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The effect of *Busseola fusca* infestation, fungal inoculation and mechanical wounding on Fusarium ear rot development and fumonisin production in maize

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ABSTRACT

Fusarium verticillioides and *Busseola fusca* are among the most significant biotic constraints to maize production in South Africa. In this study, the effect of *B. fusca* damage and mechanical wounding to maize ears on Fusarium ear rot development and fumonisin production was investigated. The effect of the interaction on Fusarium ear rot and *B. fusca* damage was studied by inoculating maize ears with *F. verticillioides* isolate MRC826 and infesting plant whorls with aliquots of 10–15 neonate larvae at the 12th leaf stage prior to tasselling. To simulate hail damage, maize ears were mechanically wounded at the blister stage with cork borers of different sizes and number of wounds, with and without *F. verticillioides* inoculation. Uninoculated, uninfested and undamaged control treatments were included. Field trials were conducted over three seasons using a randomised complete block design with six replicates per treatment. In all seasons, most Fusarium ear rot developed on ears inoculated with *F. verticillioides*. This study indicated that *B. fusca* infestation was not associated with higher Fusarium ear rot incidence in two of the three seasons. Moreover, *B. fusca* infestation was not associated with high fumonisin production in any season. This indicates that the presence of *B. fusca* was not highly associated with Fusarium ear rot or fumonisin production, possibly due to its characteristic feeding nature that causes sporadic wounds on maize ears, but also the strong impact of weather variability. Fusarium ear rot development and fumonisin production by *F. verticillioides* inoculum varied seasonally indicating the importance of environmental conditions on Fusarium ear rot and fumonisin production. Fusarium ear rot and fumonisin production significantly increased with the severity of cork borer wounding in both naturally infected and artificially inoculated maize ears. Therefore, the prevention of severe injuries to kernels, whether by mechanical damage or insects, should be considered as important in reducing Fusarium ear rot and fumonisin production in maize.

1. Introduction

Maize (*Zea mays* L.) is the most important crop cultivated in South Africa for food consumption and as livestock feed (Anonymous, 2014b; Visser, 2015; Kganyago, 2016). Production of maize in the country is, however, affected by a number of biotic and abiotic stresses. One of the most important biotic constraints is *Fusarium verticillioides* (Sacc.) Nirenberg (syn = *F. moniliforme* Sheldon), a fungus that causes Fusarium ear rot, stem rot, root rot and seedling blight (Nelson et al., 1993; Leslie and Summerell, 2006). Infection of maize plants with *F. verticillioides* takes place from contaminated seed (Foley, 1962; Munkvold and Carlton, 1997), lateral root infection by soilborne spores (Oren et al., 2003), as well as through the silk channel by airborne spores (Munkvold and Carlton, 1997; Galperin et al., 2003). Wounding, caused by insects and mechanical damage, also provides an important entry point for the fungus (Flett and Van Rensburg, 1992; Munkvold et al., 1997; Robertson et al., 2011). Fusarium ear rot symptoms become visible as single or groups of pinkish-red mouldy kernels, and/or

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pink or white streaks (White, 1999). *Fusarium verticillioides* can, however, sometimes infect kernels without showing any visible symptoms (Oren et al., 2003).

Infection of maize with *F. verticillioides* results in yield and quality losses of maize kernels (Kommedahl and Windels, 1981; Marasas et al., 1984). The most detrimental effect of the fungus is the production of fumonisin mycotoxins that are harmful to human and animal health (Marasas, 1995; Rheeder et al., 2002). Fumonisins can cause human diseases such as neural tube defects (Missmer et al., 2006), and have been associated with oesophageal cancer in the Eastern Cape province of South Africa (Sydenham et al., 1990; Rheeder et al., 1992) and in the Cixian, Linxian and Shangqiu regions in China (Chu and Li, 1994; Yoshizawa et al., 1994). Various animal diseases, such as pulmonary edema in pigs (*Sus scrofa domestica* L.), can occur as a result of ingestion of fumonisin-contaminated feed (Haschek et al., 2001; Glenn, 2007). Toxicity to chickens (*Gallus gallus domesticus* L.) and broiler chicks also occurs as a result of consumption of fumonisin-contaminated feed. The most dramatic effect of maize feed contaminated with fumonisin is equine leukoencephalomalacia, a neurotoxin disease of horses (*Equus feras caballus* L.) and donkeys (*Equus africanus asinus* L.) (Kellerman et al., 1990; Jovanović et al., 2015).

Maize hybrids grown outside their range of adaptation are more susceptible to *F. verticillioides* infection and concomitant fumonisin production (Shelby et al., 1994). Moreover, hybrid × season and season × location interactions are significant sources of variation for Fusarium ear rot and fumonisin production in maize (Schoeman, 2014; Venturini et al., 2015). Environmental conditions prior to and during silking promote both Fusarium ear rot and fumonisin production in maize (Warfield and Gilchrist, 1999; Janse van Rensburg, 2012). Adverse conditions such as drought stress and high temperature as well as cool, wet climate enhances the development of Fusarium ear rot (Miller, 2001; Ngoko et al., 2001), while temperatures exceeding 26 °C during the dough stage of kernel fill promote fumonisin production (Janse van Rensburg, 2012). Low soil moisture levels coupled with high soil and air temperatures during the later stages of ear maturity and dry down aggravate lateral splits in the kernel pericarp (Murillo-Williams and Munkvold, 2008). This loss of kernel integrity exposes the kernel tissues to *F. verticillioides* infection and concomitant fumonisin production (Odvody et al., 1997).

Infection of maize with *F. verticillioides* has been reported to be increased due to damage caused by stem borers. Infestations with *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), for instance, resulted in higher fumonisin concentrations in non-*Bt* hybrids in the USA (Munkvold et al., 1997, 1999; Hammond et al., 2004), Europe (Bakan et al., 2002; Magg et al., 2002) and Argentina (Barros et al., 2009). In South Africa, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) was associated with a high incidence of Fusarium ear rot (Flett and Van Rensburg, 1992), but its influence on fumonisin production is not known. *Busseola fusca* is regarded as the most injurious pest of maize in South Africa (Van Rensburg et al., 1988a). Its larvae reduce maize yield through feeding on leaves, destruction of the growing point of the plant (dead heart), and by causing stem damage (Van Rensburg et al., 1988a). Female moths oviposit their eggs behind the vertical edges of leaf sheaths of pre-tasselling plants (Van Rensburg et al., 1987; Calatayud et al., 2014), but oviposition can also occur after tasselling when younger plants are not readily available (Van Rensburg et al., 1987). The eggs hatch into larvae that feed on maize plants before turning into pupae, after which adult moths emerge to complete the life cycle (Calatayud et al., 2014).

Information on the effect of wounding on *F. verticillioides* infection of maize ears is lacking. Injuries in maize production systems can occur because of hail damage (Robertson et al., 2011), herbicide damage (Huang et al., 2012), mechanical cultivation equipment (Bricknell et al., 2008) and bird damage (Klosterman et al., 2012). In South Africa, the effect of hail damage to Fusarium ear rot and fumonisin production is particularly important, as the main maize-producing areas of the country are often severely affected by hailstorms (Anonymous, 2014a). Direct damage to maize ears can occur as a result of hail damage during the later reproductive stages of maize (Klein and Shapiro, 2011). The force of the hailstorm results in damage to maize kernels beneath the husks (Klein and Shapiro, 2011), thereby exposing kernels to natural infection with *F. verticillioides*. Hail damage to kernels increased the risk of Fusarium ear rot and fumonisin production in the USA (Robertson et al., 2011), but this has not been investigated under South African farming conditions.

The aim of this study was to determine the effect of damage by *B. fusca* and mechanical wounding to maize ears on Fusarium ear rot and fumonisin production. Mechanical wounding of maize ears was performed to simulate hail damage, which frequently occurs in the maize-producing region in South Africa (Le Roux and Olivier, 1996).

2. Materials and methods

2.1. Experimental design

Two field trials with a conventional non-*Bt* commercial maize hybrid (PAN6723) were planted over three growing seasons in South Africa. One of the trials was used to determine the effect of biological damage caused by *B. fusca* on Fusarium ear rot and fumonisin production, and the other to investigate the effect of mechanical wounds on Fusarium ear rot and fumonisin production. The *B. fusca* damage field trial was planted during the 2008/09, 2010/11 and 2011/12 growing seasons, and the mechanical wounding trials during the 2009/10, 2010/11 and 2011/12 growing seasons.

Planting was done under dry land conditions at the ARC-Grain Crops Institute (ARC-GCI) experimental farm in Potchefstroom, North West province, South Africa (26°73′60.7″S; 27°07′55.3″E) with hybrid seeds purchased from Pannar Seeds (Pty) Ltd (Greytown, South Africa). Soil samples were collected and analysed at the ARC-Institute of Industrial Crops, Soil Analysis Laboratory in Rustenburg, South Africa to determine the quantity of fertiliser that needed to be applied in each season as described by Du Plessis (2003). Pre-emergence (S-metolachlor) and post-emergence (thiadiazine) herbicides were applied for weed control according to the manufacturer's instructions. The *B. fusca* trial was planted with 5-m rows, and the mechanical damage trial with 20-m rows. In both trials the intra-row spacing was 30 cm and inter-row spacing 1.5 m. The experimental rows had two border rows on each side to reduce inter-row interference.

The *B. fusca* trials consisted of four treatments with six replicates, planted in a randomised complete block design. The treatments were: *B. fusca* infestation only, *F. verticillioides* inoculation only, *F. verticillioides* inoculation and *B. fusca* infestation combined, and a control treatment with neither *B. fusca* nor *F. verticillioides*. For the mechanical wounding trial, different levels of wounding were inflicted to maize ears by using cork borers. The following treatments were used with and without *F. verticillioides* inoculation: wounding of all primary ears on each experimental row using cork borers with diameters of 1.59-, 1.75-, 2.23- and 2.39 cm. The 1.59-cm-diameter cork borer was used to stab through the husk to create a single wound. To increase the severity of wounding, five wounds were made with each of the 1.75-, 2.23- and 2.39-cm-diameter cork borers on maize ears at the blister stage. An unwounded control treatment was included.