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Seed treatments enhance photosynthesis in maize seedlings by reducing infection with *Fusarium* spp. and consequent disease development in maize

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Abstract Maize seed that was either treated with the fungicide Cruiser Extreme 250 ® (fludioxonil + azoxystrobin + mefenoxam + thiamethoxam) or not treated was planted at two Iowa locations in 2007. Root, mesocotyl and crown rot severity, incidence of *Fusarium* spp. colonisation and chlorophyll florescence (CF) were assessed at growth stages V2, V4 and V6, and stalk rot severity at R6. At both locations, seed treatment reduced disease severity and incidence of *Fusarium* spp. infection at all growth stages assessed. Measurements of CF decreased significantly with increased disease severity and incidence of *Fusarium* spp. at V2 and V4 at both locations, indicating that seedling disease negatively affected photosynthetic performance. Mesocotyl rot severity at V4 predicted crown rot severity at V6 at both locations, as well as crown rot at V6 and stalk rot at R6 at one location.

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Maize is susceptible to infection by *Fusarium graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani, F. subglutinans, F. verticillioides,* and other *Fusarium* spp. Symptoms include early-season seedling blights (seed, root, and mesocotyl rot) and late-season crown, stalk and ear rots. Furthermore, infection of grain by *F. graminearum*, *F. proliferatum*, *F. subglutinans,* and *F. verticillioides* may result in mycotoxin contamination (White, 1999).

Since seedling blights result in stand loss, virtually all maize seed planted in the US Corn Belt are treated with fungicides. Efficacy of seed-treatment fungicides in field trials is usually assessed by measuring emergence, plant height (used as an indication of plant vigour), and yield. However, these data provide very little information on infection by specific pathogens and subsequent disease development. A clearer understanding of the effect of seed treatments on the *Fusarium*-maize pathosystem would enable better seedling disease management.

Stress can trigger reduced photosynthetic performance (Maxwell and Johnson 2000). In the *F. oxysporum*-tomato pathosystem, chlorophyll fluorescence (CF) indicated stress as a result of infection (Wagner et al. 2006). Similarly, CF in maize plants infected with *F. moniliforme* was reduced compared with uninfected plants (Pinto et al. 2000). Presumably, reduced photosynthesis in *Fusarium*-infected hosts

resulted from root and mesocotyl tissue damage, as well as systemic colonisation that interfered with absorption and transport of water and nutrients. If CF assessments could provide an index of plant vigour reduction related to infection of maize seedlings by soil-borne pathogens and an indirect measure of disease severity, they could be a valuable tool to assess the effectiveness of seed treatments.

The primary objective of this study was to determine the strength of relationship between CF measurements and seedling vigour, disease severity, and incidence of *Fusarium* spp. infection of maize seedlings. We also assessed the contribution of seedling disease management with seed treatment fungicides in management of late-season crown and stalk rot.

Field trials were planted at the Iowa State University (ISU) Southeast Research Farm (SERF), near Crawfordsville, Iowa and the ISU Northeast Farm (NERF) at Nashua, Iowa, USA. Fields both sites had been in a continuous-maize rotation for 4 years. Each field was tilled prior to planting. The trial at SERF was planted on April 19, 2007, when soil temperature was 10°C. At NERF, planting was done on May 1, 2007, when soil temperature was 15.5°C. Approximately 80 000 plants ha^{-1} were planted at both locations at 5 cm depth and standard agronomic practices were followed. Plots were 5.3 m long by 5.25 m (8 rows spaced 75 cm apart) wide. At each location the experiment was a randomised complete block design of five blocks in a split-plot arrangement with seed treatment as the main plot effect and the sampling date (V2, V4, and V6) as the split-plot effect.

Maize seed (var. Garst® 8545) was either treated with Cruiser Extreme 250® (azoxystrobin + fludioxonil + mefenoxam + thiomexoam) using a Gustafson® (model BLT) seed treater or was left untreated. A modified agar seed-health test was performed to determine incidence of seed-borne *Fusarium* spp. in the seed lot before applying the treatments.

Chlorophyll fluorescence measurements were conducted according to Earl and Tollenaar (1999) on 10 apparently healthy maize seedlings that were arbitrarily chosen from the centre two rows of each plot at growth stages V4 and V6 respectively, using a pulse amplitude modulation fluorometer (PAM 2000; Walz®, Effeltrich, Germany) equipped with a fibre-optic probe and leaf clip holder. Measurements were made on cloud-free days between approximately 1,100 and 1,200 h on the topmost fully expanded leaf of each plant, midway between the leaf tip and base and midway between the leaf margin and midrib. The quantum efficiency of Photosystem II (Φ_{PSII}) was calculated according to Genty et al. (1989). Values of Φ_{PSII} range from 0–1; values closer to 1 indicate better photosynthetic performance. Vigour was estimated at V4 and V6 growth stages by measuring height of each of the 10 plants on which Φ_{PSII} measurements were taken (Ritchie et al. 1992), from the soil to the top of the arch of the tallest fully developed leaf.

After measurements of Φ_{PSII} were also made on 20 arbitrarily selected seedlings in each treatment (four per plot), excluding the two middle and two edge rows, seedlings with intact root systems were carefully dug with a shovel and transported to the laboratory. Root- and mesocotyl-rot severity were assessed at growth stages V2 and V4, and crown rot severity was assessed at V2, V4, and V6 as follows: 0 = apparently healthy root or mesocotyl or crown tissue, 1=<25% of tissue with disease rot symptoms, 2=25–49% of tissue rotted, 3=50–74%, of the tissue rotted, 4=75% or greater of the roots rotted, and 5= wilted or dead seedlings/completely rotted mesocotyl or crown tissue (Soonthornpoct et al. 2000).

Stalk rot was assessed at growth stage R6 using the University of Illinois (0–5) stalk rot rating scale (Hines 2007). Thirty arbitrarily selected plants from each eight-row plot (five maize plants from each row of the plot, excluding the two middle rows of the plot) were removed with a shovel, and the lower third of the stalk was bisected longitudinally using a knife.

Isolation of *Fusarium* spp. was done from seedlings assessed for blight severity. Immediately after disease assessments, seedlings were surface-disinfested and samples of crown and mesocotyl tissue and five 1 cm-long sections of root tissue were selected arbitrarily (a total of seven samples per seedling) and placed on Nash-Snyder (NS) medium (Leslie and Summerell 2006) supplemented with 0.1 g l^{-1} Rose Bengal (Sigma® 0.1 g l^{-1}) and kept at 25°C for 12 h day/12 h night. White, septate mycelium that grew from a tissue segment was transferred to carnation leaf agar (CLA) medium (Leslie and Summerell 2006) for identification based on conidiophore and conidial morphology (Leslie and Summerell 2006). For each treatment, disease incidence was determined as the

percentage of root, mesocotyl and crown dissections colonised by *Fusarium* species.

Data analysis was conducted using PROC MIXED of SAS version 9.1 (SAS Institute, Cary, NC, USA). Analysis of variance was done by location to assess effects of seed treatment on plant height, mean root, mesocotyl and crown rot severity, proportion of root, mesocotyl and crown tissues from which *Fusarium* spp. were isolated, and CF measurements. Mean separation was conducted with Tukey's honest significant difference (HSD) test for multiple comparisons test (Ramsey and Schafer 2002). Relationships among plant height, root rot severity, mesocotyl rot severity, crown rot severity, incidence of *Fusarium* spp. and CF values were computed with PROC REG of SAS version 9.1 for each sampling date at each location.

No seed-borne *Fusarium* spp. were detected in seed and seed germination was 100% under laboratory conditions.

Seedling height was generally more uniform in plants grown from treated seed at both locations (data not shown). Plants grown from treated seed were taller ($P<0.001$) than control plants at SERF at growth stage V4; by V6, however, there was no difference in height. At NERF, no differences in seedling height occurred. Seed treatment significantly affected CF ($P<0.001$) (data not shown). Seedlings from non-treated seed had lower Φ_{PSII} values than those from treated seed, indicating that seedlings from treated seed were less stressed.

Regression analysis showed significant relationships at SERF and NERF between Φ_{PSII} and plant height at V4 and V6 (Table 1). Shorter, less vigorous plants had lower Φ_{PSII} values than taller, more vigorous plants.

Mean root-rot index was significantly ($P<0.001$) less at V2, but not at V4, in seedlings grown from treated seed (Table 2) at both locations. Mesocotyl rot was significantly lower ($P=0.030$) in the seedlings grown from treated seed at V2 at both locations (Table 2); byV4, however, mesocotyl rot was comparable to untreated controls. Seed treatment significantly ($P<0.001$) reduced crown rot at V2, V4 and V6 and significantly reduced stalk rot at both SERF ($P=0.034$) and NERF ($P=0.028$) (Table 2).

At V2 and V4, Φ_{PSII} and disease severity were significantly related at both locations (Table 1); higher disease severity was associated with lower Φ_{PSII} values. The fact that CF measurements were able to estimate root, mesocotyl and crown disease severity suggests that they could be used as a non-destructive, objective method to evaluate seed treatment effects under field conditions. Seed treatments benefited maize seedling growth by reducing disease-related stress and maintaining photosynthetic performance.

Table 1 Regression equations, correlation and *P*– values of relationships between dependent variables plant height, root rot severity, mesocotyl rot severity, crown rot severity, percent incidence of *Fusarium* spp. and the independent variable photosynthetic performance (as measured by Φ_{PSII})

Dependent variable	Growth stage	SERF[a]				NERF[a]			
		Slope	Intercept	R^2	*P* value	Slope	Intercept	R^2	*P* value
Plant height (mm)	V4	14.25	5.44	73.4	<0.001	13.66	8.70	36.0	0.005
	V6	81.64	73.11	36.0	0.005	44.80	80.64	33.4	0.008
Root Rot	V2	−5.96	3.95	53.2	<0.001	−4.29	2.91	10.3	0.017
Crown Rot	V2	−2.71	2.60	32.1	0.009	−9.68	4.78	40.8	0.002
	V4	−5.89	4.68	42.1	0.002	−15.66	7.27	66.3	<0.001
Mesocotyl Rot	V2	−5.89	3.24	61.0	<0.001	−8.57	3.99	30.5	0.012
	V4	−5.73	5.09	39.8	0.003	−8.94	4.79	53.7	<0.001
Incidence of *Fusarium* spp.	V2	−92.49	62.91	54.6	<0.001	−208.37	101.05	26.5	0.020
	V4	–	–	–	NS[b]	−306.37	157.87	43.8	0.002
	V6	–	–	–	NS	−129.87	121.71	28.3	0.016

[a] SERF—Iowa State University Southeast Research Farm; NERF—Iowa State University Northeast Research Farm

[b] NS—Not significant ($P>0.05$)

Table 2 Mean root rot, mesocotyl rot, crown rot, and stalk rot severity indices and incidence of *Fusarium* infection of maize plants grown from non-treated and seed treated with the fungicide Cruiser Extreme 250®

Location[a]	Treatment	Root rot[b]		Mesocotyl rot[b]		Crown rot[b]		Stalk rot[c]		Incidence of *Fusarium* infection (%)		
		V2	V4	V2	V4	V2	V4	V6	R6	V2[d]	V4[d]	V6[e]
SERF	Cruiser Extreme 250	0.1 b[f]	2.6 a	0.3 b	2.1 a	1.3 b	1.3 b	1.2 b	2.0 b	22.1 a	37.1 a	47.5 a
	Non-treated	2.0 a	2.6 a	1.1 a	2.7 a	1.5 a	2.0 a	2.6 a	3.6 a	48.6 b	74.3 b	100 b
NERF	Cruiser Extreme 250	1.5 b	2.0 a	0.8 b	1.4 a	1.3 b	1.0 b	1.5 b	1.8 b	19.3 a	51.4 a	52.5 a
	Non-treated	2.2 a	1.6 a	2.2 a	2.0 a	2.1 a	1.8 a	2.8 a	2.7 a	56.4 b	81.4 b	97.5 b

[a] SERF—Iowa State University Southeast Research Farm; NERF—Iowa State University Northeast Research Farm.

[b] Mean root, mesocotyl, and crown rot severity indices were estimated using the following scale where 0=apparently healthy tissue, 1=<25% of tissue with disease rot symptoms, 2=25–49% of tissue rotted, 3=50–74%, of the tissue rotted, 4=75% or greater of the tissue rotted, and 5=wilted or dead seedlings/completely rotted tissue.

[c] Stalk rot disease severity was assessed using University of Illinois (0–5) stalk rot rating scale. A total of 30 plants was assessed per treatment.

[d] Percentage of root, mesocotyl and crown tissue dissections that were infected with *Fusarium* spp.

[e] Percentage of crown tissue dissections that were infected with *Fusarium* spp.

[f] Values from the same location and column followed by the same letter are not significantly different, according to the Tukey's HSD test ($P<0.05$) performed by location.

At both locations and at all growth stages, seed treatment reduced incidence of *Fusarium* spp. colonisation ($P<0.001$) (Table 2). Although the proportion of seedling tissues colonised with *Fusarium* spp. increased over time in both treated and non-treated seedlings, incidence was always significantly less than in treated seedlings. At NERF, regression analysis showed a significant relationship between Φ_{PSII} and incidence of *Fusarium* spp. infection at each growth stage (Table 1), with greater infection associated with lower photosynthetic performance. At SERF, a significant relationship between Φ_{PSII} and infection incidence occurred at V2, but not at growth stages V4 or V6, perhaps because plants were not under significant environmental stress at growth stages V4 and V6 at this location. Differences in photosynthetic performance of drought tolerant hybrids occurred only when plants were under significant water stress (O'Neill et al., 2006). Thus, we demonstrated that CF measurements indicated soil-borne infection even when no above-ground symptoms were evident. Pinto et al. (2000) showed reduced photosynthesis in maize seedlings infected with *F. verticillioides* under controlled environmental conditions. To our knowledge, this is the first report demonstrating that *Fusarium* spp. infections reduce photosynthetic rates under field conditions.

At both locations, severity of mesocotyl rot at V4 was related to crown rot severity at V6 (Table 3). At SERF, a significant relationship was found between crown rot severity at V6 and stalk rot severity at R6 (Table 3). Maize seedling disease management practices should aim to protect mesocotyl health because

Table 3 Regression coefficient, intercept coefficient of determination (R^2) and *P* values of relationships between dependent variable crown rot severity at V6 and stalk rot severity at R6, and the independent variable mesocotyl rot severity at V4 and crown rot severity at R6, respectively

Dependent variable	Independent variable	SERF[a]				NERF[a]			
		Slope	Intercept	R^2	*P* value	Slope	Intercept	R^2	*P* value
Crown Rot	Mesocotyl rot	1.09	−0.60	77.5	<0.001	0.92	0.12	41.5	0.002
Stalk rot	Crown rot	0.68	0.93	73.4	<0.001	–	–	–	NS

[a] SERF—Iowa State University Southeast Research Farm; NERF—Iowa State University Northeast Research Farm

[b] NS—Not significant ($P>0.05$)

this tissue transports water and nutrients to developing seedlings until V6, when nodal roots become physiologically active (Ritchie et al. 1992). Although fungicide seed treatment has a limited period of activity (Mueller and Bradley 2008), we found that beneficial effects of seed treatment remained evident at harvest. Seed treatment not only suppressed seedling disease but also contributed to reduced crown and stalk rot. Since stalk rot development is complex, however, additional research under varying growing conditions is needed to verify that seed treatment fungicides sufficiently reduce stalk rot.

This preliminary study has clearly shown that seed treatments suppress infection by *Fusarium* spp., and seedling disease development in maize, resulting in enhanced photosynthesis and increased plant vigour. Furthermore, early-season protection of germinating seedlings may contribute to reduced mid- to late-season disease.

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