Evaluation of New Fungicide Seed Treatment Pre-Mixes for Penicillium Dry Rot Control in Soft White Winter Wheat

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Introduction

Penicillium dry rot of wheat (also known as Penicillium dry seed decay) occurs when wheat seed is planted into soils that are too dry to allow for timely germination of the seed. This is often done in semi-arid production regions with the expectation that precipitation will follow planting and stimulate seed germination. However, if precipitation does not occur in a timely manner the wheat seed may remain in dry soils for extended periods prior to germination and, as a result, *Penicillium* spp. can infect the seed and prevent it from germinating. The objectives of this study were to: i) determine the efficacy of experimental seed treatment EXP102 alone and in combinations with other Penicillium dry rot-effective products; and ii) evaluate each seed treatment product for negative effects on wheat germination and growth.

Materials and Methods

Greenhouse Trial

The *Penicillium* isolate (Pen-5) used in this study was obtained by incubating seed of soft white winter wheat cv. 'Stephens' in petri plates filled with autoclaved soil under conditions conducive for Penicillium dry rot (~8% soil moisture at 70°F in the dark). Pathogenicity of isolate Pen-5 was tested in a preliminary petri plate assay and it was observed to cause more dry rot on 'Stephens' than the other isolates tested.

Seed germination flats (length: 21 in; width: 15 in; depth: 2.5 in) were filled with dry, pasteurized field soil. Each treatment consisted of 20 seeds of soft white winter wheat cv. 'SY Ovation' that were treated with experimental seed treatments by Syngenta prior to planting (Table 1). Seeds were covered with 35 g of *Penicillium*-infested soil (equal to approximately 2 g of *Penicillium* spores/treatment). A non-treated, non-infested control and a non-treated, infested control were also included. The non-treated, non-infested control treatment was covered with 35 g of pasteurized soil. All seeds were covered with an additional 2 cm of pasteurized soil and moistened with water to a depth of approximately 1 in. After 14 days the flats were thoroughly watered to promote seed germination. Each treatment was replicated 4 times and the experiment was arranged as a randomized complete block design.

Treatments were assessed 7 days after germination (DAG) for stand count, crop growth stage (Feekes scale), plant height, number of leaves, % crop phytotoxicity (0% = no injury; 100% = dead plants), and % pre-emergence damping off (% of non-germinated seeds exhibiting Penicillium dry rot). At 21 DAG, treatments were assessed for stand count, crop growth stage, plant height, number of leaves, % crop phytotoxicity, fresh weight, and % post-emergence seed decay (% of plants with *Penicillium* sporulation on the germinated seed).

Petri Plate Assay

A petri plate assay was also performed to test the same seed treatments for Penicillium dry rot control under controlled conditions in the laboratory. Each experimental unit consisted of a petri plate with 20 treated seeds and each treatment was replicated 4 times. A non-treated, non-infested control and a non-treated, infested control were also included as described above.

Approximately 25 g of autoclaved soil was added to each petri plate and 20 seeds/treatment were placed on the soil with the crease side facing up. Two grams of *Penicillium*-infested soil (isolate Pen-5) was sprinkled over the seeds (~125 mg of spores/plate) and an additional 10 g of autoclaved soil was added to cover the seed completely. Sterile water was sprayed onto each plate using a hand sprayer to achieve a soil moisture level of 8.1% (v/w). Plates were placed in a 68° F incubator and the experiment was arranged as a randomized complete block design. After 18 days of incubation the seeds were assessed for % pre-emergence damping off (% of seeds exhibiting Penicillium dry rot). All seed was then placed in a petri plate lined with sterile Whatman filter paper, moistened with sterile water, and assessed for % germination.

Data analysis

Data from both trials were analyzed as randomized complete block designs. Analysis of variance (ANOVA) was performed using PROC MIXED in SAS version 9.4 and multiple pairwise comparisons were made using Tukey's test.

Results and Discussion

Greenhouse Trial

All treatments exhibited germination rates of 85% or more and pre-emergence damping-off was not observed at 7 DAG (Table 1). A significant effect on plant height ($P = 0.0006$) and leaf number ($P = 0.002$) was observed, with treatment EXP108+EXP109 exhibiting reduced plant height and leaf number. Significant effects on stand count or growth stage were not observed ($P > 0.05$). The high germination rates and lack of pre-emergence damping off at 7 DAG may have been a result of sufficient moisture content in the flats that promoted seed germination and prevented dry seed decay.

At 21 DAG, phytotoxicity was observed in two plants grown from seed treated with EXP112; the phytotoxicity symptoms consisted of chlorotic, twisting leaves. Treatment EXP108+EXP109 exhibited significantly lower plant height ($P = 0.04$) than the non-treated, non-infested control treatment (Table 2). Significant effects on stand count, growth stage, leaf number, or fresh weight were not observed ($P > 0.05$) at 21 DAG. However, *Penicillium* was observed sporulating on some of the germinated seed (post-emergence seed rot) when plants were removed from the soil. All treatments exhibited significantly ($P < 0.05$) lower levels of post-emergence seed rot than the non-treated, infested control and several treatments, including EXP101, EXP102, EXP102+EXP103, EXP102+EXP104, EXP101+EXP104, EXP110, and EXP102+EXP111, were not significantly different ($P > 0.05$) than the non-treated, non-infested control (Table 2).

Petri Plate Assay

A significant treatment effect ($P < 0.0001$) was observed on pre-emergence damping off and germination in the petri plate assay. All treatments significantly reduced pre-emergence damping off compared to the non-treated, infested control (Table 3). Treatments EXP102, EXP102+EXP103, EXP102+EXP104, EXP101+EXP104, EXP106, and EXP102+EXP111 exhibited dry rot at levels that were equal to or less than the non-infested control. Treatments EXP102+EXP103, EXP102+EXP104, EXP101+EXP104 completely prevented pre-emergence damping off. The highest germination rates were observed in treatments EXP101+EXP104 (95.0%), EXP102+EXP104 (93.8%), EXP106 (88.8%), and EXP102+EXP111 (81.3%) (Table 3).

Conclusions

All of the seed treatments, including experimental seed treatment EXP102 alone and in combination with other seed treatments, significantly reduced post-emergence seed rot in the greenhouse assay and significantly reduced pre-emergence dry rot in the petri plate assay. Treatments that combined EXP102 with other seed treatments were the most effective. Relatively few negative effects (e.g. phytotoxicity, reduced germination or stand count, reduced plant height or fresh biomass) were observed in both studies. The results from this study indicate that seed treatments can be very effective at controlling Penicillium dry rot in soft white winter wheat.

Acknowledgements

The authors would like to thank Syngenta Crop Protection LLC for providing funding and in-kind support.

Table 1. Phytotoxicity, stand count, Feekes growth stage, plant height, number of leaves, and pre-emergence damping off of winter wheat cv. 'Ovation SY' seed that was treated with fungicides to prevent Penicillium dry rot (7 days after germination)[1]

	7 days after germination					
Treatment	**Phytotoxicity (%)**	**Stand count**	**Feekes growth stage**	**Height (cm)**	**No. leaves**	**Pre-emergence damping-off (%)**
Non-infested control	0	19.0	1	9.0 a	2.0 a	0
Infested control	0	18.0	1	7.7 ab	1.7 ab	0
EXP101	0	17.8	1	8.2 a	2.0 ab	0
EXP102	0	18.0	1	8.5 a	1.9 ab	0
EXP102+EXP103	0	19.0	1	8.0 a	1.9 ab	0
EXP102+EXP104	0	17.0	1	8.2 a	1.9 ab	0
EXP101+EXP104	0	18.8	1	7.9 a	2.0 a	0
EXP106	0	18.8	1	7.3 ab	2.0 ab	0
EXP107	0	19.5	1	9.3 a	2.0 ab	0
EXP108+EXP109	0	17.0	1	5.3 b	1.6 b	0
EXP110	0	18.3	1	7.2 ab	1.7 ab	0
EXP102+EXP111	0	18.3	1	7.8 a	1.9 ab	0
EXP112	0	17.5	1	7.4 ab	1.7 ab	0
***P*-value**	1.00	0.49	1.00	0.0006	0.002	1.00

[1] Treatments followed by the same letter are not significantly different from each other using Tukey's test at $P = 0.05$.

Table 2. Phytotoxicity, stand count, Feekes growth stage, plant height, number of leaves, fresh weight and post-emergence seed rot of winter wheat cv. 'Ovation SY' seed that was treated with fungicides to prevent Penicillium dry rot (21 days after germination)[1]

	21 days after germination						
Treatment	**Phytotoxicity (%)**	**Stand count**	**Feekes growth stage**	**Height (cm)**	**No. leaves**	**Fresh weight (g)**	**Post-emergence seed rot (%)**
Non-infested control	0	19.0	2.1	23.0 a	5.5	0.72	0.0 e
Infested control	0	18.5	1.6	21.6 ab	4.0	0.51	74.4 a
EXP101	0	18.5	1.8	21.0 ab	4.5	0.56	18.9 bcde
EXP102	0	18.0	1.5	20.4 ab	4.0	0.51	9.4 bcde
EXP102+EXP103	0	19.3	1.6	20.1 ab	4.0	0.48	5.3 cde
EXP102+EXP104	0	18.0	1.9	20.1 ab	4.5	0.53	1.6 e
EXP101+EXP104	0	19.5	1.8	19.6 ab	4.3	0.49	2.6 de
EXP106	0	19.5	2.4	19.1 ab	5.5	0.59	25.6 bcd
EXP107	0	19.5	1.6	20.0 ab	4.5	0.51	31.5 b
EXP108+EXP109	0	17.8	1.6	15.8 b	4.8	0.51	28.5 bc
EXP110	0	18.8	1.6	17.0 ab	4.5	0.48	10.7 bcde
EXP102+EXP111	0	18.3	1.6	20.6 ab	4.5	0.56	6.9 cde
EXP112	2.5[2]	17.8	1.8	20.5 ab	4.5	0.46	26.3 bc
***P*-value**	0.47	0.27	0.64	0.04	0.30	0.56	<0.0001

[1] Treatments followed by the same letter are not significantly different from each other using Tukey's test at $P = 0.05$.

[2] Chlorosis and twisting of leaves observed in two plants.

Table 3. Pre-emergence damping off and germination of winter wheat cv. 'Ovation SY' seed that was treated with fungicides to prevent Penicillium dry rot[1]

Treatment	Dry rot (%)	Germination (%)
Non-infested control	8.8 d	66.3 abc
Infested control	82.5 a	5.0 f
EXP101	21.3 cd	53.8 bcd
EXP102	8.8 d	62.5 abc
EXP102+EXP103	0.0 d	75.0 ab
EXP102+EXP104	0.0 d	93.8 a
EXP101+EXP104	0.0 d	95.0 a
EXP106	1.3 d	88.8 a
EXP107	40.0 c	21.3 def
EXP108+EXP109	45.0 bc	15.0 ef
EXP110	28.8 cd	45.0 cde
EXP102+EXP111	1.3 d	81.3 ab
EXP112	23.8 cd	50.0 bcd
***P*-value**	<0.0001	<0.0001

[1] Treatments followed by the same letter are not significantly different from each other using Tukey's test at $P = 0.05$.