The Efficacy of Ethaboxam as a Soybean Seed Treatment Toward *Phytophthora*, *Phytophthium*, and *Pythium* in Ohio

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Abstract

Phytophthora, *Phytophthium*, and *Pythium* species that cause early-season seed decay and pre-emergence and post-emergence damping off of soybean are most commonly managed with seed treatments. The phenylamide fungicides metalaxyl and mefenoxam, and ethaboxam are effective toward some but not all species. The primary objective of this study was to evaluate the efficacy of ethaboxam in fungicide mixtures and compare those with other fungicides as seed treatments to protect soybean against *Pythium*, *Phytophthium*, and *Phytophthora* species in both high-disease field environments and laboratory seed plate assays. The second objective was to evaluate these seed treatment mixtures on cultivars that have varying levels and combinations of resistance to these soilborne pathogens. Five of eight environments received adequate precipitation in the 14 days after planting for high levels of seedling disease development and treatment evaluations. Three environments had significantly greater

stands, and three had significantly greater yield when ethaboxam was used in the seed treatment mixture compared with treatments containing metalaxyl or mefenoxam alone. Three fungicide formulations significantly reduced disease severity compared with nontreated in the seed plate assay for 17 species. However, the combination of ethaboxam plus metalaxyl in a mixture was more effective than either fungicide alone against some *Pythium* and *Phytophthium* species. Overall, our results indicate that the addition of ethaboxam to a fungicide seed treatment is effective in reducing seed rot caused by these pathogens commonly isolated from soybean in Ohio but that these effects can be masked when cultivars with resistance are planted.

Keywords: disease management, field crops, *Glycine max*, oilseeds and legumes, oomycetes, seed treatments

Soybean (*Glycine max* L. Merr.) seedling diseases caused by species of *Phytophthora*, *Phytophthium*, and *Pythium* are a major yield constraint in the United States and Canada (Allen et al. 2017; Bradley 2008; Broders et al. 2007; Jiang et al. 2012; Kirkpatrick et al. 2006; Koenning and Wrather 2010; Marchand et al. 2014; Radmer et al. 2017; Rizvi and Yang 1996; Rojas et al. 2017; Wei et al. 2010; Zitnik-Anderson and Nelson 2015). Reduced stands and plants that survive infection have reduced vigor because of root rot, which contributes to lower yields. Soybean fields may need replanting if stand loss is extensive, further reducing profit for farmers through added input costs and lower yield because of delayed planting. In Ohio, field surveys and subsequent pathogenicity assays have identified species of *Phytophthora* (Ph.), *Pythium* (Py., syn. *Globosporangium*), and *Phytophthium* (Pp.) that pose a serious risk to soybean (Broders et al. 2007, 2009; Dorrance et al. 2004; Vargas Loyo 2018).

Saturated soils favor infections because these conditions are conducive to the development and reproduction of soilborne pathogens and

may lead to large-scale disease epidemics (Kirkpatrick et al. 2006; Martin and Loper 1999; Schroeder et al. 2013). Soybeans at the seed and seedling stage are the most vulnerable to this disease. Crop rotation is not an effective means to manage *Pythium* seed and root rot because many *Pythium* spp. also infect commonly rotated crops such as corn or wheat (Broders et al. 2007; Ingram and Cook 1990). Because of the wide host range of many *Pythium* spp., cover crops have also been reported to increase the incidence of seedling disease of corn (Acharya et al. 2018). The combination of multiple potential hosts, poorly drained soils, and reduced tillage all promote inoculum buildup in the top 7.5 cm of soil (Workneh et al. 1998).

During surveys of symptomatic soybeans in Ohio and other regions, more than one species of *Pythium* was isolated from a single infected seedling, indicating a probable *Pythium* disease complex (Broders et al. 2007, 2009; Dorrance et al. 2004; Zitnik-Anderson and Nelson 2015). *Pythium* spp. can also form a seed and seedling complex with other soilborne pathogens such as *Phytophthora sojae* and *Rhizoctonia* spp. (Rizvi and Yang 1996). More important is the great diversity of *Pythium* spp. that have been recovered from individual fields (Broders et al. 2007, 2009; Dorrance et al. 2004; Jiang et al. 2012; Marchand et al. 2014; Radmer et al. 2017; Rojas et al. 2017; Zitnik-Anderson and Nelson 2015), indicating a need for more encompassing management tools for a pathogen complex rather than just focusing on a single species.

Integrated disease management that combines resistant cultivars and fungicide seed treatments is recommended for the management of seedling diseases of soybean (Bradley 2008; Dorrance et al. 2009; Garnica and Giesler 2019; Urrea et al. 2013). Unfortunately, few sources of host resistance to *Pythium* spp. have been identified in soybean germplasm (Bates et al. 2008; Ellis et al. 2013; Rod et al. 2018; Rosso et al. 2008; Rupe et al. 2011; Scott et al. 2019; Urrea et al. 2017). In addition, very little is known about the levels of resistance toward the individual species of *Pythium* in soybean cultivars currently planted by farmers. Because of the current limitations of the knowledge of levels of host resistance toward *Pythium*, *Pythium* seed and root rot is typically managed with fungicide seed treatments (Bradley 2008; Esker and Conley 2012; Gaspar et al. 2014, 2015, 2017; Munkvold 2009).

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The management of early season infections of soybean by *Ph. sojae* has additional challenges. The pathotype diversity of *Ph. sojae* in the north central region of the United States has increased from a few decades ago as populations have adapted to commonly deployed resistance genes. These resistance genes have become ineffective, resulting in the greater reliance on quantitative resistance (Dorrance et al. 2003, 2009, 2016). However, quantitative resistance toward *Ph. sojae* is not effective until the plant is at least in the V1 growth stage (when the seedling has one set of trifoliate leaves) (Dorrance and McClure 2001). These challenges have also led to fungicide seed treatments having greater role in the management of *Ph. sojae* (Anderson and Buzzell 1982; Bradley 2008; Dorrance and McClure 2001; Dorrance et al. 2009).

Fungicide seed treatments are a valuable means of managing early season diseases. The phenylamides (metalaxyl and its active isomer mefenoxam) have been reliable active ingredients in reducing losses in soybean because of early season stand reductions, thereby preserving yield (Anderson and Buzzell 1982; Bradley 2008; Bradley et al. 2001; Dorrance and McClure 2001; Gaspar et al. 2015; Guy et al. 1989; Lueschen et al. 1991; Radmer et al. 2017), even under reduced tillage production systems (Guy and Oplinger 1989). These active ingredients inhibit ribosomal RNA synthesis in oomycetes (Davidse et al. 1983; Randall et al. 2014). In Ohio and across the United States, isolates of several different species of *Pythium* that are insensitive to metalaxyl and mefenoxam have been recovered from corn and soybean (Broders et al. 2007; Dorrance et al. 2004; Taylor et al. 2002; Radmer et al. 2017). Quinone outside inhibitor (QoI) fungicides have also been used to manage some soybean seed and seedling diseases caused by oomycetes (Bradley 2008; Broders et al. 2007; Matthiesen et al. 2016; Radmer et al. 2017). The efficacy of individual QoI and phenylamide fungicides toward different *Pythium* species varies. Similar to phenylamide fungicides, the QoI fungicides are also at high risk for the development of resistance in the pathogen population (Broders et al. 2007; Gisi et al. 2002; Radmer et al. 2017). To maintain the ability to manage these seedling diseases and prevent poor stands, replanting, and potential yield loss, new fungicide chemistries should be evaluated for efficacy toward the complex of water molds that cause seed rot and seedling damping off.

Ethaboxam is effective in managing several foliar diseases on pepper and potato caused by different oomycete species, including *Phytophthora infestans* (Kim et al. 1999, 2004). The mode of action of ethaboxam is the disruption of microtubulin assembly, as modeled in *Ph. infestans* (Uchida et al. 2005). Ethaboxam is considered as having low to medium risk for the development of fungicide resistance (Kim et al. 2004). In our earlier studies, ethaboxam was effective against most but not all evaluated species of *Pythium* (Dorrance et al. 2012). Radmer et al. (2017) reported that 5 of 7 species of *Pythium* had significant reductions in growth at both 5 and 100 ppm of ethaboxam. Matthiesen et al. (2016) compared the growth of four species of *Pythium* on ethaboxam amended agar plates, and although it was effective in limiting mycelial growth, the EC₅₀ values were different at 13, 18, and 23°C. The EC₅₀ value for ethaboxam was lower at 13 compared with 23°C for *Pythium sylvaticum* and higher at 23 compared with 13°C for *Pythium torulosum*. In another study, the combination of ethaboxam

and metalaxyl protected soybean seedlings from infections of *Py. sylvaticum* that were enhanced by cold stress (Serrano et al. 2018). More importantly, White et al. (2019) recently reported an improvement in stand and yield of wheat when the seed treatment included ethaboxam, compared with metalaxyl alone.

Together, these studies suggest that ethaboxam may be a good candidate for effective management of seed and root disease caused by *Phytophthora*, *Phytophythium*, and *Pythium* species in the high-disease environments that occur annually in Ohio. The objectives of our study were to evaluate ethaboxam and metalaxyl alone or in combination in seed treatment mixtures, compare these mixtures with other seed treatments on cultivars with various levels and combinations of resistance to these pathogens in high-disease environments, and evaluate the efficacy of these mixtures in laboratory seed plate assays to isolates representing the breadth and diversity of pathogens that cause soybean seed and seedling diseases in Ohio. This work expands on earlier studies of this fungicide from amended agar and greenhouse assays (Dorrance et al. 2012). Preliminary reports of these studies were published previously (Dorrance et al. 2012; Scott et al. 2016).

Materials and Methods

Environments. In 2014, 2015, and 2016, eight field sites were selected to study the effects of fungicide seed treatments on early (soybean growth stages V1 and V3) plant populations, final (R8) plant populations, and yield (Table 1). In this study, an “environment” refers to a single field site within a growing season (e.g., VW-2016 is the Van Wert field in 2016). These environments were selected based on a history of soybean seedling disease, high incidence of damping off, and poor drainage caused by soil type. Soil types for the fields were Paulding clay, Latty silty clay, Hoytville clay, and a Ravenna silt loam in Defiance (DEF-2015, DEF-2016), Van Wert (VW-2014, VW-2016), Wood (NWARS-2014, NWARS-2015, NWARS-2016), and Wayne (SNY-2016) counties in Ohio, respectively. One field site was planted each year (for a total of three environments) at the Ohio Agricultural Research and Development Center Northwest Agricultural Research Station (NWARS-2014, NWARS-2015, NWARS-2016). Each year, the field at NWARS was irrigated with 4.1 to 7.4 cm of water for 2 to 3 days after planting (dap) to ensure saturated soil conditions for optimal disease development (Table 1). The other environments did not have irrigation and were dependent on natural rainfall to produce an environment conducive for oomycete seedling disease.

Field experiments. The seed for each trial was treated at Valent USA LLC’s Seed Protection Lab, in Leland, Mississippi. Additional fungicides (ipconazole, fludioxonil, prothioconazole, fluxapyroxad, or pyraclostrobin) and an insecticide (clothianidin, thiamethoxam, imidacloprid, or penflufen) were included in the base mix each year to eliminate any secondary effects from injury caused by insects or seed and seedling damping off caused by fungal pathogens (*Fusarium* spp., *Phomopsis*).

The effects of seven different fungicide seed treatments and a non-treated control were evaluated for the effects on stand and yield with the cultivars Conrad and Sloan in 2014 (Table 2). These seven fungicide seed treatment mixtures included metalaxyl or mefenoxam

Table 1. Environment (location-year), planting and harvest dates, number of cultivars, and number of seed treatment formulations evaluated in Ohio in 2014, 2015, and 2016 for efficacy toward seed and seedling diseases of soybean caused by species of *Phytophthora*, *Phytophythium*, and *Pythium*

Environment	Planting date	Harvest date	Number of cultivars	Number of seed treatment formulations ^z
VW-2014	26 May	13 Oct	2	7
NWARS-2014	3 Jun	24 Oct	2	7
NWARS-2015	22 Jun	20 Oct	3	3
DEF-2015	26 May	27 Sep	4	3
NWARS-2016	1 Jun	17, 18 Oct	7	2
SNY-2016	20 May	6 Oct	7	2
VW-2016	25 May	8 Oct	7	2
DEF-2016	26 May	15 Oct	7	2

^z Seed treatment formulations are listed in Table 2 and were provided by Valent USA; seed was treated at Valent USA research station in Leland, Mississippi.

alone at different rates and in combination with ethaboxam in different seed treatment mixtures. Conrad and Sloan have relatively low amounts of partial resistance to *Pythium* spp. and no *Rps* genes for *Ph. sojae*.

In 2015 the efficacy of ethaboxam plus metalaxyl in a seed treatment mixture (Table 2) was compared with those with mefenoxam, as well as pyraclostrobin plus metalaxyl with four cultivars (Conrad, Sloan, Kottman, and Lorain) with various levels and combinations of resistance toward *Pythium* and *Ph. sojae*. Kottman has resistance genes *Rps1k* and *Rps3a* (Dorrance et al. 2009), Lorain has resistance gene *Rps1c* to *Ph. sojae*, and both of these cultivars have moderately high levels of partial resistance to *Pythium ultimum* var. *ultimum*, *Pythium ultimum* var. *sporangiferum*, and *Pythium irregulare* (Balk 2014; Dorrance Lab, unpublished data).

In 2016, one seed treatment formulation containing ethaboxam, metalaxyl plus ipconazole, and the insecticide clothianidin (Table 2) was compared with nontreated seed with seven cultivars for early and late plant populations and yield. The cultivars were Clermont, Dennison, Kottman, Streeter, Conrad, Lorain, and Sloan; these cultivars were selected because each had different levels and combinations of resistance toward *Ph. sojae* and *Pythium* spp. (Supplementary Table S1).

Experimental design. Each field experiment (environment) was planted as randomized split plot design with the cultivar as the whole plot and the seed treatments as the subplots with four to eight replicates, depending on the space available in each field. Plots in VW-2014 and DEF-2015 were four rows spaced 76.2 cm apart and 9.1 meters long and a seeding rate of eight seeds per 30.5 cm. Data for stand were collected by counting all soybean plants in the full length of the second row and converted to plants per hectare for analyses. The remaining plots were all planted with four-row cone planters with 38.1 cm row spacing and a minimum of 7.6 m in length. The seeding rate was 4.1 seeds per 30.5 cm. Data for stand were collected by counting the soybean plants in 0.9 m of all four rows in one location of the plot and converted to plants per hectare before analysis. Data for plant populations were collected three times in each growing season, based on soybean developmental growth stages: one or two trifoliate leaves (growth stage V1 to V2), three or four trifoliate leaves (growth stage V3 to V4), and when pods were brown or tan (growth stage R7 to R8). Stand counts were converted into the number of plants per hectare before analysis.

Yield data were collected with plot combines, which also collected weights and moisture readings. Fields at NWARS (-2014, -2015, -2016) were harvested with a Kincaid 8-XP (Kincaid Equipment Manufacturing, Haven, KS) paired with a High Capacity Grain Gage weigh system. (Juniper Systems, Logan, UT). The fields at DEF

(-2015, -2016) and VW (-2014, -2016) were harvested by Tech Services, Inc. (Bluffton, IN) with a combine paired with an Almaco LRX electronic data system. (ALMACO, Nevada, Iowa). The study at SNY-2016 was harvested with an Almaco Specialized Plot Combine (Almaco SPC20) (Almaco, Nevada, IA) equipped with a Harvest-master Grain Gage system. The yield data for all locations were standardized to 13.5% moisture and converted to kilograms per hectare.

Field data analyses. The data collected from the field experiments were individually analyzed with a generalized linear mixed model (PROC GLIMMIX) and the Kenward–Roger method for calculating denominator degrees of freedom (Littell et al. 2006). Fungicides and cultivars were fixed, whereas block and block-by-cultivar were random effects. Least square mean comparisons were used to separate the treatments in SAS version 9.4.

The 2016 field data were analyzed across the four environments in order to test for significance of the environment, cultivar, seed treatment, and each of the corresponding interactions on stand and yield. For this analysis, the fixed effects were environment, cultivar, seed treatment, and their interactions, and block and cultivar-by-block nested within environment were random effects.

The seed treatment mixture of metalaxyl (3.2 µg of active ingredient [a.i./seed] plus ethaboxam (12.0 µg of a.i./seed) plus clothianidin (75.1 µg of a.i./seed) plus ipconazole (3.8 µg a.i./seed) was compared with the nontreated with environment, cultivar, and seed treatments and their interactions as fixed effects, and block and cultivar by block nested within environment were random effects in PROC GLIMMIX in SAS.

Species identification. All isolates of *Phytophthora*, *Phytophthora*, and *Pythium* used in these experiments (Table 3) originated from Ohio soybean fields and were recovered from symptomatic seedlings or from soil baiting. Each isolate was identified to the species level based on morphological characteristics from cultures grown on potato carrot agar plates or from grass blade cultures with a monographic key for the genus *Pythium* (van der Plaats-Niterink 1981; Waterhouse 1967), as well as sequence analysis of the entire internal transcribed spacer (ITS) region of the ribosomal RNA (White et al. 1990). Each isolate was grown on vegetable juice broth at ambient temperature for 2 to 6 days, and the mycelia was collected on filter paper placed in a Buchner funnel, transferred to a mortar, frozen with liquid nitrogen, ground into a powder, and stored at -20°C. DNA was extracted from the mycelia powder as described by Zelaya-Molina et al. (2011). Full length of the ITS1-5.8S-ITS4 region was amplified with ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) primers (White et al. 1990). The PCR master mix was adapted from the Promega Protocol (Promega Corp, Madison, WI, US), which consisted of 5.0 µl of GoTaq

Table 2. Fungicide seed treatments evaluated for efficacy toward seed and seedling diseases of soybean caused by *Phytophthora sojae*, *Phytophthora* spp., and *Pythium* spp. in field studies in Ohio

Environment	Active ingredient and rate (µg of a.i./seed)	Trade name
NWARS-2014, VW-2014	Metalaxyl (6.2) + clothianidin (75.1) + ipconazole (3.8)	Inovate Pro (2.47 lb of a.i./gal) + Sebring (2.65 lb of a.i./gal)
NWARS-2014, 2016; VW-2014, 2016; SNY-2016; DEF-2016	Metalaxyl (3.2) + ethaboxam (12.0) + clothianidin (75.1) + ipconazole (3.8)	Inovate Pro (2.47 lb of a.i./gal) + Intego Solo (3.2 lb of a.i./gal)
NWARS-2014, VW-2014	Metalaxyl (6.2) + ethaboxam (12.0) + clothianidin (75.1) + ipconazole (3.8)	Inovate Pro (2.47 lb of a.i./gal) + Sebring (2.65 lb of a.i./gal) + Intego Solo (3.2 lb of a.i./gal)
NWARS-2014, VW-2014	Mefenoxam (11.7) + thiamethoxam (77.8) + fludioxonil (3.8)	CruiserMaxx Plus (2.47 lb of a.i./gal)
NWARS-2014, VW-2014	Mefenoxam (23.0) + thiamethoxam (77.8) + fludioxonil (3.8)	CruiserMaxx Plus (2.47 lb of a.i./gal) + Apron XL (3.0 lb of a.i./gal)
NWARS-2014, VW-2014	Metalaxyl (24.9) + prothioconazole (8.1) + penflufen (4.0) + imidacloprid (101.0)	Evergol Energy (1.47 lb of a.i./gal) + Allegiance-FL (2.65 lb of a.i./gal) + Gaucho (5 lb of a.i./gal)
NWARS-2014, 2015; VW-2014; DEF-2015	Metalaxyl (13.2) + pyraclostrobin (16.9) + flupyrroxad (8.3) + imidacloprid (127.0)	Acceleron package: Acceleron DX 109 (0.8 fl oz/cwt) + Acceleron DX 309 (0.4 fl oz/cwt) + Acceleron DX 612 (0.240 fl oz/cwt) + Acceleron IX 409 (2.00 fl oz/cwt)
NWARS-2015, DEF-2015	Metalaxyl (3.2) + ethaboxam (12.0) + ipconazole (4.0) + clothianidin (81.0)	Intego Suite (3.37 fl oz/cwt)
NWARS-2015, DEF-2015	Mefenoxam (11.7) + fludioxonil (3.8) + thiamethoxam (77.9)	CruiserMaxx Advanced (3.2 fl oz/cwt)

Flexi, 1.5 µl of 25 mM MgCl₂, 1.0 µl containing 2.5 mM each dNTP, 0.25 µl GoTaqFlexi DNA polymerase, 1.0 µl of 10 µM of ITS1 primer, 1.0 µl of 10 µM of ITS4 primer, 1 µl of DNA template (50 ng/µl), and 12.25 µl of ultrapure distilled water (Invitrogen, Carlsbad, CA, US). The PCR conditions were 95°C for 5 min, followed by 30 cycles: denaturation at 94°C for 1 min; primer annealing at 53°C for 1 min, and extension at 72°C for 1 min. PCR products were cleaned with a ExoSAP-IT (Affymetrix Inc, Ohio, USA) kit (exonuclease I and shrimp alkaline phosphatase), and DNA quality was evaluated on 1% agarose gel. Cleaned amplicons were submitted for sequencing at the Ohio Agricultural Research and Development Center Molecular and Cellular Imaging Center in Wooster, Ohio.

Table 3. Isolates of *Phytophthora*, *Phytophthium*, and *Pythium*, survey years, and county in Ohio where they originated, used in a seed plate assays to evaluate the efficacy of three fungicide seed treatments and determine the relative aggressiveness of each isolate

Watermold	Isolate code	Year	County
<i>Pythium attrantheridium</i>	MDa4.2b	2015	Defiance
	MVa12.5b	2015	Van Wert
	17SNY111.3–	2017	Snyder
	17CLN214.4A ^z	2017	Clinton
<i>Pythium cryptoirregulare</i>	Erie 2-6-4	2006/2007	Erie
	Craw 1-1-10	2006/2007	Crawford
<i>Pythium diclinum</i>	MVa3.5b	2015	Van Wert
<i>Pythium dissotocum</i>	MDa2.1a	2015	Defiance
	D411.1	2016	Defiance
	N405.1	2016	Wood
	MVa23.5a ^z	2015	Van Wert
<i>Pythium heterothallicum</i>	MDa6.5b	2015	Defiance
	N405.4 ^z	2016	Wood
	MDa11.2a	2015	Defiance
<i>Pythium inflatum</i>	MVa3.3a	2015	Van Wert
	MVa5.3a	2015	Van Wert
	D206.1 ^z	2016	Defiance
	Mont 1-4-7	2006/2007	Montgomery
<i>Pythium irregulare</i>	Sand 1-6-22	2006/2007	Sandusky
	Wood 1-6-9	2006/2007	Wood
	MVa20.4b	2015	Van Wert
<i>Pythium lutarium</i>	MVa23.5b	2015	Van Wert
<i>Pythium nodosum</i>	MWa9.1a1	2015	Franklin
<i>Pythium oopapillum</i>	MDa2.4b	2015	Defiance
	MVa1.4a	2015	Van Wert
	N110.1	2016	Wood
	MVa13.3b	2015	Van Wert
<i>Pythium pleroticum</i>	MDa4.4b	2015	Defiance
<i>Pythium torulosum</i>	MVa7.2a	2015	Van Wert
	N205.1 ^z	2016	Wood
	MDa5.2b	2015	Defiance
<i>Pythium ultimum</i> var. <i>ultimum</i>	Miami137	2006/2007	Miami
	MVa1.2a	2015	Van Wert
	MVa21.4b	2015	Van Wert
	N508.1	2016	Wood
	N201.2(2)	2016	Wood
<i>Phytophthora sansomeana</i>	MVa2.5b	2015	Van Wert
	MVa8.4b	2015	Van Wert
	17SNY208.5–	2017	Wayne
	17CLN111.2	2017	Clinton
<i>Phytophthora sojae</i>	OH.12108.06.03	2012	Van Wert
	R25_1-97-01		Standard isolate
<i>Phytophthium helicoides</i>	MVa14.4b	2015	Van Wert
	D502.1	2016	Defiance
<i>Phytophthium litorale</i>	D206.3	2016	Defiance
<i>Phytophthium mercuriale</i>	N109.1(2)	2016	Wood
<i>Phytophthium vexans</i>	MWa12.1a	2015	Wood
	17SNY101.1+	2017	Wayne
	17CLN111.1	2017	Clinton

^z Isolate did not cause measurable seed rot in the seed plate assay and was excluded from the final analyses.

Sequence was trimmed with CodonCode Aligner and then blasted against the sequences in the NCBI database. Sequence identities of 99% to validated cultures were considered a match for species identification.

Laboratory seed plate assay. Commercial formulations of three seed treatment mixtures were compared with nontreated seed in a lab-based seed plate assay. The seed treatments were: (i) ethaboxam (12 µg of a.i./seed), metalaxyl (3.2 µg of a.i./seed), ipconazole (4.0 µg of a.i./seed), and an insecticide, clothianidin (81.0 µg of a.i./seed); (ii) mefenoxam (11.7 µg of a.i./seed), fludioxonil (3.8 µg of a.i./seed), and the insecticide thiamethoxam (77.9 µg of a.i./seed); and (iii) pyraclostrobin (16.9 µg of a.i./seed), metalaxyl (13.2 µg of a.i./seed), fluxapyroxad (8.3 µg of a.i./seed), and the insecticide imidacloprid (127.0 µg of a.i./seed). These were the same treatments that were evaluated in the 2015 field experiments. Seed of the cultivar Kottman, which is susceptible to both *Pythium* spp. and some isolates of *Phytophthora* spp., was treated at Valent USA LLC Seed Protection Lab for this study. Nontreated seeds were surface sterilized in a 10% bleach solution for 1.5 min, rinsed with deionized water for 1 min, and allowed to dry thoroughly before use.

The seed plate assay was used to compare the efficacy of the three fungicide seed treatments and the relative pathogenicity of 49 isolates of *Phytophthora*, *Pythium*, and *Phytophthium* on the nontreated soybean seed (Table 3) as previously described by Broders et al. (2007). Briefly, mycelial plugs 5 mm in diameter were aseptically removed from the growing margins of a culture and transferred to the center of a 100-mm 1.2% potato carrot agar plate. Isolates were placed in incubators to grow for 3 days at 20°C for *Pythium* and *Phytophthium* and 7 days at 25°C for *Phytophthora*. Ten seeds of one treatment were gently pushed into the agar, evenly spaced, approximately 1 cm from the edge of the plate. Plates were incubated in the dark for 7 days at 20°C or 25°C depending on the organism. From each plate, data for percentage germination of the seed and disease severity were collected. Seeds were considered germinated only if the radicle was >1 cm. A disease severity score was assigned as first described by Broders et al. (2007). Briefly, scores of 0 to 3 were assigned to the 10 seeds on each plate on the ordinal scale: 0 = 100% germination with no infection, 1 = 70 to 99% germination with lesions on the roots, 2 = 30 to 69% germination with lesions on the roots, and 3 = 0 to 29% germination with lesions on the roots (Fig. 1).

There were three replicate plates of each seed treatment–isolate combination, and the experiment was done twice, for a total of six replicate plates for each treatment–isolate combination. Subsets of the isolates were evaluated against the fungicide seed treatments over time in an incomplete block design for a total of 14 subsets with a range of 4 to 10 isolates in each. One isolate, *Py. ultimum* var. *ultimum* (Miami 137), was included in each subset to verify that the responses of the isolates were the same over time. Plates within a subset were randomized within the incubator. The seed rot score data from isolates that caused measurable seed rot (relative marginal effect score of >0.30 on the nontreated seed) were then combined for final analysis via a nonparametric relative marginal effects analysis as described by Shah and Madden (2004). Data were analyzed with PROC MIXED in SAS. Additionally, six *Py. ultimum* var. *ultimum* isolates were used to compare intraspecies variation in a separate nonparametric relative marginal effects analysis as described by Shah and Madden (2004) with PROC MIXED in order to identify variation within a species. Fungicide seed treatment, oomycete species, and their interaction were considered fixed effects, and the experiment subset and replicate were considered random effects. The slice option in SAS was used to determine whether there were any oomycete species by treatment interactions for each species included. The values shown in the seed plate assay result graphs depict the average seed rot scores for presentation purposes; statistics were based on the relative marginal effects.

Results

Each environment was considered to have disease pressure if seedlings with damping off (data not shown) were readily observed, as was a significant difference between the seed treatment fungicides

and the nontreated control. Five of the eight field studies (NWARS-2014, DEF-2015, NWARS-2016, SYN-2016, and VW-2016) all had significant differences in the V1/V2 plant populations for fungicide seed treatment (Table 4). For these environments that had significant

difference for V1/V2 plant populations, the precipitation that accumulated during the 14 dap was between 1.7 and 10.4 cm (Table 4). For four of the five environments, the yield differences were significantly different between the seed treatment fungicides (Table 4).

Fig. 1. Soybean seed rot scoring system used in a seed plate assay to evaluate the level of seed rot caused by different species of *Phytophthora*, *Phytophthora*, and *Pythium* and the effectiveness of fungicide seed treatments where 0 = 100% germination with no infection, 1 = 70 to 99% germination with lesions on the roots, 2 = 30 to 69% germination with lesions on the roots, and 3 = 0 to 29% germination with lesions on the roots.

Table 4. Significance levels (*P* value) for the effects of fungicide (F) seed treatments and nontreated on soybean stands at V1/V2, V3/V4, R7/R8, and yield (kg/ha) with different soybean cultivars^z (C) in environments with varying disease pressure during 2014, 2015, and 2016. Degrees of freedom is abbreviated as df.

Environment	df	Plant population			Yield	Precipitation 14 days after planting (cm)
		V1/V2	V3/V4	R7/R8		
VW-2014						4.8
C	1	0.6780	—	0.0370	0.1454	
F	7	0.9442	—	0.7637	0.0408	
C × F	7	0.5441	—	0.9515	0.9824	
NWARS-2014						10.4
C	1	0.0164	0.0181	0.0230	0.232	
F	7	<0.0001	<0.0001	<0.0001	<0.0001	
C × F	7	0.3150	0.2739	0.3204	0.3623	
DEF-2015						8.2
C	3	<0.0001	<0.0001	0.4290	<0.0001	
F	3	<0.0001	<0.0001	0.0141	<0.0001	
C × F	9	0.0169	0.0120	0.1985	0.0074	
NWARS-2015						14.5
C	2	0.5550	0.5897	0.4478	0.1745	
F	3	0.8061	0.7532	0.6089	0.3440	
C × F	6	0.0551	0.0451	0.0634	0.2930	
DEF-2016						2.5
C	6	0.4209	0.0154	0.0002	<0.0001	
F	1	0.0502	<0.0001	0.0094	0.2234	
C × F	6	0.3595	0.1145	0.3393	0.3586	
NWARS-2016						8.2
C	6	0.0268	0.0017	0.0014	<0.0001	
F	1	0.0001	0.0003	0.0006	0.0008	
C × F	6	0.1759	0.1386	0.1590	0.2063	
SNY-2016						1.7
C	6	0.6334	0.5337	0.4993	0.0630	
F	1	0.0138	0.0428	0.0813	0.0469	
C × F	6	0.9092	0.8956	0.9643	0.7669	
VW-2016						2.5
C	6	0.1780	0.1682	0.4258	<0.0001	
F	1	0.0063	0.2909	0.3718	0.4395	
C × F	6	0.3950	0.6601	0.8218	0.0833	

^z The cultivars in 2014 were Conrad (high partial resistance) and Sloan (moderately susceptible) to *Phytophthora sojae* and both susceptible to *Pythium*; in 2015, Conrad, Sloan, Kottman (*Rps1k*, *Rps3a*, high partial resistance to *Ph. sojae*, but susceptible to *Pythium*), and Lorain (*Rps1c*) and partial resistance to *Pythium*; and in 2016, Conrad, Sloan, Kottman, Lorain, Clermont, Dennison, and Streeter.

Field evaluations 2014. Only one of the two environments (NWARS-2014) received sufficient precipitation to discriminate the efficacy between the seven fungicide seed treatments. In the NWARS-2014 environment, the cultivar–fungicide seed treatment interaction was not significant ($P > 0.30$) for early, late plant populations and yield, and the cultivars were different from each other ($P \leq 0.0164$), with Sloan having lower plant populations than Conrad (Table 5). Seeds treated with either of the two combinations of metalaxyl (3.0 or 6.0 μg of a.i./seed) and ethaboxam (12.0 μg of a.i./seed) had significantly greater plant populations at growth stages V1/V2 (Table 5) and V3/V4 (data not shown) as well as yield (Table 5) for the more susceptible cultivar, Sloan. In addition to these two seed treatments, mefenoxam alone at the higher rate (23.0 μg of a.i./seed) had significantly greater stand than the nontreated check for the cultivar with higher level of resistance, Conrad. These three seed treatments and the combination of pyraclostrobin and metalaxyl had significantly greater yields than the nontreated based on LS means for Conrad (Table 5).

Field evaluations 2015. The NWARS-2015 environment received a combined total of 14.5 cm of water through irrigation (4.0 cm) and natural rainfall, including 7.5 cm that fell in a 48-h period, and thus flooding and soil crusting had a greater impact on stand and yield and masked any effect of fungicide seed treatments or cultivars (Supplementary Fig. S1). At the DEF-2015 environment, there was a significant cultivar–fungicide seed treatment interaction and a highly significant difference between the four cultivars and four

fungicide seed treatments for both V1/V2 plant population and yield. For the most susceptible cultivar, Sloan, seed treated with a combination of metalaxyl (3.2 μg of a.i./seed) plus ethaboxam (12.0 μg of a.i./seed) or metalaxyl (13.2 μg of a.i./seed) plus pyraclostrobin (16.9 μg of a.i./seed) had significantly greater plant populations and yield than seed that received mefenoxam (11.7 μg of a.i./seed) alone (Table 6). However, the seed treated with metalaxyl (3.2 μg of a.i./seed) plus ethaboxam (12.0 μg of a.i./seed) had greater yields than the two seed treatments and the nontreated control. The cultivars with higher levels of resistance toward *Ph. sojae* (*Rps* gene plus partial resistance [Kottman, Lorain]) were not significantly different between treated and nontreated seed for yield (Table 6).

Field evaluations 2016. These field studies focused on seven cultivars with and without seed treatment in four different environments. The fixed effect of environment was highly significantly different ($P < 0.0001$), as were the interactions between fungicide seed treatment and environment ($P < 0.01$) and cultivar and environment ($P \leq 0.03$) for plant populations at all growth stages and for yield (Supplementary Table S2). Because of the high level of significance for environment and the interactions, each environment was evaluated separately. The seed treated with the mixture of ethaboxam (12.0 μg of a.i./seed) plus metalaxyl (3.2 μg of a.i./seed) plus clothianidin (75.1 μg of a.i./seed) plus ipconazole (3.8 μg of a.i./seed) had significantly greater early stand at V1/V2 ($P \leq 0.01$) when compared with the nontreated seed at three of four environments. Seed treatments affected yield at one environment ($P = 0.0002$) (Table 4).

Table 5. Comparison of fungicide treatment combinations for the efficacy toward *Phytophthora*, *Phytophthium*, and *Pythium* compared with nontreated control on the soybean cultivar Sloan with moderate susceptibility and Conrad with high partial resistance to *Phytophthora sojae*, and both are moderately susceptible to several species of *Pythium* at one environment NWARS in 2014

Fungicide seed treatment ^y (rate in μg of a.i./seed)	Plant population/ha ^x V1/V2		Yield (kg/ha)	
	Sloan ^z	Conrad	Sloan	Conrad
Metalaxyl (6.2)	71,758 f	169,349 cd	1,074 fg	1,186 e
Metalaxyl (3.0) + ethaboxam (12.0)	299,948 a	322,910 a	2,275 ab	2,410 a
Metalaxyl (6.0) + ethaboxam (12.0)	289,902 a	295,642 a	2,200 b	2,462 a
Mefenoxam (11.7)	68,888 f	166,478 cde	776 g	1,332 def
Mefenoxam (23.0)	134,905 cdef	254,023 ab	1,114 efg	1,995 abc
Metalaxyl (24.9) + prothioconazole (8.1) + penflufen (4.0) + imidacloprid (101.0)	126,294 cdef	179,395 bc	1,144 defg	1,610 defg
Pyraclostrobin (16.9) + metalaxyl (13.2) + fluxapyroxad (8.3) + imidacloprid (127.0)	152,127 cde	196,616 bc	1,632 cd	1,879 bc
Nontreated control	87,545 ef	96,155 def	872 fg	1,034 fg
Overall mean	153,920	209,386	1,386	1,738
Least significant difference ($P < 0.05$)	66,329	94,507	358	633

^x Plant population data were collected at soybean growth state V1 to V2 when the first or second trifoliate was visible.

^y Fungicide formulations and trade names are listed in Table 2.

^z Least square means among treatments for both cultivars followed by the same letter are not significantly different based on Fisher's protected least significant difference at $P < 0.05$.

Table 6. Comparison of three fungicide seed treatment combinations for the efficacy toward *Phytophthora*, *Phytophthium*, and *Pythium* compared with nontreated control on cultivars^x Sloan, Conrad, Kottman, and Lorain in one environment (DEF-2015) in 2015

Fungicide rate (μg of a.i./seed)	Plant population ^y V1/V2				Yield (kg/H)			
	Sloan	Conrad	Kottman	Lorain	Sloan	Conrad	Kottman	Lorain
Metalaxyl (3.2) + ethaboxam (12.0) + ipconazole (4.0) + clothianidin (81.0)	221,961 a ^z	253,926 a	281,581 a	293,433 a	1,382 a	1,214 a	1,559 ab	2,030 a
Mefenoxam (11.7) + fludioxonil (3.8) + thiamethoxam (77.9)	141,150 b	183,890 b	249,616 a	286,250 a	901 bc	1,021 ab	1,346 b	1,932 a
Pyraclostrobin (16.9) + metalaxyl (13.2) + fluxapyroxad (8.3) + imidacloprid (127.0)	211,904 a	194,665 ab	258,595 a	282,659 ab	1,066 b	1,076 ab	1,635 a	1,952 a
Nontreated control	107,389 b	151,925 b	211,545 b	273,321 b	740 c	953 b	1,524 ab	1,882 a
Overall mean	170,601	196,101	250,334	283,915	1,022	1,066	1,516	1,949

^x The cultivar Sloan (S) has moderate susceptibility and Conrad (C) has high partial resistance to *Phytophthora sojae* and both are moderately susceptible to several species of *Pythium*; Kottman (*Rps1k*, *Rps3a*, high partial resistance to *Ph. sojae* and moderately susceptible to *Pythium* spp.) and Lorain (*Rps1c*, high partial resistance to both *Ph. sojae* and *Pythium* spp.).

^y Plant population data were collected at soybean growth stage V1/V2, when the soybean plants' first or second trifoliate was visible.

^z Least square mean values followed by the same letter in a column are not significantly different.

Fig. 2. Comparison of seed treated with ethaboxam (12.0 μg of a.i./seed) plus metalaxyl (3.2 μg of a.i./seed) compared with nontreated seed among seven soybean cultivars for **A**, early plant population (V1/V2) and **B**, yield (kg/ha) in a field study at the NWARS-16 environment. An asterisk indicates that there was a significant difference ($P \leq 0.05$) between the treated and nontreated seed for that cultivar.

Table 7. Comparison and significance of least squares estimates for early plant population and yield of the soybean seed of two cultivars Conrad and Sloan treated with the mixture of metalaxyl (3.2 μg of a.i./seed) plus ethaboxam (12.0 μg of a.i./seed) plus clothianidin (75.1 μg of a.i./seed) + ipconazole (3.8 μg of a.i./seed) in seven different environments in Ohio. Degrees of freedom is abbreviated as df.

	Estimate	Standard error	df	t value	P value	Lower	Upper
V1-V2 plant population	-69,183	7,041.17	50.31	-9.83	<0.0001	-83,323	-55,042
Yield	-371.92	49.44	56.02	-7.52	<0.0001	-470.96	-272.89

Fig. 3. The average seed rot score of each *Pythium* (*Py.*), *Phytophthora* (*Ph.*), and *Phytopythium* (*Pp.*) species evaluated in a seed plate assay of nontreated seed of the cultivar Kottman. Bars sharing a letter are not significantly different from each other ($P \leq 0.05$) based on relative marginal effects. Error bars are standard deviation.

There was also a significant cultivar effect for early stand ($P = 0.0206$) at V1/V2 at one environment, whereas yield was highly significant among cultivars ($P < 0.0001$) at two of the four environments. At the NWARS-2016 field environment the more susceptible cultivars with the seed treatment had greater stands and yields when compared with nontreated seed; there was no significant differences between the treatments and the nontreated seed for the more resistant cultivars (Fig. 2). For DEF-2016, the fungicide seed treatment significantly increased stand (V1/V2 and R7/R8) for the cultivar Streeter with high partial resistance to *Ph. sojae*, as well as Sloan with moderate susceptibility. All these cultivars are susceptible to species of *Pythium*. Yields of the cultivars Clermont, Streeter, and Conrad were all significantly higher. The cultivar Sloan in the DEF-2015 environment had a lower stand than the initial population, indicating additional disease development throughout the season (data not

shown), which can explain in part the lack of significance for yield. As in 2015, the seed treatment did not affect the yield of cultivars with higher levels of resistance to *Ph. sojae* (Clermont, Dennison, Kottman, and Lorain). The fungicide-treated seed for the cultivar Sloan also did not have greater yields, because of repeated losses during the season to *Phytophthora* root and stem rot.

The seed of cultivars Conrad and Sloan treated with the mixture of ethaboxam (12.0 μg a.i./seed) plus metalaxyl (3.2 μg of a.i./seed) plus clothianidin (75.1 μg of a.i./seed) plus ipconazole (3.8 μg of a.i./seed) were compared with the nontreated in seven environments. With environment as a fixed effect, the seed treated with fungicide mixture had higher plant populations and greater yields than the nontreated seed (Table 7).

Seed plate assay. Of the 49 isolates initially included in the experiments, five isolates did not cause seed rot and were not included in

Fig. 4. The average seed rot score caused by 13 species of *Pythium* and *Phytophthora* on nontreated or fungicide-treated soybean seed (n = number of isolates per species) in a seed plate assay. Rates of each of the active ingredients for each mixture are as follows (in μg of a.i./seed): mfenoxam (11.7), fludioxonil (3.8) plus the insecticide thiamethoxam (77.0), metalaxyl (13.2) plus pyraclostrobin (16.9) plus fluxapyroxad (8.3) plus the insecticide imidacloprid (127.0), or ethaboxam (12.0) plus metalaxyl (3.2) plus ipconazole (4.0) and the insecticide clothianidin (81.0 μg of a.i./seed). All data were grouped together and analyzed together, and no within-species differences were found in this group; data are separated into four graphs for visual clarity. Bars sharing a letter are not significantly different from one another ($P \leq 0.05$) based on relative marginal effect. Standard deviation is shown.

the pathogenicity or fungicide efficacy final analysis. The mean seed rot severity for the nontreated seed ranged from 1.7 to 3.0, which is an indication of the relative aggressiveness for each isolate in this study (Fig. 3). There were 44 isolates with relative marginal effect scores of >0.30 on the nontreated seed. *Pythium ultimum* var. *ultimum*, *Pythium diclinum*, *Pythium cryptoirregularare*, and *Phytophthora helicoides* had the greatest seed rot scores on the nontreated Kottman seed. In this study, four isolates of *P. sojae* and *Phytophthora sansomeana* caused a low amount of seed rot, which was expected because of the *Rps* genes in Kottman (*Rps1k* and *Rps3a*; Dorrance et al. 2009).

Soybean seed treated with any of the three fungicide mixtures had significantly less ($P < 0.05$) seed rot when compared with the nontreated seed for 24 isolates of 13 different species of *Pythium*, *Phytophthora* (Fig. 4), and six isolates of two species of *Phytophthora* (Fig. 5). All three seed treatment mixtures limited seed infection of the *Pythium* spp. compared with the nontreated seed. However, there were differences in the level of efficacy between the seed treatments for isolates of the species *Py. ultimum* var. *ultimum*, *Pythium oopapillum*, *Phytophthora vexans*, and *Pp. helicoides* (Fig. 6). For an isolate of *Pp. helicoides*, seed treated with mefenoxam (11.7 μg of a.i./seed) had significantly greater seed rot scores than metalaxyl (13.2 μg of a.i./seed) plus pyraclostrobin (16.9 μg of a.i./seed) or ethaboxam (12.0 μg of a.i./seed) plus metalaxyl (3.2 μg of a.i./seed) but significantly lower than the nontreated seed. Similarly, the mefenoxam (11.7 μg of a.i./seed) treatment reduced seed rot compared with the nontreated seed, but seed rot scores were greater

than the scores from seed treated with ethaboxam (12.0 μg of a.i./seed) plus metalaxyl (3.2 μg of a.i./seed) for *Py. ultimum* var. *ultimum* (Fig. 6).

For a comparison between the response of the three fungicide seed treatment mixtures to six isolates of *Py. ultimum* var. *ultimum*, each with a similar level of aggressiveness, the fungicides reduced the level of seed rot compared with the nontreated for four of the isolates. Mefenoxam (11.7 μg of a.i./seed) was not significantly different from the nontreated for one isolate (N508.1) and had significantly greater seed rot scores compared with seed treated with ethaboxam (12.0 μg of a.i./seed) plus metalaxyl (3.2 μg of a.i./seed) for another (N201.2) (Fig. 7). These differences in response could be because of differences in sensitivity to mefenoxam, because some isolates within Ohio are insensitive (Broders et al. 2007; Dorrance et al. 2004).

Discussion

This study evaluated the efficacy of ethaboxam in combination with metalaxyl as a soybean seed treatment in Ohio fields with a history of seedling disease caused by multiple species of *Pythium*, *Phytophthora*, and *Phytophthora* and compared them with other seed treatment formulations in a seed plate assay. Our data indicate that ethaboxam used in combination with metalaxyl improved soybean stands across multiple environments and was effective in managing a number of species of *Phytophthora*, *Phytophthora*, and *Pythium*.

The fungicide ethaboxam was initially used in Korea to manage cucumber downy mildew, potato late blight, and pepper *Phytophthora* blight starting in the late 1990s (Kim et al. 1999, 2003). Ethaboxam was recently labeled for soybean seed treatment in the United States, and efficacy toward *Ph. sojae* and several species of *Pythium* in amended agar assays have been reported (Dorrance et al. 2012; Matthiesen et al. 2016; Radmer et al. 2017). Ethaboxam was also effective in managing metalaxyl-resistant *Py. ultimum* var. *ultimum* on spring wheat, against *Phytophthora capsici* on pepper in the field and greenhouse (Matheron and Porchas 2015; White et al. 2019), and against *Pseudoperonospora cubensis* isolates that had reduced sensitivity to fluopicolide and propamocarb (Thomas et al. 2018). Earlier lab and greenhouse assays indicated that the combination of ethaboxam and metalaxyl as a fungicide seed treatment would provide protection from diverse *Pythium* spp. (Dorrance et al. 2012).

Environmental variation played a key part in the differences observed in the fungicide seed treatment effects across the individual environments in this and in other studies (Bradley 2008; Dorrance et al. 2009; Esker and Conley 2012). Specifically, the amount of rainfall in the 14 dap appears to affect the amount of disease pressure from *Phytophthora*, *Phytophthora*, and *Pythium*, as noted in previous field seed treatment studies (Bradley 2008; Dorrance et al. 2009). Esker and Conley (2012) did not find a difference for the efficacy of seed treatments in their study, but the rainfall 10 dap in the Wisconsin study ranged from 0.5 to 72.9 mm, which was less than what occurred during our studies.

Seed treatment fungicide affected early plant populations in four of the five field environments that received >2.5 cm of water 14 dap. Because the efficacy of fungicide seed treatments could be determined only with adequate disease pressure, efforts should be taken to properly irrigate research field sites to encourage pathogen development. However, overwatering or flooding may mask any effects by a fungicide or otherwise affect the study, as noted by others (Henshaw et al. 2007a, b; Kirkpatrick et al. 2006; VanToai et al. 1994). In this study, the effect of flooding overriding seed treatment effects occurred at three locations: NWARS-2015, with 14.5 cm of combined rainfall and irrigation 14 dap; and VW-2016 and DEF-2016, with nearly 48 cm of rainfall in total during the growing season. Despite these fields having a history of *Pythium* and *Phytophthora* infection, no significant fungicide effects on yield were found at these environments when excessive rainfall occurred.

The field sites in 2014 compared seed treatments containing ethaboxam and metalaxyl combinations with metalaxyl alone. When both active ingredients were present, stands were improved. Similarly, ethaboxam as part of a fungicide mixture improved stands

Fig. 5. The average root rot score of soybean seed treated with one of three fungicide seed treatments and nontreated in a seed plate assay with four isolates of *Phytophthora sojae* and two isolates of *Phytophthora sansomeana*. Rates of each of the active ingredients for each mixture are as follows (in μg of a.i./seed): mefenoxam (11.7), fludioxonil (3.8) plus the insecticide thiamethoxam (77.0), metalaxyl (13.2) plus pyraclostrobin (16.9) plus fluxapyroxad (8.3) plus the insecticide imidacloprid (127.0), or ethaboxam (12.0) plus metalaxyl (3.2) plus ipconazole (4.0) and the insecticide clothianidin (81.0 μg of a.i./seed). The isolates were analyzed together, and no within-species differences were found in this group; *Phytophthora* data are shown separately for visual clarity. Bars sharing a letter are not significantly different from one another ($P \leq 0.05$) based on relative marginal effects. Error bars indicate the standard deviation.

Fig. 6. Average seed rot scores of *Phytophthium helicoides*, *Phytophthium vexans*, *Pythium ultimum* var. *ultimum*, and *Pythium oopapillum* that exhibited a differential response in a seed plate assay to the fungicide seed treatments, with standard deviations shown. Rates of each of the active ingredients for each mixture are as follows (in μg of a.i./seed): mfenoxam (11.7), fludioxonil (3.8) plus the insecticide thiamethoxam (77.0), metalaxyl (13.2) plus pyraclostrobin (16.9) plus fluxapyroxad (8.3) plus the insecticide imidacloprid (127.0), or ethaboxam (12.0) plus metalaxyl (3.2) plus ipconazole (4.0) and the insecticide clothianidin (81.0 μg of a.i./seed). Nontreated seed was used as a control. Bars with the same letter are not significantly different ($P \leq 0.05$) based on relative marginal effects. Error bars represent standard deviation.

Fig. 7. Response of six *Pythium ultimum* var. *ultimum* isolates to three fungicide seed treatments and nontreated control used in the plate assay, with standard deviations shown. Within each isolate, the level of seed rot on the following seed treatments with the rates of each of the active ingredients for each mixture as follows (in μg of a.i./seed): mfenoxam (11.7), fludioxonil (3.8) plus the insecticide thiamethoxam (77.0), metalaxyl (13.2) plus pyraclostrobin (16.9) plus fluxapyroxad (8.3) plus the insecticide imidacloprid (127.0), or ethaboxam (12.0) plus metalaxyl (3.2) plus ipconazole (4.0) and the insecticide clothianidin (81.0 μg of a.i./seed). Bars with the same letter are not significantly different ($P \leq 0.05$). Error bars represent standard deviation.

and yield of spring wheat in northern Idaho, where metalaxyl-insensitive strains of *Pythium* were reported (White et al. 2019). However, the metalaxyl treatment alone (6.0 µg of a.i./seed) did not protect against seed and root disease for cultivars Conrad and Sloan. This finding is consistent with previous reports of low rates of metalaxyl having limited efficacy toward *Ph. sojae* (Dorrance and McClure 2001) and previous reports of the emergence of populations of *Pythium* with insensitivity to this fungicide in Ohio (Broders et al. 2007; Dorrance et al. 2004). Based on these studies, production of crops where metalaxyl-insensitive strains of *Pythium* are an issue, the addition of ethaboxam as a seed treatment component can limit losses.

At the DEF-2015 field site, there was a significant fungicide-cultivar interaction; Conrad and Sloan both had greater yields from the fungicide seed treatments, whereas cultivars Lorain and Kottman did not. This difference in fungicide seed treatment effect may be because of the host resistance of Lorain (*Rps1c*, high partial to both *Ph. sojae* and some *Pythium*) and Kottman (*Rps1k* + *Rps3a*, high partial to *Ph. sojae*) performing better against the particular pathogen population in that environment. Interestingly, for three of the four 2016 environments plant populations were greater for the one fungicide seed treatment with ethaboxam plus metalaxyl, but yields were greater in only one environment for all but one cultivar (Lorain), which has a combination of resistance toward *Ph. sojae* and *Pythium* spp. In this highly favorable environment for seedling disease, the more susceptible cultivars performed better with the ethaboxam plus metalaxyl seed treatment when compared with the nontreated seed. Garnica and Giesler (2019) reported similar trends where this same seed treatment increased the number of soybean plants per hectare in several environments in Nebraska in 2017 and 2018. Soybeans have a great potential to compensate for reduced stands, and high standard deviations are very common in these types of studies.

The effect that resistance in a specific cultivar can have on the outcomes of these studies cannot be overstated. Cultivars with both *Rps* genes and partial resistance toward *Ph. sojae* had the most consistent yield over many environments (Dorrance et al. 2003, 2009). When yields were averaged across years, with or without disease pressure, cultivar was a major factor for determining whether a seed treatment was cost effective in Wisconsin (Esler and Conley 2012). However, Esler and Conley (2012) emphasized that seed treatments are justified as insurance against loss because of the rapid changeover in soybean cultivars. This practice has been cost effective approximately 30% of the time in studies in North Dakota (Bradley 2008) and Wisconsin (Esler and Conley 2012). In this study for the highly susceptible cultivar Sloan, seed treatments were cost effective if measured by yield. The overall effects of these seed treatment fungicides were more readily measured on cultivars with greater susceptibility to species of *Phytophthora*, *Phytophythium*, or *Pythium*. Future field studies should include cultivars with a range of level of host resistance, taking care to include highly susceptible cultivars. This study is in agreement with others that suggest that soybean cultivar selection should be used in combination with fungicide seed treatment to protect against diseases caused by fungi and oomycete pathogen diseases (Dorrance et al. 2004, 2009; Esler and Conley 2012; Garnica and Giesler 2019; Kandel et al. 2016; Matthiesen et al. 2016; Marburger et al. 2014).

The seed plate assay was used to evaluate the efficacy of commercial fungicide combinations to a large number of *Pythium* spp. individually. This assay has been used previously to evaluate the relative pathogenicity of each oomycete species on nontreated seed (Broders et al. 2007) and to determine the effect of temperature on *Pythium* spp. aggressiveness (Matthiesen et al. 2016). In this study, there was a wide range of pathogenicity among the *Phytophthora*, *Pythium*, and *Phytophythium* isolates. Variation in the response to the seed treatments also was found between isolates of *Py. ultimum* var. *ultimum*. More importantly, this seed plate assay was also useful in identifying fungicide seed treatment-isolate interactions, which would be a great asset to companies as they explored new active ingredients or formulations.

Overall, our findings from the field environments support the findings from previous studies; fungicide seed treatments have stand- and yield-protecting benefits against species of *Phytophthora*, *Phytophythium*, and *Pythium* (Bradley 2008; Esler and Conley 2012; Garnica and Giesler 2019; Gaspar et al. 2014, 2017; Guy and Oplinger 1989; Guy et al. 1989; Poag et al. 2005; Urrea et al. 2013). Additionally, these results suggest that ethaboxam used in combination with metalaxyl was effective in managing a number of species of *Phytophthora*, *Pythium*, and *Phytophythium* commonly recovered from Ohio symptomatic soybean seedlings. Thus, this active ingredient could be a valuable tool for managing soybean seedling disease in Ohio and other regions where metalaxyl insensitivity is a concern.

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