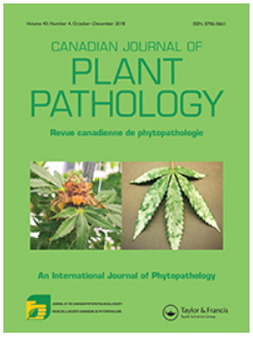

Canadian Journal of Plant Pathology

ISSN: 0706-0661 (Print) 1715-2992 (Online) Journal homepage: http://www.tandfonline.com/loi/tcjp20

Effects of fungicide seed treatments and a winter cereal rye cover crop in no till on the seedling disease complex in corn

Jyotsna Acharya, Matthew G. Bakker, Thomas B. Moorman, Thomas C. Kaspar, Andrew W. Lenssen & Alison E. Robertson

To cite this article: Jyotsna Acharya, Matthew G. Bakker, Thomas B. Moorman, Thomas C. Kaspar, Andrew W. Lenssen & Alison E. Robertson (2018) Effects of fungicide seed treatments and a winter cereal rye cover crop in no till on the seedling disease complex in corn, Canadian Journal of Plant Pathology, 40:4, 481-497, DOI: 10.1080/07060661.2018.1506503

To link to this article: https://doi.org/10.1080/07060661.2018.1506503

View supplementary material

Accepted author version posted online: 14 Aug 2018.
Published online: 07 Sep 2018.

Submit your article to this journal

Article views: 91

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=tcjp20

Can. J. Plant Pathol., 2018
Vol. 40, No. 4, 481–497, https://doi.org/10.1080/07060661.2018.1506503

Taylor & Francis
Taylor & Francis Group
Check for updates

Disease control/Moyens de lutte

Effects of fungicide seed treatments and a winter cereal rye cover crop in no till on the seedling disease complex in corn

JYOTSNA ACHARYA[1], MATTHEW G. BAKKER [2*], THOMAS B. MOORMAN[2], THOMAS C. KASPAR[2], ANDREW W. LENSSEN[3] AND ALISON E. ROBERTSON[1]

[1]*Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011, USA*
[2]*National Laboratory for Agriculture and the Environment, USDA ARS, Ames, IA 50011, USA*
[3]*Department of Agronomy, Iowa State University, Ames, IA 50011, USA*

(Accepted 26 July 2018)

Abstract: Corn seed is treated with various fungicide active ingredients that target several fungal and oomycete genera in the corn seedling disease complex. In this study, we investigated the effects of various combinations of fungicides as seed treatments on disease development, pathogen prevalence and seedling growth of corn planted after a winter cereal rye cover crop or a winter fallow. Under controlled environment conditions, corn seed was treated with a combination of metalaxyl, pyraclostrobin, fludioxonil, ipconazole and sedaxane to reduce infection by *Pythium*, *Fusarium* and *Rhizoctonia solani* (treatment ALL); metalaxyl alone to reduce *Pythium* (P); metalaxyl and pyraclostrobin to reduce *Fusarium* and *Pythium* (FP); pyraclostrobin, fludioxonil, ipconazole and sedaxane to reduce *Fusarium* and *R. solani* (FR); and non-treated seed control (NT). Complementary field trials compared the ALL seed treatment against NT, with and without winter cereal rye. Under cold, wet environmental conditions, winter cereal rye negatively affected corn seedling growth and increased seedling root disease and *Pythium* incidence. In general, seed treatments improved seed germination and seedling growth ($P < 0.05$) in controlled environment conditions. Seed treatments active against *Pythium* spp. (i.e. P, FP and ALL) reduced radicle disease index scores ($P < 0.01$) compared with FR and NT. *Pythium* spp. were recovered less frequently from corn seedlings planted after winter cereal rye when treated with P, FP and ALL compared with FR and NT. No seed treatment effect was detected on the recovery of *Fusarium* spp. In the field, no effect of a fungicide seed treatment was detected on corn seedling growth or root disease incidence following winter cereal rye. Mesocotyl disease incidence, however, was reduced when using a fungicide treatment in 2014. These data suggest that *Pythium* species play a major role in the corn seedling disease complex following a winter cereal rye cover crop. Consequently, disease management in corn systems that include a winter cereal rye cover crop should explicitly consider *Pythium* spp.

Keywords: fungicide, *Fusarium*, *Pythium*, rye cover crop, seedling disease, seed treatment

Résumé: La semence de maïs est traitée avec diverses substances actives de fongicides qui ciblent plusieurs genres de champignons et d'oomycètes du complexe des maladies des semis du maïs. Dans cette étude, nous examinons les effets de différentes combinaisons de fongicides comme traitements des semences sur le développement des maladies, la prévalence des agents pathogènes et la croissance des semis de maïs plantés à la suite d'une culture-abri de seigle d'hiver ou d'une jachère hivernale. Dans des conditions contrôlées, la semence de maïs a été traitée avec une combinaison de métalaxyl, de pyraclostrobine, de fludioxonil, d'ipconazole et de sédaxane afin de réduire l'infection causée par *Pythium* et *Fusarium* ainsi que par *Rhizoctonia solani* (traitement ALL); avec le métalaxyl seul pour réduire celle causée par *Pythium* (P); avec le métalaxyl et la pyraclostrobine pour réduire celle causée par *Pythium* et *Fusarium* (FP); avec la pyraclostrobine, le fludioxonil, l'ipconazole et le sédaxane pour réduire celle causée par *Fusarium* et *R. solani* (FR); de plus, de la semence non traitée a été utilisée comme témoin (NT). Des essais complémentaires effectués au champ ont permis de comparer le traitement ALL au traitement NT, avec et sans culture-abri de seigle d'hiver. Par temps froid et humide, la culture-abri de seigle d'hiver a nui à la croissance des semis et a

Correspondence to: Alison E. Robertson E-mail: alisonr@iastate.edu

*Present affiliation: Mycotoxin Prevention and Applied Microbiology, USDA ARS, Peoria, IA

The work was authored as part of the authors' official duties as employees of the United States Government and is therefore a work of the United States Government. In accordance with 17 U.S.C. 105 no copyright protection is available for such works under U.S. law.

Published online 07 Sep 2018

provoqué chez ces derniers une incidence accrue des maladies racinaires ainsi que de *Pythium*. En règle générale, dans les conditions contrôlées, les traitements de semences ont amélioré la germination et la croissance des semis ($P < 0.05$). Les traitements efficaces contre *Pythium* spp. (c.-à-d. P, FP et ALL) ont permis de réduire les résultats de l'indice de maladie des radicules ($P < 0.01$), comparativement à FR et NT. *Pythium* spp. ont été collectés moins souvent sur les semis de maïs plantés à la suite d'une culture-abri de seigle d'hiver lorsque la semence avait été traitée avec P, FP et ALL, comparativement à FR et NT. Aucun effet du traitement n'a été détecté quant à la collecte de *Fusarium* spp. Au champ, aucun effet du traitement des semences avec fongicide n'a été décelé sur la croissance des semis de maïs ou sur l'incidence des maladies racinaires à la suite d'une culture-abri de seigle d'hiver. Toutefois, l'incidence de la maladie sur les mésocotyles a été réduite lorsqu'un fongicide a été appliqué en 2014. Ces données suggèrent que les espèces de *Pythium* jouent un rôle important dans l'ensemble des maladies des semis du maïs à la suite d'une culture-abri de seigle d'hiver. En conséquence, la gestion des maladies du maïs, qui inclut une culture-abri de seigle d'hiver, devrait explicitement tenir compte de *Pythium* spp.

Mots clés: culture-abri de seigle, fongicide, *Fusarium*, maladie des semis, *Pythium*, traitement des semences

Introduction

Corn (*Zea mays* L.) is susceptible to infection by numerous pathogens, many of which survive in previous crop residue or are endemic to soil (Munkvold & White, 2016). Over 152.4 million metric tons of corn grain worth approximately US $27 billion were lost due to corn diseases during 2012–2015 (Mueller et al., 2016). Of this, almost 10.2 million metric tons of corn was lost to seedling diseases.

Symptoms of corn seedling diseases include leaf yellowing and necrosis, plant wilting, mesocotyl rot and root rot, which may result in plant death, delayed emergence or reduced plant growth. Corn yield is dependent on high populations and uniform crop stands (Nafziger et al., 1991). When plants are missing and populations are reduced, yield is often decreased. Similarly, if plant vigour is reduced, smaller plants produce smaller or no ears because they are outcompeted by neighbouring healthy plants for water, nutrients and sunlight (Nafziger et al., 1991).

Fusarium, Pythium and *Rhizoctonia* species are the prominent pathogens associated with seedling diseases of corn (Munkvold & White, 2016). Often, multiple genera and species are recovered from the same diseased seedling, suggesting that these pathogens form multi-species complexes, as well as complexes with other soil-borne organisms such as nematodes (Back et al., 2002). For example, Broders et al. (2007a, 2007b) recovered nine species of *Pythium* and eight species of *Fusarium* from diseased corn seedlings in Ohio. Similarly, Matthiesen et al. (2016) recovered nine species of *Pythium* from diseased corn seedlings in Iowa and in a few cases recovered multiple species from individual plants. The presence of multiple species and genera capable of infecting corn seedlings within a field undoubtedly complicates development of effective disease management strategies.

The presence of multiple pathogenic taxa also complicates the use of fungicides as a disease management strategy. Matthiesen et al. (2016) and Broders et al. (2007a, b) determined that different species of *Pythium* and *Fusarium* showed variable sensitivity to numerous fungicides. Therefore, although corn seeds have been treated with fungicide for over 50 years to protect germinating seeds and seedlings from infection by soil-borne pathogens (Cohen & Coffey, 1986), fungicide seed treatments may not be optimally targeted towards the correct mix of pathogenic taxa. In response to this and other pest problems, seed treatments today may contain up to four fungicides, and include an insecticide, a nematicide and/or biological agents. The multiple fungicides used to treat seeds belong to several chemical groups and have varying degrees of effectiveness against the individual pathogens within the pathogen complex that can cause corn seedling disease. Nonetheless, fungicide seed treatments often have a limited zone of activity around the seed, persist for a limited amount of time, and may have reduced effectiveness against novel pathogen complexes resulting from changes in management practices or soil environmental conditions.

Cover crops in corn and soybean [*Glycine max* (L.) Merr.] rotations are an expanding practice in the upper Midwest of the USA. Cover crops are grown primarily between periods of regular crop production for the purposes of protecting and improving soil by reducing soil erosion, improving water infiltration and mitigating nutrient loading of surface waters (Kaspar et al., 2001; Kaspar & Singer, 2011). In Iowa, introducing winter cover crops into corn and soybean production is being encouraged because of its potential to reduce nitrate leaching into water and sustain the productive capacity of agricultural land (Kaspar & Singer, 2011; Kladivko et al., 2014). It was estimated that in the autumn of 2015, Iowa farmers planted cover crops on more than 239.5 thousand ha (~2.6% of the total corn and soybean ha in 2015)

(Runquist & Carlson, 2017). To achieve an estimated 42% reduction in nitrate loss as detailed in one strategy of the Iowa Nutrient Reduction Strategy (IDALS (Iowa Department of Agriculture and Land Stewardship), IDNR (Iowa Department of Natural Resources), ISU (Iowa State University), 2016), cover crop use will need to increase to 5.1 million ha of cover crops (60% of corn and soybean ha).

Winter cereal rye (*Secale cereale* L.) is the most commonly used cover crop species in Iowa because it survives the Midwest winter and produces a substantial amount of biomass before planting of the next cash crop in spring (Snapp et al., 2005). However, occasionally a winter cereal rye cover crop may negatively affect corn yield (Kaspar & Bakker, 2015; Acharya et al., 2017) and consequently many farmers are reluctant to incorporate cover crops into their production systems. This occasional yield loss of corn following a winter cereal rye cover crop has been attributed to several biotic and abiotic factors, including reduced inorganic nitrogen and water availability due to cover crop uptake, inhibitory allelochemicals from living or dying winter cereal rye plants, immobilization of nitrogen during decomposition of the cover crop, poor plant performance associated with dense plant residue and near-surface roots, and increased pest pressure (Mitchell & Tell, 1977; Ebelhar et al., 1984; Wagger & Mengel, 1988; Karlen & Doran, 1991; Tollenaar et al., 1993; Kessavalou & Walters, 1997; Duiker & Curran, 2005).

Recently, Bakker et al. (2016) demonstrated that the dying roots of winter cereal rye cover crops harbour high populations of corn seedling pathogens. Moreover, Acharya et al. (2017) showed that corn following a winter cereal rye cover crop had greater seedling disease incidence than corn following a winter fallow, and that lower corn yields in one year of the study were partly explained by reduced stands and poor plant vigour caused by soil-borne pathogens. In their study, increased levels of members of *Pythium* Clade B were recovered from corn seedlings grown after winter cereal rye while no effect of winter cereal rye on *Fusarium* species was detected (Acharya et al., 2017). Moreover, Schenck et al. (2017) reported that a commercial fungicide seed treatment with activity against both oomycetes and fungi had limited efficacy against radicle rot on corn grown following a winter cereal rye cover crop, but did protect against mesocotyl rot in a controlled environment. Schenck et al. (2017) also reported that Clade B *Pythium* populations were greater in radicle and mesocotyl tissues of corn seedlings following winter cereal rye, compared with those following a winter fallow or hairy vetch (*Vicia villosa* Roth) and canola (*Brassica napus* L.) cover crops.

The main objective of this study was to identify the pathogens most likely to be involved in corn seedling disease development after cover cropping with winter cereal rye. Knowing the genera that are prevalent for seedling diseases in corn following winter cereal rye cover crop could help in improved management practices such as seed treatment targeted to specific pathogens. We also compared the effect of various fungicide active ingredients when applied to corn seed planted after a winter cereal rye cover crop or after winter fallow on seedling disease development, pathogens associated with seedlings roots and mesocotyls, and growth and yield of corn. We hypothesized that the different fungicide active ingredients would selectively inhibit the various candidate pathogen taxa and this would enable us to further identify the most important genus or genera contributing to stand loss and reduced seedling vigour in corn planted after a winter cereal rye cover crop or a winter fallow.

Materials and methods

Corn seedling growth under controlled environment conditions

Corn seedling growth in the absence of (fallow), or following a winter cereal rye cover crop, and treated with various seed applied fungicides was evaluated in a controlled environment experiment. A factorial treatment arrangement (2 × 5) was used, where factor 1 was presence or absence of the cover crop, and factor 2 was one of four seed treatment combinations consisting of different combinations of fungicides (Table 1) with activity against different genera of seedling pathogens and a no fungicide control.

Briefly, following the methods of Acharya et al. (2017), field soil (Webster silty clay loam; fine loamy, mixed, superactive, mesic Typic Endoaquolls) was collected from a continuously cropped corn field in the autumn of 2013, sieved through 4 mm and 2 mm sieves and mixed with finely chopped corn residue in a ratio of 40:1, respectively. The non-sterilized ground corn residue was added to the soil to improve drainage and increase inoculum levels. Polystyrene cups (0.5 L) with drainage-holes were filled with the field soil and 6–8 winter rye seeds 'Elbon' were planted in each cup. Cups without rye (winter fallow) were included as a check. Hereafter, winter cereal rye will be referred to as rye and winter fallow as fallow.

Rye and fallow cups were placed in a growth chamber set at 20°C/16 h light and 10°C/8 h dark for ~4 weeks. All cups, including the winter fallow cups, were

Table 1. Description of the fungicide seed treatments applied to corn seeds to evaluate corn seedling growth and seedling diseases following winter cereal rye cover crops in the controlled environment study and field study in Iowa in 2014 and 2015.

Seed treatment	Active ingredient[a]	Commercial name	Manufacturer	Rate (mL kg^{-1})	Active against
ALL[b]	Metalaxyl	Allegiance	Bayer CropScience	0.29	*Pythium* spp.
	Pyraclostrobin	Stamina	BASF	0.47	*Fusarium* spp.
	Fludioxonil	Maxim 4FS	Sygenta	95	*F. graminearum*
	Ipconazole	Rancona	Valent	0.03	*F. solani*
	Sedaxane	Vibrance 500	Sygenta	189	*Rhizoctonia* spp.
P	Metalaxyl	Allegiance	Bayer CropScience	0.29	*Pythium* spp.
FP	Metalaxyl	Allegiance	Bayer CropScience	0.29	*Pythium* spp.
	Pyraclostrobin	Stamina	BASF	0.47	*Fusarium* spp.
FR	Pyraclostrobin	Stamina	BASF	0.47	*Fusarium* spp.
	Fludioxonil	Maxim 4FS	Sygenta	95	*F. graminearum*
	Ipconazole	Rancona	Valent	0.03	*F. solani*
	Sedaxane	Vibrance 500	Sygenta	189	*Rhizoctonia* spp
NT[b]	None	None	None	None	None

[a] Active ingredients applied to corn seed treatment target specific soil-borne pathogens, such as metalaxyl = *Pythium* spp., pyraclostrobin = *Fusarium* spp., fludioxonil = *F. graminearum*, ipconazole = *F. solani* and *F. oxysporum*, and sedaxane = *Rhizoctonia* spp.
[b] Indicates these seed treatments were included in the field experiment.

sprayed with glyphosate [(*N*-(phosphonomethyl) glycine; 6.6 g a.i L^{-1}] using a hand-held spray bottle at a rate equivalent to ~1.26 kg a.i. ha^{-1}, 30–32 days after rye planting to terminate rye plants.

Corn seeds (Hybrid P1151R, DuPont-Pioneer Hybrid International, Johnston, IA) were treated with five different combinations of fungicides (Table 1) with activity against different genera of seedling pathogens. Each treatment was applied as a water-based slurry to 50 g of corn seed (7.7 mL slurry kg^{-1} seed) following the company's recommended rate for corn. To monitor seed coverage, red seed colourant (Becker Underwood, 0.22 mL kg^{-1} seed) was added to all treatments, including the non-treated control. For each treatment, the fungicide slurry and seeds were weighed using an analytical scale, poured into a plastic bag and sealed, and hand shaken for 5 min until the fungicide seed treatment was evenly distributed on the corn seed coat. Treated corn seeds were air dried and then stored at 4°C prior to use.

Corn seeds (5 per cup) were planted 3 days after rye termination. Similarly, 5 seeds were planted in each of the fallow cups 3 days after glyphosate application. Each cup was considered an experimental unit and the 5 seeds within cups were subsamples. The cups were arranged in a randomized complete block design within the controlled environment chamber. The controlled environment experiment was conducted twice (Run 1 and 2). Environmental conditions in the growth chamber were set to be conducive for seedling disease development (12°C/16 h light and 10°C/8 h dark for 32 days). Seven days after rye termination, the dead rye plants were cut at the soil surface and removed from the cups. Cups were watered (50–60 mL) every 2 to 3 days, and 80 mL of nutrient solution (Miracle-Gro, Scotts Miracle-Gro Products, Marysville, OH; 1.85 g L^{-1} water) was applied once a week.

After 32 days, whole corn seedlings were removed from the cups and soil was carefully washed from the roots. For each cup or seedling, the following data were recorded: number of seedlings emerged, shoot height, shoot dry weight, radicle length, disease incidence and disease severity (only Run 2). In both Runs 1 and 2, disease incidence (DI) was calculated as the percentage of the five seedlings within each cup with visible rot or disease symptoms on the radicle. In Run 2, in an effort to improve the detection of seed treatment effects on disease development, the severity of disease symptoms was also determined for each seedling by visually estimating the area of each radicle covered with lesions and scoring on a 1 to 5 scale, where 1 = 1–10% of roots covered with lesions; 2, 3, 4 and 5 = 11–25%, 26–50%, 51–75% and 76–100% of roots with lesions, respectively (Acharya et al., 2017). Healthy plants with no visible lesions were assigned a value of 0.

Corn seedling growth in field experiment

A field experiment testing corn growth following a rye cover crop and corn fungicide seed treatments was conducted over two years at a field site near Boone, IA, which had a history of corn-soybean rotation and no-tillage. The soils at the field site are mapped as Nicollet clay loam (fine loamy, mixed, superactive, mesic Aquic Hapludolls) and Clarion loam (fine loamy, mixed,

superactive, mesic Typic Hapludolls) (Andrews & Diderikson, 1981). A factorial treatment arrangement (2 × 2) with four treatments testing the effects of presence of a rye cover crop by fungicide seed treatment was used. The rye cover crop presence treatments were corn planted following a rye cover crop (rye) and corn planted following no cover crop (fallow). The two fungicide corn seed treatments consisted of no fungicide seed treatment applied to the seed (NT) and a combination of all fungicides (ALL) that was used in the growth chamber experiment (Table 1). The four treatments included: (i) Winter fallow with non-treated corn seed (fallow × NT), (ii) Winter fallow with treated corn seed (fallow × ALL), (iii) Winter rye cover crop with non-treated corn seed (rye × NT), and (iv) Winter rye cover crop with treated corn seed (rye × ALL). Twenty-four plots, 18.3 m long and 3.8 m wide, with five rows 0.76 m apart, were arranged in a randomized complete block design with six replications.

In the autumn of 2013 and 2014, a rye cover crop was established by broadcasting seeds (variety 'Elbon') following soybean harvest in the autumn (19 Oct. 2013 and 21 Sept. 2014) at a rate of 3.1×10^6 seeds ha^{-1}. Seeds were shallowly incorporated with a rolling stalk chopper (Buffalo Manufacturing Co., Columbus, NE). The following spring (14 May 2014 and 28 April 2015), rye was killed by spray application of glyphosate (1.35 kg a.i. in 150 L ha^{-1}). Fallow plots were also sprayed with glyphosate to manage emerged weeds and for consistency of exposure to glyphosate. After rye shoots were dead, they were collected from two arbitrary locations in each plot by clipping plants close to the soil surface inside a rectangular frame (0.76 m wide by 0.50 m long), dried at 60°C in a forced-air oven, and weighed to determine cover crop shoot biomass. Combined subsamples were finely ground and analysed for N concentration using the dry combustion-gas chromatograph method (Schepers et al., 1989) with an EA1112 Flash NC Elemental analyser (Thermo Electron Corp., Waltham, MA). Nitrogen contents were calculated by multiplying cover crop shoot biomass on an area basis by tissue N concentration.

The glyphosate resistant corn hybrid P1151R (DuPont-Pioneer Hybrid International, Johnston, IA) treated either with a combination of all fungicides (ALL; as described previously) or non-treated (NT), was planted at a rate of 88 960 seeds ha^{-1} in 0.76 m inter-row spacing using a 5-row no-till planter on 17 May 2014 and 1 May 2015, which was 3 days after herbicide application for rye termination. Application of fertilizer before planting or during planting was avoided to preclude potential effects on soil pathogens, especially in or near the seed furrow. A post-planting side dress application of N fertilizer as liquid urea-ammonium nitrate was applied next to the corn rows at a rate of 201 kg ha^{-1} on 3 June in 2014 and on 2 June in 2015.

Whole corn seedlings were sampled by extracting a soil volume of ~0.15 m^3 directly below the plant with a spade to preserve a large portion of the root system. Intact shoots and roots were then separated from the soil by soaking and agitating the plants and soil in water. In 2014, corn seedlings were sampled on 16–17 June, at stage V4 to V5. Because the root systems at this growth stage were larger than expected, it was difficult to remove the seedlings from the soil either during the initial extraction or the washing without breaking off roots. As a result, sampling in the second year was performed at growth stage V2 to V3 (1–2 June 2015). Six corn seedlings, three each from row 2 and row 4 of each plot were arbitrarily sampled and assessed for plant growth and root disease. Data were recorded for shoot height, radicle length, shoot dry weight, root disease incidence, and root rot severity (in 2015). Disease was assessed visually on all root types and mesocotyl tissue. These same plant samples were used for pathogen isolations and for assessment of pathogen density in roots and mesocotyls, as described below.

Monthly average air temperature and precipitation data were obtained from a weather station located near the experimental site (Iowa Environmental Mesonet, 2015). In 2015, thermocouples (copper-constantan) attached to a data logger were installed at a 5-cm depth in one plot of each of the rye and fallow treatments to obtain soil temperatures.

Agronomic assessments

The number of plants, ears and barren plants were counted within the entire centre row of each plot on 9 October 2014 and 21 September 2015. Corn yield data were collected from the centre three rows of each five-row plot, using a modified combine (Colvin1990) on 30 October 2014 and 19 October 2015. Grain moisture was recorded and grain yield adjusted to 0.155 g water kg^{-1}.

Isolation of pathogens from corn seedlings

For both the controlled environment and field trials, corn roots were washed with tap water and rinsed with sterile distilled water. Root tissue samples of 6 mm length were cut from either the root tip or zone of elongation from the radicles. If a lesion was present, the tissue sample included the leading edge of the lesion; if no lesion was present, an arbitrary sample was taken from the root tip or

the zone of elongation. Root samples were cut in half and pressed into the surface of each of corn meal agar and water agar plates. Corn meal agar medium was amended with pimaricin (5 $\mu g\,mL^{-1}$), ampicillin (250 $\mu g\,mL^{-1}$), rifampicin (10 $\mu g\,mL^{-1}$), pentachloronitrobenzene (50 $\mu g\,mL^{-1}$) and benomyl (10 $\mu g\,mL^{-1}$) (PARP+ B) to isolate oomycetes such as *Pythium* spp. (Matthiesen et al., 2016). Similarly, water agar medium was amended with streptomycin (300 $\mu g\,mL^{-1}$) and metalaxyl (96.6%, 14.2 $\mu g\,mL^{-1}$), to isolate *Fusarium* spp. and *R. solani* (Windham & Lucas, 1987; Vincelli & Beaupré, 1989; Bakker et al., 2016). Plates were incubated at 22–23°C for 2–3 days in the dark.

Identification of pathogens from corn seedlings

Isolates with coenocytic hyphae on PARP+ B were transferred to 4% V8 juice medium (DV8) containing neomycin sulphate (50 $\mu g\,mL^{-1}$) and chloramphenicol (10 $\mu g\,mL^{-1}$), while colonies on water agar were transferred to potato dextrose agar (PDA). Putative pathogen isolates were identified morphologically to genus using taxonomic keys (Middleton, 1943; Waterhouse, 1967; Van Der Plaats-Niterink, 1981; Leslie & Summerell, 2006). In 2015, marker gene sequencing was used to improve the resolution of identification of *Pythium* and *Fusarium* spp. Isolates of *Pythium* and *Fusarium* spp. were grown in potato dextrose broth (PDB) for 2–3 days, mycelium was washed with sterile water and freeze-dried, and tissue was pulverized by beating with a tungsten-carbide bead (3 mm diameter; Qiagen) on a Mini Bead beater (Biospec Products). DNA was extracted using the DNeasy Plant Mini Kit (Qiagen), according to the manufacturer's directions.

For *Pythium* spp., the cytochrome C oxidase subunit I (COI) gene was amplified as a useful marker for distinguishing among species (Robideau et al., 2011). For *Fusarium* spp., the translation elongation factor 1-α (TEF 1-α) gene was amplified (Geiser et al., 2004). After amplification, PCR products were cleaned using either a spin column or magnetic bead clean-up method, and were submitted for Sanger sequencing at the Iowa State University DNA Facility. Sequence chromatographs were manually trimmed using FinchTV (Geospiza). *Pythium* spp. COI gene sequences were compared with those provided by Robideau et al. (2011). *Fusarium* spp. TEF 1-α gene sequences were matched against entries in the *Fusarium*-ID database (Geiser et al., 2004).

Pathogen density in field-grown corn seedlings

The density of *Pythium* spp. present in corn radicle and mesocotyl tissues was measured using quantitative PCR (qPCR) (Acharya et al., 2017). Clade B and Clade F *Pythium* spp. were targeted, as prior work has indicated that members of these clades constituted the majority of *Pythium* spp. associated with corn seedling disease in Iowa (Matthiesen et al., 2016; Acharya et al., 2017; Bakker et al., 2017). The procedure for qPCR was as described by Acharya et al. (2017).

Standard curves were prepared from synthesized DNA (Invitrogen GeneArt Strings, Invitrogen) and spanned 6 orders of magnitude in copy number. Across qPCR runs, the standard curves always produced an $r^2 > 0.99$, and calculated PCR efficiencies were in the range of 93–106%. Three technical replicates were run for all samples and standards. Each qPCR run included no-template controls. Quantitative PCR readings below the detection limit were assigned a small non-zero value, defined as half of the calculated DNA content at a cycle threshold of 40. In order to account for the possibility of variable DNA extraction efficient among samples, which could confound estimates of absolute pathogen abundance, pathogen DNA content in corn tissues was expressed relative to corn DNA content (copies of *Pythium* ITS gene per million copies of corn tua4 gene). Resulting ratios were log-transformed prior to analysis in order to meet homogeneity of variance assumptions. In corn tissue designated for qPCR analysis, an error during sample processing left only one subsample per plot for radicles (vs. 2 subsamples for mesocotyls).

Data processing and statistical analyses

Values were averaged across subsamples originating from the same plot. Disease incidence (DI) was determined based on the percentage of seedlings in each cup or plot with visible lesions, or from which isolates of a given genus (*Fusarium, Pythium* or *Rhizoctonia*) were recovered. Disease incidence (DI) and disease severity (DS) were combined into a single disease index (DX), using the formula DX = DI × DS/5 (Li et al., 2013; Kandel et al., 2015).

Analysis of variance was performed on data for all measured parameters using PROC GLIMMIX in SAS version 9.3 (SAS Institute Inc. Cary, NC). Data from runs within the controlled environment chamber experiments and years within the field experiment were analysed separately because of differences observed in experimental variability, disease incidence levels, and treatment effects between runs and years. For both experiments, cover crop treatment, seed treatment and their interaction were considered fixed factors and replication was considered as a random factor. If a main effect was significant ($P < 0.05$), then treatment means were

compared using Fisher's LSD at P = 0.05. In some cases, for consistency between runs, years, or among variables, means of all treatment combinations were also analysed and compared as individual treatments even if the main effects interaction was not significant.

Results

Effect of rye cover crop and fungicide seed treatment on corn seedling growth under controlled environment conditions

Corn seedling growth-development. The main effects of cover crop presence or seed treatments were significant for almost all of the measured parameters for corn seedling growth (seedling emergence, shoot height, radicle length and shoot dry weight) in both runs (Table 2). Interactions between cover crop and seed treatments were not significant for any corn seedling growth measurements. Although the interaction was not significant, to be consistent with our presentation of data for root disease and infection, we also analysed and presented the data as 10 individual treatments.

Seedling emergence was improved by the fungicide seed treatments when corn followed a rye cover crop (Table 2). Following rye, the ALL, P and FP treatments had greater emergence than NT in both runs. Following fallow, seed treatments had less effect and only the ALL treatment improved emergence in both runs.

The effect of fungicide treatments was significant on shoot height for the treatment combinations following rye in both runs (Table 2). In Run 1 of the experiment, seedlings treated with fungicide treatments ALL, P and FP were taller compared with those treated with FR and NT. In Run 2, ALL, P, FP and FR treatments had taller corn seedlings than NT. In contrast, fungicide seed treatments showed no effect on shoot height following fallow except for FR having taller plants than NT in Run 2.

The effect of fungicide seed treatment was not consistent between runs for corn seedling shoot dry weight (Table 2). In general, fungicide seed treatments ALL, P and FP showed greater corn seedlings shoot dry weight than NT when corn followed rye in both runs. Corn shoot dry weight of five plants ranged from 0.1 to 0.3 g following rye. However, seedling shoot dry weight of five plants was greater and ranged from 0.3 to 0.9 g when corn

Table 2. Main effects of fungicide seed treatments following winter cereal rye cover crop or fallow on corn seedling growth in a controlled environment[a].

	Run 1				Run 2			
Treatments[b]	Emergence (%)	Shoot height (cm)[c]	Shoot dry weight (g)	Radicle length (cm)	Emergence (%)	Shoot height (cm)	Shoot dry weight (g)	Radicle length (cm)
Rye, ALL	95.8^{ad}	11.4^{abc}	0.3^{b}	1.9^{b}	90.0^{b}	11.4^{c}	0.3^{d}	1.2^{c}
Rye, P	87.5^{ac}	10.7^{cd}	0.2^{bc}	2.9^{b}	90.0^{ab}	12.2^{c}	0.3^{de}	2.7^{c}
Rye, FP	79.2^{ab}	11.0^{bc}	0.2^{b}	3.0^{b}	76.7^{bc}	11.8^{c}	0.3^{d}	1.7^{c}
Rye, FR	50.0^{c}	8.0^{e}	0.1^{d}	0.8^{b}	63.3^{c}	11.5^{c}	0.3^{de}	1.2^{c}
Rye, NT	55.0^{c}	8.3^{de}	0.1^{cd}	0.7^{b}	43.3^{d}	7.6^{d}	0.1^{e}	0.7^{c}
Fallow, ALL	100.0^{a}	13.7^{a}	0.4^{a}	13.2^{a}	100.0^{a}	17.9^{ab}	0.9^{b}	11.7^{a}
Fallow, P	87.5^{ab}	13.4^{ab}	0.4^{a}	12.3^{a}	93.3^{ab}	17.1^{b}	0.9^{b}	11.1^{a}
Fallow, FP	87.5^{ab}	11.5^{abc}	0.3^{b}	10.4^{a}	90.0^{ab}	19.4^{ab}	0.9^{b}	10.6^{ab}
Fallow, FR	95.8^{a}	13.3^{ab}	0.4^{a}	12.8^{a}	93.3^{ab}	20.8^{a}	1.2^{a}	11.9^{a}
Fallow, NT	70.8^{bc}	12.7^{abc}	0.3^{ab}	10.6^{a}	80.0^{bc}	16.6^{b}	0.6^{c}	9.6^{b}
P value	0.0002	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Main effects and interaction effect								
P value (CC)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
P value (ST)	<0.01	<0.01	0.05	<0.01	<0.01	<0.01	0.11	<0.01
P value (CC × ST))	0.14	0.72	0.88	0.96	0.15	0.25	0.41	0.16

[a]Growth chamber was set at 20°C with 16 h of light and 10°C with 8 h of darkness for growing winter cereal rye and 12°C with 16 h of light and 10°C with 8 h of darkness for growing corn.

[b]Seed treatments applied to corn seed planted in cups following winter cereal rye or no winter cereal rye (fallow): ALL = metalaxyl (Alliance; Bayer CropScience, 0.29 mL kg^{-1}), pyraclostrobin (Stamina; BASF, 0.47 mL kg^{-1}), fludioxonil (Maxim 4FS; Sygenta, 95 mL kg^{-1}), ipconazole (Rancona; Valent, 0.03 mL kg^{-1}) and sedaxane (Vibrance 500; Sygenta, 189 mL kg^{-1}); P = metalaxyl alone; FP = metalaxyl and pyraclostrobin; FR = pyraclostrobin, fludioxonil, ipconazole and sedaxane; and NT = non-treated seed control.

[c]Corn shoot height was measured from ground level to the extended leaf.

[d]Means followed by the same letter within a column and run are not significantly different at P value 0.05 using Fisher's protected least significant difference.

followed fallow. Fungicide seed treatments following fallow had no significant effect in Run 1 and a positive effect in Run 2. Fungicide seed treatments showed no effect on radicle length of corn seedlings for all treatment combinations when corn followed rye in both runs (Table 2). Radicle length was ~10 times smaller following rye than following fallow. Fungicides seed treatments following fallow had no significant effect in Run 1 and a positive effect in Run 2.

Corn root disease and infection. There was a significant interaction of cover crop presence and seed treatment on corn seedling root disease parameters. As a result, data for radicle root disease and infection are presented as individual treatment combination means rather than as main effect means (Table 3).

No effect of fungicide treatment was detected on the incidence of radicle lesions for all treatment combinations when corn followed rye (Table 3). Dark black or brown lesions near the radicle tip were observed on nearly all plants when corn followed rye, including those treatments that received fungicide seed treatments. In contrast, when corn followed fallow, there were 0–15.3% of the seedlings with lesions in Run 1 and the ALL, P, FP and FR seed treatments had significantly less disease incidence than the NT treatment. Similarly, in Run 2, radicle disease incidence when corn followed fallow ranged from 6.7 to 46.7% and the ALL, P and FP fungicide treatments had significantly lower disease incidence than the FR and NT treatments.

Fungicide seed treatment significantly affected the disease index (determined only in Run 2) when corn followed rye, but not when corn followed fallow (Table 3). Radicle disease index values were 39–97% following rye versus 0.3–6% following fallow. Following rye, the radicle disease index values of corn seedlings grown from seed treated with metalaxyl (ALL, P and FP) were lower compared with those of seed treatments where metalaxyl was not included (FR and NT; Table 3). In Run 2, disease incidence was 100% for all corn radicles regardless of seed treatment, but we noted that disease severity differed among treatments and this prompted us to add disease index as a measurement to Run 2.

Recovery of *Pythium* spp. was significantly affected by fungicides for treatments following rye in Run 1 and for treatments following fallow in Run 2 (P = 0.01; Table 3). In general, *Pythium* incidence was reduced with fungicide

Table 3. Mean radicle disease incidence (RDI), radicle disease index (RDX) and the incidence of *Pythium* and *Fusarium* spp. recovered from corn seedlings at growth stage V2 to V3 for 10 different combination of corn seed treatments and winter cereal rye presence (rye and fallow) in a controlled environment[a].

	Run 1			Run 2			
Treatments[b]	RDI (%)[c]	*Pythium* incidence (%)[d]	*Fusarium* incidence (%)[e]	RDI (%)	RDX (%)[f]	*Pythium* incidence (%)	*Fusarium* incidence (%)
Rye, ALL	100.0[ag]	26.4[b]	4.2[bc]	100.0[a]	39.3[d]	57.5[a]	86.7
Rye, P	100.0[a]	0.0[b]	36.1[ab]	100.0[a]	53.2[c]	40.8[ab]	72.5
Rye, FP	100.0[a]	27.8[b]	11.1[bc]	100.0[a]	45.7[cd]	39.2[a]	84.2
Rye, FR	100.0[a]	68.7[a]	56.3[a]	100.0[a]	70.1[b]	63.9[ab]	83.3
Rye, NT	100.0[a]	59.9[a]	50.0[a]	100.0[a]	97.3[a]	33.3[ab]	55.6
Fallow, ALL	5.0[c]	4.2[b]	10.0[bc]	6.7[c]	0.3[e]	13.3[bc]	80.0
Fallow, P	0.0[c]	0.0[b]	12.5[bc]	10.0[c]	0.4[e]	0.0[c]	47.5
Fallow, FP	0.0[c]	0.0[b]	0.0[c]	16.7[c]	2.3[e]	0.0[c]	55.8
Fallow, FR	5.6[c]	5.6[b]	20.8[abc]	44.2[b]	5.8[e]	14.2[bc]	62.5
Fallow, NT	15.3[b]	22.2[b]	15.3[bc]	46.7[b]	5.3[e]	38.3[ab]	72.2
P value	<0.01	<0.01	0.03	<0.01	0.01	<0.01	0.11

[a]Growth chamber was set at 20°C with 16 h of light and 10°C with 8 h of darkness for growing winter cereal rye and 12°C with 16 h of light and 10°C with 8 h of darkness for growing corn. DI = Disease incidence and DX = Disease index.

[b]Seed treatments applied to corn seed planted in cups following winter cereal rye or no winter cereal rye (fallow): ALL = metalaxyl (Alliance; Bayer CropScience, 0.29 mL kg^{-1}), pyraclostrobin (Stamina; BASF, 0.47 mL kg^{-1}), fludioxonil (Maxim 4FS; Sygenta, 95 mL kg^{-1}), ipconazole (Rancona; Valent, 0.03 mL kg^{-1}), and sedaxane (Vibrance 500; Sygenta, 189 mL kg^{-1}); P = metalaxyl alone; FP = metalaxyl and pyraclostrobin; FR = pyraclostrobin, fludioxonil, ipconazole and sedaxane; and NT = non-treated seed control.

[c]Radicle disease incidence was calculated as the percentage of disease seedlings with lesions on the radicle (N = 6).

[d]*Pythium* incidence was determined as the percentage of plants from which *Pythium* spp. were isolated (N = 6).

[e]*Fusarium* incidence was determined as the percentage of plants from which *Fusarium* spp. were isolated (N = 6).

[f]Radicle disease index was calculated using the formula DX = DI × DS/5. Disease severity (DS) was scored on a 1 to 5 scale where 1 = 1–10% of radicle covered with lesions; 2, 3, 4 and 5 = 11–25%, 26–50%, 51–75% and 76–100% of radicle roots with lesions.

[g]Means followed by the same letter within a column and run are not significantly different at P value 0.05 using Fisher's protected least significant difference.

treatments ALL, P and FP, which all contained metalaxyl (Table 3). In Run 1 when corn followed rye, *Pythium* incidence ranged from 0 to 69% and the ALL, P and FP fungicide treatments had lower *Pythium* incidence than the FR and NT treatments. When corn followed fallow in Run 1, *Pythium* incidence only ranged from 0 to 22% and none of the fungicide seed treatments were significantly different from each other. In Run 2, when corn followed rye, *Pythium* incidence was higher than Run 1, ranging from 33 to 64%, and there were no significant differences among seed treatments. When corn followed fallow in Run 2, *Pythium* incidence ranged from 0 to 38% and the P and FP seed treatments had lower *Pythium* incidence than the no fungicide (NT) seed treatment.

Fusarium incidence results were generally less consistent than those for *Pythium* incidence. In Run 1, when corn followed rye, *Fusarium* incidence ranged from 4 to 56% and the ALL and FP seed treatments had lower *Fusarium* incidence than the FR and NT treatments (Table 3). Both the ALL and FP treatments contain pyraclostrobin, which has activity against *Fusarium* spp., but the FR treatment also containing pyraclostrobin had no effect. When corn followed fallow in Run 1, *Fusarium* incidence ranged from 0 to 20.8% and there were no significant differences among seed treatments. In Run 2, *Fusarium* incidence was quite high for all treatment combinations (55–87%) and there was no significant effect of the treatment combinations on the recovery of *Fusarium* spp. Although results are not shown in Table 3, *Rhizoctonia solani* was not recovered from any seedling roots in this experiment.

Effect of rye cover crop and fungicide seed treatment on corn seedling growth in a field experiment

Weather data. Average air temperature and precipitation differed in each year of the study. In the second year, it was warmer from December 2014 to March 2015 by ~4°C and slightly warmer from March 2015 to May 2015 by 2°C compared with the same periods in the previous year (Table 4). More precipitation occurred from February to April 2014 than from February to April 2015. In 2015, average soil temperature at a 5 cm depth after corn planting was 16.3°C in the rye plot and 17.3°C in the fallow plot between 30 April 2015 and 30 May 2015 (day of year (DOY) 120 through 150; Supplementary Fig. 1). This demonstrates that on average the soil temperature in the rye plots was cooler than the fallow plots.

Rye shoot biomass and corn seedling growth. Rye shoot biomass was ~4 times greater in 2015 (3.45 Mg ha^{-1}) than in 2014 (0.95 Mg ha^{-1}). This may have been due in part to the rye cover crop planted 28 days earlier in the autumn of 2014 than in 2013. Additionally, March and April were considerably warmer in 2015 than in 2014. The average N concentration of rye shoot biomass at cover crop termination was 22.1 g N kg^{-1} in 2014 and 15.4 g N kg^{-1} in 2015. This resulted in rye shoots removing 21 and 53 kg N ha^{-1} from the soil prior to corn planting in 2014 and 2015, respectively.

Table 4. Average monthly air temperature (°C) and total monthly precipitation (mm), as recorded by a weather station 2 km from the experimental site in Iowa.

Months	Average monthly temperature 2013–2014 (°C)	Average monthly temperature 2014–2015 (°C)	Total monthly precipitation 2013–2014 (mm)	Total monthly precipitation 2014–2015 (mm)
October	11.2	11.7	63.6	95.0
November	1.7	−0.9	35.0	25.9
December	−7.8	−1.5	8.6	30.0
January	−8.5	−4.0	4.0	7.3
February	−10.1	−8.9	38.7	20.7
March	0.4	4.6	31.4	6.2
April	9.7	11.6	125.8	88.0
May	16.6	16.7	108.3	114.6
June	21.6	21.5	236.4	175.1
July	20.9	22.6	73.1	151.4
August	22.4	20.9	144.9	208.9
September	17.6	20.9	139.8	128.3

Seedling shoot height, radicle length and shoot dry weight were significantly affected by the presence of a rye cover crop in both years ($P < 0.01$; Table 5). Alternately, fungicide seed treatment had no effect on shoot height and radicle length and only affected shoot dry weight in 2014. Because seedlings were sampled at an earlier leaf stage in 2015 to allow more complete extraction of seedling roots, the corn seedlings in 2015 were smaller than those in 2014. Corn seedlings planted after a rye cover crop had shoots that were 9% shorter in 2014 and 33% shorter in 2015 than shoots of seedlings planted after fallow ($P < 0.01$). Corn radicle length was longer following fallow than a rye cover crop by 19% in 2014 and 44% in 2015. The interaction between cover crop and fungicide treatment was significant for radicle length and shoot dry weight in 2014. In 2014, radicle length was longest in the NT seeds (9.3 cm) compared with the ALL treated seeds (7.7 cm) from plots following rye. However, no difference in radicle length was observed among plants in the fallow treatments with or without a seed treatment. Mean radicle length following fallow was 10.4 cm and 10.0 cm with ALL and NT, respectively. Corn shoot dry weight was greater in both years when following fallow and with treated corn seeds

Table 5. Means for the main effects of cover crop presence and fungicide seed treatment on corn seedling growth, root disease incidence and root disease index of radicle, seminal and mesocotyl from corn seedlings at growth stage V4 to V5 in 2014 and at V2 to V3 in 2015 in field experiments in Iowa.

	Variables[a]	Shoot height (cm)	Radicle length (cm)	Shoot dry weight (g)	Radicle DI (%)[b]	Seminal DI (%)[c]	Mesocotyl DI (%)[d]	Radicle DX (RDX)[e]	Seminal DX (SDX)[f]	Mesocotyl DX (MDX)[g]
2014	Cover crop (CC)									
	Rye	67.3[bh]	8.5[b]	36.2[b]	95.0[a]	95.0[a]	4.8	–	–	–
	Fallow	73.5[a]	10.1[a]	47.1[a]	65.3[b]	63.9[b]	1.4	–	–	–
	P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.15			
	Seed treatment (ST)									
	ALL	71.3	9.6	44.0[a]	77.5	76.4	0.0[b]	–	–	–
	NT	69.6	9.1	39.4[b]	82.8	82.5	6.4[a]	–	–	–
	P value	0.15	0.32	0.03	0.50	0.53	0.01			
	P value (CC × ST)	0.24	0.05	0.02	0.28	0.11	0.12			
2015	Cover crop (CC)									
	Rye	20.6[b]	7.2[b]	1.4[b]	73.6[a]	65.3[a]	6.94	18.9[a]	13.0[a]	0.6
	Fallow	30.9[a]	10.4[a]	5.1[a]	8.3[b]	6.9[b]	2.77	0.4[b]	0.4[b]	0.5
	P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.40	<0.01	<0.01	0.88
	Seed treatment (ST)									
	ALL	26.5	8.9	3.3	44.4	44.4[a]	4.6	9.9	8.5	0.28
	NT	25.0	8.8	3.2	37.5	27.8[b]	5.6	9.4	4.9	0.74
	P value	0.08	0.81	0.54	0.35	0.04	0.78	0.89	0.12	0.46
	P value (CC × ST)	0.73	0.22	0.42	0.35	0.08	0.40	0.98	0.13	0.46

[a]Seed treatments applied to corn seed planted following winter cereal rye or no winter cereal rye (fallow): ALL = metalaxyl (Alliance; Bayer CropScience, 0.29 mL kg^{-1}), pyraclostrobin (Stamina; BASF, 0.47 mL kg^{-1}), fludioxonil (Maxim 4FS; Sygenta, 95 mL kg^{-1}), ipconazole (Rancona; Valent, 0.03 mL kg^{-1}) and sedaxane (Vibrance 500; Sygenta, 189 mL kg^{-1}). NT = Non-treated.

[b]Radicle disease incidence was calculated as the percentage of seedlings with lesions on the radicle (N = 6). DI = Disease incidence and DX = Disease index. Values followed by the same letter within a column and run are not significantly different at P value 0.05 using Fisher's protected least significant difference.

[c]Seminal disease incidence was calculated as the percentage of seedlings with lesions on the seminal roots (N = 6).

[d]Mesocotyl disease incidence was calculated as the percentage of seedlings with lesions on the mesocotyl (N = 6).

[e]RDX was calculated as radicle DX = DI × DS/5. It was calculated in 2015 only.

[f]SDX was calculated as seminal DX = DI × DS/5. It was calculated in 2015 only.

[g]MDX was calculated as mesocotyl DX = DI × DS/5. It was calculated in 2015 only.

[h]Means followed by the same letter within a column and year are not significantly different at P value 0.05 using Fisher's protected least significant difference.

in 2014. In 2014 the cover crop by seed treatment interaction was significant for shoot weight because when separated by cover crop, there was no effect of seed treatment on shoot weight when following rye, but there was an effect following fallow. In 2014 following rye, corn shoot weight was 35.7 g with ALL and 36.7 g with NT. Alternately, following fallow, corn shoot weight was 52.3 g with ALL and 42.1 g with NT. In 2015, corn seedling shoots following rye weighed 28% less than corn shoots following fallow.

Root and mesocotyl disease incidence and indices. Lesions on the radicle were mostly restricted to near the tip or the zone of elongation, while on the seminal roots, lesions were small (<1 mm) and scattered. On average, mesocotyl lesions were present on less than 7% of the plants (Table 5). Because lesions were few and usually very small on the nodal roots of seedlings from all treatments, these results will not be discussed. Cover crop treatment was significant for disease incidence on radicle and seminal roots in both 2014 and 2015 (Table 5), but did not significantly affect mesocotyl disease incidence. The main effect of seed treatment was only significant for mesocotyl disease incidence in 2014 and seminal root disease incidence in 2015. No interaction between use of a cover crop and fungicide seed treatment was observed for any of the measured root type or mesocotyl disease incidences. Unexpectedly, seminal root disease incidence was 60% greater for ALL in 2015 than for NT. In contrast, the incidence of mesocotyl rot in 2014 was zero for ALL versus 6.4% for NT. Following a rye cover crop, radicle and seminal root disease incidences were up to 8 or 9 times greater than fallow in 2015, but only 46% greater than those following fallow in 2014 ($P < 0.01$). Because seedlings were sampled at an earlier leaf stage in 2015, this may indicate that the difference in disease incidence was more pronounced at early growth stages than later. No difference was detected in mesocotyl disease incidence between cover crop treatments in either year.

The main effect of cover crop on the disease indices (taken only in 2015) was significant for lesions on the radicles and seminal roots, but not for the mesocotyls (Table 5). The effect of seed treatment and the interaction of seed treatment by cover crop were not significant for any of the indices. Disease index values for radicle and seminal root rot were more than 32 times greater for seedlings grown after rye compared with those grown after a fallow ($P < 0.01$; Table 5). However, like the disease incidence measurements, seed treatment had no effect on the mesocotyl disease index.

Root rot pathogens

Pythium spp. were recovered more frequently from seedlings planted after a rye cover crop in both years as compared with seedlings grown in the fallow treatment plots (Table 6). However, in 2015, this difference was marginally significant ($P = 0.06$). In either year, seed treatment did not affect the recovery of either *Pythium* or *Fusarium* species from corn seedlings (Table 6), nor did the presence of the preceding cover crop affect recovery of *Fusarium* spp. No *R. solani* isolates were recovered from seedlings planted in the field study.

Table 6. Means for the main effects of the use of a winter cereal rye cover crop presence and the use of fungicide seed treatment on the recovery of *Pythium* and *Fusarium* species from corn seedling radicles in field experiments in Iowa.

	2014		2015	
Variables[a]	*Pythium* incidence (%)[b]	*Fusarium* incidence (%)[c]	*Pythium* incidence (%)	*Fusarium* incidence (%)
Cover crop (CC)				
Rye	53.3[ad]	56.0	22.2[a]	69.4
Fallow	2.8[b]	44.4	8.3[b]	55.6
P value	<0.01	0.24	0.06	0.2
Seed treatment (ST)				
ALL	25.0	51.9	11.1	69.4
NT	31.1	48.6	19.4	55.6
P value	0.52	0.74	0.25	0.2
P value	(CC × ST)	0.95	0.37	0.69
0.79				

[a]Seed treatments applied to corn seed planted following winter cereal rye or no winter cereal rye (fallow): ALL = metalaxyl (Alliance; Bayer CropScience, 0.29 mL kg^{-1}), pyraclostrobin (Stamina; BASF, 0.47 mL kg^{-1}), fludioxonil (Maxim 4FS; Syngenta, 95 mL kg^{-1}), ipconazole (Rancona; Valent, 0.03 mL kg^{-1}) and sedaxane (Vibrance 500; Syngenta, 189 mL kg^{-1}). NT = Non-treated.
[b]*Pythium* incidence was determined as the percentage of radicles from which *Pythium* spp. were isolated.
[c]*Fusarium* incidence was determined as the percentage of radicles from which *Fusarium* spp. were isolated.
[d]Values followed by the same letter within a column and run are not significantly different at P value 0.05 using Fisher's protected least significant difference.

In 2015, 17 isolates of *Pythium* recovered from corn root tissue were identified using marker gene sequence information. From within *Pythium* Clade B, *P. aristosporum/arrhenomanes* (9 isolates) and *P. folliculosum/torulosum* (1 isolate) were recovered. From within *Pythium* Clade F, *P. sylvaticum* (6 isolates) was recovered and from within *Pythium* Clade I, *P. heterothallicum* (1 isolate) was recovered.

Clade B *Pythium* spp. were present at elevated densities (in terms of pathogen gene copies per million copies of a corn gene in DNA extracts) in the radicle and mesocotyl tissues of corn seedlings following a rye cover crop ($P < 0.01$), compared with corn seedlings in the fallow treatment (Fig. 1A). Seed treatment reduced Clade B *Pythium* densities only in the mesocotyls of the fallow treatment, and did not impact densities in the radicle or in the mesocotyl following a rye cover crop.

Clade F *Pythium* spp. were slightly less abundant in radicles from ALL treated seed following fallow, compared with untreated seed after fallow, but did not differ with seed treatment following a rye cover crop (Fig. 1B). For mesocotyl tissue, seed treatment significantly reduced Clade F *Pythium* density in the cover crop treatment, but not in the fallow treatment. Notably, cover crop presence did not result in the same consistent increase in the density of *Pythium* Clade F that was seen for Clade B.

In 2015, marker gene sequence information for 35 *Fusarium* isolates collected from corn root tissue identified five species. The majority of isolates belonged to the *F. oxysporum* species complex (30 isolates). The remaining isolates collected were identified as *F. accuminatum, F. armeniacum, F. graminearum* and *F. solani*. Quantification of *Fusarium* spp. was not attempted because our earlier results showed that *Fusarium* was not responsive to the rye cover crop treatment and in most cases showed uniformly high incidence of infection (Tables 3, 6).

Corn yield and agronomic assessments

There was no interaction between cover crop presence and seed treatment for any measured end-of-season agronomic parameter (Table 7). No effect of cover crop was detected on final plant stand in either year. In 2015, final plant stand was 6% greater when a seed treatment was used ($P < 0.01$). Significant effects of cover crop and seed treatment were detected for number of ears only in 2015. There were 7% more harvestable ears following the fallow treatment compared with the cover crop treatment ($P < 0.01$) and 7% more harvestable ears when a seed treatment was used ($P < 0.01$). In both years, the number

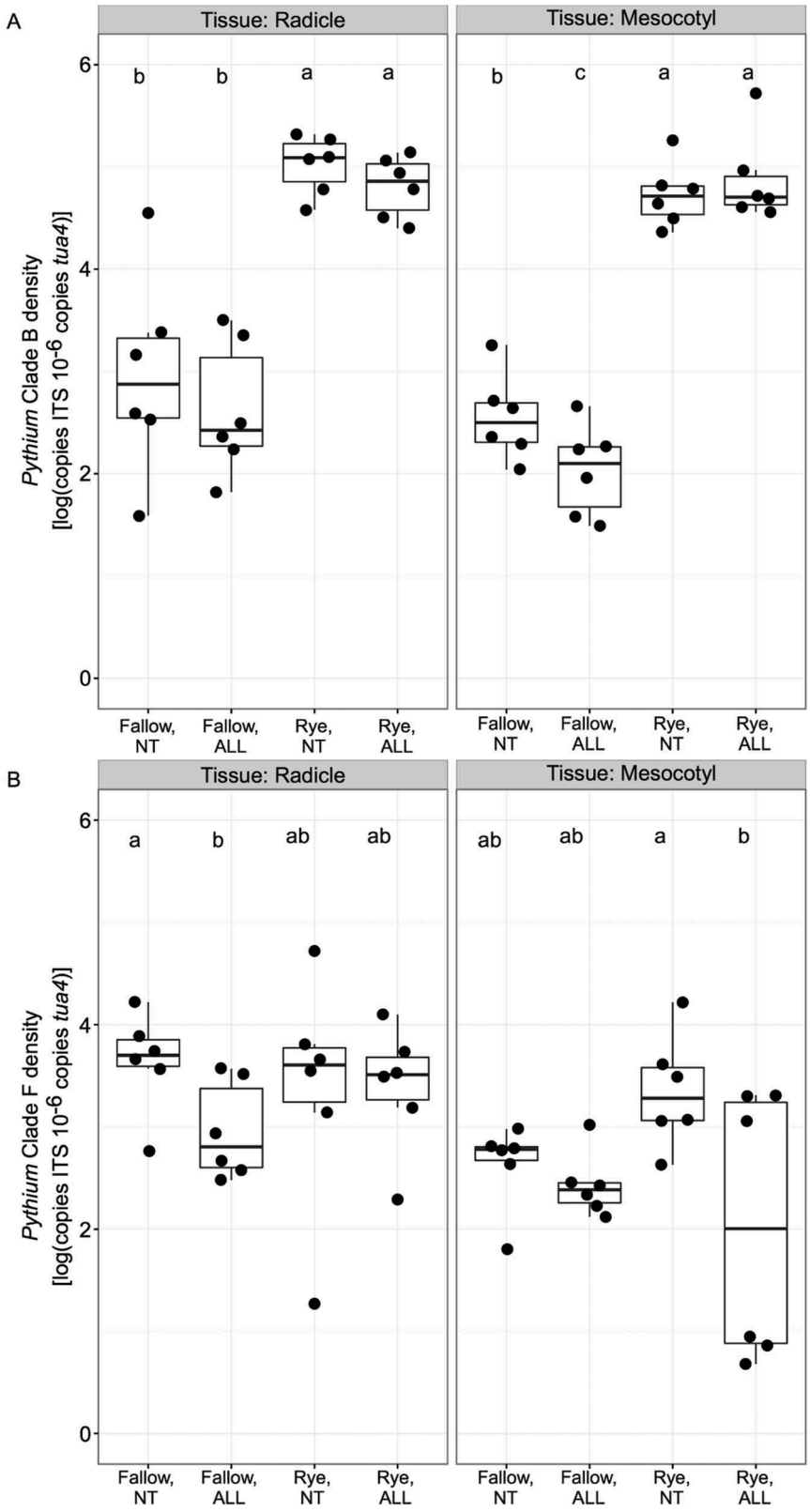

Fig. 1. Density of *Pythium* spp. belonging to Clade B (A) and Clade F (B) in corn radicles and mesocotyls following no-rye (Fallow) control or winter cereal rye treatments in the 2015 field experiment. Seed was non-treated (NT) or received chemical seed treatments (ALL). Pathogen density (copies of *Pythium* spp. ITS gene) was calculated relative to host plant DNA (copies of corn *tua*4 gene). Boxplots indicate median, first and third quartiles. Whiskers extend up to 1.5 times the interquartile range. Data points beyond this range are displayed individually. Within each panel, means do not differ significantly among treatments indicated with the same letter ($P > 0.05$).

Table 7. Means for the main effects of cover crop presence and fungicide seed treatment on final plant stand, number of ears, number of barren plants, grain moisture and yield of corn in field experiments in Iowa.

	Variables[a]	Plants/ha	Ears/ha	Barren/ha	Moisture (g kg^{-1})	Yield (Mg ha^{-1})[b]
2014	Cover crop (CC)					
	Rye	77 199	76 008	1191[ac]	209.9	13.1
	Fallow	76 725	76 302	416[b]	208.3	13.6
	P value	0.78	0.85	0.02	0.24	0.06
	Seed treatment (ST)					
	ALL	76 840	76 187	660	210.0	13.4
	NT	77 084	76 122	954	208.1	13.3
	P value	0.89	0.97	0.34	0.16	0.46
	P value (CC × ST)	0.53	0.58	0.56	0.58	0.09
2015	Rye Cover crop (CC)					
	Rye	80 851	78 038[b]	2813[a]	180.9	12.8[b]
	Fallow	84 016	83 600[a]	416[b]	180.9	15.0[a]
	P value	0.06	<0.01	<0.01	1.00	<0.01
	Seed treatment (ST)					
	ALL	84 978[a]	83 363[a]	1615	181.0	14.0
	NT	79 890[b]	78 275[b]	1615	180.7	13.8
	P value	<0.01	<0.01	1.00	0.70	0.66
	P value (CC × ST)	0.42	0.43	0.80	0.19	0.96

[a]Seed treatments applied to corn seed planted following winter cereal rye or no winter cereal rye (fallow): ALL = metalaxyl (Alliance; Bayer CropScience, 0.29 mL kg^{-1}), pyraclostrobin (Stamina; BASF, 0.47 mL kg^{-1}), fludioxonil (Maxim 4FS; Sygenta, 95 mL kg^{-1}), ipconazole (Rancona; Valent, 0.03 mL kg^{-1}) and sedaxane (Vibrance 500; Sygenta, 189 mL kg^{-1}). NT = Non-treated.
[b]Corn yield data were collected from the centre three rows of each plot.
[c]Values followed by the same letter within a column and runs are not significantly different at P value 0.05 using Fisher's protected least significant difference.

of barren plants was greater following rye ($P < 0.02$), but there was no effect of seed treatment on the number of barren plants. No effect of cover crop or seed treatment was detected on grain moisture. In 2015, the yield of corn in plots following rye was 15% lower than in the plots following fallow ($P < 0.01$). However, no effect of seed treatment on corn yield was observed in either year ($P = 0.46$; $P = 0.66$; Table 7).

Discussion

Results from the current study improve our understanding of the risk of corn seedling diseases following a rye cover crop, the presence of pathogens that contribute to corn seedling disease, and the effect of rye on the density of pathogens associated with corn seedling disease. In general, fungicide seed treatment improved emergence and seedling growth under controlled environment conditions that were conducive to disease development. However, not all combinations of fungicides were equally effective. Seedlings from treatments that contained metalaxyl (ALL, P and FP) had better emergence, were taller and had longer radicles compared with seedlings from seed treatments that did not contain metalaxyl. Because metalaxyl is active against oomycetes, this suggests that under the conditions used in our experiments, *Pythium* species played a major role in seedling disease. These results support earlier findings that *Pythium* spp. play an important role in corn seedling disease and yield loss in corn planted after a rye cover crop (Bakker et al., 2016; Acharya et al., 2017). Thus, our results indicate that seed treatments with activity against oomycetes are important to help to minimize corn seedling disease risk in cropping systems that include rye cover crops.

In our controlled environment study, seed treatments were not effective at reducing seedling disease following a rye cover crop even though some seed treatments had a positive effect on emergence and corn seedling growth. Similarly, Schenck et al. (2017) were not able to detect a seed treatment effect on radicle rot when only the presence or absence of disease symptoms was recorded. However, in Run 2 of our controlled environment experiment, when we assessed radicle disease severity and computed a radicle disease index score, we observed that fungicide seed treatments ALL, P and FP reduced the disease index, and these data were similar to the response of emergence, shoot height and radicle length to seed treatments. These results suggest that only recording disease incidence data may not allow seed treatment efficacy to be detected, and consequently disease severity assessments and disease index scores should be done in seed treatment evaluations.

Although fungicide seed treatments had a positive effect on seedling growth and reduced radicle rot severity

in our controlled experiments, no effect of fungicide seed treatment was detected on seedling growth or root rot in our field trials. Different environmental conditions in the two types of trials are likely responsible for this discrepancy. For instance, in the growth chamber, cold (12°C) and wet conditions were maintained continuously for 30–32 days. This environment was likely more favourable for seedling disease development. In the field, however, conditions such as soil temperature, air temperature and soil moisture fluctuate (Supplementary Fig. 1). Different factors also affect fungicide persistence and performance (Latin, 2011); for example, fungicides rapidly degrade at higher temperatures (Koch, 2012). It is possible that the effective period of the fungicides under cold and wet conditions in our controlled environment studies was longer than in the field studies. Similarly, under cold and wet conditions in the growth chamber, seed treatments mitigated seedling disease of soybeans caused by *Pythium* spp. (Serrano et al., 2018). In contrast, Schenck et al. (2017) reported the efficacy of seed treatment was limited on corn seedling disease after winter cereal rye in cold and wet controlled environment conditions.

The incidence of *Pythium* spp. recovered from corn radicles in both our controlled environment and field studies was significantly increased when corn followed rye. One possible reason could be that dying rye roots harbour greater *Pythium* populations (Bakker et al., 2016; Acharya et al., 2017), and this increases inoculum density in the soil. The effect of seed treatments varied depending on whether corn followed rye or fallow and on the background incidence levels of *Pythium* infection on the radicles. For example, in Run 1 the *Pythium* background levels were so low when corn followed fallow that any positive effect of the seed treatments could not be expressed. Following rye in Run 1, seed treatments with metataxyl had lower radicle *Pythium* incidence levels than the FR and NT treatments.

Fusarium spp. are often associated with the corn seedling disease complex (Broders et al., 2007b). Although we recovered *Fusarium* spp. more frequently than *Pythium* spp. in our field trials and the second run of the controlled environment study, we did not detect treatment effects of either cover crop or seed treatment on the incidence of *Fusarium* spp. Similarly, we observed no effect of rye cover crop on *Fusarium* incidence in an earlier study (Acharya et al., 2017). We primarily recovered *F. oxysporum* from seedling radicles, and very few isolates of *F. graminearum* and *F. solani*. Species of *Fusarium* vary in their sensitivities to different fungicide active ingredients (Broders et al., 2007b). The seed treatment we used in the field contained fludioxonil that has excellent activity against *F. graminearum*, and ipconazole, which has excellent activity against *F. solani* and to a lesser extent *F. oxysporum* (G. Munkvold, *personal communication*). Munkvold & O'Mara (2002) reported *Fusarium* infections were reduced, but not eliminated, when corn seed had a protective fungicide coating. Thus, our observed *Fusarium* infection rates although very high are consistent with prior reports, and do not support *Fusarium* as the primary pathogen causing corn seedling root disease following rye cover crops.

The current understanding of the efficacy of fungicides against various pathogen species is based on work done with single active ingredients (Munkvold & O'Mara, 2002; Broders et al., 2007b; Matthiesen et al., 2016). Though the FR and FP seed treatments include fungicides that target *Fusarium* species, the higher incidence of *Pythium* and *Fusarium* spp. in Run 1 of the controlled environment study could be due to variability in the efficacy of fungicides toward certain pathogens when two or more fungicides are used in combination. It is also possible that because the fungicides in ALL and FP reduced *Pythium* infection, this also reduced secondary infection by *Fusarium*.

Detection of almost equal densities of *Pythium* Clade B members from mesocotyl tissue and radicle root tissue in both the treated seed and non-treated seeds in the 2015 field trial was unexpected because the mesocotyls were considerably less diseased compared with the radicles. We suggest that we detected latent or very early stage infection in the mesocotyl. Because the radicle is the first tissue to emerge during germination (Nielson, 2013), it is exposed to soil-borne pathogens for a longer period of time than the mesocotyl. Consequently, disease on the radicles had more time to develop. Interestingly, mesocotyl lesions were observed only in the field trials.

At finer taxonomic resolution, our results also support earlier reports that species belonging to *Pythium* Clade B are more prevalent in corn radicle tissue after a cover crop of rye compared with fallow (Schenck et al., 2017). Noel et al. (2018) reported that sensitivity to fungicides active against oomycetes varied among clades of *Pythium*. Our improved understanding of the *Pythium* community affecting corn development after a rye cover crop documents the need for improved fungicides and targeted combinations to reduce the risk of corn seedling diseases associated with rye cover cropping. Further, studies on the oomycete community and specific clades in relation to specific fungicide active ingredients are warranted.

Rhizoctonia solani was not detected on corn radicle or mesocotyl tissues in this study. Molecular-based techniques were not used to detect this fungus. We suspect the environmental conditions in our studies were unfavourable for growth of *Rhizoctonia* spp. due to the cool soil temperatures present in field and controlled environments. Previous

studies reported that *Rhizoctonia* spp. grew better in warm (25–30°C) soils (Harikrishnan & Yang, 2004). The average recorded soil temperatures in our 2015 field study were 16–17°C. Another reason for not detecting *R. solani* in this study may be the selective media used to recover the pathogen. Future studies should consider using a better selective medium for *R. solani* such as Ko & Hora (1971) medium.

In our study, we detected a yield loss following rye in one year, 2015. Several published studies have also reported an occasional yield loss of corn following rye cover crop (Johnson et al., 1998; Kaspar & Bakker, 2015). One possible reason for the lower corn yields in 2015 compared with 2014 in our study may be the greater difference in disease incidence and severity that was observed in the rye cover crop treatment compared with the winter fallow treatment. In 2015, disease incidence was ~9-fold greater in the rye treatment compared with the fallow treatment; in 2014, however, disease incidence was 1.5-fold greater in the rye treatment compared with the fallow treatment.

Another reason for the reduced yield observed in 2015 may be related to cover crop biomass accumulation. In 2015, ~3-fold more rye biomass accumulated compared with 2014. This likely reduced soil temperature (Supplemental Fig. 1) and increased soil moisture through a mulching effect on the soil surface. Consequently, risk of seedling disease was increased for the rye treatment relative to the fallow treatment more in 2015 than in 2014. Lower soil temperature favours infection of corn seedlings by soil-borne pathogens that prevent or delay seed germination and emergence, reduce plant vigour, and may subsequently decrease grain yield (Munkvold, 1999).

Additional reasons for the reduced yield in the 2015 field trial may be related to plant stand and ear size. There is a direct relationship between plant stand and corn yield – corn yield is dependent on uniform crop stands (Nafziger et al., 1991). Seedling disease may reduce stand, delay emergence, or reduce plant vigour, and thus result in lower yields. When plants are uneven in size, as often occurs with seedling disease, smaller plants often produce smaller ears or no ears and thus yields may be reduced. In our 2015 field trial, we observed a greater difference in the number of barren plants between the rye and fallow treatments compared with our 2014 field trial. Reduced corn grain yield often has been attributed to increases in the number of barren plants (Buren et al., 1974; Smith et al., 1982). However, in 2015, the number of harvestable ears in corn following rye was reduced by about 7%, whereas yield was reduced by about 15%. This would seem to indicate that ear size, number of kernels and weight per kernel was also reduced for corn following rye compared with corn following fallow. Part of the difference in ear size might be explained by competition from barren plants or uneven growth. Additionally, because no nitrogen fertilizer was applied until about 30 days after planting and because the rye cover crop in 2015 took up at least 53 kg N ha^{-1} from the soil, corn following rye was more likely N limited during early growth and this may have explained part of the reduction in ear size and yield. It should be noted that nitrogen management in the current study (no nitrogen applied at or before planting) would not be considered typical management in a production setting.

The results from this study showed that the prevalence of soil-borne pathogens, particularly those belonging to *Pythium* Clade B, and seedling disease effects differed in corn following a winter cereal rye cover crop versus corn following a fallow. Bakker et al. (2016) showed that rye hosts pathogens of corn and our study supports their work. Moreover, by using different fungicide combinations and isolation of pathogens from affected tissues in the growth chamber study, we were able to confirm that *Pythium* species play a major role in corn seedling disease following a cover crop or fallow when conditions are cool and wet. The study suggests that *Pythium* spp. constitute the primary pathogens in the corn seedling disease complex following rye and suggests that seed treatments that target *Pythium* are important for corn seed planted after a rye cover crop.

Acknowledgements

The authors thank the Iowa Nutrient Reduction Research Center and the Leopold Center for funding the project, and Keith Kohler for excellent management of the field trial. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture or Iowa State University. USDA is an equal opportunity provider and employer.

Funding

This work was supported by the Iowa Nutrient Research Center and the Leopold Center.

Supplementary material

Supplementary data can be accessed online here: https://doi.org/10.1080/07060661.2018.1506503.

ORCID

Matthew G. Bakker http://orcid.org/0000-0003-0345-0587

References

Acharya J, Bakker MG, Moorman TB, Kaspar TC, Lenssen AW, Robertson AE. 2017. Time interval between cover crop termination and planting influences corn seedling disease, plant growth, and yield. Plant Dis. 101:591–600.

Andrews WF, Diderikson RO. 1981. Soil survey of Boone County, Iowa. Washington (D.C): USDA-SCS U.S. Gov. Printing Office.

Back MM, Haydock PPJ, Jenkinsen P. 2002. Disease complexes involving plant parasitic nematodes and soil-borne pathogens. Plant Pathol. 51:683–697.

Bakker MG, Acharya J, Moorman TB, Robertson AE, Kaspar TC. 2016. The potential for cereal rye cover crops to host corn seedling pathogens. Phytopathology. 106:591–601.

Bakker MG, Manter DK, Moorman TB, Kaspar TC. 2017. Isolation of cultivation-resistant oomycetes, first detected as amplicon sequences, from roots of herbicide-terminated winter rye. Phytobiomes J. 1:24–35.

Broders KD, Lipps PE, Paul PA, Dorrance AE. 2007a. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:727–735.

Broders KD, Lipps PE, Paul PA, Dorrance AE. 2007b. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:1155–1160.

Buren LL, Mock JJ, Anderson IC. 1974. Morphological and physiological traits in maize associated with tolerance to high plant density. Crop Sci. 14:426–429.

Cohen M, Coffey MD. 1986. Systemic fungicides and the control of oomycetes. Annu Rev Phytopathol. 24:311–338.

Duiker SW, Curran WS. 2005. Rye cover crop management for corn production in the northern Mid-Atlantic region. Agron J. 97:1413–1418.

Ebelhar SA, Frye WW, Blevins RL. 1984. Nitrogen from legume cover crops for no-tillage corn. Agron J. 76:51–55.

Geiser DM, Jimenez-Gasco MD, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K. 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. Eur J Plant Pathol. 110:473–479.

Harikrishnan R, Yang XB. 2004. Recovery of anastomosis groups of *Rhizoctonia solani* from different latitudinal positions and influence of temperatures on their growth and survival. Plant Dis. 88:817–823.

IDALS (Iowa Department of Agriculture and Land Stewardship), IDNR (Iowa Department of Natural Resources), ISU (Iowa State University). 2016. Iowa Nutrient Reduction Strategy, (revised 1 Oct. 2016) Ames, IA. http://www.nutrientstrategy.iastate.edu/documents (accessed 28 October 2017).

Iowa Environmental Mesonet. 2015. NWS COOP Network. Online publication. Iowa State University Department of Agronomy. http://mesonet.agron.iastate.edu/request/coop/fe.phtml

Johnson TJ, Kaspar TC, Kohler KA, Corak SJ, Logsdon SD. 1998. Oat and rye over seeded into soybean as fall cover crops in the upper Midwest. J Soil Water Conserv. 53:276–279.

Kandel YR, Bradley CA, Wise KA, Chilvers MI, Tenuta AU, Davis VM, Esker PD, Smith DL, Licht MA, Mueller DS. 2015. Effect of glyphosate application on sudden death syndrome of glyphosate-resistant soybean under field conditions. Plant Dis. 99:347–354.

Karlen DL, Doran JW. 1991. Cover crop management effects on soybean and corn growth and nitrogen dynamics in an on-farm study. Amer J Alternative Agr. 6:71–82.

Kaspar TC, Bakker MG. 2015. Biomass production of 12 winter cereal cover crop cultivars and their effect on subsequent no-till corn yields. J Soil Water Conserv. 70:353–364.

Kaspar TC, Radke JK, Laflen JM. 2001. Small grain cover crops and wheel traffic effects on infiltration, runoff, and erosion. J Soil Water Conserv. 56:160–164.

Kaspar TC, Singer JW. 2011. The use of cover crops to manage soil. In: Hatfield JL, Sauer TJ, editors. Soil management: building a stable base for agriculture Wisconsin (WI): American Society of Agronomy and Soil Science Society of America; p. 321–337.

Kessavalou A, Walters DT. 1997. Winter rye as a cover crop following soybean under conservation tillage. Agron J. 89:68–74.

Kladivko EJ, Kaspar TC, Jaynes DB, Malone RW, Singer J, Morin XK, Searchinger T. 2014. Cover crops in the upper Midwest USA: potential adoption and reduction of nitrate leaching in the mississippi river basin. J Soil Water Conserv. 69:279–291.

Ko WH, Hora FK. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology. 61:707–710.

Koch PL 2012. Environmental and cultural factors affecting the persistence and efficacy of fungicides on golf course turfgrass. Ph.D. Thesis. UW-Madison.

Latin R. 2011. A practical guide to turfgrass fungicides: disease pressure, host pathogen, and environment components performance. Chapter 2-Factors that influence fungicide performance. Minneapolis (MN): American Phytopathological Society; p. 79–106.

Leslie JF, Summerell BA. 2006. The *Fusarium* laboratory manual. 1st ed. Iowa (IA): Blackwell.

Li SJ, Zhu TH, Zhu HMY, Liang M, Qiao TM, Han S, Che GN. 2013. Purification of protein AP-toxin from *Arthrinium phaeospermum* causing blight in *Bambusa pervariabilis* × *Dendro-calamopisis grandis* and its metabolic effects on four bamboo varieties. Phytopathology. 103:135–145.

Matthiesen RL, Ahmad AA, Robertson AE. 2016. Temperature affects aggressiveness and fungicide sensitivity of four *Pythium* spp. that cause soybean and corn damping off in Iowa. Plant Dis. 100:583–591.

Middleton JT. 1943. The taxonomy, host range and geographical distribution of the genus *Pythium*. Memoirs Torrey Botanical Club. 20:1–171.

Mitchell WH, Tell MR. 1977. Winter-annual cover crops for no-tillage corn production. Agron J. 69:569–573.

Mueller D, Wise KA, Sisson AJ, Allen TA, Bergstrom GC, Bosley DB, Bradley CA, Broders KD, Byamukama E, Chilvers MI, et al. 2016. Corn yield loss estimates due to diseases in the United States and Ontario, Canada from 2012 to 2015. Plant Health Progress. 17:211–222.

Munkvold GP 1999. Corn seedling diseases causing problems. Online publication. Iowa State University Integrated Crop Management News 2156. http://lib.dr.iastate.edu/cropnews/2156

Munkvold GP, O'Mara JK. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by *Fusarium* species. Plant Dis. 86:143–150.

Munkvold GP, White DG, editors. 2016. Compendium of Corn Diseases. Fourth ed. Minneapolis (MN): American Phytopathological Society Press.

Nafziger ED, Carter PR, Graham EE. 1991. Response of corn to uneven emergence. Crop Sci. 31:811–815.

Nielson RL 2013. Root development in young corn. Corny News Network, Purdue Extension. https://www.agry.purdue.edu/ext/corn/news/timeless/roots.html

Noel Z, Rojas A, Jacobs JL, Chilvers MC 2018. Managing an oomycete community: fungicide sensitivity and evolution of resistance to etaboxam. Phytopathology 49-O (abstr.).

Robideau GP, De Cock AW, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Desaulniers N, Eggertson QA, Gachon CMM, et al. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol Ecol Resource. 11:1002–1011.

Runquist S, Carlson S 2017. Mapping cover crops on corn and soybeans in Illinois, Indiana, and Iowa, 2015-2016. Environmental Working

Group. Washington, D.C. https://www.agripulse.com/ext/resources/pdfs/EWG_CoverCropReport_C05.pdf

Schenck L, Bakker MG, Moorman TB, Kaspar TC. 2017. Effects of cover crop presence, cover crop species selection and fungicide seed treatment on corn seedling growth. Renewable Agric Food System. doi:10.1017/S1742170517000345

Schepers JS, Francis DD, Thompson MT. 1989. Simultaneous determination of total C, total N, and ^{15}N on soil and plant material. Commun Soil Sci Plant Anal. 20:949–959.

Serrano M, McDuffee D, Robertson AE. 2018. Seed treatment reduces damping-off caused by *Pythium sylvaticum* on soybeans subjected to periods of cold stress. Can J Plant Pathol. 40:1–9.

Smith CS, Mock JJ, Crosbie TM. 1982. Variability for morphological and physiological traits associated with barrenness and grain yield in the maize population, Iowa Upright Leaf Synthetic #11. Crop Sci. 22:828–832.

Snapp SS, Swinton SM, Labarta R, Mutch D, Black JR, Leep R, Nyiraneza J, O'Neil K. 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. Agron J. 97:322–332.

Tollenaar M, Mihajlovic M, Vyn TJ. 1993. Corn growth following cover crops: influence of cereal cultivar, cereal removal, and nitrogen rate. Agron J. 85:251–255.

van der Plaats-Niterink AJ. 1981. Monograph of the genus *Pythium*. Stud mycology, no. 21. Baarn (The Netherlands): Centraalbureau voor Schimmelcultures

Vincelli PC, Beaupré CM-S. 1989. Comparison of media for isolating *Rhizoctonia solani* from soil. Plant Dis. 73:1014–1017.

Wagger MG, Mengel DB. 1988. The role of nonleguminous cover crops in the efficient use of water and nitrogen. In: Halgrove WL, editor. Cropping strategies for efficient use of water and nitrogen. Wisconsin (WI): American Society of Agronomy and Soil Science Society of America, Crop Science Society of America, Soil Science Society of America; p. 115–127.

Waterhouse GM. 1967. Key to *Pythium* Pringsheim. Mycol Pap. 109: 1–15.

Windham AS, Lucas LT. 1987. A qualitative baiting technique for selective isolation of *Rhizoctonia zeae* from soil. Phytopathology. 77:712–714.