Synergistic effects of insect-resistant maize and Teretrius nigrescens on the reduction of grain losses caused by Prostephanus truncatus (Horn.)

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ABSTRACT

Prostephanus truncatus is a pest that causes serious losses in stored maize (Zea mays L.) especially in developing countries. This research was conducted to investigate the use of post-harvest insect resistance maize in combination with biological control of P. truncatus by the predator Teretrius nigrescens to reduce maize storage losses. We studied the population dynamics of P. truncatus with and without a predator in combination with susceptible maize and resistant maize to insects under laboratory conditions. This study confirms that P84c3 is a resistant variety against P. truncatus. maize resistant kernels had a reduction of 30% losses in comparison with susceptible kernels. Significant and favorable interactions were observed between P84c3 maize and presence of T. nigrescens. A dramatic reduction of 80% in progeny number, 81% grain weight loss, and 75% frass production caused by P. truncatus was observed when the predator was used in combination with P84c3. Resistant maize reduced the prey development time and consequently the insect density allowing the predator to control more effectively the population. Prey/predator proportion on resistant maize was significantly higher in comparison with susceptible kernels; thus, giving a more effective pest population control by the predator. These results demonstrated that the combination of post-harvest insect resistance maize with the predator T. nigrescens reduces grain maize losses by P. truncatus.

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1. Introduction

Post-harvest losses of maize (Zea mays, L.) due to storage insect pests such as the larger grain borer, Prostephanus truncatus (Horn) (Coleoptera: Bostrichidae), are an increasingly important constraint of food security worldwide (Borgemeister et al., 2003; FAO, 2009). P. truncatus is a woodborer and an invasive post-harvest insect pest native form Mesoamerica that damages maize granaries and storage facilities (Farrell and Schulten, 2002).

This insect has acquired the status of a serious pest in several of North, Central and South America and Asian countries. In the African continent, P. truncatus is presumably a major post-harvest insect pest (Markham et al., 1994; Tigar et al., 1994; Kumar, 2002; Gueye et al., 2008). This insect was accidentally introduced to Tanzania in the late 1970s and to Togo in the early 1980s (Golob and Hodges, 1982), from where it spread rapidly to 16 other countries in tropical Africa. It is anticipated that this invasive pest will eventually establish in all of sub-Saharan Africa (Farrell, 2000; Farrell and Schulten, 2002) with enormous consequences.

Studies in Africa and Latin America have shown that subsistence farmers in tropical and subtropical agroecologies experience 10–45% maize losses caused by P. truncatus attack (Markham et al., 1991; Tigar et al., 1994; Bergvinson, 2001). P. truncatus has gradually increased farm-losses from 5 to 30% after it was introduced in Africa (Borgemeister et al., 2003). Furthermore, P. truncatus is characterized by 35 weeks longevity, high flight activity, reproduction under wide spectrum of conditions and tolerance to insecticides such us pyrethroids or inert dusts (Fadamiro, 1997; Golob, 2002; Nansen and Meikle, 2002; Athanassiou et al., 2006, 2007).

New enhanced diatomaceous earth formulations, such as DEA-P, a mixture of crystalline silica and abamectin, proved to be very effective, when alternative control strategies are followed against this species (Athanassiou et al., 2006, 2007). Among other alternative control methods, parasitoid use and host plant resistance have been developed and described previously (Golob, 2002). A biological control agent for P. truncatus is the natural enemy and predator Teretrius nigrescens (Lewis) (Coleoptera: Histeridae). This
beetle attack a wide variety of appropriately sized prey encountered in its environment (Meikle et al., 2002) and is found commonly associated with *P. truncatus* in Mesomerica (Borgemeister et al., 1997). Studies of its biology demonstrated that *T. nigrescens* exhibits a strong preference for feeding on *P. truncatus* in choice tests indicating a relatively close association between the predator and its prey (Ayertey et al., 1999). Adults and larvae of *T. nigrescens* feed voraciously on *P. truncatus* eggs and larvae and develop at a comparable rate to *P. truncatus*. However, *P. truncatus* has a comparatively low fecundity rate compared to *T. nigrescens* (Holst and Meikle, 2003). Releases of *T. nigrescens* in six African countries have shown variable success (Schneider et al., 2004). Because biological control is not sufficient, supplementary control measures need to be included in an integrated pest management (IPM) strategy.

The use of host plant resistance is an important component of an IPM program to reduce post-harvest losses (Markham et al., 1997; Bergvinson, 2004; Bergvinson and García-Lara, 2004; García-Lara and Bergvinson, 2007). Several maize varieties, including local landraces, have been characterized as sources of resistance to *P. truncatus* (Arnason et al., 1997; Kumar, 2002). Post-harvest resistant varieties have been found to suffer only 13–50% as much grain weight loss compared to susceptible counterparts (García-Lara et al., 2007). Mechanisms of resistance in these kernels are based on two biochemical processes (Arnason et al., 1997): 1) Mechanical fortification or strengthening of the pericarp cell walls that act as physical barrier (García-Lara et al., 2004; Bergvinson and García-Lara, 2004) and 2) Antibiosis, the toxic effects of compounds localized in the endosperm aleurone layer (García-Lara et al., 2007; Winkler and García-Lara, 2010).

Important efforts have been made to develop resistant maize populations against *P. truncatus* using Caribbean accessions (Bergvinson, 2001; Kumar, 2002). Recently, significant gains in storage pest resistance have been achieved through recurrent selection. P84c3 is the result of a S2 intra-population improvement (Bergvinson and García-Lara, 2003). Our previous reports (García-Lara et al., 2004; Winkler and García-Lara, 2010) have also demonstrated that maize population 84c3 showed resistance to the attacks of the maize weevil, *Sitophilus zeamais*. This source of resistant germplasm could be used for both post-harvest insect pests.

Breeding of resistant maize varieties has been successful against *P. truncatus* (Bergvinson, 2001; Kumar, 2002; Bergvinson and García-Lara, 2003) and *T. nigrescens* had been used for the control of this pest (Markham et al., 1994; Meikle et al., 2002) However, until now host plant resistance and biological control have been studied individually and therefore the potential of an integrated control strategy has not been addressed. In this research study, we investigated the interaction and synergistic effects of the use of maize kernels obtained from insect resistant plants and the predator *T. nigrescens* for the control of the larger grain borer *P. truncatus*.

2. Materials and methods

2.1. Maize genotypes

Two maize genotypes available at the International Maize and Wheat Improvement Center (CIMMYT) were selected and used. P84c3 is an open-pollinated population developed at CIMMYT using S2 intra-population improvement under artificial infestation (Bergvinson and García-Lara, 2003). P84c3 was derived from 20 Caribbean accessions with moderate resistance to *P. truncatus*. P84c3 was used as resistant genotype to *P. truncatus* while CML244 × CML349, a highland hybrid, was considered as susceptible genotype based on previous reports (Bergvinson, 2001; García-Lara et al., 2004). The seed of P84c3 was increased during 2005 at CIMMYT’s experimental stations: Tlaltizapan, Morelos Mexico (18°41’ N lat., 940 masl). Hybrid seed was produced in 2005 at CIMMYT’s experimental station el Batan, Texcoco Mexico (19°31’ N lat., 2240 masl). After harvest, genotypes were kept at 4°C to prevent pest damage and to conserve seed quality until bioassay evaluations.

2.2. Insect pest culture

The culture and insect bioassays were performed in the Entomology Laboratory at CIMMYT’s headquarters, El Batan, Mexico. The *P. truncatus* colony was collected in 2003 from Oaxaca, Mexico, and renewed every 8 months. Insects were cultured on white maize CML460 × CML461, single cross dent highland hybrid, for four cycles at 27 ± 1°C, 70% relative humidity (rh) and 12:12 h cycles of light and dark (L:D). Cultures were maintained and renewed using the methods described by Bergvinson (2001). Briefly, *P. truncatus* was reared in 0.5 L glass jars with vented lids that were filled with 400 g of equilibrated maize (30 d at 27 ± 1°C, 70% of rh) covered with 10 g of maize frass and infested with 250 unsexed adults. Progeny were collected after 6–8 weeks. *P. truncatus* adults were obtained by sieving (#10 and #16; USA Standard Testing Sieve E–11, Seedburo Equipment Company, Chicago IL, USA) maize containing grain damage greater than 50%.

2.3. Insect predator culture

*T. nigrescens* were collected from maize storage facilities located in the state of Oaxaca, Mexico, (1500 masl) and subsequently cultured for two generations on a colony of *P. truncatus* collected from the same region. Predator was reared on *P. truncatus* in maize in 0.5 L glass jars (27 ± 1°C, 70% of rh and 12:12 L:D). Maize (400 g) was placed into each jar, to which 200 unsexed and unaged adult of *P. truncatus* were added. Two weeks later, 20 unsexed and unaged adults of *T. nigrescens* were added to the jars; these were removed two weeks later. Prior to being used in the experiment, newly emerged adult predators were removed from the culture and kept separately for a maximum of 1 week.

2.4. Insect population dynamics and susceptibility parameters

For insect bioassays, two separated and independent experiments were run during 2006. Each experiment consisted of kernels obtained from two genotypes (resistant and susceptible) infested with *P. truncatus* with the presence or absence of the predator (two treatments by genotype). All experiment were conducted under controlled conditions in an environmental chamber (27 ± 1°C, 70% of rh and 12:12 L:D). For each experiment, five replicates were performed at the same time for each variety and treatment. Each replicate was conducted on a jar with 300 g samples of maize that was allowed to equilibrate at 13% of grain humidity for 3 weeks prior to infestation with *P. truncatus*. Each jar was infested with 100 unsexed adults of *P. truncatus* (0–7 days old). Two weeks later, only treated jars were infested with 30 newly emerged and unsexed adult predators that marked day 1 of the experiment (proportion 3:1 prey: predator). The predator adults were added two weeks later to guarantee the availability of *P. truncatus* larvae so they could be attached (Ayertey et al., 1999). Susceptibility parameters on each jar were determined as well as insect dynamic populations for a period of 28 weeks. Every 4 weeks the grain was sieving using mesh sieves (#10 and #16) to separate grain, frass, *P. truncatus* adults and predators, which were return to the jar until the next estimation. Grain weight loss, frass production, adult emergence of *P. truncatus* and predator numbers were recorded. Healthy and
damaged kernels were separated and counted using a light table (Kumar, 2002). At the end of 28 weeks, all *P. truncatus* adults were counted, while the predator was counted and weighed for comparison.

### 2.5. Statistical analysis

Since genotypes were selected based on previous screening results to obtain a wide range in *P. truncatus* resistance, an ANOVA model was used. Susceptibility parameters and dynamic population traits were subjected to analysis of variance using the statistical software Statistit v.7 (Analytical Software, Tallahassee, FL) and differences among means were compared by Tukey–Kramer HSD test at *P* < 0.01.

### 3. Results

#### 3.1. Effect of insect resistant maize and predator on grain losses and frass production

Kernels from the different maize genotypes kept under controlled storage conditions differed significantly (*P* < 0.001) in terms of insect damage parameters such as grain weight loss, frass production, and *P. truncatus* progeny (Table 1). Highly significant correlations among grain weight loss, frass production, and *P. truncatus* progeny were observed (*r* > 0.9, *P* < 0.001). There was a reduction of 30% for grain weight loss, 32% for frass production, and 40% decrease for emerged adult *P. truncatus* progeny, when resistant and susceptible maize kernels were compared without the predator presence.

Maize grain infested with *P. truncatus* in combination with the predator *T. nigrescens* displayed significant differences (*P* < 0.001) in progeny number, grain weight loss, and frass production for both resistant and susceptible maize genotypes. Combination of insect resistance maize, P84c3, with biological control, *T. nigrescens*, reduced by 80% the number of *P. truncatus* adult progeny, 81% grain weight loss, and 75% frass production with predator absence, after 28-weeks storage as compared to the analogous material. A similar condition was observed for the over time for frass production associated with *P. truncatus* (Fig. 1B). Susceptible and resistant maize, without the predator, yielded more frass. There was a significant reduction of frass production on susceptible (50%) compared with resistant maize (75%) infested with *P. truncatus* in the presence of the predator.

For both grain weight loss and frass production, the major effect of the predator was clearly noticed after 16 weeks of infested storage maize over a period of 28 weeks. A sigmoid trend was observed for both parameters under absence of the predator. In contrast, a linear trend was observed when the predator was present in infested maize varieties (Fig. 1A and B).

Population dynamics of *P. truncatus* reared on resistant or susceptible maize genotypes is shown in Fig. 1C. The number of emerged adult *P. truncatus* progeny was 1.7-fold greater in susceptible versus resistant kernels. Insect population dynamics in susceptible maize showed a sigmoid curve compared with the logarithmic trend observed in resistant maize. When the predator was present in the susceptible maize, the number of adults was drastically reduced by four-fold (to a forth), while in resistant maize this reduction was five-fold (to a fifth). This difference in adults reached two-fold when both types of maize (resistant vs susceptible) were compared.

The average of *T. nigrescens* adult population at the end of the experiment in susceptible maize was 205 per kg grain, in contrast with 112 adults found in the resistant maize (Table 2). This represents an increase of 1.8 fold in the population for successive generations. Adult weight of *T. nigrescens* grown in susceptible maize was also significantly different (22% more) compared to counterparts that infested the resistant maize. Furthermore, the ratio of *P. truncatus* adults against surviving adults of *T. nigrescens* showed a significant difference for both genotypes having an insect pressure of 5.4:1 prey to predator in susceptible kernels compared to 4.6:1 in resistant kernels.

#### 3.2. Insect population dynamics

Grain weight loss over time associated with *P. truncatus* feeding on a resistant or susceptible maize genotype is depicted in Fig. 1A. During the period of the experiment, grain weight losses ranged from 0.5 to 90.7%. There was a significant correlation between grain weight loss and frass production (*r* > 0.9, *P* < 0.05) caused by *P. truncatus* infestation. Grain weight losses also increased with insect density of *P. truncatus*. The susceptible genotype in absence of the predator had greater losses in grain weight compared to the resistant maize. The presence of the predator reduced grain weight losses by 50% in susceptible maize infested with *P. truncatus* in comparison with the untreated counterpart, and by 20% in comparison with resistant maize. Surprisingly, predator in combination with resistant genotype reduced grain losses by 59% in comparison with the untreated and this reduction reached up 83% in comparison with susceptible maize.

A similar condition was observed for the over time for frass production associated with *P. truncatus* (Fig. 1B). Susceptible and resistant maize, without the predator, yielded more frass. There was a significant reduction of frass production on susceptible (50%) compared with resistant maize (75%) infested with *P. truncatus* in the presence of the predator.

#### 3.3. Interactions among resistance maize, predator, and insect pest infestation

Major parameters associated with the biological control and insect resistance maize against the attack of *P. truncatus* were analyzed for multiple interactions during 28 weeks of storage (Table 3). Maize genotypes, treatments, and time of infestation significantly affected (*P* < 0.01) progenies of *P. truncatus* and *T. nigrescens*, grain weight loss and frass production. Furthermore, there was

### Table 1

Effect of postharvest insect-resistant and susceptible maize kernels in conjunction with the predator *Teretrius nigrescens* on *Prostephanus truncatus* progeny production, grain weight loss and frass production after 28-weeks storage.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Type</th>
<th>Treatment</th>
<th>Grain weight loss (%)</th>
<th>Frass production (%)</th>
<th><em>P. truncatus</em> (no/kg of grain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML244 X CML349</td>
<td>Sus</td>
<td>-</td>
<td>90.7 ± 3.5 a</td>
<td>68.5 ± 1.1 a</td>
<td>4661 ± 141 a</td>
</tr>
<tr>
<td>P84c3</td>
<td>Res</td>
<td>-</td>
<td>62.9 ± 3.7 b</td>
<td>45.9 ± 2.3 b</td>
<td>2720 ± 127 b</td>
</tr>
<tr>
<td>CML244 X CML349</td>
<td>Sus</td>
<td>+</td>
<td>41.9 ± 3.3 c</td>
<td>35.2 ± 1.1 c</td>
<td>1095 ± 136 c</td>
</tr>
<tr>
<td>P84c3</td>
<td>Res</td>
<td>+</td>
<td>11.7 ± 5.1 d</td>
<td>11.1 ± 1.0 d</td>
<td>527 ± 91 d</td>
</tr>
</tbody>
</table>

* a, b, c Values followed by different letters were significantly different (*P* < 0.001; Tukey Test). Mean values ± SD (n = 5).
significant interaction ($P < 0.005$) between genotype, treatment, and time of infestation, and among all the sources of variation. More importantly, there was a strong interaction between genotype and predator indicating a synergistic relationship.

### 4. Discussion

Significant and positive effects were observed between insect resistant maize, P84c3, and presence of the biological control *T. nigrescens* to reduce post-harvest losses in maize infestation by *P. truncatus*. The impact of *T. nigrescens* on populations of *P. truncatus* has been established before with contradictory results (Borgemeister et al., 1997; Ayertey et al., 1999; Meikle et al., 2002). Our results demonstrated a major reduction of progeny number and grain weight loss produced by *P. truncatus* with the presence of the predator. A more dramatic effect was observed in conjunction with insect resistant maize (>80%) compared to susceptible maize (>48%). Comparable suppression of the *P. truncatus* population (reduced by 81%) and losses (reduced by 40%) were achieved by Helbig et al. (1992) using natural infestations and by Hill et al. (2003) in a comparative laboratory study where a reduction of 66% of *P. truncatus* population was achieved when *T. nigrescens* was introduced 3 weeks after the substrate was inoculated. However, in the farm scale these reductions could be different (Meikle et al., 2002).

Early studies on population dynamics of *P. truncatus* demonstrated that densities tend to rise in a nearly exponential fashion (Meikle et al., 1998) after the fourth month of storage onwards. However in our study run over a period of 28 weeks insect population dynamics in susceptible maize followed a sigmoid curve which also contrasts with the logarithmic trend observed in resistant maize with or without the predator. Final densities of *P. truncatus* in this study ranged from 527 to 4661 adults per kilogram grain, while maximum densities of 3500 *P. truncatus* have been reported in similar conditions (Meikle et al., 2002; Hill et al., 2003). Final densities of *P. truncatus* are directly impacted by dynamic populations and in consequence this affects the predator control as it was shown in this study. Previous reports have demonstrated that *P. truncatus* density is a significant factor in recovered *T. nigrescens* larval and adult densities (Ayertey et al., 1999). Holst et al. (2000) also suggested that *T. nigrescens* had difficulties controlling large populations of *P. truncatus*. Therefore, this predator prefers a relatively small *P. truncatus* population to maintain them at low densities (Nang’ayo et al., 2002). These conditions were reached only when insect-resistant kernels were used. This positive effect is due to differences in the biochemical

### Table 3

<table>
<thead>
<tr>
<th>Source</th>
<th>df $P$</th>
<th>T. nigrescens</th>
<th>Grain Weight Loss (%)</th>
<th>Frass Production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Treatment*</td>
<td>1</td>
<td>5379</td>
<td>0.001</td>
<td>29130</td>
</tr>
<tr>
<td>Genotype*</td>
<td>1</td>
<td>1627</td>
<td>0.001</td>
<td>829</td>
</tr>
<tr>
<td>Time*</td>
<td>6</td>
<td>862</td>
<td>0.001</td>
<td>168</td>
</tr>
<tr>
<td>Genotype * treatment</td>
<td>1</td>
<td>871</td>
<td>0.001</td>
<td>829</td>
</tr>
<tr>
<td>Genotype * time</td>
<td>6</td>
<td>144</td>
<td>0.001</td>
<td>95</td>
</tr>
<tr>
<td>Treatment * time</td>
<td>6</td>
<td>473</td>
<td>0.001</td>
<td>168</td>
</tr>
<tr>
<td>Genotype * treatment * time</td>
<td>6</td>
<td>48</td>
<td>0.001</td>
<td>95</td>
</tr>
</tbody>
</table>

* Genotype, susceptible and resistant maize.

* Genotype, susceptible and resistant maize.

* Genotype, susceptible and resistant maize.

* Time, weeks after infestation.
properties of kernels especially in terms of phenolic compounds and proteins (García-Lara et al., 2004, 2007). These chemical compounds are known to increase insect development time and consequently decrease population growth rate, which will allow the predator to suppress the population better when the host population is increasing at a slower rate.

There is still some doubt as to whether the numerical response of the predator is adequate to allow proper pest control (Markham et al., 1994; Holst and Meikle, 2003). In this study, T. nigerescens populations increased in parallel (but at lowest rate) compared to those of P. truncatus, when grown on susceptible kernels but not on resistant maize on which the number of T. nigerescens remained constant. Hill et al. (2003) using different substrates showed that the final prey/predator ratio on wood was much higher (4.1:1) than that on maize (11.7:1) after 12 weeks. Here we found that the prey/predator ratio on resistant kernels was significantly lower (4.6:1) compared with susceptible counterparts (5.4:1). This might translate into more effective pest population control by the predator in resistant maize compared to susceptible maize. Therefore, the reproduction of T. nigerescens was not adversely affected by the presence of insect resistance maize, suggesting that post-harvest insect resistant maize did not interfere with T. nigerescens predation and development.

The resistant maize populations did not have an adverse effect on P. truncatus population growth by lowering P. truncatus fecundity explained by the fact that resistant maize increased the developmental time for P. truncatus. This enabled T. nigerescens to functionally respond to pest population control in an enclosed system. Nevertheless, laboratory studies conducted to assess the impact of natural enemies, especially T. nigerescens, showed that conditions usually involved high densities of predators and prey (Ayertey et al., 1999; Holst et al., 2000) as occurred in this study.

Compatibility between diverse control measures, like host plant resistance and biological control, is a major issue in IPM. Thus, it is important that any control practices taken against P. truncatus do not adversely affect its predator, T. nigerescens (Golob, 2002). In this study, we established a positive synergism between insect resistant varieties and biological control.

At this step, our results provide evidence that insect resistant varieties and biological control may be useful. Comparative long term data from large scale experiments in stores is needed; however we propose to include the use of post-harvest insect resistance maize, such as derivates from P84c3, in combination with biological control with T. nigerescens, as part of an IPM program to effectively control P. truncatus. These practices can be applied and adopted by international programs to reduce and minimize post-harvest maize grain losses in tropical areas located in Africa, Asia and America.

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