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Growth Competition between *Trichoderma harzianum* and *Fusarium solani* on a Plant Residue in Non-Sterile Soil

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Plant residues serve as substrates for the proliferation and overwintering of plant pathogenic fungi in soil. Effects of the biocontrol fungus *Trichoderma harzianum* on the colonization of wheat straw by the plant pathogenic fungus *Fusarium solani* were investigated under different soil moisture regimes (–50 vs. –500 kPa) in non-sterile soil. *T. harzianum* ThzID1-M3 and/or *F. solani* were added along with wheat straw to non-sterile soils. ThzID1-M3, other *Trichoderma* species, and *F. solani* were monitored for a 21-day period using quantitative PCR. ThzID1-M3 reduced the colonization of *F. solani* on wheat straw ($p < 0.05$) under both moisture regimes, and *F. solani* reduced the colonization by ThzID1-M3 and other *Trichoderma* species ($p < 0.05$), thus suggesting competitive inhibition between ThzID1-M3 and *F. solani*. Colonization by ThzID1-M3 and generic Trichoderma was improved in the wet soil ($p < 0.05$), but colonization by *F. solani* did not differ between the two moisture conditions. Thus, the inhibitory effect of ThzID1-M3 was greater in the wet soil ($p < 0.05$). The growth competition between ThzID1-M3 and *F. solani* to colonize plant debris suggests that the biocontrol fungus *T. harzianum* may reduce the potential of the plant pathogen, *F. solani*, to survive and proliferate on crops.

Keywords: ***Trichoderma harzianum*, *Fusarium solani*, competition, colonization, wheat straw, biological control**

Introduction

Trichoderma and *Fusarium* species are common soil-inhabiting fungi and world-widely distributed [4, 11, 16]. A number of *Trichoderma* isolates have been studied as biological control agents against a wide range of soil-borne plant pathogenic fungi [8, 15, 16, 23, 31]. *T. harzianum* isolates have been shown to reduce population densities of plant pathogenic *Fusarium* spp. in soil [26, 37], and to control of plant diseases caused by the pathogens [9, 12, 26, 27, 34, 38].

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Competitive interactions among fungal species commonly take place and significantly affect their establishment [21, 36]. For instance, Kim and Knudsen [21] found the strong negative association between *T. harzianum* and *F. solani* for their growth in non-sterile soil. In general, plant residues serve as substrates for proliferation and overwintering of saprophytic fungi in soil. *Trichoderma* spp. including *T. harzianum* are a strong competitor with other organisms on plant residues [7, 32]. Harper and Lynch [17] reported a very high growth and activity of *T. harzianum* in a range of fungi isolated from decomposing straw. *T. harzianum* may inhibit colonization of plant residues by plant pathogenic fungi and thus, reduce their potential to survive and proliferate in soil. Similarly, there are a large number of *Fusarium*

propagules including *F. solani* in wheat and bean field soils [3, 28, 35]. Rodriguez *et al.* [33] reported that *F. solani* was one of the most powerful degraders of wheat straw when a total number of 82 fungal isolates were tested. *F. solani* could compete with other filamentous fungi for colonizing plant residues [10, 21]. Therefore, *T. harzianum* may interact with *F. solani* to colonize plant residues in natural environments.

Soil moisture is an important factor to influence the growth and activity of filamentous fungi in natural environments. It has been generally observed that *T. harzianum* is better in its hyphal growth and myco-parasitic activity at dry conditions (–500~–1,000 kPa) than at wet conditions (–30~–100 kPa) [19, 22, 25], while *Fusarium* spp. show better growth at –100 to –300 kPa than drier conditions [6, 30]. The objective in the present study was to investigate growth competition between the biocontrol fungus *T. harzianum* and *F. solani* on wheat straw in non-sterile soil. We first tested whether *T. harzianum* and *F. solani* compete to colonize wheat straw in a confrontation assay. We tested whether *T. harzianum* reduces colonization of *F. solani* on wheat straw, and whether *F. solani* affects colonization of *T. harzianum* and total *Trichoderma*, at two different soil moisture regimes (–500 vs. –50 kPa).

Materials and Methods

Used organisms

The isolates *Trichoderma harzianum* ThzID1-M3 and *Fusarium solani* f.sp. *pisi* (*Fsp*) were used in this study. The genetic transformant *T. harzianum* ThzID1-M3 contains three exogenous genes for green fluorescent protein (GFP), hygromycin B resistance, and β-glucuronidase [1]. The parent isolate ThzID1 was originally isolated from field soil near Moscow, Idaho. The plant pathogenic fungus *F. solani* was isolated from a diseased pea plant near Moscow, Idaho.

Confrontation assays on wheat straw

T. harzianum ThzID1-M3 and *F. solani* were grown on potato dextrose agar (PDA) plates at 23℃ for 7 days without light. Agar disks (1 cm^2) were taken from the margin of ThzID1-M3 and *F. solani* colonies and placed on water agar (distilled water and 15% agar) plates. Pieces of wheat straw in relatively similar size (approximately 0.4–0.7 cm in height and 0.2–0.4 cm in diameter) were autoclaved. They were placed between agar disks of ThzID1-M3 and *F. solani*. At 3, 5 and 7 days, we observed colonization of wheat straw by both fungi using a Nikon Eclipse E1000 epifluorescence microscope (Nikon, Melville, NY) at 100× or 200× magnification. Pieces of wheat straw were placed on slide glasses and stained with Calcofluor white solution (0.05 mg/ml). To observe colonization of wheat straw by *F. solani* and ThzID1-M3, DAPI and FITC filters, respectively, (excitation wavelengths 380–400 and 470–490; dichroic mirror wavelengths 425–470 and 500–540; and barrier wavelengths 450–465 and 510–540) were used. At day 7, colonization of wheat straw by ThzID1-M3 and *F. solani* was quantified using real-time PCR as described below. There were 5 replicates.

Formulation of *T. harzianum* ThzID1-M3

ThzID1-M3 was formulated with alginic acid as previously described [24]. ThzID1-M3 was allowed to grow for 1 week on potato dextrose agar (PDA) plates at 21℃. Three 1-cm^2 pieces from the culture were transferred to a 1-liter flask containing 500 ml potato dextrose broth. Flasks were plugged with sterile cotton and placed on a rotary shaker (120 rpm) at room temperature for 1 week with 12 hours of light per day. The cultures of ThzID1-M3 were strained through cheesecloth and rinsed thoroughly with sterile water. Thirty-seven grams of hyphal biomass was blended briefly with 2 grams of wheat bran and 100 ml of 1% aqueous sodium alginate solution. Drops of the mixture were added to 0.25 M $CaCl_2$ solution, forming a pellet precipitate. Pellets were removed by straining, placed on waxed paper to air-dry at room temperature for 2 days, and then stored at 4℃.

To make alginate pellets without the fungus, 37 ml of sterile distilled water replacing 37 g of ThzID1-M3 biomass was blended briefly with 2 g of wheat bran and 100 ml of 1% aqueous sodium alginate solution. Drops of the mixture were added to 0.25 M aqueous $CaCl_2$, forming a pellet precipitated. Pellets were removed by straining, placed on waxed paper to air-dry at room temperature, and stored at 4℃ before use.

Preparation of *F. solani* conidia

Conidia of *F. solani* produced on wheat straw were used. *F. solani* was allowed to grow on PDA plates at

21℃ for 2 weeks. Wheat straw was autoclaved for 30 min twice, then was chopped and placed into 500-ml flasks. Distilled water and malt extract were added into the flasks at rates (weight : weight) of 1:1 (water : wheat straw) and 1:20 (malt extract : wheat straw). The flasks were plugged with cotton and autoclaved for 30 min. Five 1-cm^2 pieces from the culture were transferred into the flasks. They were mixed well and incubated at room temperature for 45 days with 12 hours of light per day. The flasks were filled up with sterile distilled water and agitated. Wheat straw suspensions were strained by 4 layers of cheesecloth to retain mycelia and wheat straw. Conidia extracts were spun down for 10 min at 800× g at 4℃. The supernatant was removed and conidia were suspended with sterile distilled water. The concentration in conidial suspensions was determined with a hemocytometer. Conidia included macro- and micro-conidia.

Soil preparation

Palouse silt loam soil was obtained from the University of Idaho Plant Science Farm near Moscow in 2008. Soil analysis results (University of Idaho Analytical Services Laboratory) indicated that the soil contained 20% sand, 20% clay and 60% silt by weight, with 82.2 μg/g of plant-available iron per gram. Soil pH in soil/water (2:1) solution was approximately 5.9 [1]. Collected soil was air-dried and sieved through a 2-mm mesh prior to use.

Experimental treatments

Soil was adjusted to a soil matric potential of −50 kPa or −500 kPa with sterile distilled water or conidial suspension of *F. solani* to provide *F. solani* population at 0 or 1×10^5 conidia per gram of soil. A 250-g amount of soil was added to containers, approximately 8 × 6.3 × 6.3 cm in size. Wheat straw was autoclaved prior to use. Pieces of wheat straw, approximately 0.4–0.7 cm in height and 0.2–0.4 cm in diameter, were attached onto plastic toothpicks using cyanoacrylate glue. Alginate pellets with or without *T. harzianum* ThzID1-M3 were attached approximately 0.5 cm away from pieces of wheat straw onto the plastic toothpicks. Toothpicks were randomly assigned to containers and were placed vertically in the soil at a depth of approximately 5 cm (4 toothpicks per container). The containers were covered with lids and were sealed with parafilm to maintain a relatively constant moisture level. They were incubated at 22℃ without light. There were four treatments in the two soil moisture regimes: i) *F. solani* at 1×10^5 conidia per g of soil and single alginate pellet with ThzID1-M3; ii) *F. solani* at 1×10^5 conidia per g of soil and single alginate pellet without ThzID1-M3; iii) *F. solani* at 0 conidia per g of soil and single alginate pellet with ThzID1-M3; and iv) *F. solani* at 0 conidia per g of soil and single alginate pellet without ThzID1-M3. After 0, 3, 5, 7, 14 and 21 days, 2 containers per each treatment were randomly selected. Toothpicks were taken from the containers and wheat straw was obtained. Wheat straw was washed three times and kept at −80℃ before extracting DNA. DNA of ThzID1-M3, *F. solani*, and *Trichoderma* spp. was quantified using real-time PCR. There were 5 replicates per each treatment at each sample time.

DNA extraction

An individual piece of wheat straw was placed in a 2 ml microtube with a 3 mm tungsten carbide bead (Qiagen Inc., USA). Disruption of wheat straw was done using TissueLyser (Qiagen) for 1 min at 30 hz at room temperature. DNA was extracted using the DNeasy plant DNA extraction kit (Qiagen) according to the manufacturer's instructions. DNA was eluted twice in 75 μl of AE buffer. Both elutions were combined and stored at −20℃ before use.

ThzID1-M3 and *F. solani* allowed to grow in potato dextrose broth for 4 days at room temperature on a rotary shaker. Mycelia were harvested by filtration through cheesecloth and rinsed with sterile distilled water, then ground with liquid nitrogen. Genomic DNA was extracted from mycelia using the DNeasy plant DNA extraction kit (Qiagen). DNA was eluted twice in 75 μl of AE buffer. Both elutions were combined and stored at −20℃ prior to use.

Quantitative real-time PCR

TMeGFP2 [20], TGP4 [19], and TMFsol4 [21] primer/Taqman probe sets for quantifying *T. harzianum* ThzID1-M3, the genus *Trichoderma*, and *F. solani*, respectively, were used. The TMeGFP2 set includes the forward primer (5'-GCTGCCCGACAACCACTAC-3'), the reverse primer (5'-CGTCCATGCCGAGAGTGATC-3') and the Taqman probe (5'-FAM-CGGCGGCGGTCACGAACTCCA-TAMARA-3'). The TGP4 set includes the forward primer

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(5'-CTCCCAAACCCAATGTGAAC-3'), the reverse primer (5'-GCGAGTGTGCAAACTACTG-3') and the Taqman probe (5'-FAM-ACCAAACTGTTGCCTCGGCGG-IBFQ-3'). The TMFsol4 set includes the forward primer (5'-GGGTACTCATCAGTCACTTC-3'), the reverse primer (5'-CGATGTGGGATAGCAAGG-3') and the Taqman probe (5'-FAM-TGTGACCAACCTTCTCGAACTTC-IBFQ-3').

Real-time PCR amplifications of total DNA extracted from wheat straw were performed using the iCycler IQ (Bio-Rad). The reactions were carried out in a real-time PCR plate with a total volume of 25 μl per single reaction. The reaction mixture for *T. harzianum* ThzID1-M3 contained 2.5 μl of 10× PCR buffer (Invitrogen, Carlsbad, CA), 0.5 μl of 10 mM dNTPs mixture (Invitrogen), 2 μl of 50 mM $MgCl_2$ (Invitrogen), 0.1 μl (0.5 unit) of Platinum *Taq* DNA polymerase (Invitrogen), 0.75 μl of TMeGFP2F (10 μM), 0.25 μl of TMeGFP2R (10 μM), 0.25 μl of TMeGFP2P (5 μM), and 5 μl of DNA template. Control reactions contained the same mixtures with 5.0 μl of sterile water replacing the DNA template. The cycling condition of the real-time PCR for *T. harzianum* ThzID1-M3 was an initial denaturation at 95℃ for 2 min, followed by 40 cycles at 95℃ for 20 s and 60.5℃ for 60 s. The reaction mixture for *Trichoderma* spp. contained 2.5 μl of 10× PCR buffer, 0.75 μl of 10 mM dNTPs mixture, 2 μl of 50 mM $MgCl_2$, 0.5 μl (0.75 unit) of Platinum Taq DNA Polymerase, 0.75 μl of TGP4-F (10 μM), 0.25 μl of TGP4-R (10 μM), 0.25 μl of TGP4 probe (5 μM), and 5 μl of DNA template. Control reactions contained the same mixtures with 5.0 μl of sterile water replacing the DNA template. The cycling condition of the real-time PCR for *Trichoderma* spp. was an initial denaturation at 95℃ for 2 min, followed by 40 cycles at 95℃ for 25 s and 57℃ for 90 s. The real-time PCR reaction mixture for *F. solani* contained 2.5 μl of 10× PCR buffer, 0.5 μl of 10 mM dNTPs mixture, 2 μl of 50 mM $MgCl_2$, 0.1 μl (0.5 unit) of Platinum *Taq* DNA polymerase, 0.5 μl of TMFsol4F (10 μM), 0.5 μl of TMFsol4R (10 μM), 0.25 μl of TMFsol4P (5 μM), and 5 μl of DNA template. Control reactions contained the same mixtures with 5.0 μl of sterile water replacing the DNA template. The cycling condition for TMFsol4 was an initial denaturation at 95℃ for 2 min, followed by 40 cycles at 95℃ for 20 s and 53℃ for 60 s.

Data analysis

This study was conducted as a randomized block design, with 2 factors blocked by sample time and soil moisture levels. All analyses were performed using SYSTAT software version 11 (Systat Software, Inc., USA). General linear model in SYSTAT with significant differences occurring at the level of $\alpha = 0.05$ was used to analyze results.

Results

The TMeGFP2, TGP4, and TMFsol4 primer/Taqman probe sets for quantifying ThzID1-M3, *Trichoderma* spp. and *F. solani*, respectively, generated standard curves with a linear fit with coefficient of determination values (R^2) greater than 0.99 and with PCR efficiencies higher than 90%, over at least 6 orders of magnitude.

Wheat straw confrontation assay

Microscopic observation indicated that both ThzID1-M3 and *F. solani* hyphae grew on wheat straw and there was no mycoparasitism by ThzID1-M3. A quantitative PCR result showed that both fungi all colonized the pieces of wheat straw. ThzID1-M3 reduced the colonization extent of *F. solani* on wheat straw ($p < 0.05$), while *F. solani* did not significantly reduced the colonization extent of ThzID1-M3 (Fig. 1).

Effects of *F. solani* on colonization of ThzID1-M3

Colonization of ThzID1-M3 on wheat straw was quantified over time (Fig. 2). We did not obtain any positive signal within the range of DNA standards in the real-time PCR assays when ThzID1-M3 was not added, in either soil moisture level. Incidence (% of wheat straw colonized) and extent (colonization quantity) of colonization of ThzID1-M3 were significantly reduced by the added *F. solani* (1×10^5 conidia/g soil), and significantly greater in the wet soil than in the dry soil ($p < 0.05$). There was a significant effect of sampling time on incidence of colonization ($p < 0.05$), but not on extent. In the wet soil (–50 kPa), both incidence and extent rapidly increased for the first 5 days, and retained for the remainder of the period when *F. solani* was not added. With *F. solani*, they declined rapidly. In the dry soil (–500 kPa), incidence and extent increased for a 3–7

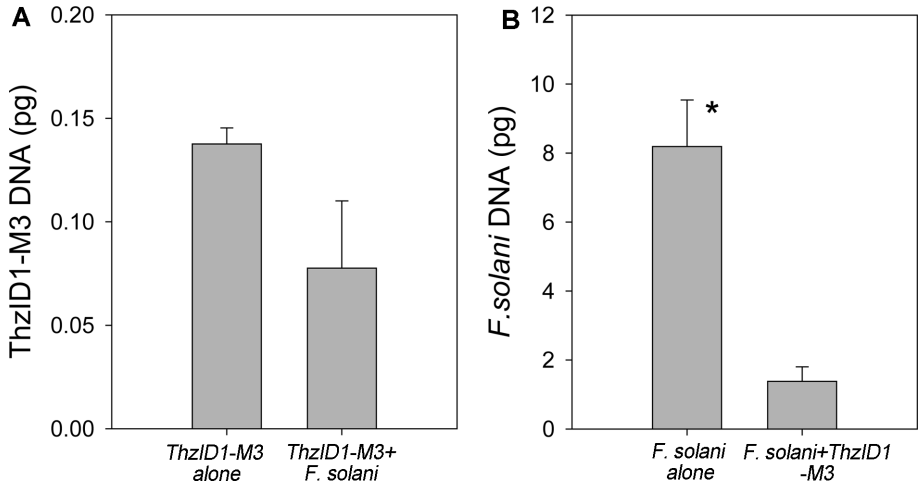

Fig. 1. Confrontation assay of *T. harzianum* ThzID1-M3 (A) and *F. solani* (B) on wheat straw. There were five replicates (n = 5), and the symbol * indicates a significant difference ($p < 0.05$).

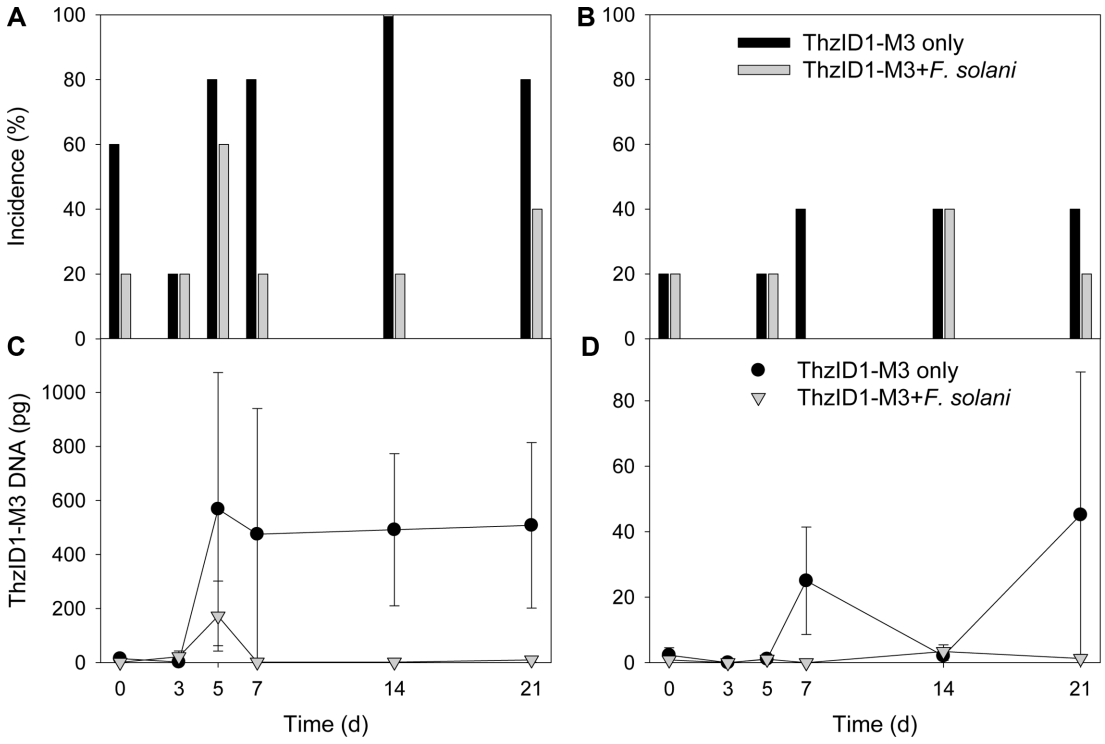

Fig. 2. Colonization incidence and extent of *T. harzianum* ThzID1-M3 on wheat straw at −50 kPa (A and C) and −500 kPa (B and D). There were five replicates per each sample time. Vertical bars represent ±1 standard error of the mean.

day period and then leveled off when *F. solani* was not added, while peaked on day 14, with *F. solani*.

Colonization of wheat straw by *Trichoderma* spp.

Colonization of *Trichoderma* spp. on wheat straw was monitored over time (Fig. 3). The added *F. solani* significantly reduced the extent of colonization of *Trichoderma* spp. ($p < 0.05$), but did not significantly reduced the incidence of colonization. Incidence and extent of colonization was significantly greater in the wet soil ($p < 0.05$).

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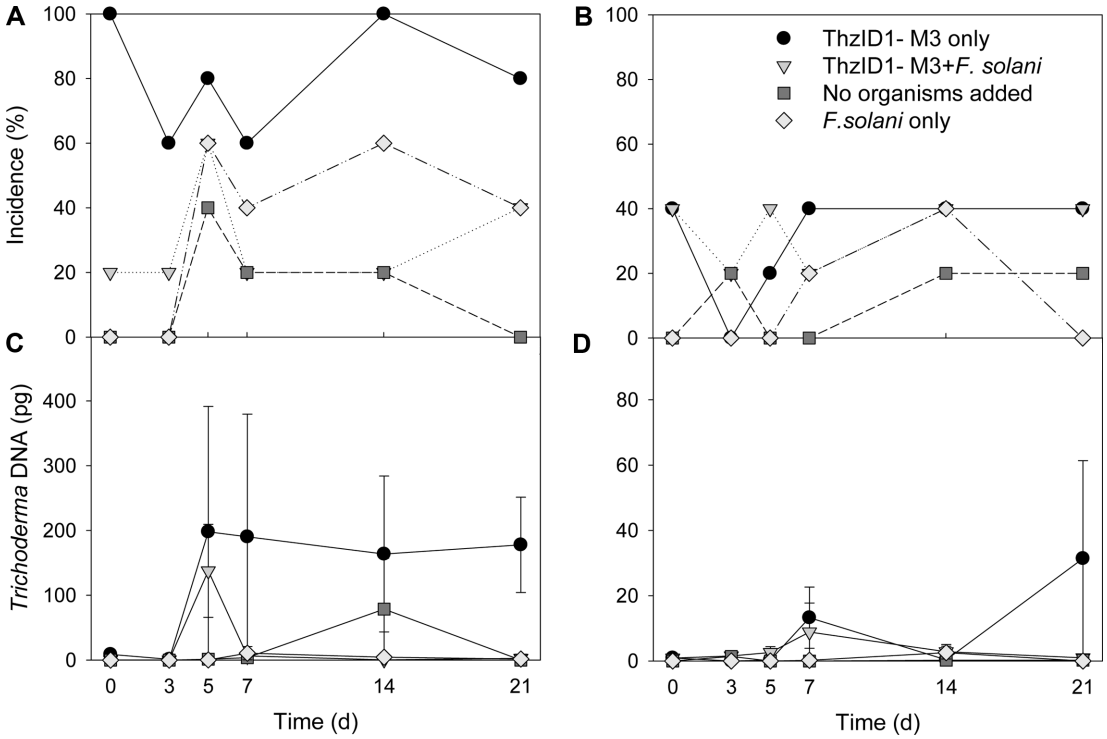

Fig. 3. Colonization incidence and extent of generic *Trichoderma* on wheat straw at −50 kPa (A and C) or −500 kPa (B and D). There were five replicates per each sample time. Vertical bars represent ±1 standard error of the mean.

There was no significant effect of sampling time on incidence and extent ($p > 0.05$). In the wet soil (−50 kPa), incidence varied from 60 to 100% with time when ThzID1-M3 alone was added. With both fungi, incidence peaked on day 5, followed by a reduction. Colonization incidence of indigenous *Trichoderma* increased to 60% by day 5 then retained over time when no fungi were added. Colonization incidence of indigenous *Trichoderma* increased to 40% by day 5 then decreased over time, when *F. solani* alone was added. Extent rapidly increased by day 5, and retained for the rest of the experimental period when ThzID1-M3 alone was added. With both fungi, extent increased by day 5, followed by a rapid reduction. Colonization extent of indigenous *Trichoderma* peaked on day 14 then reduce with time when no fungi were added. No increase in extent was observed when *F. solani* alone was added.

In the dry soil, incidence by *Trichoderma* spp. was no more than 40%. When both fungi were added, incidence varied from 40% to 20% over time. Incidence peaked at 40% on day 7 and did not change over time when ThzID1-M3 alone was added. With no added fungi, incidence was varied with time. When both fungi were added, extent increased by day 7 then reduced over time. When ThzID1-M3 alone was added, extent increased by day 7, decreased by day 14, and increased again by day 21. DNA of indigenous *Trichoderma* was near or less than the detection level when ThzID1-M3 was not added.

Effects of ThzID1-M3 on colonization of wheat straw by *F. solani*

Colonization of *F. solani* on wheat straw was monitored over time (Fig. 4). Colonization of indigenous *F. solani* was observed in soil not infested with the pathogen. Colonization extent of *F. solani* was significantly decreased by ThzID1-M3 ($p < 0.05$). Incidence of colonization also was reduced ($p = 0.068$). Soil moisture did not significantly affect the incidence and extent of colonization of the pathogen ($p > 0.05$). Inhibitory effect of ThzID1-M3 on colonization of wheat straw by the pathogen was significantly greater in the wet soil ($p < 0.05$).

There was a significant effect of sampling time on incidence and extent of colonization ($p < 0.05$). In the wet

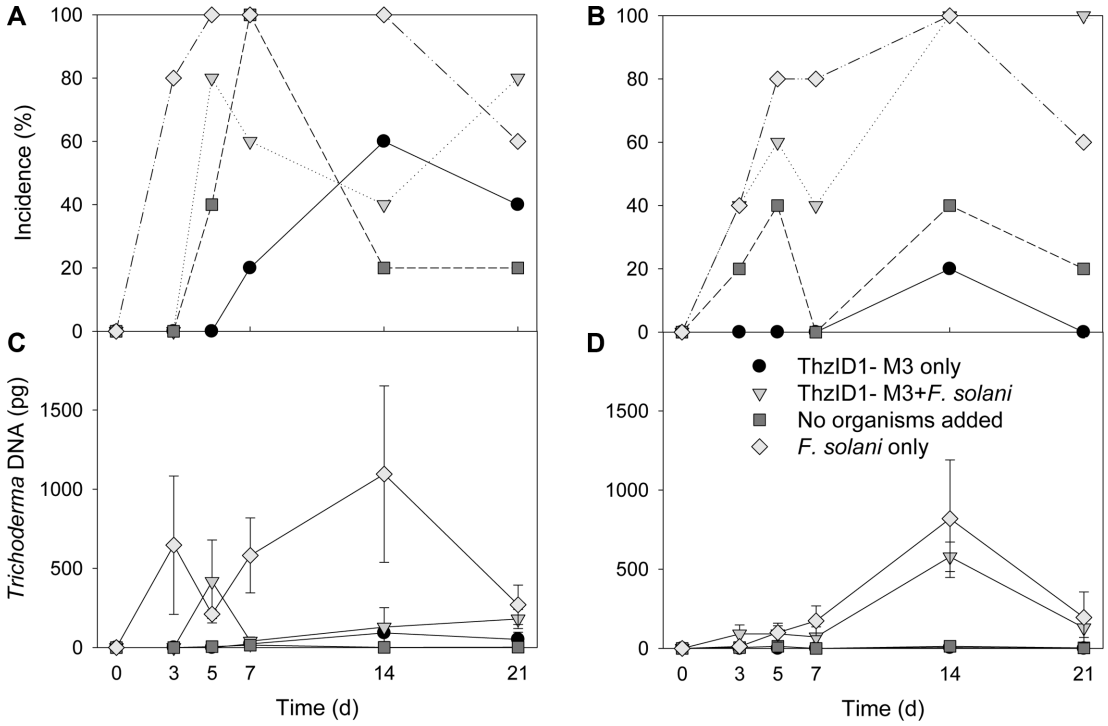

Fig. 4. Colonization incidence of *F. solani* on wheat straw at −50 kPa (A and C) or −500 kPa (B and D). There were five replicates per each sample time. Vertical bars represent ±1 standard error of the mean.

soil, colonization incidence of *F. solani* rapidly increased to 100% within the first 5 days then reduced to 60% from day 14 to day 21 when *F. solani* alone was added. It increased to 80% by day 5, followed by a reduction, and increased again to 80% on day 21 when both fungi were added. Indigenous *F. solani* DNA was detected from wheat straw in soil without the added *F. solani* in either soil moisture level. Colonization extent peaked on day 14, followed by a reduction when *F. solani* alone was added, while it peaked on day 5 then rapidly reduced when both fungi were added. When no organism was added, colonization extent of indigenous *F. solani* peaked on day 14. With ThzID1-M3 alone, indigenous *F. solani* DNA was near or less than the detection level.

In the dry soil, incidence and extent peaked on day 14 regardless of whether ThzID1-M3 was present or not, when *F. solani* was added. When no fungus was added, incidence by indigenous *F. solani* increased to 40% for a 0-5 day period then fluctuated over time. With ThzID1-M3 alone, colonization event (20%) was only observed at day 14. DNA of indigenous *F. solani* was near or less than the detection level.

Discussion

Mycoparasitism, parasitism of one fungus by another fungus, is considered the main mechanism of the antagonistic activity of *T. harzianum* [23]. The confrontation assay indicated that there was no mycoparasitism between ThzID1-M3 and *F. solani* on wheat straw, and showed that a competitive interaction between them was present. In non-sterile soil, ThzID1-M3 significantly reduced the colonization of *F. solani* on wheat straw. The added pathogen also significantly decreased the colonization of ThzID1-M3 and total *Trichoderma* spp. on wheat straw. Their interaction is categorized by interference competition that involves some form of behavioral or chemical interaction between individuals before actual use of a resource, affecting the access of a competitor to the resource [39].

The most common method to determine colonization of plant residues (e.g. wheat straw) by fungi relies on plating residues on a nutrient medium. Real-time PCR has several important advantages over culture-based methods to quantify saprophytic colonization of wheat straw.

Typically, wheat straw from which hyphae of the saprophytes grow out is presumed to be colonized. Therefore, the method provides an estimate of the incidence of colonization, though not of the extent that each piece of wheat straw is colonized. It is usually difficult to determine actual extent of colonization using culture-based methods [14]. Furthermore, they are time-consuming and labor-intensive. Real-time PCR quantifies actual extent that an individual piece of wheat straw is colonized within a day. Therefore, real-time PCR provides a better detection and measurement tool to evaluate colonization of fungi on wheat straw in natural environments.

Colonization extent of ThzID1-M3 first peaked between days 5 and 7, which is consistent with our previous observations. Hyphal growth of *T. harzianum* from the pellets was rapid for the first 5–7 days in soil [2, 22]. ThzID1-M3 biomass peaked within the first 3–7 day period, followed by a rapid reduction in soil [29]. Unlike in soil, ThzID1-M3 DNA levels did not reduce with time on wheat straw because this plant residue provides nutrients for growth. ThzID1-M3 greatly delimited the growth of *F. solani* on wheat straw (Fig. 4). The confrontation assay showed that ThzID1-M3 reduced the hyphal growth of the pathogen on wheat straw. In addition, the antagonist may inhibit germination of the pathogen in soil. Sivan and Chet [37] reported that *T. harzianum* could reduce recoverable numbers of the plant pathogenic *Fusarium* spp. by inhibiting their germination. The added *F. solani* also decreased the colonization of ThzID1-M3 (Fig. 2). *F. solani* may inhibit the initial hyphal growth of ThzID1-M3 from the alginate pellets, since *F. solani* can compete with other members of fungi [10]. *F. solani* also significantly reduced the colonization of total *Trichoderma* spp. Trends of ThzID1-M3 DNA levels over time were similar with those of generic *Trichoderma* since a majority of *Trichoderma* DNA resulted from the added ThzID1-M3 (Figs. 2, 3). *T. viride*, *T. polysporum*, *T. koningii*, *T. hamatum*, and *T. harzianum* are relatively abundant in soil [11]. From plant residues buried in soil, *Trichoderma* was one of the predominant genera, of which *T. koningii*, *T. pseudokoningii*, and other *Trichoderma* spp. were found [5].

Soil moisture is known to be a factor influencing the natural distribution, growth, and saprophytic activity of *Trichoderma* [11, 13, 14, 18]. Soil moisture significantly affected the colonization of *Trichoderma*, but did not affect the colonization of the pathogen. Colonization of ThzID1-M3 was significantly less in the dry soil and therefore, adverse effect of ThzID1-M3 on colonization of the pathogen was significantly less. We previously observed that hyphal density of *T. harzianum* growing from an alginate pellet formulation was significantly greater at a soil matric potential of –500 kPa, compared with –30 kPa or –100 kPa [22], and that colonization of *Trichoderma* spp. on sclerotia was greater at a soil matric potential of –1,000 kPa, compared with –50 kPa [19]. Optimum germ-tube growth of *Trichoderma* spp. was observed at external water potentials between –700 kPa and –1400 kPa [25]. Thus, hyphal density of ThzID1-M3 around wheat straw probably might be greater in the dry soil. An important scale difference in DNA of ThzID1-M3 and generic *Trichoderma* was apparent in the two different soil moisture regimes, although no scale difference in *F. solani* DNA was observed. Soils are densely populated with a wide variety of organisms and highly competitive habitats. These results may reflect the fact that there were potential qualitative and quantitative differences in the indigenous microbial community. Greater competitive interactions with soil biota (including the added *F. solani*) might reduce colonization of ThzID1-M3 on wheat straw in the dry soil. In addition, it is possible that colonization ability of *Trichoderma* on wheat straw may be lowered under the dry condition.

A better understanding of their ecology is necessary to improve the predictability and reliability of the biocontrol agent in crop system. Our results demonstrated that the competition between ThzID1-M3 and/or total *Trichoderma* vs. *F. solani* adversely influenced their growth and proliferation on wheat straw. Thus, the growth competition of *Trichoderma* for colonizing plant residues, other than mycoparasitism, can reduce the potential of plant pathogenic fungi to survive and overwinter in soil, and may result in the biological control of the plant diseases in crop system. In general, the growth and biocontrol activity of *Trichoderma* have been known to be better at drier conditions. However, the biocontrol effect of *T. harzianum* against *F. solani* was much greater at the wet condition because the biocontrol fungus was better in colonization on wheat straw at the wet condition. These findings can provide an insight into the applica-

tion strategy of this biocontrol agent.

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국문초록

토양 식물 잔사에서 *Trichoderma harzianum*에 의한 식물 병원균 *Fusarium solani*의 성장 저해

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토양에 존재하는 식물의 잔사는 식물 병원균의 확산 및 월동을 위한 자원으로써 역할을 수행 할 수 있다. 본 연구에서는 식물 잔사인 밀짚에서 식물 병원균인 *Fusarium solani*의 군락 형성에 대한 생물학적 방제균인 *Trichoderma harzianum*의 억제 효과를 조사하였다. 두 종류의 토양 수분 조건(–50, –500 kPa)의 멸균하지 않은 토양에서 연구를 수행하였다. *T. harzianum* ThzID1-M3와 *F. solani*를 밀짚과 함께 토양에 첨가 한 후 ThzID1-M3, *Trichoderma* spp. 및 *F. solani*를 quantitative real-time PCR을 사용하여 21일 동안 관찰하였다. 모든 토양 수분 조건에서 ThzID1-M3는 *F. solani*의 밀짚 점유를 감소하고($p < 0.05$), 반면에 F. SOLANI는 ThzID1-M3 및 *Trichoderma* spp.의 밀짚 점유를 감소시켰다($p < 0.05$). 이 결과는 *F. solani*와 ThzID1-M3 사이의 경쟁적 억제를 보여주고 있다. 높은 수분 조건의 토양(–50 kPa)에서 ThzID1-M3 및 *Trichoderma* spp.는 더 높은 밀짚의 점유를 보여주었다($p < 0.05$), 반면에 *F. solani*의 밀짚 점유는 두 수분조건에서 차이가 없었다. 따라서 ThzID1-M3의 병원균 억제 효과는 높은 수분 조건의 토양에서 더 컸다($p < 0.05$). 토양내 식물 잔사를 점유할 때 발생되는 ThzID1-M3와 *F. solani* 사이의 강력한 경쟁 관계는 생물학적 방제 균인 *T. harzianum*가 작물 시스템에서 식물 병원균의 생존과 증식을 감소시킬 수 있음을 시사한다.