

Influence of nitrogen fertilization on mycotoxin contamination of maize kernels

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Abstract

Two experiments were set up from 2000 to 2004 in North West Italy to determine the effects of the type (urea and a slow-release fertilizer) and the application rate of nitrogen (N) fertilizer (0, 100, 200, 300 or 400 kg ha⁻¹) on the susceptibility of maize hybrids to ear rot and to mycotoxin contamination in natural infection conditions. The ears were rated for the incidence and severity of disease symptoms at harvest and the harvested kernels were analysed for mycotoxin fumonisin B₁, zearalenone, deoxynivalenol, aflatoxin B₁ and ochratoxin A. The use of urea instead of a slow-release fertilizer may reduce the contamination of some mycotoxins. There were no significant differences between the two types of N fertilizer in any of the years for zearalenone, fumonisin B₁ or ochratoxin A. Fungal ear rot incidence and severity was generally higher in ears from plants fertilized with insufficient N. High N fertilizer application (> 300 kg N ha⁻¹) significantly increased the zearalenone content, while for fumonisins the highest contaminations were related to N deficiencies (+ 80%).

Deoxynivalenol and ochratoxin A, when found, did not show a clear relationship to N fertilization rate. A negative correlation was found between the N rate and aflatoxin B₁ contamination, when the climatic conditions during ripening favoured this mycotoxin. A balanced N fertilizer application (200 kg ha⁻¹) generally seems to ensure lower mycotoxin contamination and is usually the best solution for low mycotoxin contamination. This study is the first to report on the effects of N fertilizer application on mycotoxin contamination in non-inoculated conditions.

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1. Introduction

Contamination of foods and feeds with mycotoxins is a significant problem throughout the world (Charmley et al., 1995; D'Mello and Macdonald, 1997; Bennet and Klich, 2003). Mycotoxins are secondary metabolites of fungi that have adverse effects on humans and animals resulting in illnesses and economic losses (CAST, 2003). The main groups of toxins commonly found in cereals are fumonisins, mainly produced by *Fusarium verticillioides*, deoxynivalenol (DON) and zearalenone (ZEN) mainly produced by *Fusarium graminearum*, aflatoxins and ochratoxins produced by the fungi genera, *Aspergillus* and

Penicillium (Bilgrami and Choudhary, 1998; Bottalico, 1998).

Mycotoxin contamination in maize depends on the co-existence of host susceptibility and environmental conditions favourable to fungal infection, growth and toxinogenesis. Their occurrence in maize kernels, caused above all by pre-harvest diseases in the field (Abramson, 1998; Chelkowski, 1998), is clearly influenced by the climatic conditions of the year, by microclimatic conditions inside the crop and by agricultural practices (Brown et al., 1998; Doohan et al., 2003; Munkvold, 2003a; Nicholson et al., 2004).

Fungal infection and ear colonization is favoured by high levels of moisture and high relative humidity, from silking to the end of the maturation period (Sutton, 1982; Schaafsma et al., 1993; Reid et al., 1999; Logrieco et al.,

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2002). These favourable conditions could be prolonged as a consequence of rich nitrogen (N) fertilizer applications, which would lead to a longer vegetative growth and higher leaf expansion. Bottalico and Logrieco (1988) and Osunlaja (1990) reported that stalk rot is influenced by N fertilizer application and the disease incidence increases with high fertilizer applications.

On the other hand, maize plants exposed to drought or fertility stress are also more susceptible to infection by microorganisms than plants not under stress. Several authors have reported that the high incidence of maize diseases caused by toxigenic fungi on ears and stalks is often linked to the stress conditions of the crop (Shelby et al., 1994; Rodriguez-Del-Bosque, 1996; Widstrom, 1996; Miller, 2001; Munkvold, 2003a). Elevated aflatoxin levels have been associated with fertility-related stresses, particularly with N deficiency (Lisker and Lillehoj, 1991). Anderson et al. (1975) and Jones and Duncan (1981) reported that a higher rate of N fertilizer application consistently resulted in reduced aflatoxin levels.

In the same way as aflatoxins, tolerance to specific environmental stresses, such as fertility, could also be a way of reducing the vulnerability of maize hybrids to other toxins.

It is necessary to understand whether high levels of N fertilizer application, positively correlated to the microclimatic conditions inside the crop, can influence the mycotoxin content of grains.

The objective of this study was to investigate the effect of N fertilizer application, as types of fertilizers and rate of application, on the development of fungal diseases in maize ears in non-inoculated conditions and on the mycotoxin contamination of grains.

2. Materials and methods

2.1. Experimental site and treatments

The effect of dose and the type of N fertilizer application on mycotoxin contamination in maize kernels was studied in two sites in Piedmont (NW Italy), from 2000 to 2004.

The following treatments were compared each year at site A (45°14'N, 7°51'E; altitude 209 m, in a shallow and sandy soil (Typic Hapludalfs), from 2000 to 2002:

- *Three N fertilization rates:* 0, 200 and 300 kg ha⁻¹. The fertilizer was urea (46%).
- *Two types of fertilizers:* urea (granular, 46%) and a slow-release fertilizer (ENTEC 26[®]), with ammonium N (26%) and the nitrification inhibitor DMPP (3,4-dimethyl pyrazole phosphate). The dose for both fertilizers was 200 kg N ha⁻¹.

In all the treatments, the amount of N was distributed in one application at the eight unfolded leaf growth stage (GS 18, scale BBCH, Weber and Bleiholder, 1990; Lancashire et al., 1991). No N fertilizers were distributed at planting.

The treatments were assigned to experimental units using a completely randomized block design with three replicates. The plot size was 30 m × 12 m. The hybrid used each year was Pioneer 3235 (FAO rating 600; 130 d). Sowing was on April 12 in 2000, April 6 in 2001 and April 20 in 2002. Potassium fertilization was applied to the plots as potassium chloride (150 kg ha⁻¹ at harrowing). Irrigation was applied at flowering to maintain the water-holding capacity between 33 and 200 kPa. Weeds were controlled with flufenacet, isoxaflutole and terbutylazine.

Five doses of N: 0, 100, 200, 300 and 400 kg ha⁻¹ applied as urea fertilizer (46%) were compared each year at site B (44°50'N, 7°40'E; altitude 245 m, in a sandy-medium textured soil (Typic Udifluvents), from 2002 to 2004. In all the treatments, the amount of N was distributed in one application at the eight unfolded leaf growth stage (GS 18). No N fertilizers were distributed at planting.

The plots and treatments used in the experiment were established in 1992; the plots therefore received the same previously cited five doses of N each year.

The treatments were assigned to experimental units using a completely randomized block design with three replicates. The plot size was 20 m × 6 m. The hybrid used each year was Pioneer PR34G13 (FAO rating 500; 125 d) sown on April 16 in 2002, April 8 in 2003 and April 7 in 2004. Potassium fertilization was applied to plots as potassium chloride (100 kg ha⁻¹ at harrowing). Irrigation was applied at flowering to maintain the water-holding capacity between 33 and 200 kPa. Weeds were controlled with metolachlor and terbutylazine pre-emergence and sulcotrione and nicosulfuron post-emergence.

In both sites, 100 ears (included ears used for the evaluation of fungal ear rot incidence and severity and European corn borer (ECB) damage incidence) were collected by hand at the end of maturity (moisture content of grains between 22% and 26%) from five subplots from each plot and shelled using an electric sheller.

The kernels were mixed thoroughly to obtain a random distribution of the kernels, and samples (5 kg) were taken to analyse the mycotoxin content and to perform mycological analysis. These samples were refrigerated at 4 °C, while those for the mycotoxin content analyses were frozen at a temperature of -8 °C.

2.2. Entomological and mycological measurements

The ECB *Ostrinia nubilalis* Hb. damage incidence was evaluated on 30 ears randomly sampled from each plot. This parameter was calculated as the percentage of ears per plot with injury and kernel damage or apical and basal tunnels in the cob due to larval activity.

Fungal ear rot incidence and severity were calculated on 30 ears randomly sampled from each plot. The fungal ear rot incidence was calculated as the percentage of ears per plot with symptoms, while the fungal ear rot severity was calculated as the percentage of kernels per ear with symptoms. A scale of 1–7 was used in which each

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