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Fusarium ear rot and fumonisins in maize kernels when comparing a *Bt* hybrid with its non-*Bt* isohybrid and under conventional insecticide control of *Busseola fusca* infestations

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ABSTRACT

Maize production in South Africa is negatively affected by *Fusarium verticillioides*, an endophytic maize pathogen, as well as by *Busseola fusca* larval damage. *Fusarium verticillioides* causes ear, stem and root rot, and also produces fumonisin mycotoxins which are toxic to humans and livestock. The African stem borer (*Busseola fusca*) is a pest of economic importance in maize plants in South Africa. In this study, the interaction between *F. verticillioides* and *B. fusca* was investigated to elucidate its effects on Fusarium ear rot and fumonisin production in a *Bt* hybrid (MON810 event) and its *B. fusca*-susceptible non-*Bt* isohybrid. Field trials were conducted over three seasons using a randomised complete block design with six replicates per treatment. The effect of Beta-cyfluthrin (non-systemic, granular) and Benfuracarb (systemic, seed treatment) insecticide applications on the incidence of Fusarium ear rot and fumonisin production in maize was also determined in an unrelated conventional hybrid. For *B. fusca* infestations, larvae were dispensed into the whorl of each plant at the 12th leaf stage prior to tasselling, while a *F. verticillioides* MRC826 spore suspension was inoculated through the silks at the silking stage. Maize ears were harvested at physiological maturity and Fusarium ear rot, total fumonisin levels, stem borer damage and target DNA of fumonisin-producing *Fusarium* spp. quantified. Significantly less Fusarium ear rot and fumonisin were produced in the *Bt* maize hybrid compared to the non-*Bt* isohybrid under natural farming conditions, but fungal colonisation and fumonisin production under artificial *F. verticillioides* inoculation did not differ significantly between the *Bt* and non-*Bt* maize. Fumonisin production correlated moderately with the quantity of target DNA of fumonisin-producing *Fusarium* spp. extracted from maize plants. Benfuracarb application to control stem borer infestation resulted in a significant reduction in Fusarium ear rot and fumonisin production while Beta-cyfluthrin did not. Moreover, *B. fusca* damage to maize ears significantly increased when both insecticides were not applied to the *B. fusca*-infested plants. This study indicated that *Bt* maize and the application of Benfuracarb reduce *B. fusca* damage to maize ears thereby indirectly reducing Fusarium ear rot and fumonisin production. However, this was not consistent over seasons due to differences in climatic conditions.

1. Introduction

Maize (*Zea mays* L.) is a summer crop that serves as staple food to millions of Africans, with average human consumption exceeding 300 g per person per day in rural areas of South Africa (Shephard et al., 2007). The crop is affected by the cosmopolitan fungus *Fusarium verticillioides* Sacc. Nirenberg (syn = *F. moniliforme* Sheldon), which occurs in many production regions of the world (Munkvold and Desjardins, 1997; Ncube et al., 2011). *Fusarium verticillioides* causes Fusarium root, stem and ear rots, with symptoms varying from non-symptomatic infections to severe rotting of infected plant parts (Munkvold et al., 1997). The most detrimental

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effect of *F. verticillioides*, however, is that it produces fumonisin mycotoxins that have been associated with diseases of humans and livestock (Marasas, 2001). Fumonisins are naturally occurring metabolites that are produced by *F. verticillioides* beginning from the early post-silking and the dough stages in maize kernels (Janse van Rensburg, 2012).

Busseola fusca (Fuller) (Lepidoptera: Noctuidae) is considered the most injurious pest of maize in South Africa (Van Rensburg and Flett, 2010). *Busseola fusca* is endemic in the Highveld and the western maize production regions of the country (Kfir and Bell, 1993; Kfir, 2000, 2002), and can cause losses of between 10 and 60% (Kfir et al., 2002) under favourable conditions such as, cool and humid climatic conditions (Van Rensburg et al., 1987a) at altitudes ranging from sea level to 2000 m above sea level (Abate et al., 2000). *Busseola fusca* larvae feed mainly in the whorls of plants until the fourth instar, after which they tunnel into the stem (Van Rensburg et al., 1989; Calatayud et al., 2014). Once inside the stem, the larvae cause extensive damage to internal stem tissue (Van Rensburg et al., 1988a). *Busseola fusca* larvae also cause direct damage to maize ears, although this damage can be sporadic (Van Rensburg et al., 1988a). Wounds produced by lepidopteran insects provide a pathway for infection of maize ears and stems by airborne or rain-splashed *F. verticillioides* spores (Sobek and Munkvold, 1999). Maize stem borers of lesser importance in South Africa are *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Kfir et al., 2002) and *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) (Van den Berg, 1997; Van den Berg and Drinkwater, 2000), which cause similar damage as *B. fusca*.

Damage caused by *B. fusca* larvae can be reduced through the application of insecticides (Beyene et al., 2011), crop residue management (Kfir et al., 2002), push-pull habitat manipulation and trap cropping (Khan et al., 2008; Van Den Berg and Van Hamburg, 2015) and biological control (Kfir et al., 2002). However, *B. fusca* damage is most effectively controlled by planting maize genetically modified maize with *Bacillus thuringiensis* (*Bt*) genes that encode for δ-endotoxin crystal proteins that are toxic to lepidopteran insects (Hellmich et al., 2008). *Bt* maize hybrids have been found to be less prone to Fusarium ear rot and fumonisin contamination than non-*Bt* maize hybrids after infestation with *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) in Europe, north and south America (Munkvold et al., 1997, 1999; Bakan et al., 2002; Magg et al., 2002; Hammond et al., 2004; Barros et al., 2009; Bowers et al., 2014). *Bt* hybrids also contain higher concentrations of lignin (Saxena and Stotzky, 2001; Poerschmann et al., 2005; Yanni et al., 2011), which improves the constitutive defence mechanisms in the plant (Freeman and Beattie, 2008). Lignin plays a central role in plant defence against insects and pathogens (Johnson et al., 2009; Barakat et al., 2010) and its synthesis is also induced by insect herbivory or pathogen attack (Ostrander and Coors, 1997; Barakat et al., 2010). High levels of gene expression for the lipoxygenase-derived oxylipin genes protect plants against oxidative stress resulting in maize ears that are resistant to *F. verticillioides* colonisation (Maschietto et al., 2015).

Bt maize (MON810 event, expressing a single Cry1Ab gene) has been widely cultivated in South Africa to reduce *B. fusca* damage. Resistance in *B. fusca* larvae to the MON810 event has, however, occurred due to selection pressure derived from continuous exposure of larvae to sub-lethal levels of the *Bt* toxin at late plant growth stages (Van Rensburg, 2007; Kruger et al., 2011, 2012). The *Bt*-protein concentration in silks of *Bt* maize (MON810 event) is considered low enough to allow survival of some larvae until completion of the first two instars, after which the ear tips and husk leaves serve as important feeding sites (Van Rensburg, 2001). Moreover, resistance to *Bt* maize is attributed to the compromised efficacy of the refuge strategy (Kruger et al., 2012). As a result, the maize industry recently introduced stacked-gene *Bt* hybrids containing MON89034 event which expresses the Cry1A.105 and Cry2Ab2 genes (Van den Berg et al., 2013). These stacked-gene hybrids that produce two different insecticidal proteins are a much more effective insect resistance management (IRM) tool (Van den Berg et al., 2013). Seed mixtures with different ratios of non-*Bt* and *Bt* maize seed have also been suggested as an IRM tool but may not be effective to delay resistance evolution in *B. fusca* (Erasmus et al., 2016).

In regions where national restrictions on *Bt* maize cultivation are enforced, insecticides are used to control insects such as stem borers (Meissle et al., 2010). The application of the pyrethroid insecticide Beta-cyfluthrin, a granular stomach and contact insecticide, successfully controls *B. fusca* and *C. partellus* larvae (Beyene et al., 2011). A mixture of Endosulfan and Deltamethrin applied into the whorl or the sides of plants has also effectively controlled *B. fusca* in maize in South Africa (Van den Berg and Van Rensburg, 1996). The application of foliar insecticide spray formulations of Chlorpyrifos, Imidacloprid, Cypermethrin + Dimethoate and Lambda-cyhalothrin were further found to be effective in controlling *B. fusca* larvae (Adamu et al., 2015). Insecticide control can be economically applied during the vegetative growth stages when approximately 10% of plants are infected with *B. fusca* (Van Rensburg et al., 1988b). Application of insecticides in the whorl is potentially effective until shortly before tasselling, while application after tasselling appearance results in poor control of *B. fusca* because insecticides are ineffective against larvae in stems (Van Rensburg et al., 1987b). The cryptic feeding behaviour of stem borer species inside plant stems adversely affects the efficacy of whorl applied insecticides (Slabbert and Van den Berg, 2009), making the use of *Bt* maize a viable option for pest management.

Busseola fusca damage has been reported to increase the incidence of Fusarium ear rot caused by *F. verticillioides* in conventional maize hybrids in South Africa (Flett and Van Rensburg, 1992). More than 70% of maize planted on commercial farms in South Africa is genetically modified for insect resistance and herbicide tolerance (James, 2012). Subsistence farmers, however, rely on insecticides, particularly Beta-cyfluthrin, to control *B. fusca* (Ncube, 2008). This study, therefore, was conducted to elucidate the effect of *Bt* maize and insecticide application, as *B. fusca* control strategies, on Fusarium ear rot and fumonisin production in maize in South Africa.

2. Materials and methods

2.1. Experimental design

Field trials to evaluate the effect of *Bt* maize and insecticides on Fusarium ear rot and fumonisin production in maize plants infested with *B. fusca* and inoculated with *F. verticillioides* were performed in the North West province of South Africa. Field trials comprising of a commercial *Bt* maize hybrid (PAN6236B) expressing the MON810 event, and its insect-susceptible non-*Bt* isohybrid (PAN6126), were planted at the ARC-Grain Crops Institute (ARC-GCI) experimental farm in Potchefstroom (26°73′60.7″S; 27°07′55.3″E) during the 2009/10, 2010/11 and 2011/12 seasons. The MON810 event was the only commercially available *Bt* event for the control of stem borers in maize in South Africa at the time of the study.

Field trials in which insecticide efficacy was evaluated were also planted. These trials involved a non-*Bt* (conventional) maize hybrid (PAN6723), and was planted at the ARC-GCI experimental farm during October and late December 2012 (first and second trial, respectively), and at Buffelsvlei near Ventersdorp (26°49′38.6″S; 26°60′02.9″E) in November 2012. All trials were planted under conventional dry land conditions. Soil analysis was performed before planting to calculate the quantity of fertilisers required. Pre-

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