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Control of common bunt of wheat under field conditions with the biofumigant fungus *Muscodor albus*

Blair J. Goates · Julien Mercier

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Abstract Field experiments were conducted to evaluate the biological control potential of the fungus *Muscodor albus*, when applied as a seed treatment or an in furrow soil treatment, for control of common bunt (CB) of wheat caused by *Tilletia caries*. For seed treatments, dry rye grain culture of *M. albus* was ground into powder and applied, at a rate of 125 mg/g seed, to wheat seed infested with *T. caries* teliospores. The culture was also cracked into particles and applied in furrow at the rate of 4 g/m of row, along with teliospore infested seed during planting. Treatments were evaluated during two growing seasons and two planting dates beginning in early spring when soil temperatures were optimal for disease development (5–10°C), and then approximately 3 weeks later. In the first year, treatments in the first seeding date reduced CB from 44% diseased spikes in untreated controls to 12% and 9% in seed and in furrow treatments respectively, and from 6% in controls to 0% in both treatments in the second seeding date. In the second year, CB was reduced from 8% in controls to 0.5% and 0.25% for seed and in furrow treatments respectively in the first seeding date, and from 0.75% in controls to 0% in both the treatments in the second seeding date. *M. albus* may have potential for CB control in organic wheat production where options for managing the disease are very limited.

Keywords Biocontrol · *Tilletia caries* · *Tilletia tritici*

B. J. Goates (✉)
United States Department of Agriculture,
Agricultural Research Service,
1691 S. 2700 W.,
Aberdeen, ID 83210, USA
e-mail: Blair.Goates@ars.usda.gov

J. Mercier
AgraQuest Inc.,
1530 Drew Avenue,
Davis, CA 95618, USA
e-mail: jmercierca@aol.com

J. Mercier
Harris Moran Seed Company,
9241 Mace Blvd,
Davis, CA 95618, USA

Introduction

Common bunt of wheat, caused by the fungus *Tilletia caries* (= *T. tritici*) and the closely related species *T. foetida* (=*T. laevis*) caused significant losses in yield and quality prior to the advent of efficacious chemical seed treatments (Hoffmann 1982). Few modern cultivars released in the U.S. and elsewhere contain resistance to common bunt due to the common use of effective chemical seed treatments. Incorporation of host-specific resistance genes (Goates 1996) is, however, still an important part of wheat cultivar development in many areas of the world. Common bunt can be a serious production problem in some wheat growing regions when chemical seed treatments are not routinely used, are unavailable, or when their use is not permitted such as in organic agriculture.

Common bunt is primarily a seed-borne disease incited by teliospores that infest the surface of seed, but soil-borne teliospores can also cause disease. The disease is initiated when teliospores in soil or on seed germinate and produce hyphae that infect the coleoptile prior to seedling emergence. Teliospores on seed typically require several days to germinate which coincides with the emergence of the coleoptile. The disease is favoured by cool soil temperatures with an optimum of 5 to 10°C (Hoffmann 1982).

Common bunt is often a target disease for efficacy analyses of various compounds or treatments outside of traditional chemical seed treatments. Liquid manure (Borgen et al. 1995), copper sulphate plus lime (Lukanowski 2006), the mustard powder Tellecur (Koch et al. 2006; Waldow and Jahn 2007; Winter et al. 2001), skim milk powder (Winter et al. 2001), acetic acid vapour (Sholberg et al. 2006) and strains of *Pseudomonas* (Hokeberg et al. 1997) have all shown some efficacy against common bunt. Koch et al. (2006) tested numerous products for common bunt efficacy that included plant-based materials and several strains of both actinomycetes and Trichoderma. They concluded that the mustard powder Tellecur was the only product that provided a practical level of common bunt control. None of the products tested thus far have provided the disease control levels of modern seed treatment chemicals. In recent in vitro tests (Goates and Mercier 2009), we determined that the volatiles emitted by the fungus *Muscodor albus* during its growth completely prevented germination of common bunt teliospores plated on agar, which compelled further investigation.

Muscodor albus isolate CZ-620 (family Xylariaceae, Ascomycetes) was originally isolated as an endophyte from a cinnamon tree (*Cinnamomum zeylanicum*) growing in Honduras (Strobel et al. 2001). Since its discovery, several more isolates of *M. albus* or other *Muscodor* species have been found as endophytes of various plant species in other tropical locations (Strobel 2006). *M. albus* CZ-620 produces an array of low-molecular weight volatile organic compounds, acting as a biofumigant with broad spectrum antimicrobial activity (Corcuff et al. 2011; Mercier and Jiménez 2004, 2007; Strobel et al. 2001). Exposure of barley seeds infested with *Ustilago hordei* to the volatiles produced by potato dextrose agar culture of *M. albus* for four days completely controlled covered smut when the seeds were subsequently planted (Strobel et al. 2001). The incorporation of *M. albus* culture to soil or potting mix before planting controlled soil-borne diseases such as damping-off and root rot of *Brassica* spp., *Phytophthora* root rot of bell pepper and verticillium wilt of eggplant (Mercier and Manker 2005; Stinson et al. 2003; Worapong and Strobel 2009), as well as some plant parasitic nematodes (Riga et al. 2008). The volatile compounds produced by *M. albus* in soil or potting mix were shown to provide complete control of damping-off of broccoli caused by *Rhizoctonia solani* (Mercier and Jiménez 2009). There was a strong relationship between the production of isobutyric acid, the main volatile produced, and damping-off control. The production of volatiles occurs within hours of incorporating desiccated cultures of *M. albus* to soil but lasts only for a few days, after which the biofumigation process is over and the fungus, a sterile mycelium, does not persist in soil (Mercier and Jiménez 2009). Factors affecting volatile production are still poorly understood but water activity (Aw) appears to have a major effect on the type and quantity of volatiles produced (Corcuff et al. 2011).

With better understanding of the possibilities and limitations of *M. albus* as a biocontrol agent in soil, it becomes possible to investigate various use strategies for controlling bunt and smut diseases of grain. The exceptional in vitro efficacy of *M. albus* volatiles on *T. caries* teliospores mentioned previously indicates that the biocontrol fungus may have potential for controlling common bunt when *M. albus* is present in the infection court applied as an in furrow or seed treatment. Experiments were designed to test this hypothesis.

Materials and methods

Cultures of *M. albus* were produced by growing the fungus on autoclaved rye grain at ambient room temperature, starting with a potato dextrose broth culture (Mercier and Jiménez 2004). The colonized grain was air-dried at room temperature and stored at 4°C until use.

Seed of the highly susceptible spring wheat cultivar Red Bobs was heavily inoculated with *T. caries* teliospores to the extent that seed was noticeably darkened by the black spores. For the seed treatment, desiccated rye grain culture of *M. albus*

was ground into a coarse powder using a small motorized coffee grinder. The powder was moistened and mixed in a glass vial with teliospore inoculated seed at a rate of 125 mg per g of seed and then the seed was dried at ambient room temperature. The seed was held 4 days in the laboratory and then planted about 5 cm deep in field plots. For the in-furrow application, the *M. albus* rye grain formulation was cracked into 1 mm or less sized particles with a small manually operated mill, and then applied in furrow at the rate of 4 g/m along with infested wheat seed during planting.

Field plots were planted at the University of Idaho Extension and Research Center in Aberdeen, Idaho in sandy loam soil at approximately pH 8.3. Treatments were evaluated in four replicate 2 m rows seeded at a rate of 3 g seed / m on two planting dates during 2007, and the experiment was repeated in 2008. In each year, the first planting was done as soon as soil was sufficiently dry to cultivate in the spring when cool soil temperatures at planting depth typically occur, which is optimal for infection. The second planting each year was done about 3 weeks later when temperatures are typically higher and less optimal for disease development. Plots were fertilized at rates to maximize grain yields and were given periodic sprinkler irrigation during the growing season. At plant maturity, the mean percent infected spikes per replicate was determined.

Results

Plant emergence and plant stands were similar in *Muscodor* treated plots as compared to untreated teliospore-inoculated control plots. Planting dates were delayed in 2008 compared to 2007 due to wet soil conditions. Thus, soil temperatures during the experiments in 2008 were higher than in 2007 and were not as favourable for disease development. As was expected, a higher level of disease occurred on the first planting date of each year due to cooler soil temperatures during planting.

In 2007 and 2008, the untreated control plots had 43.8 and 7.75% infected spikes respectively on the first planting date, and 5.8 and 0.8% infection on the second planting date. Seed and in-furrow treatments in 2007 had 11.8 and 8.5% bunted spikes respectively for the early planting date, and 0% in both treatments for the later planting date. In 2008, the respective treatments had 0.5 and 0.3% bunted spikes in the early planting date, and 0% in both treatments for the later planting date (Table 1). The biofumigation treatments substantially reduced common bunt in all experiments and eliminated infection under lower disease pressure.

Discussion

The results of these experiments demonstrate that in vitro activity against *T. caries* teliospores determined previously (Goates and Mercier 2009) can also translate into a significant reduction or complete elimination of common bunt under field conditions. This indicates a potential for further development of methodologies that could enhance the effectiveness of the biofumigant. The preparation (grinding) and application of the *M. albus* desiccated culture in these experiments was quite simple and crude and the ground culture made for seed application did not adhere well to the seed. Inclusion of an adhesive, such as aqueous methyl cellulose, during seed application would have kept more of the powdered formulation on the seed and thus directly in the infection court. In

Table 1 Percent of wheat spikes infected with common bunt after application of *Muscodor albus* rye grain culture to seed or in furrow on two planting dates in each of 2 years

Treatment	Seeding date							
	6 April 2007		1 May 2007		18 April 2008		13 May 2008	
	Percent diseased spikes							
	Mean[a]	SD[b]	Mean	SD	Mean	SD	Mean	SD
Bunt inoculated - Untreated	43.8	4.8	5.8	5.3	7.75	4.9	0.8	0.5
Seed Treatment 0.125 g/g seed	11.8	8.1	0	0	0.5	0.6	0	0
In Furrow treatment 4 g/m	8.5	7.3	0	0	0.3	0.5	0	0

[a] Data based on 4 replicated plots

[b] Standard deviation

addition, different formulation ingredients could have been added to the seed treatment preparation to enhance the growth of *M. albus* after planting and increased biofumigation activity in the field. There is certainly room for developing and formulating *M. albus* to facilitate its use and enhance its activity over the simple methods used in these experiments. The rye grain culture is best seen as an experimental formulation for proof of concept and other approaches could be taken in formulating *M. albus* for its intended use (Mercier et al. 2007a). For example, *M. albus* was produced on perlite loaded with nutrients and was found to be effective for soil fumigation (Mercier et al. 2007b).

In the experiments presented here, there was an extremely high disease pressure from the inoculum load; far above that which would occur under normal growing conditions. It might be expected that in a situation of normal disease pressure from inoculum, control with *M. albus* might be enhanced. In these experiments, the untreated controls in second seeding dates in both years produced a level of infection that was more typical of what is found under commercial growing situations. Under these conditions, both biofumigation treatments eliminated the disease. This demonstrates the potential practical effectiveness of the treatments tested. Management options such as avoiding planting late in the fall or early in spring, when cool soil temperatures favours infection and disease development, should always be considered when attempting to control common bunt when modern chemical treatments are excluded.

In this study, *M. albus* appeared to have a longer length of activity in soil than anticipated from a previous study by Mercier and Jiménez (2009) which showed that production of isobutyric acid, the main indicator of fungicidal activity, peaked at 48 h in a loamy soil held at ambient room temperature. About 5 days are required for teliospores of *T. caries* to germinate on agar at optimal laboratory conditions at 20°C, while germination may take about 14 days at 5°C. At the time of planting during the present study, soil temperatures ranged from approximately 5 to 18°C. Teliospores of *T. caries* are only partially affected by *M. albus* when fumigated in the lab in a dry dormant state (Goates and Mercier 2009). Their susceptibility to the volatiles increases as they become physiologically active and the teliospore wall begins to break down during the process of germination at proper moisture and temperature. This would not have occurred for several days after infected seed was planted in the cool soil during the first planting date in this study. This suggests that biofumigation activity in different soils or field conditions could continue for a longer period than anticipated by the previous lab experiments (Mercier and Jiménez 2009).

We demonstrated that two approaches could be used to reduce common bunt resulting from teliospore infested seed. The efficacy of *M. albus* volatiles on soil-borne teliospores was not investigated in these studies but the biocontrol fungus could also be effective against soil-borne inoculum as well if the volatiles sufficiently penetrate the soil surrounding germinating seed. Only the hyphae originating from germinating teliospores that are in close proximity to seed have the potential to infect the coleoptile. Although the in-furrow treatment is interesting, there might be limitations for using *M. albus* in soil because the biofumigation process in soil can be short-lived and sensitive to the environment. It might also be more costly on a surface area basis. The pre-plant seed application might be more practical, as it can be more easily controlled and requires less biofumigant material.

One of the primary reasons for fungicide seed treatment of wheat and other small grains is to control smut and bunt diseases. Organic growers have few options for effective control of these diseases in the absence of modern chemical seed treatments. An effective economical treatment with *M. albus* could be especially interesting in organic production.

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