Pythium and *Fusarium* species causing seed rot and damping-off of alfalfa

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ABSTRACT

Seed rot and damping-off is an important disease of alfalfa, severely affecting stand establishment when conditions favor the disease and reducing vigor of adult plants. Globally, 15 *Pythium* species have been found to cause damping-off and seed rot of alfalfa, although surveys of species causing disease on alfalfa in the Midwestern U.S. are lacking. A survey for soil-borne seedling pathogens identified soils from commercial production fields in Minnesota with high levels of damping-off. Pathogens were isolated by a seedling baiting technique from soil of five alfalfa fields. Of the 149 organisms isolated, 93 (62%) were identified as *Pythium* species and 43 (29%) were identified as *Fusarium* species. Three species, *P. sylvaticum, P. irregulare,* and *P. ultimum* var. *ultimum,* were pathogenic on germinating alfalfa seedlings. Strains of seven species isolated from infected soybean, *P. irregulare, P. intermedium, P. sylvaticum, P. recalcitrans, P. conidiophorum, P. ultimum* var. *sporangiferum,* and *P. ultimum* var. *ultimum,* were also pathogenic on alfalfa in the plate test. The majority of the *Fusarium* isolates were identified as *F. solani* and *F. oxysporum* with a low number of *F. redolans, and F. incarnatum-equisetum*. All species caused seed rot, damping-off, and root rot. Assays with Apron XL (mefanoxam) treated seed showed that 56% of *Pythium* strains were insensitive to the fungicide. Insensitivity to Stamina (pyraclostrobin) seed treatments occurred in 94% of *Pythium* strains. The presence of broad host range species and fungicide resistance of a high percentage of *Pythium* isolates suggests that crop rotation and these widely used seed treatments are not effective tools for managing this disease. These results indicate that resistant cultivars are needed for managing damping-off in alfalfa production systems.

INTRODUCTION

Surveys in Minnesota for soil-borne seedling pathogens identified soils from commercial production fields in Becker, Fulda, Parkers Prairie, St. Hilaire, and Waseca with high levels of seedling damping-off. High levels of disease occurred when using alfalfa seed with a commercial Apron XL treatment, suggesting that the pathogens were resistant to the fungicide. Experiments were done to identify the pathogens present in the soil samples and test them for resistance to mefanoxam (Apron XL) and pyraclostrobin (Stamina).

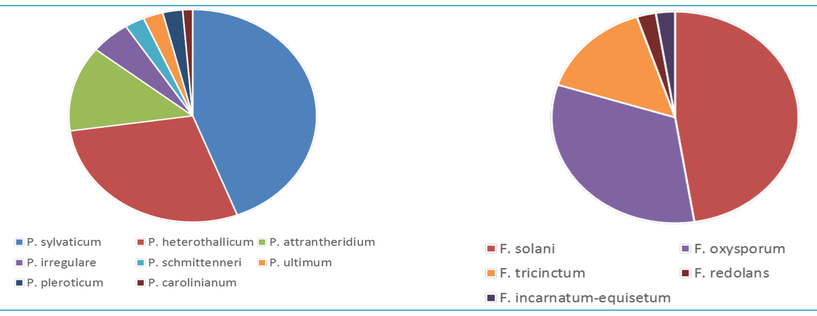
RESULTS

Isolation and identification of *Pythium* and *Fusarium* species from Minnesota soils.

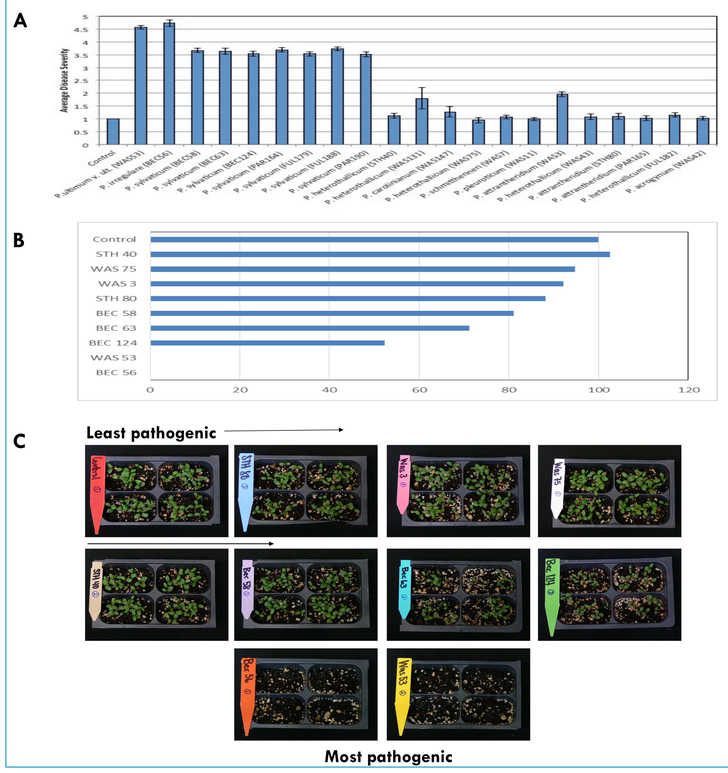
A total of 149 organisms were isolated from five soil samples taken from commercial alfalfa fields in Minnesota with a high level of damping-off.

93 isolates (62%) were *Pythium* species; 76 were identified unambiguously to species.

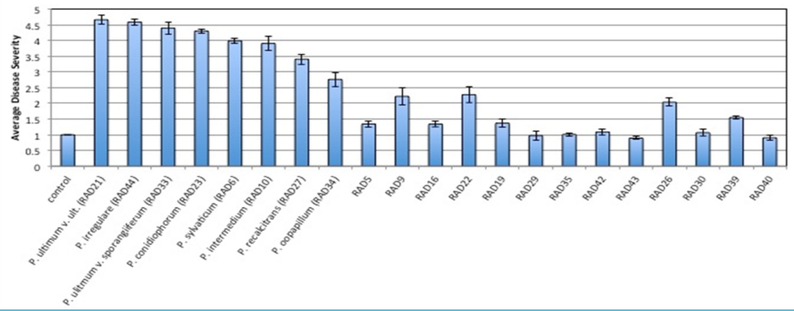
43 isolates (29%) were identified as *Fusarium* species; 40 were identified unambiguously to species.



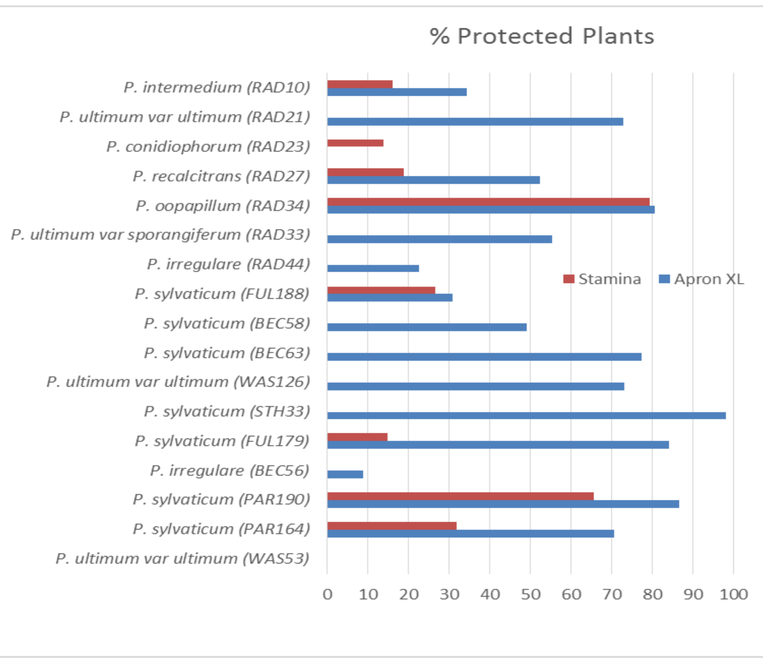
Three *Pythium* species isolated from alfalfa were pathogenic on germinating alfalfa seedlings at 21˚C using a standard agar plate test (A). These results were confirmed by the mean percent germination in the soil assay (B).



Pythium strains isolated from diseased soybean were pathogenic on alfalfa seedlings.



Seed treatments on Vernal alfalfa with the fungicides Apron XL and Stamina shows many strains are not controlled by the fungicides in the damping-off plate assays.



MATERIALS AND METHODS

Pathogen isolation

A rolled-towel method was used to bait pathogens from soil. Surface sterilized Saranac seeds were place on sieved soil on wet germination paper, rolled, and placed in a plastic bag and incubated at 18°C or 24°C for 3 days. Seeds and seedlings were rinsed with water, surfaced sterilized and placed on water agar plates. Hyphal tips were transferred to cornmeal agar. A minimum of 15 pure cultures were recovered from each soil.

Pathogen identification

DNA was isolated from 76 pure cultures and the rDNA ITS sequence amplified and sequenced. Species of *Pythium* were further distinguished by amplification and DNA sequencing of the cytochrome oxidase c subunit I gene (COI) and *Fusarium* species were identified by sequencing the elongation factor 1-a gene.

Pathogenicity assays

The NAAIC standard test for Pythium seed rot and damping-off was used to test 21 strains of *Pythium* isolated from alfalfa and 21 strains isolated from infected soybean seedlings. Surface sterilized seeds were placed on water agar containing each strain, incubated for 5 days at 21°C, then scored for disease symptoms on a 1-5 scale (Fig. 1). The same assay was used to test 8 strains of *Fusarium*.

A soil assay was developed and used to test 9 *Pythium* strains. Sungro professional growing mix (250 ml) was mixed with 5 g corn meal, autoclaved and 50 ml was placed in each well of a 4 pack. One quarter of a 15 x 100 mm agar plate with a 7-day-old *Pythium* culture was ground with 250 ml water in a blender and 25 ml inoculated into each well filled with growing mix. Twenty five Agate alfalfa seeds were planted in each well and allowed to grow 5 days at 21°C and germination assessed.

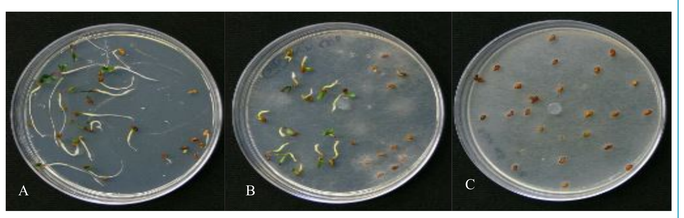


Fig. 1. The culture plate assay after 5 days at 21°C with a 16 hour photoperiod. **A**, Uninoculated control. White roots and emerging radicles are healthy = 1, late germinating seed =1, Seeds in upper right which did not imbibe = hard, are not included in average severity index (ASI) calculation. **B**, Inoculated with *P. sylvaticum*. Lower right, dead seed = 5. Upper right = 4. Left = 3, stunted infected seedlings. **C**, Inoculated with *P. ultimum* var *ultimum*. All dead seeds = 5.

Fungicide sensitivity

The standard Pythium seed rot and damping-off test was used to test 17 *Pythium* strains with Vernal seeds commercially coated with Apron XL (0.64 oz./100 pounds seeds), Stamina (3.1 oz./100 pounds seeds), and untreated seeds.

Radial growth of 10 *Pythium* strains was assessed on media containing a range of concentrations of mefanoxam or pyraclostrobin.

CONCLUSIONS

- Damping-off in the soils tested was due to the presence of highly pathogenic strains of *P. sylvaticum, P. irregulare,* and *P. ultimum* var. *ultimum,* and *Fusarium* strains capable of causing seed rot, damping-off, and root rot.
- Significant amounts of resistance to Apron XL and Stamina were observed in *Pythium* strains.
- *Pythium* strains isolated from soybean are able to cause disease on alfalfa.
- Crop rotation and seed treatments may not be efficient tools for managing this disease.
- These *Pythium* strains would be useful in selection programs to develop resistant germplasm.