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Managing fumonisin contamination in maize kernels through the timing of insecticide application against European corn borer

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8 2 **the timing of insecticide application against European corn**
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Abstract

The European corn borer (ECB), *Ostrinia nubilalis*, is the main maize pest in Central and South Europe and it has been shown to promote *Fusarium verticillioides* infection in maize grains, a well-known fungal producer of fumonisins. Field experiments were performed in 2006 and 2007 in two sites in NW Italy in order to determine the effects of the timing of insecticide applications on maize on ECB damage, fungal ear rot and fumonisin contamination in naturally infected conditions. Different insecticide application timings were compared, from maize flowering to approximately 15 days after the flight peak of the adult. At harvest, the ears were rated for incidence and severity of ECB damage, fungal ear rot symptoms and fumonisin (FB₁ + FB₂) contamination. In all the years/sites, the treatments applied at the beginning of a consistent ECB flight activity showed the best efficacy to control the insect damage on ears. Fungal ear rot and fumonisin contamination were significantly affected by ECB control. The efficacy of the best timing of insecticide application to control fumonisin contamination was on average 93%, compared to the untreated control. Contamination levels of these mycotoxins increased moving to either an earlier or later treatment; furthermore an earlier insecticide application showed lower fumonisin contamination than a treatment applied after the adult flight peak. The production of maize kernels and maize-based foods that do not exceed the maximum international and EU permitted levels for fumonisins could be enhanced by an appropriate insecticide treatment against the second generation ECB. The optimum insecticide application timing is comprised between the beginning of the consistent adult flight activity and the flight peak.

Keywords: maize, European corn borer, insecticide treatment, pyrethroids, *Fusarium*, fumonisins, mycotoxins.

Introduction

Fusarium verticillioides (Sacc.) Nirenberg is the most common toxigenic fungus in maize in temperate areas which causes ear rot disease and fumonisin accumulation in kernels (Logrieco et al., 2002; Munkvold et al., 2003b). Fumonisin are receiving increasing attention by the scientific community since fumonisin B₁ (FB₁) and B₂ (FB₂) are the most common mycotoxins found in maize kernels throughout the world (Doko and Visconti, 1994; Placinta et al. 1999; Soriano and Dragacci, 2004). 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 in some parts of the world (Yoshizawa et al., 1994; Marasas, 1995). Therefore, admissible maximum levels for fumonisins B₁ and B₂ have recently been set by the European Commission in maize and maize-based foods (EC, 2007).

Maize is consumed either directly or indirectly when processed into food and feed products. In food production, maize is generally dry milled to produce flours and grits which can be processed for snacks, breakfast cereals, cooked or extruded products (Serna-Saldivar et al., 2001). Since interesting new uses of dry milling foodstuffs can be found in gluten-free products intended for people with gluten intolerance (celiac disease) and for foods used for infants (baby food), the demand for maize for food processing has increased in the last few years.

Since fumonisin contamination starts in the field, maize grain safety can be improved through agronomic prevention strategies, which are able to reduce *Fusarium* infection and fumonisin synthesis (Munkvold, 2003a). The occurrence of fumonisins is mainly related to

1 ear injuries caused by Lepidoptera borers (Avantaggiato et al., 2002; Papst et al., 2005).
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1 ear injuries caused by Lepidoptera borers (Avantaggiato et al., 2002; Papst et al., 2005).
2 In temperate maize areas, the insect most frequently associated with *F. verticillioides* and
3 fumonisin synthesis is *Ostrinia nubilalis* Hübner (European corn borer – ECB) (Mason et
4 al., 1996; Dowd, 2003). The larvae of this insect tunnel into the stalks and ears of the
5 plant, causing a yield reduction and loss of grain quality (Godfrey et al., 1991). Two
6 generations of ECB larvae usually occur per year in North Italy: the first generation attacks
7 plants during the mid to late vegetative stages and the second generation attacks during
8 the reproductive stages (from early milk stage to maturity). The second generation larvae,
9 above all, plays an important role in the epidemiology of *F. verticillioides* in maize (Sobek
10 and Munkvold, 1999) and the insect damage of ears can increase the fumonisin
11 contamination of kernels (Logrieco et al., 2003). ECB feeding activity is crucial in maize
12 grain fumonisin contamination: damaged ears can suffer fumonisin contamination at a 40
13 times higher rate than healthy ones (Alma et al., 2005). Several studies have established
14 that the control of ECB clearly affects fumonisin levels in maize kernel at harvest; this has
15 been demonstrated through the use of methods such as insecticide treatment (Blandino et
16 al, 2008a; Folcher et al., 2009), biological control with parasitoids (Dowd, 2000) and
17 genetic control involving GMO Bt technology (Munkvold et al., 1999; Papst et al., 2005).
18 Aerial applications of spray formulations of Bt appear less interesting since Bt toxins show
19 a low persistence and is susceptible to a rapid degradation by sunlight (Tamez Guerra et
20 al., 1996). Despite the vast amount of literature available on the biology and biological
21 control of *O. nubilalis*, the use of chemical insecticides is still the main method for ECB
22 management, where Bt maize cultivation is not permitted (Saladini et al., 2008). Insecticide
23 treatment against second generation larvae of ECB plays an increasing role in maize crop
24 practices and several insecticides, mainly synthetic pyrethroids, are currently labelled for
25 ECB control in maize and generally applied after the first generation flight peak of adults

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(Blandino et al., 2008a). On the other hand, insecticide treatments are not always able to guarantee maize production with contamination levels of fumonisins below the thresholds (Saladini et al., 2008). Since fumonisin contamination is positively related to the number of tunnels in ears (Alma et al., 2005), a lower grain contamination could be guaranteed by increasing the efficacy of the insecticide treatment, to obtain a major reduction of ECB larval injury in the ears. Increasing the number of insecticide applications is not practicable, because it is not economically favourable and could have a high impact on non-target biota, in particular on the natural enemies of ECB and could lead to a high development of other pests (Ayyappath et al., 1996). The timing of the insecticide application is an important technical aspect that needs to be better understood in order to improve ECB and fumonisin control, since a correct application is crucial to reach the larvae during the few days between the time they hatch and when they enter into the stalk and ear (Mason et al., 1996).

The aim of this study was to investigate the effect of the timing of the insecticide application against the second generation larvae of ECB with pyrethroid lambda-cyhalothrin on insect damage, fungal ear symptoms and fumonisin B₁ and B₂ contamination in maize cultivated in NW Italy.

Materials and methods

Experimental site and treatments

The effect of sowing date and insecticide application on fumonisin contamination in maize kernels was studied in 2006 and 2007 in 2 sites in NW Italy: site A (Vignale, 45° 29' N, 8° 37' E; altitude 160 m., in a sandy-loamy textured soil) and site B (Vigone, 44° 51' N, 7° 30' E; altitude 256 m, in a sandy-medium textured soil).

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6 2 Seven insecticide application timings (T1-T7) were compared to an untreated control (T0),
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8 3 in each site and in each year. The insecticide treatments were applied at approximately 7-
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10 4 d intervals, starting from maize flowering [Growth stage (GS) 65, Weber and Bleiholder,
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12 5 1990; Lancashire et al., 1991], with wet silks (T1).
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18 7 The ECB flight activity was monitored by means of a cone trap placed outside the
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20 8 experimental plots, baited with sex pheromone (E:Z=97:3) to attract males and with
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22 9 phenylacetaldehyde (PAA) for females. The sex pheromones and PAA dispenser were
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25 10 replaced each 15 and 30 d, respectively. Adults were removed from the trap and counted
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27 11 every 1-2 d. The sowing, silking and harvest dates, the ECB flight peak and the insecticide
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29 12 application dates are reported in table 1 for each year and site.
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34 14 The applied insecticide was pyrethroid lambda-cyhalothrin (Karate® Zeon, formulation:
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36 15 capsule suspension, Syngenta Crop Protection, Italy) at 0.019 kg AI ha⁻¹. Treatments were
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38 16 carried out with self-propelled sprayers (Agri JS 826, Kertitox®), with a hydraulically
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40 17 adjustable working height (0.70 – 3.50 m) in order to spray the maize crop after flowering.
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42 18 Twenty fan nozzles on the air-assisted boom applied a spray volume of 400 l ha⁻¹ at a
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44 19 pressure of 200 kPa; the operation speed was 10 km h⁻¹. Air-assisted spraying uses
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46 20 relatively large volumes of low-pressure air, which is generated by a fan, to direct the
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48 21 spray onto the crop.
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56 23 Studies were carried out each year on the commercial dent corn hybrid Pioneer 3235
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58 24 (FAO 600; 130 d). The previous crop was maize each year. The treatments were assigned
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60 25 to experimental units using a completely randomized block design with 3 replicates. Each

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1 plot consisted of 20 rows, separated by two untreated buffer rows on either side; the plot
2 length was 40 m.

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4 During the crop maturation, 30 ears were visually evaluated in each plot for the ECB
5 damage incidence and severity and fungal ear rot symptoms every 10 days, starting from
6 the milk stage (GS 73), until the physiological maturity stage (GS 89).

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8 At the end of maturity, 100 ears (included ears used for the evaluation of ECB and fungal
9 ear rot incidence and severity at harvest) were collected by hand in each plot and shelled
10 using an electric sheller. The ears were collected at a grain moisture content of between
11 23-27%. The kernels in each plot were mixed thoroughly to obtain a random distribution
12 and 5 kg samples were taken to analyze the fumonisin (FB₁ and FB₂) content.

13 14 ***Entomological and mycological measurements***

15 The ECB damage incidence was calculated as the percentage of ears per plot with kernel
16 injuries or apical and basal tunnels in the cob due to larvae activity. The ECB damage
17 severity was calculated as the percentage of kernels per ear with injuries due to larvae
18 activity. A scale of 1 to 7 was used in which each numerical value corresponds to a
19 percentage interval of surfaces exhibiting visible kernel damage due to larvae activity
20 according to the following schedule: 1 = no injuries, 2 = 1-5%, 3 = 6-10%; 4 = 11-20 %, 5 =
21 21-35%, 6 = 35-60%, 7 > 60% (Blandino et al., 2008a).

22 The fungal ear rot incidence was calculated as the percentage of ears per plot with
23 symptoms, while the fungal ear rot severity was calculated as the percentage of kernels
24 per ear with symptoms. A scale of 1 to 7 was used in which each numerical value
25 corresponds to a percentage interval of surfaces exhibiting visible symptoms of the

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3 1 disease according to the following schedule: 1 = no symptoms, 2 = 1-3 %, 3 = 4-10%; 4 =
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5 2 11-25 %, 5 = 26-50%, 6 = 51-75%, 7 > 75% (Blandino et al. 2008a). The ECB damage
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8 3 severity and ear rot severity scores were converted to percentages of ears exhibiting
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10 4 symptoms and each score was replaced with the mid-point of the interval.
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15 6 The data recorded from the visual evaluation of the ears during the crop maturation were
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17 7 used to describe the increase in ECB and fungal ear rot symptoms for the insecticide
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19 8 application timings. A logistic model, based on accumulated growing degree days (GDD)
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21 9 according to an analogous approach set up by Trnka et al. (2007) and Maiorano et al.
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23 10 (2009), was used to describe the development of ECB and fungal ear rot incidence. In the
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25 11 logistic model applied, $Y = 1/[1 + A^{-1} + (B_0 * B_1^X)]^{-1}$, Y represents ECB or fungal ear rot
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27 12 incidence, A is the asymptotic maximum incidence level observed, B₀ is related to the
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29 13 initial disease, B₁ is the rate of damage and symptom progress and X is the accumulated
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31 14 GDD. Both B₀ and B₁ are estimated parameters in the logistic model. ECB and fungal ear
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33 15 rot severity were instead modelled as a linear function of the accumulated GDD. GDD
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35 16 accumulation was calculated from the 1st of January (Mason et al., 1996) by summing the
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37 17 average of the daily maximum (T_{max}) and minimum (T_{min}) temperatures. A base
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39 18 temperature (T_{base}) and a temperature cut-off (T_{cut}) were defined and set equal to 10°C
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41 19 and 30°C, respectively (Got and Rodolphe, 1989). If T_{min} and/or T_{max} were lower than T_{base},
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43 20 T_{base} was considered. If T_{min} and/or T_{max} were greater than T_{cut}, T_{cut} was considered.
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51 21 Fungal isolation and identification were performed through a morphological analysis to
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53 22 verify which *Fusarium* species were involved to the greatest extent occurrence of
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55 23 symptoms. The evaluation of *Fusarium* infections was carried out using 100 kernels
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57 24 randomly sampled from the untreated control at dough stage (GS 85). The kernels were
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59 25 surface disinfested for 3 min in a 2% solution of sodium hypochlorite, then rinsed 3 times
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3 1 with sterile water. The kernels were placed in Petri dishes containing dicloran
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5 2 cloramfenicol peptone (DCPA) and incubated at 20 °C. The *Fusarium* colonies were
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8 3 identified after 7 to 10 days through colony and conidial morphology, as reported by
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10 4 Nelson et al. (1983).
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15 6 ***Fumonisin analyses***

17 7 A 5 kg representative sample of grain from each plot was freeze-dried and milled. A
18 8 representative sub-sample of the milled material was analyzed for fumonisins according to
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20 9 the AOAC Official Method N.995.15 (AOAC, 2000). Briefly, maize samples (50 g) were
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24 10 extracted with 100 mL of methanol-water (3:1, v/v) by blending for 3 min. 10 mL of the
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27 11 filtered extracts were cleaned-up using strong anion exchange (SAX) cartridges and
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29 12 derivatized with an *o*-phthaldialdehyde (OPA)/2-mercaptoethanol solution. Fumonisin-OPA
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32 13 derivatives were analyzed by reversed-phase HPLC with fluorescence detection ($\lambda_{\text{ex.}}=335$
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34 14 nm, $\lambda_{\text{em.}}=440$ nm). The limit of detection (LOD) of the analytical method was 20 ng g⁻¹ for
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37 15 FB₁ and FB₂.
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42 17 ***Statistical analysis***

44 18 The relationship between the ECB and fungal ear rot incidence and severity and the
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46 19 accumulated GDD was studied through nonlinear and linear regression in order to obtain
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49 20 parameters for each insecticide application timing.
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53 22 The normal distribution and homogeneity of variances were verified for the data collected
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56 23 at harvest, by performing the Kolmogorov–Smirnov normality test and the Levene test,
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58 24 respectively. An analysis of variance (ANOVA) was utilized to compare ECB damage and
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60 25 fungal ear rot incidence and severity and fumonisin B₁ + B₂ content, using a completely

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3 1 randomized block design. Each experiment was analyzed separately since there were
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5 2 significant interactions of the treatment with year and site. Multiple comparison tests were
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8 3 performed according to the Student-Newman-Keuls test on the treatment means. The
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10 4 SPSS Version 13.0 for Windows statistical package, (SPSS Inc., Chicago), was used for
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12 5 the statistical analysis. The incidence and the severity values of ECB and fungal ear were
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14 6 previously transformed using $y' = \arcsin \sqrt{x} * 180 / \pi$, as percentage data derived from
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16 7 counting. The concentration of fumonisins was transformed using the equation $y' = \ln(x+1)$
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18 8 to normalize the residuals.
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23 24 25 10 **Results**

26 27 11 ***Meteorological data***

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30 12 The two years were subject to different meteorological trends, as far as both rainfall and
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32 13 temperature (expressed as growing degree days, GDDs) from flowering to harvesting are
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34 14 concerned (Table 2). The year 2007 had more rainfall in May and June, but less in
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36 15 September, compared to the previous year. In this last year, the GDDs from January to
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38 16 May were higher than those observed in 2006, while in 2007 the GDDs were lower from
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40 17 June to July and from September to October. In both years, site A had more rainfall than
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42 18 site B, while the two sites had similar GDDs.
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49 20 ***ECB flight peak, damage incidence and severity***

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51 21 In both years, the flight activity of the first-generation moths started at the beginning of July
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53 22 and peaked about 20 days later (Table 1). In the first year, the captures at flight peak were
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55 23 higher compared to those observed in the second year, while site B showed more
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57 24 captures than site A. On the basis of flight monitoring information, the T5 treatment was
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59 25 applied in each site and year at approximately the ECB flight peak, while the last treatment
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3 1 (T7) was performed about 12-15 days after the ECB flight peak.
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8 3 The increase in ECB incidence and severity during the crop maturation for some
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10 4 representative treatments (NT, T1, T3 and T6) for each site and year is reported in figures
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12 5 1 and 2, respectively. The parameters, coefficients of determination (R^2) and level of
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14 6 significance (P) for the logistic and linear models shown in figures 1 and 2 and those of the
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16 7 other insecticide application timings not shown, are reported in tables 3 and 4.
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20 8 In 2006, the first ear injuries caused by ECB larvae in the untreated plots appeared at
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22 9 GDD=1009 and GDD=1108, in site A and site B, respectively, while in 2007, the first ears
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24 10 showing damage were recorded at GDD=1141 and GDD=1334, respectively. In both sites,
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26 11 the ECB incidence increased rapidly between GDD 1200 and 1500 in 2006 and between
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28 12 GDD 1300 and 1700 in 2007 (Fig. 1). However, figure 2 shows that ECB severity
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30 13 increased linearly over the whole the crop maturation period until the physiological maturity
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32 14 stage.
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39 16 In 2006, the maximum level of ECB incidence in the untreated control (NT) was reached at
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41 17 GDD=1510 (96%) and at GDD=1569 (93%), in sites A and B, respectively. In this year,
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43 18 treatment T3 showed the lowest increase in ECB incidence and severity, while the T1 and
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45 19 T6 insecticide applications resulted in a similar ear damage development to the untreated
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47 20 control. The maximum level of ECB incidence in the T3 treatment was 34% and 38% in
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49 21 sites A and B, respectively, and was reached at approximately GDD=1700 for both sites.
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53 22 In 2007, the maximum level of ECB incidence in the untreated control was reached at
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55 23 GDD=1706 (45%) and at GDD=1810 (75%), in site A and site B, respectively. In this year,
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57 24 treatment T3 reached the maximum level of ECB incidence at approximately GDD=1761
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59 25 (16%) and GDD=1875 (43%), in site A and site B, respectively. Treatments T1 and T4
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3 1 showed a higher final ECB incidence and severity, which was reached earlier than in
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5 2 treatment T3.
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10 4 At harvest, significant differences ($P < 0.001$) between the treatments were observed for
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12 5 ECB incidence and severity in both years and sites (Table 5). In 2006, treatments T3 and
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14 6 T4 in site A and the T2 to T5 treatments in site B showed a significantly lower ECB
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16 7 incidence and severity than the untreated control (NT). In 2007, in site A, a significantly
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18 8 lower ECB incidence and severity was shown compared to NT for the timing of the
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20 9 insecticide application from 7 days after maize flowering (T2) to approximately 7 days after
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22 10 the adult flight peak (T6), while in site B, only the last time of application, applied at
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24 11 approximately 15 days after the adult flight peak (T7), was not significantly different from
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26 12 the untreated control. In both sites, in 2006, and in site A in 2007, the T3 treatment
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28 13 showed the lowest ECB incidence and severity, while both these parameters increased
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30 14 moving to an earlier or later treatment. In 2007, in site B, the highest reduction of ECB
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32 15 damage on ears was observed for treatment T4.
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41 17 ***Fungal ear rot incidence and severity***

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43 18 The most frequently isolated fungal species in the kernel samples taken at the dough
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45 19 stage (GS 85) in each year was *F. verticillioides*. The mean percentage of kernels infected
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47 20 by this species in the untreated control (T0) was on average 65% and 37% in the years
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49 21 2006 and 2007, respectively (data not shown). Other *Fusarium* species, such as *F.*
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51 22 *graminearum*, *F. subglutinans*, *F. culmorum*, *F. oxysporum*, were found each year, but
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53 23 only on some isolated samples.
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60 24 The increase in fungal ear rot incidence and severity during crop maturation for the

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different treatments is reported in figures 3 and 4, respectively. The parameters, coefficients of determination (R^2) and levels of significance (P) for the logistic and linear models are reported in tables 3 and 4. In 2006, the first mold symptoms in the untreated plots appeared at GDD=1261 and GDD=1395, in sites A and B, respectively, while in 2007, the first ears showing fungal ear rot were recorded at GDD=1301 and GDD=1430, respectively. In both sites, the fungal ear rot incidence increased rapidly between GDD 1400 and 1600 in 2006 and between GDD 1500 and 1700 in 2007 (Fig. 3). However, fungal ear rot severity increased linearly during maize ripening until the physiological maturity stage (Fig. 4). As observed for ECB, the insecticide application reduced the impact and delayed the appearance of ear rot symptoms compared to the untreated control, with an effect which was proportional to the efficacy of the ECB control. In both years and sites, treatment T3 resulted in the lowest increase in fungal ear rot incidence and severity, followed by treatment T1, while the T6 insecticide applications resulted in a fungal ear rot development that was similar to the untreated control.

At harvest, significant differences ($P<0.001$) between the treatments were observed for ECB incidence and severity in both years and sites (Table 5). In 2006, treatments T3 and T4 in site A and treatments T2 to T5 in site B showed a significantly lower fungal ear rot incidence than the untreated control (NT), while a significantly lower ear rot severity was also observed for treatments T1 and T2 in site A, and T1 and T6 in site B. In 2007, in site A, a significantly lower fungal ear rot incidence was shown compared to NT for all the insecticide application timings, while ear rot severity was significantly lower than NT in the T2 to T6 treatments. In site B, with the exception of treatments T6 and T7 for ear rot incidence, all the insecticide application timings significantly reduced the fungal ear rot incidence and severity compared to the untreated control.

As far as ECB is concerned, the T3 treatment showed the lowest fungal ear rot incidence and severity at harvest, with the exception of the experiment conducted in 2007 in site B, where the highest efficacy to control ear rot was observed for treatment T4.

Fumonisin contamination

A significant effect ($P < 0.001$) of the insecticide application timing on fumonisin occurrence in maize kernels was observed in both years (Table 5). Only treatment T6 in 2007 in site B and treatment T7 in 2007 and in 2006 in site B, were not significantly different from the untreated control.

As for ECB and fungal ear rot, the T3 treatment showed the lowest fumonisin contamination in each experiment. Contamination of these mycotoxins increased when the treatment was moved to earlier or later dates. Treatment T4 only had a significantly higher fumonisin contamination than T3 in 2007 in site A, while treatment T2 was not significantly different from the previous two insecticide application timings in either year in site B.

For the other application timings, the insecticide application at maize flowering (T1) resulted in a significantly lower contamination than the treatment applied at approximately 7 days after the ECB flight peak (T6) in 2006 in site A and in 2007 in site B.

The reduction in fumonisin level can be expressed by a parameter, efficacy (E), defined by the following ratio (Folcher et al., 2009):

$$E(\%) = \left[\frac{(\text{control fumonisin level} - \text{treatment fumonisin level})}{\text{control fumonisin level}} \right] \times 100$$

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3 1 Considering both years and sites, the average efficacy of the fumonisin contamination
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5 2 reduction was evaluated at 64, 84, 93, 89, 69, 46 and 31%, for the T1, T2, T3, T4, T5, T6
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8 3 and T7 treatments, respectively.
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13 Discussion

15 6 The results of these experiments confirm the important link between fumonisin
16
17 7 contamination, fungal development and ECB activity in the damage of maize ears (Sobek
18
19 8 and Munkvold, 1999; Dowd, 2003). ECB larvae are vectors of *Fusarium* spp.; they cause
20
21 9 entry wounds and carry fungal inoculum from the plant surface to the ears. It has been
22
23 10 demonstrated that in temperate areas *F. verticillioides* is favoured by ECB larvae feeding
24
25 11 more than other *Fusarium* species (Lew et al., 1991; Munkvold et al., 1999). The
26
27 12 development of *F. verticillioides* on damaged area of kernels caused by second generation
28
29 13 ECB is the predominant cause of fumonisin contamination in natural conditions in maize
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31 14 fields in North Italy (Blandino et al., 2008b).

32 15 When the insects were controlled by chemical applications, the fumonisin amounts clearly
33
34 16 decreased within the harvested kernels. The collected data also underline the importance
35
36 17 of timing of insecticide application in order to improve the effectiveness of the fumonisin
37
38 18 contamination control. Under natural conditions, with relatively high ECB levels, the
39
40 19 treatments applied 7-10 days before the ECB adult flight peak (T3), in correspondence to
41
42 20 the constant increase in adult captures, showed the best efficacy to control the insect
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44 21 damage on ears and the lowest fungal ear rot development and fumonisin contamination.
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46 22

47 23 At this insecticide application timing, fumonisins decreased within the maize grains with an
48
49 24 efficacy of more than 90%. This study underlines how a single insecticide treatment with
50
51 25 pyrethroids, when properly applied, is able to provide a reduction in ear mould and
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53 26 fumonisin that is almost comparable with those observed in several experiment with *Bt*
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3 1 hybrids and their isogenic counterparts (Masoero et al., 1999; Munkvold, 1999; Bakan et
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5 2 al, 2002). Since pyrethroids are only effective when the ECB is in its larval status and has
6
7
8 3 not yet penetrated the stalk or ear, and they have approximately 15-d residual activity
9
10 4 (Rinkleff et al., 1995), the correct treatment window for their application ranges between
11
12 5 the first captures of 1st generation adults and the flight peak. If the treatment is delayed
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14 6 until after the adult flight peak, larvae from the eggs deposited early in the egg-laying
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16 7 period enter the plant and are not controlled effectively by the insecticide, causing ear
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18 8 injuries are responsible for fumonisin contamination.

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20 9 Furthermore, an early insecticide treatment, applied at the end of maize flowering without
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22 10 adult captures, showed significantly lower fungal ear rot symptoms and fumonisin
23
24 11 contamination than a treatment applied after the flight peak. Thus, the higher efficacy of
25
26 12 early pyrethroid application is probably due to the control of larvae which initiate feeding
27
28 13 earlier during the maize ripening stages and which have a greater potential to cause a
29
30 14 higher *Fusarium* development and fumonisin contamination than those hatched at the end
31
32 15 of adult flight, which initiate feeding on harder and drier kernels (Mason et al., 1996).
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34 16 Moreover, moulds brought by the first larvae take advantage of a less compact and more
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36 17 easily colonizable substrate (Yates and Jaworski, 2000).
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46 19 The higher effect caused by larvae that arrive at the beginning of the maize ripening
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48 20 stages on ear moulds and fumonisin content could also be supported by data reported by
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50 21 Munkvold et al (1999). Since the *Fusarium* ear rot and fumonisin concentration were
51
52 22 higher in manually infested ECB treatments, the authors suggested that this effect was
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54 23 related to the timing of the manual infestation, which occurred at an early reproductive
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56 24 stage, thus allowing the maximum time for fumonisin accumulation before harvest
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58 25 (Munkvold et al;1999).
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6 2 In the present study, the monitoring of insect injuries during maize ripening clearly showed
7
8 3 that the chemical treatment not only reduced the ECB severity, but also delayed the
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10 4 appearance of ECB damage and shifted the insect activities to a later ear development
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12 5 stage. As Calvin et al. (1988) reported, the impact of ECB feeding declines from the
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14 6 beginning of the reproductive period to physiological maturity. At present, the pyrethroid
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16 7 treatment applied after the first generation ECB flight peak is the normal practice of
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18 8 farmers in North Italy, since they usually spray insecticide when adult catches decline,
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20 9 after the peak has been reached. In our experiments, the efficacy to reduce fumonisin
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22 10 contamination observed with a pyrethroid application at approximately 7 days after the
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24 11 ECB flight peak was between 26 and 54%, confirming values observed in previous studies
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26 12 (Alma et al., 2005; Saladini et al., 2008; Blandino et al., 2008a). Considering the higher
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28 13 efficacy on reducing fumonisin content observed in this research with earlier applications,
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30 14 it can be stated that the timing of the chemical treatment needs to be reconsidered.
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16 Rinkleff et al. (1995) reported that application timing may be particularly flexible for
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18 pyrethroids which have good control and residual activity on neonates. On the other hand,
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20 insecticides with higher contact residual toxicity could play a major role in the control of
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22 this pest and fumonisin contamination. Moreover, determining the best distribution timing
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24 for each insecticide class could lead to a differentiation of the products in function of the
25
26 spray timing, in order to obtain high efficacy treatments throughout the entire chemical
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28 ECB control period. The possible interaction between application timing and type of
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30 insecticide used still needs further investigation, in order to find the best timing of
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32 application for each insecticide and to obtain a larger window for useful chemical
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34 treatments at a farm scale.
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6 2 Since, according to the data collected, the optimum insecticide application timing begins
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8 3 after the trap catches indicated a constant adult ECB activity, the monitoring of adult flight
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10 4 activity with either pheromone or light traps could not give a timely prediction to efficiently
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12 5 drive the management of chemical applications in a certain area. ECB development and
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14 6 activity risk assessment models, based on GDD accumulation, could be used as
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16 7 instruments to help farmers estimate the correct timing of chemical applications during the
17
18 8 growing season (Got et al., 1997).
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24 10 In the present experiments, the insecticides were applied on early planted maize. It was
25
26 11 demonstrated in previous works that later sowing times caused a higher incidence of ECB
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28 12 larvae damage (Anderson et al., 2003) and this is one of the main causes of a higher
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30 13 fumonisin contaminations (Blandino et al., 2009b).
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34 14 The information on the correct application of insecticides against ECB obtained from this
35
36 15 research, should be introduced into a integrated field programme to enhance the
37
38 16 prevention of fumonisin in maize destined for the food chain (Blandino et al., 2009a).
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43 44 18 **Conclusions**

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46 19 In non*Bt* maize fields cultivated in areas with a high ECB pressure, the control of second
47
48 20 generation larvae of this insect by an insecticide application is essential to reduce
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50 21 fumonisin levels in kernels intended for human consumption.
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54 22 In order to obtain a lower yield reduction, associated with a lower fumonisin contamination,
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56 23 as a consequence of the development of ECB injuries on maize ears, the optimum timing
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58 24 of pyrethroid insecticides is comprised between the beginning of a consistent adult flight
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60 25 activity and the flight peak.

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3 1 Since European consumers have shown great opposition to the cultivation of genetically
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5 2 modified organisms and the use of transgenic maize will probably be subject to restrictions
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8 3 in food chains in the near future, the use of insecticides will remain a necessary
9
10 4 component for the Good Agricultural Practices (GAP) of maize for food processing.
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19
20
21 8 Syngenta Crop Protection S.p.A.
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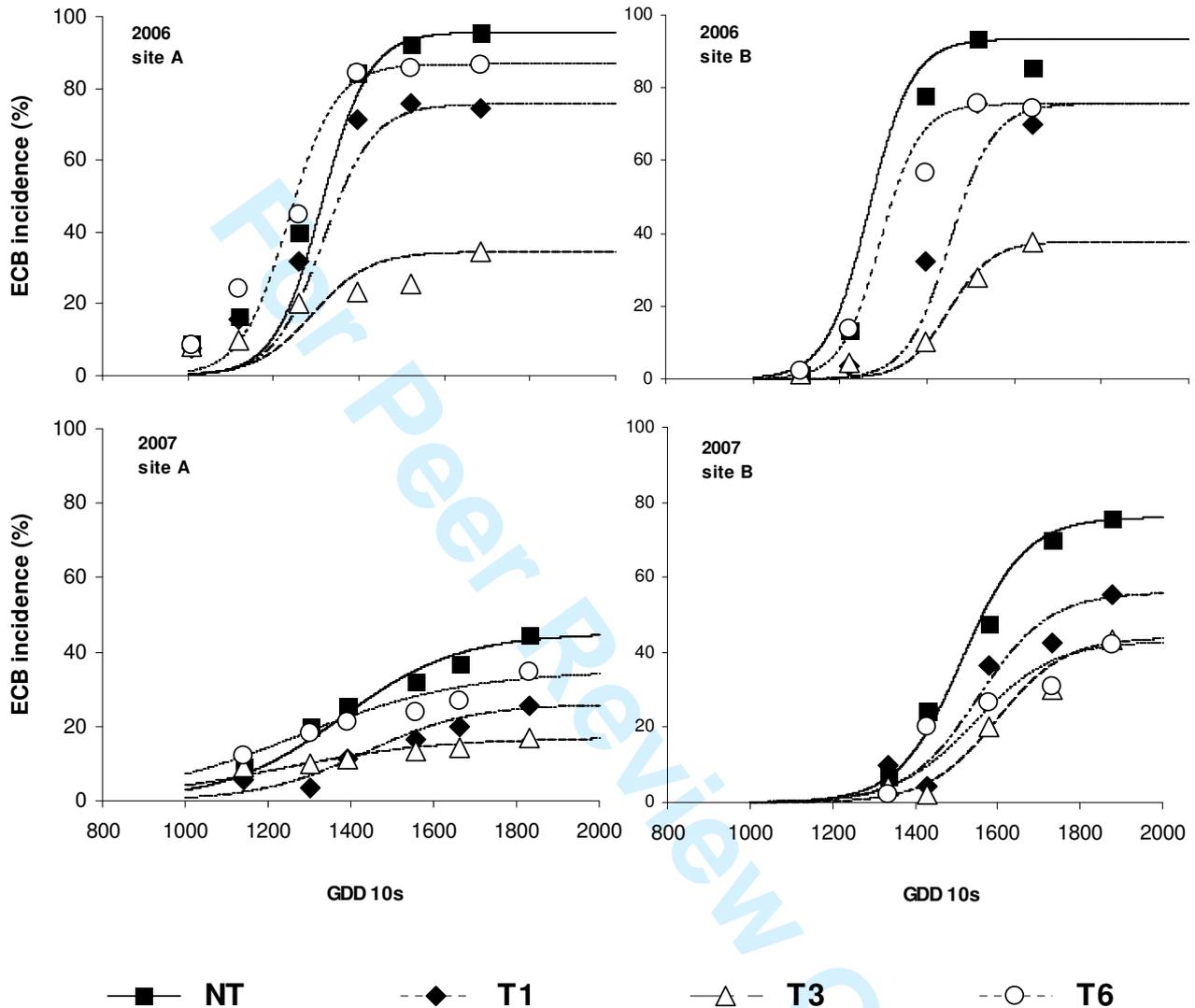
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3 **Figure 1.**

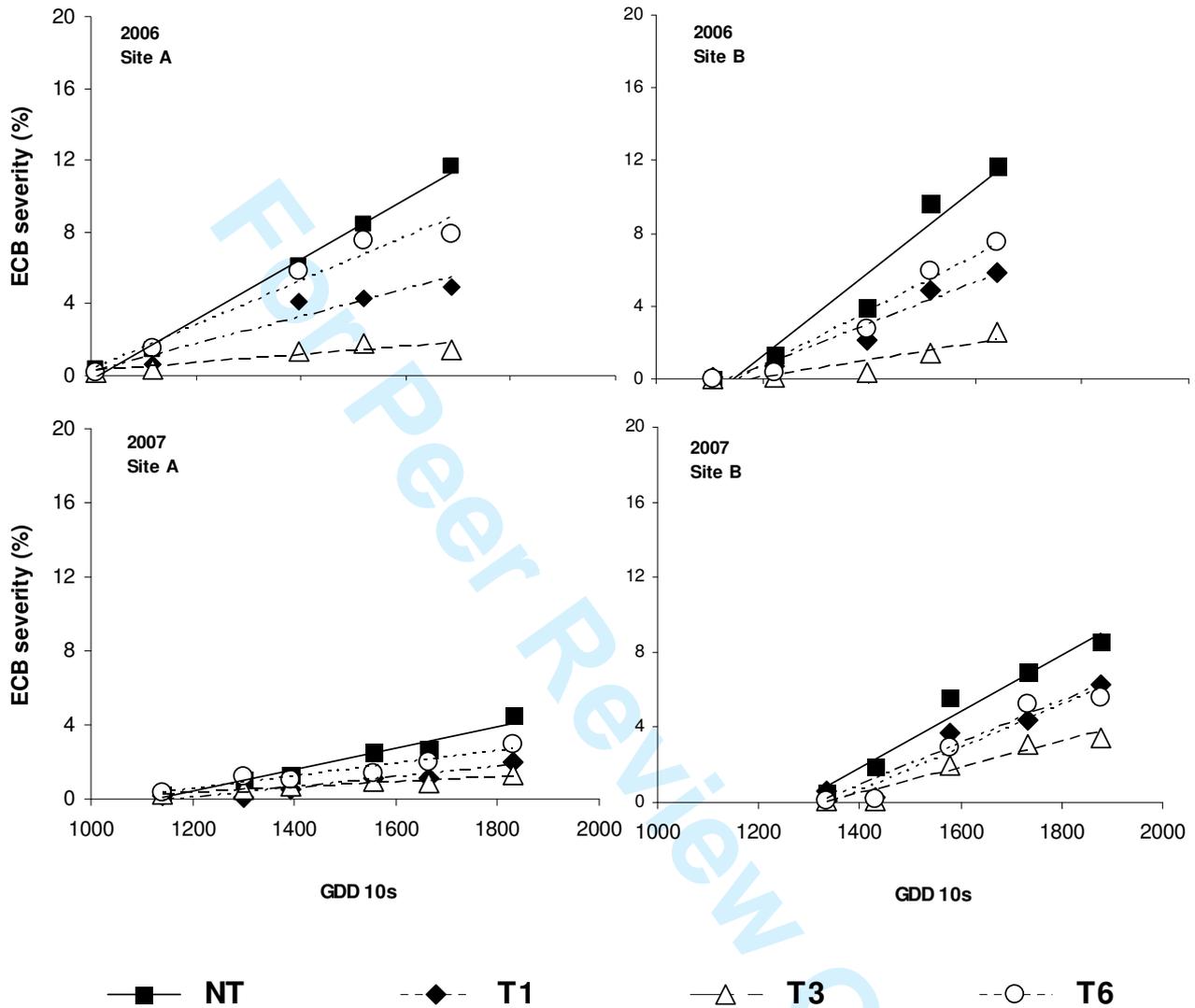
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5 2 Effect of the timing of the insecticide application on the ECB incidence evolution during
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7 3 maize maturation.



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7 ECB incidence was calculated as the percentage of ears with ECB damage, based on 3 replications of 30
8 ears each.

9 The fitted lines are logistic curves fitted to the accumulated GDD calculated from 1st of January. See table 3
10 for details of the fitted models.

1 **Figure 2.**
 2 Effect of the timing of the insecticide application on the ECB severity evolution during
 3 maize maturation.

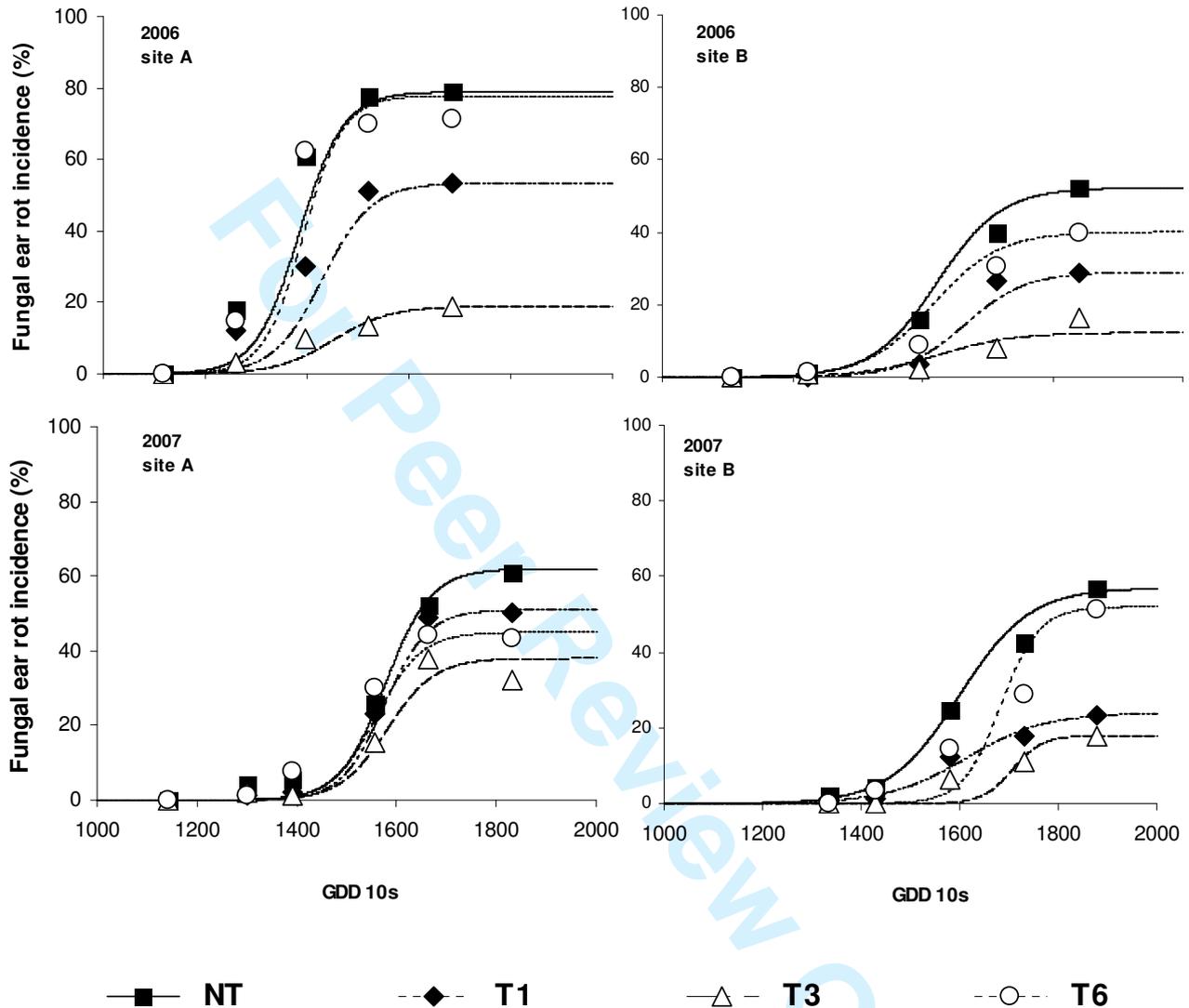


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 7 ECB severity was calculated as the mean number of tunnels per ear, based on 3 replications of 30 ears
 8 each.

9 The linear lines are fitted to the accumulated GDD calculated from 1st of January. See table 4 for details of
 10 the fitted models.

1
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3 **Figure 3.**

4
5 2 Effect of the timing of the insecticide application on fungal ear rot incidence evolution
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7 3 during maize maturation.

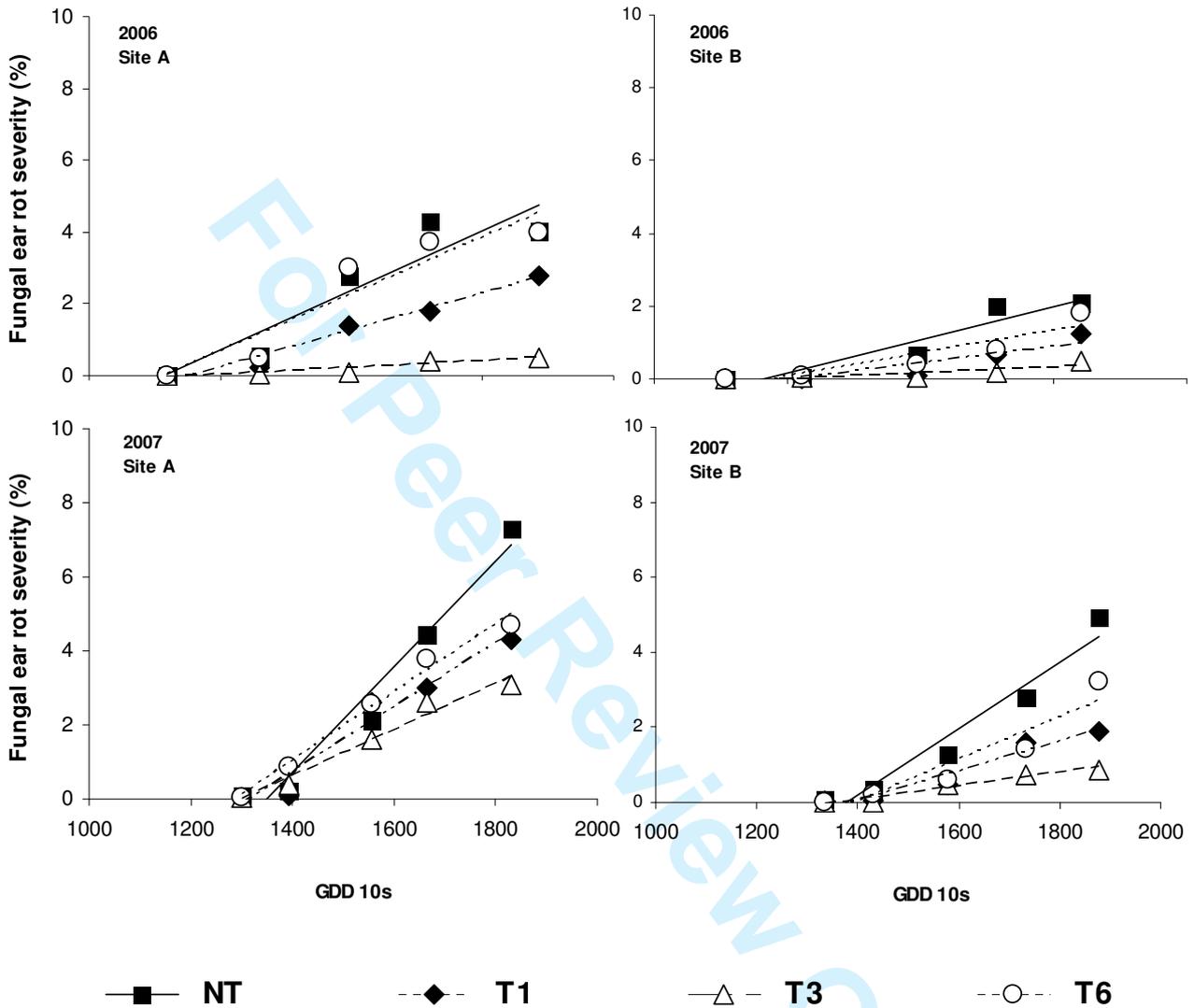


7 Fungal ear rot incidence was calculated as the percentage of ears with symptoms, based on 3 replications of
8 30 ears each.

9 The fitted lines are logistic curves fitted to the accumulated GDD calculated from 1st of January. See table 3
10 for details of the fitted models.

1 **Figure 4.**

2 Effect of the timing of the insecticide application on fungal ear rot severity evolution during
 3 maize maturation.



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5 **■** NT **◆** T1 **△** T3 **○** T6

6 Fungal ear rot severity was calculated as the mean percentage of kernels with symptoms per ear, based on
 7 3 replications of 30 ears each.

8 The linear lines are fitted to the accumulated GDD calculated from 1st of January. See table 4 for details of
 9 the fitted models.

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Table 1.

Main trial information and date of insecticide application in the field experiments conducted in the 2006-2007 period.

Year	2006		2007	
	Site A	Site B	Site A	Site B
Treatment (date of insecticide application)				
T1	June 30	June 27	June 26	June 26
T2	July 6	July 5	July 3	July 3
T3	July 13	July 12	July 10	July 10
T4	July 20	July 19	July 16	July 17
T5	July 27	July 26	July 22	July 26
T6	Aug. 3	Aug. 1	July 30	July 31
T7	Aug. 9	Aug. 8	Aug. 7	Aug. 8
Maize growth stage and ECB flight peak				
Sowing date	April 2	March 25	March 26	March 23
Silking date	June 28	June 26	June 23	June 27
Harvest date	Sept. 26	Sept. 28	Sept. 20	Sept. 24
Date of first ECB adult capture	July 4	July 6	July 3	July 7
Date of ECB flight peak	July 24	July 25	July 23	July 27
Trapping count at ECB flight peak	26	51	14	32

Table 2.

Total rainfall and accumulated growing degree days (GDD) from January to October 2006-2007 in the research sites.

Site	2006				2007			
	Site A		Site B		Site A		Site B	
Year	Rainfall	GDD ^a						
Month	(mm)	(°C d ⁻¹)						
January	37	0	20	4	74	25	35	30
February	59	2	27	7	57	34	39	37
March	45	42	50	43	77	78	47	77
April	86	127	45	131	32	205	16	200
May	72	229	62	233	142	261	111	258
June	34	397	22	352	160	351	101	333
July	62	465	42	469	55	404	37	399
August	120	336	82	348	194	368	97	366
September	115	321	123	323	73	225	52	235
October	103	201	56	199	50	141	37	149
January-October	733	2120	529	2110	913	2091	572	2084

^a Accumulated growing degree day for each month.

Table 3.

Parameters (u , B_0 and B_1)^Z, coefficients of determination (R^2) and level of significance (P) for the logistic model fitted to progress curves of ECB and fungal ear rot incidence on accumulated growing degree-days.

Year	Site	Treatment	ECB incidence					Fungal ear rot incidence				
			A	b_0	b_1	R^2	P	A	b_0	b_1	R^2	P
2006	A	NT	95.7	2.4×10^8	0.982	0.855	0.008	78.9	1.1×10^{12}	0.9771	0.672	0.046
		T1	75.7	8.5×10^7	0.983	0.768	0.022	53.4	1.6×10^{11}	0.9794	0.674	0.045
		T2	59.0	3.8×10^{10}	0.980	0.754	0.025	36.8	5.7×10^{10}	0.9807	0.703	0.037
		T3	34.5	8.7×10^6	0.985	0.671	0.046	18.9	1.1×10^{10}	0.9822	0.586	0.076
		T4	57.9	2.7×10^9	0.983	0.724	0.032	52.3	2.4×10^8	0.9834	0.679	0.086
		T5	83.4	5.6×10^7	0.983	0.743	0.027	82.3	8.3×10^{12}	0.9768	0.865	0.022
		T6	86.8	6.4×10^7	0.982	0.742	0.027	77.8	4.7×10^{13}	0.9746	0.862	0.023
	T7	95.7	2.4×10^8	0.982	0.855	0.008	71.2	4.5×10^{11}	0.9786	0.729	0.066	
	B	NT	93.4	1.4×10^{10}	0.980	0.880	0.006	52.3	5.8×10^{10}	0.980	0.967	0.003
		T1	75.7	7.4×10^9	0.980	0.896	0.004	29	1.1×10^{12}	0.979	0.760	0.045
		T2	42.3	7.7×10^{10}	0.979	0.960	0.001	16.8	1.6×10^{11}	0.981	0.758	0.048
		T3	37.8	1.4×10^{10}	0.980	0.938	0.001	12.3	3.3×10^9	0.983	0.967	0.003
		T4	33.4	4.5×10^9	0.981	0.948	0.001	15.7	1.9×10^9	0.983	0.897	0.015
		T5	55.6	4.1×10^9	0.980	0.812	0.014	37.9	4.8×10^9	0.982	0.867	0.021
T6		75.6	4.1×10^{10}	0.978	0.926	0.002	40.1	2.4×10^{10}	0.981	0.969	0.002	
T7	86.7	8.8×10^{11}	0.9761	0.903	0.004	54.5	6.0×10^{10}	0.9791	0.980	0.001		
2007	A	NT	45.1	3.8×10^2	0.993	0.819	0.013	62.2	1.7×10^{13}	0.978	0.830	0.012
		T1	26.0	2.8×10^3	0.992	0.865	0.007	50.8	1.5×10^{14}	0.977	0.883	0.005
		T2	23.3	6.1×10^2	0.994	0.759	0.024	35.3	2.2×10^{18}	0.972	0.869	0.007
		T3	17.3	3.2×10^1	0.995	0.858	0.008	38.0	7.8×10^{12}	0.979	0.750	0.026
		T4	23.9	6.7×10^1	0.994	0.871	0.007	39.1	7.6×10^{16}	0.974	0.729	0.031
		T5	31.0	1.7×10^1	0.995	0.886	0.005	42.7	6.7×10^5	0.989	0.845	0.010
		T6	35.2	1.5×10^1	0.995	0.817	0.013	45.0	3.2×10^{13}	0.978	0.827	0.012
	T7	46.0	6.7×10^3	0.991	0.808	0.015	47.2	1.6×10^{14}	0.977	0.880	0.006	
	B	NT	76.0	5.0×10^6	0.987	0.984	0.001	57.0	9.8×10^7	0.986	0.900	0.014
		T1	56.2	1.3×10^6	0.988	0.838	0.029	24.2	2.4×10^7	0.988	0.962	0.003
		T2	43.1	1.1×10^7	0.987	0.994	<0.001	24.3	5.3×10^6	0.989	0.897	0.014
		T3	44.0	5.2×10^6	0.988	0.899	0.014	18.1	1.2×10^{23}	0.968	0.874	0.020
		T4	25.9	3.8×10^4	0.991	0.890	0.016	14.9	5.0×10^{15}	0.977	0.783	0.046
		T5	31.2	4.3×10^3	0.992	0.903	0.013	22.4	1.6×10^{15}	0.977	0.789	0.045
T6		43.0	2.7×10^5	0.990	0.882	0.018	52.0	1.4×10^{17}	0.975	0.796	0.049	
T7	55.8	1.2×10^7	0.987	0.963	0.003	55.3	2.9×10^{17}	0.974	0.805	0.046		

^Z Logistic model $Y = 1/[1 + u^{-1} + (B_0 \cdot B_1^X)]^{-1}$; Y represents ECB or fungal ear rot incidence, A is the observed asymptotic maximum incidence level, B_0 is related to initial disease, B_1 is the damage progress rate and X is the accumulated GDD calculated from the 1st of January. The equations were fitted to the mean value of each treatment. The parameters were calculated by SPSS non-linear regression.

Table 4.

Slopes, intercepts and coefficients of determination (R^2) and level of significance (P) of the relationship between ECB and fungal ear rot severity (%) and accumulated growing degree-days.

Year	Site	Treatment	ECB severity				Fungal ear rot severity			
			Slope	Intercept	R^2	P	Slope	Intercept	R^2	P
2006	A	NT	0.016	-16.73	0.984	0.001	0.008	-9.31	0.869	0.021
		T1	0.008	-7.610	0.936	0.007	0.005	-5.93	0.971	0.003
		T2	0.005	-5.57	0.757	0.045	0.002	-1.92	0.694	0.046
		T3	0.002	-2.10	0.813	0.031	0.001	-1.12	0.897	0.019
		T4	0.004	-4.10	0.981	0.001	0.003	-3.25	0.727	0.031
		T5	0.010	-9.38	0.977	0.002	0.006	-6.47	0.965	0.003
		T6	0.011	-10.42	0.844	0.027	0.008	-8.89	0.890	0.019
	T7	0.013	-12.29	0.887	0.017	0.007	-6.77	0.867	0.022	
	B	NT	0.024	-26.48	0.858	0.024	0.004	-5.26	0.899	0.026
		T1	0.012	-13.12	0.846	0.019	0.002	-2.73	0.791	0.043
		T2	0.006	-7.62	0.874	0.020	0.001	-1.03	0.751	0.047
		T3	0.005	-5.48	0.846	0.027	0.001	-0.92	0.734	0.064
		T4	0.004	-4.60	0.962	0.003	0.001	-1.61	0.679	0.039
		T5	0.008	-8.68	0.930	0.008	0.003	-3.29	0.908	0.012
T6		0.015	-17.57	0.909	0.012	0.003	-3.73	0.7913	0.046	
T7	0.016	-18.27	0.983	0.001	0.004	-4.44	0.958	0.004		
2007	A	NT	0.005	-6.65	0.962	<0.001	0.014	-19.16	0.957	0.001
		T1	0.003	-3.26	0.928	0.002	0.009	-11.47	0.954	0.001
		T2	0.002	-2.05	0.717	0.044	0.006	-7.82	0.807	0.015
		T3	0.001	-1.33	0.928	0.002	0.006	-9.09	0.933	0.007
		T4	0.002	-1.54	0.916	0.003	0.007	-8.85	0.761	0.023
		T5	0.002	-1.81	0.991	<0.001	0.007	-8.47	0.784	0.019
		T6	0.003	-3.57	0.918	<0.001	0.009	-9.45	0.946	0.001
	T7	0.004	-4.47	0.965	<0.001	0.009	-11.81	0.905	0.003	
	B	NT	0.015	-19.34	0.967	0.003	0.008	-12.16	0.948	0.006
		T1	0.011	-14.81	0.934	0.007	0.004	-5.40	0.931	0.008
		T2	0.008	-11.19	0.914	0.011	0.003	-4.00	0.905	0.013
		T3	0.007	-9.29	0.936	0.007	0.002	-2.41	0.944	0.006
		T4	0.004	-4.58	0.920	0.010	0.001	-1.65	0.855	0.025
		T5	0.004	-5.50	0.883	0.018	0.002	-2.95	0.994	<0.001
T6		0.011	-15.05	0.841	0.028	0.006	-7.81	0.883	0.018	
T7	0.011	-14.00	0.814	0.036	0.008	-10.94	0.946	0.005		

The equations were fitted to the mean value of each treatment. The growing degree-days were calculated from the 1st of January. The parameters were calculated by SPSS linear regression.

Table 5.

Effect of the timing of the insecticide application on ECB incidence and severity, fungal ear rot incidence and severity at harvest and fumonisin (sum of B₁+B₂) concentration.

Year	Site	Treatment	ECB incidence ^U (%)	ECB severity ^V (%)	Fungal ear rot incidence ^X (%)	Fungal ear rot severity ^Y (%)	Fumonisin B ₁ + B ₂	
							T	N (µg kg ⁻¹)
2006	A	NT	72.0 a	21.7 a	62.9 a	12.5 a	9.1 a	9330
		T1	63.9 a	18.1 ab	51.0 abc	7.3 bc	7.9 c	2830
		T2	59.2 a	16.7 abc	46.0 abc	6.4 bc	7.7 c	2235
		T3	41.1 b	11.6 c	30.2 c	4.7 c	7.1 d	1170
		T4	42.1 b	13.4 bc	38.2 bc	6.4 bc	6.9 d	970
		T5	63.7 a	16.2 abc	55.3 ab	8.5 abc	8.0 bc	3090
		T6	71.5 a	18.9 ab	62.5 a	9.0 abc	8.4 b	4420
		T7	71.8 a	19.6 ab	61.8 a	9.9 ab	8.6 b	5513
		<i>P</i> (F)	< 0.001	0.001	0.001	0.001	< 0.001	
		SEM ^Z	2.7	2.7	0.8	0.6	0.2	
	B	NT	85.0 a	23.2 a	53.8 a	10.5 a	8.1 a	3150
		T1	66.4 a	16.0 abcd	45.1 abc	7.1 bc	6.9 c	950
		T2	46.5 b	11.7 cd	35.3 bc	5.0 cd	6.0 d	400
		T3	42.3 b	10.1 d	27.3 c	3.7 d	4.8 d	120
		T4	46.0 b	10.4 d	28.1 c	4.9 cd	5.5 d	265
T5		44.6 b	12.6 cbd	35.2 bc	6.5 bcd	7.1 c	1200	
T6		72.1 a	18.4 abc	48.0 ab	7.2 bc	7.3 bc	1435	
T7		74.8 a	20.1 ab	50.0 ab	8.8 ab	7.7 ab	2130	
	<i>P</i> (F)	< 0.001	< 0.001	0.001	< 0.001	< 0.001		
	SEM ^Z	3.5	2.3	1.1	0.5	0.2		
2007	A	NT	42.2 a	14.2 a	50.1 a	13.2 a	8.8 a	6970
		T1	39.3 a	13.6 a	42.1 b	10.8 ab	7.9 b	2790
		T2	24.4 bc	9.1 b	28.3 cd	6.0 c	7.3 c	1530
		T3	18.3 c	4.5 d	12.6 e	2.3 d	6.3 d	540
		T4	18.4 bc	5.2 cd	22.4 d	5.5 c	7.0 c	1160
		T5	28.0 bc	7.0 bc	36.6 bc	9.1 b	7.3 c	1480
		T6	28.4 b	8.4 b	34.8 bc	9.3 b	8.1 b	3380
		T7	40.6 a	13.0 a	42.2 b	12.7 a	8.9 a	7830
		<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
		SEM ^Z	2.0	2.4	0.8	0.8	0.2	
	B	NT	61.7 a	19.9 a	60.2 a	14.6 a	6.8 a	940
		T1	45.2 c	15.5 b	45.7 b	11.4 bc	6.0 b	400
		T2	42.4 c	15.3 b	42.4 bc	9.5 cd	3.7 c	40
		T3	43.6 c	11.7 c	37.6 c	6.6 e	3.7 c	40
		T4	24.6 d	9.4 c	26.3 d	6.0 e	4.2 c	70
T5		43.7 c	14.5 b	40.4 bc	8.7 d	5.7 b	290	
T6		54.2 b	14.5 b	55.3 a	11.9 b	6.6 a	700	
T7		60.2 a	18.2 a	55.6 a	12.8 b	6.6 a	755	
	<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		
	SEM ^Z	2.4	2.2	0.7	0.6	0.3		

^U ECB incidence was calculated as the percentage of plants with ECB damage, based on 3 replications of 30 plants each.

^V ECB severity was calculated as the percentage of ears with ECB damage, based on 3 replications of 30 ears each.

^X Fungal ear rot incidence was calculated as the percentage of ears with symptoms, based on 3 replications of 30 ears each.

^Y Fungal ear rot severity was calculated as the mean percentage of kernels with symptoms per ear, based on 3 replications of 30 ears each.

^Z SEM = standard error of mean

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2 1 Means followed by different letters are significantly different (the level of significance is shown in the table).
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4 2 The ECB and fungal ear rot incidence and severity means reported are values transformed using
5 3 $y' = \arcsin \sqrt{x} \cdot 180/\pi$, as percentage data derived from counting. The fumonisin contamination means reported
6 4 are transformed [T; $y' = \ln(x + 1)$] and not transformed (N) values.
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