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Integrated management of Fusarium crown rot of wheat using fungicide seed treatment, cultivar resistance, and induction of systemic acquired resistance (SAR)

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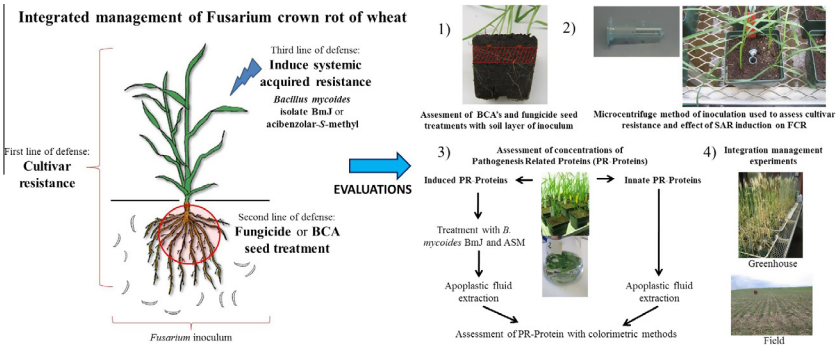
HIGHLIGHTS

- Fungicide seed treatment reduced FCR severity better than seed treatment with BCAs.
- Reduced disease severity and high levels of induced and innate PR-proteins were observed on Volt.
- *Bacillus mycoides* isolate BmJ increased peroxidase and endochitinase on wheat cultivars.
- Induction of SAR reduced the severity of FCR compared to a water control.
- Integration of seed treatment, cultivar resistance, and SAR induction reduced FCR severity.

GRAPHICAL ABSTRACT



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ABSTRACT

Fusarium crown rot (FCR) of wheat (*Triticum aestivum* L.) is a perennial problem for wheat producers worldwide. Integration of the different management tools was assessed to determine a new strategy to reduce FCR. Difenoconazole–mefenoxam fungicide seed treatment reduced FCR severity 29–50% in glasshouse trials, while seed treatment with *Bacillus pumilis* isolate 314-16-5 and *Trichoderma harzianum* T-22 provided control statistically different than the untreated or seed treated with *Bacillus* isolate L324-92 or *Pseudomonas fluorescens* isolate 2-79. Assessment of resistance in inoculated glasshouse trials and innate activity levels of three pathogenesis-related (PR) proteins in apoplastic fluids of five non-inoculated spring wheat cultivars showed the lowest disease severity on cv. Volt and the highest levels of endochitinase and β-1,3-glucanase activity compared to the other cultivars ($P < 0.05$). Induction of SAR with foliar applications of *Bacillus mycoides* isolate BmJ (1.5×10^8 cfu/ml) or acibenzolar-*S*-methyl (ASM, 1.0 mM) on the cultivars Hank, Knudson and Volt reduced the severity of FCR by 10% compared to a water control ($P < 0.05$). BmJ application increased concentrations of peroxidase and endochitinase, while ASM increased β-1,3-glucanases levels in cultivars Volt and Hank compared to water controls ($P < 0.05$). Integration of the management tools, difenoconazole–mefenoxam seed treatment, cultivar resistance, and SAR induction, showed integration of all three management tools did not reduce disease severity more than use of cultivar resistance plus fungicide seed treatment or SAR induction in glasshouse trials. In a dryland field trial, integration of all three management tools reduced disease severity and FCR populations more than individual tools ($P < 0.05$).

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1. Introduction

A diverse group of *Fusarium* spp. damage small grain cereals by rotting the seed, causing seedling blights, and roots crown, basal stems rots, and in some case, infect the heads or spikes and grains (Paulitz et al., 2002). Fusarium crown rot (FCR) of wheat (*Triticum aestivum* L.) is a perennial problem that occurs in most cereal-producing regions of the world (Cook, 2010; Moya-Elizondo et al., 2011; Poole et al., 2013). FCR is most commonly caused by *Fusarium culmorum* (W. G. Sm.) Sacc., *Fusarium pseudograminearum*, (O'Donnell & T. Aoki), and *Fusarium graminearum* Schwabe (Paulitz et al., 2002; Cook, 2010; Moya-Elizondo et al., 2011). Several other *Fusarium* species have been included as causal agents in the crown rot disease complex but these are thought to be of less importance (Smiley and Patterson, 1996; Moya-Elizondo et al., 2011; Poole et al., 2013). Adoption of conservation tillage practices has been linked to increased FCR severity by increasing the amount of inoculum survival in crop residues (Paulitz et al., 2002).

Different strategies have been suggested for management of FCR. Cook (2010) recommended the use of clean and chemically disinfected seed, adjusting the date of seeding, proper fertilization, tillage to hasten infected residue decomposition, crop rotations avoiding other host crops (primarily cereal crops and grasses), and use of cultivars with resistance to the pathogens or to water stress. Among these strategies, fungicide seed treatments are recommended for FCR management. However, efficacy of chemical control is limited to early stages of the wheat growth cycle, since fungicide seed treatments do not maintain their efficiency much beyond the seedling stage (Balmas et al., 2006). Current resistance in commercial cultivars to FCR is only partial and losses can be severe when climatic conditions are favorable for disease development even on resistant cultivars (Strausbaugh et al., 2005). Seedling and adult-plant tolerance (partial resistance) to some FCR complex pathogens have been reported (Collard et al., 2005; Bovill et al., 2006), and is associated with reduced damage to crown and stem base tissue (Wildermuth et al., 2001).

Biological control agents (BCAs) have shown promise for the control of FCR (Dal Bello et al., 2002; Johansson et al., 2003; Luongo et al., 2005; Khan et al., 2006; Singh et al., 2009). Two basic approaches have been considered for use of BCAs in the control FCR: (i) Manipulation of microbial antagonists to increase the rate of mortality of survival structures of *Fusarium* spp. in cereal residues (Wong et al., 2002; Luongo et al., 2005; Singh et al., 2009), and (ii) seed treatment with biological control agents (BCAs) (Dal Bello et al., 2002; Johansson et al., 2003; Khan et al., 2006). Seed treatments with *Burkholderia cepacia* (Huang and Wong, 1998) pseudomonads and *Pantoea* sp. (Johansson et al., 2003), *Stenotrophomonas maltophilia*, *Bacillus cereus* and isolates of *Trichoderma harzianum* (Dal Bello et al., 2002) have significantly reduced FCR infections caused by different *Fusarium* species on wheat and have increased grain yield and seedling stand in field experiments (Johansson et al., 2003). Also, Khan et al. (2006) working with a pseudomonad seed treatment against *F. culmorum* reported the induction of a wheat class III plant peroxidase gene, suggesting that part of the biocontrol activity of these bacteria could be due to the induction of systemic acquired resistance (SAR) in host plants.

Induction of SAR by chemicals or BCAs has proven to be a valuable tool in plant disease control (Vallad and Goodman, 2004; Jacobsen et al., 2004). While wheat germplasm with a high level of resistance to FCR has not been described, the induction of defense genes through application of methyl jasmonate or acibenzolar-S-methyl (Bion or Actigard, Syngenta) enhanced resistance and delayed symptom development (Desmond et al., 2006). Several BCAs have been shown to induce SAR and Induced Systemic Resistance (ISR) (Vallad and Goodman, 2004; Bargabus et al., 2004; Walters et al., 2005). An example of a BCA that induces SAR is *Bacillus mycoides* isolate BmJ, which has provided control of diseases caused by fungal, bacterial, and viral pathogens in different plant species (Bargabus et al., 2003, 2004; Neher et al., 2008; Neher, 2008; Jacobsen et al., 2014). BmJ is able to induce PR-proteins through a salicylic acid (SA)-independent but NPR1-dependent pathway in sugar beet (Bargabus-Larson and Jacobsen, 2007) and NPR1 plus jasmonic acid (JA)/ethylene dependent pathways in *Arabidopsis thaliana* (Neher, 2008). This novel signaling defense described for BmJ may activate the defense response to necrotrophic fungal pathogens such as that mediated through JA pathway described by Desmond et al. (2006, 2008a).

Jacobsen and Backman (1993) stated the importance of integrating host resistance, biological and cultural controls in integrated management systems both as alternatives and supplements to pesticides. Combinations of agents that induce resistance with fungicides or biological control agents or with disease resistant hosts can provide effective disease control, especially in situations where achieving acceptable disease control is difficult (Walters et al., 2005; Jacobsen et al., 2004). Since FCR is difficult to control and immunity-type host-disease resistance is not available, this article addresses the testing of the ability of an SAR-inducing bacterium to be integrated with seed treatments and cultivar resistance in an integrated disease management program for FCR. Different biological control agents were compared to difenocona zole–mefenoxam seed treatment for control of FCR. The level of FCR resistance and the innate activity level of three PR-Proteins on five spring wheat cultivars were assessed by greenhouse experiments as well as the level of induction of SAR with *B. mycoides* BmJ and acibenzolar-*S*-methyl (ASM) was investigated. Finally, the integration of host plant resistance, fungicide seed treatment and BCA induced resistance was evaluated relative to FCR control in both glasshouse and field experiments.

2. Materials and methods

2.1. Assessment of seed treatments for control of Fusarium crown rot (FCR) of wheat

2.1.1. Preparation of pots

Pots (10 cm square, 0.70 L) containing 400 g of pasteurized soil substrate MSU mix (1/3 sand, 1/3 peat, and 1/3 topsoil (Bozeman silt loam) plus wetting agent [Aquagrow 2000, Aquatrols, Cherry Hill, NJ]) were used in glasshouse experiments. Pathogen inoculated pots were inoculated with *F. culmorum* isolate 2279 using a 100 g macroconidia infested MSU mix placed in a layer immediately below the planted seeds. Soil above and below this layer was pasteurized soil MSU mix. The soil infestation was done by mixing pasteurized MSU mix with the suspension of macroconidia in a tumbler machine to obtain concentrations of 10,000 macroconidia per gram of soil (1.0×10^6 per pot). Macroconidia were obtained by growing the fungus in 25% Potato Dextrose Broth for 10 days followed by filtering through cheesecloth. The number of macroconidia was determined by counting using a hemacytometer.

2.1.2. Preparation of seeds and seed treatments

Seeds (cv. Hank) were disinfected for 30 s by immersion in 70% ethanol followed by two rinses with sterile distilled water. Seeds were air dried for 3 h in a fume hood at room temperature 23 ± 1C. Seed were untreated or treated with: *Bacillus* sp. L 324-92; *Pseudomonas fluorescens* strain 2-79 (both isolates courtesy Dr. David Weller, USDA/ARS, Pullman, WA); *Bacillus pumilis* MSU