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Concentration and cultivar effects on efficacy of CLO-1 biofungicide in controlling Fusarium head blight of wheat

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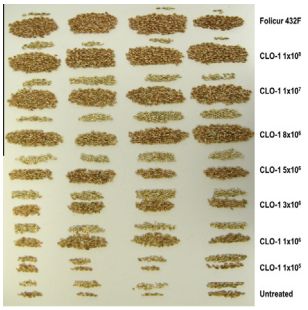
HIGHLIGHTS

- CLO-1 (*Clonostachys rosea* strain ACM941) controls Fusarium head blight in wheat.
- CLO-1 biofungicide was to large extent as effective as conventional fungicides.
- CLO-1 biofungicide was most effective on moderately resistant cultivars.

GRAPHICAL ABSTRACT



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ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat in Canada and *Clonostachys rosea* strain ACM941 has been identified as a promising biological control agent for managing FHB. In the present research the concentration and cultivar effects on the efficacy of CLO-1, a formulated product of *C. rosea* strain ACM941, in controlling FHB and deoxynivalenol (DON) contamination in wheat was studied. Of the eight concentrations ranging from 10^4 to 10^8 cfu mL^{-1} evaluated, significant effects were generally observed for concentrations at or above 10^6 cfu mL^{-1} in the greenhouse and field trials in 2009 and 2010. In the greenhouse, CLO-1 reduced the area under the disease progress curve (AUDPC) by 65–83%, Fusarium damaged kernels (FDK) by 68–92%, and DON by 51–95%. Under field conditions, CLO-1 reduced FHB index by 30–46%, FDK by 31–39%, and DON by 22–33%. These effects were numerically lower but not significantly different from those of the registered fungicide Folicur® (tebuconazole) used in these trials. When applied onto wheat cultivars differing in resistance to FHB in field trials in 2009 and 2010, CLO-1 was most effective on the moderately resistant cultivar AC Nass (representing the highest level of resistance commercially available) and least effective on the highly susceptible cultivar AC Foremost. Results of this study suggest that CLO-1 is a promising biocontrol product that may be used in combination with cultivar resistance for managing FHB in wheat.

1. Introduction

Fusarium head blight (FHB), caused by *Gibberella zeae* (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe), is the most important disease of wheat (*Triticum aestivum* L.) throughout

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the world (Gilbert and Tekauz, 2000; McMullen et al., 1997, 2012). The disease causes reduced grain yield and quality, and contamination of harvested grains with the mycotoxin deoxynivalenol (DON), which is harmful to livestock and poses a safety concern in food and feed (Del Ponte et al., 2012; Desjardins, 2006; Miller, 1995; Placinta et al., 1999).

The use of genetic resistance is an important strategy for the control of FHB, but cultivars with high levels of resistance are not yet commercially available (Bai and Shaner, 2004; McMullen et al., 2012; Gilbert and Haber, 2013). Fungicides have been used to control FHB under high inoculum pressure and favorable environmental conditions, to sustain productivity and improve grain quality in many wheat production regions (Jones, 2000; Matthies and Buchenauer, 2000; Tekauz et al., 2003). However, these fungicides have not been consistently effective in controlling FHB and DON contamination (Blandino et al., 2006; McMullen et al., 1997, 2012) and their long-term usage has become a public concern because their residues may remain in food and feed products, causing potential risk to human and animal health and the environment (Fantke et al., 2011; Herrero-Hernández et al., 2011; Imfeld and Vuilleumier, 2012; Moser et al., 2001; Muñoz-Leoz et al., 2011).

There is a need for alternative FHB disease management strategies and to augment host resistance. Recent studies have suggested biological control as an important tool to reduce FHB and DON contamination and reduce fungicides' negative impacts, such as pathogen resistance development, harmful effects on beneficial microorganisms in the soil and damage to the environment and human health (Khan and Doohan, 2009; Schisler et al., 2011; Yuen and Schoneweis, 2007).

Previous studies reported that a fungal strain of *Clonostachys rosea* (Link:Fr.) Schroers, Samuels, Serfert and Gams (syn. *Gliocladium roseum* Bainier), strain ACM941 (ATCC #74447), was a mycoparasite of certain fungal pathogens in plants (Xue, 2002, 2003a,b). Recent research also demonstrated that strain ACM941 holds considerable promise as an effective alternative to fungicides for the control of FHB, by foliar application to reduce FHB disease severity, Fusarium damaged kernel (FDK) and DON in harvested grain (Xue et al., 2009a,b, in press) and by crop residue treatment to inhibit the perithecial production of *G. zeae* and reduce the initial inoculum (Chen et al., 2012; Xue et al., 2009a,b). The objectives of this study were to examine the effect of concentration and cultivar resistance on the efficacy of the formulated product of *C. rosea* strain ACM941, CLO-1, in controlling FHB and DON contamination in wheat.

2. Materials and methods

2.1. CLO-1 biofungicide

CLO-1 biofungicide is an experimental commercial formulation of the *C. rosea* strain ACM941 bioagent (ATCC #74447). The product is a wettable powder containing more than 8×10^9 cfu g^{-1} and was produced and provided by ICUS Canada Inc., a biopesticide company located in St. John's, Newfoundland. The ACM941 strain used for the product development was originally recovered from a field pea plant in 1994 (Xue, 2002). The fungus was stored by freeze-drying and kept at −20 °C in ampoules. Cultures of the fungus were established by transferring freeze-dried fungal material at approximately 0.01 g per Petri dish containing potato dextrose agar (PDA) and incubating these dishes at 22–25 °C under mixed UV and fluorescent lighting.

2.2. Pathogen inoculum production

Three isolates of *G. zeae*, DAOM 178148, DAOM 212678, and DAOM 232369, obtained from the Canadian Collection of Fungal Cultures at the Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa, Canada, were used for this study. These isolates were chosen as they are known to be aggressive (Xue et al., 2004).

For the greenhouse inoculation, the isolates were cultured on a modified PDA (10 g L^{-1} of dextrose amended with 34 μmol L^{-1} streptomycin sulfate) and incubated at 22–25 °C under mixed 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 vigor, and to increase spore production by the pathogen (Xue et al., 2004). To prepare inoculum, 0.5 mL of a concentrated macroconidial suspension (approx. 10^7 - spores mL^{-1}), obtained from the above, was spread over the surface of the modified PDA in 9-cm Petri dishes and incubated as described above for 48 h. Ten milliliters of sterile distilled water containing 0.01% Tween 20 (polyoxyethylene sorbitan monolaurate) were then added to each dish, and the surface was scraped gently with a sterile microscope slide to dislodge spores. The resulting conidial suspension was filtered through two layers of cheesecloth and adjusted to a concentration of 5×10^4 spores mL^{-1} using a haemocytometer. Separate conidial suspensions were prepared for each isolate. The final suspension used for greenhouse consisted of 1:1:1 mixture of each of the three *G. zeae* isolates.

For the field inoculation, infected barley and corn kernels were infected separately with the three isolates of *G. zeae* (Xue et al., 2006). The infected kernels were scattered evenly by hand between the two rows of each plot. Sprinkler irrigation was applied daily for 0.5 h in the morning and afternoon, excluding rainy days, starting with the first inoculation and continuing until about 3 weeks after anthesis, when plants were at the soft dough stage. Appropriate herbicides for efficient weed control were applied according to standard management practices (Ontario Ministry of Agriculture, Food and Rural Affairs, 1999).

2.3. CLO-1 concentration effects in greenhouse trials

Two greenhouse trials (Trial I and Trial II) were conducted at ECORC, Ottawa, Ontario, from January to June each year in 2009 and 2010. The winter wheat cultivar Pioneer 25R was used in trial I and the spring wheat cultivar AC Brio used in trial II in both years.

Uninoculated seed was planted in 15-cm diameter pots containing a mixture of loam soil, sand, and composted cow manure (1:1:1, *v*/*v*/*v*) and maintained at 23–25 °C during the day and 18–20 °C at night in a greenhouse. The winter wheat cultivar was vernalized for eight weeks at 4 °C prior to planting. Supplemental light was provided by 300 W metal halide lamps to ensure a 16 h photoperiod and a minimum intensity of 360 mol m^{-2} s^{-1}. Plants were fertilized with a 1% solution of 20–20–20 (N–P–K) at the fifth week after planting and once per week thereafter.

At the anthesis stage (Zadoks' growth stage (ZGS) 65) (Zadoks et al., 1974), wheat spikes were sprayed with CLO-1 at five concentrations ranging from 10^4 to 10^8 cfu mL^{-1} in 2009 (Table 1) and at seven concentrations raging from 10^5 to 10^8 cfu mL^{-1} in 2010 (Table 2), 65 μmol L^{-1} tebuconazole (292 mL of the fungicide Folicur® per 100 L), or sterile distilled water as the untreated control. All treatments were applied to incipient runoff using a DeVilbiss model 15 atomizer. Following the treatment application, plants were kept at room temperature for 3 h to allow the treated spikes to air dry and then were inoculated with a spore suspension of 5×10^4 conidia mL^{-1} from equal parts of the three isolates of *G. zeae*. The inoculum was sprayed on all spikes as described above. Thirty minutes later, plants were transferred to a polyethylene humidity chamber in a growth room operated at 25 °C with a 12 h photoperiod at a light intensity of 250 mol m^{-2} s^{-1} for 48 h. The humidity chamber was maintained at or near 100% relative humidity by the continuous operation of two ultrasonic humidifiers, and air temperature and humidity in the chamber were mon-