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Rotting in the Inoculated Internodes of Maize (*Zea mays*) Stem Aided by Wounding due to Inoculation Method for Antagonists (*Trichoderma* species)

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Trichoderma **species that were isolated from maize plant parts and identified at IMI, England, and which successfully inhibited growth of *F. verticillioides in vitro* were taken to the screenhouse and field, to examine any probable pathogenic effect they might exert in the tissues of maize (*Zea mays*) stem; and to also examine the effect of nail punch method (combined with toothpick method of inoculation) on rot formation within the inoculated internodes of maize (*Zea mays*) plant. The *Trichoderma* species include *T. pseudokoningii* strain 1; *T. harzianum* strain 2; *T. hamatum*; *T. pseudokoningii* strain 2; *T. pseudokoningii* strain 3; *T. pseudokoningii* strain 4 and *T. pseudokoningii* strain 5. Maize seeds (DMR-LSRW) were planted in the screenhouse, and in the field. Seven weeks after planting, sterilized nail on a wooden handle was used to pierce the 2nd internodes of the maize stems, both in screenhouse and field, and toothpicks, previously treated with the *Trichoderma* species and control were inserted therein using a sterile forceps. Data collection was done 5 weeks after inoculation. All inoculated internodes for all treatments of *Trichoderma* species and control, in screenhouse and field were rotten. Rot formation was localized in inoculated internodes for all treatments except control, in screenhouse and field. All internodes above and below inoculated internodes for all treatments except control, in screenhouse and field were not rotten. All the *Trichoderma* species were however recovered from varying points in the internodes above and below inoculated internodes where there was no rot formation in screenhouse and field. In some control, fungi different from *Trichoderma* species were recovered from inoculated internodes. In screenhouse and field, some *Trichoderma* species moved farther in upper internodes than in lower internodes and their spores were visible therein; yet such stem tissues were neither rotten nor discoloured. The *Trichoderma* isolates could thus be said to be non-pathogenic to the maize stems. The rot formation within the inoculated internodes could also be said to have been aided by the wounding due to nail punch for toothpick method of inoculation.**

Key words: Nail punch method, Toothpick method, *Trichoderma* species, Inoculated internodes, Lower internodes, Upper internodes.

Generally, maize has been reported to be of global economic importance and stalk rot, as one of the most important widespread diseases of maize (Bunting *et. al.*, 1978; MacDonald and Chapman, 1996). Amongst several biocontrol agents of maize diseases are the *Trichoderma* species (Howell, 2003). However, a good antagonist, amongst other characteristics, should be non pathogenic to the host in/on which it is administered (Sharma & Sankaran 1988).

There are different methods of administering the antagonist (and indeed, the pathogen) into the host plant. Drepper and Renfro (1990) submitted that efficiency of any antagonist

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in vivo depends, to a large extent, on its inoculation method. Such methods for inoculation of maize's stalks with microorganisms (either pathogen or antagonist) include shooting a BB pellet into the stem, the drill/toothpick method, nail punch/ sponge method, insertion of infested toothpicks (i.e. punching straight with the infested toothpicks), injection of a spore suspension, wheat kernels, agar disks and insertion of infested pipe cleaners. (Drepper and Renfro, 1990).

Koehler, (1960) reported that wounding types of inoculation causes more ear and stalk rot than non wounding types. In the experiments of Drepper and Renfro (1990) as well, ear and stalk rot severities were found to be positively correlated with the diameter of the inoculation tool. More often than not, large wounds had been reported to increase the chance of infection by other pathogens at the site of the wound, an effect which may not be desirable. However, non wounding methods of inoculation have been reported to closely simulate natural infection, while wound-type inoculations was also reported to simulate to some extent, insect attack (Drepper and Renfro, 1990).

The objectives of the study therefore was to examine probable rotting effect that may be exerted by the *Trichoderma* species, that were previously successful against *Fusarium verticillioides in vitro* (Sobowale *et al*., 2005), in the tissues of maize (*Zea mays*) stem, both in the screenhouse and the field. Probable rotting effect that is aided by the nail punch method for the inoculation of antagonists, such as *Trichoderma* species in the tissues of inoculated internodes of maize (*Z. mays*) plant, both in the screenhouse and in the field were also examined.

MATERIALS AND METHODS

Planting maize (*Z. mays*) in the screenhouse and field

Maize seeds (DMR-LSRW) were planted in twenty four pots in three replicates in the screenhouse, with the experimental design being a Complete Randomized Design (CRD). Four seeds were planted in each pot and they were later thinned down to two. The soil used was the normal untreated soil from the field. In the field, maize seeds (DMR-LSRW) were also planted in three replicates of 61 rows per replicate. Experimental design in the field was randomized complete block (RCBD).

Nail punch method of inoculation

The *Trichoderma* species (antagonist) that were isolated from maize plant parts and identified at IMI, England, and which successfully inhibited growth of *F. verticillioides in vitro* (Sobowale *et al*., 2005) were cultured on toothpicks (Sobowale *et al*., 2007). The seven *Trichoderma* species include *T. pseudokoningii* strain 1; *T. harzianum* strain 2; *T. hamatum*; *T. pseudokoningii* strain 2; *T. pseudokoningii* strain 3; *T. pseudokoningii* strain 4 and *T. pseudokoningii* strain 5. Toothpicks dressed with sterile distilled water (H_2O) were used as control. Seven weeks after planting, sterilized nail on a wooden handle was used to pierce the stems of the potted plants at the 2nd internodes and the treated toothpicks were inserted therein using a sterile forceps. There were eight treatments in all for the 7 *Trichoderma* species including the control (H_2O). Inoculation of antagonists on the second internode per stalk as done in the screenhouse was also done in the filed.

Scoring for stalk rot and data collection

Five weeks after inoculation (12 weeks after planting), the stems, both from the screenhouse and field were cut and brought to the laboratory for scoring. Stalk rot was evaluated on the basis of the extent of rot developed in the inoculated internodes as well as internodes above and below the inoculated internodes. This was done by centrally splitting the stalks of the inoculated plants open lengthwise and recording the degree of rotting according to the scale below:

1 = 0% of inoculated internode rotten
2 = 1-25% of inoculated internode rotten
3 = 26-50% of inoculated internode rotten
4 = 51-75% of inoculated internode rotten
5 = 76-100% of inoculated internode rotten

Rating was done on individual plant basis. The lengths of inoculated internodes (inocint), and internodes above and below the inoculated internodes (upint and lowint respectively) were measured, after which stem tissues of specific distances within the inocint, upint and lowint were plated out using acidified Potato Dextrose Agar (APDA). Incubation at 37^0C was done for 2-7 days to allow for any growth

whatsoever; of the antagonist from both the rotting and non-rotting stem tissues. The Petri plates were later scored for presence or absence of the antagonists.

RESULTS

The mean rot scores for rot formation within the internodes of the inoculated maize stems for all the treatments, both in the screenhouse and field are given in Tables 1 and 2 respectively.

Rot formation in the screenhouse

Almost all the inoculated internodes (inocint) for all the treatments of *Trichoderma* species were heavily rotten. The rot formation was found to be localized in the inoculated internodes (Plate 1a) for all the *Trichoderma* species, although the inoculated internodes for *T. pseudokoningii* strains 1, 3, and 4 were only slightly rotten (Table 1) and the rotting were around the point of toothpick insertion within the inoculated internode. All the internodes above (upint) and below inoculated internode were not rotten in any way (Plate 1a). However, all the *Trichoderma* species were re-isolated from varying points in the inoculated internodes, as well as internodes above and below inoculated internodes (Plate 1b). Some of the antagonists such as *T. hamatum*, were found to have moved farther in the upper internodes than in the lower internodes.

In some treatments such as *T. harzianum*

Table 1. Mean rot formed within the three internodes of maize stems after inoculation of *Trichoderma* species by nail punch and toothpick method in the screenhouse

Treatment	Inocint	Upint	Lowint
T. pseudokoningii strain 1	2	1	1
T. pseudokoningii strain 2	4	1	1
T. pseudokoningii strain 3	2	1	1
T. pseudokoningii strain 4	2	1	1
T. pseudokoningii strain 5	3	1	1
T. harzianum strain 2	5	1	1
T. hamatum	4	1	1
H_2O (Control)	5	2	3

Inocint: Inoculated internode
Upint: Internode above inocint
Lowint: Internode below inocint

Table 2. Mean rot formed within the three internodes of maize stems after inoculation of *Trichoderma species* by nail punch and toothpick method in the field

Treatment	Inocint	Upint	Lowint
T. pseudokoningii strain 1	3	1	1
T. pseudokoningii strain 2	5	1	1
T. pseudokoningii strain 3	5	1	1
T. pseudokoningii strain 4	4	1	1
T. pseudokoningii strain 5	4	1	1
T. harzianum strain 2	3	1	1
T. hamatum	4	1	1
H_2O (Control)	5	2	1

Inocint: Inoculated internode
Upint: Internode above inocint
Lowint: Internode below inocint

strains 2 and 3, spores of the antagonist were visible in the upper and lower internodes of the inoculated stems. There was no rotting of the tissues where they were found in the internodes above and below inoculated internodes and these internodes were also not discoloured in any way. In the first replicate of the control, only the inoculated internodes of all the plant stands were rotten; both the inoculated and the two lower internodes were rotten in the second replicate while the inoculated internode as well internodes above and below inoculated internodes were rotten in the third replicate (Plate 2a). Fungi different from *Trichoderma* species were recovered from all the plated stem sections from the inoculated internodes of the control (Plate 2b).

Rot formation in the field

Just like the results obtained in the screenhouse, the inoculated internodes were rotten for all the treatments of *Trichoderma* species. Unlike what was obtained in the screenhouse, all the inoculated internodes for all

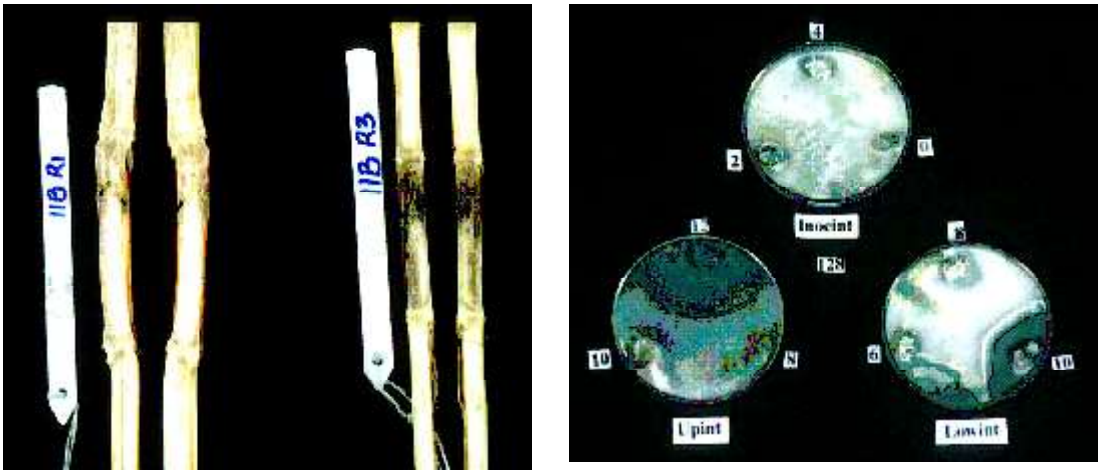

Plate 1. Stems inoculated with one of the *Trichoderma* isolates. Only inoculated internodes are seen rotten (a), yet the *Trichoderma* was recovered, even from upper and lower internodes (b), which were not rotten (a)

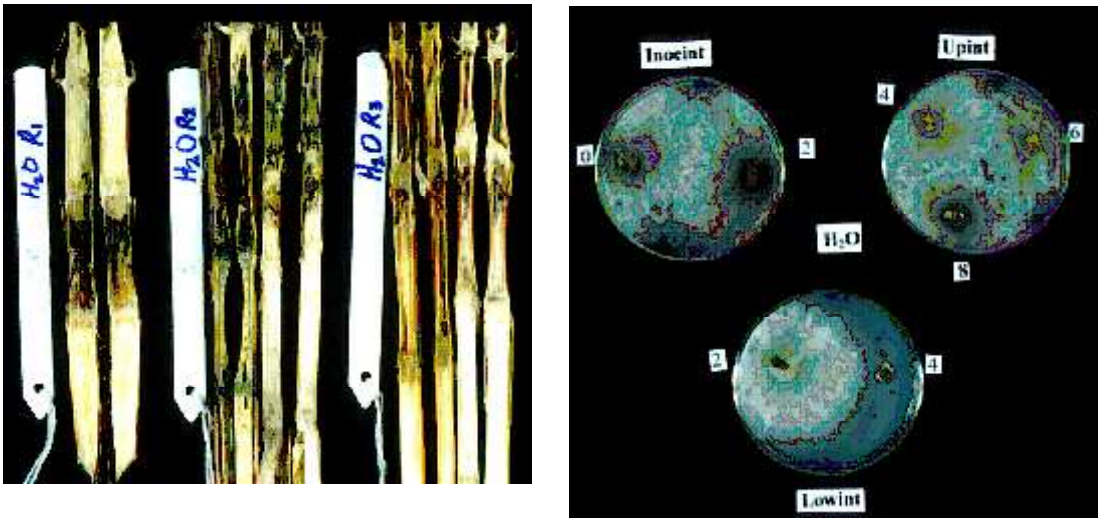

Plate 2. Stems inoculated with sterile toothpicks (control). Inoculated internodes and upper and lower internodes are seen heavily rotten (a), and fungi other than *Trichoderma* species were isolated from them (b)

the treatments in the field were heavily rotten (Table 2). Rot formation here was also found to be localized in the inoculated internodes for all treatments. All the internodes above (upint) and below inoculated internode were also not rotten in any way, just like in the case of the screenhouse. All the *Trichoderma* species were also re-isolated from varying points in the inoculated internodes, and internodes above and below inoculated internodes. Some of the *Trichoderma* species were also found to have moved farther in the upper internodes than in the lower internodes.

As observed in the screenhouse, some treatments in the field also had their spores being visible at varying points in the internodes above and below inoculated internodes. However, there was neither rotting nor discolouration of the tissues of internodes above and below inoculated internodes wherein the *Trichoderma* spores were located. In the control, some internodes above the inoculated internodes were also slightly rotten apart from the rotten inoculated internode (Table 2). Some plant stands here had tissues of their upper internodes dotted with rot formation. Fungi different from *Trichoderma* species in the control were also recovered from all the plated stem cuttings from the inoculated internodes.

DISCUSSION

The recovery of all the antagonists from the internodes above and below the inoculated internodes of plant stands which were not rotten, both in the screen house and field showed that the rot formed mostly within the inoculated internodes could not have been caused by the *Trichoderma* isolates. That spores of some of the *Trichoderma* species were visible in the tissues of the upper and lower internodes and without any accompanying rot formation also lends credence to the fact that the *Trichoderma* species could not have been the cause of the rotting. Thus it might be said that none of these antagonists were pathogenic to the maize stems and this is characteristic of a good antagonist as concluded by Sharma and Sankaran, (1988). The rot formed within the inoculated internodes therefore, more often than not, could have been due to the wounding caused to the maize stem by the nail punch for the toothpick insertion.

The nail punch might have aided invasion and infection of the maize stem by more than one rot causing organisms (Christensen and Wilcoxson, 1966; Munkvold and Desjardins 1997; Cardwell *et. aI*., 2000). This also agreed in a way with the work of Koehler (1960), who concluded that wounding types of inoculation caused more rot than nonwounding types. This was made more evident in the rot observed in the inoculated internodes of the control which was inoculated with sterile toothpicks, more so as fungi other than *Trichoderma* species were recovered therefrom. This corroborated the findings of Drepper and Renfro (1990), who recorded very high stalk rot levels on using BB pellets with and without inoculum thereby suggesting the importance of 'size of wound'. They concluded that the size of the wound may be more important than the amount of inoculum introduced. According to them, large wounds usually increase chances of infection by other pathogens at the site of infection just as was observed in the inoculated internodes, both in the screenhouse and field.

This also might not be unconnected with the occurrence of the stem borer observed in some instances within the internodes as wound-type inoculations simulate insect attack to some extent (Drepper and Renfro, 1990). However nail punch method of inoculation can still be said to be efficient since it allowed for injection of high amount of inoculum into the stem tissues as argued by Drepper and Renfro, (1990). The recovery of an entirely different fungus from all the plated stems from all the rotted plant stands in the control also suggested the involvement of some other rot forming organisms in the rotting of some of the plant stands in some of the treatments.

Conclusively, all the *Trichoderma* species examined both in the screenhouse and field could be said to be non pathogenic in the tissues of maize (*Zea mays*) stems, a major characteristic of a good antagonist. Although, nail punch method of inoculation of microorganisms could be said to be an efficient method of inoculum delivery, care still has to be taken regarding appropriate size of wounding to guard against, or at least minimize as good as possible, occurrence of secondary infection(s) at sites of inoculum delivery.

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