Identification, Pathogenicity, and Fungicide Sensitivity of \textit{Pythium} spp. Associated with Corn and Soybean Seed and Seedling Disease in Ohio.

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Abstract
Cool moist conditions in combination with minimum tillage, earlier planting, and recent changes in commercial seed treatment active ingredients have led to an increase in corn and soybean stand establishment problems. Persistent reduction in stands over the years has led to the current investigation of \textit{Pythium} species associated with seed and seedling disease of corn and soybean in Ohio. Samples of diseased corn and soybean were collected from 40 locations in Ohio and northeastern Indiana. All isolates of \textit{Pythium} recovered were identified to species, evaluated in an \textit{in vitro} pathogenicity assay on both corn and soybean seeds, and a subset of the isolates were tested for their sensitivity to fungicides currently used as seed treatments. Twelve species and two distinct morphological groups of \textit{Pythium} were identified, of which 6 species were moderately to highly pathogenic on corn seeds and 9 species were highly pathogenic on soybean seeds. There was significant variation ($P<0.01$) in sensitivity to mefenoxam, azoxystrobin, triloxystrobin, and captan both across species and within species of \textit{Pythium}. This research has shown that multiple species of \textit{Pythium} have the capacity to reduce germination of both corn and soybean seed leading to decreased emergence. In addition, mefenoxam, azoxystrobin, trifloxystrobin, and captan, as seed treatments, may not be able to inhibit infection by all pathogenic species of \textit{Pythium}.

Introduction

Objectives

Isolate and identify \textit{Pythium} spp. associated with corn and soybean seed and seedling disease symptoms

Determine the pathogenicity \textit{in vitro} of each \textit{Pythium} isolate on both corn and soybean seeds

Determine the level of sensitivity of \textit{Pythium} species to mefenoxam, azoxystrobin, trifloxystrobin, and captan

Results & Conclusions

Isolation & Identification

105 isolates were recovered from 32 locations. From these isolates, 14 species of \textit{Pythium}, as well as 2 distinct morphological groups, were identified by morphology and sequence analysis.

The phylogeny produced 3 major clades within the genus \textit{Pythium} and a fourth clade for the \textit{Phytophthora} species (Fig 6). All species with globose sporangia were in Clade A, filamentous sporangia were in Clade B, proliferating sporangia in Clade D, and \textit{Phytophthora} species in Clade C.

Pathogenicity

All species were more pathogenic on soybean seeds than on corn seeds. Of the 14 \textit{Pythium} species, 6 were moderately to highly pathogenic on corn seeds and 9 were highly pathogenic on soybean seeds. (Fig 3)

This is the first report of \textit{P. inflatum} and \textit{P. atrantheridium} as pathogens of corn and soybean seedlings, and the first report of \textit{P. sylvaticum} as a pathogen of corn seeds and seedlings.

These results demonstrate that several phylogenetically diverse species of \textit{Pythium} have the capacity to reduce the germination of corn and soybean seeds.

Fungicide Sensitivity

There were significant differences ($p<0.01$) in sensitivity to mefenoxam, azoxystrobin, trifloxystrobin, and captan across species (Fig 4) and among isolates of a species

Sensitivity to mefenoxam and azoxystrobin were associated with the sporangial morphology of the species. (Fig 5)

It appears that mefenoxam, azoxystrobin, trifloxystrobin, and captan alone are not able to effectively control all pathogenic species of \textit{Pythium}, due to varying sensitivities to the fungicides among species.
Materials and Methods

Isolations & Identification
- Diseased seeding were collected from 40 locations in 20 counties in Ohio and Indiana during the spring of 2005 and 2006.
- Isolates were identified by morphological characters (Fig 1a-f) using standard keys and by sequence analysis.
- Sequence data were aligned using Clustal-W, and parsimony analysis to create a phylogenetic tree (Fig. 6) was done with Molecular Evolutionary Genetic Analysis (MEGA) version 3.1.

Pathogenicity Assays
- An in vitro pathogenicity assay was utilized to determine the pathogenicity of each isolate on LG2540 corn and Sloan soybean seeds.
- The seeds were scored on a 0-3 rating scale (Fig 2) for pathogenicity with 0=100% germination, 1=70-99% germination, 2=30-69% germination, and 3=0-29% germination.
- Each isolate had 3 replications per experiment and the experiment was repeated 3 times. The entire experiment was carried out for both corn and soybean seeds.

Fungicide Sensitivity
- Mefenoxam, azoxystrobin, trifloxystrobin, and captan were amended to PCA in a series of dilutions depending on the application rate recommended on the label.
- Sixty isolates representing all the species and locations were selected to be tested.
- The diameter of the mycelial growth of each isolate at each concentration was measured at 48, and 72 hours.
- The diameter of each isolate on the amended media was divided by the growth on the control to calculate the percent inhibition of mycelial growth.
- The experiment was repeated 3 time.

Literature Cited

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