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# Impact of the insecticide application to maize cultivated in different environmental conditions on emerging mycotoxins

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ABSTRACT

Maize can be competitively colonized by several fungi that are capable of producing a variety of mycotoxins, mainly fumonisins B (FBs), with a negative impact on maize safety and quality.

The aim of this study was to investigate the impact of the insecticide application against European Corn Borer on the contamination of "emerging" mycotoxins and other fungal metabolites co-occurring with the legislated ones in maize for human consumption in North-West Italy from 2009 to 2015.

The insecticide application on average significantly increased the yield by 5%, and significantly reduced the ECB incidence and severity and the fungal ear rot incidence and severity compared to the untreated control.

Overall, 25 *Fusarium* mycotoxins and metabolites were detected. The results underline that the use of the insecticide, the most common FBs control practice in temperate areas on food maize, also resulted in significant reductions of other mycotoxins produced by *Fusarium* spp. of the *Liseola* section. However, this practice was not generally able to reduce the contents of *Fusarium* spp. section *Discolor* and *Roseum* mycotoxins. In environmental and agronomic conditions that favor deoxynivalenol and other metabolites produced by the previous mentioned *Fusarium* spp., the insecticide treatment could even increase their contamination.

## 1. Introduction

Maize (*Zea mays* L.) is a very versatile and multipurpose cereal grain that is used throughout the world as a raw material for feed, food, industrial and energy purposes. This cereal is mainly used in Africa and South America for the preparation of traditional food, such as tortillas, arepas, couscous and porridge.

The consumption of this crop has recently increased in developed countries, as it is used as an ingredient for breakfast products, snacks, dietetic products and, in particular, for baby food and gluten-free food, whose consumption is rising (Escobar et al., 2013).

Unfortunately, this agricultural commodity can be colonized competitively by several spoilage fungi of the *Fusarium*, *Aspergillus*, *Alternaria* and *Penicillium* species that are capable of producing a large variety of mycotoxins as a result of fungal ear rot on maize ears (Marin et al., 2012).

*Fusarium* mycotoxins, which develop mainly in the field under appropriate environmental conditions, are the most common mycotoxins that contaminate maize in temperate areas (Logrieco et al., 2002). Since the same plant tissue can be colonized by various mycotoxigenic species, it is possible that several mycotoxins could co-occur in the same food or feed matrix, with the consequent possible additive or synergic toxicological effects due to their co-presence (Sanhueza and Degrossi, 2004).

Approximately 400 mycotoxins or potential risky fungal metabolites are known to date (Berthiller et al., 2007). Nevertheless, only a very limited number of these mycotoxins are subject to legislation and regular monitoring.

As far as maize is concerned, fumonisins B (FBs), aflatoxins (AFs), zearalenone (ZEA) and deoxynivalenol (DON) are the ones that are reported and monitored the most (Binder, 2007). Among them, FBs are by far the most abundant mycotoxins and constantly present over the

*Abbreviations:* 3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; AFs, aflatoxins; ANOVA, analysis of variance; AUR, aurofusarin; BEA, beauvericin; BIK, bikaverin; BUT, butenolide; CULM, culmorin; DON, deoxynivalenol; DON-3-G, deoxynivalenol-3-glucoside; EC, European Commission; EQU, equisetin; FA, fusaric acid; FBs, fumonisins B; FUS, fusaproliferin; GDD, accumulated growing degree days; GS, growth stage; HPLC, high performance liquid chromatography; JECFA, joint expert committee on food additives; LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry detection; LOD, limit of detection; LOQ, limit of quantification; MON, moniliformin; NIV, nivalenol; PMTDI, provisional maximum tolerable daily intake; ZEA, zearalenone; ZEA-4-S, zearalenone-4-sulphate; α-ZEAol, alpha-zearalenol; β-ZEAol, beta-zearalenol

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years in Mediterranean countries as well as in Italian maize (Marin et al., 2012; Munkvold, 2003; Pietri et al., 2004; Blandino et al., 2015).

The toxicological effects of the regulated mycotoxins are well known: $FB_1$ was classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC, 2002); $AFB_1$ is the most potent known hepatocarcinogen to which humans are widely exposed (group 1B) (IARC, 1993); DON is responsible for serious mycotoxicosis in humans and animals (Pestka and Smolinski, 2005); and ZEA is a non-steroidal estrogenic mycotoxin that causes hormonal effects in animals and humans (Zinedine et al., 2007).

Because of the toxicity of these substances, the European Commission (EC) has set maximum regulatory limits for different mycotoxins in unprocessed maize used for food purposes, through the EC (2007, 2010) regulations.

The other mycotoxins and fungal metabolites, which until now have not received detailed scientific attention, are commonly indicated as "novel" or "emerging" mycotoxins (Streit et al., 2013).

The European Food Safety Authority (EFSA) is currently working on establishing a scientific opinion on the risks to public health related to the presence of "emerging" mycotoxins in feeds and food (EFSA, 2010, 2014).

Little is known about the toxicological effects of these compounds, some of which could be potentially toxic to humans and livestock. In addition, limited information is known about the synergistic or additive toxic effects related to the co-presence of different mycotoxins, both emerging and regulated. Furthermore, although fungal ear rot in maize depends on climatic factors and the susceptibility of a hybrid to the disease, it has been demonstrated that *Fusarium* ear rot is closely correlated to insect injury, and in particular to ear damage caused by Lepidoptera borers (Avantaggiato et al., 2003).

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* and *F. proliferatum* infection in maize grains, which are well-known fungal producers of FBs (Sobek and Munkvold, 1999; Ostry et al., 2010). Moreover, several studies have established that the control of ECB clearly affects FBs levels in maize kernels at harvesting, since the injuries produced by this pest on kernels during ripening appear to be the most important infection pathway in North Italy (Alma et al., 2005; Camardo Leggieri et al., 2015). In 2015, Blandino et al. reported that ECB promotes contamination by the other mycotoxins produced by *Fusarium* spp. of the *Liseola* section in temperate areas, in the same way as for FBs, while it does not affect those produced by *Fusarium* spp. of the *Discolor* and *Roseum* sections.

Since genetic control involving GMO *Bt* technology is forbidden in Italy, as well as in other European countries, the control of ECB is usually conducted through the use of insecticide treatments in order to protect maize and maize-based food products from FBs contamination (Folcher et al., 2009; Blandino et al., 2009a; Mazzoni et al., 2011).

At present, insecticide treatments against ECB in maize and an early sowing date are the two main crop techniques required in the chain agreements between the food processing industry and farmers in Northern Italy (Vanara et al., 2005).

Although insecticide application is a practice widely used in the maize food chain to control the occurrence of FBs, there is still a lack of information about the effect of this strategy on contamination by emerging mycotoxins and other fungal metabolites. Therefore, the aim of this study was to investigate the impact of the insecticide application on the control of emerging mycotoxins and other fungal metabolites co-occurring with the legislated ones in raw maize used for human consumption in North-West Italy over 7 growing seasons under field conditions.

## 2. Materials and methods

### 2.1. Chemicals

Methanol and acetonitrile (both LC gradient grade) were purchased from J.T. Baker (Deventer, The Netherlands); ammonium acetate (MS grade) and glacial acetic acid (p.a.) were obtained from Sigma–Aldrich (Vienna, Austria). Water was purified successively by reverse osmosis, using a Milli-Q plus system from Millipore (Molsheim, France). Fungal metabolite standards were obtained from the following commercial sources: Biopure Referenzsubstanzen GmbH (Tulln, Austria), Sigma-Aldrich (Vienna, Austria), Iris Biotech GmbH (Marktredwitz, Germany), Axxora Europe (Lausanne, Switzerland) and LGC Promochem GmbH (Wesel, Germany).

### 2.2. Experimental design and samples

Field trials were carried out, from 2009 to 2015, over 7 growing seasons and in 2 sites in North-West Italy:

- Trials A, B, C and E: at Cardè, (44° 44′ N, 07° 28′ E; altitude 258 m) in the 2009, 2010, 2011 and 2012 growing seasons, respectively, in a deep and fertile sandy soil, Typic Eutrochrepts (USDA classification);
- Trials D, F, G, H and I: at Carmagnola (44° 50′ N, 7° 40′ E; altitude 245 m) in the 2011, 2012, 2013, 2014 and 2015 growing seasons, respectively, in a loam soil, Typic Udifluvents (USDA classification).

During all the investigated period, rainfall and temperature data were recorded daily from a weather station, located next to the experimental field.

Studies were conducted using maize hybrids that are suitable in the food chain for the production of both flaking grits and meal: Pioneer P1543 (FAO rating 600; 130 days) in trials A, B, C, D and E and Pioneer P1547 (FAO rating 600; 130 days) in trials F, G, H and I.

An insecticide was applied in each experiment (growing season × location) to minimize the ear injuries caused by ECB activity, and it was compared with an untreated control, grown under naturally-infected field conditions.

The insecticides used were: alpha-cypermethrin (pyrethroid) [Contest®, formulation: water dispersible granules, BASF, Italy, applied at 0.044 kg of active ingredient (AI) $ha^{-1}$] in trials A, B, C and E and a lambda-cyhalothrin (pyrethroid) + chlorantraniliprole (diamide) mixture [Ampligo®, formulation: suspension concentrate, capsule suspension, Syngenta Crop Protection S.p.A., Italy, applied at 0.015 and 0.030 kg (AI) $ha^{-1}$, respectively] in trials D, F, G, H and I.

The ECB flight activity was monitored by means of a cone trap placed outside the experimental plots, baited with sex pheromone (E:Z = 97:3) to attract males and with phenylacetaldehyde (PAA) for females. The sex pheromones and PAA dispenser were replaced every 15 and 30 days, respectively. Adults were removed from the trap and counted every 1-2 days. The insecticide was applied during the milk stage, [growth stage (GS) 75] (Zadoks et al., 1974), after noting the presence of the ECB flight peak, by means of a self-propelled ground sprayer (GT7, Grim), according to the conditions described in Blandino et al. (2009a). The sowing, flowering, harvest and the insecticide application dates are reported in Table 1 for each field trial.

The treatments were assigned to experimental units, using a completely randomized block design with 3 replicates. Each plot consisted of 12 rows of a length of 25 m spaced 0.75 m apart, and separated by two untreated buffer rows on either side. The plot alleys, orthogonal to the maize rows, were 1 m wide.

Conventional agronomic techniques were adopted for the field experiments in all of the growing seasons. Briefly, the previous crop was always maize, mechanical sowing was carried out after an autumn ploughing (30 cm) and disk harrowing was conducted to prepare a