and quadratic coefficients was tested for each pair of treatments. The analysis was made using the MIXED procedure of SAS [15]. All factors were considered random except treatment which was considered fixed. Finally, to analyze larval survival, the Kaplan–Meier estimates of the survival function were calculated for each treatment, and curves were compared using the log rank test [14]. Survival functions were significantly different if they deviated from the expected values of the null hypothesis (that survival functions are equivalent in all treatments). The statistic determines whether differences between survival functions are significantly different at any probability level, thus indicating that larvae have significantly different survival between treatments (LIFETEST procedure of SAS). Missing larvae were censured for larval survival analysis, which means that the analysis considered that larvae lived at least until they disappeared. When larvae reached pupal stage, the larvae survival was scored as reaching the end of the experiment.

3. Results

Significant differences were found for leaf ratio, leaf damage and individual leaf weight. No differences were found for number of live larvae, and number of pupae. The genotype with the lowest proportion of eaten leaves was P. Saltillo, followed by Yellow T, which was not significantly different (Figure 1). The highest leaf damage was found for Black T and control treatments that were not significantly different. Nevertheless, the proportion of eaten leaves with Black T was twice that of the control treatment and was not significantly different from that of Yellow T.

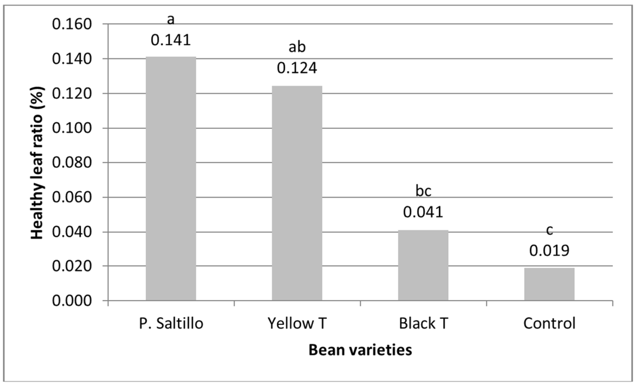

Figure 1. *Healthy leaf ratio (%) of maize plants treated with extracts of three bean genotypes under infestation of Mithymna unipuncta in greenhouse. Means followed by the same letter are not significantly different (P= 0.05) (LSD=0.094).*

Damage scale followed a similar pattern than healthy leaf ratio, although differences among treatments were of lower magnitude (Figure 2). The genotype with lowest leaf damage scale was Yellow T, followed by P. Saltillo, which were not significantly different. Control and Black T had the worst values on damage scale, being Black T not significantly different from P. saltillo.

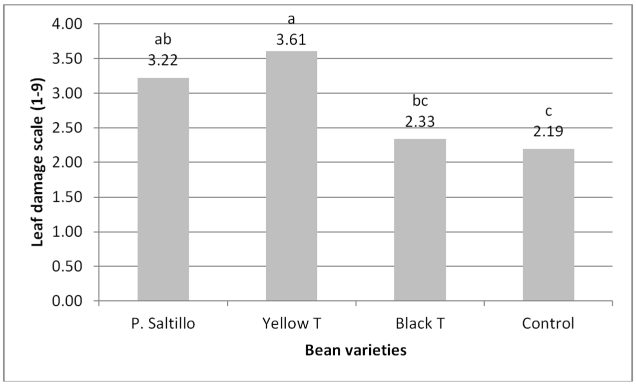

Figure 2. *Leaf damage scale (1-9) of maize plants treated with extracts of three bean genotypes under infestation with Mithymna unipuncta in a greenhouse. Means followed by the same letter are not significantly different (P= 0.05) (LSD=0.977).*

Individual leaf weight was highest for Yellow T, P. Saltillo, and Black T treatments, which were not significantly different (Figure 3). The control treatment was significantly below P. Saltillo and Yellow T, although not significantly different from Black T. No significant differences were found for number of live larvae per plant or for pupae per plant. Overall, the genotype with best control of *M. unipuncta* damage was Yellow T.

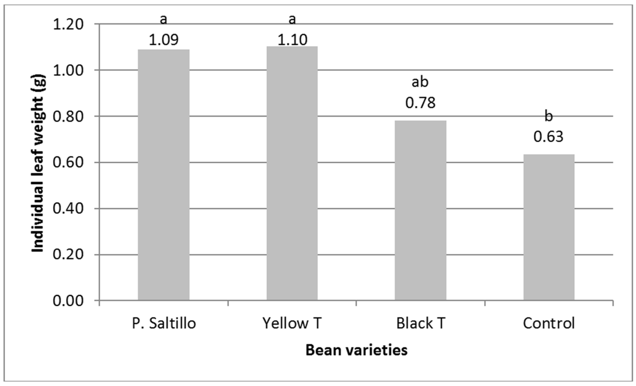

Figure 3. *Individual leaf weight (g) of maize treated with extracts of three bean genotypes under Mithymna unipuncta infestation a greenhouse. Means followed by the same letter are not significantly different (P= 0.05) (LSD=0.332).*

No significant differences were found between genotypes for larval weight, and the repeated measures analyses were not significant (data not shown). Differences were neither significant for survival probability (0.36) in days (data not shown), although the trend confirmed previous results, as larvae lived longer (-0.66) under Yellow T treatment than in the control (0.33) and Black T (0.33) treatments.

Significant differences were found between treatments for number of holes in the maize grains. The treatment with the lowest number of holes in maize grains caused by *S. cerealella* was Yellow T, while the number of holes was highest for PS-AZH-15; moreover, with this treatment, there were more holes than in the control (Figure 4). The number of holes in maize grains under Black T treatment was significantly higher than for Yellow T treatment, but it was also significantly lower than under PS-AZH-15 treatment. Differences among treatments for number of adults emerged were not significant (data not shown).

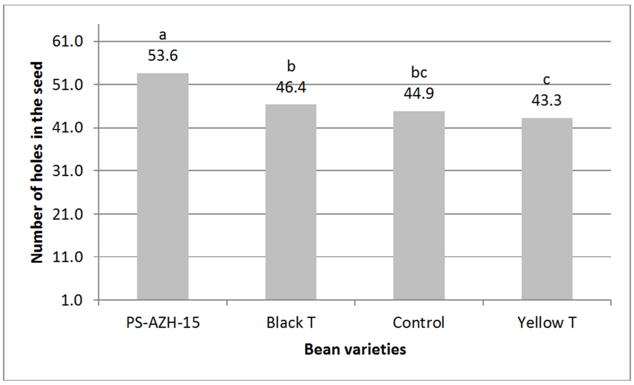

Figure 4. *Number of holes in maize seed with three bean genotypes under Sitotroga cerealella infestation in a greenhouse. Means followed by the same letter are not significantly different (P= 0.05) (LSD=2.57).*

4. Discussion

P. Saltillo had protective effects against leaf feeding by *M. unipuncta*. P. Saltillo was obtained from a multiple cross performed in CIAT and selected and released in Mexico [10]. The origin and the environment where this genotype was selected could explain why P. Saltillo could have repellence effects against defoliating insects as *M. unipunta* in this study.

Conversely, the other *Phaseolus vulgaris* genotype, PS-AZH-15, had a stimulating effect for seed feeding by *S. cerealella*. PS-AZH-15 came from a cross between P. Saltillo and Azufrado Higuera, this last variety is one of the most nutritious beans in Mexico [13]. We speculate that the possible mechanisms for stimulating the reproduction and consumption of maize by *S. cerealella*, could be related to its high nutritional value.

Yellow T is a very interesting genotype because a previous study has found that this bean has resistance to *Acanthoscelides otectus* [9], and previous observation indicate that it has also resistance and antixenosis effects against *Brachystola magna*, one of the most important defoliating pest of beans in North Mexico. Contrarily, the other *P. acutifolius* genotype, Black T, and the *P. vulgaris* genotype, P. Saltillo, behaved as susceptible in this bioassay.

Yellow T is one of the most interesting genotypes for future studies because it has resistance to stored grain pests of beans as *A. obtectus* and defoliating pest as *B. magna*. Furthermore, our results suggest that Yellow T can protect maize against a defoliating pest as *M. unipuncta*, and a seed pest as *S. cerealella*.

Further research could try to figure out optimum doses of leaf extract for using Yellow T as natural protectant against maize pests. It would also be interesting to test whether there are antioxenosis in adults, when maize has been sprayed with macerated extracts of P. Saltillo or Yellow T. In other words if the application of macerates causes antixenosis in the adults and therefore do not lay eggs in the protected maize. Also another possibility for short-term control is to test whether the associated cultivation of Yellow T or P. Saltillo with maize helps to protect against *M. unipuncta* and *S. cerealella*.

5. Conclusion

Yellow T showed antixenoxis effects against *M. unipuncta* and *S. cerealella* and it can be used as natural repellant for controlling some pest of maize. The protective mechanism has not insecticide properties, as it does not affect larvae survival; conversely, it decreases feeding rate, indicating that we should look for substances that increase the hardness or reduce the palatability of tissues.

Interestingly Yellow T was consistently the best genotype for controlling both pests; suggesting that there is a clear genetic regulation of this antixenotic effect.

Considering the *P. vulgaris* genotypes, P. Saltillo also has some protective effects that could be worthwhile to study, while PS-AZH-15 has some stimulating effects for *S. cerealella*.

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