

Incidence and spatial distribution of *Rhizoctonia* and *Pythium* species determined with real-time PCR

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ABSTRACT

Populations of *Rhizoctonia* and *Pythium* are diverse in eastern Washington, with multiple species/anastomosis groups present throughout the region and within individual fields. Recent evidence suggests that species composition may be influenced by crop rotation. The Cook Agronomy Farm near Pullman, WA was established in 1999 to test direct-seed cropping systems on a field scale, with 369 GPS sampling locations spaced every 30 m. A three-year rotation was established to include winter wheat-alternate crop-spring wheat, with each rotation occurring every year. The alternate crops consisted of a winter and/or spring variety of pea, lentil, barley or canola. Soil samples were collected from about 116 sites from the spring wheat portion of the field following the alternate crop. DNA was extracted from each soil sample using a Barocycler™ and Mo-Bio Soil DNA kit, and quantified with species-specific primers and real-time PCR. *Rhizoctonia solani* AG-2-1 occurred more frequently in fields with a history of canola, with a higher frequency following spring canola (48% of sites) compared to winter canola (28%). *Rhizoctonia oryzae* was detected more often following winter alternate crops (39-44%) than spring crops (24-37%). Of four *Pythium* species examined, *P. irregulare* group IV and *P. rostratifungens* had the highest incidence of occurrence (66% and 67% respectively). These species of *Pythium* were also higher following winter canola and pea versus spring canola and pea.

INTRODUCTION

The population dynamics of *Rhizoctonia* and *Pythium* spp. in eastern Washington is complex with at least seven species/anastomosis groups of *Rhizoctonia* and ten species of *Pythium* occurring in the dryland regions of the state. This high degree of diversity makes the study of individual species of these soilborne pathogens challenging since identification to species or anastomosis group using classical techniques is laborious and technically challenging. Using species-specific primers and real-time PCR (1,4), each species can be identified and quantified from a soil sample. Previous work on the Cook Agronomy Farm demonstrated that *Rhizoctonia solani* and *R. oryzae* have aggregated distributions across the farm (2). However, less is known about the distribution of *Pythium* spp. in dryland eastern Washington, partially because of the difficulty in studying individual species in complex populations.

The purpose of this study was to examine the distribution of several *Pythium* and *Rhizoctonia* species across a 36 ha Cook Agronomy Farm near Pullman, WA using real-time PCR and to attempt to correlate pathogen distribution with previous cropping histories. The Cook Agronomy Farm is a unique site where cropping systems and precision agriculture are tested on a field scale with each plot encompassing 2 ha. The research farm also has 369 georeferenced sampling sites dispersed over the 36 ha.

MATERIALS AND METHODS

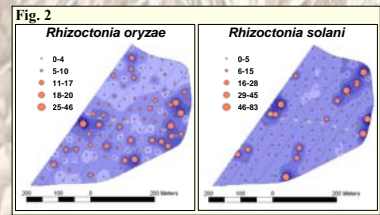
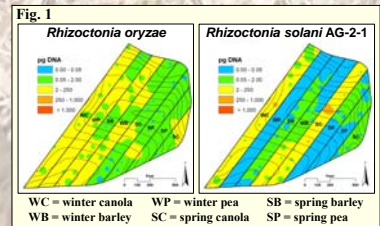
Soil samples were collected from each of 116 georeferenced sites at the Cook Agronomy Farm north of Pullman, WA in early June of 2006. Each sample consisted of about 500 g of soil removed from the root zone to a depth of about 15 cm. After thorough mixing, a 0.5 g subsample was removed from each soil sample and DNA was extracted using a Barocycler™ NEP3229 (Pressure Biosciences, Inc., West Bridgewater, MA) for cell disruption in combination with the UltraClean Soil DNA Isolation kit (MoBio Laboratories, Solana Beach, CA) as previously described (1). Species-specific PCR primers were used with real-time PCR to detect and quantify two species of *Rhizoctonia* (*R. oryzae* and *R. solani* AG-2-1) (1) and four species of *Pythium* (*P. abapressorium*, *P. irregulare* grp. I, *P. irregulare* grp. IV and *P. rostratifungens*) (4). Amplification was detected by use of SYBR Green I fluorescent dye in combination with the Roche LightCycler and use of melting curve profiles to verify target. Data were plotted using ArcGIS version 9.3 (ESRI, Redlands, CA) after interpolation using the inverse distance weighted (IDW) method.

The effect of previous cropping history was tested by mean comparison using Fisher's LSD ($P=0.05$). To examine the distribution of each species, frequency distributions were generated in SigmaPlot version 10. In addition, an index of dispersion and Lloyd's index of patchiness were calculated to examine the degree of aggregation.

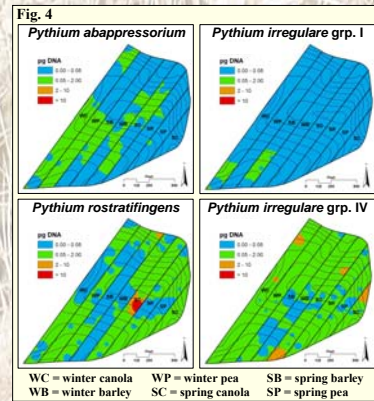
RESULTS

Rhizoctonia oryzae and *R. solani* AG-2-1 represent the most commonly identified groups of *Rhizoctonia* in the higher rainfall (>500 mm annual precipitation) dryland production regions of Washington. *Rhizoctonia oryzae* was distributed throughout the field, being observed in all rotations and occurring at 36% of the sample sites (Fig. 1). *Rhizoctonia solani* AG-2-1 occurred almost exclusively in portions of the field that were previously cropped to spring or winter canola (39% of canola sites versus 8% of non-canola sites). *Rhizoctonia solani* AG-2-1 was recently described as a pathogen of canola in Washington (3) and is observed to cause wirestem and damping-off of canola seedlings (Fig. 3). This pathogen is contributing to problems in plant establishment and winter survival of canola in Washington.

For both groups of *Rhizoctonia*, the incidence and pattern of distribution with respect to cropping history was very similar when compared to data collected with traditional plating assay 3 years previous to this study (Fig. 2).



All four species of *Pythium* were found on the Cook Agronomy Farm to varying degrees (Fig. 4). *Pythium abapressorium* occurred at 44% of sites and *P. irregulare* grp. I at only 9% of the sites. Data from separate studies indicates that there is a strong correlation between quantifiable levels of *P. irregulare* grp. I with lentil production or fields that have been previously planted to lentils. Lentils have not been planted on this farm in over 10 years, so the scarcity of this species is not surprising. *Pythium rostratifungens* and *P. irregulare* grp. IV are the two of the most abundant species of *Pythium* found in dryland production areas of eastern Washington and were also very abundant on the Cook Agronomy Farm (66 and 67%, respectively).



Average DNA concentrations were calculated for each pathogen/crop combination (Table 1). No significant differences in DNA quantities were found for *P. abapressorium*, *P. irregulare* grp. I or *P. rostratifungens* with respect to previous cropping history. However, significantly more DNA of *P. irregulare* grp. IV and *R. solani* AG-2-1 was observed for fields with a history of canola. In addition to higher DNA quantities of *R. solani* AG-2-1 in spring canola, there was also a higher incidence in spring canola (48%) than in winter canola (28%). *Rhizoctonia oryzae* DNA quantities were significantly higher following winter seeded rotation crops versus spring seeded crops.

Table 1. Average pg of DNA for each crop/pathogen.

Crop	ABA*	IRRI	IRRV	ROS	ORY	AG2-1
Spring barley	0.04 ^a	0.06	0.18 ^b	0.19	5.31 ^b	0.32 ^b
Winter barley	0.03	<0.01	0.27 ^b	0.10	6.84 ^{ab}	7.61 ^b
Spring canola	0.07	<0.01	0.78 ^a	1.29	1.62 ^b	52.86 ^a
Winter canola	0.12	0.02	0.80 ^a	0.12	14.83 ^a	30.83 ^{ab}
Spring pea	0.01	<0.01	0.24 ^b	0.11	1.19 ^b	0.10 ^b
Winter pea	0.04	<0.01	0.65 ^{ab}	0.20	9.62 ^{ab}	0.00 ^b

* ABA = *P. abapressorium*, IRRI = *P. irregulare* grp. I, IRRV = *P. irregulare* grp. IV, ROS = *P. rostratifungens*, ORY = *R. oryzae*, AG2-1 = *R. solani* AG-2-1.
 * Values with the same letter are not significantly different using Fisher's LSD ($P=0.05$).

Frequency distributions were plotted for each of the pathogens and found to be highly skewed for all species (Fig. 5). Lloyd's index of patchiness was calculated for each of the species and all *Pythium* and *Rhizoctonia* species were found to have an aggregated pattern of distribution (Table 2). However, the values for *P. abapressorium*, *P. irregulare* grp. IV and *R. oryzae* were closer to 1, indicating that degree of aggregation was lower and was approaching a random pattern. This is evident in Figs. 1 and 4, particularly for *P. irregulare* grp. IV. The index of dispersion also showed that each of the species has an aggregated distribution.

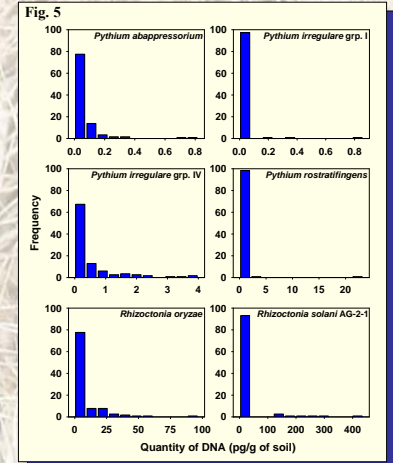


Table 2. Analysis of spatial distribution.

Species	Lloyd's Index of Patchiness	Index of Dispersion
<i>P. abapressorium</i>	6	25
<i>P. irregulare</i> grp. I	146	57
<i>P. irregulare</i> grp. IV	3	130
<i>P. rostratifungens</i>	40	1348
<i>R. oryzae</i>	5	3082
<i>R. solani</i> AG-2-1	16	25005

CONCLUSIONS

- Populations of *Pythium* and *Rhizoctonia* species in eastern Washington are diverse, with multiple species occurring within a single sample.
- Sites with a history of canola had higher DNA quantities of *P. irregulare* grp. IV and *R. solani* AG-2-1.
- Sites with a history of fall planted rotation crops had a higher incidence and higher DNA quantity of *R. oryzae* than spring seeded crops.
- All species of *Pythium* and *Rhizoctonia* in this study displayed an aggregated pattern of spatial distribution.

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