THE INFLUENCE OF SOWING DATE AND INSECTICIDE TREATMENTS ON OSTRINIA NUBILALIS (HÜBNER) DAMAGE AND FUMONISIN CONTAMINATION IN MAIZE KERNELS

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ABSTRACT - Fusarium verticillioides, a known producer of fumonisins, has been reported to be the most common pathogen of maize causing Fusarium ear rot and grain fumonisin contamination. A field experiment was conducted from 2005 to 2007 in North Italy to determine the effects of sowing date and insecticide treatment against ECB on the susceptibility of maize to Fusarium ear rot and to fumonisin contamination in natural infection conditions. Three sowing dates and two insecticide applications were compared for each year. The late sown maize showed significantly higher insect damage to both the plants (stalks) and the ears (kernels and cobs). The ECB damage severity was 23% higher for the later sowing date than for the earlier. The insecticide treatment significantly reduced the ECB infestation compared to the untreated control. A significant effect of the sowing date and of the insecticide application on Fusarium ear rot was highlighted. The earlier sowing date reduced the ear rot incidence and severity by 25% and 49%, respectively, compared to the later dates. The insecticide application led to 25% lower ear rot severity than the untreated control. The fumonisin contamination was significantly reduced by an earlier sowing date (62%) and by the treatment against ECB (51%). The plots sown earlier and treated with insecticide resulted in a 79% lower concentration of fumonisins in kernels compared to plots characterized by later sowing and a lack of treatment.

KEY WORDS: Maize; Sowing date; European corn borer; Fusarium ear rot; Fumonisins.

INTRODUCTION

Recently, fumonisins, produced mainly by F. verticillioides, have received a greater deal of attention because they are the most common mycotoxins found in maize kernels at harvest in North Italy (VISCONTI, 1996; PIETRI et al., 2004) and France (BARRIER-GUILLOT et al., 2007). These toxins have been shown to cause a number of health problems in livestock and laboratory animals (MARASAS et al., 2000) and have been associated with an increased incidence of human esophageal cancer (YOSHIZAWA et al., 1994). Many nations, like the E.U., have established regulatory standards on permissible fumonisin levels in food, while regulatory standards for the feed of the main domestic animal categories are under development (CAST, 2003; VERSTRAETE, 2006).

Several authors (SOBEK and MUNKVOLD, 1999) reported that the 2nd-generation larvae of European corn borer (ECB) Ostrinia nubilalis (Hübner), are closely related to Fusarium verticillioides (Sacc.) Nirenb., development in maize in temperate areas. ECB facilitates the infection of this fungus in two ways: (i) the larvae directly damage kernels, break the pericarp and give the fungus a direct point of entry; (ii) the same larvae can be vectors of inoculum and can bring the fungus inside the ears. Moreover the ECB larva movement on kernels carries endogenous and exogenous mycelium to different parts of the ear. The occurrence of fumonisins is clearly related to the ear injuries caused by ECB (PAPST et al., 2005): damaged ears can have a 40 times higher fumonisin contamination than undamaged ones (ALMA et al., 2005); thus control measures against ECB larva activity are necessary to guarantee a less contaminated production (DOWD, 2003).
The reduction of ECB injury, and consequently of fumonisins contamination, is effective in maize genetically engineered for ECB with genes of Bacillus thuringiensis Berliner (Bt hybrids), that can express Cry1A proteins, which have lethal effects against lepidopteran species (Masoero et al., 1999). Although the use of some transgenic crops has been approved by the E.U., they are currently subject to restriction in Italy and chemical treatments are the main ECB control method (Rice and Ostlie, 1997). Insecticide treatment against 2nd-generation larvae of ECB has an increasing importance in maize crop practices and several insecticides, mainly synthetic pyrethroids and organophosphates, are currently labelled for ECB control in maize (Blandino et al., 2005; Saladini et al., 2008).

In temperate areas, an interaction between the maize sowing date and ECB damage on maize ear was also been shown. Pilcher and Rice (2001) and Anderson et al. (2003) reported that later sowing times, compared to an earlier date, led to a higher incidence of ECB larvae damage. A late sowing shifts the ear development phase of the crop to a period with greater insect activity and this produces more kernel injuries (Derridj et al., 1989). Damage to stalks and ears and yield loss are higher in maize sown late (Myers and Wedberg, 1999). For this reason, the practice of insecticide treatment against ECB was first introduced on maize sown late for silage.

The sowing date could also play a significant role on reducing fungal ear rot development, as the sowing time in temperate regions determines the environmental conditions to which the maize crop is exposed during anthesis and grain filling, which are the phenological stages that are involved in the most by fungal infection and development (Blandino et al., 2009).

The objective of this study was to investigate the effect of insecticide treatments against the 2nd-generation ECB larvae at different sowing dates on insect injury in maize ears, on Fusarium ear rot development and on the fumonisins contamination of grains in non inoculated conditions.

**MATERIALS AND METHODS**

**Experimental site and treatments**

The experiment was carried out from 2005 to 2007 in Chieri (44° 54’ N, 7° 24’ E; altitude 262 m.), in a loamy-medium textured soil, Typic Fragudalsf (USDA classification).

![Image](image.png)

**TABLE 1 - Main trial information of the field experiments.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Time of sowing</th>
<th>Sowing date</th>
<th>Harvest date</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>S1</td>
<td>March 22</td>
<td>Sept. 13</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>April 16</td>
<td>Sept. 13</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>May 15</td>
<td>Sept. 30</td>
</tr>
<tr>
<td>2006</td>
<td>S1</td>
<td>March 25</td>
<td>Sept. 7</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>April 4</td>
<td>Sept. 20</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>May 20</td>
<td>Oct. 5</td>
</tr>
<tr>
<td>2007</td>
<td>S1</td>
<td>March 20</td>
<td>Sept. 21</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>April 4</td>
<td>Sept. 21</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>May 11</td>
<td>Oct. 10</td>
</tr>
</tbody>
</table>

The treatments were a factorial combination of:
- three sowing dates: March (S1), April (S2) and May (S3). The maize was sown at approximately 30-d intervals;
- two insecticide application treatments: untreated control (NT); insecticide application between the ECB flight peak and 10 days after this moment (T).

A three replicate split-plot design was used with the sowing date as the main-plot treatment and the insecticide application as the subplot treatment. The subplots consisted of twenty 60-m long rows spaced 0.75 m apart and separated by two untreated buffer rows on either side.

The experiment fields received 200, 100 and 90 kg ha⁻¹ of N, P₂O₅ and K₂O each year. Irrigation was applied at flowering to maintain the water-holding capacity between 33 and 200 kPa. Weed control was conducted with metholachlor and terbutylazine. The previous crop was maize each year. The hybrid used was Pioneer PR34N43 (FAO 500, 128 days). The sowing and harvest dates for each year are reported in Table 1.

The applied insecticide was pyrethroid deltamethrin (Decis® Jet, formulation: emulsifiable concentrate (EC), Bayer Crop Science, Italy) at 0.012 kg active ingredient (AI) ha⁻¹. Treatments were applied using self-propelled sprayers (Agri Jet 826®), with a hydraulically adjustable working height (0.70 - 3.50 m). An air-assisted boom with twenty fan 04 nozzles applied a spray volume of 400 l ha⁻¹ at a pressure of 200 kPa; the operation speed was 10 km h⁻¹. Air-assisted spraying uses relatively large volumes of low-pressure air, generated by a fan, to direct the sprays into the crop. The timing of the insecticide applications was chosen by monitoring the ECB flight activity using a cone trap placed outside the experimental plots, and baited with sex pheromone (E,Z=97:3) to attract males and with phenylacetaldehyde (PAA) for females. The sex pheromone and PAA dispensers were replaced each 15 days. The adults were removed from the trap and counted every 1-3 days.

In each plot, 100 ears (included the ears used for the evaluation of fungal ear rot incidence and severity) were collected by hand at the end of maturity and shelled using an electric sheller. All the treatments were collected at a grain moisture content of between 22-28%. At harvest, three replications of 30 undamaged ears each (ears without any visible ECB damage) were collected after a visual evaluation from the S1/T plots.

The kernels from each plot were mixed thoroughly to obtain a random distribution and samples (5 kg) were taken to analyze the fumonisins (FB₁ and FB₂) content.
Entomological and mycological measurements

The ECB damage and Fusarium ear rot incidence and severity were evaluated on 30 ears randomly sampled at harvest from each plot.

The ECB damage incidence was calculated as the percentage of ears per plot with kernel injuries or apical and basal tunnels in the cob due to larva activity. The ECB damage severity was calculated as the percentage of kernels per ear with injuries due to larva activity. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of surfaces exhibiting visible kernel damage due to larva activity according to the following schedule: 1 = no injuries, 2 = 1-5%, 3 = 6-10%; 4 = 11-20%, 5 = 21-35%, 6 = 35-60%, 7 > 60%.

The Fusarium ear rot incidence was calculated as the percentage of ears per plot with symptoms, while the Fusarium ear rot severity was calculated as the percentage of kernels per ear with symptoms. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of surfaces exhibiting visible symptoms of the disease according to the following schedule: 1 = no symptoms, 2 = 1-3%, 3 = 4-10%; 4 = 11-25%, 5 = 26-50%, 6 = 51-75%, 7 > 75% (REID et al., 1999).

The ECB severity and ear rot severity scores were converted to percentages of the ear exhibiting symptoms and each score were replaced with the mid-point of the interval, as suggested by CAMPBELL and MADDEN (1990).

Fumonisins analyses

A 5 kg representative sample of grain from each plot was freeze-dried and milled. A representative sub-sample of the milled material (50 g) was extracted with 100 ml methanol-water (80/20) by blending for 15 min. The supernatant was filtered through filter paper (0.45 µm), diluted with 40 ml of PBS (8.0 g of NaCl + 1.2 g of Na2HPO4 + 0.2 g of KCl + 0.2 g of KH2PO4 in 1 l of water) and was cleaned using a FumoniTest column (Vicam®) by rinsing with 10 ml of PBS. The FB1 and FB2 were then eluted using 1.5 ml of methanol and 200 µl of methanol/water (50/50) and derivatized with o-phthalaldehyde (OPA)/2-mercaptoethanol solution. The Fumonisins-OPA derivatives (10 µl) were analyzed by reversed-phase HPLC with fluorescence detection (λex = 335 nm, λem = 440 nm). Toxin quantification was performed using external standards and peak height measurements.

The detection limit of the analytical method was 1.0 µg kg⁻¹ for FB1 and FB2.

Statistical analysis

The normal distribution and homogeneity of variances were verified performing the Kolmogorov-Smirnov normality test and the Bartlett-box test, respectively. The effect of the treatments on ECB and Fusarium ear rot incidence and severity and Fumonisin B1 + B2 content was tested by the analysis of variance (ANOVA) using a randomized complete block split plot design in which the experimental block factor was the year effect, the main-plot factor was the effect of sowing date (S1/S2/S3) and the sub-plot factor was the effect of the insecticide application (NT/T). When significant, the block and the main-plot factor means were compared using Bonferroni’s test at P ≤ 0.05.

The SPSS statistical package for Windows, Version 13.0 (SPSS Inc., Chicago) was used for the statistical analysis. The incidence and the severity values of ECB and Fusarium ear rot were previously transformed using y' = arcsin(√x*180/π) as percentage data derived from counting.

RESULTS

The 3 years were subject to different meteorological trends, as far as both rainfall and temperature (expressed as growing degree days) from flowering to harvest are concerned (Table 2). The meteorological conditions were similar for rainfall in 2005 and 2006, while 2006 had more growing degree days (GDDs) than 2005, both during the flowering and the final ripening period.

The year 2007 had more rainfall in June, but less in September, compared to the previous years. In
this last year, the GDDs observed from June to October, and particularly in June and in September, were lower than those observed in 2005 and 2006.

**ECB flight peak, damage incidence and severity**

The ECB flight peak was recorded in all three years between the end of July and the beginning of August (Table 3). In the third year the captures at flight peak were higher compared to those observed in the first and second years. The sowing date (main factor) did not show an effect on the ECB incidence (Table 4), while the differences in damage severity due to the insect was significant (P<0.05); the ECB damage severity was in fact 37% and 23% higher in S3 respectively than in S1 and S2.

The insecticide application (sub-plot factor) resulted in significant effects on the ECB incidence and severity (P<0.001); thus the insecticide treatment (T) reduced the ECB incidence (21%) and severity (22%) compared to the control (NT). No significant effects of the interaction of sowing date and insecticide treatment were observed.

**Fusarium ear rot incidence and severity**

A significant effect of the sowing date on Fusarium ear rot incidence (P<0.05) and severity (P<0.05) was clearly shown (Table 4); they were respectively 25% and 49% higher in S3 than in S1. The S1 plot only showed significant differences from S2 for Fusarium ear rot incidence, which was 17% lower.

The insecticide application showed significant effects on the Fusarium ear rot incidence and severity (P<0.001); the insecticide treatment in fact reduced the ear rot incidence and severity compared to the control (NT) by 22% and 25%, respectively.

### TABLE 3 - ECB flight peak and date of insecticide applications for the field experiments conducted in the 2005-2007 period in the research site.

<table>
<thead>
<tr>
<th>Year</th>
<th>Date of ECB flight peak(*)</th>
<th>Trapping count at ECB flight peak</th>
<th>Date of insecticide application(**)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>July 27</td>
<td>12</td>
<td>Aug. 5</td>
</tr>
<tr>
<td>2006</td>
<td>Aug. 4</td>
<td>10</td>
<td>Aug. 8</td>
</tr>
<tr>
<td>2007</td>
<td>July 30</td>
<td>35</td>
<td>Aug. 1</td>
</tr>
</tbody>
</table>

(*) ECB first generation flight peak was observed with pheromone trap catches of adult ECB males and females at the experimental site.

(**) A.I. delthametrin was applied at 0.012 kg (A.I.) ha⁻¹.

### TABLE 4 - Effect of sowing date and insecticide application on ECB and Fusarium ear rot incidence and severity and fumonisin (sum of FB₁ and FB₂) contamination at harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>ECB incidence</th>
<th>ECB severity</th>
<th>Fusarium ear rot incidence</th>
<th>Fusarium ear rot severity</th>
<th>Fumonisin B₁+B₂ (µg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>73.9 a</td>
<td>17.5 b</td>
<td>69.9 a</td>
<td>17.7 b</td>
<td>2827 a</td>
</tr>
<tr>
<td>2006</td>
<td>74.1 a</td>
<td>21.9 ab</td>
<td>62.8 a</td>
<td>15.1 b</td>
<td>2095 a</td>
</tr>
<tr>
<td>2007</td>
<td>79.3 a</td>
<td>23.3 a</td>
<td>68.7 a</td>
<td>21.7 a</td>
<td>10285 b</td>
</tr>
<tr>
<td>P (F)</td>
<td>0.196</td>
<td>0.00***</td>
<td>0.096</td>
<td>0.00***</td>
<td>0.00***</td>
</tr>
<tr>
<td>Main-plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>73.4 a</td>
<td>18.0 b</td>
<td>56.9 c</td>
<td>11.5 b</td>
<td>2797 a</td>
</tr>
<tr>
<td>S2</td>
<td>72.7 a</td>
<td>20.1 b</td>
<td>68.2 b</td>
<td>15.4 b</td>
<td>5030 a</td>
</tr>
<tr>
<td>S3</td>
<td>81.2 a</td>
<td>24.7 a</td>
<td>76.3 a</td>
<td>22.6 a</td>
<td>7569 b</td>
</tr>
<tr>
<td>P (F)</td>
<td>0.208</td>
<td>0.017*</td>
<td>0.036*</td>
<td>0.042*</td>
<td>0.029*</td>
</tr>
<tr>
<td>Sub-plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>87.8 a</td>
<td>23.5 a</td>
<td>75.4 a</td>
<td>18.8 a</td>
<td>6782 a</td>
</tr>
<tr>
<td>T</td>
<td>66.8 b</td>
<td>18.3 b</td>
<td>58.8 b</td>
<td>14.1 b</td>
<td>3354 b</td>
</tr>
<tr>
<td>P (F)</td>
<td>0.000***</td>
<td>0.000***</td>
<td>0.000***</td>
<td>0.000***</td>
<td>0.000***</td>
</tr>
<tr>
<td>Main X Sub-Plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (F)</td>
<td>0.191</td>
<td>0.532</td>
<td>0.464</td>
<td>0.208</td>
<td>0.095</td>
</tr>
<tr>
<td>sem</td>
<td>2.2</td>
<td>0.9</td>
<td>2.4</td>
<td>1.2</td>
<td>696</td>
</tr>
</tbody>
</table>

Means followed by different letters are significantly different (the level of significance is shown in the table: * P<0.05; ** P<0.01; *** P<0.001).

The ECB and Fusarium ear rot incidence and severity means reported refer to values transformed using \( y’ = \arcsin(\sqrt{x \times 180/\pi}) \), as percentage data derived from counting.

\( \text{Y} \) S1 = sowing date from March 10 to March 25; S2 = sowing date from April 4 to April 16; S3 = sowing date from May 11 to May 20; NT = control not treated; T = insecticide treatment.

\( \text{Z sem} \): standard error of mean.
The interaction between sowing date and insecticide treatment was never significant. Even when the insecticide treatment was effective, a further reduction of moldy ears and kernels was found in the ears not infested by ECB, compared to those harvested from plots treated with insecticides (Table 5). The undamaged ears on average had 54% and 51% lower values of Fusarium ear rot incidence and severity than naturally infested plots and 32% and 24% lower than treated plots.

**Fumonisin contamination**

The occurrence of fumonisin in kernel at harvest in 2007 was higher than in 2005 and 2006. A significant effect (P<0.05) of the sowing date on fumonisin occurrence in maize kernels was observed (Table 4). The contamination of the S1 treatment was 62% lower than in S3. No significant difference between the S1 and S2 treatments was observed.

As for ECB and Fusarium measurements, the effect of the insecticide application was highly significant (P<0.001). The insecticide treatment reduced the fumonisin contamination compared to the untreated control (NT) by 51%. The interaction between sowing date and insecticide treatment was not significant.

On the whole, the plots sown earlier and treated with insecticide resulted in a 79% lower concentration of fumonisin in kernels compared to untreated plots characterized by a later sowing (Fig. 1).

The kernels from the undamaged ears clearly had a lower fumonisin contamination each year than the kernels from plots treated against second generation larvae (Table 5). The contamination of this mycotoxin in undamaged ears was 6 and 10 times lower than the average value of the treated plots and untreated control, respectively.

**DISCUSSION**

A significant effect of the sowing time on the fumonisin concentration in maize kernels was observed, thus confirming data of a previous research on maize untreated with insecticide (Blandino et al., 2009). The reduction of Fusarium ear rot severity observed with the earlier sowing date could first be related to the direct effect that the sowing date has on the length of maturity and, as a consequence, on the harvest date. The sowing delay led to a later harvest, with a higher probability of having the final
part of ripening in climatic conditions more favourable to the development of *F. verticillioides* (Lacey and Magan, 1991) and with a more prolonged kernel drydown (Nielsen et al., 2002), both of which guarantee higher fungal growth.

Moreover, the elevated fumonisin contamination of the late sowing is also a consequence of the higher severity of the ECB damage that was observed. As previously reported, the ECB larvae that arrive on kernels with a higher moisture content and digestibility are more dangerous than those hatched on the kernel at the end of the maturity process and they could promote a higher *Fusarium* development and fumonisin contamination. The higher ECB feeding capacity on late sown maize promotes fungal infection and mycelium growth through the ear. The fungal development also takes advantage of a less compact and more easily colonizable substrate (Headrick et al., 1990; Hoenisch and Davis, 1994; Yates and Jaworski, 2000).

This research has confirmed that with a sowing delay, ECB infestation occurs in a physiological stage that is more attractive for the insects (Mason et al., 1996). Maize sown late showed significantly higher insect damage. In Serbia, Bača et al. (1995) reported that delayed maize planting can result in large ECB populations and that early planted maize (mid April) had a 70% infestation level compared to 86% in later planting (late May). Similar results were reported in Iowa, which also has two generations of ECB (Showers et al., 1989). This higher insect infestation for a later sowing could be related to the emission of phenylacetaldehyde with flowering during the insect flight peak (Derridj et al., 1989), which makes the plants more attractive to the females (Burgio and Maini, 1994).

Moreover, the capacity of insect larvae to create holes and injury in cob and kernels clearly increases with late sown maize, confirming data by Mason et al. (1996).

Feeding activity is influenced by the characteristics of the maize grain and it slows down for harder textured and lower moisture levels (Reardon et al., 2006; Velasco et al., 2007), as occurs for early sown maize. Buedgen et al. (1990) and Buxton et al. (1996) reported that plant material with a high digestibility is more susceptible to ECB larva feeding.

The data collected in our experiment showed that controlling ECB with insecticides also reduced Fusarium ear rot and fumonisin contamination, confirming data by Dowd (1998) and Dowd et al. (2000). No interaction between the insecticide appli-

The data concerning fumonisin occurrence in grains from undamaged ears are in agreement with those of Alma et al. (2005), who found a fumonisin 40 times higher contamination in damaged ears than undamaged ones. Our experiment showed a 82% fumonisin occurrence in ears without any visible ECB damage than that found in those from treated plots.

Since fumonisin contamination is positively related to the number of tunnels on ears (Alma et al., 2005), better grain health could be guaranteed with an early sowing time and a correct insecticide application, in order to obtain a remarkable reduction of ECB larva injury in the ears. In this experiment on a maize crop sown late, the treatment against ECB was not in fact sufficient to obtain kernels with a contamination level which does not exceed the maximum E.U. regulation levels for food. On the other hand, an early sowing of maize, without an insecticide application to prevent ECB damage, could lead to elevated fumonisin contamination, in years and locations with high insect pressure and larva injury.

**CONCLUSION**

The results confirm the important role of the sowing date and the insecticide treatment on ECB management and consequently on Fusarium ear rot control and fumonisin contamination of kernels.

In temperate climates, where ECB attack is consistent and with non-Bt hybrids, the production of maize kernels with a low fumonisin content may be enhanced by an early sowing date and a correct insecticide application against 2nd-generation ECB larvae. At present, the early sowing time and the treatment against ECB are the main crop techniques required in the chain agreements between the food processing industry and farmers in North Italy (Vanara et al., 2005). Since certain livestock species (pigs, horses, rabbits) in the future could have regulatory standards concerning permissible fumonisin levels in feed similar to those for food, these crop techniques could also be extended to animal feeding maize production.

Since ECB control is a key factor in fumonisin contamination prevention in maize production, studies to obtain better efficacy results concerning
insect management are urgently required in order to help farmers achieve maize kernels that contain mycotoxins which do not exceed the maximum international and UE regulation levels.

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