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Probable effects of dual inoculation of maize (*Zea mays*) stem with *Fusarium verticillioides* and *Trichoderma* species on fumonisin content of maize seeds

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Seeds from maize (*Z. mays*) plants whose stems received various treatment combinations of pathogen (*F. verticillioides*) and four antagonists (i.e. *Trichoderma harzianum* strain 2, *T. hamatum*, *T. pseudokoningii* strains 2 and 11) in the field were subjected to fumonisin analysis. Three pairing methods were employed for the inoculation of pathogen and the antagonists into stem of the maize plant, viz., 'Pathogen inoculated before Antagonist', 'Antagonist inoculated before Pathogen', and 'Antagonist and Pathogen inoculated simultaneously'. Controls include 'Inoculation of pathogen alone', 'Inoculation of antagonist alone', and 'Inoculation of sterile toothpicks'. Inoculation method used was the toothpick method. Seeds were harvested five weeks after inoculation and subjected to fumonisin analysis. Resulting data were subjected to ANOVA using the GLM procedure of SAS. There was a high significance among treatments i.e. there were varying levels of fumonisin occurrence among the treatments and varying *Fusarium* occurrences within the blocks. Seeds from treatments involving 'Inoculating *T. pseudokoningii* strain 5 alone' and 'Inoculating *T. harzianum* strain 2 alone' had the highest mean fumonisin content (P>0.01) which were not significantly higher than in control. Seeds from treatments involving 'Inoculating *T. pseudokoningii* strain 5 and pathogen simultaneously' and 'Inoculating *T. harzianum* strain 2 before pathogen' were significantly low in fumonisin content compared to seeds from other treatments. Seeds which received 'Inoculation of *T. hamatum* alone' were also significantly low (P>0.01) in fumonisin content compared to others. It could thus be said that treatments involving *Trichoderma* species applied in the maize stem might have effect on the fumonisin content and hence *Fusarium* occurrence in the seeds depending on the occurrence pattern of the *Trichoderma* within the maize stem.

Key words: Fumonisins, antagonist, *Trichoderma* species, pathogen, *Fusarium verticillioides*

INTRODUCTION

F. verticillioides is known to pose a severe threat to human and animal health due to its potent mycotoxigenic and carcinogenic characteristics (Marasas, 1988). Generally, the pathogen is known to produce different mycotoxins which pose severe threats to both human and animal health. Such mycotoxins include fusaric acid, trichothecenes, moniliformins, zearalenones, fumonisin, amongst others (Porter *et al*., 1995; Amoah, 1994; Julian *et al*., 1995; Nguen-Khong-Min and Smirnov, 1992). The toxicity of the corn contaminated by *F. verticillioides* has been well-documented for more than a hundred years, but the fumonisins were discovered only recently.

The fumonisins is a group of structurally related secondary metabolites, which has been found to contain fumonisin B_1(FB_1) and B_2 (FB_2) with cancer-promoting activity in rat liver and they have been found to occur naturally in maize-based feed samples associated with field outbreaks of equine leukoencephalomalacia (ELEM), a fatal brain disease in horses, donkeys, mules, and rabbits (Gelderblom *et al*., 1988; Thiel *et al*., 1991; Munkvold and Desjardins, 1997). FB_1 was reported to cause liver cancer in rats (Gelderblom *et al*., 1991), ELEM in horses (Marasas *et al.,* 1988) and porcine pulmonary edema (PPE) in pigs in which the principal lesions, is not in the brain but in the lungs (Harrison *et al.,* 1990; Munkvold and Desjardins, 1997).

Because *F. verticillioides* infects maize worldwide, fumonisins contamination of maize from all geographic regions is not unlikely. In maize grown in temperate regions*, F. verticillioides* and *F. proliferatum* represent the greatest threat for fumonisin production (Munkvold and Desjardins, 1997). In the experiment conducted by Yates *et al* (2000), *Trichoderma viride* isolated from the roots of corn plants was able to suppress the radial colony extension of *Fusarium verticillioides* on agar as well as the fumonisin B_1 (FB_1) on corn kernels.

Trichoderma species are known to be commonly used as biocontrol agents against several pathogens (Paavanen-Huhtala *et al*., 2000). The genus *Trichoderma* had actually produced tested fungal antagonists against several pathogens of many crops (Etebarian *et al*., 2000). The experiment was therefore conducted to examine the fumonisin content of maize (*Z. mays*) seeds whose stems had received dual inoculations of *F. verticillioides* and some *Trichoderma* species.

MATERIALS AND METHODS

Planting and inoculation in the field

Maize seeds (DMR-LSRW) were planted in the field in three replicates, with each replicate representing a block of 30 rows (14.5m by 15m). There were 25 plant stands per row and 90 rows in all the three replicates, experimental design being randomized complete block (RCBD). Seven weeks after planting, sterile 1.8mm-diameter nails on wooden handles were used to puncture each stem on either sides at the 3rd internode (Drepper and Renfro 1990). Four of the antagonists employed in Sobowale *et al* (2007) viz., *Trichoderma harzianum* strain 2, *T. hamatum*, *T. pseudokoningii* strains 2 and *T. pseudokoningii* 11 were used. The pathogen (*F. verticillioides*) employed was isolated and identified in the pathology laboratory, International Institute of Tropical Agriculture, Ibadan, Nigeria. Toothpicks that have been dressed separately with pathogen and the antagonists were inserted into the holes on either side of the stems using toothpick method as employed by Sobowale *et al* (2007). Three pairing methods were used for the inoculation of pathogen (*F. verticillioides*) and the antagonists into the stem of the maize (*Z. mays*) plant, viz., 'Pathogen inoculated before Antagonist', 'Antagonist inoculated before Pathogen', and also 'Antagonist and Pathogen inoculated simultaneously'. Controls include 'Inoculation of Pathogen alone', 'Inoculation of Antagonist alone', and 'Inoculation of sterile toothpicks alone. There were thus 6 treatments in a block of 30 rows (5 rows per treatment) while border rows were not inoculated.

Fumonisin Assay

Five weeks after inoculation (12 weeks of planting), seeds from maize (*Z. mays*) plants whose stems received the various treatment combinations of pathogen (*F. verticillioides*) and the four antagonists, *Trichoderma harzianum* strain 2, *T. hamatum*, *T. pseudokoningii* strains 2 and 11 in the field, were collected and subjected to fumonisin analysis.

The Veratox for fumonisin test kit was used, this being a direct competitive enzyme-linked immunosorbent assay (ELISA) for the quantitative analysis of fumonisin in such commodities as corn, corn meal and rice. The kit was stored refrigerated at 2-8°C.

Maize sample preparation and fumonisin extraction

The maize samples for each treatment were ground to a fine blend and thoroughly mixed. All the samples were stored at 2-8°C until analysis was completed. 70% methanol was used for the extraction processes. 25 grams of the ground sample was blended with 125ml of 70% methanol for 2 minutes in a high-speed blender. The extract was filtered by passing 10ml through a Whatman #1 filter and the filtrate was collected in beaker. The filtrate sample was then subjected to analysis using the standard fumonisin test kit (VERATOX). After analysis, the bottoms of the microwells were wiped with dry towels and later read in a microwell reader using a 650nm filter. Air bubbles were eliminated as they could affect analytical results. The result was read within 20 minutes of completion of the test. The results were manually calculated without Neogen's Log/Logit Software and the data obtained were subjected to ANOVA using the GLM procedure of SAS.

RESULTS

Table 1 shows the summary of the fumonisin analysis done on the maize seeds from the plants that received treatment combinations of the pathogen and the selected antagonists. The seeds from the maize plants whose stems received treatments of *T. pseudokoningii* strain 5 alone and *T. harzianum* strain 2 alone had the highest mean fumonisin content (6.90 and 6.88 respectively) though not significantly higher than those of the controls ('Inoculating pathogen alone' and 'sterile toothpick alone') which had mean fumonisin content of 5.40 and 5.30 respectively. On the other hand, seeds from plants that received treatments containing the same *T. pseudokoningii* strain 5 and *T. harzianum* strain 2 (i.e 'Inoculating *T. pseudokoningii* strain 5 and pathogen simultaneously' and also 'Inoculating *T. harzianum* strain 2 before pathogen') were also among the ones with the lowest fumonisin content. However seeds from plants that received treatments involving 'Inoculation of *T. hamatum* alone', 'Inoculation of *T. pseudokoningii* strain 5 and pathogen simultaneously', and 'Inoculation of *T. harzianum* strain 2 before pathogen' were significantly low in fumonisin content (i.e. 2.48, 2.69 and 2.91 respectively) compared to those from most of the other treatments. There was a high significance among treatments and within the blocks (replicates), i.e. there were varying levels of fumonisin occurrence among the treatments and varying *Fusarium* occurrences within the blocks (replicates) (Table 2).

Table 1: Fumonisin concentrations in seeds of maize plant that received treatment combinations of pathogen and the selected antagonists

Treatment	Fumonisin concentration (μgkg^{-1})
'Inoculating *T. pseudokoningii* strain 5 alone'	6.90^{a}
'Inoculating *T. harzianum* strain 2 alone'	6.88^{a}
'Inoculating pathogen before *T. harzianum* strain 2'	6.13^{ab}
'Inoculating *T. pseudokoningii* strain 2 alone'	5.99^{ab}
'Inoculating pathogen before *T. pseudokoningii* strain 5'	5.97^{ab}
'Inoculating *T. pseudokoningii* strain 5 before pathogen'	5.80^{ab}
'Inoculating *T. hamatum* and pathogen simultaneously'	5.41^{ab}
'Inoculating pathogen alone'	5.40^{ab}
'Inoculating sterile toothpick alone'	5.30^{ab}
'Inoculating *T. harzianum* strain 2 and pathogen simultaneously'	5.20^{ab}
'Inoculating pathogen before *T. hamatum*'	5.04^{abc}
'Inoculating *T. pseudokoningii* strain 2 before pathogen'	4.69^{bcd}
'Inoculating *T. hamatum* before pathogen'	4.52^{bcde}
'Inoculating *T. pseudokoningii* strain 2 and pathogen simultaneously'	4.35^{bcde}
'Inoculating pathogen before *T. pseudokoningii* strain 2'	4.30^{bcde}
'Inoculating *T. harzianum* strain 2 before pathogen'	2.91^{cde}
'Inoculating *T. pseudokoningii* strain 5 and pathogen simultaneously'	2.69^{de}
'Inoculating *T. hamatum* alone'	2.48^{e}
$LSD_{0.01}$	2.16

Where:
P = *Fusarium verticillioides*

Table 2: ANOVA Table for Fumonisin analysis of maize seeds whose stems received treatment combinations of pathogen and selected antagonists

SV	DF	MS	Pr>F
Block	2	26.67	0.0001**
Trt	17	10.14	0.0001**
Error	87	2.03	
Total	106		

**Highly Significant

DISCUSSION

The fumonisin analysis showed that the maize seeds from all the maize plants that received the treatments most likely contained fumonisin though at varying levels. This might be part of the reasons for the arguments of Mckeen (1953) as well as Visconti and Doko, (1992) that *F. verticillioides* is perhaps the best known ear rot pathogen. It might also be part of the reasons for the conclusion of Kedera *et al*., (1992) that *F. verticillioides* is almost a constant companion of maize infecting the roots, stalk and kernels. This also agreed with the belief of close relationship between maize plant and *F. verticillioides* by Munkvold and Desjardins (1997). They submitted that because of the close relationship, technology is not available to ensure that all maize-based foods and feeds are completely free of fumonisins. It is such a serious case that, according to them, there can not be complete elimination without banning maize as a food and feed ingredient in the United States, an economically and politically unrealistic option.

The high fumonisin content in the seeds of the plants that received treatments involving 'Inoculation of *T. pseudokoningii* strain 5 alone' and 'Inoculation of *T. harzianum* strain 2 alone, as well as 'Inoculation of sterile toothpicks' (control) is in line with the conclusion of Kedera *et al* (1992). In other words the high fumonisin content is likely indicative of the resident *Fusarium verticillioides* in the seeds. This is because Munkvold and Desjardins, (1997) opined that *F. verticillioides* and *F. proliferatum* represent the greatest threat for fumonisin

production in maize. The symptomless expression of almost all the seeds (even though they contained fumonisin) corroborates the findings of Munkvold and Desjardins, (1997) who were of the opinion that *F. verticillioides* is not only the most common pathogen of maize, but also the most common fungi found colonising symptomless maize plants. The high significance within the blocks might be due to the ubiquitous nature of fumonisin-producing *Fusarium* as concluded by Munkvold and Desjardins (1997) which naturally will be at varying levels in different places.

Even though *T. harzianum* strain 2 seemed to be the most effective antagonist against the pathogen within the stem tissues in another experiment (Sobowale *et al*., 2007), seeds from the plants that received 'Inoculation of *T. harzianum* strain 2 alone' and two other different treatment combinations of the fungus (i.e. 'Inoculation of pathogen before *T. harzianum* strain 2' and 'Inoculation of *T. harzianum* strain 2 and pathogen simultaneously') contained a comparatively high content of fumonisin. The same could be said of two of the treatment combinations of *T. hamatum* (i.e. 'Inoculating pathogen before *T. hamatum*' and 'Inoculating *T. hamatum* and pathogen simultaneously') even though 'Inoculating *T. hamatum alone*' gave the lowest fumonisin concentration in the seeds. Even though, this might be interpreted that the treatments applied on the stem has little or no effect on the fumonisin content and hence *Fusarium* occurrence in the seeds, this, however can not be said to be totally true. This is because seeds with the lowest fumonisin content were also obtained from plants whose stems received treatments involving the same fungi (i.e. 'Inoculation of *T. hamatum* alone' and 'Inoculation of *T. harzianum* strain 2 before pathogen). This low fumonisin content might not be unconnected with the presence of the two *Trichoderma* species. The antagonists might have moved up the stem through the parenchyma tissue into the cob as concluded by Lawrence e*t al* (1981) thereafter reducing *Fusarium* occurrence in the seeds. Perhaps *T. hamatum* and/or *T. harzianum* strain 2 isolated from maize plant parts might be used, just like *T. viride* employed by Munkvold and Desjardins (1997) to suppress the radial colony extension of *Fusarium verticillioides* on agar as well as the fumonisin B_1 (FB_1) on corn kernels. In line with their suggestions for *T. viride*, perhaps *T. hamatum* and/or *T. harzianum* strain 2 isolated from the maize plant parts might be useful in biological control as a pre-harvest agent (used separately or combined) to prevent disease during plant development and /or as a post-harvest agent to suppress FB_1 production during seed storage. This will be an adventurous challenge going by the opinions of Munkvold and Desjardins (1997) that there will be many obstacles to successful fumonisin reduction by biological control because of the numerous pathways for infection by the fungi. They added that the ubiquitous nature of naturally occurring, fumonisin-producing *Fusarium* strains will make this approach extremely challenging and Environmental Protection Agency (EPA) approval of such practice may also pose an obstacle.

REFERENCES

Amoah, B.K. 1994. Pathogenicity and Genetic Studies of *Fusarium moniliforme* Sheld.Cambridge, UK: University of Cambridge, Ph.D thesis.

Etebarian, H.R., Scott, E.S. and Wicks, T.J. 2000. *Trichoderma harzianum* T39 and *T.virens* DAR 74290 as potential biological control agents for *Phytophthora erythroseptica. European Journal of Plant Pathology* 106:329-337.

Drepper, W.J. and Renfro, B.L. 1990. Comparison of Methods for Inoculation of Ears and Stalks of Maize with *Fusarium moniliforme. Plant Disease* 74:952-956.

Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M.,Vleggaar, R. and Kriek, N.P.J. 1988. Fumonisins-novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. *Applied and Environmental Microbiology* 54: 1806-1811.

Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O. and Thiel, P.G. 1991. Toxicity *and carcinogenicity of the* Fusarium moniliforme *metabolite, fumonisin B1 in rats.* Carcinogenesis *12: 1247-1251*

Harrison, L.R., Colvin, B.M., Greene, J.T., Newman, L.E. and Cole, J.R. 1990. Pulmonaryedema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. *Journal of Veterinary Diagnostic Investigation* 2: 217-221.

Julian, A.M., Wareing, P.W., Philips, S.I., Medlock, V.F.P., MacDonald, M.V., and Del Rio, L.E. 1995. Fungal contamination and selected mycotoxins in pre- and postharvest maize in Honduras. *Mycopathologia* 129: 5-16.

Kedera, C.J., Leslie, J.F., and Claflin, L.E. 1992. Systemic infection of corn by *Fusariummoniliforme*. (Abstr.) *Phytopathology* 82:118.

Lawrence, E. B., Nelson, P. E., and Ayers, J. E. 1981. Histopathology of sweet corn seed and plants infected with *Fusarium moniliforme* and *F. oxysporum Phytopathology* 71:379-386.

Marasas, W.F.O. 1988. Medical relevance of mycotoxins in Southern Africa. *Microbiol. Aliments Nutrition* 6: 1-5.

McKeen, W.E. 1953. Preliminary studies of root and basal stalk rot of maturing corn inOntario. *Canadian Journal of Botany* 31:132-141.

Munkvold, G.P., and Desjardins, A.E. 1997. Fumonisin in maize: Can we reduce their occurrence? Am. Phytopathol. Soc: *Plant Disease* 81: 556–565.

Nguen-Khong-Min, and Smirnov, V.A. 1992. Somaclonal variation under treatment with Fusaric acid intomato. *Genetika Moskva* 28, 113-20.

Paavanen-Huhtala, S., Avikainen, H., and Yli-Mattila, T. 2000. Development of strain-specific primers for a strain of

Gliocladium catenulatum used in biological control. *European Journal of Plant Pathology* 106: 187-198.

Porter, J.K., Rimando, A.M., Bacon, C.W., and Voss, K.A. 1995. Identification of Fusaric acid in the stomach colostrum of neonate rats and effects on growth. In *Fusarium*, Mycotoxins, Taxonomy and Pathogenicity, May 9-13, 1995. Italy: Martina Fanca.

Sobowale, A.A., Cardwell, K.F., Odebode, A.C., Bandyopadhyay, R., and Jonathan, S.G. 2007. Persistence of *Trichoderma* species within maize stem against *Fusarium verticillioides*. *Archives of Phytopathology and Plant Protection* 40, 3: 215-231.

Thiel, P.G., Shephard, G.S., Sydenham, E.W., Marasas, W.F.O., Nelson, P.E. and Wilson,T. M. 1991. Levels of Fumonisin B1 and B2 in feeds associated with confirmed cases of equine leuko-encephalomalacia. *Journal of Agricultural and Food Chemistry* 339:109-111.

Visconti, A. and Doko, M. 1994. Survey of Fumonisin Production by *Fusarium* isolated fromcereals in Europe. Journal of AOAC International Vol.77, No. 2.

Yates, I.E., Meredith, F., Bacon, C.W., and Jaworski, A.J. 2000. *Fusarium moniliforme* production of fumonisin B_1 suppressed by *Trichoderma viride. Journal of Food Protection* 62: 1326-1332.