Rhizoctonia in Idaho Wheat, Barley and Common Bean Crops: Isolate Characterization and Potential Chemical Control

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Lara Brown

Major Professor: James Woodhall, Ph.D.

Committee Members: Juliet Marshall, Ph.D.; Michael Thornton, Ph.D.;

Kurtis Schroeder, Ph.D.

Department Administrator: Edwin Lewis, Ph.D.

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Authorization to Submit Thesis

This thesis of Lara Brown, submitted for the degree of Master of Science with a Major in Plant Pathology and titled "*Rhizoctonia* in Idaho Wheat, Barley and Common bean crops: Isolate Characterization and Potential Chemical control," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____
James Woodhall, Ph.D.

Committee Members: _____ Date: _____
Juliet Marshall, Ph.D.

_____ Date: _____
Michael Thornton, Ph.D.

_____ Date: _____
Kurtis Schroeder, Ph.D.

Department Administrator: _____ Date: _____
Edwin Lewis, Ph.D.

Abstract

Rhizoctonia and associated fungi are an important group of soil-borne pathogens. Three groups of *Rhizoctonia* fungi are important for plant diseases, these are the multinucleate *Rhizoctonia solani* and *Waitea circinata*, as well as binucleate *Rhizoctonia species* (BNR). The *Rhizoctonia* species complex is characterized through the affinity for hyphal fusion and isolates can be assigned to anastomosis groups (AGs) based on hyphal fusion affinity. The *Rhizoctonia solani* complex is comprised currently of thirteen known AGs, with twenty-nine subgroups. The BNR complex consist of twenty AGs with eight subgroups, including the most recently found AG W, found in China on potatoes (*Solanum tuberosum*) in 2015. There are five varieties that are known within *Waitea circinata* in the newly placed *Corticacaeae* family. AGs can differ in, means of dispersal, host range, fungicide sensitivity (Kataria and Gisi 1999) and aggressiveness. Even certain plant parts, such as roots of potatoes are almost exclusively infected by *R. solani* AG 8 (Woodhall et al. 2008). Multiple AGs have been implicated causing disease in wheat, barley and common beans. Presently there is limited knowledge of the AGs present on wheat, barley and common bean crops in Idaho. Furthermore, there is very limited knowledge of the AGs present in Idaho and the specific diseases they cause, particularly for Southern Idaho where there is a diverse range of crops grown.

Optimum disease management based on the knowledge of the AG, or even AG subgroup present, is essential for correct diagnosis. Therefore, this study aimed to determine which species, AGs or subgroups, are associated with Rhizoctonia diseases were present in wheat, barley and common bean crops in Idaho. 118 commercial wheat and barley fields and 102 commercial common bean fields were sampled in 2018, 2019 and 2020 resulting in 238 wheat and barley isolates and 188 common bean isolates of *Rhizoctonia* and related groups. Isolate identity was determined using rDNA ITS sequencing and phylogenetic analysis with known reference strains. Of the isolates collected, *Waitea circinata* varieties were most prevalent in wheat and barley, while AG 4 HG-II was the most prevalent in common beans. Phylogenetic placement showed three unidentified BNR isolates and five unidentified *Waitea circinata* varieties for wheat and barley. Phylogenetic placement showed two unidentified AG 11 clades for beans. For cereal fields, the relative incidence of *Rhizoctonia solani* AGs 2-1, 4 HG-II, 5, 8 and BNR AG D was also determined using real-time PCR on directly extracted soil DNA samples. This determined that AG2-1 was widely present in cereal fields prior to planting. With 62 wheat and barley isolates and 61 common bean isolates, replicated glasshouse experiments

were undertaken to determine their relative aggressiveness to each host. For cereals BNR AG D was the most aggressive, whilst AG 4 HG-I was most aggressive for beans. Finally, the effectiveness of various fungicides to control Rhizoctonia was evaluated in a laboratory based EC_{50} assay to 23 isolates. For AG 11, which was determined to significantly reduce bean yields in preliminary experiments, a field and glasshouse experiment was undertaken to determine the effectiveness of serval fungicides to control the pathogen in pinto beans. These experiments indicate that inpyrfluxam and prothioconazole showed most potential to manage the pathogen.

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Dedication

Firstly, I want to thank my parents for their unwavering love and support throughout the many years. They have supported my love for agriculture from an early age and it continues even into adulthood. I want to thank my amazing daughter for her support and understanding in the late nights, stressful and busy days. I can only hope it serves as an inspiring role model she can look up to as she looks to go to college. I want to thank my family who supported me from the beginning of the bachelor's degree and all the way through this journey. I want to thank all my friends who helped me along this journey, as well with their support and love.

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Chapter 1: Literature Review

Introduction

The Genus *Rhizoctonia*

Since the discovery of the *Rhizoctonia* genus in 1815 by De Candolle (Ogoshi 1987), *Rhizoctonia* species have been described worldwide in cultivated and uncultivated soils (González García et al. 2006). This ubiquitous group of fungi is primarily soil borne, initiating a wide variety of root and stem diseases (Ajayi-Oyetunde et al. 2017). However, many reports show this genus can be saprophytic and symbiotic as well (González García et al. 2006), initiating mycorrhizal relationships with orchids (Carling et al. 1999). Isolates within *Rhizoctonia* can be assigned to specific anastomosis groups (AGs) based on hyphal fusion affinity (Yang et al. 2012). Three groups of fungi associated within the wider species complex of *Rhizoctonia* are considered important for plant diseases, these are the multinucleate *Rhizoctonia solani* (*R. solani*) and *Waitea circinata*, *as* well as binucleate *Rhizoctonia* species (BNR) (Yang & Li 2012). The *R. solani* species complex is comprised of thirteen currently known AGs, with twenty-nine subgroups (Yang & Li 2012). The BNR complex consist of twenty AGs with eight subgroups, including the most recently found AG W, found in China on potatoes (*Solanum tuberosum*) in 2015 (Yang et al. 2015). *Waitea circinata* spp. are *Rhizoctonia*-like fungi that were originally described as anastomosis groups known as WAG-O for *Rhizoctonia oryzae* and WAG-Z for *Rhizoctonia zeae* (Sneh et al. 1991). Leiner and Carling proposed to describe *Waitea* spp. as varieties in 1994 over anastomosis groups (1994). In 2008, *Waitea circinata* was shown to be phylogenetically divided into four distinct clades placed in *Corticacaeae* family (Lawrey et al. 2008). Currently, there are five varieties that are known within *Waitea circinata* (Arakawa & Inagaki, 2014).

Classification

Vegetative characteristics of *Rhizoctonia* spp. are the basis for placement within the *Rhizoctonia* genus. *Rhizoctonia* species were one of the many plant pathogenic fungi to have been assigned more than one scientific name based off sexual and asexual stages (Ajayi-Oyetunde et al. 2017). However, dual nomenclature of pleomorphic fungi was discontinued in 2013 due to advancing technology verifying the weakness of using morphological characteristics to assign scientific names

(Hawksworth, 2011; Ajayi-Oyetunde et al. 2017). The new rule set forth by the International Code for Botanical Nomenclature (ICBN), states that precedence of the first published generic name to be used regardless of teleomorph or anamorph identification (Hawksworth, 2011). Since De Candolle first identified the species in 1815, the generic name for *Rhizoctonia* species is now solely under *Rhizoctonia* (Ajayi-Oyetunde and Bradley, 2017).

Within the *Rhizoctonia* spp., there are three separate names associated with the teleomorph or perfect sexual stage. *Thanatephorus cucumeris* (Frank) Donk, was used for *R. solani* (anamorph name), while *Ceratobasidium* was used for BNR species (Ogoshi, 1987). *Waitea circinata* has been used for *Waitea circinata* as a teleomorph for these species (Sharon et al. 2006). However, the teleomorphic stages are difficult to reproduce under laboratory conditions and many species are characterized under their anamorphic stages (González García 2006). Characterization of anamorphic stages includes the listed key attributes (Ogoshi, 1987; Sneh et al. 1991):

1. Young, vegetative hyphae with branching near the distal septum.
2. Origin of the branches has hyphal constriction and septa formation near it.
3. Dolipore septum
4. Lack of conidia, clamp connections or rhizomorphs
5. Undifferentiated sclerotia

Ceratobasidium and *Thanatephorus* belong in the family *Ceratobasidiaceae*, Order *Ceratobasidiales* in the Class Basidiomycota (Roberts 1999). *Waitea* belongs in the family *Corticacaeae*, Order *Waitea circinata* in the Class Basidiomycota. *Waitea* is classified as a *R. solani*, however, phylogenetic distance estimated by molecular analysis separates it from *Ceratobasidium* and *Thanatephorus* (Lawrey et al. 2008; Arakawa and Inagaki 2104). Furthermore, species of the *Waitea* genus possess determinate sterigmata up to 6 µm in length and self-replicating basidiospores, which distinguishes them from *Thanatephorus* (Roberts 1999). *Rhizoctonia* spp. have sections of hyphae separated by a septa, containing one to multiple nuclei (Roberts 1999). The presence of nuclei between the septa can be used to identify between binucleate and multinucleate species (Staplers and Andersen 1996). *Thanatephorus cucumeris* are classified as multinucleate species, with each section having at least three nuclei and *Ceratobasidium cereale* are classified as binucleate species with two nuclei per section.

Life Cycle

Unlike other basidiomycete species, *R. solani* does not have straightforward monokaryotic and dikaryotic life cycles (Cubeta and Vilgalys 1997). All groups of *R. solani* lack clamp connections resulting in a lack of morphological characteristics that allow mating reaction detection (Cubeta and Vilgalys 1997). Identification of mating behavior is further complicated by a lack of sporulation of *R. solani* isolates under laboratory conditions (González García et al. 2006). Lack of sporulation under laboratory conditions could be due to the predominantly asexual functionality of the species in nature (Ajayi-Oyetunde and Bradley 2017). Research on interactions between homokaryons of aerial tuft hyphae within a laboratory culture has been used to assess heterokaryon formation (Whitney 1963; Puhalla 1976). Extensive studies on homogenic or heterokaryon incompatibility systems has resulted in an evident bipolar mating system, where a single locus containing multiple alleles controls sexual compatibility (Whitney 1963; Puhalla 1976; Anderson 1972; Yang et al. 1992). Two closely linked genes, termed the heterokaryon incompatibility factor (H factor), were identified in AG 4 as genetic determinants for homogenic incompatibility (Anderson 1972). This mating system is heterothallic and at least two AGs, AG1-IC and AG 8 have evidence of being heterothallic (Adams Jr. 1996; Yang et al. 1992). Further, it has been estimated that 17 different H factors exist in natural populations (Anderson 1972). Heterokaryon formation occurs when homokaryons carrying different H factors are paired, creating tufts of heterokaryotic hyphae at the pairing junction characterized as heterokaryotization (Cubeta and Vilgalys 1997). Tuft formation has been found to be only slightly reliable and not always associated with heterokaryon formation since heterkaryotization was evident in cases without tuft formation (Cubeta and Vilgalys 1997; Cubeta 1993). Further, identification through tuft formation can be difficult if tuft-like growth occurs between heterokaryons (Yang et al. 1992). Even with these disadvantages, analysis through amplified fragment length polymorphism (AFLP) supported the heterokaryotic observations within tufts (Julián et al. 1999). Observed *R. solani* field isolates are mostly heterokaryotic and form morphologically and pathogenically distinct heterokaryons when paired with compatible heterokaryons (Ajayi-Oyetunde and Bradley 2017).

Several AGs that exhibit heterothallic mating systems, also exhibit homothallic behavior through the process of homokaryotic fruiting when primary hyphae produce basidia (Adams Jr. and Butler 1982). This process has been observed in other basidiomycetes and is known as haploid fruiting or monokaryotic fruiting (Julián et al. 1999). Progeny through homothallic self-mating can create population structure consequences through lack of genetic diversity (Cubeta and Vilgalys 1997). To

increase genetic diversity, homothallic AG can undergo recombination with a heterothallic AG through the process of heterokaryon-homokaryon (di-mon) mating (Cubeta and Vilgalys 1997). Production of a viable homokaryotic mycelium, from a germinated basidiospore on soil or plant tissue, must be capable of interacting with heterokaryotic mycelium on the structure (Cubeta and Vilgalys 1997). AGs 1 to 5 have been observed creating hymenia, consisting of basidia, basidiospores and sterigmata, within the canopy of crops (Cubeta and Vilgalys 1997).

The mating system of *Ceratobasidium* is unknown. It could be hypothesized that it is similar, if not the same as *R. solani* (Brown 2016). A recent review on environmental factors that can affect sexual reproduction of *Rhizoctonia* spp. does not specify differences between *R. solani* and BNR isolates, while stating that little is known about uninucleate and *Rhizoctonia*-like isolates mating system (Gonzalez 2006).

Anastomosis Groups

Anastomosis can be defined as the fusion between hyphal pairings of separate *Rhizoctonia* isolates. The ability of the hyphae to anastomose has been used as the traditional classification of *Rhizoctonia* spp. To date, the work originated by Carling and co-workers categorizing hyphal fusion that forms the basis of assignment of AGs to isolates is widely used (1987; 1988). Hyphal fusion with a tester isolate of a known AG to an unknown AG isolate is generally used for classification (Naito 2006). Hyphal pairing is categorized based on four different reactions during fusion (Table 1.1). Reactions are listed as C0 to C3, where C3 is perfect hyphal fusion of genetically identical or near identical isolates. Perfect fusion between isolates places them within the same vegetative compatibility group (VCG).

Table 1.1. Previous anastomosis reaction categorization systems for *Rhizoctonia* isolates (adapted from Carling, 1996)

Present Class (Carling et al., 1988)	**Matsumoto *et al.* (1932)**	**Flentje and Stretton (1964)**	**Parmeter *et al.* (1969)**
C0	No reaction	NR (No reaction)	0
C1	Contact	WF (Wall fusion)	1
C2	Imperfect	K (Killing)	2-Imperfect
C3	Perfect	S (Self)	2-Perfect

AGs exist as a higher taxonomic unit over VCGs, with subgroups within multiple AGs. To date there are thirteen AGs characterized in the *R. solani* species complex (Carling et al. 2002). Additionally,

subgroups within AG 1, 2, 3, 4, 6 and 9 have been identified, with AG 2 showing the most diversity with nine subgroups and five subsets (Table 1.2). There are no reports of subgroups within AG 5, 10, 11, 12, 13. In 2017, Ajayi-Oyetunde and Bradley (2017) identified two different clades within AG 7 through rDNA ITS phylogeny that could potentially be new subgroups, though there is no further published research.

To date there are twenty AGs characterized in the BNR species complex that are identified as AG A through AG W (Table 1.3) (Ogoshi et al. 1991; Sneh et al. 1991; Yang et al. 2015). Recent research with rDNA ITS phylogeny of the BNR species complex proved AG T was inaccurately identified and was actually AG A, therefore AG T is excluded from the currently characterized AGs (Sharon et al. 2008). The BNR species complex does not have as many subgroups identified as compared to *R. solani*. AGs D and F have the most with 3 subgroups identified within each AG. AG D, also known as *Rhizoctonia cerealis*, subgroups are designated I, II, III (Toda et al. 1999; Hayakawa et al. 2006), with subgroup AG D-I being the main causal agent in sharp-eye species in cereal crops (Li et al. 2014). rDNA ITS sequencing is used for designation of subgroups I, II, and III (Sharon et al., 2008). AG F subgroups are designated Fa, Fb and Fc. Designation between Fa and Fb subgroups has been based upon hyphal fusion and rDNA-ITS sequencing (Sharon et al. 2008), while subgroup Fc was based on rDNA-ITS sequencing between all AG F subgroups (Hua et al. 2004). A further taxonomic review on the BNR species needs to be done. Additional BNR isolates have been described as species rather than an AG. For example, *Ceratobasidium theobromae* has been characterized as a novel species, though through phylogeny placement the isolates are closely related to AG A and AG K (Samuels et al. 2012). Furthermore, multiple *Ceratobasidium* isolates found on several trees in Brazil causing white thread blight were characterized as new novel species, *C. niltonsouzanum* and *C. chavesanum* (de Melo et al. 2018). Phylogenetic placement of these isolates does not support the characterization as a new novel species, though does support the characterization of a new AG (Samuels et al. 2011; de Melo *et al.* 2018). Some BNR isolates have gone uncharacterized and could add further AGs or subgroups to the species (Woodhall et al. 2011; Schroeder et al. 2012).

Waitea species were first identified in 1934 as *Rhizoctonia zeae* (Voorhess 1934) and in 1938 as *Rhizoctonia oryzae* (Ryker and Gooch 1938). The teleomorph stage for both *R. zeae* and *R. oryzae* was described as *Waitea circinata* by Warcup and Talbot (1962). In 1985, *R. zeae* and *R. oryzae* were linked to their teleomorphs resulting in two anastomosis groups, WAG-O for *R. oryzae* and WAG-Z for *R. zeae* (Oniki et al. 1985). In 1994, Leiner and Carling proposed *Waitea* spp. be described as a variety

over anastomosis groups when they characterized *Waitea circinata* var. *circinata* from Alaskan soils (1994). In 2008, phylogenetic analysis placed *Waitea* varieties in the *Corticiaceae* family, where they are closely related to *Laetisaria* (also known grass pathogens) species (Lawrey et al.). Recently two additional varieties have been found, *Waitea circinata* var. *agrotis* (Toda et al. 2007) and *Waitea circinata* var. *prodigus* (Table 1.4) (Kammerer et al. 2011).

Table 1.2 Currently described *Rhizoctonia solani* anastomosis groups and subgroups with details of differentiation methods, pectic zymogram group, host range and thiamine requirement (adapted and updated from Kuninaga 2002)

AG	Subgroup	Subset	Differentiation Method[1]		Zyogram[2]	Host Range	Thiamine (where known)	Reference
1	IA		ITS	FA		Corn, bean, rice, barley, turf grass, potato, lima bean, leaf lettuce, cabbage, soybean, snap bean	autotrophic	Sneh *et al.* (1991); Fenille *et al.* (2002); Naito (2004); Yang and Li (2012); Quadros, *et al.* (2019)
	IB		ITS	FA		Common bean, adzuki bean, corn, sugar beet, onion, leaf lettuce, soybean, bent grass	autotrophic	Sneh *et al.* (1991); Naito (2004); Yang and Li (2012)
	IC		ITS	FA		Spinach, radish, carrot, sugar beet, soybean, bean, onion	autotrophic	Sneh *et al.* (1991); Naito (2004); Yang and Li (2012)
	ID		ITS	FA		Coffee	autotrophic	Priyatmojo *et al.* (2001)
	IE		ITS			Common bean	autotrophic	Godoy-Lutz *et al.* (2003)
	IF		ITS			Common bean, cowpea, soybean	autotrophic	Godoy-Lutz *et al.* (2003); Chavarro-Mesa *et al.* (2019)
	IG		ITS			Kale, Chinese flowering cabbage, chickpea	autotrophic	Hua *et al.* (2014); Chen *et al.* (2020)
2	1		ITS		5, 6	Potato, pepper, wheat, sugar beet, canola, spinach, strawberry, cauliflower, onion	autotrophic	Sneh *et al.* (1991); Naito, 2004; Pannecoucque *et al.* (2008); Yang and Li (2012)
		Subset-1 Japanese culture type II and Dutch AG-2T	ITS			Tulip, radish, pea, barley, strawberry	autotrophic	Kuninaga *et al.* (2000); Das et al. (2014), Misawa *et al.* (2018)
		Subset 2-Alaskan and Australian isolates	ITS			Barley, potato, broccoli	autotrophic	Misawa et al. (2018)
		Subset 3-AG 2-1/NT	ITS			Tobacco, tomato, potato	autotrophic	Nicoletti *et al.* (1999); Carling *et al.* (2002); Misawa and Kuninaga (2010); Das *et al.* (2014); Misawa et al. (2018)
		HK Clade	ITS			Cauliflower, Welsh onion, cabbage, Chinese cabbage, mustard	autotrophic	Misawa et al. (2018)
		UK Potato Clade	ITS			Potato	autotrophic	Woodhall *et al.* (2007), Misawa *et al.* (2018)
	2-IIIB		ITS	FA	4, 10	Onion, sugar beet, rice, corn, soybean, common bean, wheat	auxotrophic	Engelkes *et al.* (1996); Sneh *et al.* (1991); Zhao *et al.* (2014); Yang and Li (2012); Misawa *et al.* (2017)

	2-IV	ITS	FA	4, 10	Sugar beet, common bean, soybean, carrot, pepper, spinach	auxotrophic	Engelkes *et al.* (1996); Sneh *et al.* (1991); Yang and Li (2012)
	2-V	ITS	FA		Creeping bentgrass	auxotrophic	Abad et al. (2003)
	2-LP	ITS	FA		Turf grass, chickpea	auxotrophic	Hyakumachi *et al.* (1998);
	2-WB	ITS	FA		Common bean	auxotrophic	Godoy-Lutz *et al.* (2008); Nerey *et al.* (2009)
	3	ITS			Soybean, chickpea	auxotrophic	Naito and Kanematsu (1994); Youssef *et al.* (2010)
	4	ITS			Corn, carrots	auxotrophic	Sumner *et al.* (2003);
	BI	ITS			None-Bridging isolate	auxotrophic	Yang and Li (2012)
3	PT	ITS	FA	7	Potato, tomato, corn	autotrophic	Carling and Leiner (1986); Misawa and Kuninaga (2010); McCormack *et al.* (2013)
	TB	ITS	FA		Tobacco	autotrophic	Kuninaga *et al.* (2000)
	TM	ITS	FA		Tomato	autotrophic	Misawa *et al.* (2020)
4	HG I	ITS	FA		Soybean, common bean, onion, swiss chard, sugar beet, potato	autotrophic	Yang *et al.* (2007); Nerey *et al.* (2010); Strausbaugh *et al.* (2011); Muzhinjii *et al.* (2015); Ajayi-Oyetunde and Bradley (2017); Misawa *et al.* (2017)
	HG II	ITS	FA	8	Potato, snow pea, barley, sugar beet, common bean	autotrophic	Strausbaugh *et al.* (2011); Woodhall *et al.* (2012); Keshavarz Tohid and Taheri (2014); Woodhall *et al.* (2019); Pizolotto *et al.* (2020)
	HG III	ITS	FA		Soybean, onion, potato, common bean	autotrophic	Muzhinjii *et al.* (2015); Ajayi-Oyetunde and Bradley (2017); Misawa *et al.* (2017)
5		ITS			Pea, potato, barley, wheat, sugar beet, tobacco, turf grass, soybean, onion, bean	auxotrophic	Anderson *et al.* (1982); Eken and Demirci (2004); Mathew *et al.* (2011); Yang and Li (2012); Muzhinjii *et al.* (2015); Misawa *et al.* (2017)
6	HG I	ITS			Mycorrhizal with orchid, wheat, apple	autotrophic	Review research done by Sharon *et al.* (2006) shows phylogenetic placement of subgroup GV within four different subgroups. Most research shows all GV subgroups to be nonpathogenic and mycorrhizal with orchids, though it has been found to cause wheat crater disease (Meyer *et al.* 2007) and cause apple replant disease (Mazzola 1997).
	GV1	ITS				autotrophic	
	GV2	ITS				autotrophic	
	GV3	ITS				autotrophic	
	GV4	ITS				autotrophic	
7		ITS	FA		Soybean, potato, cotton, radish	autotrophic	Homma *et al.* (1983); Rothrock *et al.* (1993); Abd-Elsalam *et al.* (2009); Abd-Elsalam *et al.* (2010)

8		ITS	1-1 to 1-5	Potato, barley, wheat, pea, onion	autotrophic	Ogoshi *et al.* (1990); Patzek *et al.* (2013); Woodhall *et al.* (2008); Sharma-Poudyal *et al.* (2015)
9	TP	Thiamine		Potato, lettuce, carrot	autotrophic	Carling *et al.* (1987)
	TX	Thiamine		Potato, lettuce, carrot	auxotrophic	Carling *et al.* (1987)
10		ITS		Canola, pea, wheat, barley	auxotrophic	MacNish *et al.* (1996); Schroeder and Paulitz (2010); Sharma-Poudyal *et al.* (2015); Jaaffar et al. (2015)
11		ITS	9	Soybean, sugar beet, lupine, wheat, rice, lily, bean	auxotrophic	Carling *et al.* (1994); Jaaffar et al. (2015); Sharma-Poudyal *et al.* (2015); Misawa et al. (2017); Moliszewska *et al.* (2020), Woodhall et al. (2020)
12		ITS	3	Radish, cauliflower, mycorrhizal with orchid	autotrophic	Carling *et al.* (1999)
13		ITS		Corn, cotton		Carling *et al.* (2002); Tomaso-Peterson and Trevathan (2004)

[1]Differentiation method: ITS, rDNA ITS sequence and/or RFLP analysis; FA, fatty acids. [2]Pectic zymogram pattern group (MacNish and Sweetingham, 1993; MacNish et al., 1994

Table 1.3 Currently described Binucleate *Rhizoctonia* (BNR) anastomosis groups and subgroups with details of differentiation methods and host range

AG	Subgroup	Differentiation Method[1]		Host Range	References
A		ITS	HF	Potato, swiss chard, pea, strawberry, sugar beet	Sharon *et al.* (2007); Yang *et al.* (2007); Nerey *et al.* (2009); Yang and Li (2012); Miles *et al.* (2013); Zhang *et al.* (2016);
B	Ba	ITS	HF	Rice, foxtail millet	Sneh *et al.* (1991)
	Bb	ITS	HF	Rice, foxtail millet	Sneh *et al.* (1991)
C		ITS	HF	Sugar beet, strawberry	Ogoshi (1982); Fang *et al.* (2013)
D	I	ITS	HF	Barley, zoysia grass, wheat	Toda *et al.* (1999); Yang and Li (2012); Ünal and Kara (2017)
	II	ITS	HF	Zoysia grass	Toda *et al.* (1999)
	III	ITS	HF	Zoysia grass	Hayakawa *et al.* (2007)
E		ITS	HF	Onion, kale, sugar beet, soybean, tomato, radish, bean	Ogoshi *et al.* (1982); Sneh et al. (1991); Yang and Li (2012); Patzek *et al.* (2013); Türkkan *et al.* (2020)
F	a	ITS	HF	Common bean, kale, strawberry, corn, sugar beet, tobacco, onion	Burpee et al (1980); Eken and Demirci (2004); Sharon *et al.* (2007); Tolga Gurkanli and Ozkoc (2011); Türkölmez *et al.* (2019); Türkkan *et al.* (2020); Woodhall *et al.* (2020)
	b	ITS	HF	Tobacco	Tolga Gurkanli and Ozkoc (2011)
	c	ITS	HF	Chinese cabbage	Hoang Hua *et al.* (2014)
G		ITS	HF	Strawberry, miniature roses, potato, peanut, sugar beet, tomato, bean	Sneh et al. (1991); Demirci and Döken (1995); Eken and Demirci (2004); Sharon *et al.* (2007); Yang and Li (2012); Yang *et al.* (2013)
H		ITS	HF	Orchids (symbiosis)	Yang and Li (2012)
I		ITS	HF	Pea, strawberry, sugar beets	Ogoshi *et al.* (1982); Martin (2007); Sharma-Poudyal *et al.* (2015)
K		ITS	HF	Pea, kale, strawberry, tomato, carrot, radish, wheat, chickpea	Sneh et al. (1991); Sharon *et al.* (2007); Yang and Li (2012); Sharma-Poudyal *et al.* (2015); Türkkan *et al.* (2020);
L		ITS	HF	Soil	Ogoshi *et al.* (1990)
M		ITS	HF	Soil	Ogoshi *et al.* (1990)
O		ITS	HF	Soil	Ogoshi *et al.* (1990)
P		ITS	HF	Red birch, tea	Sneh et al. (1991); Yang *et al.* (2006)
Q		ITS	HF	Turf grass	Oniki *et al.* (1986)
R		ITS	HF	Potato, ginger, red birch, radish, pea, onion, bean, tomato, peanut	Burpee et al (1980); Sneh et al. (1991); Yang et al. (2006); Sharon *et al.* (2007); Muzhinjii *et al.* (2015); Yang *et al.* (2018)
S		ITS	HF	Wheat, barley, azalea	Yang and Li (2012)
U		ITS	HF	Potato, carrot	Misawa and Toda (2013); Misawa and Kurose (2018)
V		ITS	HF	Taro, ginger	Dong *et al.* (2017)

W	ITS				HF	Potato, sugar beet	Yang *et al.* (2015); Zhao *et al.* (2019)

[1]Differentiation method: ITS, rDNA ITS sequence and/or RFLP analysis; HF, Hyphal fusion. (MacNish and Sweetingham, 1993; MacNish et al., 1994)

Table 1.4 Currently described *Waitea circinata* variety groups with details of differentiation methods and host range

	Variety	**Differentiation Method[1]**				**Host Range**	**References**
Waitea circinata	*agrostis*	ITS				Bentgrass	Toda *et al.* (2007)
	circinata	ITS	FA	β-tubulin	HF	Turfgrass, wheat, barley	DeMirci (1998); Toda *et al.* (2007)
	oryzae	ITS	FA	β-tubulin	HF	Rice, bentgrass, carrots, pea, wheat, barley, maize	Mazzola et al. (1996); Ali *et al.* (1998); Paulitz *et al.* (2007); Paulitz (2007); Guo *et al.* (2006); Toda *et al.* (2007); Ünal *et al.* (2013)
	prodigus	ITS				Seashore paspalum, kikuyugrass	Kammerer et al. (2011); Chen *et al.* (2011)
	zeae	ITS	FA	β-tubulin	HF	Cabbage, rapeseed, Bermudagrass, soybeans, bean bent grass, wheat, onion	Erper *et al.* (2003); Toda *et al.* (2007); Kerns *et al.* (2017); Vojvodić *et al.* (2021), Erper *et al.* (2007)

[1]Differentiation method: ITS, rDNA ITS sequence and/or RFLP analysis; FA fatty acids; β-tubulin, beta tubulin; HF, Hyphal fusion. (MacNish and Sweetingham, 1993; MacNish et al., 1994)

Importance of Anastomosis Groups

The importance of AGs is clearly expressed in the statement by Cubeta and Vilgalys (1997): "Anastomosis grouping still represents the single most important advance in our understanding of the genetic diversity within *Rhizoctonia*". *Rhizoctonia* spp. have been placed in genetically related AGs based off hyphal fusion affinity, though they can also differ in genotypic and phenotypic characteristics (Kuniaga and Carling 2000). These phenotypic characteristics, which have an influence on understanding and managing plant diseases, including host range and aggressiveness, optimal growth temperature, means of survival and disease transmission, and fungicide sensitivity which are discussed individually below.

Rhizoctonia spp. can cause a wide range of disease symptoms from damping off to foliar lesions depending on the diversity of AGs and subgroups. Damping off, caused by a variety of AGs, can be described as a seed or seedling that fails to germinate or emerge due to infection from a soilborne pathogen (Schumann and D'Arcy 2019). *Rhizoctonia* spp. are capable of infecting seeds and seedlings since they are more vulnerable prior to emergence (Schumann and D'Arcy 2019). The major AGs associated with damping off include AG 2-1, AG 2-2 IIIB, AG 4 HG-II (Dorrance et al. 2003; Paulitz et al. 2016, Hanson and McGrath 2011). Root rot disease occurs when *Rhizoctonia* spp. attack the newly

emerged roots of the seedling, resulting in stunted crops with a reduction in vigor (Schroeder and Paulitz 2007). The most common AGs associated with root rot include AG 2-2 IIIB and AG 8 (Nerey et al. 2010; Schroeder and Paulitz 2007). Bare patch disease can be a combination of both, damping off and root rot, in wheat (*Triticum aestivum L.*) and barley (*Hordeum vulgare*) crops if there is a long history of a no-till, production practices (MacNish and Neate 1996; Schroeder and Paulitz 2007). The two primary pathogens for bare patch are *R. solani* AG 8 and *Waitea* var. *oryzae* (Schroeder and Paulitz 2007; Ogoshi et al. 1990). Crown rot in sugar beets (*Beta vulgaris*) occurs from the pathogen entering the crown of the crop and moving down the root, whereas root rot starts at the tip of the crop and moves upward (Neher and Gallian 2011). Crown rot can occur from AG 2-2 IIIB and AG 2-2 IV (Buhre et al. 2008). Web blight disease, which infects many bean (*Phaseolus vulgaris* L.) species, is common in environments that are warm and humid (Costa-Coelho et al. 2014). Symptoms include cobweb like mycelium along with leaf, pod, stem and petiole lesions (Singh and Kumar, 2018). AGs 1-IA, AG 1-IB, AG 1-IE, AG 1-IF, AG 2-2 and AG 4 have been associated with web blight disease (Yang et al. 1990; Valentín Torres et al. 2016). Rice sheath blight is a disease caused by AG 1 IA on rice (*Oryza sativa*) leaf sheaths causing lesions that can enlarge and spread to other leaf blades (Uppala and Zhou 2018). Multiple patch diseases can occur in turfgrasses (*Poaceae* family) caused by different *Rhizoctonia* spp. Brown patch, large patch, yellow patch and leaf and sheath spot are diseases that cause patches or rings in the grass due to infection from the pathogen (Tredway and Burpee 2001). Symptoms occur as lesions on the foliar parts of the grass (Tredway and Burpee 2001).

Rhizoctonia spp. are able to infect a wide range of plants from ornamentals to crops (Table 1.2, 1.3, 1.4). Previous research has shown that there is genetic and host diversity within individual AGs and their ability to produce specific symptoms on specific hosts (Muzhinji et al. 2015). *R. solani* AG 3 was originally characterized as a homogeneous population only infecting potato (Das et al. 2014). To date AG 3 has been characterized on potatoes, tomatoes (*Solanum lycopersicum*), corn (*Zea mays*) and tobacco (*Nicotiana tabacum*) (Table 1.2). Currently, AG 3 is subdivided into three subgroups based on host specificity. Individual AGs and subgroups are capable of infecting multiple hosts with different symptoms. For example, AG 1-IA can cause web blight on snap beans (*Phaseolus vulgaris* L.) (Ferreira Quadros et al. 2019), while causing rice sheath blight on rice (Uppala and Zhou 2018). *R. solani* AG 8 has been found to severely infect potato roots, with no other symptoms presenting on potatoes (Woodhall et al. 2008). A previous characterization of AG 8 reported that is also caused bare patch disease of wheat and barley (Ogoshi et al. 1990).

Aggressiveness of individual AGs and subgroups can vary as well. Woodhall et al. (2007) showed diverse aggressiveness within the AG 2-1 strains in the UK. Temperatures can also affect aggressiveness in AGs and subgroups. Carling and Leiner (1990b) reported AG 5 caused disease on potato stem when temperatures were 15.5 – 21.1 °C compared to AG 3-PT which caused greater damage at 10 °C.

Optimal growing temperatures and environments of individual AGs may influence geographical distribution. AG 2-2 IIIB has optimal growth at 35 °C in cultures, while AG 2-2 IV will not grow at that temperature (Sneh et al. 1991). AG 3-PT has been commonly associated with a cooler environment and high altitudes, while AG 4 has been associated with warmer temperatures and lower altitudes in Peru (Anguiz and Martin 1989). Environment is a key component of the disease triangle, though more than just temperature can play a role. A recent study found that fluctuating temperatures and moisture in the soil, along with the soil type can influence disease severity along with disease symptoms (You and Barbetti 2017). This study also indicated that fluctuating temperatures likely plays a key role in relative expression of host plant resistance to *R. solani* in subterranean clover (You and Barbetti 2017). Soil type can contribute to disease severity through increased presence of pathogens in certain soils (Jager and Velis 1983). Furthermore, moisture content of soil can affect AGs. A study on AG 8 causing disease on wheat seedlings showed severe disease at 75% WHC in lower temperatures over warmer temperatures with lower WHC (Gill et al. 2001).

Rhizoctonia spp. are able to infect plant hosts through either vegetative mycelium or sclerotia. Sclerotia are compact masses of monilioid cells that are enlarged through repeated branching and formation of new cells (Sumner 1996). Some AGs, such as AG 3 on tomatoes, are able to infect plants through basidiospores (Bartz et al. 2010), though this type of inoculum source is under studied. Of these inoculum sources, the vast majority of plant diseases are thought to be initiated from sclerotia (Anderson 1982). The formation of sclerotia allows the pathogen to survive in debris and/or organic matter in the soil (Papavizas et al. 1975). Individual AGs can produce different amounts of sclerotia (Anderson 1982). AG 3-PT has been shown to produce large amounts of sclerotia in culture, as well as on potato tubers as black scurf, compared to other AGs (Woodhall et al., 2008). This seed tuber borne sclerotia will serve as inoculum in subsequent crops. Through a specific PCR assay targeted to detect AG 3-PT in soil, the study showed an increase in the presence of AG 3-PT in the soil post-harvest over pre-harvest (Woodhall et al. 2013). Furthermore, AG 2-1 was found in the soils and on tubers through direct DNA detection more frequently than through isolation methods, suggesting

that AG 2-1 can survive on tubers as hyphae or a-typical blemishes that are difficult to isolate (Woodhall et al. 2013). AG 2-1 does not produce sclerotia as abundantly as AG 3-PT, thus making it more difficult to isolate from tubers (Woodhall et al. 2013).

The sensitivity of individual AGs of *Rhizoctonia* to several fungicides can also vary (Kataria and Gisi 1999). This is inherent sensitivity or insensitivity and should be considered separate to the development of resistance. Resistance occurs from repeated applications of the same fungicide resulting in selection pressure. This has occurred in *R. solani* with resistance to tolclofos-methyl (van Brugeen and Arneson 1984) with cross resistance occurring to iprodione and tolclofos-methyl on AG 2 isolates (Gullino et al. 1984) and resistance to flutolanil on AG 4 HG-I isolates with cross resistance to thifluzamide, penthiopyrad and tebuconazole (Zhao et al. 2019a).

Variation in inherent sensitivity of different AGs to fungicides has been observed with triadimenol, propiconazole, carboxin, furmecyclox, and the pyrimidine fungicides, fenarimol and nuarimol (Kataria and Gisi 1999). However, the phenylurea fungicide, pencycuron, probably exhibited the highest selectivity amongst AGs as variability in sensitivity can even differ between individual subgroups of AG4 (Kataria and Gisi 1999). Binucleate *Rhizoctonia* isolates are insensitive to pencycuron, while a AG1, AG2-1, AG2-2, AG3, AG6 and AG9 are sensitive. In contrast, pencycuron exhibits little or no activity against AG5, AG7 and AG8 (Kataria and Gisi 1989; 1999). The existence of such fungicide selectivity has led to the speculation that their continued use may force a change in the pathogen population towards insensitive AGs.

Rhizoctonia Diseases

As an important group of soil-borne diseases, many AGs of all *Rhizoctonia* species can cause damping off on a wide range of species. However, it could be argued that a degree of host specialization by individual AGs can be observed with specific diseases and AGs. Two examples are AG 3-PT with potato stem canker and black scurf, as well as AG D with sharp eyespot in cereals. In this study, the *Rhizoctonia* AG complex was investigated on Idaho wheat and barley, as well as on beans and this is discussed below.

Rhizoctonia on Wheat and Barley

Global production of wheat crops increased to a record high in March of 2021 due to a record crop in Australia (The Foreign Agricultural Service USDA 2021). The increased demand for wheat, due to

residual use in China and higher feed use, accounted for the increase in wheat crops grown (The Foreign Agricultural Service USDA 2021). 776.8 million metric tons of wheat was produced worldwide, with the U.S. producing ~6.4% or 49.7 million metric tons in 20/21 (The Foreign Agricultural Service USDA 2021). 42 states in the U.S. grow wheat, though Idaho is one of the select few states that can grow five market classes of wheat (ISDA 2021; U.S. Wheat Associates 2021). Idaho is ranked 5th in the country for wheat production and wheat ranks 2nd behind potatoes within the state agricultural economy (ISDA 2021). The U.S. exported 553,000 metric tons of barley in 2018/2019 (U.S. Grains Council) for malt and food use. Idaho is the top producing state for barley, with 75% of production going to malt production (ISDA 2021). Both wheat and barley crops, jointly ranking above potatoes in economic value, are important to the agricultural economy of Idaho and worldwide consumption.

Rhizoctonia spp. can cause yield loss in both wheat and barley, though published literature that has quantified yield losses within an actual field of wheat and barley is limited. Literature on yield loss caused by *R. solani* and BNR AGs mostly focuses on AG 8 and *R. cerealis*, the most common *Rhizoctonia* spp. on wheat and barley. A yield loss of 25% or higher was attributed to AG 8 when combined with no-till production practices in Australia (MacNish and Neate 1996). The Pacific Northwest region found similar results with AG 8 accounting for a higher yield loss and reduced quality of wheat and barley when no-till practices were used (Smith et al. 2003). The no-till method allows proliferation of hyphal networks through lack of soil disturbance, which also greatly increases the numbers of weeds and volunteer crops that can act as a host if not controlled in a timely manner (MacNish 1985; Pumphrey et al. 1987; Smiley et al. 1996; Paulitz et al. 2002). Weeds are defined as grassy weeds and some broad leaf weeds, while volunteer crops are mainly barley and wheat from previous season(s) (Veseth 1992). Yield loss from *R. cerealis* is highest when severe sharp eyespots lesion occurs, though moderate lesions have been shown to cause yield loss as well (Hamada et al. 2011).

Control practices for *Rhizoctonia* spp. in wheat and barley include cultural, chemical and biological methods. Elimination of grassy weeds and volunteer plants have been reported as the most significant cultural control option regarding control of AG 8 within no-till fields (Cook, 2001). Although it should be pointed out, this control method heavily relies on chemical control since many grassy weeds and volunteer plants are treated with a glyphosate herbicide prior to planting (Baley et al. 2008; Babiker et al. 2011). Additional chemical control methods include foliar and seed

treatments with various fungicides (Hamada et al. 2011). Screening soils prior to planting to determine *Rhizoctonia* spp. present will determine fungicide use and rate. This method is currently used in Australia as the PredictaB system (Ophel-Keller et al. 2008). Biological control methods are not widely available for commercial use, though many studies are looking to use this option as a means to be more sustainable. Biological control agents include *Pseudomonas* spp. (Jaaffar et al. 2017) and *Trichoderma* spp. (Howell et al. 2007). Biological agents control the pathogen through siderophore production, antibiosism induced resistance and competition (Kloepper et al. 1980; Cardoso and Echandi 1987; Harris et al. 1997).

***Rhizoctonia* on Common Bean Crops**

Idaho is ranked 1st in the U.S. for common bean seed production and 5th for common bean (Ellis 2020). With nationwide production at 33.0 million hundred weight for 2020 (USDA). Production of common beans from Idaho is forecasted at 1.67 million cwt for 2020, which is a 56% increase from 2019 (USDA 2020).

Rhizoctonia spp. in common beans has resulted in up to a 100% loss (Hagedorn 2005; Singh and Schwartz 2010). A study on AG 2-2 IIIB and AG 4 HG-I showed from 13 to 100% loss from damping off, root rot and post-emergence diseases in Egypt (Rashad et al. 2012). In the U.S., losses from *Rhizoctonia* spp. of more than 10% in tilled fields and 20-30% in no-till fields has been shown due to root rot caused by AG 2-2 and AG 4 (Hagedorn 2005). Web blight disease has been estimated to have caused a $7.1 million dollar loss in one season in El Salvador in 1993 (Godoy-Lutz et al. 2008). Not only does web blight cause a reduction in yield through defoliation at any stage (Valentín Torres et al. 2016), it can occur with other abiotic and biotic stresses, such as poor seed quality (Godoy-Lutz Costa-Coelho et al. 2014).

Cultural and chemical methods are commonly used to control *Rhizoctonia* spp. in common beans. Cultural control includes reduced compaction of soil, shallow seed placement, planting in warm soil, and rotation with nonhost crops. Soil compaction has been found to compound hypocotyl rot and stem cankers through increased stress to the plant that results in a reduced root development (Harveson et al. 2005). Shallow seed placement reduces infection from *Rhizoctonia* root rot pathogens by reducing emergence time (Manning et al. 1967; Leach and Garber 1970). Because damping off pathogens are favored by lower soil temperatures and excessive soil moisture, planting into moist soils at a temperature of at least 20 °C or greater, helps to reduce disease (Hagedorn

2005). Planting bean crops after potatoes or sugar beets can increase disease in the bean crop (Hagadorn 2005). Rotations with nonhost crops breaks pathogen life cycles (Hagadorn 2005). Chemical control utilizes seed treatments and in-furrow sprays. In-furrow treatment is not commonly used due to the longevity of seed treatments (Rideout 2002; Tvedt 2017). In-furrow fungicides are diverse with different classes being used (Tvedt 2017). As with wheat and barley, biological methods are being studied for control by utilizing yeast (de Tenório et al.), bacterial, e.g., *Bacillus pumilus* and fungal, e.g., *Trichoderma harzianum* and *Rhizophagus intaradices* agents (Nasir Hussein et al. 2018).

Objectives

Presently there is limited knowledge of the extent of AGs present on wheat, barley and common bean crops in Idaho and the specific associated diseases, particularly for Southern Idaho where there is a diverse crop rotation. Knowledge of fungicide sensitivity in *Rhizoctonia* isolates found in Idaho is also crucial for disease management and to help reduce resistance development in the AGs.

Therefore, the specific objectives of this research project were to:

- Determine the diversity of AGs present in Idaho by surveying wheat and barley crops and soil. Characterize of isolates collected from wheat and barley crops throughout the state, determining AGs using rDNA ITS sequencing and phylogenetic placement. The aggressiveness caused by selected representative isolates of each AG will be determined in two glasshouse experiments. The relative incidence of root and stem diseases in Idaho crops analyzed, as well as the prevalence of AGs 2-1, 4 HG-II, 5, 8 and D, detected in wheat and barley field soils prior to planting using direct soil DNA extraction and real-time PCR.
- Determine the diversity of AGs present in Idaho common bean crops utilizing a soil and plant survey. Characterize isolates collected from common bean crops throughout Southern Idaho production areas, with AGs determined using rDNA ITS sequencing and phylogenetic analysis. The aggressiveness and type of disease caused by selected representative isolates of each AG will be determined in two glasshouse experiments. The relative incidence of root and stem diseases in Idaho crops will be analyzed and reported.
- Determine the effectiveness of fungicides to isolates of *Rhizoctonia* spp. collected in Idaho through *in vitro* EC_{50} determination. Field and glasshouse experiments will be conducted to determine the effectiveness of fungicides in controlling *Rhizoctonia solani* AG 11 in pinto beans.

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Chapter 2: Characterization and Prevalence of Individual *Rhizoctonia* Species Associated with Wheat and Barley in Idaho

Abstract

238 isolates associated with the *Rhizoctonia* species complex were recovered from Idaho wheat and barley plants between 2019 and 2020. Isolates were assigned to species or anastomosis group (AG) through sequencing of the rDNA internal transcribed spacer region. The most frequently isolated species were *Waitea circinata* varieties with 75 isolates recovered, the binucleate *Rhizoctonia* AG D (*Ceratobasidium cereale*) accounting for 68 isolates followed by *Rhizoctonia solani* AG4 HG-II (38 isolates). In addition, *R. solani* isolates representing AG2-1, AG2-2, AG3, AG 5 and AG11 were present, as well as the binucleate *Rhizoctonia* species AG C, D, E, H, K and 3 isolates of a previously unidentified BNR species. Cereal fields were sampled prior to planting for AG 2-1, 4 HG-II, 5, 8 and AG D using real-time PCR. AG2-1 was most frequently present in Idaho cereals fields followed by AG 4 HG-II. In a glasshouse experiment, AG D was determined to be the most aggressive to winter wheat stems (cv Stephens), followed by AG K. This is the first study to determine the relative incidence of individual AGs of *Rhizoctonia* over the whole state of Idaho, previous studies only considered northern Idaho. Knowledge of the relative incidence of individual *Rhizoctonia* species and AGs present will enable appropriate disease management practices to be applied.

Introduction

Rhizoctonia, is considered a species complex composed of several groups. These groups include multinucleate *Rhizoctonia solani* (*Thanatephorus cucumeris*), binucleate *Rhizoctonia* species (BNR or *Ceratobasidium*) and a range of *Rhizoctonia*-like species, originally characterized as *Rhizoctonia oryzae* (WAG-O) and *Rhizoctonia zeae* (WAG-Z) (Sneh et al. 1991). *Rhizoctonia oryzae* and *Rhizoctonia zeae* are now considered *Waitea circinata* within the *Corticacaeae* family (Lawrey et al. 2008). Considerable diversity exists within both *Rhizoctonia solani* and BNR species. The basis of hyphal fusion can be used to assign isolates to numerous anastomosis groups (AGs). AGs of *R. solani* have been designated AG1 to 13 (Carling et al. 2002) and for BNR species AG A to AG W (Yang et al. 2015). Furthermore, many subgroups exist within individual AGs. *R. solani* contains 28 subgroups, while BNR contains 8 subgroups (Sharon et al. 2006; Yang and Li 2012; Hua et al. 2014; Misawa et al. 2018). *Waitea circinata* are currently described as varieties in concordance with Leiner and Carling after

characterization of a new variety, *Waitea circinata* var. *circinata* from Alaskan soils (1994). There are five varieties characterized to date for *Waitea circinata*, variety *agrotis*, *circinata*, *oryzae*, *prodigus* and *zeae* (Arakawa & Inagaki, 2014). Isolates are regularly assigned to AGs using rDNA ITS sequence analysis (Sharon et al., 2008) and various PCR assays for detection and identification (Budge et al. 2009; Woodhall et al. 2013; 2017). Knowledge of the AG, or even AG subgroup present is essential for optimum disease management and correct diagnosis. AGs can differ in fungicide sensitivity (Kataria and Gisi, 1999), means of dispersal, host range, aggressiveness, even to particular plant parts, such as *R. solani* AG8 almost exclusively infecting the roots of potatoes (Woodhall et al., 2008).

The *Rhizoctonia* species complex contains several species associated with disease in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) capable of causing serious economic losses. One member of the species complex, *Rhizoctonia cerealis* (*Ceratobasidium cereale* or (BNR) AG-D), has been associated with yield losses up to 26% in the UK (Clarkson and Cook, 1983) and up to 40% in China (Hamada et al, 2011). Subgroup AG D (I) is characterized as the causal agent for sharp eyespot symptoms in cereal crops (Li et al. 2017). As well as sharp eyespot lesions, other diseases associated with *Rhizoctonia* spp. in cereals include root rots, stem rots, foliar blights and damping off (Brown et al. 2021). Disease from *Rhizoctonia* spp. usually occur at the soil line and below, however there are some AGs known to cause lesions on the stem above the soil line. AG 5 was found causing brown lesions on wheat stems in England (Woodhall et al. 2012).

The second most common pathogenic *Rhizoctonia* AG to wheat and barley crops is AG 8. *R. solani* AG 8 is commonly known as the bare patch disease that affects wheat and barley through infection in the roots causing “pinched-off” or “spear-tipping” symptoms that leave the roots severely pruned (Dada 2017). AG 8 has been reported in the Pacific Northwest, Australia and Turkey (Ogoshi et al. 1990; MacNish and Neate, 1996; Ünal and Dolar, 2012). *Waitea circinata* var. *oryzae* has also been associated with root rot, bare patch, and damping off in the Pacific Northwest (Okubara et al. 2014).

BNR AGs, with the exception of AG D, have not been extensively studied, so there is little to report on disease symptoms caused by these AGs. AG E and H were found in fields of the Pacific Northwest, though Ogoshi et al. (1990) did not specify if the AGs were pathogenic or if they were isolated specifically from plant material or soils (1990).

Presently there is limited knowledge of the AGs present on wheat and barley crops in Idaho and the specific diseases they cause, particularly for Southern Idaho. Although *R. solani* AG4 HG-II was

recently reported infecting barley stems in East Idaho, previous surveys only considered North Idaho (Ogoshi et al., 1990) and did not use DNA sequencing or PCR to aid in identification. Therefore, the aim of this study was to characterize isolates collected from wheat and barley crops throughout the whole state, with AG determined using rDNA ITS sequencing. The aggressiveness and type of disease caused by selected representative isolates of each AG was also determined in two glasshouse experiments. The relative incidence of root and stem diseases in Idaho crops is presented here as well as the prevalence of AGs 2-1, 4 HG-II, 5, 8 and D detected in wheat and barley fields prior to planting.

Materials and Methods

Sample Collection

Representative fields in Northern, Southern (Western and Central) and Eastern Idaho were chosen, resulting in a range of climate conditions, soils textures and irrigation methods. Soil was taken from fields designated for spring wheat and barley in the months of March through April, while soil was collected in October through November for winter wheat and barley. Each field was sampled within a one-hectare area of the field. This area was selected away from the headland and edge rows. Samples removed were 20 m spaced parallel transects that were 100 m x 5. A GPS unit (Garmin, Schaffhausen, CH) was used to record coordinates of the sampled area. A gator probe (AMS, American Falls, ID) was used to collect 25 cores each up to a depth of 30 cm in a grid pattern over the hectare. Individual cores were mixed in a clean bucket and approximately 500 g of soil was placed in a polyethylene bag and stored at 4° C for up to 3 days prior to processing.

Plant material was collected from the same hectare in each field in the months March through April for winter crops and May through June for spring crops. A trowel was used to lift 30 entire plants, with as much root material as possible. Plant samples were placed in a separate polyethylene bag and stored 4 ° C for up to 36 hours prior to assessment.

Disease Assessments, Isolation and Storage of *Rhizoctonia* Isolates

Plant samples were washed in tap water to remove all soil prior to assessment. Plants were visually assessed for root and stem disease symptoms. Root disease severity was assessed on a percentage scale, 0% to 100%, 0 = no disease to 100% necrosis of root structure based off root infected area (RIA). Stem disease severity, which included outer leaf blades, were assessed on a scale adapted

from Woodhall et al. (2008). 0 to 4; 0 = no lesion, 1 = any lesion, 2 = lesion above 5 mm, 3 = lesion above 10 mm, 4 = girdled lesion. Stem disease severity are presented as stem severity index (SSI). Severity of lesions were also assessed on penetration of a lesion to the stem with a scale of 0 to 1; 0 = no penetration and 1 = penetration. Any variance in symptoms were noted.

Symptomatic and border asymptomatic plant material, roughly 5-7 mm in size, was surface disinfested in sodium hypochlorite (1% NaOCl) for at least one minute, rinsed in two changes of sterile distilled water and allowed to dry before being placed onto 1.5% water agar (TWA) and potato dextrose agar (PDA) amended with streptomycin (0.8 g/liter) and penicillin (0.2 g/liter). After a 48–72 h incubation at 21° C, hyphal tips from colonies of *Rhizoctonia* were identified under a dissecting microscope and transferred to PDA and incubated at 21° C until DNA extraction or being placed into storage on PDA slopes in 50 ml tubes stored at 21° C.

DNA Extraction from Cultures, Sequencing and Phylogenetic Analysis

Isolates were given an individual 'C' code designation prior to DNA extraction. DNA was extracted from 10-day old PDA culture hyphae, within a roughly 20 mm x 20 mm area, using a Wizard food DNA purification kit (Promega) in conjunction with a KingFisher ML magnetic particle processer in accordance with manufacturer's instructions (Woodhall et al. 2017). Extracted DNA was kept at -20ºC.

The rDNA internal transcribed spacer (ITS) region was used for sequencing of extracted DNA. PCR amplification was first performed with ITS4 and ITS5 primers at an annealing temperature of 58 °C. Amplified products were purified using the Qiagen purification kit in accordance with manufacturer's protocol (Woodhall et al. 2017). Purified products were observed on a 1% (w/v) low melting point agarose gel (containing 0·5 μg mL^{-1} ethidium bromide) in TAE buffer (40 mm tris-acetate, 1 mm EDTA, pH 8). Samples with visible bands were sent to Eurofins genomics for sequencing.

Sequencing results were analyzed using Geneious Prime (Geneious). DNA alignments for each isolate were performed using Geneious alignment and visually edited to create a consensus sequence (Thompson et al. 1994). Consensus sequences were run through NCBI BLAST for AG identification. Furthermore, DNA alignments were performed using ClustalW. Exemplar isolates were used as a representative isolate to 100% identical isolates within multiple AGs. Exemplar isolates were used to create clean phylogenetic trees for *R. solani*, BNR, AG D, and *Waitea circinata* variety isolates.

Phylogenetic trees were constructed using Geneious Prime. Reference sequences from known species/AGs were obtained from GenBank and used in the construction of all phylogenetic trees (Table 2.1). Neighbor joining method for distance matrix values were constructed and distances in the rDNA ITS region were calculated using the Tamura-Nei model (Tamura and Nei, 1993). Bootstrap analysis was performed with 1000 re-samples of data. rDNA ITS sequences from opposite *Rhizoctonia* species were used as an outgroup for each phylogenetic tree. *Rhizoctonia solani* AG 11 (GenBank accession NO. LC215406) was used for the BNR phylogeny, while *Ceratobasidium* sp. AG-E (GenBank accession NO. AB290019) was used for the *R. solani* and the *Waitea circinata* phylogenies.

Table 2.1 Reference Accession numbers used in phylogenetic trees

Reference Accession #	AG	Host	Origin	Collector
AB054845	Subset 1	*Raphanus sativus*	Japan	Kuninaga, S.
AJ238166	2-2 IIIB	*Zea mays*	Japan	Salazar, O.
AB019023.1	3-PT	*Solanum tuberosum*	Japan	Kuninaga, S.
AF153774.1	3-TB	*Nicotiana tabacum*	USA	Pope, E.J.
AY152704	4 HGI	*Lycopersicum esculentum*	Brazil	Kuramae, E.
AY154308	4 HGII	Unknown	Brazil	Kuramae, E.
DQ102449	4 HGIII	Soil	Israel	Sharon, M.
KF870931	5	*Triticum aestivum* L.	Canada	Broders, K.D.
AF354112	5	*Glycine max*	USA	Gonzalez, D.
DQ356413	8	Soil	USA	Gonzalez, D.
DQ356410	10	*Triticum aestivum* L.	USA	Okubara, P.A.
LC215406	11	*Oryza sativa*	Japan	Misawa, T.
AF153802	11	Soil	Australia	Pope, E.J.
DQ102407	A	*Fragaria ananassa*	Israel	Sharon, M.
DQ102421	A	*Fragaria ananassa*	Israel	Sharon, M.
AB290021	C	*Beta vulgaris*	Japan	Uchino, H.
AB198700	DI	*Zoysia matrella*	Japan	Hayakawa, T.
AB198709	DII	*Zoysia japonica*	Japan	Hayakawa, T.
AB198705	DIII	*Zoysia japonica*	Japan	Hayakawa, T.
AB290019	E	*Oxalis*	Japan	Kuninaga, S.
DQ102433	Fa	*Fragaria ananassa*	Israel	Sneh, B.
AB219145	Fb	Soil	Japan	Ogoshi, A.
DQ102395	G	*Fragaria ananassa*	USA	Martin, F.N.
MT380177	H	*Fragaria ananassa*	Turkey	Genc Kesimci, T.
AB290023	I	*Beta vulgaris*	Japan	Uchino, H
AB196652	K	Soil	Japan	Hyakumachi, M.

JQ859871	K	*Fragaria ananassa*	Australia	Fang, X.
HQ424246	*Ceratobasidium theobromae*	*Theobroma cacao*	Indonesia	Samuels, G.J.
JQ247572	*Ceratobasidium spp.*	*Brassica napus*	USA	Schroeder, K.L.
AB213580	*Waitea circinata* var. *circinata*	*Agrostis stolonifera*	Japan	Toda, T.
AB213583	*Waitea circinata* var. *circinata*	*Agrostis stolonifera*	Japan	Toda, T.
KT428732	*Waitea circinata* var. *circinata*	*Beta vulgaris*	China	Zhao, C.
AB213571	*Waitea circinata* var. *agrotis*	*Poa pratensis*	Japan	Toda, T.
HM597143	*Waitea circinata* var. *prodigus*	*Paspalum vaginatum*	USA	Kammerer, S.J.
MN160232	*Waitea circinata* var. *zeae*	*Brassica oleracea* var. *capitata*	Serbia	Vojvodic, M.
MN160242	*Waitea circinata* var. *zeae*	*Brassica napus*	Serbia	Vojvodic, M.
AJ000195	*Waitea circinata* var. *oryzae*	*Oryza sativa*	Japan	Johanson, A.
AB213589	*Waitea circinata* var. *oryzae*	*Oryza sativa*	Japan	Toda, T.

Reference isolates used in the construction of phylogenetic trees. Isolates represented by their DNA accession numbers available on GenBank. Many accession numbers were from Misawa et al. 2018, Sharon et al. 2006 and 2008.

DNA Extraction from Soil and Real-time PCR

DNA was extracted from a 250 g sample of soil. Soil DNA extraction was done as described by Woodhall et al. (2012). Soil was tested using real-time PCR for AG 2-1, AG 4 HG-II, AG 5, AG 8 and AG D (*Rhizoctonia cerealis*). Using the assays described in Table 2.2, Real-time PCR was undertaken as described in Woodhall et al. (2012), with the exception a 20 µl reaction size was used instead of 25 µl and a Quant Studio 3 used for thermocycling. Synthetic DNA standards (Eurofins Genomics) were used as positive controls.

Table 2.2 Details of primers in real-time qPCR testing of soils

Target Group	Primer/Probe	3' Modification	Sequence 5' -3'	Target Region	Source
AG 2-1	AG 2-1_F		CTTCCTCTTTCATCCCACACA	ITS1	Budge et al. 2009
	AG 2-1_R		TGAGTAGACAGAGGGTCCAATAACCTA	ITS1	
	AG 2-1_P	MGB	AAGTAAATTCCCCATCTGT	ITS1	
AG 4 HG-II	AG 4 HGII_F		GCAAAGAGGCTGAGGGCTGT	β-tubulin	Budge et al. 2009
	AG 4 HGII_R		CGGTCTGGGTACTCTTCACGAA	β-tubulin	
	AG 4 HGII_P	BHQ1	TACAGGGCTTCCAGATTACCCACTC	β-tubulin	
AG 5	AG 5_F		TGATCAGGTGCTCGATGTCGT	β-tubulin	Budge et al. 2009
	AG 5_R		CCCTGCAAGCAGTCGGTT	β-tubulin	
	AG 5_P	MGBE	CGCAAAGAGGCCGAG	β-tubulin	
AG 8	AG 8_F		AGTTGGTTGTAGCTGGTCCATTAAT	ITS1	Budge et al. 2009
	AG 8_R		AGTAGACAGAGGGGTCCAATAAATGA	ITS1	
	AG 8_P	MGB	TGTGCACACCTCCTC	ITS1	
Rc (AG D)	RcF		AAAGCATCGTCGCCATGAG	ITS1	Woodhall et al. 2017
	RcR		CTGCCAACACACCGACATGT	ITS1	
	RcP	BHQ1	ATAAAATGGAAGGTAGGTGCGGGTGCATAG	ITS1	

All forward (_F) and reverse (_R) primers and probes (_P) used in this study. Duel labelled fluorescent probes were labelled with 6-carboxyfluorescein (FAM) 5' modification and either minor groove binding (MGB) or Black hole Quencher (BHQ1) 3' modification.

Aggressiveness of Cereal Isolates

To test the aggressiveness of the *Rhizoctonia* isolates, two glasshouse experiments were setup under 16hr light and 8hr dark cycle conditions at an average of 18 ° C. The experiment was duplicated with a difference of two-days between inoculation and different placement within the greenhouse. Experiments were labeled A and B. 10 winter wheat seeds, cv Stephens, were planted in a 16 cm^3 plastic pot with Ferti·lome Ultimate Potting Mix to mimic row conditions. For each isolate and experiment, soil was removed from the roots of ten 2-week-old plants and inoculated with a $10\ \mathrm{mm}^2$ plug from a 10-day old PDA culture at the base of the stem and roots. A uninoculated PDA plate was used to create control plants. Plants were harvested 4 weeks post-inoculation, washed and visually assessed for symptoms as described above.

Based on the number of AGs collected, 60 isolates were used to represent the diverse AGs present in the survey. The number of isolates used for individual AGs was in proportion to the number of isolates per AG recovered in the survey. Additionally, two unidentified BNR isolates found in the Southwest region and the Northern region in 2020 were used. A BNR isolate of AG W, found on an Idaho potato in 2020, was used as an unknown culture control.

Statistical Analysis

Plant assessment stem severity index (SSI) and % root area infected (% RAI) were calculated for the mean scores of all fields within a region. The pathogenicity experiment SS and RS values were calculated for the mean scores of all isolates within an individual AG. Statistical analyses were performed using ARM 2021 software. Data analysis for SSI values were performed using automatic rank transformation followed by a Kruskal-Wallis test. Post hoc mean separation was performed with Fisher's LSD with an of alpha 0.05. Data analysis for % RIA and penetration values were performed using ANOVA followed by Tukey's HSD for means comparison with an alpha of 0.05. % RIA raw values were log transformed (log of X + 1) in ARM before statistical analysis.

Results

Sampling and Plant Assessment

A total of 118 commercial cereal fields from twenty-one counties were sampled across Idaho. In the 2019 season, 16 fields in Southwest Idaho, 20 fields in East Idaho, 13 fields in North Idaho, and 3 fields in Southcentral Idaho were sampled. In the 2020 season, 26 fields in Southwest Idaho, 24 fields in East Idaho, 9 fields in North Idaho, and 7 fields in Southcentral Idaho were sampled. In total, 42 fields in Southwest Idaho, 46 fields in East Idaho, 22 fields in North Idaho, and 6 fields in Southcentral Idaho were sampled.

Plant assessments in 2019 had a mean SSI of 0.28 for the Southwest region, 0.60 for the Eastern region, 0.69 for the Northern region and 1.02 for the Southcentral region on a disease severity scale of 0 to 4. Mean stem penetration of 0.09 for the Southwest region, 0.15 for the Eastern region, 0.23 for the Northern region and 0.32 for the Southcentral region on a key lesion scale of 0 to 1. % RAI of 13% for the Southwest region, 35% for the Eastern region, 39% for the Northern region and 19% for the Southcentral region on a disease scale of 0 – 100%.

Plant assessments in 2020 had a mean SSI of 0.35 for the Southwest region, 0.62 for the Eastern region, 1.21 for the Northern region and 0.93 for the Southcentral region on the lesion scale. Mean stem penetration of 0.14 for the Southwest region, 0.24 for the Eastern region, 0.48 for the Northern region and 0.44 for the Southcentral region on the lesion scale. % RAI of 14% for the Southwest region, 35% for the Eastern region, 32% for the Northern region and 22% for the Southcentral region on the disease scale.

Isolate Collection and Phylogenetic Analysis

Over 1600 isolates were obtained from symptomatic and asymptomatic plant material. Of these isolates, 238 *Rhizoctonia* spp. were identified through rDNA ITS sequencing. Most isolates (*n* = 93) identified as BNR, with AG D being the prominent group. The second most predominant was *Waitea circinata* varieties (*n* = 75). The remaining multinucleate *R. solani*. isolates (*n* = 72) were observed with white to brown mycelium and typical right-angle branches at the distal septae of cells. Assignment of isolates to the various AGs were based on BLAST and phylogenetic analysis of the ITS region.

A majority of the isolates were obtained from the Northern region (Table 2.3). Isolates obtained from the Eastern region were the second highest. Sixteen fields had multiple AGs isolated over the course of the survey. Eleven different AGs, W*aitea circinata* varieties and 3 unidentified BNR isolates were isolated from Idaho wheat and barley plant material. W*aitea circinata* varieties had the highest presence and was found in all regions. AG D was the second highest group and found in all regions as well. The remaining groups were found varied throughout the state.

Table 2.3 *Anastomosis Group Breakdown by Region*

AG	North Idaho	East Idaho	Southcentral Idaho	Southwest Idaho	Total
AG 2-1	5	1	-	4	10
AG 2-2	-	-	-	2	2
AG 3	2	1	-	4	7
AG 4 HG-II	-	19	11	8	38
AG 5	-	-	1	5	6
AG 11	-	4	-	3	7
AG C	2	-	-	-	2
AG D	6	43	5	14	68
AG E	2	-	-	-	2
AG H	9	-	-	-	9
AG K	1	4	-	4	9
Waitea circinata varieties	66	3	1	5	75
Unidentified BNR	1	-	-	2	3
Total:	94	75	18	51	238

Anastomosis group breakdown within the individual region. Individual isolates were identified through rDNA ITS sequencing. A total of 94 isolates from the Northern region, 75 isolates from the Eastern region, 18 isolates from the Southcentral region and 51 isolates from the Southwest region.

AGs were broken down by the symptom (Table 2.4). A majority of the isolates were isolated from stem material, with only forty-two isolates from root material. AG D had the highest presence in stem material. Isolates were obtained from symptomatic sharp eyespot lesions, as well as non-symptomatic lesions and sharp eyespot-like lesions on the outer basal leaves. Minimal isolates were obtained from root symptoms. *Waitea circinata* had the second highest presence in stem material, with only twelve isolates from root material. The remaining groups were obtained from various symptoms.

Table 2.4 *Anastomosis Group Breakdown by Symptom on Plant Samples*

AG	Root	Stem	Total
AG 2-1	5	5	10
AG 2-2	2	-	2
AG 3	4	3	7
AG 4 HG-II	5	33	38
AG 5	2	4	6
AG 11	3	4	7
AG C	-	2	2
AG D	4	64	68
AG E	-	2	2
AG H	1	8	9
AG K	2	7	9
Waitea circinata	12	63	75
Unidentified BNR	1	2	3
Total:	42	196	238

Anastomosis group breakdown within the individual symptom. Individual isolates were identified through rDNA ITS sequencing. Root symptoms presented as lesions and stem symptoms ranged in lesion severity.

Isolates were further broken down into unique isolates based on year, origin field and symptoms. For example, if there were two isolates under field A, symptom B, only 1 isolate was counted towards a unique isolate for that field and symptom. Based on these criteria, there were 104 unique *Rhizoctonia* isolates in this survey (see Appendix 1.1). Exemplar isolates were used for 100% identical sequences to help create clean phylogenetic trees. AGs with exemplars used in the construction of phylogenetic trees are listed in Table 2.5.

Table 2.5 List of exemplar isolates used in construction of phylogenetic trees

Representative Exemplar C#	Pathogen	Identical Isolates C#:
C172	AG 2-1	C173, C174, C284
C379	AG 2-1	C76, C257, C375
C105	AG 3	C102, C103, C104
C278	AG 3	C353
C209	AG 4 HG-II	C37, C106, C111, C127, C128, C193, C204, C208, C213, C214, C261, C262, C263, C264, C265, C319, C320, C321, C322, C331, C332, C333, C334, C335, C336, C337, C338, C339, C343, C344, C349, C35, C351, C377, C382
C201	AG 5	C184
C323	AG 5	C79, C81
C85	AG 11	C80, C82
C203	AG 11	C202
C370	AG C	C288, C309, C371
C63	AG D	C54, C58, C95, C307
C94	AG D	C56, C57, C60, C238
C124	AG D	C250, C251, C284
C176	AG D	C170, C244, C248
C183	AG D	C177, C178, C182, C230, C235, C242
C211	AG D	C55, C160, C210, C217, C245
C218	AG D	C236, C239, C240, C259
C227	AG D	C175, C188, C229, C231, C233, C237
C234	AG D	C180, C187, C207, C241
C378	AG D	C32, C62, C171, C179, C181, C185, C186, C205, C206, C212, C215, C216, C219, C220, C221, C228, C232, C243, C258
C63	AG D	C54, C58, C95, C307
C249	AG E	C376
C272	AG H	C266, C310
C312	AG H	C271, C383
C341	AG K	C191, C340
C14	*Waitea circinata* var. *circinata*	C267
C47	*Waitea circinata* var. *circinata*	C255, C269, C293, C294, C299, C300, C326, C327, C357
C121	*Waitea circinata* var. *circinata*	C122
C125	*Waitea circinata* var. *circinata*	C253
C133	*Waitea circinata* var. *circinata*	C296, C303

C84	*Waitea circinata* var. *zeae*	C83, C169
C290	*Waitea circinata* var. *circinata*	C134, C252, C289, C384
C297	*Waitea circinata* var. *circinata*	D1, D2, C268, C281, C282, C298, C301, C315
C318	*Waitea circinata* var. *circinata*	C273, C274, C275, C276, C277, C283, C287, C292, C295, C296, C305, C314, C317, C328

Exemplar isolate represents 100% identical isolate sequences within the same AG. Alignment of all isolates within an AG, done using ClustalW, were visually assessed for identification.

Phylogenetic analysis of the *R. solani* isolates shows placement within AG 3, AG 4 HG-II and AG 5 (Figure 2.1). AG 4 HG-II isolates placed into two separate subclades, with one isolate placed with the previously reported isolate from a diagnostic barley sample (GenBank accession #MT444151). This isolate was the first report of AG 4 HG-II on barley in Idaho (Pizolotto et al. 2020). The survey isolate was from a spring barley field. AG 3 isolates group into a subclade of AG 3-PT, with two separate subclades. AG 5 isolates were placed in two separate subclades of AG 5 reference isolates. Isolates within the AG 2 subgroups were placed in the AG 2 phylogenetic tree in the next chapter with the bean survey isolates (Chapter 3, Figure 3.1). One AG 2-1 isolate clustered with common bean (DB) isolate in a subgroup of AG 2-1 subset 3. The second AG 2-1 isolate was placed in AG 2-1 subset 2. One AG 2-2 isolate was placed in a subclade of AG 2-2 IIIB, while the other isolate was placed with a DB isolate in a subclade of AG 2-2. AG 11 isolates were placed in the AG 11 bean survey phylogenetic tree in the previous chapter (Chapter 3, Figure 3.3). Two isolates are placed in the same clade as reference isolates from *Glycine max* from the U.S., *Oryza sativa* from Japan and an unknown host from Brazil. Two isolates are placed in the same clade as the *Lilium* spp. reference isolate from Japan. One cereal isolate forms a separate clade with no reference isolate to identify host range.

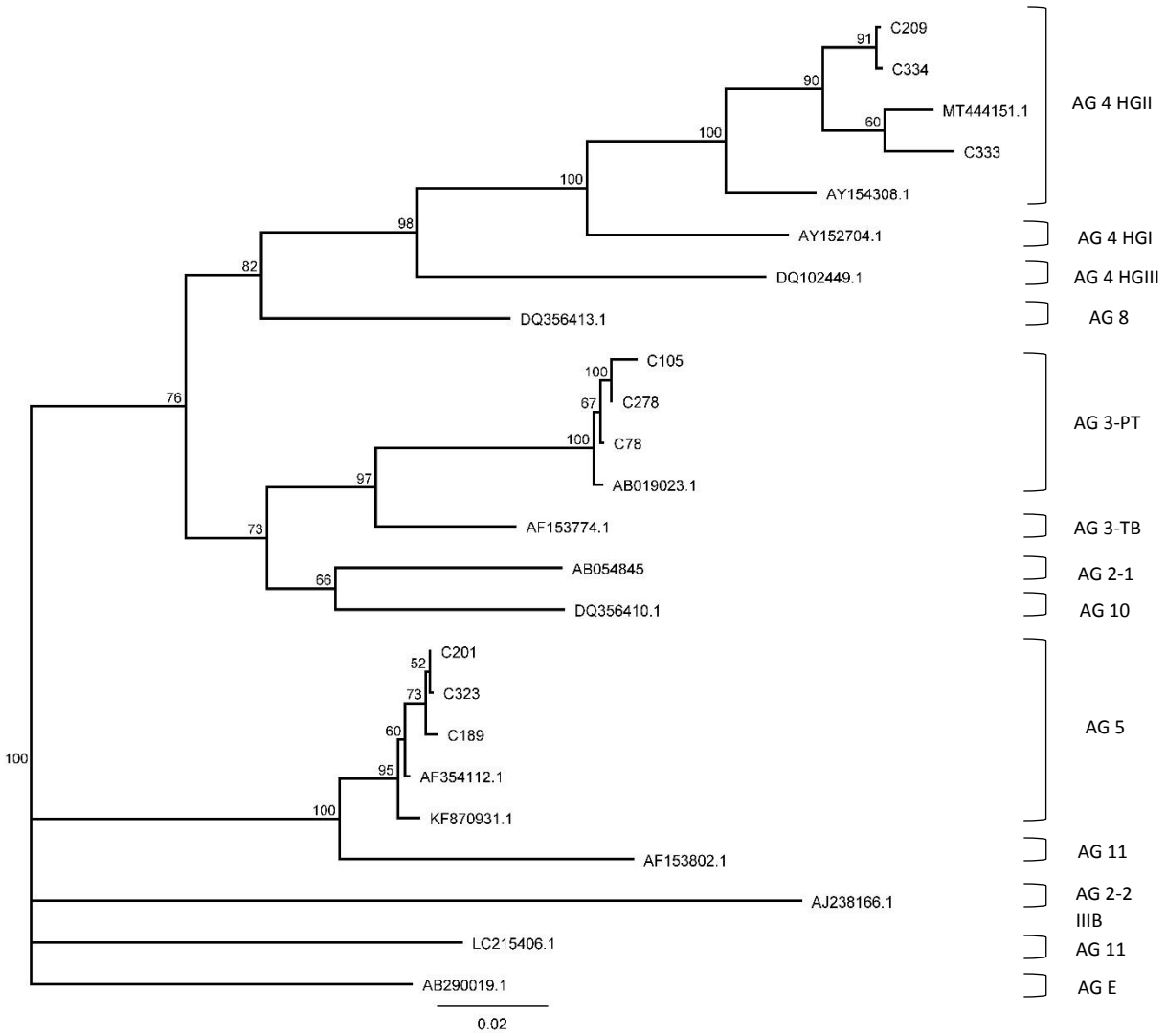

Figure 2.1 A neighbor-joining tree of *R*. *solani* anastomosis group isolates (represented by their C numbers) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 17 trials are positioned alongside the branches with values over 50. Isolate AB290019 [AG E (*Ceratobasidium*)] was used as an outgroup. The AGs and subgroups for the clusters are indicated. Bar indicates 0.2 base change per 10 nucleotide positions.

Phylogenetic analysis of the BNR isolates, not including AG D, shows placement within AG C, AG E, AG H and AG K (Figure 2.2). The AG C isolate is placed with the AG C reference isolate in a clade off the AG I reference isolate. The AG E isolate is placed with the AG E reference isolate in a clade off the AG Fa reference isolate. One AG H isolate is placed within a clade with the AG H reference isolate, while three other AG H isolates are placed within two separate subclades below. One AG H isolate is placed within a separate clade below the top groups. One AG K isolate is placed with the one of the AG K reference isolates, while the second AG K isolate is placed in a subclade below. The second reference

AG K isolate forms a separate clade below. One of the unidentified BNR (Un) isolates was placed with a reference *Ceratobasidium* spp. isolate causing root rot of canola in Washington State. The second unidentified BNR isolate is placed in a clade below.

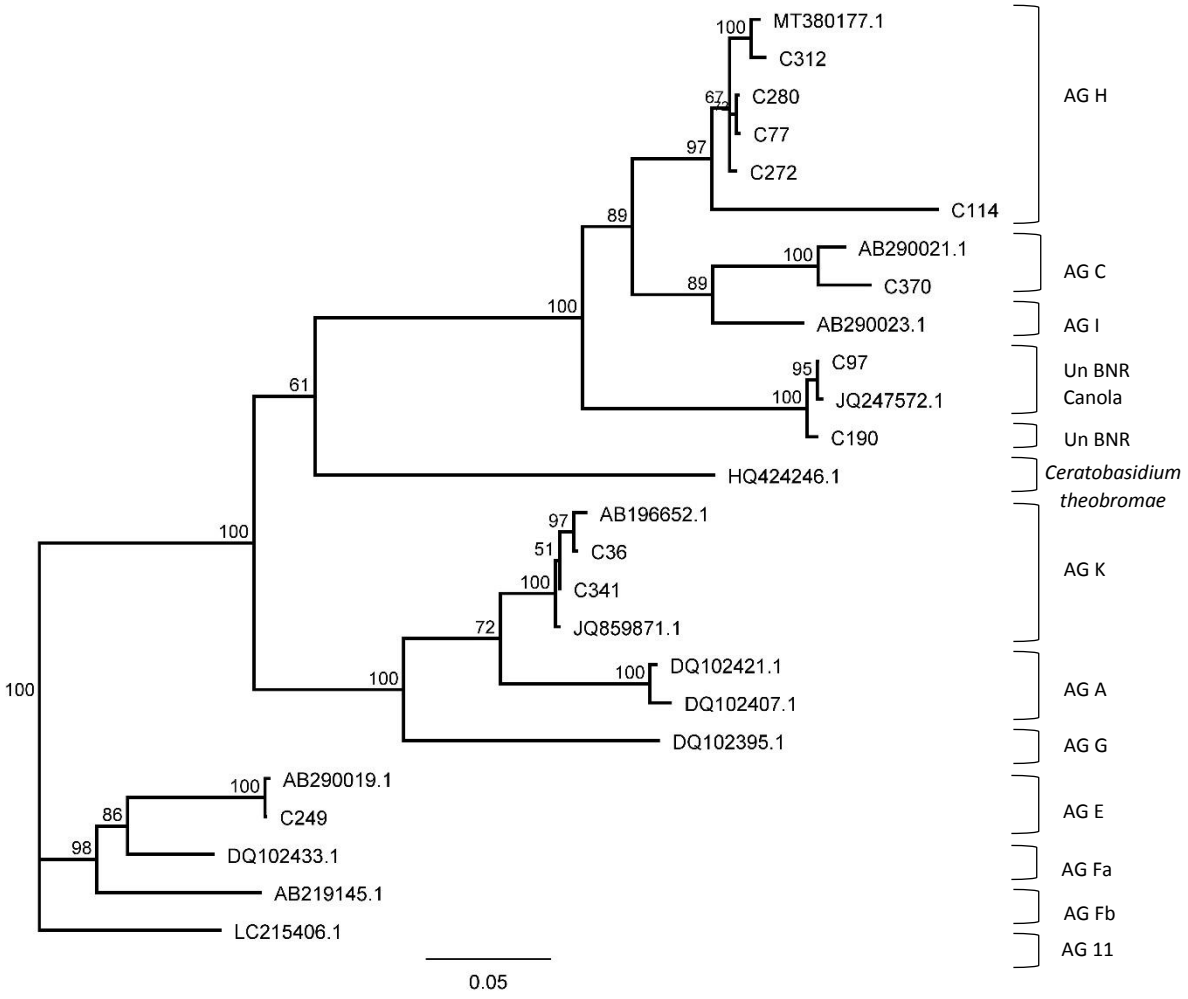

Figure 2.2 A neighbor-joining tree of binucleate anastomosis group (*Ceratobasidium* spp.) isolates (represented by their C numbers) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 17 trials are positioned alongside the branches with values over 50. Isolate LC215406 [AG 11 (*R. solani*)] was used as an outgroup. The AGs and subgroups for the clusters are indicated. Bar indicates 0.5 base change per 10 nucleotide positions.

Phylogenetic analysis of the AG D isolates were placed within AG D (I) (Figure 2.3). Five isolates are placed with the AG D (I) reference isolate, while three isolates form two separate subclades above.

The last two isolates are placed in a separate clade below. AG D (II) and AG D (III) are placed in individual clades below the AG D (I) clades.

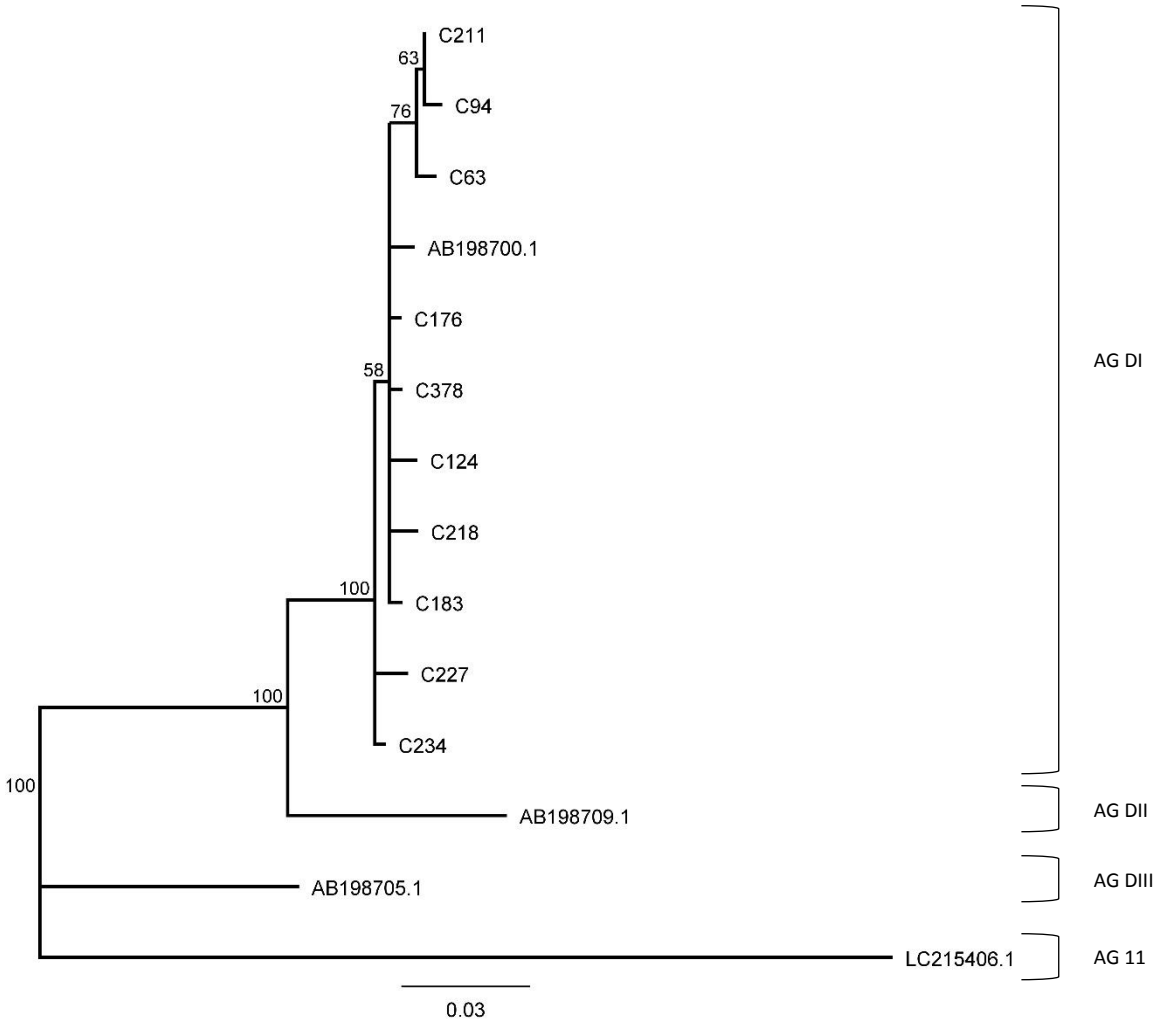

Figure 2.3 A neighbor-joining tree of AG D anastomosis group (*Ceratobasidium* spp.) isolates (represented by their C numbers) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 17 trials are positioned alongside the branches with values over 50. Isolate LC215406 [AG 11 (*R. solani*)] was used as an outgroup. The AGs and subgroups for the clusters are indicated. Unidentified BNR isolates (Un BNR) Bar indicates 0.3 base change per 10 nucleotide positions.

Phylogenetic analysis of the *Waitea circinata* variety isolates shows placement within *Waitea circinata* var. *circinata*, var. *zeae* and 3 unidentified clades (Figure 2.4). Eight isolates clustered with two var. *circinata* reference isolates forming the main var. *circinata* clade. A subclade is placed above the main var. *circinata* clade that has four subgroups within it. One isolate is placed below a subgroup with two isolates forming a separate subgroup above one isolate. Another isolate is placed

above the subgroup with three more isolates placed within two subgroups above. Another subclade is placed below the main var. *circinata* clade that has five isolates placed within three individual subgroups. Three isolates are placed in an individual clade, with two subgroups, below the var. *circinata* subclade with no reference isolates to identify host or variety. Two isolates are placed in a subgroup off the clade with two var. *zeae* reference isolates from a cabbage (*Brassica oleracea* var. *capitata*), oilseed (*Brassica napus*) from Serbia and a DB isolate from the common bean survey. An isolate is placed in an individual clade below with no reference isolate to identify host or variety. No isolates were placed with var. *oryzae* reference isolates, though one isolate is placed in an individual clade below. Furthermore, no isolates were placed with var. *agrostis* or var. *prodigus*, which is placed in a clade below var. *oryzae*.

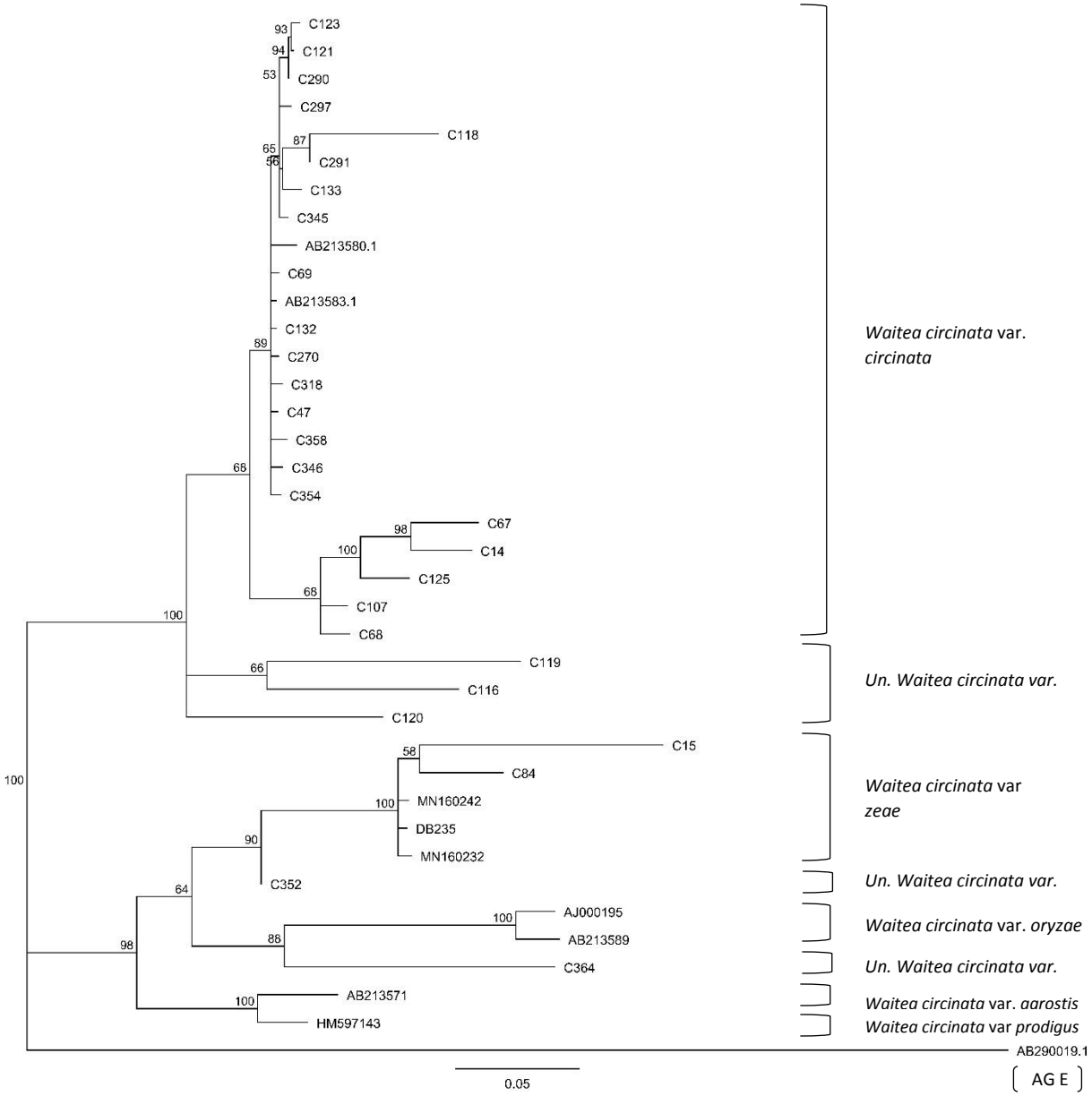

Figure 2.4 A neighbor-joining tree of *Waitea circinata* varieties anastomosis group isolates (represented by their C and DB numbers) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 17 trials are positioned alongside the branches with values over 50. Isolate AB290019 [AG E (*Ceratobasidium*)] was used as an outgroup. The AGs and subgroups for the clusters are indicated. Unidentified *Waitea circinata* variety clade (Un *Waitea circinata* varieties) Bar indicates 0.5 base change per 10 nucleotide positions.

Soil

A total of 97 soil samples from fields in the Northern, Eastern, Southcentral and Southwestern regions of Idaho were tested AG 2-1, AG 4 HG-II, AG 5, AG 8 and AG D (*R. cerealis*) through real-time PCR (Table 2.6). A total of twenty-two fields for the Northern region, thirty-four fields for the Eastern

region, nine fields for the Southcentral region and thirty-two fields for the Southwestern region. The Eastern region tested positive for all targeted AGs. The other locations had varied positive results. AG 2-1 had the highest presence throughout the state and AG 4 HG-II had the second highest presence.

Table 2.6 Detection of individual Anastomosis Groups of *Rhizoctonia* using real-time PCR on soil DNA samples by Idaho region

Target	Fields Tested	AG 2-1	AG 4 HG-II	AG 5	AG 8	AG D (*R. cerealis*)	Total
North	22	12	4	-	10	-	26
East	34	21	14	2	3	2	42
Southcentral	9	4	6	4	-	1	15
Southwest	32	14	9	5	-	-	28
Total	97	51	33	11	13	3	111

A total of 97 soil samples from fields in the Northern, Eastern, Southcentral and Southwestern regions of Idaho were tested for targeted anastomosis groups AG 2-1, AG 4 HGII, AG 5, AG 8 and AG D (*Rhizoctonia cerealis*) through Real-time PCR. A breakdown of fields tested in each region with the region total to the left, number of fields positive for individual AGs with the total below.

Pathogenicity Experiment

A total of 63 isolates were used in the pathogenicity experiment (Appendix 1.1). BNR isolates accounted for 23 isolates or 38.3%, *R. solani* isolates accounted for 18 isolates or 30% and *Waitea circinata* variety isolates accounted for 19 isolates or 31.7%. Two unidentified BNR isolates were used, as well as AG W to make up 63 isolates. A total of 614 plants were assessed for run A of the experiment, with 16 plants dying before assessment. Of these plants, 3 were inoculated with AG 4 HG-II, 2 with AG 5, 5 with AG D, 1 with AG E, 1 with AG H, 1 for unidentified 190C and 3 for *Waitea circinata* varieties. A total of 619 plants were assessed for run B of the experiment, with 11 plants dying before assessment. Of these plants, 1 was inoculated with AG 4 HG-II, 7 with AG D, 1 with AG K and 2 with *Waitea circinata* varieties.

Overall, there was no statistical significance between SSI for experiment A and experiment B (Kruskal-Wallis test, $P < 0.01$). No stem lesions were observed on the control plants in either run A or B experiments. Stem disease was not observed in every AG for either experiment (Figure 2.5, Figure 2.6). AG D isolates caused the most significant SSI with a mean average of 0.56 for experiment A and 0.72 for experiment B. There was one outlier with a mean of 2 in both experiment A and experiment B. Lesions from AG D isolates had started forming sharp eyespots on around 30% of the plants, with only 5 plants showing no lesions between experiment A and experiment B. Remaining isolates caused varied symptoms for both runs.

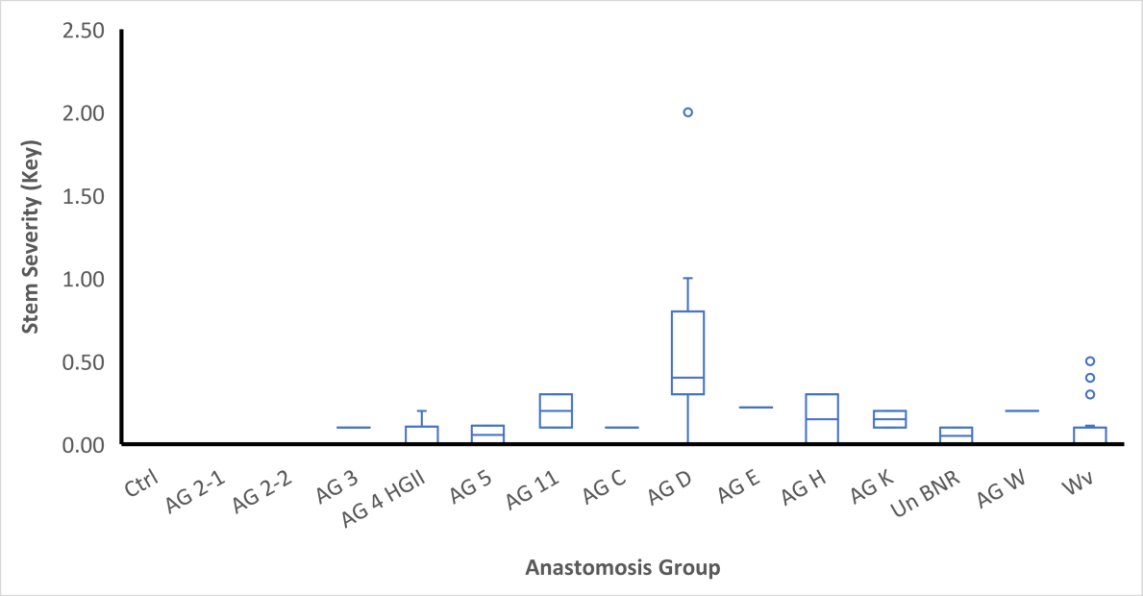

Figure 2.5 Mean stem disease severity (SSI), key scale of 0 – 4, of anastomosis groups for pathogenicity experiment 20GH5 A. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. Waitea circinata variety isolates are denoted by 'Wv'. Unidentified BNR by Un 'BNR'.

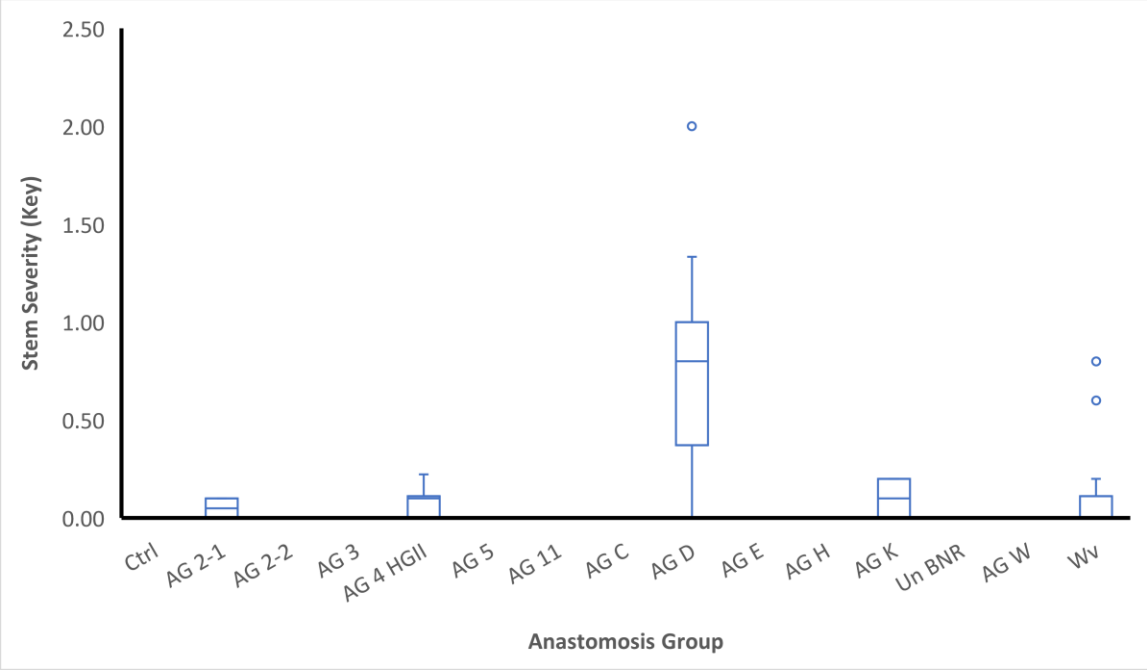

Figure 2.6 Mean stem disease severity (SSI), key scale of 0 – 4, of anastomosis groups for pathogenicity experiment 20GH5 B. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. *Waitea circinata* variety isolates are denoted by 'Wv'. Unidentified BNR by Un 'BNR'

Overall, there was no statistical significance between penetration for experiment A or experiment B (Tukey's test, $P < 0.05$). No stem penetration was observed on the control plants in either run A or experiment B. Stem penetration was not observed in every AG for either experiment (Figure 2.7, Figure 2.8). AG D isolates again showed the highest severity with a mean of 0.18 for both experiments A and B. Remaining isolates caused varied symptoms for both runs.

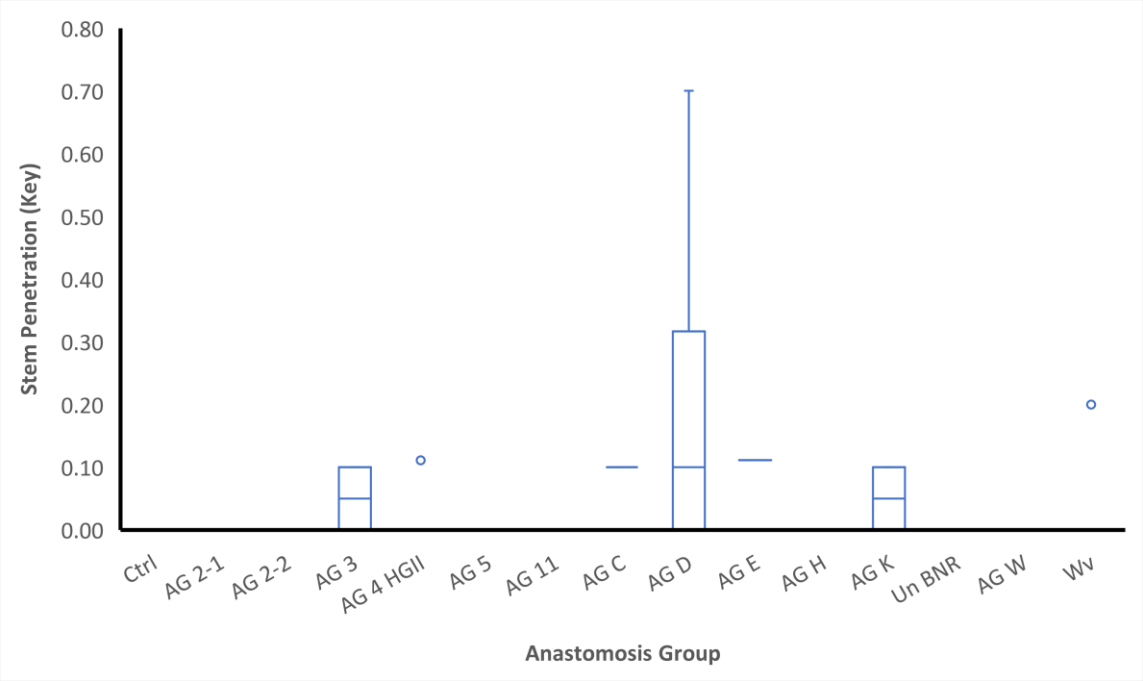

Figure 2.7 Mean stem penetration, key scale of 0-1, of anastomosis groups for pathogenicity experiment 20GH5 A. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. *Waitea circinata* variety isolates are denoted by 'Wv'. Unidentified BNR by Un 'BNR'

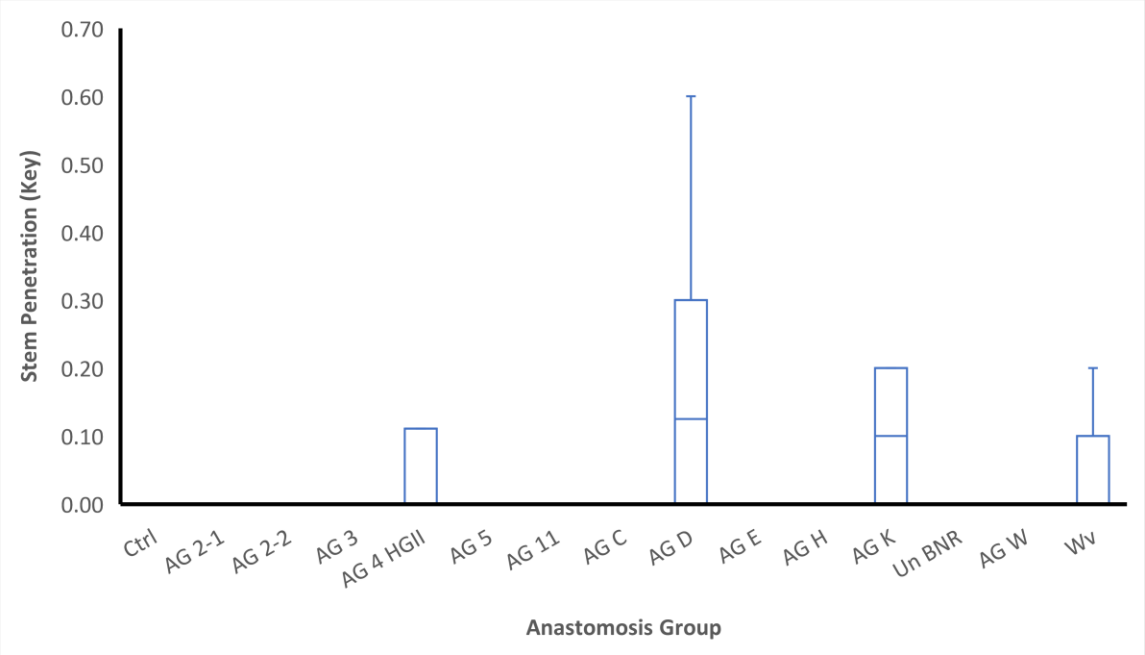

Figure 2.8 Mean stem penetration, key scale of 0 – 1, of anastomosis groups for pathogenicity experiment 20GH5 B. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. *Waitea circinata* variety isolates are denoted by 'Wv'. Unidentified BNR by Un 'BNR'

Overall, there was no statistical significance between % RIA for experiment A and experiment B (Tukey's test, $P < 0.05$). No % RIA was observed on the control plants in either A or B experiments. % RIA was observed in every AG for both experiments (Figure 2.9, Figure 2.10). Severity of disease varied within AGs and runs.

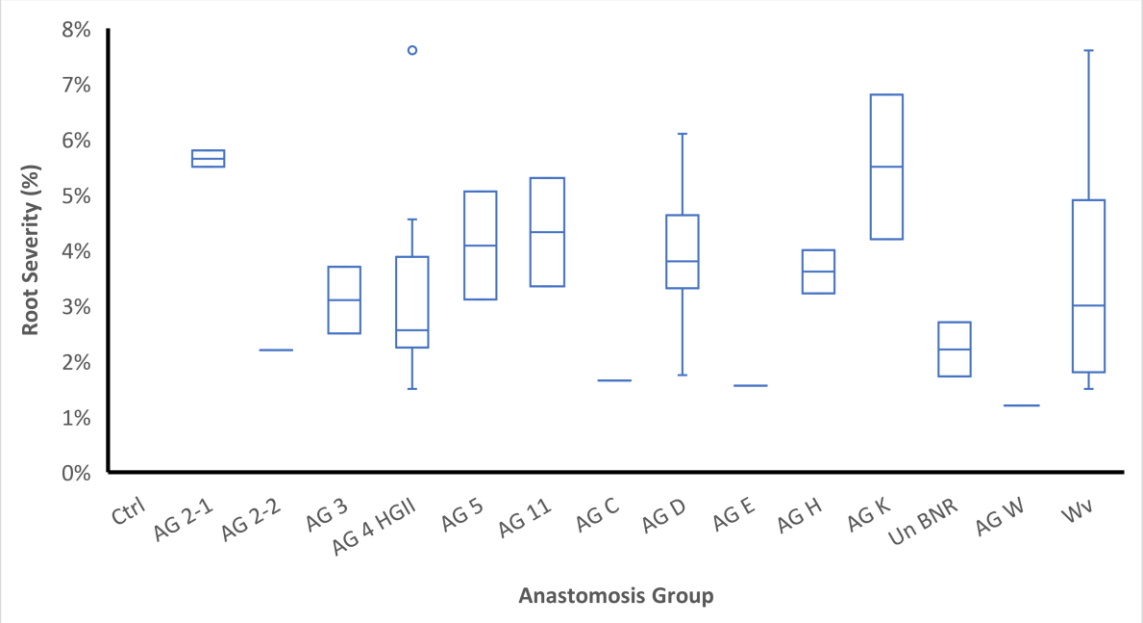

Figure 2.9 Mean % root infected area (%RIA), 0 – 100% necrosis of roots, of anastomosis groups for pathogenicity experiment 20GH5 A. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. *Waitea circinata* variety isolates are denoted by 'Wv'. Unidentified BNR by Un 'BNR'.

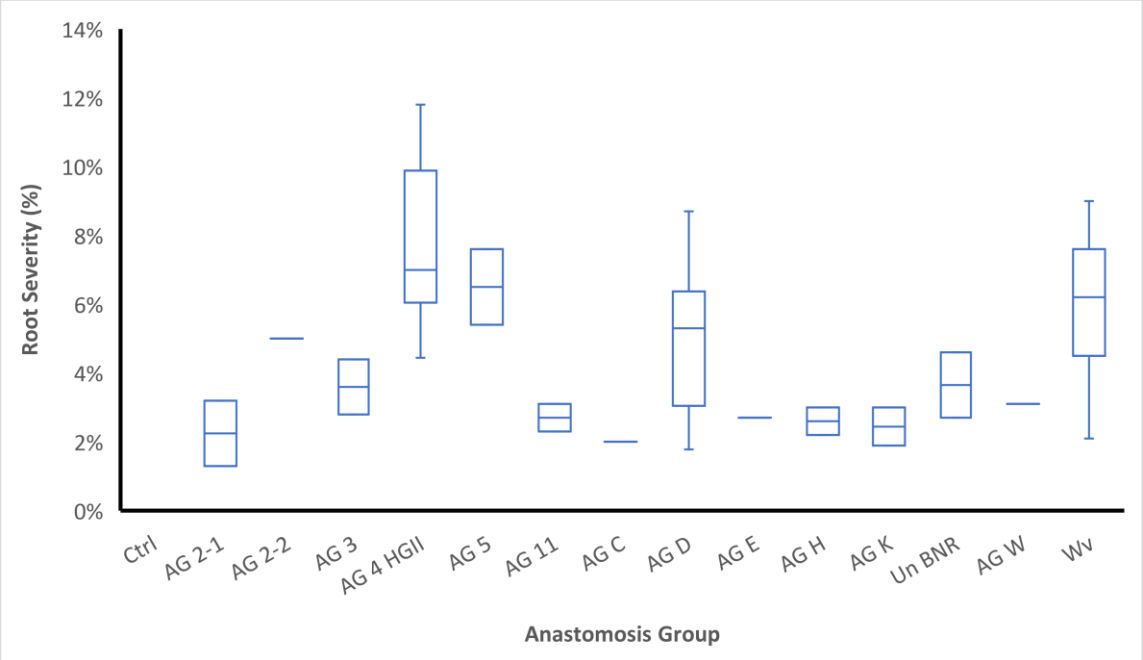

Figure 2.10 Mean % root infected area (RIA), 0 – 100% necrosis of roots, of anastomosis groups for pathogenicity experiment 20GH5 B. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. *Waitea circinata* variety isolates are denoted by 'Wv'. Unidentified BNR by Un 'BNR'.

Discussion

This is the first investigation to provide evidence of the relative AG composition of *Rhizoctonia* spp. populations causing disease in wheat and barley crops in the State of Idaho. 238 *Rhizoctonia* isolates were collected from wheat and barley samples from 118 fields. Through rDNA ITS sequencing and phylogenetic placement, isolates were characterized as eleven anastomosis groups and three *Waitea circinata* varieties. The most prominent AG was AG D, followed by *Waitea circinata* varieties.

Phylogenetic placement of the survey isolates placed them with the AG D (I) reference isolates, the causal agent of sharp eyespot in wheat and barley crops (Li et al. 2017, Ünal and Kara 2017). This is consistent with results from field sample assessments and the pathogenicity experiment. AG D (I) was isolated from sharp eyespot symptoms and non-sharp eyespot lesions from 10 fields throughout Idaho. Results from the pathogenicity experiment show around 30% of the plants starting to show symptomatic sharp eyespot lesions developing within 28 days post inoculation. The difference in phylogenetic placement between the isolates used in the pathogenicity experiment could explain the difference in the disease progression between the isolates, leaving only 30% of the plants having symptomatic sharp eyespot lesions. Results from the soil sample extraction did not reflect the presence of AG D (I) obtained through isolation from plant material, with only 3 fields testing positive. The assay primers and probe used in the real-time PCR testing are from the Woodhall et al. (2017) study on *Rhizoctonia cerealis* present in the UK. Therefore, the primers and probe may be targeted specifically for UK strains. However, one isolate from the U.S. obtained from turfgrass (*Agrostis* L.) was used in the survey (Woodhall et al. 2017). The results did reflect 3 fields that had the highest number of AG D (I) isolates obtained from plant material, suggesting a high presence of the pathogen in the soil. It is likely that a more robust sampling strategy could be required, greater sensitivity with the assay or the fungus could have been introduced on seed or equipment. It is thought that the Woodhall et al. (2017), assay targets a single copy gene whilst assays to other genes target the rDNA ITS region which is multi-copy and therefore likely to be much more sensitive.

Waitea circinata varieties had the second highest presence of isolates, including *Waitea circinata* var. *circinata*, var. *oryzae* and var. *zeae*. These varieties have all been described as pathogenic to wheat (Demirci 1998; Paulitz et al. 2003; Ünal et al. 2003; Ünal and Kara 2017), while varieties, *circinata* and *oryzae* have been described as pathogenic to barley (Demirci 1998; Paulitz et al. 2003; Ünal and Kara 2017). Among the *Waitea circinata* isolates, var. *circinata* had the highest presence, with many

isolates obtained from lesions off stem material. *Waitea circinata* var. *oryzae* (Paulitz et al. 2003) and *Waitea circinata* var. *zeae* (Ünal et al. 2003) varieties have been described as a causal root rot and stunting pathogens in wheat. Isolates of *Waitea circinata* var. *zeae* obtained in this study are consistent with these findings. However, *Waitea circinata* var. *zeae* also caused stem disease in the field samples, as well as the greenhouse experiment. These findings suggest *Waitea circinata* var. *zeae* is pathogenic to wheat stems, as well as roots. The findings for *Waitea circinata* var. *oryzae* were not consistent with the findings in this study. The *Waitea circinata* var. *oryzae* isolate was obtained from stem material. Additionally, 3 unidentified isolates were phylogenetically placed in a separate clade below the *Waitea circinata* var. *circinata* clade with no reference isolates to identify variety. Further characterization will be needed for these isolates as well. A recent characterization of *Waitea circinata* var. *zeae* obtained from cabbage (*Brassica oleracea var. capitata*) and oilseed rape (*Brassica napus*) used rDNA ITS, *RPB*2 (a large rDNA subunit) and β-tubulin genes to identify the variety through phylogenetic placement (Vojvodić et al. 2021). These methods could be beneficial in further characterizing the *Waitea circinata* var. isolates obtained in this study.

AG 4 HG-II isolates had moderate presence in this study. The isolates caused moderate root and stem disease. AG K has been described as nonpathogenic to wheat and barley (Demirci 1998; Ünal et al. 2014). These findings are not consistent with the findings in this study. AG K isolates were obtained from root and stem material in the field, while they caused mild stem and root disease in the greenhouse. These findings suggest AG K is moderately pathogenic to wheat. The 3 unidentified BNR isolates will be characterized further to determine AG. Ogoshi et al. (1990) found AG K in the Pacific Northwest, though did not indicate if the isolate was obtained from plant or soil material. AG 11 has been associated with severe disease on wheat and barley seedlings (Demirci 1998), though other studies have shown the AG to cause slight to no disease (Carling et al. 1994). The findings by Carling et al. were consistent with the findings in this study. The isolates obtained caused relatively low stem and root disease in field and in the pathogenicity experiment. No AG 8 isolates were obtained in this study, possibly because AG 8 is difficult to isolate off plant material. Direct testing of soil DNA using real-time PCR show the presence of AG 8 in 10 fields in the Northern region and 3 fields from the Eastern region. AG8 was not detected in fields Southcentral or Southwestern regions. All other AGs were isolated in low levels throughout Idaho.

In summary, this work provides knowledge of a diverse group of *Rhizoctonia* spp. present in Idaho wheat and barley crops, with *Waitea circinata* varieties being the most dominate group and AG D

(*Rhizoctonia cerealis*) being the most virulent. AG K was identified as a pathogen in Idaho wheat crops with no association of prior disease. Further characterization of 3 unidentified BNR isolates and 5 *Waitea circinata* variety isolates will need to be conducted, as well as characterizing the Waitea isolates found causing stem lesions. These results are useful in management strategies of Idaho fields and crops.

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Chapter 3: Characterization of *Rhizoctonia* Isolates from Idaho Common Bean Crops

Abstract

188 isolates of *Rhizoctonia* species complex were isolated from Idaho common bean crops (*Phaseolus vulgaris* L.) between 2018 and 2020. Isolates were assigned to anastomosis group (AG) using rDNA internal transcribed spacer region. Most of the isolates (72.9%) belonged to multinucleate *R. solani*, with AG 4 HG-II isolates having the highest presence (45.2%). The remaining isolates were binucleate (27.1%) and a *Waitea circinata* var. *zeae* (0.01%). This is also the first report of AG 3-PT, AG E, AG K and *Waitea circinata* var. *zeae* on common beans. Pathogenicity tests were carried out on bean seedlings, with a diverse representation of isolates, which revealed a wide range of aggressiveness amongst isolates. AG 4 HG-I was the most aggressive AG on stems, while AG 4 HG-II was the most aggressive on roots. This is the first investigation which provides evidence of the relative AG composition of *Rhizoctonia* species populations causing disease in common bean crops in Idaho.

Introduction

Rhizoctonia root rots have been associated with yield losses up to 100% in the US (Schwartz et al 2005), though 10-20% is typical for common bean crops. Root rot symptoms can range from water-soaked to reddish-brown sunken lesions in a range of sizes (Oladzad et al. 2019). As well as root rots, *Rhizoctonia* species are associated with other diseases including stem rots, stem cankers and web blight on common beans (Harveson 2021). *Rhizoctonia*, in its widely used but artificial sense, is considered a species complex comprised of several groups including the multinucleate *Rhizoctonia solani* (*Thanatephorus cucumeris*) and binucleate *Rhizoctonia* species (BNR) both within the *Ceratobasidiaceae. A*lso, a range of species originally categorized as *Rhizoctonia*, such as *Rhizoctonia oryzae* (*Waitea* anastomosis group WAG-O) and *Rhizoctonia zeae* (*Waitea* anastomosis group WAG-Z), both of which are now considered *Waitea circinata* varieties within the *Corticacaeae* family (Sneh et al. 1991; Lawrey et al. 2008; Kammerer et al. 2011). Considerable diversity exists within both *Rhizoctonia solani* and BNR species and isolates can be assigned to numerous anastomosis groups (AGs) on the basis of hyphal fusion. AGs of *R. solani* are designated AG1 to 13 (Carling et al. 2002) and for BNR species AG A to AG W (Yang et al. 2015). Furthermore, many subgroups exist within individual AGs. *R. solani* contains 28 subgroups, while BNR contains 8 subgroups (Sharon et al. 2006;

Yang and Li 2012; Hua et al. 2014; Misawa et al. 2018). Isolates of *Rhizoctonia* are now routinely assigned to AGs using rDNA ITS sequence analysis (Sharon et al. 2008) and various PCR assays employed for detection and identification (Budge et al. 2009; Woodhall et al. 2013;2017).

Correct diagnosis based on the knowledge of the AG, or even AG subgroup present, is essential for optimum disease management. AGs can differ in, means of dispersal, host range, fungicide sensitivity (Kataria and Gisi 1999) and aggressiveness, even to certain plant parts, such as *R. solani* AG 8 almost exclusively infecting the roots of potatoes (Woodhall et al. 2008). Multiple AGs have been implicated causing diseases in bean crops including AGs 1, 2, 4, 5, and 11 for *R. solani* (Ajayi-Oyetunde and Bradley 2017; Woodhall et al. 2020), and AGs F and G for BNR species (Yang and Li 2012). AGs 1-IA, 1-IB, 1-IE, 1-IF, 2-2 and 4 have been associated with web blight disease of common beans (Valentín Torres et al. 2016). AGs 1-IA and AG 1-IB also cause web blight on soybeans in Louisiana (Yang et al. 1990). Symptoms of web blight include leaf and pod lesions, stem and petiole lesions, cobweb like mycelium and abundant microsclerotia (Sin and Kumar, 2018). The fungus is able to spread in the canopy through mycelial bridges, airborne basidiospores, rain-splashed sclerotia, infested seed, soil and/or debris (Godoy-Lutz et al. 2003), although means of transmission is likely to vary with AG. Root rots symptoms include water-soaked, sunken, reddish-brown lesions that range in size causing damping off in bean seedlings (Reddy et al. 1993; Hagedorn, 2005). Severe disease can lead to stunting, as well as premature plant death (Oladzad et al. 2019). *R. solani* AGs 2-2, 4 and 5 have been associated with root rot of beans (Eken and Demirci, 2004; Valentín Torres et al. 2016). BNR AG F and AG G are considered weak pathogens to the roots and stems (Eken and Demirci, 2004). Of the causal AGs, AG 4 is the most aggressive (Phillips 1991; Eken and Demirci 2004), though AG 5 caused the second highest aggressiveness in a study by Eken and Demirci (2004). Stem cankers can develop on bean stems from *Rhizoctonia* spp. causing sunken, reddish-brown lesions that can start on the hypocotyl, with the ability to grow up the stem causing lesions and/or girdling resulting in stunting or plant death (Harveson et al. 2005). Water stress in mid to late season can further increase the lesions (Harveson et al. 2005). AGs 4 HG I, 4 HG-II (Taheri and Daroodi 2018) and 11 (Woodhall et al. 2020) are associated with stem cankers on beans.

Presently there is limited knowledge of the AGs present on bean crops in Idaho and the specific diseases they cause. The aim of this study was therefore to characterize isolates collected from common bean crops, with AGs determined using rDNA ITS sequencing. The aggressiveness and type of disease caused by selected representative isolates of each AG was also determined in two

glasshouse experiments. The relative incidence of root and stem diseases in Idaho crops is presented here.

Materials and Methods

Sample Collection

Soil was taken from fields designated for bean production prior to planting in the months of March and April in the years 2018, 2019 and 2020. Each field was sampled within a one-hectare area of the field. Larger fields (over 30 acres) were sampled twice with the second sampling area in the opposite corner of the field. The sampling areas were away from the headland and edge rows. 25 sub samples were taken in a grid pattern over the one-hectare area. A GPS unit was used to record coordinates of each sampled area. A gator probe (AMS, American Falls, ID) was used to collect cores each up to a depth of 30 cm. Individual cores were mixed in a clean bucket and approximately 500 g of soil was placed in a polyethylene bag and stored at 4 °C for up to 3 days prior to processing.

Plant material was collected from the same area in each field in the months of July through August of each year 2018, 2019 and 2020. A trowel was used to lift 30 entire plants, with as much root material as possible. Plant samples were placed in a separate polyethylene bag and stored 4 °C for no more than 36 hours prior to assessment.

Disease Assessments and Storage of *Rhizoctonia*

Plant samples were washed in tap water to remove all soil prior to assessment. Plants were visually assessed for root and stem symptoms. Root disease was assessed on the presence of disease. The number of plants with any symptomatic root disease were counted. Root disease is presented as incidence (RI). Stem disease severity were assessed on a scale adapted from Woodhall et al. (2008). 0 to 4, 0 = healthy/no lesion, 1 = any lesion, 2 = lesion above 5 mm, 3 = lesion above 10 mm, 4 = girdled lesion. Any variance in symptoms was noted. *Rhizoctonia* diseases on stems were presented as SS.

Symptomatic and border asymptomatic plant material, roughly 5-7 mm in size, was surface disinfested in sodium hypochlorite (1% NaOCl) for at least one minute, rinsed in two changes of sterile distilled water and allowed to dry before being placed onto 1.5% water agar (TWA) and potato dextrose agar (PDA) amended with streptomycin (0.8 g/liter) and penicillin (0.2 g/liter). After 48–72 h incubation at 21° C, hyphal tips from colonies of *Rhizoctonia* were identified under a dissecting

microscope and transferred to PDA and incubated at 21° C until DNA extraction or being placed into storage on PDA slopes in 50 ml tubes stored at 21° C.

DNA Extraction from Cultures, Sequencing and Phylogenetic Analysis

Isolates were given individual 'DB' isolate codes prior to DNA extraction. DNA was extracted from 10-day old PDA culture hyphae, within a roughly 20 mm x 20 mm area, using a Wizard food DNA purification kit (Promega) in conjunction with a KingFisher ML magnetic particle processer in accordance with manufacturer's instructions (Woodhall et al. 2017). DNA was kept at -20ºC.

The rDNA internal transcribed spacer (ITS) region was used for sequencing of extracted DNA. PCR amplification was first performed with ITS4 and ITS5 primers at an annealing temperature of 58 °C. Amplified products were purified using the Qiagen purification kit in accordance with manufacturer's protocol (Woodhall et al. 2017). Purified products were observed on a 1% (w/v) low melting point agarose gel (containing 0·5 μg mL^{-1} ethidium bromide) in TAE buffer (40 mM tris-acetate, 1 mM EDTA, pH 8). Samples with visible bands were sent to Eurofins genomics for sequencing.

DNA Sequences were analyzed using Geneious Prime (Geneious, New Zealand). Sequence alignments for each isolate were performed using Geneious alignment and visually edited to create a consensus sequence (Thompson, Higgins and Gibson 1994). Consensus sequences were run through NCBI BLAST for AG identification. Furthermore, DNA alignments were performed using ClustalW. Exemplar isolates were used as a representative isolate to 100% identical isolates within an AG. Exemplar isolates were used to create clean phylogenetic trees for BNR, all AG 2 and AG 4 isolates. AG 2 isolates from the cereal survey were included in the AG 2 phylogenetic tree. All AG 11 isolates, including any isolates from the cereal survey, were included in the AG 11 phylogenetic tree.

Reference sequences were obtained from GenBank and used in the construction of all phylogenetic trees (Table 3.1). Neighbor joining method for distance matrix values were constructed and distances in the rDNA ITS region were calculated using the Tamura-Nei model (Tamura and Nei, 1993). Bootstrap analysis was performed with 1000 re-samples of data. rDNA ITS sequences from opposite *Rhizoctonia* species were used as an outgroup for each phylogenetic tree. AG 1-IB *Rhizoctonia solani* (GenBank accession NO. MT568768.1) was used for the BNR phylogeny, while *Ceratobasidium* sp. AG-E (GenBank accession NO. AB290019.1) was used for the AG 2, AG 4 and AG 11 phylogenetic trees.

Table 3.1 *Phylogenetic Tree Reference Accession numbers*

Reference Accession #	AG	Host	Origin	Collector
MT568768	1-IB	*Brassica oleracea*	Turkey	Turkkan, M.
MN106363	2-1 Subset 1	*Brassica oleracea var. capitata*	Turkey	Ozer, G.
AB054845	2-1 Subset 1	*Raphanus sativus*	Japan	Kuninaga, S.
AB054849	2-1 Subset 2	*Hordeum vulgare*	Australia	MacNish, G.
JX161871	2-1 Subset 3	*Solanum tuberosum*	New Zealand	Das, S.
AB547384	2-1 HK Clade	*Brassica oleracea var. botrytis*	Netherlands	Misawa, T.
JX161892	2-1 UK PT Clade	*Solanum tuberosum*	UK	Das, S.
AF354116	2-2 IIIB	*Lomandra longifolia*	Japan	Gonzalez, D.
AJ238166	2-2 IIIB	*Zea mays*	Japan	Salazar, O.
AY270014	2-2 IV	*Beta vulgaris*	Brazil	e, R.C.
AJ238164	2-2 IV	*Beta vulgaris*	Japan	Salazar, O.
AB054866	2-2 LP	*Zoysia tenuifolia*	Japan	Hyakumachi, M.
AJ238163	2-2 LP	*Zoysia grass*	Japan	Salazar, O.
AF308622	2-2 WB	*Phaseolus vulgaris* L.	USA	Godoy-Lutz, G.
AB054879	2-4	*Daucus carota*	USA	Sumner, D.
AB054871	2-3	*Glycine max*	Japan	Naito, S.
AB054873	2-BI	Soil	Japan	Kuninaga, S.
AY152704	4 HG-I	*Lycopersicum esculentum*	Brazil	Kuramae, E.
AY154308	4 HG-II	Unknown	Brazil	Kuramae, E.
DQ102449	4 HG-III	Soil	Israel	Sharon, M.
AF354114	11	*Glycine max*	USA	Gonzalez, D.
LC215406	11	*Oryza sativa*	Japan	Misawa, T.
AY154313	11	Unknown	Brazil	Kuramae, E.
LC215402	11	*Lilium* spp.	Japan	Misawa, T.
AF153802	11	Soil	Australia	Pope, E.J.
DQ102407	A	*Fragaria ananassa*	Israel	Sharon, M.
DQ102421	A	*Fragaria ananassa*	Israel	Sharon, M.
AB290021	C	*Beta vulgaris*	Japan	Uchino, H.
AB290019	E	*Oxalis*	Japan	Kuninaga, S.
DQ102433	Fa	*Fragaria ananassa*	Israel	Sneh, B.
AB219145	Fb	Soil	Japan	Ogoshi, A.
DQ102395	G	*Fragaria ananassa*	USA	Martin, F.N.
MT380177	H	*Fragaria ananassa*	Turkey	Genc Kesimci, T.
AB290023	I	*Beta vulgaris*	Japan	Uchino, H.
AB196652	K	Soil	Japan	Hyakumachi, M.
JQ859871	K	*Fragaria ananassa*	Australia	Fang, X.
HQ424246	*Ceratobasidium theobromae*	*Theobroma cacao*	Indonesia	Samuels, G.J.

Reference isolates used in the construction of phylogenetic trees. Isolates represented by their DNA accession numbers available on GenBank. Many accession numbers were from Misawa et al. 2018, Sharon et al. 2006 and 2008.

Aggressiveness of Bean Isolates

To test the aggressiveness of the *Rhizoctonia* isolates, two glasshouse experiments were setup under 16hr lit and 8hr dark cycle conditions at an average of 18° C. The experiment was duplicated with a difference of two-days between inoculation and different placement within the greenhouse. experiments were labeled A and B. 5 pinto bean seeds, cv Windbreaker, were planted in a 16 cm^3 plastic pot with Ferti·lome Ultimate Potting Mix (Fert·lome, Bonham, TX) to mimic row conditions. For each isolate, soil was removed from the roots of ten 14-day old plants and inoculated with a $10\ mm^2$ plug from a 10-day old PDA culture at the base of the stem and roots. A non-inoculated PDA plate was used to create control plants. Plants were harvested 4 weeks post-inoculation, washed and visually assessed for symptoms as described above for stem disease. Root disease was assessed on the percentage of disease present on the whole root structure for root severity (RS).

60 isolates were used in the glasshouse experiment and were chosen to represent the diversity of AGs present in proportion to their relative incidence in the survey. Additionally, a *Waitea circinata* var. *zeae* isolate found on a Southwest bean plant in 2019 was used, as well as a BNR isolate of AG W, found on an Idaho potato stem in 2020.

Statistical Analysis

The plant assessment and pathogenicity stem severity (SS) means were calculated using the formula $SS = \sum[0(n_0) + 1(n_1) + 2(n_2) + 3(n_3) + 4(n_4)]/(N_{total})$, where n_x = number of stems in the *x* rating class and *N* = total number of stems. The mean root incidence (RI) for plant assessment was calculated by region. The mean root severity (RS) for the pathogenicity experiment was calculated by the average of all isolates within individual AGs. Pathogenicity statistical analyses were performed using the r statistical package (r development core team 2017). Data analysis from both experiments was performed using ANOVA followed by Tukey's HSD for means comparison with an alpha of 0.05.

Results

Sampling and Plant Assessment

A total of 102 common bean fields from five Idaho counties were sampled. In the 2018 season, 2 fields with 2 points were surveyed in the Southcentral region and 21 fields with 44 points were surveyed in the Southwestern region. In the 2019 season, 15 fields with 15 points were surveyed in

the Southcentral region and 22 fields with 39 points were surveyed in the Southwestern region. In the 2020 season, 17 fields with 22 points surveyed in the Southcentral region and 25 fields with 42 points were surveyed in the Southwestern region. In total, 34 fields in Southcentral Idaho and 68 fields in Southwest Idaho were sampled. Of these fields, 164 points were sampled with 39 points in Southcentral Idaho and 125 points in Southwest Idaho.

Plant assessments in 2018 had a mean SS score of 1 and a RI of 10 plants in the Southcentral region, while the Southwest region had a mean SS score of 1.8 and a RI of 15 plants. Plant assessments in 2019 had a mean SS score of 2.4 and a RI of 7 plants in the Southcentral region, while the Southwest region had a mean SS score of 2.6 and a RI of 17 plants. Plant assessments in 2020 averaged a SS score of 3.2 and a RI of 30 plants in the Southcentral region, while the Southwest region had a mean SS score of 1.2 and a RI of 30 plants.

Isolate Collection and Phylogenetic Analysis

Over 527 isolates were obtained from symptomatic plant material. Of these isolates, 188 isolates were confirmed to the *Rhizoctonia* spp. Most isolates identified as *R. solani* and isolates were observed with white to brown mycelium and typical right-angle branches at the distal septae of cells. The remaining isolates were identified as binucleate spp. Assignment of isolates to the various AGs was based on phylogenetic analysis of the rDNA ITS region. Isolates representing thirteen different AGs and *Waitea circinata* var. *zeae* were identified. The total presence of AGs obtained from the regions was 124 isolates from Southwest Idaho and sixty-four isolates from Southcentral Idaho (Table 3.2). Twenty fields had multiple AGs isolated over the course of the survey. AG 4 HG-II was the most frequently isolated AG in both regions, while AG 2-2 was the second most widely prevalent group found although its presence was limited to the Southwestern region. Remaining isolates varied throughout the two regions.

Table 3.2 *Anastomosis Group Breakdown by Region*

AG	Southcentral Idaho	Southwest Idaho	Total
AG 1 IB	-	1	1
AG 2-1	-	3	3
AG 2-2	-	26	26
AG 3	-	2	2
AG 4 HG-I	12	7	19
AG 4 HG-II	30	32	62
AG 4 HG-III	1	7	8
AG 5	-	10	10
AG 11	1	5	6
AG A	9	10	19
AG E	-	1	1
AG F	-	11	11
AG K	11	8	19
Waitea circinata var. *zeae*	-	1	1
Total	64	124	188

Anastomosis group breakdown within the individual region. Individual isolates were identified through rDNA ITS sequencing. A total of 53 fields were assessed for the Southcentral region, while 49 fields were assessed for the Southwestern region.

155 isolates were obtained from stem material, with minimal isolates obtained from root material (Table 3.3). A majority of the isolates were obtained from symptomatic stem material, with minimal isolates obtained from root material. AG 4 HG-II had the highest presence in stem material, with AG 2-2 isolates being the second highest. Remaining isolates were obtained from various symptomatic material.

Table 3.3 *Anastomosis Group Breakdown by Symptom*

AG	Roots	Stem	Total
AG 1 IB	-	1	1
AG 2-1	2	1	3
AG 2-2	3	23	26
AG 3	-	2	2
AG 4 HG-I	5	14	19
AG 4 HG-II	7	55	62
AG 4 HG-III	1	7	8
AG 5	-	10	10
AG 11	1	5	6
AG A	9	10	19
AG E	-	1	1
AG F	4	7	11
AG K	1	18	19
Waitea circinata var. *zeae*	-	1	1
Total	33	155	188

Anastomosis group breakdown within the individual symptom. Individual isolates were identified through rDNA ITS sequencing. Root symptoms presented as lesions and stem symptoms ranged in lesion severity.

Isolates were further broken down into unique isolates based off year, origin field and symptoms. For example, if there were two isolates under field A, symptom B, only 1 isolate was counted towards a unique isolate for that field and symptom. Overall, there were 106 unique *Rhizoctonia* isolates in this survey (Appendix 2.1). AG 4 HG-II had the highest presence, with AG A having the second highest presence. Exemplar isolates were used for 100% identical sequences to help create clean phylogenetic trees. AGs with exemplars used in the construction of phylogenetic trees are listed in Table 3.4.

Table 3.4 *List of exemplar isolates used in construction of phylogenetic trees.*

Representative Exemplar DB#	AG	Identical Isolates DB#
DB52	AG 2-1	DB39
DB49	AG 2-2	DB53, DB62, DB83
DB61	AG 2-2	DB72
DB74	AG 2-2	DB40, DB54, DB56, DB59, DB66, DB67, DB68. DB69, DB71, DB73, DB78
DB76	AG 4 HG-I	DB44, DB152, DB169, DB170, DB171, DB182, DB185, DB195, DB196, DB208
DB205	AG 4 HG-I	DB207, DB210
DB209	AG 4 HG-I	DB185, DB218, DB219
DB4	AG 4 HGII	DB3, DB5, DB6
DB112	AG 4 HG-II	DB36, DB37, DB42, DB43, DB47, DB75, DB95, DB96, DB97, DB98, DB99, DB100, DB103, DB104, DB105, DB111, DB119, DB120, DB122, DB123, DB125, DB126, DB137, DB138, DB139, DB140, DB141, DB142, DB146, DB147, DB148, DB149, DB150, DB151, DB154, DB156, DB158, DB159, DB160, DB161, DB162, DB163, DB164, DB166, DB167, DB168, DB186, DB187, DB194, DB199, DB200, DB202, DB203, DB204, DB216, DB251, DB240, DB241, DB249
DB227	AG 4 HG-III	DB7, DB117, DB121, DB179, DB215, DB228, DB229, DB253
DB10	AG-A	DB15, DB106, DB108, DB134, DB248
DB65	AG A	DB86, DB102
DB79	AG A	DB213
DB143	AG A	DB225, DB234
DB192	AG A	DB107, DB116, DB190, DB220
DB18	AG Fa	DB20, DB29, DB30, DB38, DB45, DB64, DB87, DB115, DB188
DB193	AG K	DB12, DB13, DB14, DB41, D#20-38, DB128, DB136, DB144, DB145, DB189, DB221, DB223, DB224, DB226

Representative exemplar isolate represents 100% identical isolate sequences within the same AG. Alignment off all isolates within an AG, using ClustalW, were visually assessed for identification.

Phylogenetic analysis of the AG 2 subgroups shows the survey isolates clustered within AG 2-1 and AG 2-2 subgroups (Figure 3.1). Most isolates are divided between two subgroups, 2-2 IIIB and placement within an unknown clade. The unknown clade is placed between AG 2-2 IIIB and 2-2 IV

and 2-2 LP clades. Isolates within AG 2-1 are divided between two subgroups. One isolate is placed directly with an AG 2-1 subset 3 isolate, while a bean and cereal isolate are placed within a subset below subset 3. One cereal isolate is placed within the AG 2-1 subset 2.

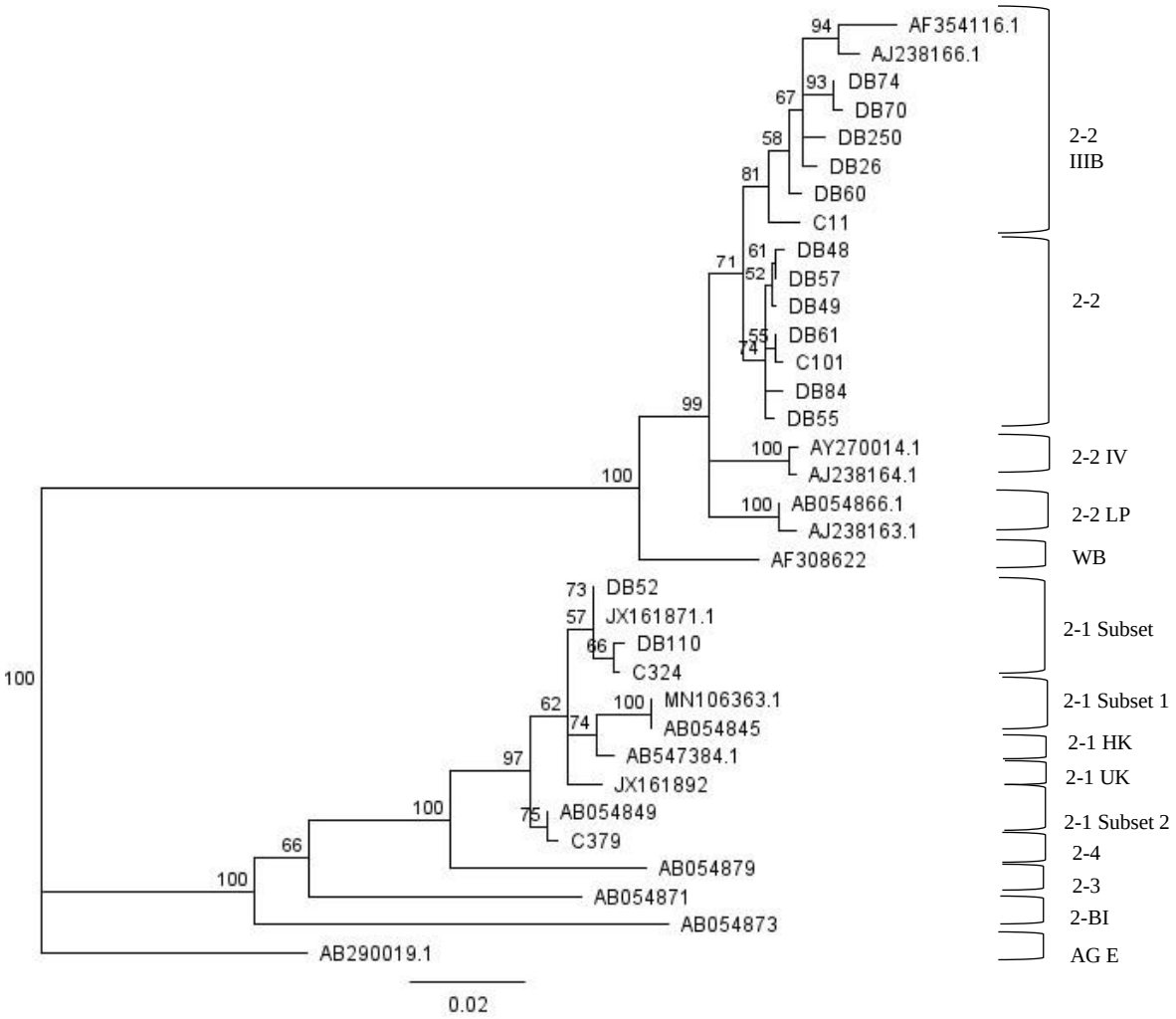

Figure 3.1 A neighbor-joining tree of AG 2 anastomosis group isolates (represented by their DB and C numbers) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 17 trials are positioned alongside the branches with values over 50. Isolate AB290019 [AG E (*Ceratobasidium*)] was used as an outgroup. The AGs and subgroups for the clusters are indicated. Bar indicates 0.2 base change per 10 nucleotide positions.

Phylogenetic analysis of the AG 4 subgroups shows the survey isolates placed within AG 4 HG-I, AG 4 HG-II and AG 4 HG-III (Figure 3.2). Four isolates are placed within the AG 4 HG-I clade, with two isolates showing split variance in relativity and two isolates showing farther relative distance. Two

isolates are placed in the AG 4 HG-II clade slit variance in relativity. One isolate is placed within the AG 4 HG-III clade.

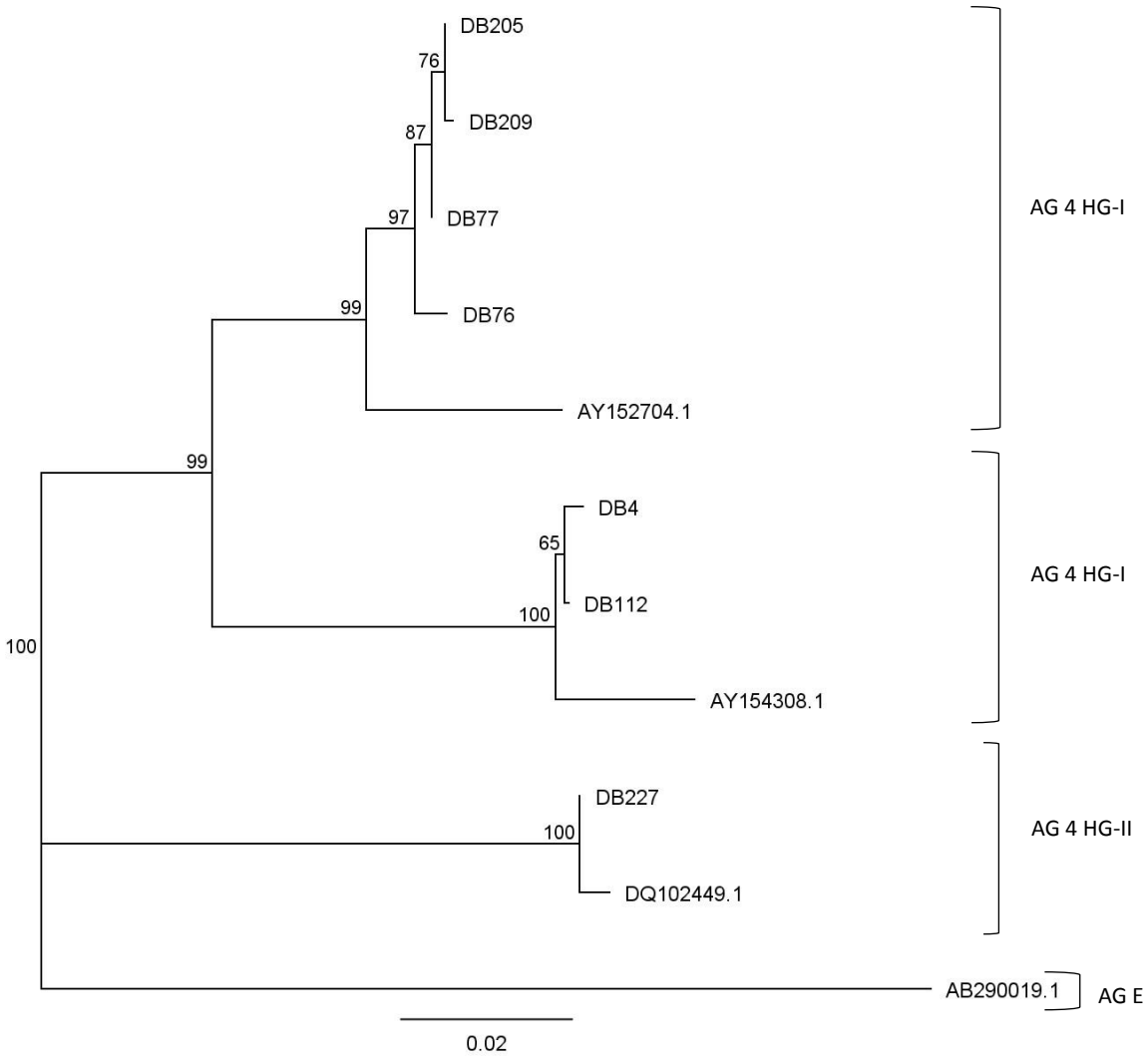

Figure 3.2 A neighbor-joining tree of AG 4 anastomosis group isolates (represented by their DB numbers) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 7 trials are positioned alongside the branches with values over 50. Isolate AB290019 [AG E (*Ceratobasidium*)] was used as an outgroup. The AG and subgroups for the clusters are indicated. Bar indicates 0.2 base change per 10 nucleotide positions.

Phylogenetic analysis of the AG 11 shows the survey isolates placed within five separate clades (Figure 3.3). Five isolates, both cereal and bean, are placed within the same clade as reference isolates from *Glycine max* from the U.S., *Oryza sativa* from Japan and an unknown host from Brazil. Two bean isolates branch off this clade to form a subclade with no reference isolate to identify host

range. Three isolates, cereal and bean, are placed within the same clade as the *Lilium* spp. reference isolate from Japan. One cereal isolate is placed within the same clade as the soil reference isolate from Australia. One cereal isolate forms a separate clade with no reference isolate to identify host range.

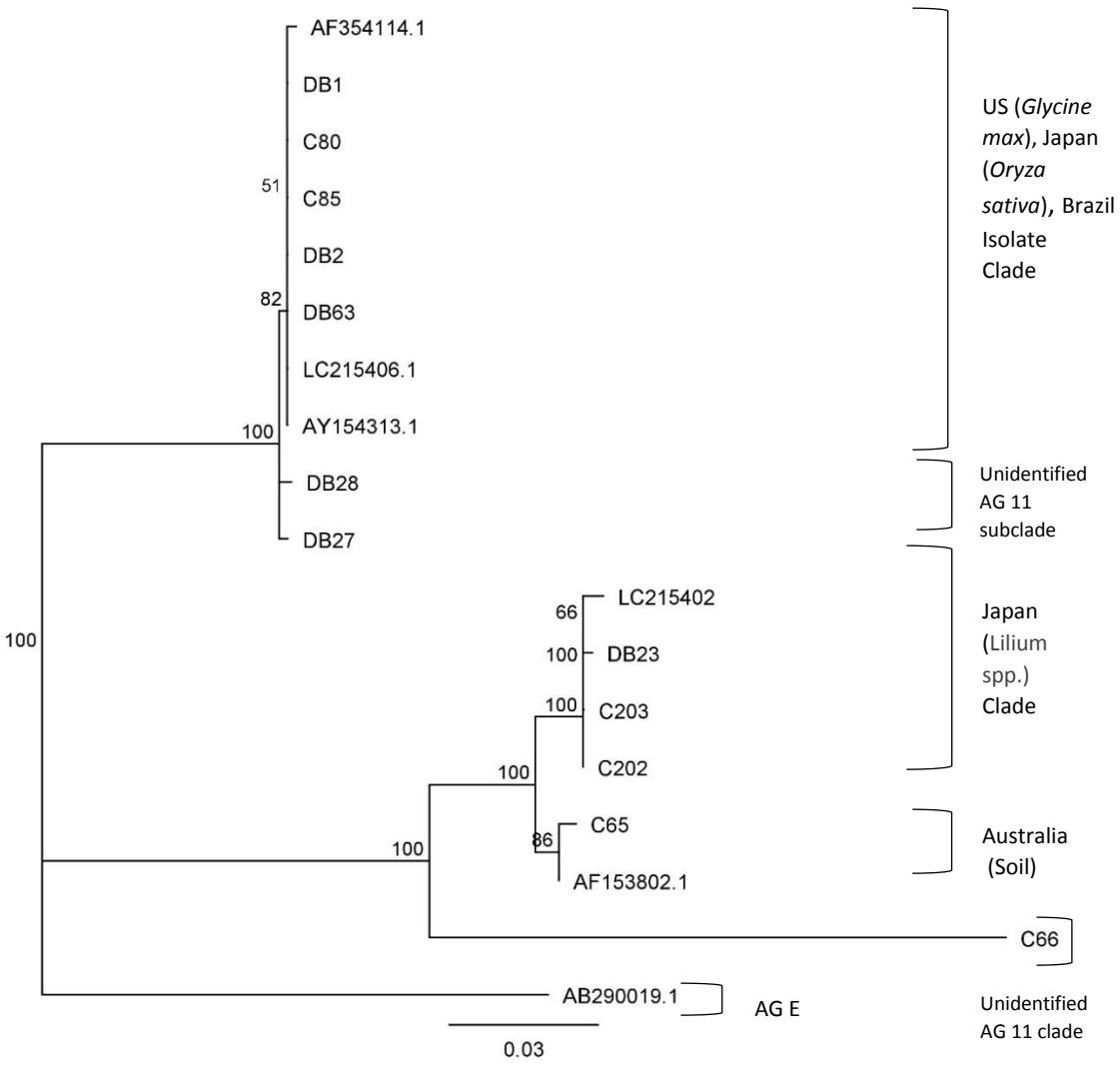

Figure 3.3 A neighbor-joining tree of AG 11 anastomosis group isolates (represented by their DB and C number) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 12 trials are positioned alongside the branches with values over 50. Isolate AB290019 [AG E (*Ceratobasidium*)] was used as an outgroup. The reference clades for the clusters are indicated. Bar indicates 0.3 base change per 10 nucleotide positions.

Phylogenetic analysis of the BNR AGs and subgroups shows isolates placed within AG A, AG E, AG F and AG K (Figure 3.4). Five isolates are placed within the AG A clade with variance in relativity between branches. One isolate is placed within the AG E clade. One isolate is placed within AG Fa after grouping with an AG Fa reference isolate. Six isolates are placed within the AG K clade. Most isolates are grouped with a general clade with slit variance in branching, while two isolates form two separate subsets.

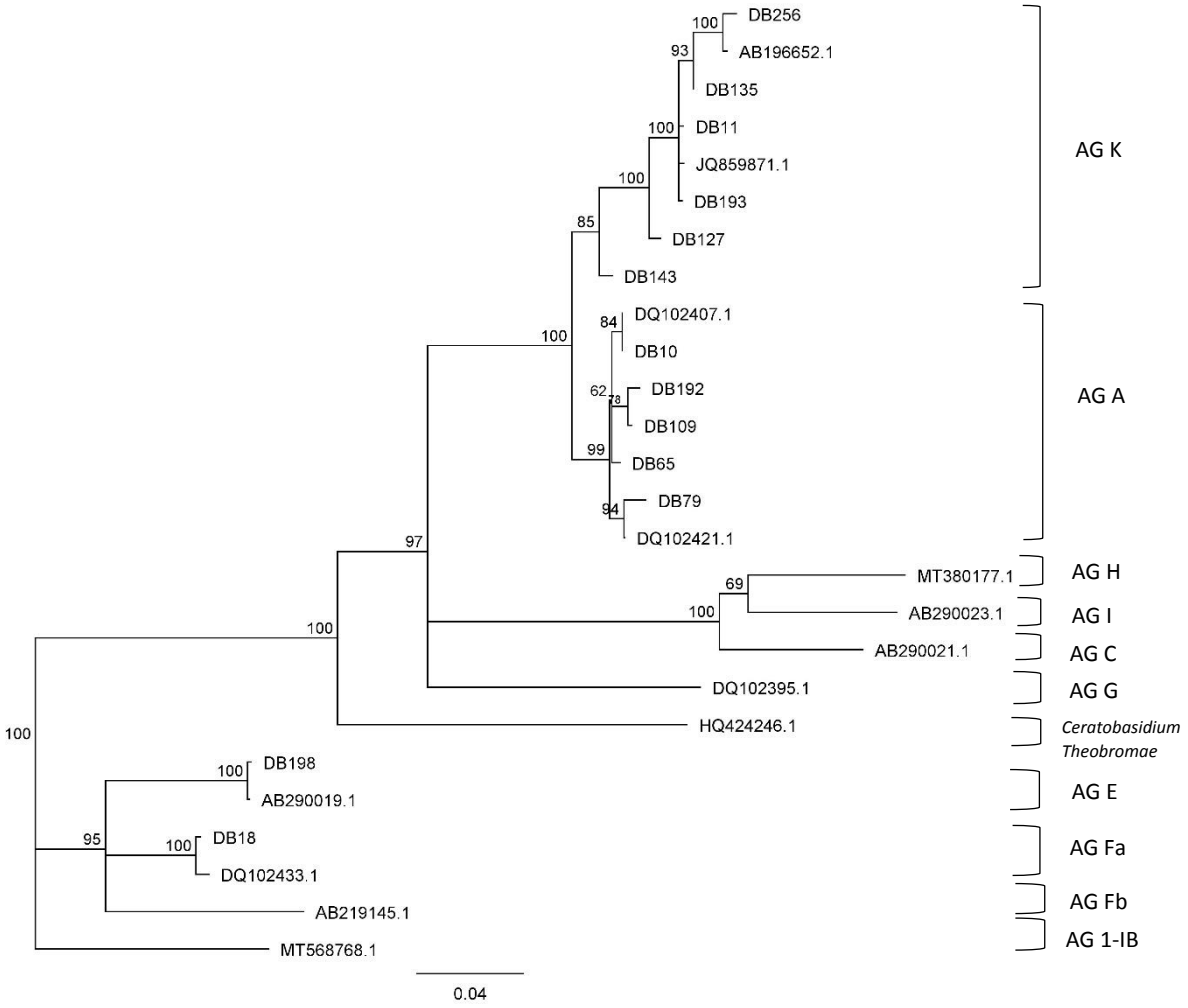

Figure 3.4 A neighbor-joining tree of binucleate *Rhizoctonia* spp. (*Ceratobasidium* spp.) anastomosis group isolates (represented by their DB) with reference isolates (represented by accession numbers available in GenBank), clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Tamura-Nei's model. Bootstraps of 13 trials are positioned alongside the branches with values over 50. Isolate MT568768 [AG 1-IB (*R. solani*] was used as an outgroup. The AGs and subgroups for the clusters are indicated. Bar indicates 0.4 base change per 10 nucleotide positions.

The *Waitea circinata* var. isolate was placed in the cereal survey *Waitea circinata* var. phylogenetic tree in the previous chapter (Chapter 2, Figure 2.4). DB235 was placed in the same clade as the reference isolates *Waitea circinata* var. *zeae* on oilseed (*Brassica napus*) (GenBank accession # MN160242) and cabbage (*Brassica oleracea var. capitata*) (GenBank accession # MN160232) from Serbia.

Pathogenicity Experiment

A total of 62 isolates were used in the pathogenicity experiment (Appendix 2.1). *R. solani* isolates accounted for 45 isolates for 75% and BNR isolates accounted for 15 isolates or 25%. One *Waitea circinata* var. *zeae* isolate and one AG W to used to make up 62 isolates. A total of 619 plants were assessed for run A of this experiment, with 1 plant dying before assessment. This plant was inoculated with an AG 4 HG-II isolate. A total of 613 plants were assessed for run B of this experiment, with 7 plants dying before assessment. Of these plants, 2 were inoculated with AG A isolate, 2 with 4 HGI isolates and 3 with 4 HGII isolates.

Overall, there was no statistical significance between SSI for experiment A and experiment B (Tukey's test, $P < 0.05$). No stem disease was observed on the control plants in either A or B experiments. All isolates caused disease on the stems (Figure 3.5, Figure 3.6). The six AG 4 HG-I isolates caused the most sever stem disease, with a mean SSI score of 3.65 for experiment A and 3.52 for experiment B. Lesions from AG 4 HG-I caused severe girdling into the vascular system of the stem. The AG 1-IB isolate caused the second highest with a mean SSI score of 2.8 for experiment A and 2.5 for experiment B. Lesions often had sclerotia was forming around them on the stems. Remaining isolates showed varied symptoms for both runs.

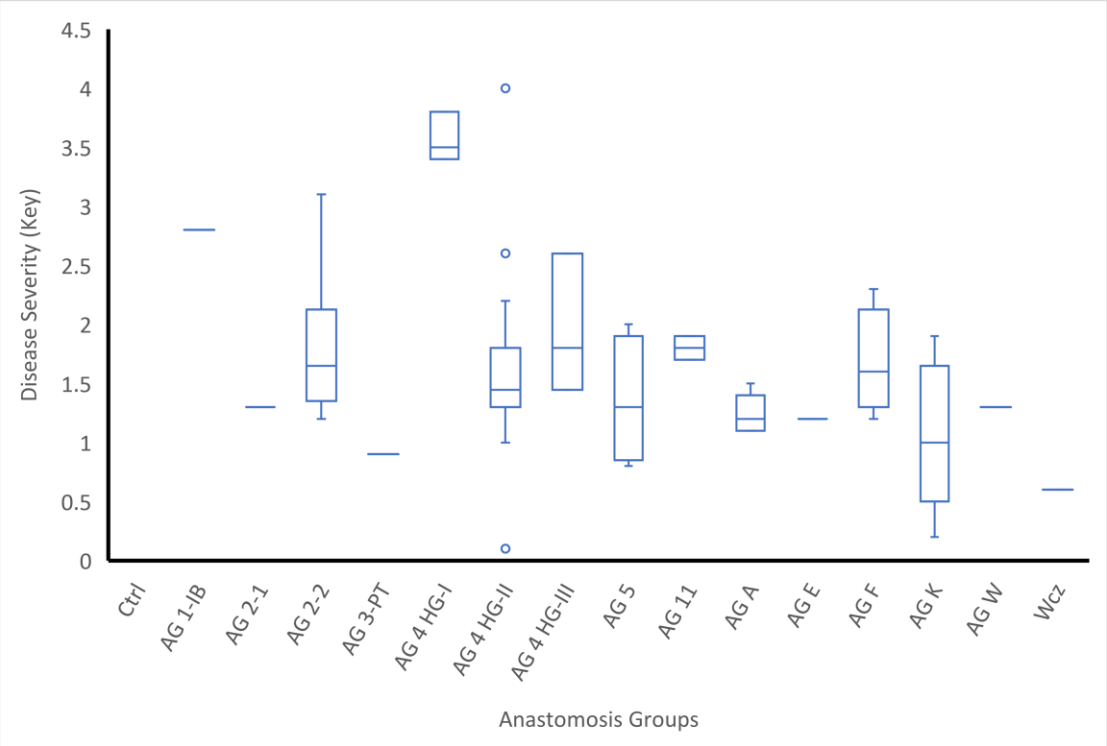

Figure 3.5 Mean stem severity index (SSI), key scale of 0 – 4, of anastomosis groups for pathogenicity experiment GH206 A. Equation SS = $\sum[0(n_0) + 1(n_1) + 2(n_2) + 3(n_3) + 4(n_4)]/(N_{total})$, where n_x = number of stems in the x rating class and N = total number of stems was used to calculate the SS per isolate within an anastomosis group. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the Median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. *Waitea circinata* var. *zeae* isolate denoted by 'Wcz'.

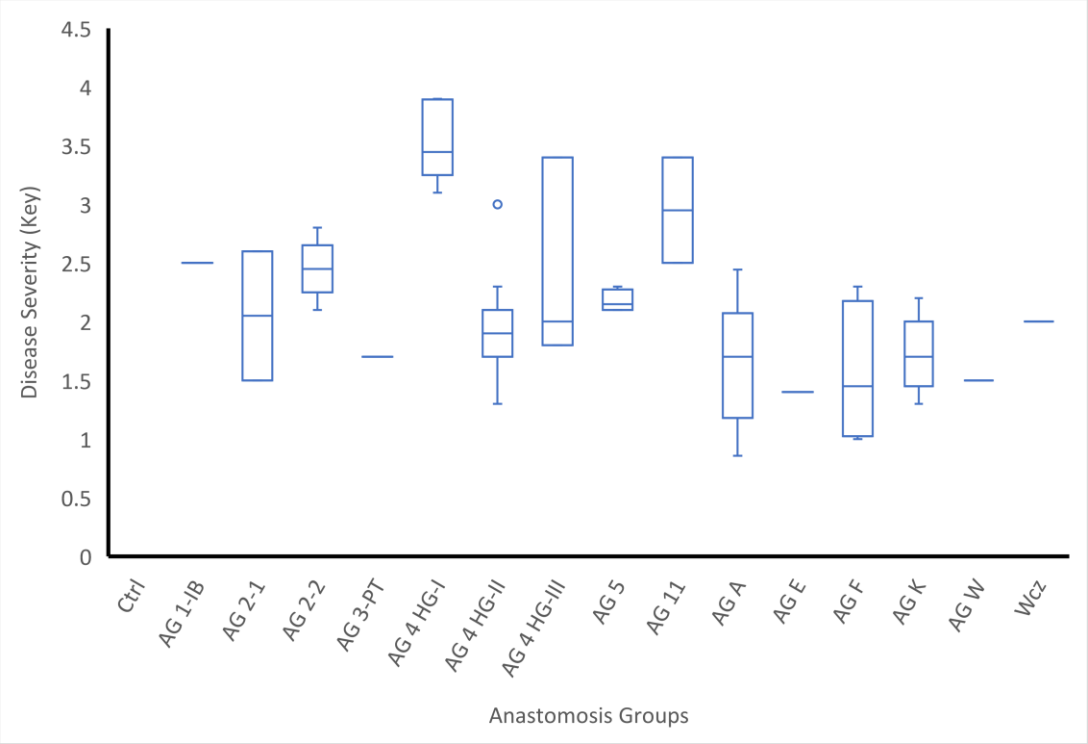

Figure 3.6 Mean stem severity index (SSI), key scale of 0 – 4, of anastomosis groups for pathogenicity experiment GH206 B. Equation SS = $\sum[0(n_0) + 1(n_1) + 2(n_2) + 3(n_3) + 4(n_4)]/(N_{total})$, where n_x = number of stems in the x rating class and N = total number of stems was used to calculate the SS per isolate within an anastomosis group. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the Median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. *Waitea circinata* var. *zeae* isolate denoted by 'Wcz'.

Overall, there was no statistical significance between RIA for A and B experiments (Tukey's test, $P < 0.05$). No RIA was observed on the control plants in either A or B experiments. All isolates caused some disease on the roots (Figure 3.7, Figure 3.8). The AG 4 HG-II isolates caused the highest root disease. AG 4 HG-II had three outlier points for assessment experiment A, with mean scores of 15% for one isolate, 24% for one isolate and 51% for one isolate. Furthermore, AG 4 HG-II had one outlier point for assessment experiment B with a mean of 45% for one isolate. Remaining isolates showed varied symptoms for both runs.

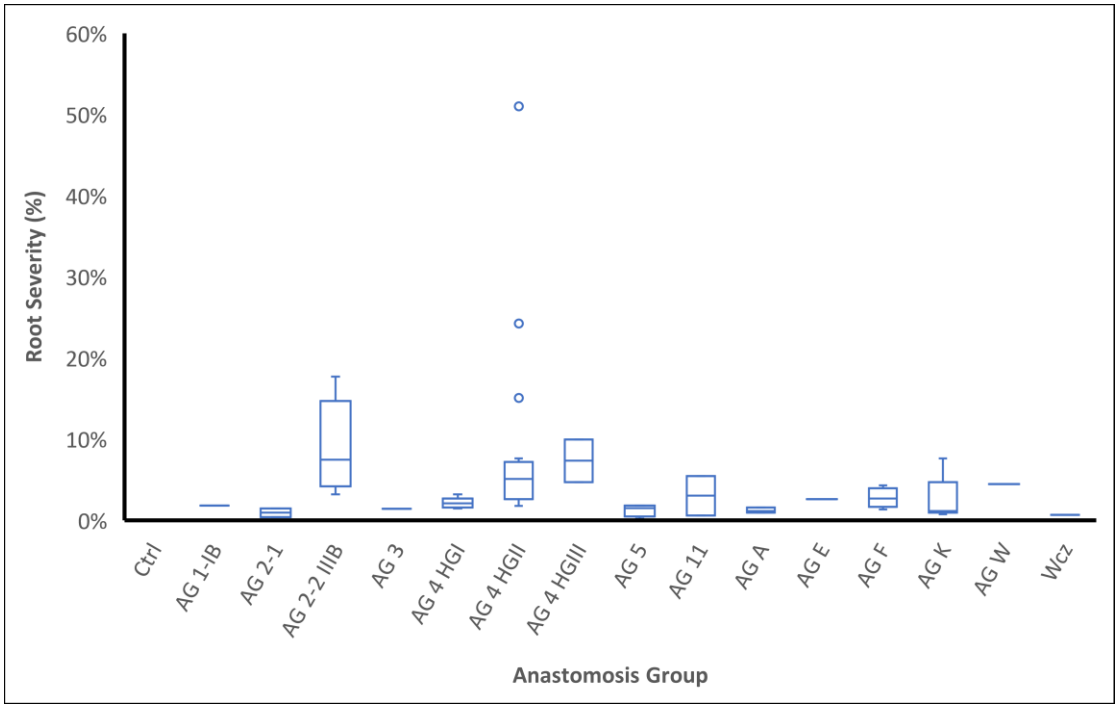

Figure 3.7 Mean root infected area (RIA), 0 -100 % necrosis of roots, of anastomosis groups for pathogenicity experiment GH206 A. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. Waitea circinata var. zeae isolate denoted by 'Wcz'.

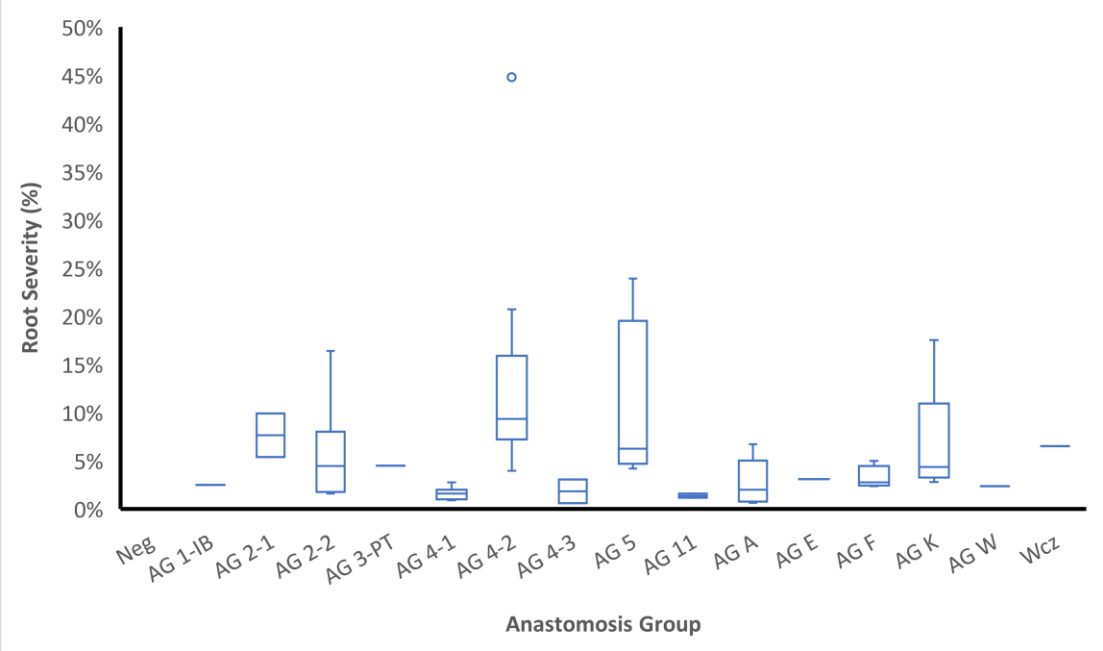

Figure 3.8 Mean root infected area (RIA), 0 -100 % necrosis of roots, of anastomosis groups for pathogenicity experiment GH206 B. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual AG isolates. Circles outside of the whisker points represent outlier mean data. Line in the box represents the median of the mean averages within an AG. Lines without box and whisker represent individual mean average if only 1 isolate had a mean within the AG. Waitea circinata var. zeae isolate denoted by 'Wcz'.

Discussion

This is the first investigation to determine the relative incidence of individual AGs of *Rhizoctonia* spp. causing disease common bean crops in Idaho. 188 *Rhizoctonia* isolates were collected from common bean from 102 fields throughout Southcentral and Southwestern Idaho over three years. Through rDNA ITS sequencing and phylogenetic placement, isolates were characterized to one of 13 AGs of *R. solani* and BNR and *Waitea circinata* var. *zeae*. The isolates obtained during this survey showed the majority of isolates to be characterized as *R. solani*, while the rest of the isolates were characterized as binucleate *Rhizoctonia* spp. and one *Waitea circinata* var. *zeae*.

There are many reports of AG 4 causing stem and root disease on bean, though few identify which subgroup is the causal agent (Phillips 1991; Eken and Demirci 2004). The most prevalent AG 4 isolates were AG 4 HG-II with 62 isolates recovered. The dominance of AG 4 HG-II was consistent with previous root and stem rot virulent report by Taheri and Daroodi (2019). The dominance of AG 4 HG-II was also consistent with the pathogenicity experiment root disease results. However, results from the pathogenicity experiment show AG 4 HG-I being the most virulent on stems. The pathogenicity results are consistent with results in Cuba and Iran (Nerey et al. 2010; Taheri and Daroodi 2018). The presence of AG 4 HG-I with 19 isolates from stem material, also indicates virulence on Idaho beans. AG 4 HG-I was not frequently isolated from root material, with only 3 isolates, and minimal root disease, with a 2% RS mean, in the pathogenicity experiment. These results are unlike the study in Cuba, which also found AG 4 HG-I to be highly virulent to bean roots (Nerey et al. 2010). Variation in phylogenetic placement may suggest variation in pathogenicity in bean roots. Unlike AG 4 HG-I and AG 4 HG-II, AG 4 HG-III had very little presence. AG 4 HG-III has been found causing root rot on beans in Nebraska (Venegas et al. 2008). These findings are consistent with the pathogenicity experiment, which showed a higher disease of roots over stems by AG 4 HG-III. However, only one of the eight isolates were from root material. The AG 4 HG-III isolates may need a longer incubation period before stem disease occurs. Optimum growth temperature, 25-30 ° C, was not met in the greenhouse during the pathogenicity experiments (Stojšin et al 2011). This could account for the difference in symptoms between field and experiment plants. AG 2-2 has also been shown to be highly virulent to bean stems and roots (Nerey et al. 2010). The seven AG 2-2 isolates used in the pathogenicity experiment did not show a high virulence on stems, though did show a high RS which is also consistent with the findings in Cuba (Nerey et al. 2020). However, a majority of the isolates collected

were obtained from stem material. Differences in phylogenetic placement may suggest a variation in pathogenicity within the AG 2-2 isolates collected in Idaho. A majority of the isolates obtained were phylogenetically placed with AG 2-2 IIIB. AG 2-2 IIIB has an optimum growth temperature of 35 ° C, which is higher than other 2-2 subgroups (Dorrance et al 2003). The temperature in the greenhouse aggressiveness experiment may have been too low to initiate disease on the stems.

AG 1-IB has been reported causing web blight of soybeans (*Glycine max*) and common beans (Yang et al. 1990; Valentín Torres et al. 2016). Recently, AG 1-IB has been reported causing root rot on common beans as well (Valentín Torres et al. 2016). This is consistent with our findings in the pathogenicity experiment, though AG 1-IB was only isolated from stem material. This isolate was obtained from a field with multiple AGs present and could have been outcompeted in the root structures by AG 4 HG-II that was present. With AG 1-IB being one of the main causal agents for web blight, it may suggest that the subgroup may prefer infecting aerial parts of the plant over soil structures.

AG 2-1, AG 5, AG 11, AG A and AG F have been reported on beans (Eken an Demirci 2004; Nerey et al. 2010; Woodhall et al. 2020). AG 2-1 has been reported as nonpathogenic (Eken and Demirci 2004). This report is not consistent with the findings from this study. AG 2-1 was isolated from both stem and root material from plants within the Southwest region. Disease was observed on the stem and root material in the pathogenicity experiment as well. To my knowledge, there are no other reports of AG 2-1 on beans. AG 5 has been reported as being virulent to bean stems in Turkey (Eken and Demirci 2004). This is consistent with the ten isolates that were obtained from stem material. However, the pathogenicity experiment showed low disease in the stem and and mean of 6% root disease between both pathogenicity assessments. Again, variance may be from temperature or a short incubation period, since disease was present on the stems. AG 11 was first reported on beans in 2020 causing stem rot (Woodhall et al. 2020). To my knowledge, are no other reports of AG 11 on beans. Stem rot is consistent with the findings in the pathogenicity experiment and the material the isolates were obtained from. AG A has been reported as a weak root pathogen (Nerey et al. 2010) and as non-pathogenic (Eken and Demirci 2004). This is not consistent with the findings from this study. 19 isolates were obtained from plant material throughout both regions in Idaho with stem and root disease present in the pathogenicity experiment. AG F has been reported as a weak pathogen (Eken and Demirci 2004) and moderately pathogenic to roots in calcisols soils (Nerey et al. 2010). These reports are inconsistent with the findings from this study. AG F was isolated from both stem

and root material from plants within the Southwestern region. Within the pathogenicity experiment they caused both stem and root disease.

To my knowledge, AG 3-PT, AG E, AG K and *Waitea circinata* var. *zeae* have not been reported on common beans. AG 3-PT has been reported on soybeans in the U.S. and Canada (Ajayi-Oyetunde and Bradley 2017), on maize in the UK (McCormack et al. 2013) and on potatoes in the U.S., UK and China (Carling and Leiner 1990; Woodhall et al. 2007; Yang et al. 2015). The presence of AG 3-PT on beans is unexpected, though not surprising since potatoes and corn are grown in rotation with beans in the Southwest and Southcentral regions. AG E has been reported causing damping off in soybeans in Indonesia (Naito et al. 1993). AG K has been reported causing root rot on chickpea (*Cicer arietinum*) in Turkey (Basbagci and Dolar 2020). Waitea *circinata* var. zeae has been found causing root rot on faba beans (*Vicia faba L.*) in Turkey (Eken et al. 2011).

In this study, we provide for the first time, knowledge about the *Rhizoctonia s*pecies and AGs associated with bean diseases in Idaho. These results can be useful when deciding crop rotation strategies. Furthermore, different *Rhizoctonia* species and AGs possess a different sensitivity towards fungicides (Kataria and Gisi, 1996), which complicates chemical control strategies. The identification and pathogenicity determination of *Rhizoctonia* isolates, as described in this study, is the first step towards an efficient control strategy for bean diseases caused by *Rhizoctonia* species in Idaho.

In summary, this work provides knowledge of a diverse group of *Rhizoctonia* spp. present in Idaho common bean crops, with AG 4 HG-II being the most dominate group and AG 4 HG-I being the most virulent. Three AGs, AG 3-PT, AG E and AG K, and *Waitea circinata* var. *zeae,* were identified as pathogens in Idaho common bean crops with no association of prior disease. These results are useful in management strategies of Idaho fields and crops.

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Chapter 4: Comparison of Fungicides Used for the Control of *Rhizoctonia* Species Associated with Diseases of Common Bean (*Phaseolus vulgaris* L.)

Abstract

Control of *Rhizoctonia* diseases in common beans is based predominantly on the application of seed treatments, however in-furrow fungicides are also used. Little is known about the fungicide response variability of different *Rhizoctonia* AGs associated with common beans diseases in Idaho. Sensitivity to fungicides *in vitro* were evaluated on a total of twenty-three *Rhizoctonia* isolates chosen on the basis of ensuring a suitable range of AGs and isolate-host combinations. Sensitivity to fungicides *in vivo* were evaluated on an *R. solani* AG 11 isolate in field and greenhouse experiments. The *in vitro* fungicides comprised seven chemical formulations representing three Fungicide Resistance Action Committee groups. *In vitro* fungicides comprised four chemical formulations representing four Fungicide Resistance Action Committee groups. All *Rhizoctonia* AGs were extremely sensitive to inpyrfluxam (EC_{50}: < 0.1 mg l^{-1}), while sedaxane and pentiopyrad values varied between extremely sensitive to intermediately sensitive (EC_{50}: < 0.1 to 5.0 mg l^{-1}). Flutolanil values varied between extremely sensitive to sensitive (EC_{50}: < 0.1 to 1.0 mg l^{-1}). Prothioconazole and azoxystrobin showed values varied between sensitive to less sensitive (EC_{50}: 1.0 to > 5.0 mg l^{-1}). Pyraziflumid had the largest variation in values ranging between extremely sensitive to less sensitive (EC_{50}: < 0.1 to > 5.0 mg l^{-1}). In the greenhouse experiment, prothioconazole exhibited significantly greater control of stem and root disease compared to other fungicides, while PCNB was the least effective. In field experiments, there was no statistical significance in stand counts, cover grid, root severity and yield for any treatment. Penthiopyrad and prothioconazole were the most effective on stem disease.

Introduction

Rhizoctonia solani spp. can cause severe root rots (RRR) in common beans resulting in 52% (Naseri 2008) up to a 100% (Schwartz et al. 2005) yield loss through pre-emergence damping off or stunting and premature plant senescence (Oladzad et al. 2019). *Rhizoctonia* spp. are also associated with web blight, stem cankers and rots (Harveson et al. 2021).

Strategies to manage *Rhizoctonia* spp. in common beans include cultural and chemical methods. Cultural control can include planting in warm soil, rotation with nonhost crops, shallow seeding and

reduced compaction of soil. *Rhizoctonia* spp. capable of causing damping off have been associated with lower soil temperatures and excessive soil moisture, thus planting in 20 °C moist soils may help reduce disease as well as suitable crop rotation strategies (Hagadorn 2005). Decreasing the emergence time of bean seedlings, through shallow seeding, can reduce the infection of *Rhizoctonia* root rot spp. (Manning et al. 1967; Leach and Garber 1970). Reduction of soil compaction can reduce stress to the plant though compounded hypocotyl rot and stem cankers that result in a reduced root development (Harveson et al. 2005). Research into sustainable management, such as resistant cultivars and biological control, has been limited. Recent research found reduced disease severity of *R. solani* AG 4 HG-I with the combination of rhizobacteria, *Bacillus amyloliquefaciens*, with metalaxyl and fludioxonil as a seed treatment (Martins et al. 2018). New insight and research into sustainable management will benefit growers in the future, though until these management strategies become commercially available, *Rhizoctonia* pathogens have been limited to chemical fungicides in furrow and as seed treatments (Martins et al. 2018).

Common in-furrow treatments in the PNW include azoxystrobin (PNW Handbook 2021). This fungicide is a Quinone outside inhibitors (QoI) class of fungicide that inhibit ATP production through restricting the transfer of electrons from cytochrome b to cytochrome c by binding to the quinone oxidizing (Qo) site of cytochrome bc1 complex I (Balba 2007; Gisi et al. 2002). Prominent fungicides used for seed treatment in North Dakota are mefenoxam and fludioxonil (Tvedt 2017), while seed treatments used in the Pacific Northwest (PNW) are captan, fludioxonil, fluxapyroxad, pyraclostrobin, metalaxy, thiamethoxam and sedaxane (PNW Hadbook 2021). Fluxapyroxad and sedexane are a succinate-dehydrogenase inhibitor (SDHI) that alters the respiratory chain involved in ATP generation (Avenot and Michailides 2010). Due to the longevity of seed treatments, this method is generally preferred over In-furrow treatment (Rideout 2002; Tvedt 2017).

Rhizoctonia isolates are showing increased insensitivity to common fungicides (Olaya et al. 2013). The sensitivity of individual AGs of *Rhizoctonia* spp. to several fungicides can vary (Kataria and Gisi 1999). This is insensitivity and should be considered separate phenomena to the development of resistance. Repeated applications of the same fungicide and selection pressure results in resistance. Fungicides such as triadimenol, propiconazole, carboxin, furmecyclox, and the pyrimidine fungicides, fenarimol and nuarimol have shown variation in inherent sensitivity to different AGs (Kataria and Gisi 1999). The existence of such fungicide selectivity has led to the speculation that their continued use may force a change in the pathogen population towards insensitive AGs.

The aim of the study is to find out which fungicides are most effective for managing Rhizoctonia, particularly in common beans. This was done through a combination of field and greenhouse experiments for common beans using an isolate of AG 11. This isolate was chosen due to yield reduction in previous studies. On a wider range of isolates, including a representative of all AGs on various hosts, we determined the EC_{50} for several fungicides available for disease management in beans.

Material and Methods

Isolates

Isolates were obtained from the culture collection at Parma Research and Extension Center. Isolates from diagnostic activities, as well from cereal, bean and potato surveys were used. Long term, storage was on potato dextrose agar (PDA) slopes amended with streptomycin (0.8 g/liter) and penicillin (0.2 g/liter) at room temperature. Isolates were sub-cultured from slopes on to PDA plates amended with streptomycin (0.8 g/liter) and penicillin (0.2 g/liter) at room temperature. Isolate identity was confirmed through real-time PCR and/or ITS sequencing, in addition to a visual check for colony morphology.

The AG 11 isolate used in both the greenhouse and field experiments originated from the 2019-2020 bean survey from a 2018 bean plant in Southwest Idaho. Isolate numbered as DB27.

Isolates were chosen for EC_{50} testing on the basis of ensuring a suitable range of AGs and isolate-host combinations. Isolates, AGs, host range and region isolate were obtained from are presented in Table 4.1. *R. solani* isolates (*n* = 13) consisted of two AG 2-1 isolates, two AG 2-2 isolates, two AG 3 isolates, one AG 4 HG-I isolate, one AG 4 HG-II isolate, one AG 4 HG-III isolate, one AG 5 isolate and three AG 11 isolates. AG 3 isolate C105 was contaminated prior to the EC_{50} experiment on prothioconazole, flutolanil and pyraziflumid, in which isolate D20-336 was used in place of. BNR isolates (*n* = 9) consisted of two AG A isolates, one AG C isolate, one AG D isolate, one AG E isolate, one AG H isolate, one AG F isolate, one AG K isolate and one AG W isolate. One *Waitea circinata* var. c*ircinata* was used. A total of twenty-two isolates were used.

Table 4.1 List of isolates used in EC50 experiments.

Isolate #	AG	Host	Region
D20-49	AG 2-1	*Humulus lupulus*	SW
C172	AG 2-1	*Triticum aestivum*	SW
DB54	AG 2-2	*Phaseolus vulgaris* L.	SW
DB55	AG 2-2	*Phaseolus vulgaris* L.	SW
C105	AG 3	*Triticum aestivum*	SW
D20-336	AG 3	*Avena sativa*	SE
DB208	AG 4 HG-I	*Phaseolus vulgaris* L.	SW
C209	AG 4 HG-II	*Triticum aestivum*	E
DB179	AG 4 HG-III	*Phaseolus vulgaris* L.	SC
C184	AG 5	*Triticum aestivum*	SW
DB28	AG 11	*Phaseolus vulgaris* L.	SW
C65	AG 11	*Triticum aestivum*	E
C203	AG 11	*Triticum aestivum*	E
DB107	AG A	*Phaseolus vulgaris* L.	SC
D20-407	AG A	*Echeveria elegans*	SW
C309	AG C	*Triticum aestivum*	N
C186	AG D	*Triticum aestivum*	SW
C249	AG E	*Triticum aestivum*	N
C271	AG H	*Triticum aestivum*	N
DB87	AG F	*Phaseolus vulgaris* L.	SW
DB189	AG K	*Phaseolus vulgaris* L.	SC
P150	AG W	*Solanum tuberosum*	SC
C325	*Waitea circinata* var. *circinata*	*Triticum aestivum*	N

Cereal survey isolates (C#), bean survey isolates (DB#), potato isolates (P#) and diagnostic isolates (D#). Region Eastern (E), Southcentral (SC), Southwestern (SW) and Northern (N).

Fungicides Used in Laboratory, Greenhouse and Field Experiments

Fungicides were supplied by the relevant chemical company and stored at room temperature until used. Fungicides were no more than 9 months old when used in experiments. Eight fungicides: pentachloronitrobenzene (PCNB), penthiopyrad, prothioconazole, azoxystrobin, inpyrfluxam, sedaxane, pyraziflumid and flutolanil were used in either the field and greenhouse experiment, the fungicide EC_{50} sensitivity experiment or all experiments. Details of fungicides for each experiment is given in Table 4.2.

Table 4.2 Details of fungicides used in the lab, greenhouse and field experiments.

Proprietary name	Active ingredient (Abbreviation)	Target Site of Action	Group Name	FRAC Code	EC_{50}	Greenhouse	Field
Blocker 4-F	Pentachloronitrobenzene (PCNB)	lipid peroxidation (proposed)	AH	14	-	x	x
Fontelis	Penthiopyrad (Pent)	complex II in fungal respiration (succinate-dehydrogenase)	SDHI	7	x	x	x
Proline	Prothioconazole (Pro)	C14-demethylation in sterol biosynthesis	DMI	3	x	x	x
Quadris	Azoxystrobin (Azo)	complex III of fungal respiration: ubiquinol oxidase, Qo site	Qol	11	x	x	x
Excalia	Inpyrfluxam (Inpy)	complex II in fungal respiration (succinate-dehydrogenase)	SDHI	7	X	-	-
Vibrance	Sedaxane (Sed)	complex II in fungal respiration (succinate-dehydrogenase)	SDHI	7	X	-	-
NA	Pyraziflumid (Pyra)	complex II in fungal respiration (succinate-dehydrogenase)	SDHI	7	X	-	-
Moncut	Flutolanil (Flut)	complex II in fungal respiration (succinate-dehydrogenase)	SDHI	7	X	-	-

Modified from Fungicide Resistance Active Committee. Fungicides listed by proprietary name, active ingredient, mode of action, Fungicide Resistance Active Committee (FRAC) code. Mode of action Aromatic Hydrocarbons (AH), succinate-dehydrogenase inhibitor (SDHI), DeMethylation Inhibitors (DMI), Quinone outside Inhibitors (Qol)

***In vitro* Determination of Fungicide EC_{50} of *Rhizoctonia* Isolates**

Assays were based on the poisoned food technique (Dhingra and Sinclair, 1995). PDA was the media used for all EC 50 assays. After autoclaving, media was allowed to equilibrate to 45°C for one hour in a water bath before amending with the appropriate fungicide dilution. Each fungicide was diluted to a 40,000 mg l^{-1} stock solution with sterile distilled water (SDW). From stock solution dilutions, concentrations of 100, 10, 1, 0.1, 0.01, 0.001 mg l^{-} were poured into separate 90 mm diameter petri-dishes. Control plates contained no amendments. For azoxystrobin, salicylhydroxamic acid (SHAM, Sigma-Aldrich, St. Louis, MO) at 100 mg l^{-1} was added to block the use of an alternative pathway for cellular respiration (Amiri et al. 2010). Dilution of SHAM was in methanol.

Plates were poured in a biosafety cabinet. Plates were allowed to solidify for an hour prior to placement of isolate plug. A 6mm cork-borer plug from 6-day old isolates was placed hyphal side down on each dilution plate and placed in 1 mil polyethylene bag that was lightly closed to allow some ventilation. After four days incubation at 21 ° C, colony diameter was recorded over two perpendicular axes.

For each fungicide-isolate concentration, the percent inhibition of growth was compared to the control using the formula: % Inhibition = 100 – ((T/C) x 100)), with T = the mean treatment measurement and C = the mean control measurement. For each isolate, the average percent inhibition was plotted against the logarithm of the fungicide concentration and a linear regression equation was determined. For all assays, isolates were considered 'extremely sensitive' if the EC_{50} values were less than 0.1 mg l^{-1}, 'sensitive' if values ranged between 0.1 to 1 mg l^{-1}, 'intermediately sensitive' with values over 1 mg l^{-1} to 5 mg l^{-1} and isolates were considered 'less sensitive' if the EC_{50} values exceeded 5 mg l^{-1} (Martin et al. 1984).

Greenhouse Experiment

To test the effectiveness of the fungicides on *R. solani* AG 11 isolate, a glasshouse experiment was setup under 16hr light and 8hr dark cycle conditions at an average of 18 °C. 1 g cornmeal to 113 g sand was placed in sterilizable spawn bags (Fungi Perfecti) and autoclaved twice (24-hour interval between autoclaves) prior to planting. The sand/cornmeal mixture was added to Ferti·lome Ultimate Potting Mix (Ferti·lome, Bonham, TX), along with *R. solani* AG 11 isolates. Mixture for each pot was 1,350 cc Ferti·lome Ultimate Potting Mix, 150 cc cornmeal: sand and one ten-day old AG 11 culture (1

petri dish). The mixture was homogenized in a sterilized cement mixer prior to placement in 16 cm^2 plastic pots. Eight pinto bean seeds, cv Windbreaker, were planted per pot. All in-furrow treatments were applied prior to covering the bean seeds. Treatments were applied as 7.14 g active ingredient per .40 hectare in a 100 ml whole pot drench. Azoxystrobin foliar treatment was applied as 7.14 g active ingredient per .40 hectare using a spray bottle. Control plants were planted in amended soil mixture with no fungicide treatment. Plants were harvested 4 weeks post-inoculation, washed and visually assessed for root and stem symptoms. Root disease was assessed on the percentage of disease present on the whole root structure for root infected area (% RIA). Stem severity index (SSI) were assessed on a scale adapted from Woodhall et al. (2008). 0 to 4, 0 = healthy/no lesion, 1 = any lesion, 2 = lesion above 5 mm, 3 = lesion above 10 mm, 4 = girdled lesion. The number of plants per stem severity were recorded.

Field Experiment

Prior to planting, a 1 g cornmeal: 113 g sand ratio was weighed out to 2.3 kg with 2 ml water added. The cornmeal/sand was placed in a sterilizable spawn bags, autoclaved twice (24-hour interval between autoclaves) and allowed to cool for 24 hours prior to inoculation. Five ten-day old AG 11 culture (petri dish) were cut into cm^2 piece prior to being placed in each bag. Inoculation was performed in a biosafety hood. Inoculum incubated for 14 days before being placed in the field to ensure a fully colonized cornmeal/sand inoculum.

Pinto bean, cv Windbreaker, were planted with air seed planter (John Deere, Moline, IL) using a 13.97 cm seed spacing to a depth of 4.4 cm. The middle two press wheels of the planter were disengaged during plot planting, allowing 1 kg AG-11 cornmeal/sand inoculum to be spread by hand directly into individual open seed furrows. In-furrow at-plant fungicide applications were made with a hand-held CO_2 boom sprayer held a few inches above the open furrows, which were closed immediately afterwards by dragging a chain across the length of the rows. Plots were 3.4 x 7.6 m consisting of six rows 55.9 cm. apart with a 1.5 m alleyway between plots. Each treatment was replicated in five plots. Plots were arranged in a randomized complete-block design. Plots were surface irrigated for a 24-hour set at 7 to 10-day intervals.

A foliar treatment of azoxystrobin was applied at the 6-8 leaf stage at a pressure of 21 psi and a rate of 56.8 L per hectare. Plots were sprayed using a four-row hand-held CO_2 sprayer with one TeeJet XR110.02-VS nozzle directly over each row. Treatments were applied at a.

Stand counts were performed every 7 days for a month. Stand counts are the mean number of plants emerged per 7.6 m row for each treatment. Foliar cover grids were performed every 7 days starting 4 weeks post planting until the end of the growing season. Foliar cover grids are the percentage of foliar cover within a 3.0 m^2 grid centered in the center of the inoculated rows. After 6 weeks post planting, sixteen plants from each plot were taken at random from the middle-inoculated rows and assessed for disease. Plants were washed in tap water to remove soil and debris. Plants were visually assessed for root and stem disease symptoms as described above. Plants were harvested 4.5 months after planting. The harvested area measured yield from the center two rows of each 3.4 x 7.6 m plot.

Statistical analysis

In vitro fungicide EC_{50} data was analyzed using mean formula in Excel (Microsoft 2021). The stem severity from the greenhouse experiment data was analyzed with ARM software (ARM 2020.5 software) that performed automatic rank transformation before using ANOVA to produce Kruskal-Wallis X2 for means comparison (P=0.01). All field experiment and the root severity from the greenhouse experiment data was analyzed using ANOVA (P=0.05) and Fisher's LSD at P=0.05 (ARM 2020.5 software).

Results

In vitro Determination of Fungicide EC_{50} of *Rhizoctonia* Isolates

Variation in sensitivity to eight fungicides was observed between twenty-three isolates (Table 4.3). Azoxystrobin (Azo) showed sensitivity variations between isolates with values between 'sensitive', 'intermediately sensitive', and 'less sensitive'. One AG 2-1, AG C, AG D, AG E AG F and AG W isolates had sensitive EC_{50} values. One AG 2-1, two AG 11 and AG K had 'immediately sensitive' values. AG 2-2, AG 3, AG 4 subgroups, AG 5, one AG 11, AG A, AG H and *Waitea circinata* var. *circinata* isolates had 'less sensitive' values.

Inpyrfluxam (Inpy) showed no sensitivity variation between isolates. All AGs had an 'extremely sensitive' EC_{50} value. Penthiopyrad (Pent) had sensitivity variation between isolates with values between 'extremely sensitive', 'sensitive' and 'intermediately sensitive'. AG 2-1, AG 3, AG D, AG H and *Waitea circinata* var. *circinata* isolates had 'extremely sensitive' values. AG 2-2, all AG 4 subgroups, AG 5, AG 11, AG A, AG C, AG E and AG W isolates were considered 'sensitive' based on the values of Martin et al (1984) with AG F and AG K determined to be 'intermediately sensitive'.

Sedaxane (Sed) showed sensitivity variation between the isolates with values between ‘extremely sensitive’, ‘sensitive’ and ‘intermediately sensitive’. AG 2-1, AG 2-2, AG 3, AG 4 HG-II, AG 5, one AG 11, AG A, AG H, AG W and *Waitea circinata* var. *circinata* isolates had ‘extremely sensitive’ values. AG 4 HG-I, AG 4 HG-III, one AG 11, AG C, AG D, AG E, AG F and AG K isolates had ‘sensitive’ values. One AG 11 had an ‘intermediately sensitive’ value.

Prothioconazole (Pro) showed sensitivity variation between the isolates with values between ‘sensitive’, ‘intermediately sensitive’ and ‘less sensitive’. One AG 2-1, AG 3, one AG 11, AG C and AG D isolates had ‘sensitive’ values. One AG 2-2, AG 4 HG-I, one AG 11, AG F, AG H and AG W isolates had ‘intermediately sensitive’ values. One AG 2-1, one AG 2-2, AG 4 HG-II, AG 4 HG-III, AG 5, one AG 11, AG A, AG E, AG K and *Waitea circinata* var. *circinata* isolates had ‘less sensitive’ values.

Flutolanil (Flut) showed sensitivity variation between the isolates with values between ‘extremely sensitive’, and ‘sensitive’. One AG 2-1 and AG H had extremely sensitive values. One AG 2-1, AG 2-2, AG 3, AG 4 subgroups, AG 5, AG 11, AG A, AG C, AG D, AG E, AG F, AG K and *Waitea circinata* var. *circinata* isolates had ‘sensitive’ values.

Pyraziflumid (Pyra) showed sensitivity variation between the isolates with values between ‘extremely sensitive’, ‘sensitive’, ‘intermediately sensitive’ and ‘less sensitive’. AG D had an ‘extremely sensitive’ value. AG 2-1, one AG 2-2, AG 3, AG 4 HG-I, AG 4 HG-II, AG 11, one AG A, AG E, AG H, AG K and AG W isolates had ‘sensitive’ values. One AG 2-2, AG 4 HG-III, AG 5, one AG A, AG F and *Waitea circinata* var. *circinata* isolates had ‘intermediately sensitive’ values. The AG C isolate had a ‘less sensitive’ value.

Table 4.3 Sensitivity of *Rhizoctonia* spp. isolates to different fungicides. Shown are mean EC_{50} values.

AG	Isolate	Azo	Inpy	Pent	Sed	Pro	Flut	Pyra
AG 2-1	C172	1.06	0.004	0.01	0.08	0.93	0.09	0.11
AG 2-1	D20-49	0.84	0.007	0.02	0.01	9.47	0.10	0.11
AG 2-2	DB54	8.93	<0.001	0.26	0.08	13.07	0.66	2.57
AG 2-2	DB55	>100	0.008	0.40	0.08	2.83	0.87	0.87
AG 3	C105	97.63	0.007	0.08	0.02	-	-	-
AG 3	D20-336	-	-	-	-	0.42	0.17	0.69
AG 4 HG-I	DB208	>100	0.010	0.75	0.66	3.82	0.22	0.72
AG 4 HG-II	C209	>100	0.008	0.16	0.08	>100	0.44	0.84
AG 4 HG-III	DB179	>100	0.040	0.26	0.50	9.47	0.85	2.97
AG 5	C184	>100	0.008	0.22	0.08	49.10	0.38	1.02
AG 11	C65	1.53	0.047	0.39	1.01	15.12	0.28	0.84
AG 11	C203	11.43	0.001	0.35	0.07	0.97	0.16	0.25
AG 11	DB28	1.03	0.005	0.49	0.22	1.22	0.43	1.00
AG A	DB107	>100	0.010	0.75	0.02	96.24	0.56	1.31
AG A	D20-407	>100	0.005	0.32	0.09	>100	0.31	0.98
AG C	C309	1.00	0.040	0.78	0.58	0.57	0.58	8.45
AG D	C186	0.12	0.008	0.09	0.16	0.81	0.13	0.08
AG E	C249	0.76	0.010	0.75	0.21	7.68	0.35	0.44
AG F	DB87	0.76	0.010	1.94	0.42	1.26	1.00	2.80
AG H	C271	77.61	0.004	0.08	0.02	2.35	0.05	0.11
AG K	DB189	2.04	0.009	1.05	0.10	87.82	0.15	0.27
AG W	P150	0.26	0.006	0.15	0.08	4.29	0.10	0.30
Waitea circinata var. *circinata*	20GH5 A	>100	0.010	0.05	0.09	40.24	0.57	3.97

The percent inhibition of growth = 100 - ((T/C) x 100)), with T = the mean treatment measurement and C = the mean control measurement. For each isolate, the average percent inhibition was plotted against the logarithm of the fungicide concentration and a linear regression equation was determined. For all assays, isolates were considered sensitive if the EC_{50} values were less than 0.1 mg l^{-1}, isolates were considered intermediately sensitive with EC_{50} values ranging between 0.1 and 1 mg l^{-1} and isolates were considered less sensitive if the EC_{50} values exceeded 5 mg l^{-1}.

Greenhouse Experiment

A majority of the fungicides were effective at reducing root and stem disease on bean plants against an AG 11 isolate, with the exception of PCNB and penthiopyrad in stem disease (Table 4.4).

Table 4.4 Root and stem severity means of Pinto beans (cv Windbreaker) planted in soil artificially inoculated with AG 11 and treated with different fungicides for the Greenhouse Experiment.

Treatment	Root Severity (mean %)	Stem Severity (Mean Scale)
Control	70.0a	3.5a
Pentachloronitrobenzene	57.7ab	3.1a
Penthiopyrad	41.8bc	3.1ab
Prothioconazole 480 SC	27.9c	2.0d
Azoxystrobin foliar	49.6b	2.8bc
Azoxystrobin in-furrow	40.2bc	2.7c
P=	0.0001	< 0.01
LSD P=.05	19.85	0.4r
CV (%)	31.39	–
Kruskal-Wallis	–	28.993

Column numbers followed by the same letter are not significantly different at P=0.05 as determined by Fisher's LSD test. Mean stem disease severity, key scale of 0 – 4. Mean root severity, 0 – 100% necrosis of roots.

The prothioconazole (Pro) treatment was the most effective at reducing root severity with a % RIA mean of 27.9% (Figure 1). The azoxystrobin (Azo) in-furrow treatment had the second most effective results with a %RIA mean of 40.2%. The penthiopyrad (Pent) treatment had a RS mean of 41.8%. The azoxystrobin foliar treatment had a %RIA mean of 49.6%. PCNB treatment had a RS mean of 57.7%. The control treatment had a %RIA of 70%.

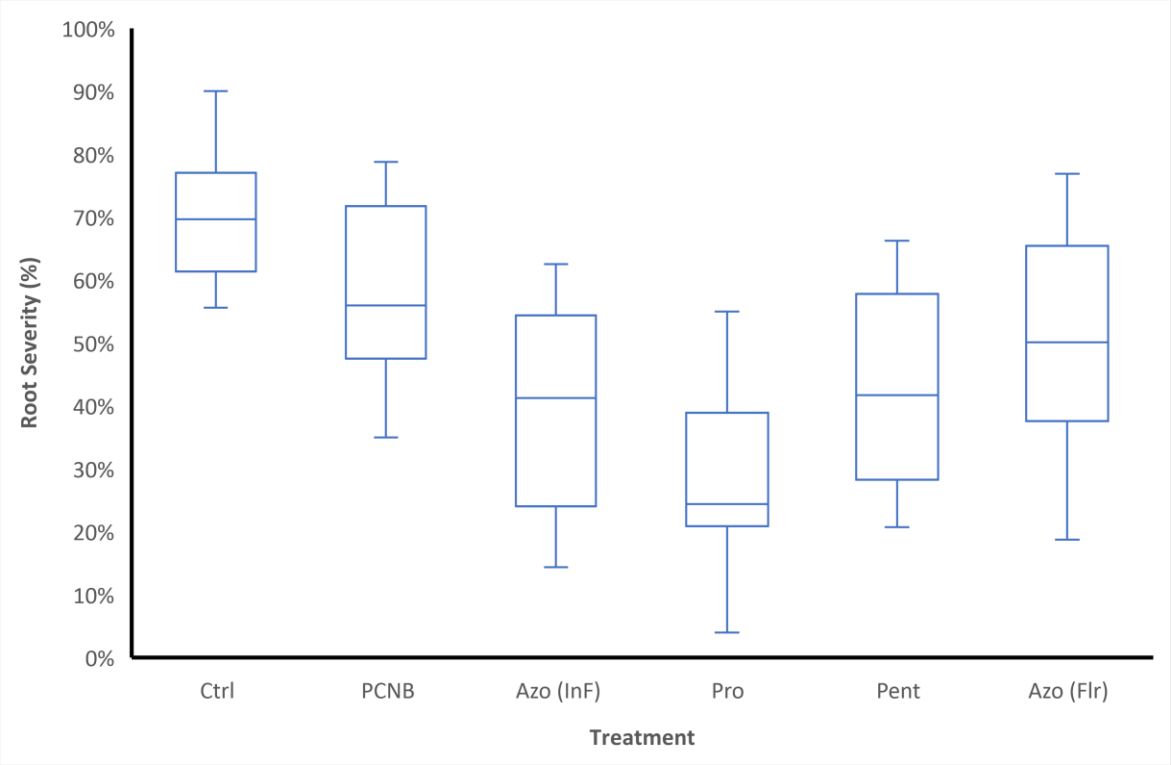

Figure 4.1 Mean percentage root infected area (RIA), 0 – 100% necrosis of roots for Greenhouse Experiment. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual treatment. Line in the box represents the median of the mean averages within a treatment. In furrow treatment (InF). Foliar treatment (Flr).

The prothioconazole treatment was the most effective at reducing stem severity with an SSI mean of 2.0 (Figure 4.2). The azoxystrobin in-furrow treatment had the second most effective result with an SSI mean of 2.7. The azoxystrobin foliar treatment had an SSI mean of 2.8 with one outlier of 1.3. The penthiopyrad treatment had SSI mean of 3.1. PCNB treatment had an SSI mean of 3.1. The control treatment had an SSI mean of 3.5.

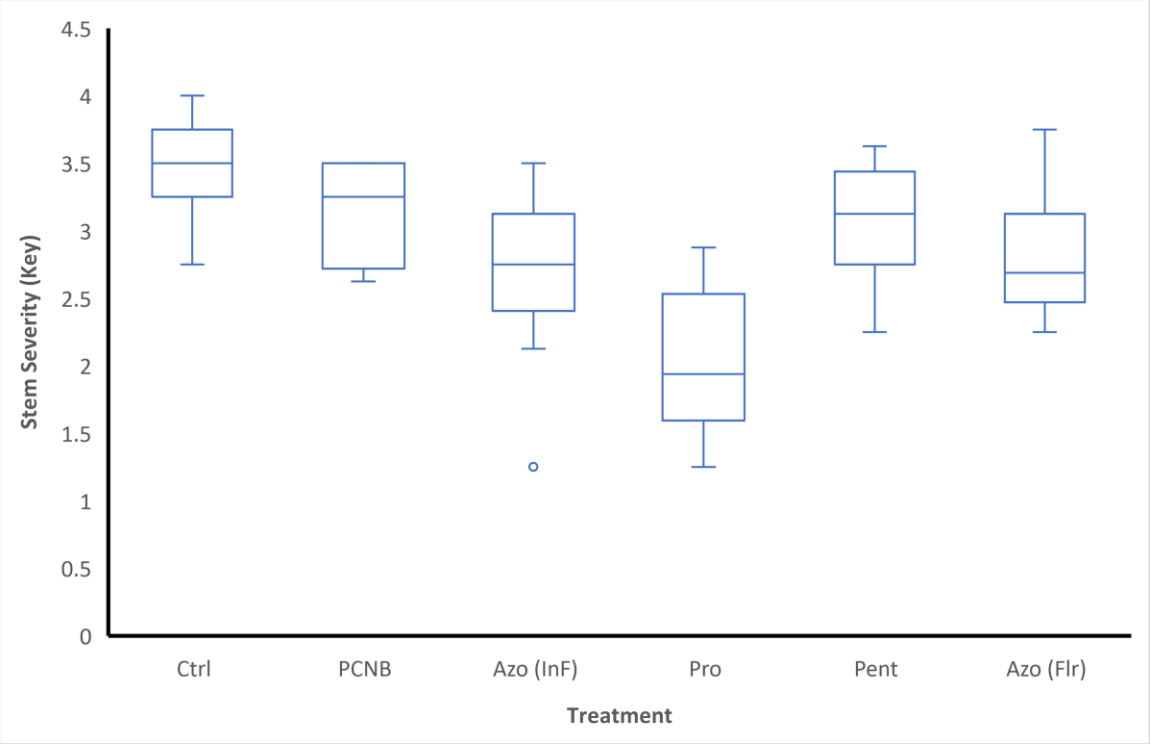

Figure 4.2 Mean stem severity index (SSI), 0 – 4 key scale for Greenhouse Experiment. Whisker points represent the highest (top whisker) and lowest (bottom whisker) points with the individual treatment. Line in the box represents the median of the mean averages within a treatment. Circles outside of the whisker points represent outlier mean data. In furrow treatment (InF). Foliar treatment (Flr).

Field Experiment

None of the five fungicides improved stand count compared to the non-treated control (Table 4.5). Likewise, mean percentage cover grid counts were not statistically significant (LSD P=0.05) compared to the non-treated control (Table 4.5).

SSI means were statistically significant compared to the non-treated control for penthiopyrad, prothioconazole and azoxystrobin foliar treatment with a mean of 2.4 (LSD P= 0.5). The PCNB and the azoxystrobin in furrow treatment were not statistically significant against the non-treated control (Table 4.5).

RIA means of five fungicides were not statistically significant (LSD P=0.05) compared to the non-treated control. Likewise, yields were not affected by fungicide treatments (Table 4.5)

Table 4.5 Stand count, cover grid, root severity, stem severity and yield means of Pinto beans (cv Windbreaker) planted in soil artificially inoculated with AG 11 and treated with different fungicides for the field experiment

Treatment	Stand Count (mean)	Cover Grid (%)	Stem Severity (0-4)	Root Severity (%)	Yield (g/ha)
Untreated control	79.8	55.5	2.7 a	56.5	2154.6
Pentachloronitrobenzene 4 pt/a IFAP	82.2	57.1	2.6 ab	63.3	1929.3
Penthiopyrad 30 oz/a IFAP	80.7	56.1	2.1 c	53.1	2186.9
Prothioconazole 480 SC 5 oz/a IFAP	78.6	55.5	2.2 c	53.1	1936
Azoxystrobin 15.5 oz/a IFAP	81.3	56.5	2.5 abc	58.8	2000.6
Azoxystrobin 15.5 oz/a foliar	78.9	55.5	2.4 bc	50.5	2331.3
P=	0.66	0.9	0.02	0.18	0.47
LSD	3.22	3.5	0.34	10.55	488.4
CV (%)	7.43	4.7	10.69	14.31	17.72

Column numbers followed by the same letter are not significantly different at P=0.05 as determined by Fisher's LSD test. Stand counts mean number of plants emerged per 7.62 m row, foliar cover grids % incidence cover grid, stem disease severity, root disease severity, yield in pounds by acre. In furrow application (IFAP).

Discussion

Management of *Rhizoctonia* pathogens in common bean crops utilize cultural and chemical methods. In the present study, five fungicides were used in field and greenhouse experiments to evaluate their efficacy to control on *Rhizoctonia solani* AG 11. Additionally, eight fungicides were used in an *in vitro* EC_{50} experiment to evaluate their efficacy to control of *Rhizoctonia* spp. obtained from various Idaho crops.

In vitro fungicide sensitivity to the selected fungicides on the various *Rhizoctonia* AGS showed a majority of the AGs were 'extremely sensitive' to 'sensitive', while majority of the AGs were 'less sensitive' to the azoxystrobin and prothioconazole fungicides. Inpyrfluxam proved to be the most efficient with all AGs and the *Waitea circinata* var. *circinata* isolates having 'extremely sensitive' values. Penthiopyrad, sedaxane and flutolanil showed 'extremely' to 'intermediate' sensitivity values

for all AGs and the *Waitea circinata* var. *circinata* isolate. Campion *et al.* (2003) also observed flutolanil causing low EC_{50} values in AG 2-1, AG 3 and AG 5. Sedaxane has been observed causing similar EC_{50} values in AG 2-2. AG 3, AG 4 and AG 11 (Ajayi-Oyetunde et al 2017).

A majority of AGs were 'sensitive' to Pyraziflumid with values and one isolate, AG C, had a 'less sensitive' value. Interestingly, one AG 2-2 isolate had a 'sensitive value', while the second AG 2-2 isolate had an 'intermediately sensitive' value. This could be due to the different subgroups of the isolates used. Phylogenetic placement of DB54, which is represented by exemplar DB74, placed in the AG 2-2 IIIB clade and DB55, placed it in the AG 2-2 clade, in Chapter 3 (Figure 3.1). One recent study showed *R. solani* (AG 2-1) was sensitive to pyraziflumid (Kikutake et al. 2020), though there is no current research on the efficacy of pyraziflumid on AG 2-2 due to the recent launch of the product in Japan in 2018 (Nihon Nohyaku Co., Ltd.). Current research on other SDHI fungicides, such as sedaxane, used on AG 2-2 IIIB on soybeans (*Glycine max*) and sugar beets shows a high sensitivity to these fungicides (Ajayi-Oyetunde et al 2017; Sharma et al. 2021), which is consistent with the findings in this study. These isolates also showed a large variance in sensitivity to azoxystrobin and prothioconazole. Azoxystrobin has been shown to cause variation in sensitivity of AG 2-2 isolates, indicating the possibility of an additional mechanism of alternative oxidation becoming active over time (LaMondia 2012; Arabiat and Khan 2016; Sharma et al. 2021). Prothioconazole has been shown to cause variation in sensitivity within this AG as well (Ajayi-Oyetunde et al 2017). Why these fungicides effect one AG 2-2 isolate more than other isolates should be further researched given the high production of common beans and sugar beets in the Southwest region of Idaho.

AG 11 has been described as nonpathogenic on common beans in Turkey (Eken and Demirci 2004). These findings are not consistent with the findings in this study or the observed stem rot on common beans caused by AG 11 in Idaho (Woodhall et al. 2020). No fungicide treatments effectively reduced root disease in the field experiment and only three treatments were effective on stem disease compared to the control treatment. However, the yield g/ha was not significantly different between any treatments. Four of the fungicide treatments in the greenhouse experiment were effective in controlling AG 11 root disease infection and only three treatments were effective at controlling stem disease compared to the control. PCNB did not effectively control root or stem disease in the field or greenhouse. Penthiopyrad did not control stem disease in either experiment or *in vitro* EC_{50} sensitivity showed values of 'sensitive' to 'intermediately sensitive'. AG 11 isolates also showed

variable sensitivity values with azoxystrobin and prothioconazole. A current study by Ajayi-Oyetunde et al. (2017) showed variation in soybean AG 11 isolate Ec_{50} values for prothioconazole, although no information is available for azoyxystrobin.

In summary, fungicides azoxystrobin and prothioconazole had minimal efficacy on the *Rhizoctonia* spp. in the EC_{50} fungicide sensitivity experiment this study. Inpyrfluxam proved to be the most efficient at reducing growth of the isolates in the fungicide sensitivity experiment, with all isolates having values of 'extremely sensitive'. While inpyrfluxam proved to be the most efficient at reducing growth, this product is also newly registered and there has been no previous exposure to the isolates. Since there was variance in the field and greenhouse values between the fungicides, inpyrfluxam's efficacy should be further evaluated to validate its efficacy under field conditions. Research will continue the 2021 growing season with the field experiment being repeated.

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Appendix 1

Appendix 1.1 Complete wheat and barley isolate list by symptom, region collected, crop, AG, unique Isolate and used in pathogenicity experiment

Isolate C#	Symptom	Region	Crop	Pathogen	Unique Cultures	Used in Pathogenicity Trial
71	Stem	N	WW	AG 2-1	X	
76	Stem	N	WW		X	
172	Roots	SW	WW		X	X
173	Roots	SW	WW			
174	Roots	SW	WW			
257	Stem	N	WW			X
324	Stem	E	Sp. B		X	
375	Stem	N	WW		X	
379	Roots	N	WW		X	
381	Roots	SW	WW			
11	Stem	SW	WW	AG-2-2	X	
101	Stem	SW	WW		X	X
78	Stem	E	Sp. B	AG 3	X	
102	Stem	SW	WW			
103	Stem	SW	WW			
104	Stem	SW	WW			
105	Stem	SW	WW		X	X
278	Stem	N	WW		X	X
353	Stem	N	WW			
D1	Stem	E	WB	AG 4 HG-II	X	
37	Stem	SW	WW		X	X
106	Stem	SW	WW			
111	Stem	SW	WW		X	
127	Stem	SW	WW			
128	Stem	SW	WW			
129	Stem	SW	WW			
193	Stem	SW	WW		X	X
204	Roots	E	Sp. W		X	
208	Stem	E	WW		X	
209	Stem	E	WW			X
213	Stem	E	WW		X	
214	Stem	E	WW		X	
261	Stem	SC	Sp. W		X	X
262	Stem	SC	Sp. W			
263	Stem	SC	Sp. W			
264	Stem	SC	Sp. W			
265	Stem	SC	Sp. W			

319	Roots	SC	Sp. W		X	X
320	Roots	SC	Sp. W			
321	Stem	SC	Sp. W		X	
322	Stem	SC	Sp. W			
331	Stem	E	Sp. B		X	X
332	Stem	E	Sp. B			
333	Stem	E	Sp. B		X	
334	Stem	E	Sp. B			
335	Roots	E	Sp. B		X	
336	Stem	E	Sp. B			
337	Stem	E	Sp. B			
338	Stem	E	Sp. B			
339	Stem	E	Sp. B			
343	Stem	E	Sp. W			X
344	Stem	E	Sp. W		X	
349	Stem	SC	Sp. B		X	X
350	Stem	SC	Sp. B		X	X
351	Roots	SC	Sp. B		X	
377	Stem	E	WW			
382	Stem	SW	WW		X	
79	Stem	SW	WW	AG 5	X	
81	Stem	SW	WW			
184	Stem	SW	WW		X	X
189	Roots	SW	WW			X
201	Stem	SW	WW			
323	Stem	SC	Sp. W		X	
65	Stem	E	WW	AG 11	X	X
66	Stem	E	WW			
80	Stem	SW	WW			
82	Stem	SW	WW		X	
85	Stem	SW	WW		X	
202	Stem	E	Sp. W		X	
203	Stem	E	Sp. W			X
288	Stem	N	WW	AG C		X
309	Stem	N	WW		X	
32	Stem	E	WW	AG D	X	X
54	Stem	E	WW			X
55	Stem	E	WW		X	
56	Stem	E	WW			
57	Stem	E	WW		X	
58	Stem	E	WW			
60	Stem	E	WW			
62	Stem	E	WW		X	X

63	Stem	E	WW				
94	Stem	E	WW				
95	Stem	E	WW				
124	Stem	E	WW		X		
160	Stem	SW	WW		X		
170	Stem	SW	WW		X		
171	Stem	SW	WW		X		X
175	Stem	SW	WW				
176	Stem	SW	WW				
177	Stem	SW	WW				X
178	Stem	SW	WW				
179	Stem	SW	WW				
180	Stem	SW	WW				X
181	Stem	SC	WB				X
182	Stem	SC	WB				
183	Stem	SC	WB				
185	Stem	SW	WW		X		
186	Stem	SW	WW				X
187	Stem	SW	WW		X		
188	Stem	SW	WW				
205	Stem	SC	WB		X		
206	Stem	SC	WB				
207	Stem	SW	WW				
210	Stem	E	WW				
211	Stem	E	WW		X		X
212	Stem	E	WW				
215	Stem	E	WW		X		
216	Stem	E	WW				
217	Stem	E	WW		X		
218	Stem	E	WW				
219	Stem	E	WW				
220	Stem	E	WW				
221	Stem	E	WW				
227	Stem	E	WW		X		
228	Stem	E	WW				
229	Stem	E	WW				
230	Stem	E	WW				
231	Stem	E	WW				X
232	Stem	E	WW				
233	Stem	E	WW		X		X
234	Stem	E	WW				
235	Stem	E	WW				
236	Stem	E	WW				

237	Stem	E	WW				
238	Roots	E	WW		X		
239	Stem	E	WW				
240	Stem	E	WW				
241	Roots	E	WW				
242	Stem	E	WW				X
243	Stem	E	WW				
244	Stem	E	WW				
245	Roots	E	WW				
248	Stem	E	WW				
250	Stem	N	WW		X		X
251	Stem	N	WW				X
258	Stem	N	WW		X		X
259	Stem	N	WW				X
284	Stem	N	WW				
307	Stem	N	WW		X		
378	Stem	E	WW				
249	Stem	N	WW	AG E	X		X
376	Stem	N	WW				
77	Stem	N	WW	AG H	X		
114	Stem	N	WW				
266	Stem	N	WW				
271	Stem	N	WW		X		X
272	Stem	N	WW				
280	Stem	N	WW		X		X
310	Stem	N	WW				
312	Stem	N	WW				
383	Stem	N	WW				
19	Stem	SW	WW	AG K	X		
36	Roots	SW	WW		X		X
191	Stem	SW	WW		X		
192	Stem	SW	WW				
330	Stem	N	WW		X		
340	Stem	E	Sp. W		X		X
341	Stem	E	Sp. W		X		
342	Stem	E	Sp. W				
380	Stem	E	Sp. W				
14	Roots	N	WW	*Waitea circinata* varieties			X
15	Stem	SW	WW				X
47	Stem	N	Sp. W		X		X
67	Stem	N	WW		X		
68	Roots	N	WW		X		X

69	Stem	N	Sp. B	X	
83	Stem	SW	WW	X	
84	Roots	SW	WW	X	
107	Roots	N	WW	X	
116	Stem	N	Sp. W	X	X
118	Stem	N	WW	X	
119	Stem	N	Sp. W		
120	Stem	N	Sp. W	X	
121	Stem	N	Sp. W		
122	Roots	N	Sp. W	X	
123	Stem	N	Sp. B	X	
125	Stem	N	Sp. B	X	X
132	Stem	N	Sp. W	X	
133	Stem	N	Sp. W		
134	Stem	N	Sp. B	X	X
169	Roots	SW	WW	X	
D1	Roots	E	WW		
D2	Stem	E	WW		
252	Stem	N	WW	X	X
253	Stem	N	WW		X
255	Stem	N	WW	X	X
267	Roots	N	WW		
268	Stem	N	WW	X	X
269	Roots	N	WW	X	
270	Roots	N	WW		
273	Roots	N	WW	X	
274	Stem	N	WW		X
275	Stem	N	WW	X	
276	Stem	N	WW	X	
277	Stem	N	WW		
281	Stem	N	WW		
282	Stem	N	WW		
283	Stem	N	WW		
287	Stem	N	WW		
289	Stem	N	WW		
290	Stem	N	WW		
291	Stem	N	WW		
292	Stem	N	WW		
293	Stem	N	WW	X	X
294	Stem	N	WW	X	
295	Stem	N	WW	X	X
296	Stem	N	WW		X
297	Stem	N	WW	X	

298	Stem	N	WW				
299	Stem	N	WW		X		
300	Stem	N	WW				
301	Stem	N	WW				
302	Stem	N	WW				
303	Stem	N	WW				
305	Stem	N	WW				X
313	Stem	N	WW				
314	Stem	N	WW				
315	Stem	N	WW				
316	Stem	N	WW				
317	Stem	N	WW				
318	Stem	N	WW				
325	Roots	N	WW		X		X
326	Stem	N	WW				
327	Stem	N	WW		X		
328	Stem	N	WW				
345	Stem	N	WW				
346	Stem	E	WW		X		X
352	Stem	SW	Sp. W		X		
354	Stem	N	WW				
355	Stem	N	WW				
356	Stem	N	WW				
357	Stem	N	WW				
358	Stem	N	WW				
364	Stem	E	Sp. B		X		
384	Stem	N	WW				
190	Stem	SW	WW	Unidentified BNR	X		X
97	Roots	SW	WW		X		
260	Stem	N	WW		X		X

Region: North (N), East (E), Southcentral (SC) Southwest (SW). Crop: Winter wheat (WW), spring wheat (Sp. W), winter barley (WB), spring barley (Sp. B)

Appendix 2

Appendix 2.1 Complete bean isolate list by symptom, region collected, AG, unique isolate and used in pathogenicity experiment

Isolate DB#	Symptom	Region	AG	Unique Culture	Used in Pathogenicity Experiment
231	Stem	SW	AG 1 IB	X	X
39	Roots	SW	AG 2-1	X	
52	Stem	SW		X	
110	Roots	SW		X	X
26	Stem	SW	AG 2-2	X	X
40	Stem	SW			
48	Stem	SC		X	X
49	Stem	SW			X
53	Stem	SW			
54	Roots	SW			X
250	Stem	SW		X	
55	Stem	SW		X	
56	Roots	SW		X	
57	Stem	SW			
59	Stem	SW			X
60	Stem	SW			
61	Stem	SW		X	
62	Stem	SW			
66	Stem	SW			
67	Stem	SW			
68	Stem	SW			
69	Stem	SW			X
70	Roots	SW			
71	Stem	SW			
72	Stem	SW			
73	Stem	SW			
74	Stem	SW			
78	Stem	SW			
83	Stem	SW		X	
84	Stem	SW		X	
92	Stem	SW	AG 3-PT		
222	Stem	SW		X	X

44	Stem	SC	AG 4 HG-I	X	X
76	Stem	SC		X	
77	Stem	SC		X	X
152	Stem	SC			X
169	Stem	SC		X	
170	Stem	SC			
171	Stem	SC		X	X
182	Roots	SC		X	
184	Stem	SC		X	
185	Stem	SC		X	
195	Roots	SC		X	X
196	Stem	SC		X	
205	Stem	SW		X	
207	Stem	SW		X	X
208	Stem	SW		X	
209	Stem	SW			
210	Stem	SW			
218	Stem	SW			
219	Stem	SW			
3	Stem	SW	AG 4 HG-II		
4	Stem	SW			
5	Stem	SW		X	
6	Stem	SW			X
36	Stem	SW		X	
37	Stem	SW			X
42	Roots	SC		X	X
43	Roots	SC			
75	Stem	SW		X	
95	Stem	SC		X	
96	Stem	SC			
97	Stem	SC			X
98	Stem	SW		X	X
99	Stem	SW			
100	Stem	SW			
103	Stem	SC		X	
104	Stem	SC		X	X
105	Stem	SC		X	X
111	Stem	SW		X	X
112	Stem	SW			

119	Stem	SW	X	X
120	Stem	SW		
122	Roots	SW	X	
123	Roots	SW		
125	Stem	SC	X	
126	Stem	SC		X
137	Stem	SC	X	X
138	Stem	SC		
139	Stem	SC		
140	Stem	SC		
141	Roots	SC		
142	Stem	SC	X	
146	Stem	SC	X	X
147	Stem	SC		X
148	Stem	SC		
149	Stem	SC		
150	Stem	SC		
151	Roots	SC	X	X
154	Stem	SW	X	
156	Stem	SC	X	X
158	Stem	SW	X	X
159	Stem	SW		
160	Stem	SW	X	
161	Stem	SW		
162	Stem	SW		X
163	Stem	SW		X
164	Stem	SW		
166	Stem	SW		
167	Stem	SW		
168	Stem	SW		
186	Stem	SW		X
187	Stem	SW		
194	Roots	SC	X	X
199	Stem	SC		X
200	Stem	SC		
202	Stem	SW	X	
203	Stem	SW		
204	Stem	SW	X	
216	Stem	SW		

251	Stem	SC		X	
240	Stem	SW		X	
241	Stem	SW		X	
7	Stem	SW	AG 4 HG-III	X	X
117	Stem	SW		X	
121	Stem	SW		X	X
179	Stem	SC		X	
215	Stem	SW			
227	Stem	SW			
253	Roots	SC		X	
229	Stem	SW			
16	Stem	SW	AG 5	X	X
88	Stem	SW			X
89	Stem	SW		X	
93	Stem	SW			
94	Stem	SW		X	
113	Stem	SW		X	X
114	Stem	SW			X
131	Stem	SW			
132	Stem	SW		X	
133	Stem	SW			
1	Stem	SW	AG 11	X	
2	Stem	SW		X	
23	Stem	SC		X	
27	Stem	SW		X	
28	Stem	SW			X
63	Roots	SW		X	X
10	Stem	SW	AG A	X	X
15	Stem	SW		X	
65	Stem	SW		X	
79	Roots	SW		X	
86	Roots	SW		X	
102	Roots	SW		X	
106	Stem	SC			
107	Roots	SC		X	X
108	Stem	SC		X	X
109	Stem	SC			
116	Stem	SW		X	X
134	Roots	SW		X	

143	Stem	SC		X	
190	Stem	SW			
192	Roots	SC		X	
213	Stem	SW		X	
220	Roots	SC		X	X
225	Roots	SC		X	
234	Roots	SC			
198	Stem	SC	AG E	X	X
18	Stem	SW	AG F		X
20	Stem	SW		X	
29	Stem	SW		X	
30	Stem	SW		X	
38	Stem	SW		X	
45	Roots	SW		X	
46	Roots	SW			X
64	Roots	SW		X	
87	Stem	SW		X	X
115	Roots	SW		X	
188	Stem	SW			X
11	Stem	SW	AG K	X	
12	Stem	SW		X	X
13	Stem	SW		X	
14	Stem	SW			
256	Stem	SW		X	
41	Stem	SC		X	X
D#20-238	Stem	SC		X	
127	Stem	SC			
128	Stem	SW		X	X
135	Stem	SW		X	
136	Stem	SW			
144	Stem	SC			
145	Roots	SC		X	
189	Stem	SC		X	X
193	Stem	SC		X	X
221	Stem	SC		X	
223	Stem	SC			
224	Stem	SC		X	
226	Stem	SC			

235	Stem	SW	*Waitea circinata* var. *zeae*	X	X

Region: Southcentral (SC), Southwest (SW)