

Effect of seed treatment with novel strains of *Trichoderma* spp. on establishment and yield of spring wheat

Allen Xue^{1*}, Wei Guo^{1,2}, Yuanhong Chen¹, Yasamin Al-Rewashdy¹

¹Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, ON K1A 0C6, Canada

²College of Plant Sciences, Heilongjiang Bayi Agricultural University, 5 Xinfeng Road, Daqing, Heilongjiang 163319, China

*Correspondence to e-mail: allen.xue@agr.gc.ca



Introduction

Fusarium head blight (FHB), caused by *F. graminearum* Schwabe, is a destructive disease of wheat (Fig. 1). Seeds obtained from fields affected by FHB become the source of seed-transmitted inoculum which may initiate epidemics. In addition, the cultivation of Fusarium-infected seeds may lead to poor stand establishment as a result of reduced seed vigor and germination (McMullen et al., 2012). Seed treatments with microbial biocontrol agents have been explored as a possible alternative to fungicides for seedling blight caused by *F. graminearum* (seed-borne phase of FHB) in wheat (Bello et al., 2002; Hasan et al., 2012). These biocontrol efforts, however, were mostly limited to laboratory and glasshouse studies. The objective of this study was to evaluate the efficacy of selected strains of *Trichoderma* spp. seed treatments for controlling the seed-borne phase of FHB under field conditions.



Figure 1. Wheat spikes with symptoms of FHB.

Materials and methods

One isolate of *F. graminearum*, DAOM232369, obtained from the Canadian Collection of Fungal Cultures at the Ottawa Research and Development Centre (ORDC), Ottawa, Canada, was used for this study. The isolate was cultured on a modified potato dextrose medium (PDA, 10g/L of dextrose amended with 34 μmol/L streptomycin sulfate). Twenty-two strains of *Trichoderma* spp. That were recovered from roots of seedling plants in agricultural soil were examined for their antagonistic effects on mycelium growth of *F. graminearum* in dual culture assays. Inhibition of growth was calculated using the formula: % inhibition = (a-b)/a x 100, where a = *F. graminearum* colony radius in the untreated control and b = colony radius in *Trichoderma* strain treatments.



Figure 2. The 0-5 scale used for rating root rot severity, where 0 = no visible lesion on lower stem and seed attachment area; 1 = slight necrosis or few small lesions on lower stem and seed attachment area; 2 = moderate necrosis and large lesions scattered over the lower stem and seed attachment area, fewer roots developed; 3 = extensive necrosis, fewer and shorter roots developed; 4 = seedling growth reduced, extensive necrosis, few or no roots developed; and, 5 = seedling growth seriously reduced or died shortly after emergence.

Six of the 22 strains were selected for field trials at ORDC in 2008, 2009, and 2011 to test the effects of seed treatments on emergence, root rot severity, and yield. A FHB-susceptible cultivar Roblin was used for the field trials. The seed lots used in each year were produced from the FHB nursery that was inoculated with *F. graminearum* in previous years. Seeds were treated with a spore suspension of each *Trichoderma* strain at 10⁷ spores mL⁻¹ at 5.0 mL kg⁻¹ seed. Percent emergence was calculated by dividing the total number of seedlings by the average of 225 seeds sown per row. The severity of the root rot was recorded on a scale of 0 to 5 2-3 weeks after emergence (Figure 2). Dry weight was measured after the plants were air-dried for 48 hr. After harvesting the plants at maturity, the yield was determined after seeds were air-dried to 13% moisture content.

Results and discussion

All 22 strains of *Trichoderma* spp. significantly inhibited mycelial growth of *F. graminearum* after 6 days of dual culturing (Table 1). All six selected strains, Trich12, TrichC06, TrichC39, TrichC70, TrichPine, and TrichMM7 significantly reduced root rot severity and increased yield, three strains increased emergence and four strains increased plant dry weight, compared with the untreated controls over 2008, 2009, and 2011 (Table 2).

TrichC70 was the only strain that showed a significant improvement to all four parameters, increasing emergence by 10.9%, dry weight by 51.7%, and yield by 11.0% and reducing root rot severity by 51.7%. These effects were not significantly different from that of Vitaflo-280, suggesting that TrichC70 may be used for controlling the seed-borne phase of FHB in organic wheat production or as a natural alternative to fungicides.

Table 1. Effect of the six most effective strains (>50% inhibition) of *Trichoderma* spp. from 22 strains evaluated on inhibition of mycelial growth of *Fusarium graminearum* in dual culture experiments.

Trichoderma strain	Species	Radius (mm)			Inhibition (%)	Origin		
		Trial 1	Trial 2	Mean		Crop	Location	Year
Trich12	<i>T. citrinoviride</i>	16.8	13.9	15.4	53.2	Soybean	Ottawa, ON	2006
TrichC06	<i>T. harzianum</i>	6.5	6.4	6.5	80.2	Corn	Ottawa, ON	2006
TrichC39	<i>T. harzianum</i>	6.3	10.3	8.3	74.7	Corn	Ottawa, ON	2006
TrichC70	<i>T. harzianum</i>	5.2	10.5	7.8	76.1	Corn	Ottawa, ON	2006
TrichMM7	<i>T. asperellum</i>	4.0	8.4	6.2	81.1	Soybean	Ottawa, ON	2006
TrichPine	<i>T. harzianum</i>	3.5	9.1	6.3	80.8	Pine tree	Ottawa, ON	2007
Control†		33.7	32.0	32.8				
Total 22 strains								
Mean		9.7	10.9	10.2				
Range		3.5-27.2	8.4-24.0	6.2-22.5				
LSD		2.5	2.1	1.6				

†Control = plates inoculated with *F. graminearum* and a potato dextrose agar disk. Colony radius was measured after 6 days of incubation in dual culture.

Table 2. Effects of seed treatments with strains of *Trichoderma* spp. on seedling emergence, root rot severity, dry weight, and yield of spring wheat compared with Vitaflo-280 fungicide and untreated control in field trials in Ottawa, Ontario in 2008, 2009, and 2011.

Treatment	Emergence (%)				
	2008	2009	2011	Mean	% increase
Trich12	73.4 abc†	74.5 ab	66.0 c	71.3 bc	12.3
TrichC06	68.6 bc	70.1 ab	60.8 c	66.5 cd	4.7
TrichC39	71.3 abc	70.5 ab	60.2 c	67.3 cd	6.0
TrichC70	69.8 abc	74.5 ab	67.0 bc	70.4 bc	10.9
TrichMM7	68.7 bc	69.1 ab	63.3 c	67.1 cd	5.7
TrichPine	75.1 ab	66.9 b	75.7 ab	72.6 ab	14.3
Vitaflo-280	77.2 a	77.9 a	76.6 a	77.2 a	21.6
Untreated	65.9 c	66.0 b	58.6 c	63.5 d	
Root rot severity (0-5)					
Trich12	0.29 bc	0.27 c	1.25 b	0.60 b	-58.6
TrichC06	0.26 bc	0.59 abc	0.88 bc	0.57 b	-60.7
TrichC39	0.28 bc	0.60 abc	1.13 b	0.67 b	-53.8
TrichC70	0.38 abc	0.85 a	0.88 bc	0.70 b	-51.7
TrichMM7	0.31 bc	0.83 ab	0.75 bc	0.63 b	-56.6
TrichPine	0.51 ab	0.76 ab	0.88 bc	0.72 b	-50.3
Vitaflo-280	0.13 c	0.27 c	0.25 c	0.22 c	-84.8
Untreated	0.66 a	0.44 bc	3.25 a	1.45 a	
Dry weight (g/plant)					
Trich12	0.18 a	0.95 c	0.42 a	0.52 c	-1.9
TrichC06	0.22 a	1.47 a	0.43 a	0.71 a	34.0
TrichC39	0.20 a	1.41 a	0.44 a	0.68 a	28.3
TrichC70	0.18 a	1.43 a	0.43 a	0.68 a	28.3
TrichMM7	0.18 a	1.38 a	0.40 a	0.65 a	22.6
TrichPine	0.19 a	1.24 abc	0.38 a	0.60 abc	13.2
Vitaflo-280	0.18 a	1.29 ab	0.44 a	0.64 ab	20.8
Untreated	0.17 a	1.03 bc	0.39 a	0.53 bc	
Yield (kg/ha)					
Trich12	2450.8 b	2992.0 ab	3280.4 ab	2907.7 ab	6.6
TrichC06	2486.3 ab	3099.7 ab	3122.0 ab	2902.6 b	6.4
TrichC39	2445.9 b	3235.4 a	3278.0 ab	2986.4 ab	9.5
TrichC70	2456.5 b	3276.6 a	3347.7 a	3026.9 ab	11.0
TrichMM7	2489.8 ab	3069.5 ab	3212.5 ab	2923.9 ab	7.2
TrichPine	2422.1 b	3290.1 a	3255.7 ab	2989.3 ab	9.6
Vitaflo-280	2577.4 a	3218.3 a	3302.4 ab	3032.7 a	11.2
Untreated	2245.2 c	2843.6 b	3091.8 b	2726.9 c	

† Means within a column by the same letter within each parameter are not significantly different at $P \leq 0.05$ (LSD).

Conclusion

All of the 22 *Trichoderma* spp. strains significantly inhibited the mycelial growth of *F. graminearum* after 6 days of dual culturing. Six of the 22 strains including Trich12, TrichC06, TrichC39, TrichC70, TrichPine, and TrichMM7 significantly reduced root rot severity and increased yield. TrichC70 was the most effective strain that may be used as an alternative to fungicide seed treatments to control the seed-borne phase of FHB.

References

- Bello, G.M.D., Monaco, C.I., Simon, M.R., 2002. Biological control of seedling blight of wheat caused by *Fusarium graminearum* with beneficial rhizosphere microorganisms. *World J. Microbiol. Biotechnol.* 18, 627-636.
- Hasan, M.M., Rahman, S.M.E., Kim, G.H., Abdallah, E., Oh, D.-H., 2012. Antagonistic potentiality of *Trichoderma harzianum* towards seed-borne fungal pathogens of winter wheat cv. Protiva *in vitro* and *in vivo*. *J. Microbiol. Biotechnol.* 22, 585-591.
- McMullen, M., Bergstrom, G., DeWolf, E., Dill-Macky, R., Hershman, D., Shamer, G., Van Sanford, D., 2012. A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Dis.* 96, 1712-1728.

Acknowledgements

This research was supported in part by grants from Grain Farmers of Ontario, AAFC-RBPI Project No. 845, and AAFC-CFCRA Project No. DIAP05987.