

The Role of Alternate Hosts in the Epidemiology of *Ascochyta* Blight of Chickpea in Reduced Tillage Cropping Systems in the Pacific Northwest

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PROJECT OBJECTIVES:

Objective 1. To quantify *Ascochyta rabiei* infection of wheat and other alternate hosts grown in rotation with chickpea in rotational, minimum tillage or direct seeding cropping systems.

Objective 2. To determine the ability of *A. rabiei* to reproduce on wheat in rotational, minimum tillage and direct seeding cropping systems.

Objective 3. To develop a specific PCR primer to detect and quantify *Ascochyta rabiei* infection of wheat and other hosts.

KEYWORDS: host specificity, rotation, inoculum, alternate host

STATEMENT OF PROBLEM: In us pacific northwest cropping systems, wheat is typically grown in rotation with barley and/or legumes. Pathogens that cause disease on grasses do not generally cause disease on legumes and this specificity forms the basis for rotations as a disease control strategy. If pathogens have a wide host range and are able to colonize all hosts in the rotation, inoculum will build up resulting in high levels of disease and economic losses. The wheat-chickpea rotations used in the pnw are thought to reduce initial inoculum of the ascochyta blight pathogen of chickpea. However, artificial inoculation studies indicate that the fungus may have a much wider host range than previously thought and this host range may include wheat. If the pathogen can colonize and/or reproduce on wheat, this may provide a potential mechanism for the buildup of initial inoculum and a means for the pathogen to "bridge" across all crops in the rotation.

ZONE OF INTEREST: high rainfall zone - annual cropping systems

ABSTRACT OF RESEARCH FINDINGS: Several greenhouse inoculation experiments have been performed that were designed to test the hypothesis that *A. rabiei* isolates originally isolated from chickpea as well as from several other presumed non-hosts are able to infect and colonize plants other than chickpea. Results from both experiments clearly demonstrate that *A. rabiei* can infect and colonize wheat, pea, alfalfa, and lentil without causing obvious disease symptoms. We have established a transformation system for *A. rabiei* and this procedure has been used to develop green and red fluorescent isolates of *Ascochyta*, which in turn has provided us with a powerful tool to observe the infection process of wheat and legumes *in planta*. The transformants have been validated as normal by observation of culture characteristics and morphology as well as ability to cause disease on chickpea hosts. We have used this tool to study the infection process in resistant and susceptible cultivars of chickpea, to establish a benchmark for assessing non-host plant infection. And in non-host infection studies we have demonstrated that *A. rabiei* spores are capable of attaching to non-host plant surfaces and subsequently germinating, which provides

supporting evidence of our observations to date. At this point, we do not know how the fungus penetrates the plant or if it is able to extensively colonize wheat as an endophyte or latent pathogen. How the fungus penetrates these other species is also not understood. Our preliminary data indicates that *A. rabiei* has a wide host range (at least in terms of plant penetration) and this may have important implications for the management of Ascochyta blight of chickpea, particularly chickpea grown in reduced tillage agro-ecosystems in rotation with wheat.

RESULTS AND INTERPRETATION: In a series of replicated greenhouse experiments we have inoculated chickpea (varieties Dwelley and Spanish White), wheat (winter wheat variety Madsen), lentil (variety Crimson), field pea (variety Columbian), and other potential hosts with *A. rabiei*. The *A. rabiei* isolates used represent the diversity of known strains in the PNW according to molecular markers and virulence testing, as well as historical isolates obtained by W. Kaiser from a number of supposed non-host plant spp. in infected chickpea fields. California isolate CAB-00-12 is highly virulent on chickpea, and was used as a positive control for pathogenicity assays. Successful infection of plants was confirmed by re-isolation from plant tissue at 4 wk post-inoculation after intensive bleach surface-sterilization.

Chickpeas begin showing lesions within one week of inoculation. Spanish White, a susceptible chickpea variety, showed moderate to severe lesions and disease response to all tested *A. rabiei* strains. Dwelley, a resistant variety, showed a range of response from mild to severe depending on the *A. rabiei* isolate, confirming findings of W. Chen. Other potential host plants generally appeared healthy and developed only small spots at a slower pace. These spots may be due to hypersensitive response to the pathogen. All tested *A. rabiei* strains recently isolated in the PNW were re-isolated from chickpea, wheat, and pea, and most were re-isolated from lentil, and/or alfalfa (Table 1). All tested *A. rabiei* strains isolated from plants other than chickpea were re-isolated from chickpea and pea, and most were re-isolated from wheat and/or lentil (Table 2). Therefore, it appears that *A. rabiei* can infect and colonize several plant species in addition to chickpea without causing any obvious disease symptoms, and viable *A. rabiei* is maintained in these alternate host tissues. This gives support to the hypothesis that reduced tillage may increase Ascochyta blight by maintaining residues of chickpea, wheat, and other crops that all may serve as disease reservoir.

Table 1. Summary of greenhouse trials, addressing the potential of plants other than chickpea to support *A. rabiei*. Note: wheat plants demonstrated small white lesions, most likely due to hypersensitive response of the plant to *A. rabiei*. Disease rating 1=healthy, 10=dead; *A. rabiei* re-isolated *=yes.

Isolate	Source	Chickpea	Wheat	Lentil	Pea	Alfalfa
C-12	California	7 *	3 *	1 *	1 *	1
EV-22	Evans Farm, Genesee, ID	6 *	3 *	1 *	1 *	1 *
EV-3	Evans Farm, Genesee, ID	3 *	3 *	1 *	1 *	1 *
SC-20	Schaefer Farm, WA	3 *	3 *	1 *	1 *	1
SC-22	Schaefer Farm, WA	4 *	3 *	1 *	1 *	1 *
SF-12	Silflow Farm, Genesee, ID	4 *	3 *	1	1 *	1 *
SP-41	Spillman farm, Pullman, WA	8 *	3 *	1 *	1 *	1 *
SP-42	Spillman farm, Pullman, WA	7 *	3 *	1 *	1 *	1 *

SP-55	Spillman farm, Pullman, WA	4 *	3 *	2	1 *	1
water		2	2	1	1	1

Table 2. Summary of greenhouse trials, addressing the potential of *A. rabiei* isolated from plants other than chickpea to infect chickpea or other common rotation crops. Disease rating 1=healthy, 10=dead; *A. rabiei* re-isolated *=yes. Chickpea cultivars “Dwelley” and “Spanish White” (Sp. White) are resistant and susceptible to PNW isolates of *A. rabiei*, respectively.

Isolate	Source and Location	Dwelley	Sp. White	Wheat	Lentil	Pea
		Chickpea	Chickpea			
AR240	Tumble pigweed, ID	2 *	6 *	3 *	1	1 *
AR265	Alfalfa, WA	2 *	8 *	3 *	1	2 *
AR28	Wheat, ID	3 *	9 *	2	1 *	1 *
AR639	Lentil, WA	6 *	6 *	2 *	1	1 *
AF40	faba bean, Lebanon	2 *	8 *	3 *	1 *	1 *
water		2	1	2	1	1

*In addition, where possible the isolates of Trial 2 were also inoculated onto their original host plant, i.e. AR265 was also inoculated onto alfalfa, AF 40 was also inoculated onto Faba bean.

Fifteen cultures of *A. rabiei* inoculated to and re-isolated from wheat, pea, lentil, or alfalfa were subsequently inoculated onto Dwelley chickpea. Dwelley again developed lesions and disease ratings were similar to infection with initial isolate (data not shown). This demonstrates that *A. rabiei* retains its pathogenicity to chickpea after colonizing “non-host” plants. Although these non-hosts remain generally healthy and productive they may serve as a significant reservoir of infective *A. rabiei*.

We have also attempted to infect chickpea and other potential hosts by allowing plants to germinate and grow through heavily infested chickpea residue, placed on the soil surface. Interestingly, when seeds were planted beneath chickpea field residue heavily infected with *A. rabiei*, only the highly susceptible Spanish White chickpea variety showed serious disease symptoms and this was the only cultivar from which *A. rabiei* was isolated in the laboratory (Table 3). Results were similar regardless of whether plants were kept under high humidity conditions or low humidity conditions in the greenhouse.

Table 3. Summary of greenhouse trials, addressing the potential of *A. rabiei*-infected chickpea residue to infect chickpea or other common rotation crops. Disease rating 1=healthy, 10=dead; *A. rabiei* re-isolated *=yes.

Inoculant source	Dwelley	Sp. White	Wheat	Lentil	Pea
	Chickpea	Chickpea			
Highly infected field chickpea residue	3	6 *	2	1	1

This result may indicate that infectivity of *A. rabiei* on different hosts may differ depending on spore type and/or life stage of the fungus or plant. These observations suggest that further research is necessary to determine the cause of these differences due to inoculum source.

Our STEEP-funded research has clearly demonstrated that *A. rabiei* can infect plants in addition to chickpea. Most significantly, this fungus can infect wheat, which is an important component of

crop rotations in the PNW. The challenge remaining is to determine how the fungus infects non-hosts, how extensively it colonizes these plants and the significance of wheat infection in terms of the epidemiology and control of *Ascochyta* blight of chickpea. To undertake studies on the infection and reproduction behavior on non-host plants we have developed a transformation procedure using a polyethylene glycol-mediated method with hygromycin B and geneticin resistance genes as selectable markers. *Ascochyta rabiei* strains are transformed with either red (pTEFDsRed2) or green (pTEFEGFP) reporter plasmids to express red or green fluorescence, respectively. To represent the two *A. rabiei* chickpea pathotype groups two isolates of *A. rabiei* AR20 and AR628, which are weakly and highly pathogenic on chickpea, respectively have been transformed for infection studies. Protoplasts prepared from each fungal isolate were transformed or co-transformed with plasmids indicated in Table 4 and 5.

Table 4. Transformation frequency (Tf).

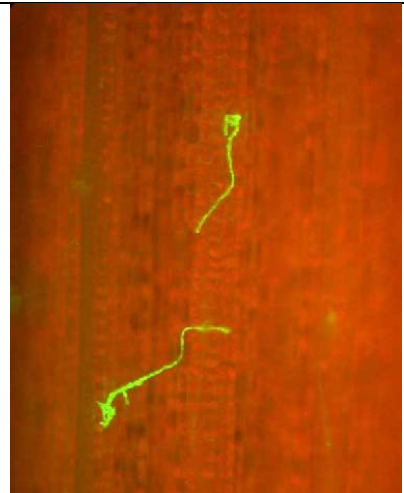
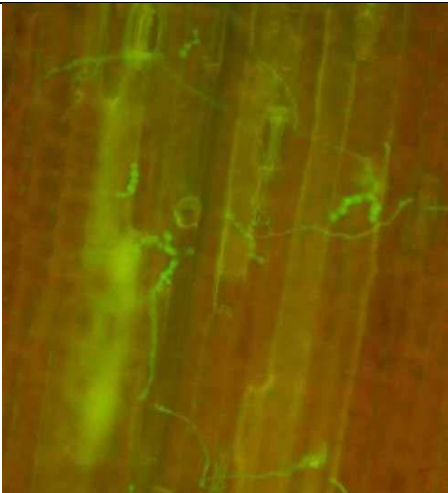
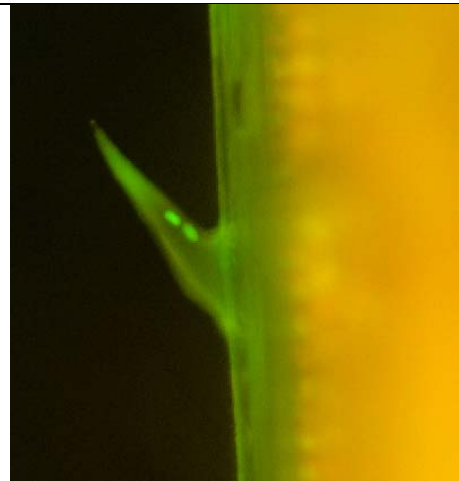
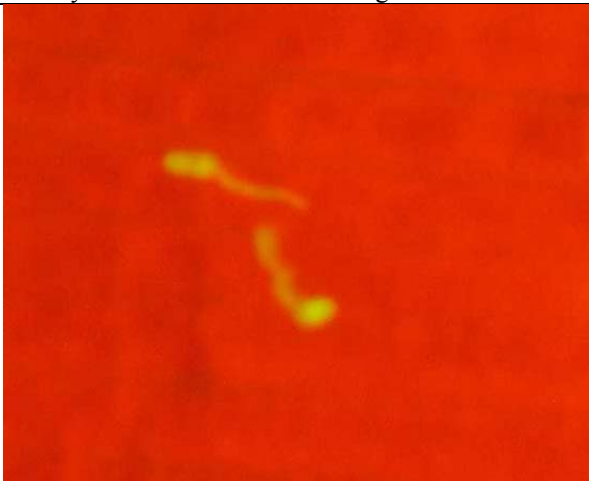
Plasmid	Selectable marker	Reporter gene	Total no. of transformants	Tf/ μg plasmid/ 10^7 protoplasts (%)
pSH75	<i>hph</i>	-	216	2.12
pII99	<i>nptII</i>	-	127	2.82
pHygEGFPex	<i>hph</i>	GFP	376	4.65
pTEFEGFP	-	GFP	-	-
pTEFDsRed2	-	DsRed2	-	-

Table 5. Co-transformation frequency (CTf).

Selection plasmid	Reporter plasmid	No. of co-transformants	Total no. of transformants	CTf (%)
pSH75	pTEFEGFP	80	103	77.7
pSH75	pTEFDsRed2	146	228	64.0
pII99	pTEFEGFP	18	29	62.1
pII99	pTEFDsRed2	16	28	57.1

The GFP-expressing strains are currently being used to investigate the infection process of *A. rabiei* on chickpea and non-host plants (Figures 1-4). Inoculated plants were observed using an Olympus BH-2 fluorescence microscope equipped with 490 nm and 545 nm excitation filters.

Figures 1-4. Infection assays of wheat (cv. Chinese Spring) with GFP transformed *A. rabiei*

	
<p>Figure 1. <i>A. rabiei</i> conidia germinated with extensive germ tubes, on detached wheat leaf 4 days after inoculation.</p>	<p>Figure 2. <i>A. rabiei</i> on detached wheat leaf 9 days after inoculation. As the leaf begins to senesce, <i>A. rabiei</i> hyphae begin to swell, perhaps as they draw nutrients. Fluorescence from the wheat plant begins to fade from red to yellow-brown as the cells begin to senesce.</p>
	
<p>Figure 3. <i>A. rabiei</i> conidia attached to the trichome of a wheat leaf, 24 hr after inoculation. Green fluorescence is exhibited by the edge of the wheat leaf.</p>	<p>Figure 4. <i>A. rabiei</i> conidia germinating on the leaf surface of an intact wheat plant 2 days after inoculation. Red fluorescence of the wheat leaf is exhibited. Non-germinated spores are also found on the non-host leaves days after inoculation, unlike inoculation of host plants where the majority of spores have germinated.</p>

We have demonstrated spore attachment to non-host surface and the germination of *A. rabiei* on non-host surface, together with the swelling of hyphae on non-host tissue indicating the growth and taking up of nutrients from this non-host. We are still characterizing the infection of non-hosts in a quantitative manner i.e. how many spores are germinating. And we will continue to investigate in further detail the infection process, determining when the fungus penetrates into the non-host and how it grows, either as an endophyte or as a latent pathogen.

Summary

1. Both GFP and DsRed2 genes were expressed in *A. rabiei* and transformed strains behaved normally in culture and on plant surfaces.
2. Fluorescence-expressing strains of *A. rabiei* are a powerful tool to observe the fungal infection process *in planta*.
3. Fungal strains expressing DsRed2 might be useful to analyse fungal behaviour in stem and seed where an intense green fluorescence is emitted by the host.
4. We have begun characterizing *A. rabiei* infection of resistant and susceptible chickpea varieties.
5. We have demonstrated attachment and germination of *A. rabiei* conidia of non-hosts in both detached leaf assay and whole plant inoculations and continue to characterize the non-host infection process.

Fluorescent strains together with fluorescence microscopy allow the study of *A. rabiei* infection of host and non-host plants, the extent to which the fungus colonizes and reproduces on these plants and the epidemiological significance of this behavior. We propose to determine the mechanism by which *A. rabiei* infects ‘non-host’ plants at the microscopic level by continuing to compare host infection with non-host infection in more detail and quantifying the interactions. Inoculations of host and non-host plants are being performed in growth chambers with the GFP transformed *A. rabiei* isolates. We also propose to investigate the potential transmission of *A. rabiei* from non-host plants to chickpea plants, i.e. by production of conidia or possibly by direct mycelial growth from infested wheat debris/plants to chickpea.

Studies of non-host infection and the development of a quantitative PCR assay are continuing projects.

INTERACTION WITH OTHER SCIENTISTS CONDUCTING RELATED ACTIVITY:

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Research groups of Dr.’s Peever and Muehlbauer interact regularly with those of Dr. Weidong Chen and Dr. Frank Dugan (USDA-ARS, Washington State Univ., USA). Dr. Chen developed the inoculation method used in these trials and performed the initial virulence testing of PNW isolates on chickpea. Dr P. Okubara (USDA-ARS, Washington State Univ., USA) assisted with quantitative PCR theory and techniques.

PUBLICATIONS AND PRESENTATIONS:

Akamatsu, H., Stone, J.L., Sigler, A.A., Chilvers, M.I., Arie, T. and Peever, T.L. 2005. Development of an integrative transformation system in the phytopathogenic fungus *Ascochyta rabiei* and visualization of the fungus *in planta* through expression of fluorescent proteins. To be

presented Dec. 2005 at the XII International Congress on Molecular Plant-Microbe Interactions in Cancun, México.

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