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Use of resistance elicitors to reduce Fusarium ear rot and fumonisin accumulation in maize

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ABSTRACT

Fusarium ear rot of maize, caused by *Fusarium verticillioides*, reduces grain yield and quality and contaminates it with mycotoxins (fumonisin). As maize cultivars with complete resistance to Fusarium ear rot and fumonisin contamination have not been identified, and since the disease is difficult to control by conventional means, novel strategies for disease control and fumonisin reduction are required. One such strategy could involve the use of chemical elicitors that induce resistance in plants against a broad spectrum of pathogens, insects and abiotic stresses. In this study the ability of resistance elicitors to reduce infection by *F. verticillioides* and prevent fumonisin contamination in maize was investigated, and their impact on yield assessed. Five elicitors were selected based on their ability to activate different pathways in plant defence systems. The elicitors included β-amino butyric acid (BABA), benzothiadiazole (BTH), harpin protein, 2,6-dichloroisonicotinic acid (INA), and methyl jasmonate (MeJA). A fungicide containing difenoconazole (triazole) and azoxystrobin (strobilurin) as active ingredients and that has known fungicidal activity in other plant-pathogen interactions, but not specifically Fusarium ear rot, was also included. The plant resistance elicitors and fungicide were evaluated in multi-site field trials. Following artificial inoculation with *F. verticillioides* isolate MRC 826, a high fumonisin producer, visual rating of Fusarium ear rot severity was performed and fumonisin B_1, B_2 and B_3 content of the grain quantified with high performance liquid chromatography. None of the five elicitors or the fungicide consistently reduced Fusarium ear rot and/or fumonisin contamination significantly. Treatment of maize with BTH resulted in a significant reduction in ear filling and yield in field trials. Treatment effects on fumonisin content were influenced by maize genotype and trial location, since significant interactions were observed between treatment, maize genotype and trial location. While the evaluated elicitors might be useful for prevention of maize foliar diseases, they were not effective at reducing Fusarium ear rot or fumonisin contamination. However, optimisation of elicitor application method, dose, frequency and timing could possibly yield improved results.

1. Introduction

Plants possess a variety of defence mechanisms to protect themselves against microbial attack, including pre-existing physical and chemical barriers, as well as inducible defence mechanisms (Guest and Brown, 1997; Hematy et al., 2009). Induced resistance may be expressed locally at the site of attack, or systemically in plant tissue away from the point of attack. Systemically induced plant responses can be triggered by biological, chemical and environmental stimuli (Walters et al., 2005; Lyon, 2007). When the defence response leads to limited infection at the site of attack, as is the case with hypersensitive necrosis, a plant response known as systemic acquired resistance (SAR) is established (Sticher et al., 1997; Durrant and Dong, 2004). In contrast, when the resistance response does not involve cell necrosis, for instance when plant roots are colonised by selected strains of non-pathogenic rhizobacteria, a plant response referred to as induced systemic resistance (ISR) is initiated (Van Loon et al., 1998). Exogenous treatment of plants with a variety of biotic and abiotic elicitors in the form of fungal and bacterial products, plant extracts and plant hormones, minerals and ions, and others such as synthetic compounds can also induce these responses (Lyon, 2007).

Despite the expected potential for the use of elicitors to induce resistance against diseases of agricultural crops, limited reports of their use in the Monocotyledoneae, which include the most important cereals such as maize, wheat and rice, are available

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(Kogel and Langen, 2005). Examples of the successful use of resistance elicitors on cereal crops such as wheat do exist, but have mostly been restricted to foliar diseases, such as powdery mildew (Görlach et al., 1996; Vechet et al., 2009). One of only a few published studies on induced resistance in maize involved benzothiadiazole (BTH) and 2,6-dichloroisonicotinic acid (INA) being used successfully to reduce downy mildew, caused by *Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw (Morris et al., 1998). Monocotyledonous crops, such as maize, share conserved elements of the induced resistance signalling pathways with dicotyledonous crops (Kogel and Langen, 2005), for which significantly more information exists on the use of resistance elicitors for disease control (Sticher et al., 1997; Pieterse and Van Loon, 2004; Conrath et al., 2006; Walters and Heil, 2007; Walters and Fountaine, 2009). This suggests that induced resistance could potentially be used as part of an integrated crop protection approach on cereal crops.

Maize is an important staple food crop to millions of people in southern Africa (Pingali and Pandey, 2001). A disease of major importance to maize producers and processors, due to its reduction in grain quality, is Fusarium ear rot caused by *Fusarium verticillioides* (Sacc.) Nirenberg (Afolabi et al., 2007). In addition, *F. verticillioides* often contaminates maize kernels with fumonisins (Rheeder et al., 2002), a group of fungal secondary metabolites associated with mycotoxicoses of animals and humans (Marasas, 2001; Stockmann-Juvala and Savolainen, 2008). Complete resistance to Fusarium ear rot and fumonisin contamination in maize cultivars planted in southern Africa has not been found (Rheeder et al., 1990; Schjøth et al., 2008), and no fungicides are registered for its control (Nel et al., 2003). An integrated disease management strategy, therefore, is required to prevent Fusarium ear rot development in the field. Strategies to prevent infection by toxigenic fungi and their associated mycotoxin contamination prior to harvest generally involve a combination of cultural practices, such as crop rotation, tillage, adherence to optimal planting date and plant densities, and management of irrigation and fertilisation (Munkvold, 2003). However, when environmental conditions are favourable for Fusarium ear rot and fumonisin contamination, cultural practices alone are not sufficient to prevent unacceptable levels of fumonisin contamination (Robertson et al., 2005).

The possibility of enhancing plant resistance to Fusarium ear rot and fumonisin contamination in susceptible commercial maize hybrids with resistance elicitors would provide maize producers with an economically viable and environmentally sound means to enhance their crop's quality and safety. This disease management tool, if effective, could then be applied alone or form part of an integrated disease management strategy. Therefore, the objective of this study was to investigate the ability of known resistance elicitors, and a fungicide, to reduce Fusarium ear rot and fumonisin contamination of maize under field conditions. Their impact on yield and the response of different maize cultivars to treatment with chemical elicitors was also evaluated.

2. Materials and methods

2.1. Treatments, maize cultivars and fungal inoculum used

Five resistance elicitors, namely β-amino butyric acid (BABA) (Sigma–Aldrich, Inc, St. Louis. USA) (Zimmerli et al., 2000), methyl jasmonate (MeJA) (Sigma–Aldrich) (Wasternack, 2007), INA (Sigma–Aldrich), BTH (Syngenta SA, Johannesburg, South Africa) (Durrant and Dong, 2004; Vlot et al., 2009), harpin (Insect Science, Tzaneen, South Africa) (Wei et al., 1992) and a systemic fungicide containing difenoconazole (triazole) and azoxystrobin (strobilurin) as active ingredients (Syngenta SA) were evaluated for their ability to reduce Fusarium ear rot and fumonisin contamination of maize in the field (Table 1). The elicitors were chosen based on their known ability to induce resistance in other host-pathogen systems and their activation of a variety of host signalling pathways and defence mechanisms. The fungicide was selected for its antifungal activity, as well as for the beneficial effects reported to occur with use of strobilurin-based fungicides, such as yield increase (Bartlett et al., 2002). The field trials were carried out on three commercial maize cultivars (CRN 3549, PAN 67, and PAN 6479) under two different field production environments. Three controls were included in the field studies. An ethanol treatment control was included since INA and MeJA were dissolved in absolute ethanol (Merck Chemicals, Gauteng, South Africa) and the surfactant Tween 20 (polyoxyethylene 20-sorbitan monolaurate; Fischer Biotech, Fairlawn, NJ) before dilution in de-ionised water (Table 1). Two inoculation controls were used: one a positive control (inoculated control) where the maize ears were artificially inoculated with a spore suspension of *F. verticillioides* at a concentration

Table 1
Active ingredients and application rates of resistance elicitors and a fungicide evaluated for their ability to reduce Fusarium ear rot severity and fumonisin accumulation in maize.

Treatment	Active ingredients	Application rate	Inoculation	Reference
Fungicide	Difenoconazole (triazole) & azoxystrobin (strobilurin) (125 g L^{-1}/200 g L^{-1})	7.14 ml L^{-1} (500 ml per 70 L ha^{-1})	Spore suspension[a]	Supplier's recommendations
BABA	DL-3-aminobutyric acid (97%)	1.00 g L^{-1}	Spore suspension	(Amzalek and Cohen, 2007)
BTH	Benzothiadiazole (BTH) (500 g kg^{-1})	0.50 g L^{-1} (35 g per 70 L Ha^{-1})	Spore suspension	Supplier's recommendations (Oostendorp et al., 2001)
Harpin protein	Harpin protein (3%)	0.70 g L^{-1} (49 g per 70 L Ha^{-1})	Spore suspension	Supplier's recommendations
INA[b]	2,6-dichloroisonicotinic acid (98%)	50 mg L^{-1} INA + 0.13 ml L^{-1} ethanol (99.5%) + 0.1 ml L^{-1} Tween 20	Spore suspension	(Dann et al., 1998) (Morris et al., 1998)
MeJA[b]	Methyl jasmonate (95%)	0.12 ml L^{-1} MeJA (0.55 mM) + 0.13 ml L^{-1} ethanol (99.5%) + 0.1 ml L^{-1} Tween 20	Spore suspension	(Deepak et al., 2007) (Desmond et al., 2005) (Walters et al., 2002)
Ethanol + Tween 20 (ethanol treatment control)	–	0.13 ml L^{-1} ethanol (99.5%) + 0.1 ml L^{-1} Tween 20	Spore suspension	–
Untreated (inoculated control)	–	–	Spore suspension	–
Untreated (water-inoculated control)	–	–	Sterile water	–
Untreated (natural infection)	–	–	Non-inoculated	–

[a] Silk channel inoculation with 2 ml of conidial suspension (2×10^6 spores ml^{-1}) of *Fusarium verticillioides* or sterile water (inoculation control).
[b] Active ingredient was dissolved by adding 2.5 ml L^{-1} absolute ethanol + 0.1 ml L^{-1} Tween 20 before dilution.