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Evaluation of biological seed treatments in combination with management practices for the control of Fusarium dry rot of potato

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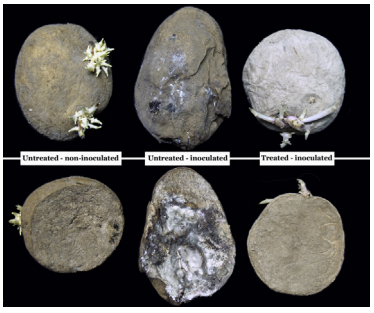
HIGHLIGHTS

- Biocontrol agents provide good control of seed piece decay under optimal conditions.
- Biocontrol agents provide good control of sprout rot under optimal conditions.
- Biocontrol agents provided no disease control under poor storage conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Seed-borne diseases of potato represent a significant constraint to potato production in the US. The use of an effective fungicide in combination with good management practices during cutting and storage, prior to planting, is essential to reducing disease. The efficacy of two biocontrol agents (*Bacillus subtilis* and *Trichoderma harzianum*), and a commercially formulated mixture of the chemicals fludioxonil plus mancozeb, applied as seed treatments in combination with different management practices, were evaluated over two years for the control of seed piece decay and sprout rot caused by *Fusarium sambucinum*. Treatments were made 10 days prior to planting and at planting, and tubers were re-stored at either 18 °C and 95% RH with forced air ventilation at 5950 l min^{-1} (optimal conditions), at 25 °C in the dark without ventilation (sub-optimal), or not stored at all prior to planting. Seed piece and sprout health were evaluated *in vitro* and agronomic impacts evaluated in field experiments. Results showed that the biological control agents *B. subtilis* and *T. harzianum* provided good control of sprout rot and seed piece decay caused by *F. sambucinum*, when seed was re-stored under optimal conditions or not re-stored at all. Under optimal conditions, treatment with *B. subtilis* reduced sprout rot and seed piece decay on average by 66% and 84%, respectively. Treatment with *T. harzianum* reduced sprout rot and seed piece decay on average by 70% and 81%, respectively. Treatment with fludioxonil + mancozeb reduced sprout rot and seed piece decay under both re-storage regimes. Under optimal conditions, disease incidence and severity was reduced on average by 81% and 97%, respectively. Neither biological control agent reduced seed piece decay incidence under either re-storage regime compared to the untreated controls.

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1. Introduction

Seed-borne diseases of potato (*Solanum tuberosum* L.) represent a significant constraint to potato production in the US (Secor and Salas, 2001) and in a recent study fungal seed-borne diseases were identified as a major constraint to healthy seed production (Frost et al., 2013). Fusarium dry rot of potato is a major postharvest disease worldwide and is caused by several Fusarium species with *Fusarium sambucinum* Fuckel being the most aggressive species in Michigan (Boyd, 1972; Secor and Salas, 2001; Wharton et al., 2007a; Gachango et al., 2011a,b). *F. sambucinum* is readily transmitted by seed-borne inoculum (Secor and Salas, 2001; Carnegie et al., 1998). Dry rot affects tubers in storage and seed tuber pieces in the field (Wharton et al., 2007a; Gachango et al., 2012a,b; Kirk et al., 2013). Losses associated with dry rot have been estimated to range from 6% to 25%, and occasionally losses as great as 60% have been reported during long-term storage (Estrada et al., 2010; Secor and Salas, 2001). In the spring, *F. sambucinum* typically relies on seed infection to establish a moderate level of sprout infection, thereby initiating seasonal epidemics on stems either above or below ground (Hanson et al., 1996; Gachango et al., 2012a). In severe cases, the pathogen can kill developing sprouts outright, resulting in delayed or non-emergence, which is usually, expressed as poor and uneven stands with weakened plants (Wharton et al., 2006). *F. sambucinum* may also rot seed pieces completely (Ray and Hammerschmidt, 1998; Wharton et al., 2007). Furthermore, bacterial pathogens such as *Pectobacterium* spp. are known to frequently invade through *Fusarium* dry rot lesions causing soft rot decay of seed pieces and blackleg. Blackleg is infection of the stem which begins with bacteria moving up from an infected seed piece. Dry rot infected seed pieces are known to have a high incidence of blackleg (Secor and Salas, 2001). In late summer/autumn, infection of potato tubers by dry rot pathogens occurs through wounds inflicted during harvesting, grading, cutting, and handling of tubers (Wharton et al., 2007a; Powelson and Rowe, 2008).

In the past ten years in the potato growing regions of the midwestern states of the US, three factors have enhanced seed-borne disease problems, a lack of information on effective fungicides for both post-harvest and pre-planting use against seed-borne pathogens, an increase in the acreage of potatoes grown by fewer growers leading to management issues such as timing of pre-cutting of seed prior to planting (MPIC Pesticide Survey, 1995–1999), and thirdly climatic factors such as increased rainfall during the planting phase of the season (Andresen et al., 2001; Baker et al., 2005). In combination, these factors can delay planting and increase the impact of fungal and secondary bacterial seed piece decay and sprout rot during the early part of the growing season, subsequently affecting yield and quality of the crop. Dry rot has traditionally been managed by implementing practices that reduce tuber bruising and provide conditions for rapid wound healing (Secor and Salas, 2001) and by application of the benzimidazole fungicide thiabendazole (TBZ) either as tubers are going into storage or before planting (Hide et al., 1992). Isolates of *F. sambucinum* resistant to TBZ and other benzimidazoles were first discovered in Europe in 1973 (Hide et al., 1992) and in the United States in 1992 (Desjardins, 1995). Gachango et al. (2012b) reported that all samples of *F. sambucinum* isolated from Michigan potato seed were resistant to TBZ. Other fungicides used to control Fusarium dry rot in US include fludioxonil alone (Maxim™ Seed Potato, Syngenta Inc. Greensboro, NC, USA) or in combination with mancozeb (Maxim MZ®, Syngenta). Fludioxonil has been shown to reduce seed piece decay and sprout rot resulting in increased plant stands and the production of healthy progeny tubers (Wharton and Kirk, 2007).

In the past five years, fludioxonil-resistant isolates of *Fusarium* spp. have been reported in Canada and the US and included isolates of *F. sambucinum*, *F. coeruleum*, and *F. oxysporum* (Peters et al., 2008a,b; Gachango et al., 2011a,b, 2012a). This has resulted in fewer alternatives for controlling potato seed piece decay and sprout rot caused by these pathogens (Gachango et al., 2012b). Thus, the use of an effective seed treatment in combination with good management practices during the cutting process and storage of cut seed prior to planting are essential to reducing these diseases in cut seed prior to planting.

In the past five years several new biological control agents based on the biocontrol bacteria *Bacillus subtilis* (Serenade Max®; Agra-Quest Inc.) and the biocontrol fungus *Trichoderma harzianum* (T-22 Planter Box®; Bioworks Inc.) have been registered for use on potato. These have shown promise in the control of seed and soil borne diseases such as late blight, black scurf and dry rot and silver scurf (McBeath and Kirk, 2000; Sadfi et al., 2002; Brewer and Larkin, 2005; Johnson, 2007; Wharton et al., 2012). None of these products has been evaluated as seed treatment for the control of Fusarium seed piece decay and sprout rot on potato. However, in greenhouse studies *B. subtilis* and *T. harzianum* were shown to have some efficacy in controlling *Rhizoctonia* stem canker and black scurf (Brewer and Larkin, 2005). Preliminary trials using a related *Trichoderma* species *T. atroviride*, as a seed treatment showed that the fungus was as effective in controlling seed-borne late blight as the conventional fungicide mancozeb (McBeath and Kirk, 2000). These biological control agents have the additional benefit of being endophytic and persisting on the host surface and thus may protect emerging sprouts from disease. Research with the biological control agent *B. cereus* showed that colonization of the potato surface increased until 61 days after planting (Sadfi et al., 2002).

Previous studies showed that cutting and treating potato seed with fludioxonil + mancozeb up to ten days before planting could significantly reduce Fusarium seed piece decay, improve plant establishment and subsequent crop vigor and early development (Wharton et al., 2007). However, the presence of fungal inoculum and poor seed storage conditions after seed cutting and prior to planting can negatively impact plant establishment, crop vigor and development (Wharton et al., 2007c). *Fusarium* insensitivity in laboratory studies may not translate directly to commercial production. This disparity may result from interactions not experienced in mixed populations or within a living host. There has been no compelling evidence to suggest that fludioxonil has failed to perform because of insensitivity to Fusarium (Gachango et al., 2012a). However, in light of the discoveries of fungicide resistant *Fusarium* isolates, the objectives of this investigation were twofold. First, to evaluate *in vitro* the effectiveness of the biological control agents as seed treatments for controlling seed piece decay and sprout rot caused by *F. sambucinum* prior to planting. Secondly, to determine the best conditions to re-store seed after cutting and treating with these biological seed treatments in order to minimize seed piece decay and sprout rot and maximize early plant development and vigor.

2. Materials and methods

2.1. Fungal cultures

A mixture of 10 virulent single spore isolates of *F. sambucinum*, which produces dry rot symptoms in potato tuber tissue and was determined to be resistant to thiabendazole but sensitive to fludioxonil, was used. Conidia of the isolates were maintained at 4 °C in the dark on filter paper and axenic cultures of the isolates were produced by placing a 1 mm^2 section of filter paper containing