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

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ABSTRACT

Fusarium head blight (FHB) is the most important disease of wheat in Canada. FHB reduces grain yield and quality and results in seed contamination with *Fusarium* spp. that is associated with reduced seed vigor and poor stand establishment in wheat. The effect of seed treatments with six strains representing three species *Trichoderma*, selected based on their superior antagonistic ability on mycelium growth of *F. graminearum* in dual culture assays, on wheat seed lots contaminated with *Fusarium* spp. (28–43%) was examined in field trials in 2008, 2009, and 2011. None of the six strains of *Trichoderma* spp. showed a significant seed treatment effect for all parameters measured each year, but over the three years, all six strains significantly reduced root rot severity and increased yield, three stains (Trich12, TrichC70 and TrichPine) increased emergence and four strains (Trich06, TrichC39, TrichC70, and TrichMM7) increased plant dry weight, compared with the untreated control. 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 less but not significantly different from that of the registered fungicide Vitaflo-280 (carbathiin + thiram) used as the positive control in the field trials. The results indicate that *Trichoderma* stain TrichC70 may be used as an alternative to fungicide seed treatments to alleviate the detrimental effect of the seed-borne phase of FHB in wheat.

1. Introduction

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* Schwabe, is a destructive and widespread disease of wheat (*Triticum aestivum* L.) in North America (Liddell, 2003; McMullen et al., 2012). In Canada, frequent FHB epidemics in Manitoba and eastern Canada in the past two decades have caused extensive losses due to the reduced yield and the discounted price of grains contaminated with Fusarium-damaged kernels (FDK) and their associated mycotoxins (Gilbert and Tekauz, 2000; Gilbert and Haber, 2013).

Wheat can be infected by *F. graminearum* from the flowering (anthesis) stage up through the soft dough stage of kernel development. Yield losses occur from failed kernel development or from infected kernels that are shriveled, discolored, and light in test weight (McMullen et al., 2012). Seeds obtained from fields affected by FHB become the source of seed-transmitted inoculum which may initiate epidemics (Arseniuk et al., 1998) and also the cultivation of *F. graminearum*-infected seeds may lead to poor stand establishment as a result of reduced seed vigor and germination (Gilbert and Tekauz, 2000; Inch and Gilbert, 2003).

Seed treatments with fungicides are recommended for wheat to manage seed- and soil-borne pathogens, and increase seed viability in Canada. However, most currently registered fungicides target pathogens other than *F. graminearum*, leading to variable and inconsistent effectiveness against seed-borne *F. graminearum* in cereal crops (Gilbert and Tekauz, 1995; Schaafsma et al., 2001). Seed treatments with microbial biocontrol agents have been explored as a possible alternative to synthetic fungicides (Bello et al., 2002; Hasan et al., 2012). These biocontrol efforts, however, were

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mostly limited to laboratory and greenhouse studies. There are currently no biocontrol agents registered for use as seed treatments to control the seed-borne phase of FHB in Canada.

Trichoderma spp. are endophytic plant symbionts that are widely used as seed treatments for controlling seed- and soil-borne pathogens and enhancing plant growth and yield (Whipps and Lumsden, 2001; Mastouri et al., 2010; Samuels and Hebbar, 2015). Bello et al. (2002) and Hasan et al. (2012) reported that seed treatment with *T. harzianum* holds considerable promise for controlling seedling blight caused by *F. graminearum* in wheat. However, there have been no comparative studies on possible differences among strains of *Trichoderma* spp. and their relative effectiveness compared with registered fungicides. The objectives of this study were to examine the antagonistic effect of strains from three species of *Trichoderma* (*T. citrinoviride*, *T. asperellum*, and *T. harzianum*), recovered from roots of field crops, against *F. graminearum* in co-culture and to further evaluate the selected novel strains for their effectiveness as seed treatments in reducing root rot severity and increasing seedling emergence, plant growth and yield under field conditions.

2. Materials and methods

2.1. Strains of F. graminearum and Trichoderma spp

One isolate of *F. graminearum,* DAOM 232369, obtained from the Canadian Collection of Fungal Cultures at the Ottawa Research and Development Centre (ORDC), Ottawa, Canada, was used for this study. This isolate was chosen because it is known to be aggressive (Xue et al., 2009). The isolate was cultured on a modified potato dextrose agar (PDA, 10 g/L of dextrose amended with 34 μmol/L streptomycin sulfate) and incubated at 22–25 °C under mixed ultraviolet (UV) and fluorescent lighting on a 12 h light: 12 h dark cycle for 14 days. The modified PDA medium was used to reduce mycelium growth, possible mutation and poor vigour, and to increase spore production by the pathogen (Xue et al., 2004).

A total of 22 strains of *Trichoderma* spp. were used for a dual culture test against *F. graminearum*. Only six strains, Trich12, TrichC06, TrichC39, TrichC70, TrichPine and TrichMM7, were used as seed treatments for the field trials. TrichC06, TrichC39, TrichC70, and TrichPine were selected among the 20 *T. harzianum* strains based on their superior ability to produce inhibition zones against *F. graminearum* in dual cultures, while Trich12 and TrichMM7 were each the only isolate from *T. citrinoviride* and *T. asperellum*, respectively (Table 1). Of the 22 strains, 21 were isolated from wheat, corn and soybean roots in a long-term rotation study at the Central Experimental Farm in Ottawa, Ontario in 2006 and 2007, whereas one (TrichPine) was isolated from roots of a pine tree in a tree nursery in Ottawa in 2007. To isolate *Trichoderma* strains, plant seedlings were dug out of the soil. Roots were washed and cut into 5-mm pieces, surface sterilized with 1% sodium hypochlorite then rinsed with sterilized distilled water, and placed on potato dextrose agar (PDA) in a 9-cm petri dish amended with 50 ppm of streptomycin sulfate. The culture plates were incubated for 10 days at 22-25 °C, under mixed UV and fluorescent lighting on a 14-h light: 10-h dark cycle. *Trichoderma* isolates were purified by single spore isolation and identified following standard taxonomic keys (Ulloa and Hanlin, 2012; Dugan, 2008; Samuels and Hebbar, 2015). Single spore cultures of *Trichoderma* spp. were freeze-dried and stored at −20 °C in ampoules until required. Fresh cultures of these fungi were established by transferring freeze-dried fungal material to PDA medium and incubating at 22–25 °C, under mixed UV and fluorescent lighting.

2.2. Dual culture of F. graminearum and Trichoderma strains

The *in vitro* antagonistic effect of strains of *Trichoderma* spp. against *F. graminearum* was tested using a dual culture protocol by placing two 5-mm mycelial disks, each from margins of 7-day-old PDA cultures of a *Trichoderma* strain and *F. graminearum* isolate DAOM 232369, on PDA in 9-cm Petri dishes with 3 replications. The two mycelial disks were spaced 4 cm apart and deposited within 30 min. A PDA disk, instead of strains of *Trichoderma* spp. was used as the negative control. The test plates were incubated at 25 °C with a 12 h light: 12 h dark cycle and on the sixth day the radius of the *F. graminearum* colonies was measured on a straight line between the two disks. The experiment was repeated twice. Inhibition of growth was calculated using the formula: % inhibition = (a-b)/a × 100, where a = *F. graminearum* colony radius in the untreated control and b = colony radius in *Trichoderma* strain treatments.

2.3. Seed treatment with selected strains of Trichoderma spp. in field trials

The effects of seed treatments with six selected strains of *Trichoderma* spp. on emergence, root rot severity and yield were evaluated under field conditions at the Central Experimental Farm, ORDC, Ottawa, in 2008, 2009 and 2011. Fusarium head blight-susceptible wheat cultivar Roblin was used for the field trials. The seed lots used in each year were produced from the same cultivar, grown during the previous year in the FHB nursery that was inoculated with *F. graminearum* at the Central Experimental Farm, Ottawa. The percentages of seed-borne infection by *F. graminearum* were 42.8, 30.9, and 28.2% for 2008, 2009, and 2011, respectively, based on 300 seeds randomly selected from each seed lot.

Seeds were treated with a spore suspension of each *Trichoderma* strain containing 10^7 spores mL^{-1} at the rate of 5.0 mL kg^{-1} seed. Seed treatment with the currently recommended fungicide Vitaflo-280 (carbathiin + thiram) at the labelled rate (0.44 + 0.39 g a.i. kg^{-1} seed) and untreated seed were used as controls. The spore suspensions of strains of *Trichoderma* spp. were prepared by washing a 10-day-old colony, grown on potato dextrose agar (PDA), with sterile distilled water containing 0.01% Tween 20 (polyoxyethylene sorbitan monolaureate), scraping gently with a sterile microscope slide to dislodge spores, and filtering through two layers of cheesecloth. The concentration of the resulting spore suspensions was determined with a haemocytometer. Seeds were treated in a 250-mL Erlenmyer flask and shaken vigorously after the addition of the treatments to insure uniform coverage of the seeds. Treated seed was spread-out in a thin layer on clean paper to air dry overnight and stored in an open paper bag until planted, usually within 24–48 h. Trials were seeded at a rate of 250 seeds m^{-2} and a depth of 5 cm, using a plot seeder on May 2, May 6, and May 13, for 2008, 2009, and 2011, respectively. Plots consisted of six rows, 5.0 m long with 20-cm row spacing and 50 cm between plots. A randomized complete block design with four replications was used each year. The soil type was loam in 2008 and 2009 and clay-loam in 2011. Soybean was the preceding crop for all test years. Plots were fertilized based on soil test recommendations and an herbicide treatment with Buctril M (bromoxynil 280 g L^{-1}, MCPA 280 g L^{-1}) was used once at 1.0 L ha^{-1} for weed control at the tillering stage of crop growth each year.

Emerging seedlings were counted in the two central rows 2–3 weeks after planting, when plants were at the 2–3 leaf stage (Zadoks 12) (Zadoks et al., 1974). Percent emergence was calculated for each plot by dividing the total number of seedlings by the average of 225 seeds sown per row. Approximately 20–30 seedlings from a 50 cm length of a side row in each plot were carefully removed 3–4 weeks after emergence to assess root rot severity and