Temperature Affects Aggressiveness and Fungicide Sensitivity of Four *Pythium* spp. that Cause Soybean and Corn Damping Off in Iowa

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Abstract

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Damping off of soybean and corn, caused by *Pythium* spp., is favored by cool temperatures and wet soil conditions and is primarily managed using fungicide seed treatments. The goal of this research was to determine the effect of temperature on aggressiveness and fungicide sensitivity of *Pythium* spp. recovered from soybean and corn in Iowa. A total of 21 isolates of four of the most prevalent *Pythium* spp. in Iowa were screened. Seed and seedling assays were used to quantify the aggressiveness of *P. lutarium*, *P. oopapillum*, *P. sylvaticum*, and *P. torulosum* on soybean and corn at 13, 18, and 23°C. Isolates recovered from soybean or corn were equally pathogenic on both hosts. *P. torulosum* was more aggressive at

13°C compared with 18 and 23°C. Conversely, *P. sylvaticum* was more aggressive at 18 and 23°C than at 13°C. A plate assay was used to assess fungicide sensitivity to seven fungicides that are commonly used as seed treatments, and EC₅₀ values at each of the three temperatures were determined and compared. EC₅₀ values for *P. torulosum* were higher for all fungicides tested at 13°C, compared with 18 or 23°C, whereas EC₅₀ values for *P. sylvaticum* were higher for all fungicides at 18 and 23°C compared with 13°C. These data contribute to our understanding of the effect of soil temperature on the risk of soybean and corn damping off, which may aid in the development of more effective management practices.

Pre- and post-emergence damping off in soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays* L.), caused by various species in the genus *Pythium*, is influenced considerably by weather conditions in the spring, and is more prevalent when soil temperatures are cool (<18°C) and abundant rainfall has occurred (Robertson and Munkvold 2012; Robertson et al. 2014). *Pythium* spp. are oomycetes and require saturated soil conditions for the production and movement of zoospores that are chemotactically attracted to roots where infection occurs (Stanghellini 1974). Damping off can significantly reduce crop yields due to reduced seedling emergence associated with seed and root rot, injury to the hypocotyl or mesocotyl, and reduced plant growth (Dodd and White 1999; Hartman et al. 1999).

Global climate change predictions suggest increases in average precipitation and temperatures across the corn belt that could substantially increase disease risk (Hatfield et al. 2011). Farmers in Iowa are planting corn earlier (mid-April) (Elmore 2013), when soil temperatures are typically below 13°C (http://www.nass.usda.gov/Statistics_by_State/Iowa/Publications). Soybean planting closely follows corn planting in Iowa, and thus, soybeans are also being planted earlier, when soil temperatures may be below 18°C. At these low temperatures, seed germination is delayed, thereby increasing the length of time when infection may occur (Martin and Loper 1999). Furthermore, when abundant rainfall occurs soon after planting, risk of damping off caused by *Pythium* is high (Yang 1997).

Iowa is a major producer of soybean and corn with 9.9 million and 13.2 million acres planted in 2014, respectively (http://www.nass.usda.gov/Statistics_by_State/Iowa/); therefore, economic losses associated with damping off can be significant to both soybean and corn production in Iowa. In 2012, corn pathologists from the United States and Ontario, Canada estimated yield losses due to *Pythium* to total 93.6 million bushels (ranked third out of 34 diseases) (Mueller 2014a),

while in 2013, seedling blight caused by *Pythium* spp. was estimated to have resulted in 149.8 million bushels (ranked first) (Mueller 2014b). In southeastern Iowa, increased corn seedling disease since 2008, especially after abundant rainfall and low soil temperatures (A. Robertson, unpublished), prompted research to determine what species of *Pythium* are prevalent in causing damping off. Damping off of soybean has been reported less frequently in Iowa than other soybean diseases, but economic losses do occur, especially if conditions are conducive soon after planting. Estimated yield losses due to *Pythium* in soybean-producing states in the United States were ranked 4th and 2nd out of all diseases evaluated in 2012 and 2013, respectively (http://extension.crops.illinois.edu/fieldcrops/diseases/yield_reductions.php). A recent survey of soybean seedling diseases in 2011 determined that 19 *Pythium* spp. were present in Iowa (Rojas et al. 2013).

Fungicide seed treatments are often used to protect germinating seed and seedlings from infection by soilborne pathogens to reduce stand losses caused by damping off. Although corn seed has been treated with fungicides, such as the phenylamides mefenoxam and metalaxyl (e.g., Apron and Allegiance, respectively) since the late 1970s (Cohen and Coffey 1986), seed treatments on soybean are relatively new and usage has been increasing (Bradley 2008; Esker and Conley 2012). More recently, strobilurins or quinone outside inhibitors (QoI) (e.g., trifloxystrobin [Trilex], azoxystrobin [Dynasty], and pyraclostrobin [Stamina]) have been used in addition to the phenylamides to control *Pythium* and other fungal pathogens (Broders et al. 2007a; Ypema and Gold 1999). Both the phenylamides and QoIs are considered high risk for the development of fungicide resistance (Mueller et al. 2013). For instance, reduced sensitivity of *Pythium* spp. recovered from soybean and corn to fungicide seed treatments, which include mefenoxam and some of the QoIs, has been reported (Broders et al. 2007a; Dorrance et al. 2004). Recently, ethaboxam, a thiazole carboxamide fungicide, has shown excellent efficacy against several oomycetes (Dorrance et al. 2012). Currently, there is no known resistance to ethaboxam.

We undertook the following research to increase our understanding of the biology of the corn/soybean-*Pythium* interactions in Iowa. The objectives of this study were to: (i) identify *Pythium* spp. affecting corn in Iowa, (ii) determine the pathogenicity of four of the most prevalent *Pythium* spp. in Iowa recovered from soybean on corn and vice versa, (iii) quantify the aggressiveness of those four *Pythium*

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* The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure is included in the online edition.

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spp. on soybean and corn at three different temperatures, two temperatures of which are similar to those that occur at planting in Iowa, and (iv) quantify the sensitivity of those four *Pythium* spp. to fungicides at the three temperatures. Results obtained from this research can be incorporated into disease management practices for *Pythium* damping off affecting soybean and corn.

Materials and Methods

Isolation and identification of *Pythium* spp. from corn. Symptomatic corn seedlings were collected during May 2012 from fields in southern Iowa with stand losses from seedling blight. The seedlings were washed with tap water for 30 min and rinsed with sterile distilled water. Sections of mesocotyl tissue, 2 to 3 mm from the edge of a lesion, were cut aseptically, placed between two sterile paper towels, and pressed to remove excess water (Broders et al. 2007a). Sections of symptomatic seedling tissue were placed underneath corn meal agar media containing pimarin (5 µg/ml), ampicillin (250 µg/ml), rifampicin (10 µg/ml), pentachloronitrobenzene (50 µg/ml), and benomyl (10 µg/ml) (PARP+B), which is selective for oomycetes (Jeffers and Martin 1986). Plates were incubated at 23°C for 3 to 4 days in the dark. Isolates with coenocytic hyphae were transferred to 4% V8 juice media containing neomycin sulfate (50 µg/ml) and chloramphenicol (10 µg/ml) (DV8) for isolate identification. Putative isolates were identified to genus through morphological characteristics and cultural features using standard keys by Waterhouse (1967), Middleton (1943), and van der Plaats-Niterink (1981).

DNA extraction, PCR amplification, and sequencing. To confirm identification of each putative species of *Pythium* recovered from symptomatic corn seedlings, the internal transcribed spacer (ITS) of rDNA of each isolate was amplified and sequenced. Each isolate was grown on DV8 at 23°C for 3 to 4 days in the dark. Mycelium from the edge of each colony was removed by scraping with a sterile toothpick and lysed using *Lyse and Go PCR reagent* (Thermo Scientific Inc., Waltham, MA). Following the manufacturer's protocol, the cell lysates were used directly for PCR amplification of the nuclear rDNA region of the ITS including the 5.8 S rDNA. PCR amplification was performed in 50 µl reactions using 2 µl of lysate and 2.5 units of Taq polymerase with supplied buffer (Promega, Madison, WI), 50 µM dNTPs and 0.4 µM of each universal

primer, ITS-6 (5'-GAAGGTGAAGTCGTAACAAGG-3') (Cooke et al. 2000) and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). Thermocycling conditions were: 94°C for 2 min, 35 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 10 min. Each PCR product (approximately 800 bp) was purified with the QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, CA) and sequenced with the primer ITS-4. Identification of each isolate was done by searching sequence data against the GenBank database using BLAST and comparing with ITS sequences of related *Pythium* spp. obtained from the National Center of Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov).

Seed and seedling assays. A total of 21 isolates representative of four of the most prevalent species of *Pythium* recovered from diseased corn seedlings in the aforementioned survey or diseased soybean seedlings in the Rojas et al. (2013) survey were screened for pathogenicity and aggressiveness on soybean and corn (Table 1). We define pathogenicity as the ability of a *Pythium* spp. to cause disease, whereas we define pathogen aggressiveness as the degree to which disease injury is caused by a *Pythium* spp. (D'Arcy et al. 2001). Pathogenicity and aggressiveness assays were assessed on two cultivars of soybean, Sloan and Archer, and one corn hybrid (proprietary hybrid) using a seed assay similar to that described by Broders et al. (2007a) and a seedling assay described by Rojas et al. (2013). Hereafter, hosts from which *Pythium* isolates were recovered will be referred to as "recovered hosts" and hosts inoculated with each isolate of *Pythium* in the aggressiveness assay (soybean cultivars Sloan and Archer, and the corn hybrid) will be referred to as "inoculated hosts." For the seed assay, isolates were grown on DV8 at 23°C for 3 to 4 days in the dark and a 2 mm plug from the edge of the colony was transferred to the center of a 100 mm petri dish containing DV8. After 72 h, 10 seeds of the inoculated hosts were surface sterilized in 0.525% sodium hypochlorite solution for 3 min and rinsed in sterile water, placed 3 to 5 mm from the edge of the dish, and incubated in the dark in growth chambers at 13, 18, or 23°C. Plates were arranged in a randomized complete block design at each temperature with three replicates per isolate and each replicate as an individual block. Seven days after placing seeds on plates, each ungerminated or germinated (radicle >3 mm long) seed was assessed for disease incidence and disease severity. Disease incidence was assessed as the percentage of seeds with symptoms of seed and/or root rot. Seed rot severity was measured as the percentage of ungerminated seed that were rotted. Root rot severity was visually assessed as an estimate of the percentage of root tissue on each germinated seed that was rotted. Each experiment screened one species on all inoculated hosts and was conducted three times with each run separated by at least 24 h.

A seedling assay, similar to that described by Rojas et al. (2013), was also used to assess pathogenicity and aggressiveness of the *Pythium* isolates. Inoculum of each isolate was prepared on rice using a modified protocol developed by Stewart and Robertson (2011). In a vented autoclavable plastic bag, 453 g of parboiled rice and 323 ml of distilled water were autoclaved for 40 min twice with each cycle separated by 24 h. Rice was inoculated with a 3- to 4-day-old *Pythium* culture grown on DV8, incubated for 7 to 10 days at 23°C in the dark, and dried for 2 to 3 days. Ten milliliters of 7- to 10-day-old inoculum was placed in a layer approximately 2 to 3 cm below 10 seeds of the inoculated hosts planted in an 8 ounce polystyrene cup filled with coarse vermiculite and incubated in growth chambers set at 13, 18, or 23°C on a diurnal cycle of 16 h light and 8 h dark. Cups were arranged in a randomized complete block design at each temperature with three replicates per isolate and each replicate as an individual block. After 14 days, seedling roots were washed and assessed for disease incidence (percent plants with rotted roots) and disease severity (percent root tissue rotted for each seedling). Each experiment screened one species on all inoculated hosts and was conducted three times with each run separated by at least 24 h.

Koch's postulates were performed for all isolates using the above-mentioned seedling assay. After 14 days, sections of symptomatic seedling tissue of both soybean and corn were placed under PARP

Table 1. *Pythium* isolates recovered from symptomatic soybean seedling tissue in 2011 or symptomatic corn seedling tissue in 2012 in Iowa, which were used in aggressiveness and fungicide sensitivity experiments

Isolate ^y	Species	Host	Location ^z
IASO 4-46	<i>P. lutarium</i>	Soybean	Harrison
IASO 5-35	<i>P. lutarium</i>	Soybean	Osceola
IASO 6-2.2r	<i>P. lutarium</i>	Soybean	Osceola
IACO 12-3	<i>P. lutarium</i>	Corn	Lee
IACO 12-5	<i>P. lutarium</i>	Corn	Lee
IASO 1-7.28	<i>P. oopapillum</i>	Soybean	Harrison
IASO 2-11.8	<i>P. oopapillum</i>	Soybean	Poweshiek
IASO 3-44	<i>P. oopapillum</i>	Soybean	Marshall
IACO 8-7	<i>P. oopapillum</i>	Corn	Lee
IASO 1-16rt	<i>P. sylvaticum</i>	Soybean	Harrison
IASO 2-8.18	<i>P. sylvaticum</i>	Soybean	Poweshiek
IASO 3-37	<i>P. sylvaticum</i>	Soybean	Marshall
IACO 12-1	<i>P. sylvaticum</i>	Corn	Lee
IACO 14-4	<i>P. sylvaticum</i>	Corn	Wayne
IACO 22-6	<i>P. sylvaticum</i>	Corn	Madison
IASO 1-13.10h	<i>P. torulosum</i>	Soybean	Harrison
IASO 2-26	<i>P. torulosum</i>	Soybean	Poweshiek
IASO 3-6	<i>P. torulosum</i>	Soybean	Marshall
IACO 1-8	<i>P. torulosum</i>	Corn	Mahaska
IACO 8-1	<i>P. torulosum</i>	Corn	Lee
IACO 12-4	<i>P. torulosum</i>	Corn	Lee

^y Isolates recovered from symptomatic soybean (SO) seedling tissue in 2011 or symptomatic corn (CO) seedling tissue in 2012 in Iowa (IA).

^z Counties in Iowa where symptomatic seedlings were collected.

+B and incubated at 23°C for 3 to 4 days in the dark. *Pythium* isolates were identified to species using morphological characteristics and cultural features as described above (Ali-Shtayeh and Dick 1985; Bala et al. 2010; Middleton 1943; van der Plaats-Niterink 1981; Waterhouse 1967).

Fungicide sensitivity assay. All isolates (Table 1) were screened for fungicide sensitivity to metalaxyl (Alliance; Bayer CropScience), ethaboxam (Valent U.S.A.), azoxystrobin (Dynasty; Syngenta Crop Protection Inc.), pyraclostrobin (Headline, BASF Corp.), trifloxystrobin (Trilex, Bayer CropScience), captan (Captan 400; Bayer CropScience), and thiram (Bayer CropScience). Fungicides were dissolved in dimethylsulfoxide (DMSO) to provide the correct concentration of active ingredient and added to clarified DV8 after autoclaving when media temperature was approximately 50°C (Broders et al. 2007a). All fungicides were assessed at 0, 0.1, 1.0, 10, and 100 µg a.i./ml of commercial-grade product. For all strobilurin fungicides, 50 µg/ml of salicylhydroxamic acid (SHAM) was added to the media to inhibit the alternative oxidase respiratory pathway (Olaya et al. 1998). This concentration of SHAM was determined for each *Pythium* spp. by testing various concentrations of SHAM to determine the concentration required for 20 to 40% mycelial inhibition (Broders et al. 2007a). A set of control plates amended with SHAM was included for each isolate.

Isolates were grown on DV8 at 23°C for 3 to 4 days in the dark and a 2 mm plug from the colony edge was placed 1 to 2 mm from the edge of a 100 mm petri dish containing clarified DV8 nonamended or amended with each concentration of fungicide. Plates were arranged in a randomized complete block design at each temperature with three replicates per isolate and each replicate as an individual block. After 48, 72, and 96 h at 13, 18, or 23°C, colony length was measured from the edge of the plug to the furthest edge of the colony. For each isolate, percent mycelial growth inhibition was calculated for each concentration by subtracting the average colony length on the fungicide-amended plates from the average colony length of the nonamended plates, then dividing by the average colony length of the nonamended plates, and multiplying by 100. For the strobilurins, percent mycelial growth inhibition was calculated for each concentration of plates amended with SHAM by subtracting the average colony length of the fungicide-SHAM-amended plates from the average colony length of the SHAM-amended control plates, then dividing by the average colony length of the SHAM-amended control plates, and multiplying by 100. Each experiment screened one species on all fungicides and was conducted three times, with each run separated by at least 24 h.

Statistical analysis. Pathogenicity and aggressiveness data among species and isolates at each temperature was analyzed using PROC MIXED of SAS (version 9.4; SAS Institute Inc. Cary, NC) where temperature, species, and isolates were treated as fixed effects. When a treatment effect was detected by ANOVA, Fisher's least significant difference (LSD) test at $P = 0.05$ was used to compare treatment means (Sokal and Rohlf 1989) of pathogenicity and aggressiveness data. Regression analysis of probits was used to calculate the EC_{50} value of the percentage of mycelia inhibition against a logarithm to base 10 value of fungicide concentrations (Zadoks and Schein 1979). Variation in sensitivity across temperatures to seven different fungicides based on EC_{50} values was analyzed using PROC GLM of SAS (SAS Institute). Treatment means of fungicide sensitivity data were separated using the Tukey's honestly significant difference (HSD) test at $P = 0.05$.

Results

***Pythium* spp. isolation and identification from symptomatic corn seedlings.** Symptomatic corn seedlings were collected from 29 commercial fields (approximately 10 seedlings per field) in 10 counties in southern and southeastern Iowa. A total of 87 *Pythium* isolates were recovered from 59 seedlings that had been collected from 19 fields. From each, an amplicon approximately 800 bp long was amplified using the primer pair ITS-6 and ITS-4. Comparison of sequence data with data in the NCBI database identified nine *Pythium* spp. The most prevalent species was *P. torulosum* (72 of

87 isolates), which was recovered from 53 (90%) seedlings. Other species present in order of prevalence were: *P. sylvaticum* ($n = 3$), *P. oopapillum* ($n = 2$), *P. lutarium* ($n = 2$), *P. heterothallicum* ($n = 2$), *P. schmittneri* ($n = 2$), *P. arrhenomanes* ($n = 2$), *P. spinosum* ($n = 1$), and *P. irregulare* ($n = 1$). Coinfections of *P. torulosum* and either *P. sylvaticum* ($n = 1$), *P. lutarium* ($n = 1$), or *P. heterothallicum* ($n = 2$) occurred on four seedlings, while one isolate of each of *P. sylvaticum* and *P. irregulare* were recovered from one seedling.

Seed and seedling assays. Isolates recovered from soybean were pathogenic on corn and isolates recovered from corn were pathogenic on soybean in each assay. All *Pythium* spp. were pathogenic on each of the inoculated hosts at each of the temperatures in each assay compared with the noninoculated control ($P < 0.0001$). No difference in aggressiveness, as measured by seed and root rot severity, of *Pythium* isolates recovered from soybean for each of the four species in each assay was detected; therefore, severity scores for all isolates of each species were combined for each assay. Similarly, no significant difference in aggressiveness of *Pythium* isolates recovered from corn for each species in each assay was detected, so all isolates for each species were combined in each assay. No difference between experimental runs at each temperature was detected; therefore, all runs per species were combined for each assay.

Seed assay - seed rot. No effect of temperature on aggressiveness of *P. lutarium* was detected ($P = 0.0713$); and seed rot severity ranged from 43 to 75% across all temperatures (Fig. 1A to C). For *P. oopapillum*, an effect of temperature on aggressiveness was detected across all temperatures ($P < 0.0001$). At 13°C, seed rot severity was greater on all three inoculated hosts than at 18 and 23°C (Fig. 1D to F). Temperature affected the aggressiveness of *P. sylvaticum* across all temperatures ($P < 0.0001$). At the higher temperatures evaluated, 18 and 23°C, seed rot severity was greater on all three inoculated hosts than at 13°C (Fig. 1G to I). At 18°C, seed rot severity was lower on Archer when compared with Sloan and corn for isolates recovered from soybean ($P = 0.0005$), but not those recovered from corn ($P = 0.0645$) (Fig. 1H). Furthermore, seed rot severity was lower on Archer compared with Sloan and corn at 23°C for isolates recovered from each host ($P < 0.0001$) (Fig. 1I). For *P. torulosum*, an effect of temperature on aggressiveness was detected for all three inoculated hosts across all temperatures ($P < 0.0001$). Seed rot severity was greatest at 13°C on all three inoculated hosts compared with 18 and 23°C (Fig. 1J to L). At 13°C, seed rot severity was lower on Archer than on Sloan or corn for isolates recovered from soybean ($P = 0.0004$), but no difference in seed rot severity for isolates recovered from corn was observed ($P = 0.0552$) (Fig. 1J).

Seed assay - root rot. For *P. lutarium*, no effect of temperature on aggressiveness was detected ($P = 0.1328$) and root rot severity ranged from 48 to 82% across all three temperatures (Fig. 2A to C). An effect of temperature on aggressiveness was detected across all temperatures for *P. oopapillum* ($P < 0.0001$). Root rot severity was greater at 13°C on all three inoculated hosts compared with 18 and 23°C (Fig. 2D to F). Similarly to seed rot severity, root rot severity of *P. sylvaticum* at 18 and 23°C was greater on all three inoculated hosts than at 13°C (Fig. 2G to I) and an effect of temperature on aggressiveness was detected across all temperatures ($P < 0.0001$). At 18 and 23°C, root rot severity of isolates recovered from soybean was lower on Archer than Sloan and corn ($P = 0.0004$ and $P < 0.0001$, respectively) (Fig. 2H and I). For isolates recovered from corn, root rot severity was lower on Archer than on Sloan and corn at 23°C ($P = 0.0020$) (Fig. 2I). For *P. torulosum*, an effect of temperature on aggressiveness was detected for all three inoculated hosts across all temperatures ($P < 0.0001$). Root rot severity was greatest at 13°C on all three inoculated hosts compared with 18 and 23°C (Fig. 2J to L). At 13°C, root rot severity was lower on Archer than on Sloan and corn for isolates recovered from soybean ($P = 0.0011$), but no difference in root rot severity for isolates recovered from corn was observed ($P = 0.0204$) (Fig. 2J).

Seedling assay - root rot. No effect of temperature on aggressiveness of *P. lutarium* was detected ($P = 0.2211$) and root rot severity ranged from 44 to 84% across all temperatures (Fig. 3A to C). For *P. oopapillum*, an effect of temperature on aggressiveness was

detected across all temperatures ($P < 0.0001$). Root rot severity was greatest at 13°C on all three inoculated hosts compared with 18 and 23°C (Fig. 3D to F). Temperature affected aggressiveness of *P. sylvaticum* across all temperatures ($P < 0.0001$). At 18 and 23°C, root rot severity was greater on all three inoculated hosts than at 13°C (Fig. 3H and I). Isolates recovered from corn at 18 and 23°C caused lower root rot severity on Archer than on the other hosts ($P < 0.0001$ and $P = 0.0002$, respectively) (Fig. 3H and I). Similar to the in vitro assays, an effect of temperature on aggressiveness was detected for all three inoculated hosts across all temperatures ($P < 0.0001$) for

P. torulosum. Root rot severity was lower on Archer than on Sloan and corn at 13°C for isolates recovered from corn ($P = 0.0002$) (Fig. 3J). At 18°C, isolates recovered from corn showed lower root rot severity on Archer than on the other hosts ($P = 0.0007$) (Fig. 3K).

Fungicide sensitivity assay. Sensitivity to all fungicides varied among species. All species were more sensitive to metalaxyl, ethaboxam, Captan, and thiram (Table 2), than the strobilurins (Table 3), especially pyraclostrobin. A temperature effect on EC_{50} values was detected for *P. oopapillum*, *P. sylvaticum*, and *P. torulosum*, but not *P. lutarium* (Tables 2 and 3).

Fig. 1. Percent seed rot severity of four species of *Pythium* recovered from soybean (S_) or corn (C_) seedlings (recovered host) on two soybean cultivars, Archer (_a) and Sloan (_s), and one corn hybrid (_c) (inoculated host) using a seed assay. Isolates of *Pythium lutarium* (A-C); *P. oopapillum* (D-F); *P. sylvaticum* (G-I); and *P. torulosum* (J-L), incubated at 13, 18, and 23°C.

No effect of temperature on sensitivity of *P. lutarium* to five of the fungicides was detected, except for azoxystrobin ($P < 0.0001$) and pyraclostrobin ($P < 0.0001$) (Table 3). At 18 and 23°C, *P. lutarium* was approximately two times less sensitive to azoxystrobin than at 13°C (Table 3).

Temperature affected the sensitivity of *P. oopapillum* to Captan ($P < 0.0001$), azoxystrobin ($P < 0.0001$), pyraclostrobin ($P = 0.0400$), and trifloxystrobin ($P < 0.0001$). At 13 and 18°C, *P. oopapillum* was 23 times and 2.4 times less sensitive to Captan than at 23°C, respectively (Table 2). For the strobilurins that were tested,

P. oopapillum was 1.2 to 2.2 and 1.4 to 2.4 times less sensitive at 13°C compared with at 18 and 23°C, respectively (Table 3).

The effect of temperature on EC_{50} values of *P. sylvaticum* to all fungicides was dramatic. This species was far less sensitive to all fungicides at 18 and 23°C compared with 13°C (Tables 2 and 3). For example, at 18°C, *P. sylvaticum* was more than 150 times less sensitive to metalaxyl, and more than 250 times less sensitive at 23°C, than at 13°C (Table 2). For ethaboxam, the EC_{50} of *P. sylvaticum* was 5.5 and 11 times greater at 18 and 23°C, respectively, than the EC_{50} at 13°C (Table 2). Similarly, EC_{50} values for Captan were more than

Fig. 2. Percent root rot severity of four species of *Pythium* recovered from soybean (S_) or corn (C_) seedlings (recovered host) on two soybean cultivars, Archer (A_) and Sloan (S_), and one corn hybrid (C_) (inoculated host) using a seed assay. Isolates of *Pythium lutarium* (A-C); *P. oopapillum* (D-F); *P. sylvaticum* (G-I); and *P. torulosum* (J-L), incubated at 13, 18, and 23°C.

100 times greater at 18°C and 162 times greater at 23°C compared with 13°C (Table 2). Sensitivity to the strobilurins was affected by temperature to a lesser extent; for example, at 18°C, *P. sylvaticum* was 4.5 times less sensitive to trifloxystrobin than at 13°C, and 7 times less sensitive at 23°C (Table 3).

Similarly, temperature strongly influenced the sensitivity of *P. torulosum*. This species, however, was less sensitive to all fungicides at 13°C compared with 18 or 23°C (Tables 2 and 3). For metalaxyl, the EC₅₀ of *P. torulosum* at 18 and 23°C were 22 times and 50 times greater, respectively, than the EC₅₀ at 13°C (Table 2). At 13°C, *P.*

torulosum was 73 and 225 times less sensitive to Captan and thiram compared with 18°C, respectively, and 125 and 343 times less sensitive at 23°C than at 13°C, respectively (Table 2). At 13°C, *P. torulosum* was 1.5 and 2.2 times less sensitive to pyraclostrobin than at 18 and 23°C, respectively (Table 3).

Discussion

There are numerous reports of various *Pythium* spp. infecting soybean and corn in Iowa and the Midwest (Broders et al. 2007a; Dorrance et al. 2004; Rizvi and Yang 1996; Rojas et al. 2013; Zhang

Fig. 3. Percent root rot severity of four species of *Pythium* recovered from soybean (S_) or corn (C_) seedlings (recovered host) on two soybean cultivars, Archer (_s) and Sloan (_s), and one corn hybrid (_c) (inoculated host) using a seedling assay. Isolates of *Pythium lutarium* (A-C); *P. oopapillum* (D-F); *P. sylvaticum* (G-I); and *P. torulosum* (J-L), incubated at 13, 18, and 23°C.

and Yang 2000; Zhang et al. 1996; Zitnick-Anderson and Nelson 2015). In our study, we evaluated the effect of temperature on pathogen aggressiveness of *Pythium* spp. on soybean and corn. Our data showed that aggressiveness of some species on soybean and corn was influenced by temperature, which suggests that future studies evaluating pathogenicity of *Pythium* species should be performed at more than one temperature. We found that *P. sylvaticum* was more aggressive at higher temperatures (18 and 23°C), while *P. torulosum* was more aggressive at lower temperatures (13°C). Temperature, however, did not affect the aggressiveness of *P. lutarium* and *P. oopapillum*. Other studies have also reported that temperature can impact aggressiveness of *Pythium* species. Thomson et al. (1971) reported *P. aphanidermatum* was more aggressive on soybean at 24 and 36°C, whereas *P. debaryanum* and *P. ultimum* were more aggressive at lower temperatures (15 and 20°C). More recently, Wei et al. (2011) evaluated the aggressiveness of eight species of *Pythium* recovered from soybean in Ontario and Quebec at 4, 12, 20, and 28°C. Temperature did not affect aggressiveness of *P. ultimum*, which was aggressive at all four temperatures. At higher temperatures, *P. aphanidermatum* caused greater seed rot, while *P. irregulare*, *P. macrosporum*, and *P. sylvaticum* caused more seed rot at lower temperatures (Wei et al. 2011). Temperature effects on pathogen aggressiveness suggests that soil temperature at planting will determine the degree to which *Pythium* species would cause greater severity to a

germinating soybean or corn seed. In some pathosystems, adjusting planting date may reduce disease risk (Bekkerman et al. 2008; Díaz et al. 2005; Kuruppu et al. 2004). Our data showed that while adjusting planting date may be possible for certain species of *Pythium*, (e.g., *P. torulosum* and *P. sylvaticum*), this practice would not be useful for other species, such as *P. oopapillum* and *P. lutarium*.

Seed rot often leads to pre-emergence damping off, while root rot may result in post-emergent damping off. Seed and seedling assays used in this study were effective at assessing the ability of each *Pythium* species we evaluated to cause seed or root rot. All four species caused both seed and root rot, although *P. lutarium* was found to cause less seed rot in the seed assay than root rot in the root assay at all three temperatures. Similarly, Rojas et al. (2013) and Wei et al. (2011) demonstrated that some species of *Pythium* were more aggressive at rotting seed than at rotting roots. This data should assist soybean breeders in evaluating germplasm for damping off resistance.

As with prior research (Broders et al. 2007a; Jiang et al. 2012; Rao et al. 1978; Rojas et al. 2013; Zhang et al. 1996), we found the *Pythium* species we evaluated to be equally pathogenic on both soybean and corn. This complicates management of *Pythium* damping off in Iowa where farmers typically use a soybean-corn rotation, or grow continuous corn, thereby limiting the effectiveness of this management tactic.

Table 2. Fungicide sensitivity of *Pythium lutarium*, *P. oopapillum*, *P. sylvaticum*, and *P. torulosum* to metalaxyl, ethaboxam, Captan, and thiram at three temperatures in a plate assay

<i>Pythium</i> spp.	°C	EC ₅₀ values (µg/ml) ± SE (range) ^x			
		Metalaxyl	Ethaboxam	Captan	Thiram
<i>P. lutarium</i> (n = 5) ^z	13	0.41 ± 0.02 (0.31-0.52) a ^y	0.03 ± 0.008 (0.009-0.05) a	0.26 ± 0.08 (0.09-0.53) a	0.19 ± 0.02 (0.15-0.29) a
	18	0.47 ± 0.02 (0.39-0.57) a	0.01 ± 0.002 (0.008-0.02) a	0.23 ± 0.09 (0.08-0.47) a	0.13 ± 0.03 (0.09-0.21) a
	23	0.69 ± 0.02 (0.59-0.77) a	0.01 ± 0.002 (0.008-0.02) a	0.25 ± 0.08 (0.08-0.56) a	0.13 ± 0.02 (0.08-0.23) a
<i>P. oopapillum</i> (n = 4)	13	0.19 ± 0.03 (0.32-0.10) a	0.09 ± 0.012 (0.06-0.14) a	1.25 ± 0.18 (0.82-1.81) a	0.11 ± 0.02 (0.07-0.19) a
	18	0.09 ± 0.007 (0.11-0.06) a	0.07 ± 0.02 (0.03-0.15) a	0.13 ± 0.03 (0.04-0.19) ab	0.09 ± 0.01 (0.03-0.12) a
	23	0.07 ± 0.01 (0.03-0.13) a	0.02 ± 0.01 (0.009-0.09) a	0.05 ± 0.02 (0.009-0.11) b	0.09 ± 0.02 (0.04-0.14) a
<i>P. sylvaticum</i> (n = 6)	13	0.03 ± 0.01 (0.01-0.07) b	0.02 ± 0.02 (0.009-0.11) b	0.004 ± 0.001 (0.001-0.009) b	0.10 ± 0.03 (0.04-0.20) b
	18	4.75 ± 0.30 (3.22-5.11) ab	0.13 ± 0.02 (0.008-0.21) ab	0.47 ± 0.11 (0.22-0.93) ab	0.17 ± 0.02 (0.09-0.21) ab
	23	8.78 ± 0.65 (5.90-10.33) a	0.26 ± 0.08 (0.12-0.60) a	0.70 ± 0.14 (0.46-1.10) a	0.75 ± 0.01 (0.40-1.13) a
<i>P. torulosum</i> (n = 6)	13	2.23 ± 0.22 (1.94-3.30) a	0.66 ± 0.17 (0.25-1.13) a	0.61 ± 0.08 (0.35-0.90) a	0.65 ± 0.12 (0.30-1.22) a
	18	0.10 ± 0.02 (0.06-0.20) ab	0.04 ± 0.02 (0.01-0.16) ab	0.008 ± 0.001 (0.003-0.02) ab	0.003 ± 0.001 (0.001-0.007) ab
	23	0.04 ± 0.01 (0.01-0.09) b	0.02 ± 0.02 (0.007-0.14) b	0.005 ± 0.002 (0.001-0.01) b	0.002 ± 0.008 (0.001-0.003) b

^x EC₅₀ = fungicide concentration that reduced mycelial growth by 50% compared with the control plate. Values are least square means of six replicates per isolate and temperature over three experimental runs.

^y Values followed by the same letter within species and fungicide are not significantly different according to Tukey's test (α = 0.05).

^z Number of isolates per species evaluated for fungicide sensitivity.

Table 3. Fungicide sensitivity of *Pythium lutarium*, *P. oopapillum*, *P. sylvaticum*, and *P. torulosum* to azoxystrobin, pyraclostrobin, and trifloxystrobin at three temperatures in a plate assay

<i>Pythium</i> spp.	°C	EC ₅₀ values (µg/ml) ± SE (range) ^x		
		Azoxystrobin	Pyraclostrobin	Trifloxystrobin
<i>P. lutarium</i> (n = 5) ^z	13	3.79 ± 0.58 (2.20-5.26) b ^y	11.96 ± 1.66 (10.03-20.20) ab	6.55 ± 1.28 (3.94-12.71) a
	18	8.03 ± 1.20 (4.71-12.84) a	10.56 ± 1.56 (7.44-18.62) b	4.79 ± 0.64 (1.79-6.11) a
	23	7.97 ± 0.70 (5.34-10.37) ab	14.74 ± 1.02 (10.11-17.58) a	5.15 ± 0.80 (2.27-8.42) a
<i>P. oopapillum</i> (n = 4)	13	6.64 ± 0.81 (4.66-10.40) a	14.12 ± 1.27 (9.80-19.60) a	8.52 ± 0.90 (5.15-11.98) a
	18	3.01 ± 0.69 (0.92-6.15) ab	11.41 ± 1.98 (7.79-21.22) ab	4.76 ± 1.05 (1.97-9.61) ab
	23	2.82 ± 0.46 (0.79-4.30) b	10.28 ± 1.04 (8.13-15.26) b	3.70 ± 0.56 (1.32-5.44) b
<i>P. sylvaticum</i> (n = 6)	13	3.45 ± 0.84 (1.12-7.38) b	4.34 ± 1.12 (2.40-9.85) b	2.13 ± 0.42 (1.09-4.14) b
	18	10.87 ± 1.43 (5.40-15.32) ab	15.01 ± 1.76 (8.67-22.22) ab	9.66 ± 0.58 (7.77-12.04) ab
	23	15.45 ± 1.81 (7.17-21.59) b	20.51 ± 1.06 (17.67-25.45) a	15.44 ± 1.05 (13.45-20.55) b
<i>P. torulosum</i> (n = 6)	13	2.66 ± 0.57 (0.67-4.90) a	14.63 ± 0.89 (10.66-17.34) a	2.94 ± 0.48 (1.13-4.79) a
	18	1.21 ± 0.50 (0.55-3.75) ab	9.56 ± 1.01 (4.76-11.06) ab	1.41 ± 0.30 (0.94-2.67) ab
	23	0.96 ± 0.31 (0.39-1.92) b	6.80 ± 1.04 (3.33-11.22) b	1.20 ± 0.36 (0.88-3.07) b

^x EC₅₀ = fungicide concentration that reduced mycelial growth by 50% compared with the control plate; salicylhydroxamic acid (SHAM) was added to all concentrations at 50 µg/ml to inhibit the alternative oxidase pathway. Values are least square means of six replicates per isolate and temperature over three experimental runs.

^y Values followed by the same letter within species and fungicide are not significantly different according to Tukey's test (α = 0.05).

^z Number of isolates per species evaluated for fungicide sensitivity.

We recovered *Pythium* from symptomatic corn seedlings from 19 of the 29 fields with stand loss due to damping off in southeast Iowa in mid-May 2012 (Robertson and Munkvold 2012). In some of the fields we visited, the seedlings collected were severely rotted and this affected our ability to isolate causal organisms. Moreover, we did isolate fungi from many of the diseased seedlings, but we did not identify these isolates to genus. *P. torulosum* was the most prevalent species of *Pythium* we recovered from the corn seedlings collected, most likely because of the temperature and soil moisture conditions that occurred in this region. Most of the affected fields were planted between April 23 and 27, immediately prior to a 10-day period of cold (10 to 16°C) and wet (84 to 152 mm precipitation) conditions (<https://mesonet.agron.iastate.edu/agclimate/>). Since we found that *P. torulosum* was more aggressive at 13°C, it is probably not surprising that it was the most prevalent species recovered in our survey. Similarly, weather conditions soon after planting may explain why *P. sylvaticum* was the most prevalent species recovered from diseased soybean seedlings. Soybean fields from which diseased plants were collected in 2011 and 2012, were planted in mid-May when soil temperatures were greater than 18°C, and our data showed that *P. sylvaticum* was more aggressive at these warmer temperatures.

Seed and root rot severity of *Pythium* spp. on soybean cv. Archer was typically lower than that on soybean cv. Sloan or corn at all three temperatures. Previous research has demonstrated that Archer has some resistance to *P. ultimum*, *P. irregulare*, *P. aphanidermatum*, and *P. vexans* (Bates et al. 2008; Kirkpatrick et al. 2006; Rosso et al. 2008) as well as tolerance to waterlogged soils (VanToai et al. 2001). Similarly in our research, Archer was less susceptible than Sloan to the four species of *Pythium* we screened, suggesting that this cultivar may also have some resistance to *P. lutarium*, *P. oopapillum*, *P. sylvaticum*, and *P. torulosum*.

Variation in fungicide sensitivity of *Pythium* spp. at room temperature on soybean and corn (Broders et al. 2007a; Dorrance et al. 2004) as well as sugar beet, potato, carrot, and forest nursery stock (Brantner and Windels 1998; Lu et al. 2012; Taylor et al. 2002; Weiland et al. 2014) has been reported. However, our research is novel in that we report fungicide sensitivity of *P. sylvaticum* and *P. torulosum* was affected by temperature. We found that *P. sylvaticum*, which is more aggressive at 18 and 23°C, was less sensitive to all fungicides evaluated at 18 and 23°C compared with 13°C. Likewise, *P. torulosum*, which is most aggressive at 13°C, was less sensitive with a higher EC₅₀ at 13°C than at 18 and 23°C.

The reason for the role of temperature in fungicide sensitivity was not investigated in this study, but we propose temperature plays a role in quantitative fungicide resistance (Deising et al. 2008). Mechanisms that keep the intracellular fungicide concentration low are often responsible for quantitative fungicide resistance. A pathogen may be less sensitive to a fungicide at its optimal temperature for growth due to the ability to metabolize and detoxify the compound before it reaches the site of action. In our study, optimal growth of *P. sylvaticum* occurred at 23°C, while the optimal temperature for growth of *P. torulosum* was 13°C (Supplementary Fig. S1); thus, optimal growth may explain the relative insensitivity of these two species to the fungicides tested at each respective temperature. Since temperature change affects membrane fluidity causing membranes to leak (Los and Murata 2004), temperature could alter fungicide permeability of the membrane. Furthermore, changes in temperature could affect the activity of membrane proteins that translocate various substrates across membranes including fungicides. Several studies have demonstrated that fungicides affect the activity of ATP-binding cassette transporters (ABC transporters) (Coleman and Mylonakis 2009; de Waard 1997; de Waard et al. 2006; Judelson and Senthil 2006; Krishnamurthy and Prasad 1999). Thus, uptake or export of a fungicide may be affected before reaching the site of action. Further research is needed to determine the effect of soil temperature on fungicide sensitivity in the field.

The results of this study greatly expand our knowledge of damping off of soybean and corn caused by the most prevalent species of *Pythium* in Iowa. Since we found that pathogen aggressiveness and fungicide sensitivity of some species can be significantly affected by temperature, this may compromise the effectiveness of traditional

management practices such as rotation, planting date, or use of a seed treatment. Our data suggest that seed treatment “failures” in the field may be a result of fungicide insensitivity to pathogenic *Pythium* spp. at planting. We acknowledge, however, that current seed treatments usually package two or more fungicides, insecticides, and/or nematocides active ingredients. It is unclear from this study how these pesticides may interact with each other and protect or not the germinating seed and seedling. Furthermore, previous research has shown multiple pathogens may be recovered from a single soybean or corn plant (Broders et al. 2009; Griffin 1990; Mao et al. 1998; Wei et al. 2011). It is uncertain from our research how *Pythium* species may interact with each other, or with fungal species commonly associated with damping off (Broders et al. 2007b; Díaz Arias et al. 2013). Future research investigating the interactions between pathogens causing damping off on soybean and corn as well as their sensitivity to fungicide mixtures is in progress.

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Literature Cited

- Ali-Shtayeh, M. S., and Dick, M. W. 1985. Five new species of *Pythium* (Peronosporomycetidae). Bot. J. Linn. Soc. 91:297-317.
- Bala, K., Robideau, G. P., Desaulniers, N., de Cock, A., and Levesque, C. A. 2010. Taxonomy, DNA barcoding and phylogeny of three new species of *Pythium* from Canada. Persoonia. Mol. Phylogeny Evol. Fungi 25:22-31.
- Bates, G. D., Rothrock, C. S., and Rupe, J. C. 2008. Resistance of the soybean cultivar Archer to *Pythium* damping-off and root rot caused by several *Pythium* spp. Plant Dis. 92:763-766.
- Bekkerman, A., Goodwin, B. K., and Piggott, N. E. 2008. Spatio-temporal risk and severity analysis of soybean rust in the United States. J. Agric. Resour. Econ. 33:311-331.
- Bradley, C. A. 2008. Effect of fungicide seed treatments on stand establishment, seedling disease, and yield of soybean in North Dakota. Plant Dis. 92:120-125.
- Brantner, J. R., and Windels, C. E. 1998. Variability in sensitivity to metalaxyl in vitro, pathogenicity, and control of *Pythium* spp. on sugar beet. Plant Dis. 82: 896-899.
- Broders, K. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007a. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:727-735.
- Broders, K. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007b. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:1155-1160.
- Broders, K. D., Wallhead, M. W., Austin, G. D., Lipps, P. E., Paul, P. A., Mullen, R. W., and Dorrance, A. E. 2009. Association of soil chemical and physical properties with *Pythium* species diversity, community composition, and disease incidence. Phytopathology 99:957-967.
- Cohen, M., and Coffey, M. D. 1986. Systemic fungicides and the control of oomycetes. Annu. Rev. Phytopathol. 24:311-338.
- Coleman, J. J., and Mylonakis, E. 2009. Efflux in fungi: la piece de resistance. PLoS Pathog. 5:e1000486.
- Cooke, D. E. L., Duncan, J. M., Williams, N. A., Weerd, M. H., and Bonants, P. J. M. 2000. Identification of *Phytophthora* species on the basis of restriction enzyme fragment analysis of the internal transcribed spacer regions of ribosomal RNA. EPPO Bull. 30:519-523.
- D’Arcy, C. J., Eastburn, D. M., and Schumann, G. L. 2001. Illustrated Glossary of Plant Pathology. The Plant Health Instructor, doi:10.1094/PHI-I-2001-0219-01
- de Waard, M. A. 1997. Significance of ABC transporters in fungicide sensitivity and resistance. Pestic. Sci. 51:271-275.
- de Waard, M. A., Andrade, A. C., Hayashi, K., Schoonbeek, H., Stergiopoulos, I., and Zwieters, L. 2006. Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. Pest Manag. Sci. 62:195-207.
- Deising, H. B., Reimann, S., and Pascholati, S. F. 2008. Mechanisms and significance of fungicide resistance. Braz. J. Microbiol. 39:286-295.
- Díaz, C. G., Ploper, L. D., Galvez, M. R., Gonzalez, V., Zamorano, M. A., Jaldo, H. E., Lopez, C., and Ramallo, J. C. 2005. Effect of late season diseases on the growth of different soybean genotypes in relation to planting date. AgriScientia 22:1-7.
- Díaz Arias, M. M., Munkvold, G. P., Ellis, M. L., and Leandro, L. F. S. 2013. Distribution and frequency of *Fusarium* species associated with soybean roots in Iowa. Plant Dis. 97:1557-1562.
- Dodd, J. L., and White, D. G., eds. 1999. Compendium of Corn Diseases. American Phytopathological Society, St. Paul, MN.

- Dorrance, A. E., Berry, S. A., Bowen, P., and Lipps, P. E. 2004. Characterization of *Pythium* spp. from three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Online. Plant Health Prog.: doi:10.1094/PHP-2004-0202-01-RS.
- Dorrance, A. E., Ellis, M. L., McDuffee, D., and Arthur, K. 2012. Efficacy of ethaboxam toward species of *Phytophthora* and *Pythium*. *Phytopathology* 102:S4.31.
- Elmore, R. W. 2013. Advances, vulnerabilities, and opportunities for corn: A perspective from Iowa. Pages 3-15 in: *Climate Vulnerability: Understanding and Addressing Threats to Essential Resources*. D. Niyogi, ed. Vol. 2. Elsevier, The Netherlands.
- Esler, P. D., and Conley, S. P. 2012. Probability of yield response and breaking even for soybean seed treatments. *Crop Sci.* 52:351-359.
- Griffin, G. J. 1990. Importance of *Pythium ultimum* in a disease syndrome of cv. Essex soybean. *Can. J. Plant Pathol.* 12:135-140.
- Hartman, G. L., Sinclair, J. B., and Rupe, J. C., eds. 1999. *Compendium of Soybean Diseases*. American Phytopathological Society, St. Paul, MN.
- Hatfield, J. L., Boote, K. J., Kimball, B. A., Ziska, L. H., Izaurralde, R. C., Ort, D., Thomson, A. M., and Wolfe, D. 2011. Climate impacts on agriculture: implications for crop production. *Agron. J.* 103:351-370.
- Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis.* 70:1038-1043.
- Jiang, Y. N., Haudenshield, J. S., and Hartman, G. L. 2012. Characterization of *Pythium* spp. from soil samples in Illinois. *Can. J. Plant Pathol.* 34:448-454.
- Judelson, H. S., and Senthil, G. 2006. Investigating the role of ABC transporters in multifungicide insensitivity in *Phytophthora infestans*. *Mol. Plant Pathol.* 7: 17-29.
- Kirkpatrick, M. T., Throck, C. S., Rupe, J. C., and Gbur, E. E. 2006. The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. *Plant Dis.* 90: 597-602.
- Krishnamurthy, S. S., and Prasad, R. 1999. Membrane fluidity affects functions of Cdr1p, a multidrug ABC transporter of *Candida albicans*. *FEMS Microbiol. Lett.* 173:475-481.
- Kuruppu, P. U., Schneider, R. W., and Russin, J. S. 2004. Factors affecting soybean root colonization by *Calonectria ilicicola* and development of red crown rot following delayed planting. *Plant Dis.* 88:613-619.
- Los, D. A., and Murata, N. 2004. Membrane fluidity and its roles in the perception of environmental signals. *BBA Biomembr.* 1666:142-157.
- Lu, X. H., Davis, R. M., Livingston, S., Nunez, J., and Hao, J. J. 2012. Fungicide sensitivity of *Pythium* spp. associated with cavity spot of carrot in California and Michigan. *Plant Dis.* 96:384-388.
- Mao, W., Carroll, R. B., and Whittington, D. P. 1998. Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in causing red root rot of corn. *Plant Dis.* 82:337-342.
- Martin, F. N., and Loper, J. E. 1999. Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. *Crit. Rev. Plant Sci.* 18:111-181.
- Middleton, J. T. 1943. The taxonomy, host range and geographical distribution of the genus *Pythium*. Published for the Club by Lancaster Press, Lancaster, PA.
- Mueller, D. 2014a. Corn disease loss estimates from the United States and Ontario, Canada 2012. Purdue Extension publication BP-96-12-W. Purdue University, West Lafayette, IN.
- Mueller, D. 2014b. Corn disease loss estimates from the United States and Ontario, Canada 2013. Purdue Extension publication BP-96-13-W. Purdue University, West Lafayette, IN.
- D. Mueller, K. Wise, N. Dufault, C. Bradley, and M. Chilvers, eds. 2013. *Fungicides for Field Crops*. APS Press, St. Paul, MN.
- Olaya, G., Zheng, D., and Köller, W. 1998. Differential responses of germinating *Venturia inaequalis* conidia to kresoxim-methyl. *Pestic. Sci.* 54:230-236.
- Rao, B., Schmitthenner, A. F., Caldwell, R., and Ellett, C. W. 1978. Prevalence and virulence of *Pythium* species associated with root rot of corn in poorly drained soil. *Phytopathology* 68:1557-1563.
- Rizvi, S. S. A., and Yang, X. B. 1996. Fungi associated with soybean seedling disease in Iowa. *Plant Dis.* 80:57-60.
- Robertson, A. E., Matthiesen, R., and Ahmad, A. 2014. Nine species of *Pythium* associated with corn seedling blight in southeastern Iowa. *Integrated Crop Management News*. Iowa State University, Ames, IA.
- Robertson, A. E., and Munkvold, G. P. 2012. Seedling diseases reported in corn and soybean. *Integrated Crop Management News*. Iowa State University, Ames, IA.
- Rojas, A., Jacobs, J. L., Napieralski, S., Bradley, C. A., Chase, T., Esler, P. D., Giesler, L., Jardine, G., Nelson, B. D., Malvick, D., Markell, S., Robertson, A. E., Rupe, J. C., Sweets, L., Wise, K., and Chilvers, M. I. 2013. Diversity of oomycetes associated with soybean seedling diseases in the U.S. *Phytopathology* 103:S2.123.
- Rosso, M. L., Rupe, J. C., Chen, P., and Mozzoni, L. A. 2008. Inheritance and genetic mapping of resistance to *Pythium* damping-off caused by *Pythium aphanidermatum* in "Archer" soybean. *Crop Sci.* 48:2215-2222.
- Sokal, R. R., and Rohlf, F. J. 1989. *Introduction to Biostatistics*, 2nd Ed. W. H. Freeman & Co., New York.
- Stanghellini, M. E. 1974. Spore germination, growth and survival of *Pythium* in soil. *Proc. Am. Phytopathol. Soc.* 1:211-214.
- Stewart, S., and Robertson, A. E. 2011. A modified method to screen for partial resistance to *Phytophthora sojae* in soybean. *Crop Sci.* 52:1181-1186.
- Taylor, R. J., Salas, B., Secor, G. A., Rivera, V., and Gudmestad, N. C. 2002. Sensitivity of North American isolates of *Phytophthora erythroseptica* and *Pythium ultimum* to mefenoxam (metalaxyl). *Plant Dis.* 86:797-802.
- Thomson, T. B., Athow, K. L., and Laviollette, F. A. 1971. The effect of temperature on the pathogenicity of *Pythium aphanidermatum*, *P. debaryanum*, and *P. ultimum* on soybean. *Phytopathology* 61:933-935.
- van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. *Stud. Mycol.* 21:1-242.
- VanToai, T., St. Martin, S., Chase, K., Boru, G., Schnipke, V., Schmitthenner, A. F., and Lark, K. 2001. Identification of a QTL Associated with tolerance of soybean to soil water-logging. *Crop Sci.* 41:1247-1252.
- Waterhouse, G. M. 1967. Key to *Pythium* Pringsheim. *Mycol. Pap.* 109:1-15.
- Wei, L., Xue, A. G., Cober, E. R., Babcock, C., Zhang, J., Zhang, S., Li, W., Wu, J., and Liu, L. 2011. Pathogenicity of *Pythium* species causing seed rot and damping-off in soybean under controlled conditions. *Phytoprotection* 91:3-10.
- Weiland, J. E., Santamaria, L., and Grunwald, N. J. 2014. Sensitivity of *Pythium irregulare*, *P. sylvaticum*, and *P. ultimum* from forest nurseries to mefenoxam and fosetyl-al, and control of *Pythium* damping-off. *Plant Dis.* 98:937-942.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis et al., eds. Academic Press, San Diego.
- Yang, X. B. 1997. Soybean damping off. Iowa State University Extension publication PM936. Iowa State University, Ames, IA.
- Ypema, H. L., and Gold, R. E. 1999. Kresoxim-methyl: modification of a naturally occurring compound to produce a new fungicide. *Plant Dis.* 83:4-19.
- Zadoks, J. C., and Schein, R. D. 1979. *Epidemiology and Plant Disease Management*. Oxford University Press Inc., New York, NY.
- Zhang, B. Q., and Yang, X. B. 2000. Pathogenicity of *Pythium* populations from corn-soybean rotation fields. *Plant Dis.* 84:94-99.
- Zhang, B. Q., Yang, X. B., and Chen, W. D. 1996. First report of the pathogenicity of *Pythium torulosum* on soybean. *Plant Dis.* 80:710.
- Zitnick-Anderson, K. K., and Nelson, B. D., Jr. 2015. Identification and pathogenicity of *Pythium* on soybean in North Dakota. *Plant Dis.* 99:31-38.