POPULATION DYNAMICS OF COEXISTENCE BY PLANT PATHOGENS OF THE RHIZOSPHERE OF SPRING WHEAT

by

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DEDICATION

To my father Brett, for pushing me to continue my education beyond a bachelor's degree, and my mother Elaine, for pushing me to do more than sit at my desk all day.

To my husband Austin, who pretends to understand what I'm talking about when I tell him about my day.

To my wonderful friend Thea, understands why we love science even though it's hard.

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GLOSSARY and NOMENCLATURE

Anamorph: the asexual reproductive stage of a fungus
Crown: basal zone of the wheat stem
Rhizosphere: the region of soil in the vicinity of plant roots, the chemistry and microbiology of which is affected by plant exudates and growth
Teleomorph: the sexual reproductive stage of a fungus

Due to changes in nomenclature, several of the fungi referenced in this document may have other synonyms that are no longer in use. For consistency, the first name listed for a fungus will be used throughout this thesis. Alternative nomenclature is listed below, following the name that will be used in this document.

Cochliobolus sativus (teleomorph)*: Bipolaris sorokiniana* (anamorph);
B. californica;
Helminthosporium sativum;
H. sorokinianum;
H. californicum;
Drechslera sorokiniana

Fusarium pseudograminearum (anamorph): *Gibberella coronicola* (teleomorph)
F. graminearum Group 1
F. roseum 'Graminearum' (Group I)

Fusarium graminearum (anamorph): *Gibberella zeae* (teleomorph)
F. graminearum Group 2
F. roseum 'Graminearum' (Group II)

Fusarium culmorum (anamorph): no known teleomorph
F. roseum 'Culmorum'
F. roseum f. sp. *cerealis* 'Culmorum'

ABSTRACT

The dryland root rot complex is a collection of root pathogens that significantly affect small grain production in the semiarid regions world-wide. The complex includes *Cochliobolus sativus, Fusarium pseudograminearum, Rhizoctonia solani, Pythium ultimum*, and *Penicillium* sp. The purpose of this thesis was to document the interactions among these pathogens that commonly coexist within the wheat rhizosphere. The thesis had two objectives: 1) examine variation in interactions amongst isolates of *C. sativus* and *F. pseudograminearum* within the wheat crown and 2) identify interactions among pathogens in the dryland root rot complex (*C. sativus, F. pseudograminearum, R. solani, P. ultimum*, and *Penicillium* sp.*)* in-field, as reflected in plant response variables. For objective 1, wheat in both field and greenhouse settings were inoculated singly and in all pathogen isolate combinations. Both *C. sativus* and *F. pseudograminearum*, alone and in combination, reduced yield ($P<0.001$, $P=0.003$, respectively), but *C. sativus* isolates had a greater effect on both yield and emergence ($P<0.001$). Inoculations with some isolates of *C. sativus* and *F. pseudograminearum* were suppressive on populations of each other. Significant variability in suppressiveness was observed among isolates for both species. For objective 2) plant health as measured by emergence, vigor, plant height and yield, was observed in response to inoculations with all single, pairwise, four-pathogen and five-pathogen combinations of *C. sativus, F. pseudograminearum, R. solani, P. ultimum*, and *Penicillium* sp. Antagonistic relationships that favored overall plant health were observed between several pathogens, including *P. ultimum, Penicillium* sp. and *C. sativus.* These antagonistic interactions affected seedling emergence and plant vigor. Conversely, *F. pseudograminearum* in combination with *R. solani* significantly reduced emergence beyond expected ($P=0.002$). Within the community interaction studies overall, *F. pseudograminearum* was the dominant pathogen, causing more disease and more consistent disease than any other pathogen, and generating increased damage to emergence when inoculated in the community ($P=0.017$). In conclusion, interactions among members of the dryland root rot complex is a dynamic process, one that varies by species and pathogen isolate. These interactions are more often antagonistic, with one pathogen suppressing another. Additional studies may improve how disease control measures are deployed in the future.

CHAPTER 1

LITERATURE REVIEW

Ecology of Wheat Production Systems

Wheat, *Triticum* spp., is the most widely adapted crop in the world, and is grown on 20% of the world's cultivated land (Bikram, 2010). Wheat comprises 75% of the carbohydrates humans consume, and 50% of the protein; it is the primary source of plant protein in the world (Bikram, 2010). U.S. production of wheat in 2014 was over 50 million metric tons (http://quickstats.nass.usda.gov/), and worldwide production of wheat has increased consistently since the 1960s (Curtis et al., 2002), to a record 715.5 million metric tons produced in 2014 (Food and Agriculture Organization of the United Nations). It is estimated that by 2030 the world will require 1 billion metric tons of wheat annually (Bikram et al., 2006). However, 25-30% of wheat crops worldwide are lost to disease and insect pests. Among crops lost to disease, fungi are the dominant plant pathogens. Agriculture is not functioning at peak efficiency on a global scale. Because fungal pathogens are such a universal plant problem, improved understanding of their dynamics will improve efficiency of crop production (Mathre, 1997) and better support future improvements to worldwide agriculture.

Wheat is grown primarily under dryland conditions worldwide (Curtis et al., 2002). The arid, low-water dryland growth conditions exacerbate losses in wheat due to a complex of disease commonly referred to as the dryland root rot complex (DRRC). The DRRC is a persistent, difficult-to-diagnose condition that occurs predominantly in

cereal crops where growing conditions are sub-optimal due to low water availability. Global warming and its predicted effects of increasingly arid conditions threaten world food production, particularly when water resources become limited (Cline, 2007). Reduced rainfall and increased temperatures frequently result in dryland root rot, and have been associated with changing pathogen species in the DRRC (Moya-Elizondo et al., 2011b). With perennial threats to global agriculture, it is now, and historically has been, imperative to gain an improved understanding of fungal pathogen dynamics to enable more effective management of disease threats. This topic has been studied for several decades, but there is still much to discover regarding fungal pathogen dynamics, and modern molecular methods may now enhance this quest.

Ecology of Plant Pathogens

Early exploration of pathogen dynamics often consisted of descriptions of pathogen effects on the host (Hynes, 1938, Hynes, 1935, Ledingham, 1942). This provided a broad picture of ecological dynamics, but one that lacked precise detail. The advent of advanced molecular techniques, including nested polymerase chain reactions, high-throughput sequencing methods, and quantitative real-time polymerase chain reactions, have greatly enhanced the scientific community's abilities to identify and enumerate fungal pathogen communities (Lindahl et al., 2013, van Elsas et al., 2000). They have allowed us to explore the complex interactions of pathogen communities in unprecedented detail. This is essential since changes in the pathogen community are due to myriad factors, such as the production of plant defense compounds. For example,

Fusarium species dominate in maize plants that produce benzoxazinoid defense compounds, and fungal diversity is higher in maize plants that do not produce benzoxazinoid compounds (Saunders and Kohn, 2009). Fungal dynamics may also be affected by combative interactions between fungi. These antagonisms may involve toxins or antibiotics, hyphal interference in which hyphal death occurs upon contact with a combatant, or mycoparasitism. Outcomes may involve replacement of one fungus by another, or deadlock (Boddy, 2000). In other situations, defensive mutualisms may be elicited in which an endophyte limits colonization by other microbes; the endophyte may have little to no effect on the host unless threatened by a microbial parasite (May and Nelson, 2014).

Zero sum dynamics is a term often used when describing competitive interactions in which the interspecific dynamics of the community are limited by resource availability, which imposes a finite limit on populations (Hubbell, 2001). Zero-sum dynamics are the concept that as populations of an organism increase, populations of competitors must decrease in equivalent amounts. However, zero-sum dynamics may be the exception and not the rule, as the zero-sum model only applies if the community is at or near carrying capacity, which may be uncommon (Houlahan et al., 2007). Zero-sum dynamics may also not apply if (1) taxa are not at the same trophic level, (2) if severe disturbance has occurred in the environment which results in the community abundance remaining below carrying capacity, or (3) if there is spatial variability in productivity (Hubbell, 2001, Cooper, 2003).

Synergism, or an increase in damage to a host which exceeds the damage generated by pathogens individually, has historically been considered to be rare; even spores of the same pathogen species are not typically synergistic with one another (Garrett, 1970). Antagonism is the opposite of synergism, in which a decrease in damage to a host compared to independent attacks is observed. In a summary of studies which analyzed the dynamics of two plant pathogens, 74% of pathogen interactions are independent, 22% are antagonistic, and just 4% are synergistic (Stephens et al., 2013). Attacks on the same plant part at the same time increases the likelihood of interactions that reduce damage to the host (antagonism) in contrast to independent attacks. Ultimately, increasing the number of plant pathogens in the dynamic increases the chances that interactions will occur (Stephens et al., 2013).

Previous research has examined the effect of the host on microbe community dynamics. As previously stated, plant defense compounds may also affect fungal diversity in corn. Host effects on biodiversity have also been noted in other crops. In the potato rhizosphere, it has been shown that plant growth stage has the greatest effect on the fungal community in soil; fungal biomass (Ascomycete, Basidiomycete, and Glomeromycete) increased in the late growth stages and changes in the community were greatest before and after plant flowering (Hannula et al., 2010). In this study, the effect of growth stage was greater than the effects of field site, soil type, and genotype, such that the rhizosphere community associated with a cultivar genetically modified for starch production was not significantly different than that of the parent cultivar.

Plant hosts are not the only contributors to changing pathogen dynamics. In the root and crown rot community of wheat, there is evidence that fungal pathogens of the rhizosphere may significantly affect community dynamics. In the rhizosphere, numerous pathogens are present throughout the year as propagules or in plant residue (Hogg et al., 2009, Fernandez and Jefferson, 2004), and any number of them may attack the root and crown tissue at any given time if environmental conditions are appropriate. Thus, implications of these potential fungal interactions should be considered when developing controls for a single member of the DRRC, particularly if control of a single community member may result in an increase in damage by another community member. One such antagonistic interaction has been observed between *Cochliobolus sativus* (the causal agent of common root rot) and *Fusarium pseudograminearum* (the causal agent of Fusarium crown rot), both common causal agents and components of the dryland root rot complex.

Interaction Between Fusarium Crown Rot and Common Root Rot

Studies concerning the interaction between *Cochliobolus sativus* and *Fusarium* spp. more frequently report antagonism between these pathogens than synergism. In the Canadian Prairies of Saskatchewan, isolation frequency of *C. sativus* is negatively correlated with isolation frequency of *Fusarium* spp. (including *F. culmorum* and *F. equiseti*), indicating possible antagonism between the two species (Fernandez and Jefferson, 2004). Less seedling blight and root rot in barley is observed for plants inoculated with both *C. sativus* and *Fusarium* spp. than for plants inoculated with

Fusarium alone (Scardaci and Webster, 1981). *Priori*, or the concept of "prior colonization-possession," may be important in the dynamics of the two pathogens; i.e., the pathogen that is inoculated first is isolated more frequently (Scardaci and Webster, 1981). However, this significance of *priori* may extend solely to *Fusarium* species, however (Scardaci and Webster, 1981). Prior infection of wheat plants with either *F. culmorum* or *F. acuminatum* suppresses subsequent invasion by *C. sativus*, but no evidence has been found that *C. sativu*s suppresses infection by *Fusarium* species (Tinline, 1977, Fernandez and Jefferson, 2004). Similarly, inoculation with *F. pseudograminearum* results in a decrease in populations of *C. sativus* in wheat crown tissues based on quantitative polymerase chain reaction (qPCR) population quantification (Moya-Elizondo et al., 2011a). In contrast to results reporting antagonism, there is only one report of synergism between the genera, observed between *F. acuminatum* and *C. sativus*, in which damage by common root rot was augmented by inoculation with *F. acuminatum* (Fernandez et al., 1985).

Both *C. sativus* and *F. pseudograminearum* experience wide distributions in Montana (Moya-Elizondo et al., 2011b). As stated previously, in this study inoculation of wheat with single isolates of *F. pseudograminearum* and *C. sativus* resulted in suppression of *C. sativus* populations by *F. pseudograminearum*, based on qPCR. No studies in Montana have examined how the variability among isolates of *F. pseudograminearum* or *C. sativus* may affect their interspecies competition, and there is only preliminary research on the population dynamics of the community as a whole. For the research presented in this thesis, variability among isolates of *F. pseudograminearum*

and *C. sativus* in their ability to suppress the opposing pathogen were examined; three additional DRRC pathogens were chosen to develop a global understanding of disease dynamics in that community, based on their established potential to suppress or be suppressed in the rhizosphere of wheat.

Pathogens in the Spring Wheat Rhizosphere

Five pathogens that inhabit the rhizosphere of spring wheat are known to interact with other members of the rhizosphere. The causal agents of common root rot (*Cochliobolus sativus*), Fusarium crown rot (*Fusarium* spp.), dry seed decay and other *Penicillium* diseases (*Penicillium* spp.), Pythium root rot (*Pythium* spp.), and Rhizoctonia root rot (*Rhizoctonia solani*) have all been observed to suppress other pathogens or to be suppressed in the rhizosphere. In general, suppression of *C. sativus* (Moya-Elizondo et al., 2011a, Ledingham, 1942), *Pythium* species (Tedla and Stanghellini, 1992, Fukui et al., 1994), and *R. solani* (Smiley et al., 1996a, MacNish, 1988) is commonly reported. Conversely, *Fusarium* and *Penicillium* species are more frequently observed as suppressors (Notz et al., 2002, Lutz et al., 2003, Yang et al., 2008). Because non-independent dynamics have been observed for causal agents of all five diseases, further investigation into the interspecies dynamics of these various community members of the DRRC is necessary. The biology and ecology of these fungal and fungus-like pathogens will be detailed further.

Biology of Common Root Rot

Cochliobolus sativus, the causal agent of common root rot (CRR), is a frequent member of the root and crown rot complex in spring wheat. It has been a noted problem in wheat-growing regions of the world for close to a century (Hynes, 1935). The Ascomycete *Cochliobolus sativus* (S.Ito & Kuribayashi) Drechsler ex Dastur (= *Bipolaris sorokiniana* (Sacc.) Shoemaker) is the sole causal agent of CRR. The teleomorph stage is exceedingly rare in nature, and is typically only observed in the laboratory on media; the sexual stage has only been observed in the wild in Zambia (Raemaekers, 1988) and plays no role in the epidemiology of CRR (Piening, 1997). In the anamorph stage, conidia of *C. sativus* are elliptical to oblong (60-120µm x 12-20µm) and dark brown to dark olive green in color with three to ten cells; mycelium is white to dark gray depending on media and isolate, and may bear simple, erect single or clustered conidiophores (6-8µm x 110-150µm) (Stein, 2010, Kumar et al., 2002). *In vitro,* colonies may develop putative spontaneous mutations, at which point sterile white hyphae and aerial mycelium may develop (Hynes, 1935).

Losses due to CRR have been noted to range from 5-42%, depending on cultivar, with an average losses of 10.3% in the Canadian Prairies in the 1970s and 9.5% losses in barley in the 1980s (Piening, 1997). More recent calculations estimate average yearly losses of 3-5% in the United States and Canada, and these may be substantially higher in drought-stressed plants (Stein, 2010).

Symptoms of infection with CRR include seedling damping-off, dark brown lesions on the roots or subcrown internode that may eventually cover the entire subcrown

internode, dark brown lesions on culm tissue, and chlorosis of leaf tissue near the crown (Piening, 1997). Whiteheads, or aborted tillers, may result under drought stress, although the cause of these may be hard to visually differentiate from Fusarium crown rot (Piening, 1997). Plants may dry out without producing seed if severe infection has occurred (Kumar et al., 2002), historically called "haying-off" (Hynes, 1935). CRR may cause thinner stands, reduce the number of tillers on a plant, and cause lower-weight and fewer kernels (Duczek, 1990, Ledingham et al., 1973). Symptoms may be difficult to visually assess or be absent from the plant (Stein, 2010, Conner et al., 1987). In addition to root and crown rot, *C. sativus* may also cause kernel blight and the foliar disease Spot Blotch, which may cause further plant damage; however, foliar disease is heavily dependent on environmental conditions (Stein, 2010). *C. sativus* is typically the most concentrated in the subcrown internode and culm bases of hosts; it may be recovered from culms with up to 80% frequency in non-inoculated fields (Fedel-Moen and Harris, 1987).

In the wheat-growing areas of Saskatchewan, *C. sativus* is one of the most frequently isolated pathogens, and is isolated from a third to half of crowns and subcrown internodes in those areas (Fernandez and Jefferson, 2004). Prominence of the pathogen may vary on a yearly basis depending on environmental conditions (Smiley and Patterson, 1996). In Montana, *C. sativus* has been detected in 93% of the fields sampled, and the pathogen has been detected in 15% of individual tillers from sampled fields (Moya-Elizondo et al., 2011b).

Ecology. *C. sativus* overwinters primarily as thick-walled conidia in stubble and plant debris or in infected seed (Piening, 1997, Mead, 1942). The pathogen is not as strong a saprophyte as other members of the root and crown rot complex (Burgess and Griffin, 1968). It survives and infects hosts in a largely necrotrophic manner (Kumar et al., 2001, Kumar et al., 2002). The spores of *C. sativus* may disperse aerially during combine harvest in substantial quantities, and conidia may still be viable despite apparent desiccation (Friesen et al., 2001). The availability of airborne conidia may affect the frequency of kernel infections with *C. sativus* (Stevenson, 1981). In barley and spring wheat, a linear relationship between *C. sativus* conidia concentration in soil and disease incidence (number of plants infected) has been observed (Duczek et al., 1985). However, while disease incidence may reach 100% with sufficient conidial availability, disease intensity (severity of affliction of infected plants, recorded as percent of the plant affected) may not reach comparable levels (Duczek et al., 1985). Others have found no evidence that inoculum density directly influences disease severity (Tinline and Spurr, 1991); however, in this study only inoculum potentials ranging from 39-140 propagules/cm^3 were examined, whereas Duczek et al., established a linear relationship using the broader range of 0-256 propagules/cm^3.

Infections with *C. sativus* are more common in barley than in wheat (Fedel-Moen and Harris, 1987); *C. sativus* is the most commonly isolated pathogen from barley subcrown tissues (Fernandez et al., 2007). In Canada, the pathogen is exceedingly common in barley and may be isolated from the majority of barley subcrown internodes (Fernandez et al., 2009).

Tilling has a significant effect on CRR. CRR severity is reduced under no-till farming compared to conventional tilling (Bailey and Duczek, 1996, Fernandez et al., 2007). Furthermore, fields that are not tilled experience significantly lower levels of CRR (Tinline and Spurr, 1991, Wildermuth et al., 1997, Conner et al., 1987). Minimum-tilled plots, however, do not show a consistent difference in disease levels than conventionally tilled plots (Conner et al., 1987, Wildermuth et al., 1997). Minimum-till plots have been observed to have intermediate disease levels, between the low CRR in no-till and high CRR in conventional till (Tinline and Spurr, 1991). Deeper seed planting is consistently associated with an increase in common root rot as well (Greaney, 1946, Wildermuth et al., 1997).

Tilling also affects the concentration of *C. sativus* spores in soil. Minimum tillage concentrates *C. sativus* spores in the top 5 cm of soil, close to plants; under those conditions, 90% of viable *C. sativus* conidia are found in the top 10 cm (Duczek, 1981). However, minimum tillage also reduces total spore count in the top 20 cm of soil as compared to conventional tillage (Reis, 1983).

Mycotoxins and Pathogenicity. *C. sativus* makes a number of sesquiterpenoid toxins; the most common of which is prehelminthosporol, a hydrophobic sesquiterpene found in conidia and hyphae (Carlson et al., 1991). Helminthosporol and sorokinianin are also commonly produced by the fungus (Kumar et al., 2002). Filtrates isolated from the fungus have been observed to induce a burst of H_2O_2 in plant tissue when certain genes were suppressed, which encourages plant tissue apoptosis and enables easier infection by *C. sativus* (Kumar et al., 2001). The fungus also produces sterigmatocystin,

typically when infecting as a storage mold; the toxin is considered to be a carcinogen (Jacobsen, 2010).

In 2013, a novel toxin was purified and characterized from *C. sativus*, called 'Bipolaroxin' (Jahani et al., 2014). Like other toxins commonly produced by the species, it is a sesquiterpene (bicyclical). The toxin causes symptoms of necrosis on wheat in foliar Spot Blotch infections; wheat and barley are the most sensitive hosts of the eight grass crops and four weeds that have been tested. The toxin appears to be universal among *C. sativus* isolates tested for its production (Jahani et al., 2014). While toxin production is considered to be an important component of pathogenicity, it is not believed to be the sole component; a loose correlation between pathogenicity and toxin production has been reported (Ludwig, 1957). Several genes have been reported to affect pathogenicity (Nelson, 1960, Nelson, 1961).

Control of Common Root Rot. While some resistance to CRR is available, no barley cultivars are resistant to both CRR and foliar Spot Blotch (Arabi et al., 2006). Most tested cultivars of hard red spring wheat tend to react consistently to the fungus over planting time and environmental conditions (Tobias et al., 2009). While resistance to both diseases by the same pathogen is conferred by similar mechanisms, the mechanisms of resistance are unknown. Infection by *C. sativus* has been linked to the *Mlo* gene, a powdery mildew resistance gene; the *Mlo* gene confers reduced sensitivity to H_2O_2, which encourages plant tissue death and subsequent infection by *C. sativus* (Kumar et al., 2001). Pre-planting applications of glyphosate in barley fields reduce the incidence of CRR (Fernandez et al., 2007).

Seed treatments have been a standard control method for decades, but their efficacy only extends to protection against seedling blight and not against infections later in the season (Hynes, 1938, Piening, 1997, Stein, 2010, Wiese, 1977, Wiese, 1987). Crop rotation that includes fallow and non-hosts may reduce disease levels as well (Stein, 2010, Hynes, 1938). However, the effects of tillage on *C. sativus* populations in barley have been found to be more important than the effects of rotation (Fernandez et al., 2007).

Biology of Fusarium Crown Rot

Fusarium crown rot (FCR) is a serious problem in grain production areas worldwide (Backhouse and Burgess, 2002, Wildermuth et al., 1997, Cassini, 1981, Smiley and Patterson, 1996, Moya-Elizondo et al., 2011b, Klassen et al., 1992, Marasas et al., 1988, Nicol et al., 2001). It commonly affects wheat, barley, rye, and oats (Miedaner, 1997, Cook, 2010, Piening, 1997). Symptoms include seedling damping-off, darkening of the culm tissue (crown rot) that may extend up to the third node, and the development of aborted seed heads, called whiteheads (Cook, 2010).

In the Pacific Northwest, crown rot has been observed to reduce winter wheat yield by up to 35%, with an average of 9.5% (Smiley et al., 2005). Artificial inoculation reduces yield up to 61%. Other losses reported range from 7-17% for seedling blight to 10-30% for crown and foot rot (Dodman and Wildermuth, 1987). In Montana, high populations of *Fusarium* spp. are associated with yield losses of 25-35% in dryland fields and 21% in irrigated fields (Moya-Elizondo et al., 2011b). Numerous studies of losses due to FCR and CRR have been conducted for decades.

Fusaria are divided into five phylogenetic clades (Schroers et al., 2009). In grains, the Gibberella clade is of particular interest. Economically important species such as *F. graminearum* and *F. culmorum* are included in the Gibberella clade, section Discolor, based on 28s and ITS rDNA sequencing (O'Donnell et al., 1998). Those species, including *F. pseudograminearum,* are sometimes referred to as the Roseum group due to their prior classification as a single species (Cook, 1970).

Fusarium crown rot is caused by a pathogen complex, including: *F. pseudograminearum* (O'Donnell & T.Aoki; group I) (= *Gibberella coronicola*), *F. graminearum* Schwabe (group II) (=*Gibberella zeae* (Schwein.) Petch), *F. culmorum* (W.G. Sm.), *F. avenaceum* (Fr.) Sacc., *F. acuminatum* Ellis & Everh., *F. equiseti* (Corda) Sacc., *F. crookwellense*, and *Microdochium nivale* (Fr.) Samuels & I.C.Hallett (=*F. nivale*) (Smiley and Patterson, 1996, Cook, 1980, Cook, 1981, Wiese, 1977, Wiese, 1987). The three most important causal agents of Fusarium Crown Rot are *F. pseudograminearum, F. graminearum,* and *F. culmorum* (Cook, 2010, Smiley and Patterson, 1996), although the dominance of secondary *Fusarium* species have been observed on a regional basis (Pettitt et al., 2003, Fernandez et al., 1985). All species may produce asexual septate macroconidia, and *F. culmorum* favor durable chlamydospores (Cook, 2010). Teleomorph *Gibberella* spp. develop globose, 125-265µm-diameter perithecia on plant tissue. These perithecia produce asci which typically contain eight ascospores; ascospores are hyaline with one to three septa, 3.3-6.5µm x 13-17µm in size, and ellipsoidal (Cook, 2010).

Fusarium species worldwide are frequently found in soil (Backhouse et al., 2001). The genus is characterized as endophytic root colonizers, although the genus can be highly transitory and colonize tissue as a saprophyte or a parasite on a wide range of hosts; they are hemibiotrophs and typically live in the host for some time before living in the necrotized host tissue as a saprophyte after the host dies (Bacon and Yates, 2006, Burgess et al., 1988). *Fusarium* species, including *F. graminearum, F. oxysporum,* and *F. culmorum* have been observed to cause symptomless infections of host tissue (Bacon and Yates, 2006).

F. culmorum is common in the Pacific Northwest, particularly northern wheat-growing regions in Washington (Cook, 2010, Smiley et al., 1996a, Smiley and Patterson, 1996, Cook, 1980, Piening, 1997), whereas *F. graminearum* and *F. pseudograminearum* thrive in warmer soils (Cook, 2010). These temperature-driven distribution dynamics persist in Australian wheat-growing regions as well (Backhouse and Burgess, 2002). Historically, *F. pseudograminearum* has been found to be dominant in Australia (Wearing and Burgess, 1977, Obanor and Chakraborty, 2014), and some evidence suggests that the species may be more common in regions of the Pacific Northwest than *F. culmorum* (Paulitz et al., 2002). In Montana, *F. pseudograminearum* has a wide distribution in warm soils, whereas *F. culmorum* is limited to the cooler soils of the Rocky Mountain front (Moya-Elizondo et al., 2011b).

F. pseudograminearum. Of the three species, *F. pseudograminearum* is considered to be the superior crown rot pathogen (Tunali et al., 2012, Dyer et al., 2009). It has demonstrated the potential to infect the entire wheat plant (Obanor and

Chakraborty, 2014), and has been found to survive in stubble as a superior residue-dwelling fungus (Tunali et al., 2012, Cook, 1980). In Montana, the species does not demonstrate the capacity to expand populations in stubble during fallow, but populations remain stable for the duration of a year in fallow (Hogg et al., 2009). There is some evidence that *F. pseudograminearum* is limited ecologically to narrow moisture ranges for successful saprophytic colonization (Burgess et al., 2001). Senescence of heavily diseased tissues may inhibit further mycelial growth, and even decrease overall fungal biomass. In durum wheat, barley, and hard white wheat, fungal biomass of *F. pseudograminearum* peaks at 16 weeks after planting (WAP) to 22 WAP in Australia, and biomass declines as plants senesce (Knight and Sutherland, 2015).

F. graminearum. Infection of wheat crowns by *F. graminearum* is typically associated with preexisting or concurrent infection with Fusarium head blight (Cook, 2010). The pathogen thrives under conditions that are conducive to FHB, and the higher-precipitation requirements for that disease often preclude or reduce the incidence of FCR (Obanor and Chakraborty, 2014). Heavy perithecia and ascospore production by the teleomorph lends it more predominantly to infection of the wheat flower (Obanor and Chakraborty, 2014). This leads to species spread and establishment in new wheat production areas. However, the species may survive and produce ascospores on wheat residue in the field at least 2 years after harvest (Pereyra et al., 2004). In inoculated trials in Montana, the species has demonstrated the capacity to cause substantial crown rot (Dyer et al., 2009). However, the development of perithecia and ascospores, *F. graminearum*'s most virulent inoculum source, is highly dependent on mild, moist

conditions (Summerell et al., 2001) that occur infrequently in Montana, and the species may functionally be less competitive than *F. pseudograminearum.*

F. culmorum. *F. culmorum* is unusual due to its lack of a sexual cycle. The species survives in soil as chlamydospores for 2-4 years, or by colonizing wheat stubble (Cook, 1980). These chlamydospores predominate in the top 10-15 cm of soil (Wiese, 1987, Cook, 2010). *F. culmorum* has demonstrated the ability to cause storage rot and Fusarium Head Blight (Scherm et al., 2013). In inoculated trials, it may generate less crown rot than *F. pseudograminearum* and *F. graminearum,* but greater seedling blight than either species (Dyer et al., 2009); this is likely due to its adaptation to lower temperatures than to lower virulence.

Ecology. The incidence of FCR has been positively associated with no-till farming (Burgess et al., 2001, Smiley et al., 1996a, Wildermuth et al., 1997). The wheat-growing regions of Montana have steadily adopted no-till farming practices with increasing frequency to conserve moisture in the dry climate, conserve fuel and labor input, and to reduce soil erosion (Dyer et al., 2009, Hogg et al., 2009). The transition to no-till has been observed at a global scale as well. This transition to no-till has been linked to an increase in the prevalence of the more heavily residue-borne *F. graminearum* and *F. pseudograminearum,* with a corresponding decrease in frequency of the more soil-borne *F. culmorum* (Dyer et al., 2009). Global climate change may be associated with increasingly arid conditions, particularly when water resources become limited (Cline, 2007). Water and temperature changes are linked to changing populations in the dryland

root rot complex, as *F. pseudograminearum* is most common in warmer soils in Montana and *F. culmorum* is limited to the cooler Rocky Mountain front (Moya-Elizondo et al., 2011b). Increasingly arid conditions lend towards greater FCR severity as drought stress increases, and warmer conditions make *F. culmorum* less competitive in the rhizosphere. More access to stubble occurs in warmer conditions as well, leading to greater inoculum potential of *F. pseudograminearum;* evidence of substantial populations of *F. pseudograminearum* have already been observed in no-till wheat-growing regions of Montana (Dyer et al., 2009).

Mycotoxins and Pathogenicity. *Fusarium* spp. generate a number of mycotoxigenic compounds, including fumonisins, trichothecenes, and fusaric acid. Fumonisins may play a role in the pathogenicity of some *Fusarium* species as well, but expression of the toxin depends substantially on the host genotype (Bacon and Yates, 2006). Most Fusaria make fusaric acid in some capacity (Bacon and Yates, 2006). Fusaric acid has the capacity to suppress a number of biocontrol agents, including inhibition of most *Bacillus* species (Bacon et al., 2006) and suppression of 2,4-diacetylphloroglucinol production, a key antimicrobial compound produced by biocontrol agent *Pseudomonas fluorescens* (Notz et al., 2002), which has been deployed in the control of several *Fusarium* species (Lemanceau and Alabouvette, 1991).

Trichothecene mycotoxins produced by *Fusarium* species include DON, NIV, T-2 toxins, and derivative compounds, as well as zearalenone (ZEA) (Vesonder, 1989). Deoxynivalenol (DON) - and nivalenol (NIV)-type trichothecenes are the most prominent mycotoxins of the Fusarium crown rot species complex, as all three of the prominent

species have the capacity to make it (Miller et al., 2001, Ichinoe, 1984). Trichothecenes inhibit protein synthesis and damage cell membranes; swine are particularly sensitive to the compound (Miller et al., 2001). DON is considered to be the most important *Fusarium* trichothecene, as it occurs in a broad range of hosts and has been isolated from plant florets and grains (Bacon and Yates, 2006).

Expression of the *Tri5* DON production gene occurs during crown rot infection by *Fusarium* spp., and expression is fairly constant throughout FCR development (Mudge et al., 2006). Some studies have found that production of mycotoxins, particularly DON, is not the sole component of pathogenicity. While the most aggressive crown rot isolates of *F. pseudograminearum* tend to produce the most DON, a number of the isolates of *F. graminearum* tested for crown rot pathogenicity made as much or more DON as the *F. pseudograminearum* isolates, yet were not as effective as pathogens of the crown (Tunali et al., 2012). The most virulent FHB isolates of *F. graminearum* also produce the highest amount of NIV-type mycotoxins (Atanassov et al., 1994), and DON production is a notable virulence factor in FHB (Miller, 1989). Higher DON levels correlate with higher levels of disease and greater reduction in grain test weight (Snijders and Krechting, 1992). *Fusarium* species that do not produce DON may generate comparable symptoms on the host as DON-producers, but are recovered less often, indicating less successful infection frequency, or reduced competitiveness in the crown (Mudge et al., 2006).

Studies have found that *F. culmorum* has two distinct chemotypes: Type I produces deoxynivalenol (DON), and Type II produces nivalenol (NIV) and/or Fusarenon-X (FUS) (Scherm et al., 2013, Minervini et al., 2004, Gang et al., 1998). Both

chemotypes have been verified as belonging to a single species based on phylogenetic analysis of a multilocus sequence, in spite of their differing toxin production (Obanor et al., 2010). The ability to break down inhibitory plant metabolites, such as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), a hydroxamic acid that may inhibit some bacteria and fungi, has been correlated with virulence (Friebe et al., 1998). Secretion of secondary plant metabolites allows *F. culmorum* to overcome plant inhibition of colonization; it was theorized that the plant metabolites encouraged production of toxins (Ojala et al., 2000, Scherm et al., 2013). The distribution of *F. culmorum*'s chemotypes worldwide is unknown, and geographic differences in chemotype may affect how the pathogen is managed (Scherm et al., 2013). The DON-producing chemotype I (most common in Europe and North America) is more aggressive than chemotype II, found largely in Asia and New Zealand (Tóth et al., 2004); other studies have observed that DON is a more effective virulence factor than NIV. A single study has observed that amount of DON and 15-ADON produced does not correlate with virulence in *F. graminearum* (Adams and Hart, 1989); however, those results have not been replicated and a greater number of studies associate DON and mycotoxin production with virulence in some capacity.

Mycotoxin production, particularly DON production, may do more than simply help the fungus colonize a host. Isolates of *F. culmorum* and *F. graminearum* that produce DON suppress the expression of the nag1-gox chitinase gene in *Trichoderma atroviride*, a fungal biocontrol; isolates that do not produce DON did not suppress the expression of this gene (Lutz et al., 2003). Synthetic DON also reduces expression of the

nag1-gox gene by up to 40%, and that the addition of DON to a non-DON-producing isolate reintroduce suppression of the gene (Lutz et al., 2003). This suppression may help *Fusarium* spp. maintain control of crown stubble during overwintering and exclude competitors.

Production of DON is variable and is subject to a complex interaction between water availability, temperature, and incubation time (Hope et al., 2005). Fungal biomass does not correlate with production of DON or zearalenone in *F. graminearum*, however; the production of zearalenone and DON by *F. graminearum* are correlated (Ryu, 1999). DON production by *F. culmorum in vitro* may be substantially higher than production in the field, and toxin production between the field and *in vitro* may not be significantly correlated (Gang et al., 1998).

Control of Fusarium Crown Rot. Cultural controls have historically been the preferred management strategy for Fusarium crown rot (Cook, 1980). Rotation to reduce in-field populations is typically a standard control method; use of resistant germplasm is also recommended (Cook, 2010). Wheat-pea rotations are the most effective in reducing the incidence of FCR (Smiley et al., 2012), and avoidance of oats in crop rotations is suggested, as oats support substantial sporulation by FCR fungi (Cook, 1980). Reduction of stubble may reduce FCR severity, but the effects may be offset by the loss of topsoil and soil water from tilling (Burgess et al., 1996). Control of seedling damping-off may be achieved with shallower planting (Cook, 2010).

Resistance to infection by *F. graminearum* and *F. culmorum* in the form of Fusarium Head Blight is generally understood to be due to resistance to spread within the

head and trichothecene resistance in plant membranes, as opposed to resistance to initial infection (Snijders and Krechting, 1992, Miller, 1989). Wheat seedling resistance to FCR has been linked to crown depth development, such that plants that develop the crown deeper in soil are correlated with higher levels of the disease (Cook, 2010, Wildermuth et al., 2001). The amount of research into the mechanisms of resistance to *Fusarium* species causing FHB far outweighs the research into resistance to the same species causing FCR. However, histopathological examination of *F. pseudograminearum* during infection has found that the pathogen may infect through stomates on leaf tissue, and that silica cells within plant tissue may be immune to penetration by fungal hyphae (Knight and Sutherland, 2013).

Some research has been conducted into control of FCR via the use of integrated pest management. In dryland field conditions, use of fungicide seed treatment, cultivar resistance, and induction of systemic acquired resistance together reduces FCR populations more than the use of a single method (Moya-Elizondo, 2010). Foliar application of the biocontrol agent BmJ (*Bacillus mycoides* isolate BmJ) reduces FCR severity and induces higher concentrations of peroxidase and endochitinase in wheat, the first such use in monocots; peroxidases may strengthen cell walls against penetration by *F. culmorum* (Moya-Elizondo, 2010). There is no silver-bullet control approach to FCR management, however, and the disease continues to threaten wheat growers globally.

Biology of *Penicillium* Diseases

Penicillium spp. commonly infect stored grain as storage molds. Grain infected with *Penicillium* spp. may suffer reduced germination, seed discoloration, heating and

fermentation, clumping of grain, and “bin burning,” or a burned, blackened appearance (Jacobsen, 1997). The genus may also cause dry seed decay, in which soil is too dry for a seed to germinate, but soil moisture levels are sufficient for *Penicillium* spp. to be active and infect seed. When this happens, the seed may rot and fail to germinate (Mathre and Johnston, 1991). Dry seed decay is a noteworthy problem in wheat, barley, and oats (Mathre et al., 2006). The disease is of particular importance in winter wheat, when winter wheat seeds are planted into soils that may be too dry to support germination, and may remain that way for weeks; *Penicillium* spp. are ubiquitous in Montana soils, and the world (Dyer et al., 2012).

While planting into dry soil is suboptimal, it may not always be avoided. Once below the soil water threshold required for germination, no differences in the incidence of dry seed decay have been observed at various below-germination soil moisture levels, meaning that once soil moisture is below seed germination requirements, dry seed decay is consistent (Mathre and Johnston, 1991). In 1991, most seed treatments tested inhibited infection by *Penicillium* spp. compared to the uninoculated control, with imazalil providing the most effective control (Mathre and Johnston, 1991). Puroindolines, the main component of grain hardness, have a notable antimicrobial effect against fungi and bacteria and have been linked to resistance to infection by *Penicillium* spp. (Kim et al., 2012).

The development of storage rot in grain bins results from improper climate control within the bins. Moisture and temperature control are essential to avoidance of storage rot, with safe grain moisture levels being below 14%, and safe temperatures

below 20°C (Woloshuk, 2010). In particular, *Penicillium* spp. dominate as storage rot causal agents in lower temperatures and at grain moisture above 16% (Jacobsen, 1997). Condensation in head space, leaks, or insect activity may all introduce unsafe moisture levels in grain bins (Woloshuk, 2010).

Mycotoxins and Pathogenicity. Species of the genus *Penicillium* generate a wide variety of mycotoxigenic compounds. Penicillic acid is produced by a number of species, and has been observed to cause malignant tumors in rats; it was first isolated in 1913 (Magan and Olsen, 2004). Penicillic acid and citrinin, another toxin, are typically recorded in grain when wet weather delays harvest (Jacobsen, 2010). Citrinin was one of the earliest discovered antibiotic compounds, but its toxicity in mammals prevented its functional use (Magan and Olsen, 2004). Ochratoxins are another commonly-produced group of toxins, particularly by *Penicillium viridicatum* and *P. verrucosum* (Jacobsen, 2010). Ochratoxin production is common where crops have lodged or when wet weather delays harvest, and it may enter mammalian systems through the consumption of infected grain or the inhalation of contaminated grain dust (Jacobsen, 2010). Production of ochratoxin, in particular ochratoxin A, has been associated with grain moisture levels of 19% or higher, but not at grain moisture levels of 15% (Abramson et al., 1990). Numerous other mycotoxins are associated with *Penicillium* spp., including roquefortine C, PR toxin, penitrem A (a neurotoxin), citreoviridin, luteoskyrin, secaonic acid D, rubratoxin, and others (Magan and Olsen, 2004). The fungus also has the capacity to directly antagonize competitors; a strain of *Penicillium oxalicum* with antagonistic tendencies has been observed to generate antifungal substances that suppress *Sclerotinia*

sclerotiorum (Yang et al., 2008). While *Penicillium* spp. are typically contaminants, some research has found that infection with *Penicillium* spp. has inconsistent effects on seed germination (Wicklow, 1995). Surface sterilization of *Oryzopsis miliacea*, a perennial rice grass native to the Mediterranean, reduced germination, whereas inoculation of the sterile seed with *Penicillium funiculosum* increased germination rates (Probert, 1981). However, this may be due to *P. funiculosum*'s hydrolytic enzymes degrading the seed coat and not a synergistic effect.

Biology of Pythium Root Rot

Currently there are 140 documented *Pythium* species, of which 41 have been discovered or elucidated from existing species since 2000 (Kageyama, 2014). They include saprophytes and parasites on plants, animals, algae, and fungi (Kageyama, 2014). *Pythium* spp. are fungus-like organisms classified as Stramenopiles in the class Oomycota (Dick, 2001). They produce spherical to lemon-shaped or lobate sporangia. Oospores, the sexual spores of the genus, are typically smooth and range from 15-40µm in diameter. As an Oomycete, *Pythium* species have diploid, coenocytic hyphae that help distinguish them and other Oomycetes from the true fungi (Chamswarng and Cook, 1985).

One of the most common plant pathogens in this genus, *P. ultimum*, generates spherical to lemon-shaped sporangia which may contain 10 to 40 zoospores (Paulitz, 2010a, Chamswarng and Cook, 1985). They cause damping off of seedlings and root rot in older plants; symptoms of infection with Pythium root rot in wheat may include fewer tillers, stunted plant development, and lower yield that result from reduced root length

and destruction of fine feeder roots (Paulitz, 2010a, Paulitz et al., 2002). All crops are susceptible to one species or another within the genus (Paulitz et al., 2002). While seedling disease is often properly attributed to *Pythium* species, losses from Pythium root rot often go unnoticed or are wrongly attributed to nutrient deficiencies due to the uniformity of the disease throughout affected fields and a lack of distinctive disease symptoms (Paulitz, 2010a). However, when Pythium root rot is controlled, plants are an average of 3-10 cm taller, produce 20-25% more tillers, and yield 13-36% higher (Cook et al., 1987). Seedling infections are thought to occur within the first 24-48 hours after a seed has been planted in moist soil, as seedlings pre-germinated for 48 hours prior to planting do not appear to be susceptible to infection (Hering et al., 1987). While toxin production is not a prominent field of *Pythium* research, the plant pathogen *P. aphanidermatum* generates necrosis and ethylene-inducing peptide 1-like proteins (NLPs) that are conserved virulence factors (Ottmann et al., 2009).

Early accounts of *Pythium* spp. in the Pacific Northwest (PNW) detected ten parasitic species, eight of which were homothallic (Chamswarng and Cook, 1985). *P. ultimum* and *P. irregulare* were later found to be the most prominent pathogens in the region and are active to temperatures as low as 10°C and 5°C, respectively (Ingram and Cook, 1990). Fourteen species of *Pythium*, with one to six species recovered from each site, have been identified in eastern Washington using ITS sequencing (Paulitz and Adams, 2003). In eastern Washington, *P. abappressorium* is recovered with 50% frequency, followed by 40% frequency of *P. rostratum*. *P. ultimum* is recovered with

18% frequency (Paulitz and Adams, 2003). Multiple species of common *Pythium* species are typically recovered from a single field (Schroeder et al., 2006).

Ecology. *Pythium* spp. are the first colonizers of green manure or crop stubble (Cook et al., 1990), but are reportedly unable to colonize residues that are already colonized by other microbes (Schmitthenner, 1970). Bacterial inhibition of *Pythium* species appears to be a particularly important natural method of biocontrol of the pathogen (Tedla and Stanghellini, 1992). Application of antibiotics that suppress bacterial antagonists have been shown to increase *Pythium* colonization of seedlings from 25% to 83% (Tedla and Stanghellini, 1992).

Generally, incidence of *Pythium* spp. is encouraged by lower soil pH (Fukui et al., 1994), older seed (3-7 years old), and heavy nitrogen fertilization (Paulitz, 2010a). *Pythium* spp. are only active in soil when soil moisture is at or above field capacity, or when a plow pan 10-15 cm deep replicates those conditions at the root level (Allmaras et al., 1988). The number of pathogen propagules may also be augmented by high clay-content soils, which hold more moisture (Fukui et al., 1994). Severity of Pythium root rot symptoms, adult plant height in particular, have been negatively correlated with number of *Pythium* spp. propagules in the soil (Cook et al., 1987).

Historically, it had been erroneously believed that wheat residue had a phytotoxic effect that resulted in stunted, low-yielding plants in no-till fields (Paulitz, 2010a). This reduction in plant success is now attributed to Pythium root rot. Plant matter may host populations of *Pythium* spp. which infect emerging seedlings; this has been observed as amendment of soil with fresh chaff increases the *Pythium* population tenfold over the

course of 4 days (Cook et al., 1990). Likewise, higher populations of *Pythium* spp. have been reported in no-till soil than in conventionally tilled fields (Cook et al., 1990).

Biology of Rhizoctonia Root Rot

Rhizoctonia root rot and bare patch affects wheat in all temperate regions of the world (Paulitz, 2010b). Symptoms of the disease include lesions and pruning of the crown and seminal (seed-originating) roots (Paulitz et al., 2002). The fungus rots away the root cortex, causing a condition called "speartipping." The loss of root tissue may be acute, in which bare patches of dead plants result from loss of seedlings, or chronic, in which stunted, unhealthy plants occur in the patches instead (Pumphrey et al., 1987, Lucas et al., 1993). The fungus may also delay maturity in the plant host, resulting in semi-senesced plants that may still have green leaves at or close to harvest (Paulitz, 2010b). Conservative calculations of losses due to bare patch have been estimated to be 8% overall in spring and winter wheat in the PNW (Smiley et al., 1989). Severity of disease has been significantly correlated with yield losses and may account for 20% of yield values (Smith et al., 2002).

Causal Agent and Distribution. The causal agent of Rhizoctonia root rot and bare batch is *Rhizoctonia solani* Kühn (=*Thanatephorus cucumeris*). *R. solani* is a genetically diverse Basidiomycete species that infects a wide variety of hosts, both dicotyledonous and monocotyledonous. *R. solani* does not produce spores in its asexual state, and is only characterized by its mycelial characteristics; the cells of the hyphae are multinucleate, 4-15µm wide, and branch at right angles (Parmeter and Whitney, 1970). The mycelium is

white to dark brown and may produce brown black, irregularly shaped sclerotia, although this is rare in the AG-8 anastomosis group; these microsclerotia survive in crop and root residue in the field, although the pathogen may also survive as hyphal fragments (Weller et al., 1986).

Anastomosis groups are classified based on the ability of a strain of *R. solani* to anastomose, or fuse and exchange nuclear material (Broders et al., 2014). One anastomosis group in particular, *R. solani* AG-8, became recognized as a serious problem in grains in the 1970s and 1980s (Weller et al., 1986, Paulitz et al., 2002). While most common in the United States, (Smiley et al., 1992, Paulitz, 2010b), AG-8 is not the only anastomosis group associated with wheat; six anastomosis groups are associated with wheat fields in the Canadian provinces of Alberta, Manitoba, and Saskatchewan, none of which are AG-8 (Broders et al., 2014). These different anastomosis groups may infect numerous hosts, including non-cereals. Current divisions into anastomosis groups have been largely supported with ITS sequencing, with few corrections (Broders et al., 2014). The significance of these numerous anastomosis groups lies in the difficulty of utilizing crop rotations as a method of control for Rhizoctonia root and crown rot. A single anastomosis groups may infect several hosts, and the presence of multiple AGs in a single field may ensure economically dangerous pathogen levels when practically any crop is grown. Because the species has so many hosts, it may survive over numerous cropping systems.

Ecology. *R. solani* thrives when a continuous supply of green host material is available; this host material may include volunteers, weeds, or grasses at the edge of a

field (Smiley et al., 1992, Paulitz et al., 2002). Controlling volunteers in or around a field may substantially curb the population of the pathogen; however, controlling with glyphosate 2-3 days before planting has been associated with up to 50% losses compared to spraying with glyphosate in autumn or early spring (Smiley et al., 1992). Application of glyphosate three or more weeks before planting is considered to be the safest course of action when controlling weeds and volunteers. When sprays occur a few days before planting, *R. solani* may invade the dying tissue and establish much higher populations. This effect has been observed to be countered with tilling 2 days before planting, however, even if glyphosate was sprayed previously. This improvement with tilling may occur when tilling breaks up host plant tissue, encouraging invasion by the microbial community and pushing *R. solani* out (Smiley et al., 1992).

Regardless of glyphosate use, tilling is an effective method of managing Rhizoctonia root rot. Numerous studies have established that severity of infection with *R. solani* is substantially greater in no-till fields than in conventionally-tilled fields (Roget et al., 1996, Weller et al., 1986, Pumphrey et al., 1987, Smiley et al., 1996a). *R. solani* is active in the top 10-15 cm of soil, close to the plant (Paulitz, 2010b), and tilling is thought to break up inoculum potential and hyphae in the upper soil profile (Paulitz et al., 2002).

The hyphal spread of *R. solani* within soil is linked to soil connectivity and soil physical characteristics, including pore space, tortuosity, and gas availability in soil architecture. The substantial effect of tilling on incidence of bare patch has been linked to the reduction in pore space available for fungal colonization and corresponding

increase in compaction (Glenn and Sivasithamparam, 1990). The bulk density of soil has a significant effect on the hyphal growth of *R. solani* in soil (Harris et al., 2003); well-connected, air-filled soil is associated with larger colonies of *R. solani*, and compacted soil is associated with smaller, denser colonies (Otten et al., 1999). Soil water content also affects *R. solani* colony size and density; dry soil experiences greater hyphal growth and greater potential for secondary infection (Otten et al., 1999), whereas smaller, denser colonies are found in wet soil, although growth in aerobic wet soils is still possible if gas-filled pores are available (Glenn and Sivasithamparam, 1990). The fungus thrives in large pores typical of no-till fields and avoids dense soil areas, and is not hindered by small gaps in soil that prevent diffusion of nutrients through soil water, as the fungus translocates nutrients through hyphal networks (Otten et al., 2004). Tilling breaks up these large pores and may reduce the mycelial growth of *R. solani*. However, some research has found that in water-restricted areas, evaporative losses due to tilling may damage yield potential as much as losses to *R. solani* (Smiley and Wilkins, 1993). This offset to the effects of *R. solani* may also be due to the increased incidence of other diseases in the DRRC associated with tilled fields.

Soil disturbance has been found to be inversely correlated to severity of Rhizoctonia root rot (Pumphrey et al., 1987). Both direct drilling that disturbs soil below the seed and cultivation reduce Rhizoctonia root rot levels compared to fields that are directly drilled without soil disturbance (Roget et al., 1996). Rotation to other crops, such as canola and mustard, is not associated with a decrease in the severity of Rhizoctonia root rot (Smiley et al., 1994). This is due to the broad range of hosts that *R.*

solani attacks; AG-8 may attack broadleaf hosts in addition to cereals (Smiley et al., 1994, Cook et al., 2002).

Control of Rhizoctonia root rot and bare patch may be the result of a condition called Rhizoctonia decline, which may result from several sources, including antibiosis by the microbial community, mycoparasitism by soil-dwelling fungi, and double-stranded RNA infection of the fungus (Castanho and Butler, 1968). Rhizoctonia decline is associated with fields continuously cropped to cereals which experience increasing levels of Rhizoctonia root rot for several years, after which the pathogen level drops precipitously (MacNish, 1988, Lucas et al., 1993, Smiley and Wilkins, 1993). This decrease in pathogen levels occurs after year 4 of continuous cropping (Cook et al., 2002). The decline is due to the increasing levels of competitors that build up and push *R. solani* out of the community over time. The severity of Rhizoctonia root rot has been inversely correlated with microbial biomass in the field (Smiley et al., 1996a). Hyphae and sclerotia in soil may be invaded by soil-borne bacteria and fungi, which degrade cell walls and decompose *R. solani* tissue over the course of 8 months, although this effect varies among isolates of *R. solani* (Naiki and Ui, 1975). *R. solani* may also be affected by hyperparasitism; numerous *Trichoderma* species (Grosch et al., 2006) and *Verticillium biguttatum* parasitize *R. solani*, reducing its hyphal and sclerotial viability (Velvis et al., 1989).

Rhizoctonia decline may also occur *in vitro* in a condition described as cytoplasmically-transmitted hypovirulence that is correlated with the presence of double-stranded RNA (dsRNA) within fungal cells (Bharathan and Tavantzis, 1991, Castanho

and Butler, 1968). dsRNA is common in field isolates tested, with up to 7 or 8 segments per isolate (Zanzinger et al., 1984). *In vitro*, hyphal tipping may help *R. solani* "escape" the dsRNA (Castanho and Butler, 1968), and the removal of the dsRNA is linked to a dramatic increase in isolate virulence (Bharathan and Tavantzis, 1991).

Unlike several other members of the DRRC, *R. solani* may be susceptible to numerous methods of suppression. However, these methods of reduction may not be easy to utilize, particularly because rotation is not an effective means of control, and because cultivation practices are trending away from conventional tilling.

Conclusions

Each of the five pathogens described have been documented as interacting with other microbes in the rhizosphere. *Fusarium* spp. and *Penicillium* spp. are often observed as antagonists in the rhizosphere (Notz et al., 2002, Lutz et al., 2003, Yang et al., 2008). Certain isolates *F. pseudograminearum* may suppress populations of its competitor *C. sativus* in wheat crown tissue (Moya-Elizondo et al., 2011a). *Fusarium* spp. have the potential to dominate in hosts. They may produce toxins including deoxynivalenol, which suppresses chitinase gene expression (Lutz et al., 2003) and is a notable virulence factor (Miller, 1989). *Pythium* spp. and *R. solani* may be antagonized in the rhizosphere as well. *Pythium* spp. in particular may be suppressed in the rhizosphere by microbial populations (Fukui et al., 1994, Tedla and Stanghellini, 1992). Given these observations, it is logical to examine how these rhizosphere pathogens interact with one another. The DRRC is characterized by several pathogens attacking the root tissue of wheat at any

given time, and dozens of members of the DRRC may be available in a single field. While ecologically, independent interactions are most common, and synergism is rare (Stephens et al., 2013), as DRRC pathogens target the same plant tissue, this increases the likelihood of interactions due to competition for the same host tissue. As the described five pathogens or closely-related species have been observed interacting with other organisms, there is substantial support for the exploration of their dynamics.

Two studies were conducted to explore pathogen dynamics among members of the DRRC: the first built on the substantial prior research which has been conducted with *Fusarium* spp. and *Cochliobolus sativus* and explored population dynamics of both species by examining isolate variability with regards to the ability to suppress populations of their competitor (Chapter 2). The discrepancy in type of interactions observed – from the most frequent observations of antagonism of *C. sativus* by *Fusarium* species to observations of mutual antagonism and even of synergism by *C. sativus* and *F. acuminatum* – between the two pathogens may indicate that substantial variability among isolates is present. The second study examined disease dynamics of all five pathogens and the effect of interactions among pathogens on host plant responses throughout the growing season (Chapter 3). This study sought to identify the types and frequency of interactions in the DRRC, as well as to explore disease dynamics and effects on the plant host both in pairwise inoculations and inoculations done in the presence and absence of the DRRC community.

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CHAPTER 2

POPULATION DYNAMICS OF COEXISTENCE BETWEEN *COCHLIOBOLUS SATIVUS* AND *FUSARIUM PSEUDOGRAMINEARUM*

Introduction

Fusarium crown rot (FCR) and common root rot (CRR) are serious problems for small grain production worldwide and prominent components of the dryland root rot complex. FCR has been observed in most grain production areas of the world, including Australia (Backhouse and Burgess, 2002, Wildermuth et al., 1997), Europe (Cassini, 1981), North America (Smiley et al., 1996a, Smiley et al., 2005, Moya-Elizondo et al., 2011b), South Africa (Klassen et al., 1992, Marasas et al., 1988), and West Asia (Nicol et al., 2001). CRR has been a documented problem for close to a century (Hynes, 1935). This causal organism is one of the most frequently isolated pathogens in the wheat-growing areas of Saskatchewan, the Pacific Northwest, and the Intermountain West (Fernandez and Jefferson, 2004, Smiley and Patterson, 1996, Moya-Elizondo et al., 2011b). Fusarium crown rot is caused by a disease complex that encompasses several *Fusarium* species that can include: *F. pseudograminearum* (O'Donnel & T.Aoki; group I) (= *Gibberella coronicola*), *F. graminearum* Schwabe (group II) (=*Gibberella zeae* (Schwein.) Petch), *F. culmorum* (W.G. Sm.), *F. avenaceum* (Fr.) Sacc., *F. acuminatum* Ellis & Everh., *F. equiseti* (Corda) Sacc., *F. crookwellense*, and *Microdochium nivale* (Fr.) Samuels & I.C.Hallett (=*F. nivale*) (Smiley and Patterson, 1996, Cook, 1980, Cook, 1981, Wiese, 1977, Wiese, 1987). In the western United States, the three most important

causal agents are *F. pseudograminearum, F. graminearum,* and *F. culmorum.* (Cook, 2010, Smiley and Patterson, 1996). CRR is caused solely by *Cochliobolus sativus* (S.Ito & Kuribayashi) Drechsler ex Dastur (= *Bipolaris sorokiniana* (Sacc.) Shoemaker). Numerous studies of losses due to FCR and CRR have been conducted for decades. In 1994 and 1995, FCR reduced winter wheat yield by up to 35%, with average yield losses of 9.5% in the Pacific Northwest (Smiley et al., 2005). Other losses reported range from 7-17% for seedling blight, to 10-30% for crown and foot rot (Dodman and Wildermuth, 1987).

The primary causal agent of FCR has been documented to vary due to temperature, plant residue in-field, and moisture conditions. The causal agent *F. culmorum* prefers cooler soils and is consistently found in cooler areas of the Pacific Northwest (Moya-Elizondo et al., 2011b, Cook, 2010, Piening, 1997, Smiley et al., 1996a, Smiley and Patterson, 1996, Cook, 1980). Conversely, *F. graminearum* and *F. pseudograminearum* thrive in warmer soils (Cook, 2010). The presence of CRR is also known to vary based on environmental conditions; *C. sativus* may vary substantially in detection frequency, from detection in 3% of fields sampled in 1993 to 12% the following year (Smiley and Patterson, 1996). In Montana, however, *C. sativus* has been detected by qPCR in 93% of the fields sampled in the wheat belt, and the pathogen was detected in 15% of individual tillers sampled from those fields (Moya-Elizondo et al., 2011b). Members of the FCR complex have been detected in 57% of fields sampled in Montana; *F. culmorum* was isolated from 13% of individual tillers, and *F. pseudograminearum* from 8% (Moya-Elizondo et al., 2011b).

Wheat grown in Montana is dispersed over 2.29 million ha annually, 43.5% of which is concentrated in the "Golden Triangle" wheat belt of the state (http://www.nass.usda.gov). The region has steadily adopted no-till farming practices with increasing frequency to conserve moisture in the dry climate, conserve fuel and labor input, and to reduce soil erosion. Likewise, the use of no-till is gaining popularity throughout the United States. However, no-till agriculture is associated with increasing incidence of FCR (Smiley et al., 1996a, Burgess et al., 2001, Wildermuth et al., 1997), and this transition to no-till has been linked to an increase in prevalence of the more heavily plant residue-dependent *F. graminearum* and *F. pseudograminearum,* with a corresponding decrease in frequency of the more soil-borne *F. culmorum* (Dyer et al., 2009). Agriculture in arid conditions in particular benefits from no-till, and increasingly arid conditions may result from global climate change, particularly when water resources become limited (Cline, 2007). Water and temperature changes have been associated with changing populations in the dryland root rot complex, as *F. pseudograminearum* is most common in warmer soils in Montana and *F. culmorum* is limited to the cooler Rocky Mountain front (Moya-Elizondo et al., 2011b). More arid conditions lend themselves towards greater FCR severity as drought stress increases, and warmer conditions make *F. culmorum* less competitive in the rhizosphere. Warmer, milder winters permit greater access to stubble by fungi as well, leading to greater inoculum potential of *F. pseudograminearum;* there is already substantial evidence of populations of *F. pseudograminearum* in no-till wheat-growing regions of Montana (Dyer et al., 2009).

Conversely, CRR severity is reduced under no-till (Conner et al., 1987, Tinline and Spurr, 1991), although the pathogen does have the capacity to colonize residue (Wildermuth et al., 1997). The differing effects of tilling on these members of the dryland root rot complex affects the competitiveness of these pathogens in the rhizosphere, and these changing rhizosphere dynamics may affect the success of control measures, as there is no silver bullet control for either pathogen. Improving scientific understanding fungal dynamics may illuminate a path to effective disease control.

Pathogen dynamics are subject to a number of interactions with the host, the environment, other competitors, and mycoparasitic organisms. Changes in the pathogen community, incited by the host, may be due to production of plant defense compounds; for example, *Fusarium* spp. dominate in maize plants that produce benzoxazinoid plant defense compounds, and fungal diversity is higher in maize plants that do not produce benzoxazinoid compounds (Saunders and Kohn, 2009). In this case, the host's defenses allow *Fusarium* spp. to drive the community composition. Fungal dynamics may be affected by combative interactions or antagonisms between fungi. These antagonisms may involve toxins or antibiotics, hyphal interference in which hyphal death occurs upon contact with an enemy, or mycoparasitism. Outcomes may involve replacement of one fungus by another or deadlock (Boddy, 2000). Antagonism is more likely when pathogens are targeting the same plant tissue at the same time (Stephens et al., 2013). *Fusarium* species produce a number of toxigenic compounds that have well-established suppressive abilities. Fusaric acid, common to several *Fusarium* spp., may suppress a number of biocontrol agents, and inhibit most *Bacillus* species (Bacon et al., 2006) and

suppress antimicrobial compounds produced by biocontrol agent *Psuedomonas fluorescens* (Notz et al., 2002*)*. Deoxynivalenol (DON)- and nivalenol (NIV)-type trichothecenes are the most prominent mycotoxins of the FCR species complex, as all three of the prominent *Fusarium* species have the capacity to make trichothecenes (Miller et al., 2001, Ichinoe, 1984). DON is considered to be the most important *Fusarium* trichothecene, as it is produced in a broad range of hosts and has been isolated from plant florets and grains, but not from root tissue (Bacon and Yates, 2006). Expression of the *Tri5* gene, which encodes DON production, occurs during crown rot infection by *Fusarium* spp., and expression is fairly constant throughout FCR development (Mudge et al., 2006). DON production by *F. culmorum* and *F. graminearum* has been linked to suppression of the chitinase gene *nag1-gox* in *Trichoderma atroviride*, a fungal biocontrol; non-DON-producing isolates do not suppress the expression of this gene, but addition of synthetic DON to non-producers reintroduces suppression of the gene (Lutz et al., 2003). The suppression of chitinases may help *Fusarium* spp. maintain control of crown stubble during overwintering and exclude competitors.

Studies examining the relationship between *C. sativus* and *Fusarium* spp. have produced inconsistent conclusions, but most report antagonism between the pathogens and suppression of *C. sativus* by *Fusarium* species. *Priori*, or the concept of "prior colonization-possession," is important to the dynamics between the two pathogens; when pathogens are inoculated in sequence, the pathogen applied first is isolated more frequently (Scardaci and Webster, 1981). Prior infection of wheat plants by *F. culmorum* and *F. acuminatum* have been observed to suppress subsequent invasion by *C. sativus*,

but no evidence has been found that *C. sativus* infection suppresses infection by *Fusarium* spp. (Tinline, 1977, Fernandez and Jefferson, 2004). In the Canadian Prairies of Saskatchewan, isolation frequency of *C. sativus* is negatively correlated with isolation frequency of *Fusarium* spp. (including *F. culmorum* and *F. equiseti*), indicating possible antagonism between the two species (Fernandez and Jefferson, 2004). Less disease in barley (i.e., reduced levels of seedling blight and root rot) is observed in plants inoculated with both *C. sativus* and *Fusarium* spp. than in plants inoculated with *Fusarium* alone (Scardaci and Webster, 1981). Reports of synergism between the pathogen complexes are rare, but it has been reported that inoculation with *F. acuminatum* enhances the severity of *C. sativus*, and that the two fungi have a greater effect together than separately (Fernandez et al., 1985).

Both *C. sativus* and *F. pseudograminearum* are documented to have a wide distribution in Montana (Moya-Elizondo et al., 2011b). Preliminary work, which utilized a single isolate each of *F. pseudograminearum* and *C. sativus*, has shown that inoculation with *F. pseudograminearum* at both high and low rates is associated with a decrease in populations of *C. sativus* based on qPCR analysis (Moya-Elizondo et al., 2011a). Similar inoculation with *C. sativus* has not been observed to reduce *Fusarium* populations; however, high native populations of *C. sativus* in the field soils prevented researchers from establishing control plots that were not infected with *C. sativus* (Moya-Elizondo et al., 2011a). The goal of this study was to address how different isolates of each species affect the dynamics between *F. pseudograminearum* and *C. sativus* in wheat in tilled Montana fields and in the greenhouse. This was assessed through the use of real-time

quantitative polymerase chain reactions to quantify fungal populations in wheat crown tissue and assessment of wheat health using several plant responses throughout the growing season.

Materials and Methods

Pathogen Isolates and Wheat Cultivar

Field and greenhouse trials were conducted to explore the population dynamics between *F. pseudograminearum* and *C. sativus.* For these trials, two isolates of *C. sativus* were used in the field, and a third was added for greenhouse trials. Isolate Cs1033 was only incorporated into greenhouse trials due to space constraints in the field locations. Four isolates of *F. pseudograminearum* were selected (see Table 2.1). All *F. pseudograminearum* isolates used in the trials were tested for deoxynivalenol (DON) production in 2008 (A.T. Dyer, *unpublished data*).

Isolates Fp2228 and Cs2344 were selected due to their prior publication in the study of population dynamics between *F. pseudograminearum* and *C. sativus* (Moya-Elizondo et al., 2011a). The other *F. pseudograminearum* isolates were chosen because they were known to be at least moderately virulent, and their DON production has been tested (A.T. Dyer, *unpublished data*). Likewise, the *C. sativus* isolates were chosen because they were known to be moderately or aggressively virulent (A.T. Dyer, *unpublished data*).

Both field and greenhouse trials were planted to the hard red spring wheat cultivar ‘McNeal’ (Montana Agricultural Experiment Station [Montana State University], 1995).

TABLE 2.1. Year collected, location and county of collection, and deoxynivalenol production of isolates of *F. pseudograminearum* and *C. sativus* which were used in greenhouse and field trials. All isolates were collected in Montana.

Species	Isolate	Year	Location	County	DON
F. pseudograminearum	Fp2228	2002	Loma	Liberty	0.55 PPM
F. pseudograminearum	Fp2234	2002	Loma	Liberty	not detected
F. pseudograminearum	Fp2278	2003	Conrad	Pondera	65.6 PPM
F. pseudograminearum	Fp2317	2004	Loma	Liberty	2.9 PPM
C. sativus	Cs1033	1994		Glacier	n/a
C. sativus	Cs2016	1997	Moccasin	Judith Basin	n/a
C. sativus	Cs2344	2007	Carter	Choteau	n/a

Field Trials

In the summer of 2014, three trials were planted to evaluate the population dynamics between several isolates of *F. pseudograminearum* and *C. sativus* as quantified at senescence. The trials were conducted at three locations centered near Bozeman, Montana; the MSU-Arthur H. Post Research Farm, the Fort Ellis Research Farm, and the Lutz Research Farm. These locations were chosen because of historical differences in average annual precipitation, temperature, and soil type. The Post Farm averaged 44 cm annual precipitation; Fort Ellis averaged 56 cm annual precipitation, and Lutz Farm averaged 40 cm annual precipitation. High temperatures from May to August at the Post Farm averaged 23.7 °C, with average lows of 8.4°C; temperatures at Fort Ellis averaged highs of 23.3°C and lows of 8.3°C. Lutz Farm averaged high temperatures of 24.4°C and lows of 7.1°C (www.ncdc.noaa.gov/cdo-web; all locations). Soil at the Post Farm is classified as an Amsterdam-Brodyk silt loam, 4-8% slopes. The Lutz Farm soil is classified as a Blackdog silt loam, 0-4% slopes, and Fort Ellis as a Blackmore silt loam, 4-8% slopes (SoilWeb: UCDavis, NRCS-USDA, University of California). At each trial location, four replicates were planted in a randomized block design.

For both fungi, oat kernel inoculum was produced and inoculated at a rate of ~18 g/row per pathogen in-furrow at planting (Mathre and Johnston, 1975). Inoculum was grown until the oats were fully colonized, and inoculum was made within 30 days of planting. The experimental unit was a four-row plot 3 m long with 30 cm centers. Rows were seeded with 'McNeal' at a rate of 150 seeds per 3-m row approximately 2.5 cm deep. Plots were planted using a Marvin Berg seeder. All of the field locations were tilled in the fall of 2013. Post Farm was planted 14 May, 2014, Fort Ellis on 15 May 2014, and Lutz Farm was planted on 21 May 2014. No fertilizer was applied at any of the field locations. Weeds were controlled at all locations by hand hoeing and through the application of broadleaf herbicides as necessary; Post Farm was sprayed with a single application of "Huskie" (Bayer CropScience) on 2 June 2014 at a rate of 1.6 L/ha (22 oz/acre), and Lutz Farm was sprayed with a single application of "Curtail" (Dow AgroSciences) on 34 June 2014 at a rate of 2.3 L/ha (32 oz/acre). Herbicides were only applied to alleyways between plots for broadleaf weed control. No herbicides were applied at Fort Ellis.

The plots were planted such that each isolate of *C. sativus* was co-inoculated with each isolate of *F. pseudograminearum* in all permutations (see Appendix A: Table 4.1 for treatments). The isolates were also inoculated singly, and each replicate included an uninoculated control, for a total of 15 treatments per replicate, and 60 plots per field location.

<u>Pre-Harvest Response Variables.</u> Plant emergence was recorded between single- and two-leaf growth stages (Feeke's growth stage 1) at 9 to 13 days after planting (DAP).

Emergence was recorded for the rightmost center row (row 3 in the plot) on 27 May 2014 at Post Farm and Fort Ellis, and 30 May at Lutz Farm. Vigor ratings were measured on 23 June 2014 at all locations, 40 DAP at Post Farm, 39 DAP at Fort Ellis, and 33 DAP at Lutz Farm, when rows had begun to close and leaves began to overlap (during the tillering stage of development and before Feeke's stem extension stages had begun). Vigor ratings were used as a measure of the overall mid-season health of the plots. The rating takes into account overall plot health, fullness of the rows, gaps in rows, and stand. Vigor ratings were assigned on a scale of 1 to 5; a score of 0 was only assigned to plots with no plants, and a 5 to a plot that exhibited no gaps, no stunting, no reduction in stand, and whose rows had begun to overlap. Counts of whiteheads, or aborted tillers, were not done due to their infrequent appearance.

Tiller counts were taken in August, 23 days prior to harvest at Post Farm, 20 days prior to harvest at Fort Ellis, and 12 days prior to harvest at Lutz Farm. All locations were at the end of flowering at the beginning of ripening when tiller counts were taken. Tiller counts were taken for 1 meter of row. Whiteheads that were empty of seed were not counted as a functional seed-bearing head.

Harvest and Sample Collection. The middle two rows of each plot were harvested with a single-row Suzue rice binder (Mitsubishi Agricultural Machinery Co., Ltd., Tokyo) and threshed using a Vogel thresher (Bill's Welding, Pullman, WA). Harvest occurred at the Post Farm on 29 August 2014, on 26 August 2014 at Fort Ellis, and on 27 August 2014 at Lutz Farm. One week after harvest, stem and crown samples were taken from all field locations. This sampling process involved collecting a random selection of

5 bundles of stems from the middle 2 rows of the plot, to provide more than 100 stem and crown samples. Plot stem samples were cleaned of soil, leaf sheaths, and leaf matter to expose the culms. When a plot's stem samples had been cleaned, they were evaluated for disease severity.

Disease severity score, or DSS, is a weighted score of crown rot disease rating based off of visual symptoms of crown rot. The rating was assigned to a sampling of crowns from the plots just after harvest had occurred. Individual tillers were rated on a scale of 1 to 4, with the main area of focus being the crown to first node of the plant. It is a standard method of measuring severity of infection in wheat (Dodman and Wildermuth, 1987, Wildermuth et al., 2001). Positive correlation between fungal biomass and DSS has been reported (Hogg et al., 2007, Knight and Sutherland, 2015). The tillers were rated: 1 = 0-25% discoloration, 2 = 25-50%, 3 = 50-75%, and 4 = 70-100% discoloration, which may extend above the first node to the second or third nodes. DSS was calculated by summing the number of tillers in each severity category and multiplying by the value of the category, then dividing by the total number of tillers scored. Observations made at the first internode have previously been correlated with yield loss and populations of *Fusarium* spp. (Hogg et al., 2007).

Greenhouse Trials

During the fall and winter of 2014, trials were conducted in the greenhouse to complement the field trials and evaluate *F. pseudograminearum* and *C. sativus* population dynamics at senescence. As in the field, McNeal wheat was planted, and the same fungal isolates were used; the sole change in pathogens being that *C. sativus* isolate

1033 was added to all experiments. The experiment was planted three times semi-concurrently, such that as one trial was at heading the next was planted. Experiment length was 107 days for trial 1, 95 days for trial 2, and 78 days for trial 3.

Pots were planted such that each isolate of *C. sativus* was co-inoculated with each isolate of *F. pseudograminearum* in all permutations. The isolates were also inoculated singly, and each replicate included an uninoculated control, for a total of 20 treatments per replicate, and 80 pots per experimental repetition.

Pot and Inoculum Preparation. Pots were 10 cm x 10 cm and held 400 g of pasteurized greenhouse soil mix (1/3 sand, 1/3 peat, and 1/3 topsoil with a wetting agent [Aquagrow 2000, Aquatrols, Cherry Hill, NJ], pH = 6.2-6.8). Seeds and oat kernel inoculum were placed 4.5 cm deep in the pot and covered with a ~ 4cm layer of autoclaved soil to reduce the potential spread of spores, and the remainder of the pot was filled with MSU mix. McNeal wheat seed was surface sterilized with 10% bleach for 60 seconds, rinsed with distilled water, and left to dry overnight prior to planting. Four seeds were placed in each pot: one near each corner.

For both fungi, oat kernel inoculum was used at planting (Mathre and Johnston, 1975). Greenhouse trials were also inoculated with 5 ml of spore suspension at a rate of 2.0 to 2.6 x 10^4 spores/ml 14 days after planting to ensure sufficient disease pressure. The spore suspension was pipetted 5mm below the soil surface and was applied such that spores were evenly distributed between the four seedlings.

Greenhouse Conditions. Photoperiod was extended to 16 hours using GE Multi-Vapor MVR1000/C/U lighting (General Electric Company, Cleveland, OH) with a daytime temperature of 25 degrees Celsius during the day and a nighttime temperature of 23 degrees Celsius.

Response Variables and Sample Collection. At the end of each trial, all surviving plants were harvested from pots by hand after they had reached heading (Feeke's growth stage 10.1). The upper stem was cut off, and leaf tissue and soil was removed from the lower stem. Stems from a single pot were bundled and dried to be prepared for DNA extraction. Yield data were not taken as the pot size used in the trials does not reliably generate usable yield or biomass data.

DNA Isolation and Sampling

To isolate *Fusarium* spp. and *Cochliobolus sativus* DNA within host crown tissue taken from the field, leaf sheath tissue was removed from the crown during cleaning and 30 stems were then randomly selected from the entire field plot sample. Three-mm stem sections were cut from the lower stem at the first internode of the stem using fabric cutters that had been modified to hold two blades to ensure precise sample size. The samples were processed following the protocol detailed by Hogg et al. (2007) using FastDNA kits (QBiogene Inc, Irvine, CA). The resulting DNA samples were diluted 1:10 with molecular-grade water prior to qPCR analysis.

For greenhouse samples, DNA extractions followed the same protocol with the exception that equal numbers of stem sections were taken from each plant in a pot to total

30 stem sections. This was necessary as there were 4 or fewer plants per pot. The same protocols and reagents were used in the DNA extractions.

Quantification of Pathogen Populations

Real-time PCR was used to quantify populations of *F. pseudograminearum* and *C. sativus* from wheat crown tissues after DNA extraction and dilution. The protocol utilized probes dual-labeled with 6-carboxyfluorescein fluorescent reporter dye and 6-carboxyltetramethylrhodamine fluorescence quencher (a TaqMan® TAMRA probe). The primer-probe combination used to quantify *F. pseudograminearum* is based on the trichodiene synthase (*tri5*) gene and has been described previously (Hogg et al., 2007, Strausbaugh et al., 2005). For *C. sativus*, the primer-probe combination is based on the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene, which has been described previously (Moya-Elizondo et al., 2011a).

All qPCR assays were conducted using the Bio-Rad CFX Connect Real-Time System (BioRad USA) and a two-step protocol with the following cycle parameters: 55°C for 2 minutes, 94°C for 10 minutes, followed by 43 cycles of 15 seconds at 94°C and 62.0°C for 60 seconds. The 25µl qPCR reaction contained 12.5 µl of Universal TaqMan Master Mix (Applied Biosystems, Carlsbad, CA), 2.5 µl of 2 µM TaqMan probe, 2.25 µl of a 1:1 mixture of 20 µM forward and reverse primers (Integrated DNA Technologies, Inc. Coralville, IA), 2.75 µl of molecular-grade water, and 5 µl of DNA sample. Each plate included positive controls consisting of mycelial DNA extracted from either *F. pseudograminearum* or *C. sativus* grown on potato dextrose agar and V8 agar, respectively. Each plate also included negative controls consisting of DNA from

Cephalosporium gramineum and the competitor fungus (e.g., *C. sativus* on *tri5* plates) to verify that master mix contamination had not occurred. Each run also included three replicates of five DNA standards, a dilution series ranging from 10^2 to 10^6 copies. Standard curves produced by plotting the log of concentration of the DNA standards versus the cycle threshold values showed strong relationships (R^2 values always exceeded 0.990). For *F. pseudograminearum*, the DNA standards were derived from a cloned fragment of the *tri5* gene taken from *F. culmorum* 2223 (Hogg et al., 2007). For *C. sativus*, the standards were cloned fragments of the *gpd* gene from *C. sativus* 2344 (Moya-Elizondo et al., 2011a). All unknown field DNA reactions were replicated three times, and mean population values were averaged between the three technical replicates. For quality control among technical replicates, the standard deviation between *Cq* values of the three technical replicates, or the number of cycles it took for a sample to amplify above the detection threshold of fluorescence, was examined to ensure that standard deviation did not exceed 0.5 cycles. Excessive variation in *Cq* values was not an issue. DNA samples were not augmented with internal controls to test for inhibition because prior work has documented no evidence of inhibition of qPCR reactions using DNA extracted in the method detailed in the "DNA Isolation and Sampling" section (Hogg et al., 2009, Moya-Elizondo et al., 2011b, Moya-Elizondo et al., 2011a). The quantification process followed the protocol detailed by Moya et al. (Moya-Elizondo et al., 2011b). For analysis purposes, DNA copy numbers of the *gpd* and *tri5* genes were interpreted as the quantified crown population of *C. sativus* and the *Fusarium roseum* group (*F. culmorum*, *F. graminearum*, and *F. pseudograminearum)*, respectively.

Statistical Analyses

Analyses of field trials were conducted using linear models which included location (Post Farm, Fort Ellis, and Lutz Farm), *F. pseudograminearum* isolate, *C. sativus* isolate, and interactions between these three factors with regard to emergence, vigor, disease severity score, tiller count, yield, and fungal populations. Multifactor ANOVAs were performed R Studio (version 0.98.932 [based on R i386 version 3.0.2]). Location was always a significant factor that affected response variables ($P < 0.05$) and was always included in the statistical models. For all response variables except yield, locations were analyzed together to generate a larger picture of pathogen dynamics, rather than location-specific dynamics. Although they did not suffer from a lack of homogeneity of variance, yield was not combined between the three locations because mean yield was substantially different (yield values at Post Farm were threefold greater than at Fort Ellis and Lutz Farm) and therefore had the potential to mask disease dynamics which affected yield at each location. No such differences were observed for other responses.

Fisher's protected least significant difference (LSD) ($\alpha < 0.05$) was utilized to compare response variable means. Population values were log-transformed after adding 1 to the values, as some of the populations had zero values, prior to analysis. For population quantifications, *t*-tests in place of using LSD values; like the Fisher's protected LSDs, *t*-tests were only used when *P* values indicated that this was appropriate. Other response variables were not transformed for analysis as their residuals graphs did not show a need for transformation. Chi-squared (χ^2) tests of homogeneity ($\alpha < 0.05$)

were conducted to verify that there was no evidence of a violation of homogeneity of variance prior to combining trial data (Ramsey and Schafer, 2002). Regression analysis was conducted using ARC software (University of Minnesota) to analyze the relationship between pathogen populations. During modeling with ARC, response variables underwent normalizing transformations based on Box-Cox analyses.

The three greenhouse trials were analyzed separately, due to violation of the assumption of homogeneity of variance (Chi-squared tests of homogeneity ($\alpha \leq 0.05$)). The three trials were first analyzed using regression analysis in ARC software to determine the effect of pathogen population on the opposing pathogen populations. None of the trials exhibited evidence that population size had an effect on the opposing pathogen's populations, and the rest of the analyses were conducted using linear models to generate multifactor ANOVAs in R Studio, as with the field data. Population values were log-transformed after adding 1 to the values, as some of the populations had zero values, prior to analysis. Emergence values were not transformed. As with the field trials, Fisher's protected least significant difference (LSD) ($\alpha < 0.05$) was utilized to compare response variable means when ANOVA results indicated significant differences. For qPCR population quantifications, *t*-tests were used to do pairwise comparison of the means between isolates.

Results

Field Trials

There were significant differences in yield among locations ($P < 0.001$), with the greatest yields occurring at the Post Farm (mean = 67.38 bu/acre), and significantly lesser yields occurring at Fort Ellis and Lutz Farm (mean = 26.60 bu/acre, 25.89 bu/acre, respectively). Yields at Fort Ellis and Lutz Farm were not significantly different from each other. There was no block effect for yield at Post Farm or Fort Ellis. There was a significant effect of block for yield at Lutz Farm ($P = 0.001$), likely due to elk damage that affected some blocks more than others. Location was a significant factor in all other response variables ($P < 0.001$ for emergence, vigor, tiller count, and disease severity score; $P = 0.009$ for *C. sativus* copy number), with the exception of *Fusarium* copy number, although there was weak evidence for a location effect on that response as well ($P = 0.057$). Block did not significantly affect these measurements at any of the locations ($P > 0.05$).

TABLE 2.2: Effect of inoculation by *F. pseudograminearum* isolates on vigor and yield at Post Farm, Fort Ellis, and Lutz Farm field trials. Yields were analyzed separately for each location, and vigor values show a location by isolate interaction effect.

Isolate	Vigor			Yield (bu/acre)		
	Post	Ellis	Lutz	Post	Ellis	Lutz
Uninoculated control	4.88_{A}	4.75_{A}	4.50_{AB}	77.78_{A}	28.28_{A}	24.60_{A}
Fp2228	2.79_{F}	4.17_{BC}	3.92_{CD}	62.09_{C}	27.07_{A}	25.38_{A}
Fp2234	3.63_{DE}	4.17_{BC}	3.88_{CD}	68.14_{B}	24.81_{A}	26.82_{A}
Fp2278	3.63_{DE}	4.17_{BC}	4.04_{C}	68.97_{BC}	26.74_{A}	29.03_{A}
Fp2317	3.42_{E}	3.58_{E}	3.58_{E}	65.44_{BC}	27.83_{A}	22.95_{A}
Fisher's LSD ($\alpha = 0.05$)		0.39		5.12	---	---

Response variables that share a letter indicate no significant difference at ($\alpha = 0.05$)

Yield. Inoculation with *F. pseudograminearum* significantly affected yield at the Post Farm ($P = 0.004$), where all four isolates significantly reduced yield compared to the uninoculated control (see Table 2.2); average loss to FCR at that location was 8%. Inoculation with *F. pseudograminearum* did not reduce yield at Fort Ellis or Lutz Farm ($P = 0.54$, $P = 0.074$, respectively). Inoculation with *C. sativus* significantly affected yield at the Post Farm (11% loss to FCR) and Fort Ellis ($P < 0.001$, $P = 0.047$, respectively) (9% loss to FCR). At both locations, both isolates significantly reduced yield compared to the uninoculated control (see Table 2.3). Inoculation with *C. sativus* did not reduce yield at Lutz Farm ($P = 0.51$). Regression analysis did not establish

TABLE 2.3: Effect of inoculation by *C. sativus* isolates on emergence, vigor, and yield at Post Farm, Fort Ellis, and Lutz Farm field trials. Yields were analyzed separately for each location, and emergence and vigor values show a location by isolate interaction effect.

Isolate	Emergence			Vigor			Yield (bu/acre)		
	Post	Ellis	Lutz	Post	Ellis	Lutz	Post	Ellis	Lutz
Uninoculated control	43.50_{D}	75.50_{B}	92.50_{A}	4.88_{A}	4.75_{AB}	4.50_{B}	77.78_{A}	28.28_{A}	24.60_{A}
Cs2016	66.35_{BC}	57.25_{C}	58.60_{C}	2.85_{F}	3.73_{CD}	3.50_{E}	62.63_{B}	25.25_{B}	24.92_{A}
Cs2344	60.45_{C}	64.55_{C}	65.90_{C}	3.33_{E}	3.95_{C}	3.63_{DE}	66.39_{B}	25.90_{B}	26.79_{A}
Fisher's LSD ($\alpha = 0.05$)		9.27			0.30		3.97	2.84	--

Response variables that share a letter indicate no significant difference at ($\alpha = 0.05$)

TABLE 2.4: Effect of inoculation with *C. sativus* isolates on plant responses and on qPCR copy numbers of *Fusarium* spp. and *C. sativus* in field trials. Data from three field locations was combined

Isolate	Emergence	Vigor	Tiller Count	Dis. severity score	*Fusarium* copy	*Cochliobolus* copy
No *C. sativus*	70.50_{A}	4.71_{A}	77.75_{A}	1.48_{A}	102493_{A}	1280_{A}
Cs2016	60.77_{B}	3.36_{C}	78.75_{A}	1.54_{A}	122160_{A}	1872_{B}
Cs2344	63.63_{B}	3.63_{B}	76.13_{A}	1.55_{A}	81939_{B}	3040_{B}
Fisher's LSD ($\alpha = 0.05$)	5.35	0.17	--	--	-- [Z]	-- [Z]

Response variables that share a letter indicate no significant difference at ($\alpha = 0.05$)
[Z] = Letter designations for these response variables were generated using contrasts instead of LSD values, and were different at a statistical level of $P < 0.05$

evidence of pathogen populations correlating significantly with yield.

Emergence and Vigor. Both *C. sativus* isolates reduced emergence and vigor compared to the uninoculated control at the combined locations (both $P < 0.001$) (see Table 2.4). Isolate Cs2016 reduced vigor significantly more than isolate Cs2344 according to Fisher's protected LSD values (LSD = 0.17, $\alpha < 0.05$), and both isolates reduced emergence equally (LSD = 5.35). Inoculation with *F. pseudograminearum* reduced vigor as well ($P < 0.001$) (see Table 2.5). All four *F. pseudograminearum* isolates reduced vigor; isolates Fp2228 and Fp2317 reduced vigor significantly more than isolates Fp2234 and Fp2278 according to LSD values (LSD = 0.23). None of the isolates of *F. pseudograminearum* had a significant effect on emergence.

Pathogen Populations. In spite of high indigenous populations of *C. sativus* in-field, both isolates of *C. sativus* significantly increased populations compared to the uninoculated control; isolates Cs2016 and Cs2344 increased populations 1.5 times and

TABLE 2.5: Effect of inoculation with *F. pseudograminearum* isolates on plant responses and on qPCR copy numbers of *Fusarium* spp. and *C. sativus* in field trials. Data from three field locations was combined

Isolate	Emergence	Vigor	Tiller Count	Dis. severity score	*Fusarium* copy	*Cochliobolus* copy
No *F. pseudogram.*	70.50_A	4.71_A	77.75_A	1.48_A	7392_A	2095_A
Fp2228	66.25_A	3.60_C	74.83_A	1.80_B	299187_D	1260_B
Fp2234	69.58_A	3.89_B	79.89_A	1.51_A	117306_C	2922_A
Fp2278	66.47_A	3.94_B	76.94_A	1.46_A	33163_B	1829_A
Fp2317	63.00_A	3.53_C	79.92_A	1.49_A	53938_C	2215_A
Fisher's LSD ($\alpha = 0.05$)	**---**	**0.23**	**--**	**0.097**	**--** [z]	**--** [z]

Response variables that share a letter indicate no significant difference at ($\alpha = 0.05$)
[z] = Letter designations for these response variables were generated using contrasts instead of LSD values, and were different at a statistical level of $P < 0.05$

2.4 times greater, respectively, than the populations in plots not inoculated with *C. sativus* ($P = 0.006$, $P < 0.001$, respectively) (see Table 2.4). Inoculation with *C. sativus* also had a significant effect on *Fusarium* populations ($P = 0.036$). Inoculation with isolate Cs2344 reduced *Fusarium* copy number to populations 2.6 times less than plots not inoculated with *C. sativus* ($P = 0.014$) (see Table 2.4). Isolate Cs2016 did not affect *Fusarium* populations ($P = 0.57$). Based on regression analysis, correlation of populations of *C. sativus* and *Fusarium* spp. was negligible and better explained by other factors ($R^2 = 0.018$, $P = 0.075$).

Inoculation with *F. pseudograminearum* increased populations of *Fusarium* isolated from crown samples ($P < 0.001$). In-field levels of *Fusarium* spp., as indicated by the uninoculated control, averaged 11,778 copies; inoculation with isolates Fp2228, Fp2234, Fp2278, and Fp2317 significantly increased mean populations compared to plots not inoculated with *F. pseudograminearum* ($P < 0.001$, all) (see Table 2.5). Inoculation with *F. pseudograminearum* also had a significant negative effect on *C. sativus* populations ($P = 0.034$). Inoculation with Fp2228 reduced *C. sativus* populations compared to plots not inoculated with *F. pseudograminearum* ($P = 0.002$) (see Table 2.5). *C. sativus* populations were not reduced by isolates Fp2234 ($P = 0.46$), Fp2278 ($P = 0.42$), or Fp2317 ($P = 0.25$).

<u>Disease Severity Score</u>. A significant effect was observed between the *F. pseudograminearum* isolates on disease severity score (DSS) ($P < 0.001$). Isolate Fp2228 significantly increased DSS to 1.80 in comparison to 1.45 for the uninoculated control to (see Table 2.5). None of the other *F. pseudograminearum* isolates significantly

affected disease severity score. Overall, *Fusarium* populations were positively correlated with an increase in disease severity score ($R^2 = 0.14$, $P < 0.001$). None of the isolates of *C. sativus* had a significant effect on disease severity score.

Tiller Count. Tiller count correlated significantly with yield ($R^2 = 0.16$, $P < 0.001$). Tiller count had a small negative correlation with *Fusarium* population ($R^2 = 0.024$, $P = 0.039$) but was not affected by inoculation with *C. sativus* ($P = 0.30$) (see Table 2.4) or by *C. sativus* population ($R^2 = 0.007$, $P = 0.25$).

Isolate by Location Interactions. A significant interaction effect was observed between *C. sativus* isolate and location in emergence ($P < 0.001$) and vigor ($P = 0.006$) (see Figures 2.1A and 2.1B). In general, similar patterns of isolate performance were observed with regard to emergence and vigor, with some isolates performing better at one location than others. At Post Farm, emergence of the uninoculated controls was poor,

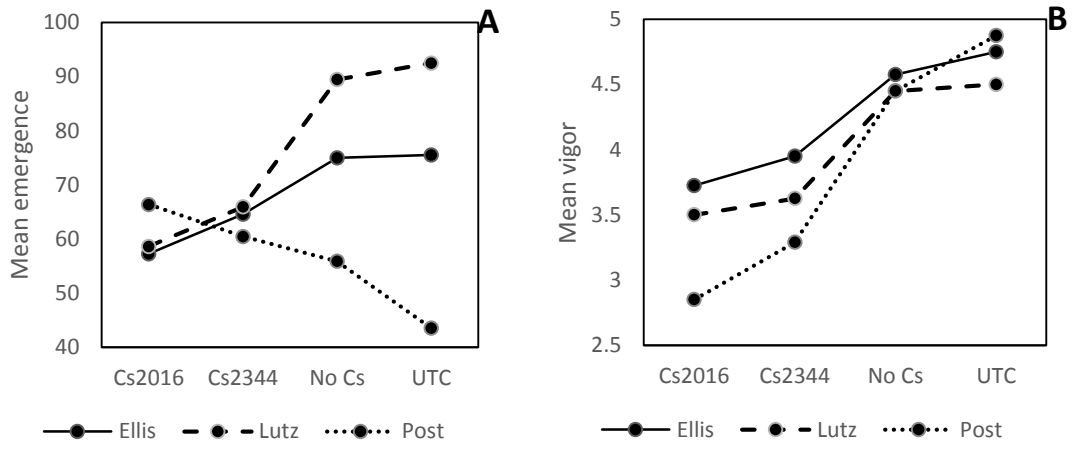

Fig. 2.1. Interaction effect between *C. sativus* isolate and location. The effect of inoculation with *C. sativus* isolates varied between locations and affected **A**, emergence ($P < 0.001$) and **B**, vigor ($P = 0.006$) in the field.

and this contributed differing dynamics between the locations.

Similarly, a significant interaction effect that affected vigor was observed between *F. pseudograminearum* isolate and location ($P < 0.001$) (see Figure 2.2). The overall pattern of isolate performance was not changed, but some isolates performed better at one location than another.

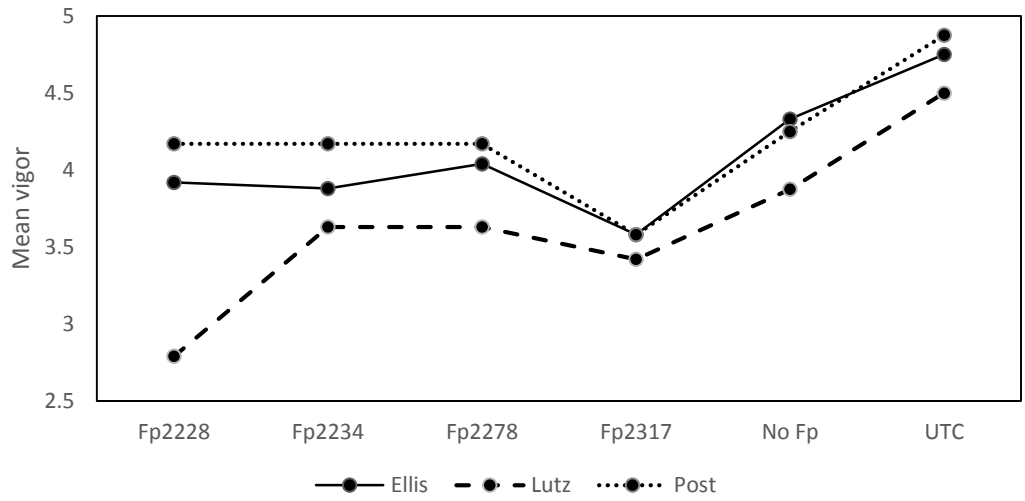

Fig. 2.2. Interaction effect between *F.pseudograminearum* isolate and location. The effect of inoculation with *F. pseudograminearum* isolates varied between locations and affected vigor ($P < 0.001$) in the field.

Greenhouse Trials

Block only had a significant effect on *C. sativus* copy numbers in greenhouse trial 1 ($P = 0.005$), possibly due to insufficient height of the surrounding border rows to reduce edge effects. No other significant block effects occurred in any of the trials (for table of *P* values, see Appendix A: Table 4.2).

Inoculation with *F. pseudograminearum* significantly increased *Fusarium* populations in all three trials ($P < 0.001$, trials 1 and 3; $P = 0.002$, trial 2) (see Table 2.6).

Of the *F. pseudograminearum* isolates, Fp2228 increased *Fusarium* spp. populations the most in all three trials ($P < 0.001$, all), from 643 copies in pots not inoculated with *F. pseudograminearum* to 37,909 copies in trial 1, from 4 copies to 2,808 copies in trial 2, and from 0 to 127,568 copies in trial 3. Isolate Fp2317 also significantly increased *Fusarium* spp. populations in all three trials ($P < 0.001$, trials 1 and 3; $P = 0.003$, trial 2) from 643, 4, and 0 copies in pots not inoculated with *F. pseudograminearum* in trials 1, 2, and 3, respectively, to 30,316 copies in trial 1, 2,168 copies in trial 2, and 199,764 copies in trial 3. Isolate Fp2234 had a weak effect, but significantly increased *Fusarium* spp. populations in two trials ($P = 0.002$ trial 1; $P = 0.002$, trial 3), increasing populations to 2,591 copies in trial 1 and 4,731 copies in trial 3, and approached significant increases in the third trial ($P = 0.063$, trial 1), increasing populations to 277 copies. Isolate Fp2278 only significantly increased *Fusarium* spp. populations in one trial ($P = 0.014$, trial 1), to 11,939 copies, and had a questionable or negligible effect in the other two trials ($P = 0.059$, trial 2; $P = 0.28$, trial 3). Populations of *Fusarium* spp. were approximately tenfold lower than those recorded in the field, although this varied by isolate.

Inoculation with *F. pseudograminearum* isolates did not have a significant effect on *C. sativus* populations in any of the greenhouse trials ($P = 0.35$, trial 1; $P = 0.47$, trial 2; $P = 0.36$, trial 3) (see Table 2.6). As inoculations with several *C. sativus* isolates largely did not increase *C. sativus* populations, however, this was only part of a larger problem with low *C. sativus* copy numbers.

TABLE 2.6: Effect of inoculation with *F. pseudograminearum* isolates on *Fusarium* spp. and *C. sativus* qPCR copy numbers in three greenhouse trials.

Isolate	Trial 1		Trial 2		Trial 3	
	Fusarium	*C.sativus*	*Fusarium*	*C.sativus*	*Fusarium*	*C.sativus*
No *F. pseudo.*	643_{A}	4105	4_{A}	100	1_{A}	200
Fp2228	37909_{C}	1642	2808_{D}	341	127568_{B}	369
Fp2234	2591_{B}	3339	277_{BC}	211	4731_{B}	113
Fp2278	11939_{B}	2618	2248_{BC}	283	$7510\ ^{z}{}_{A}$	283
Fp2317	30316_{C}	2059	2168_{CD}	172	199764_{B}	277

Response variables that share a letter indicate no significant difference at ($P \leq 0.05$)

While inoculation with *F. pseudograminearum* isolates did not significantly reduce *C. sativus* copy numbers overall, some interactions between individual isolates were observed which resulted in the suppression of *C. sativus* populations by *F. pseudograminearum* isolates. In one of the trials, a statistical interaction effect between

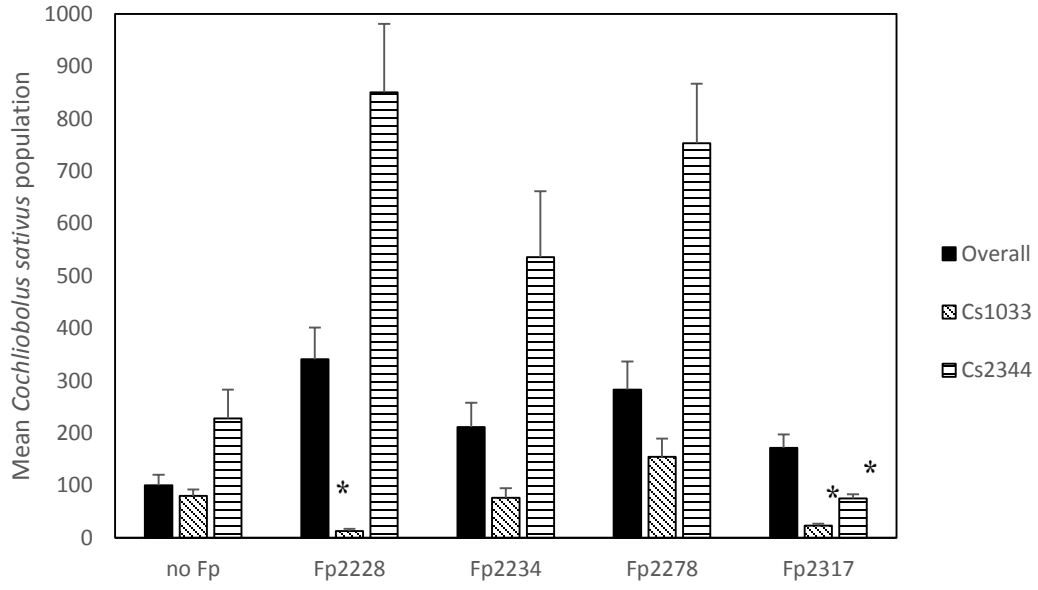

Fig. 2.3 Antagonistic interaction between isolates of *F. pseudograminearum* and *C. sativus*, which affected *C. sativus* qPCR copy number in greenhouse trial 2. Significant ($P < 0.05$) interactions are starred (*). Significance was determined through use of contrasts.

F. pseudograminearum and *C. sativus* isolates, affecting *C. sativus* copy number, was observed (P = 0.015, trial 2). Significant antagonistic interactions were observed between Cs1033 and Fp2228 (P = 0.007), Cs1033 and Fp2317 (P = 0.013), and Cs2344 and Fp2317 (P = 0.023) (see Figure 2.3), in which *C. sativus* populations were suppressed by *F. pseudograminearum* isolates.

Inoculation significantly increased *C. sativus* populations in two of the three trials (P = 0.14, trial 1; P < 0.001, trial 2; P = 0.009, trial 3) (see Table 2.7). Only inoculation with Cs2344 significantly increased populations of *C. sativus* (P = 0.035, trial 2; P = 0.0012, trial 3). Isolate Cs2344 increased populations from 156 copies in pots not inoculated with *C. sativus* to 488 copies in trial 2, and from 168 copies in pots not inoculated with *C. sativus* to 458 in trial 3. Isolate Cs1033 did not significantly increase populations (P = 0.096, trial 2; P = 0.32, trial 3). Isolate Cs2016 did not significantly increase populations (P = 0.66, trial 2, P = 0.094, trial 3). Populations of *C. sativus* in the greenhouse were approximately tenfold lower than those recorded in the field.

Due to the high numbers of *C. sativus* in uninoculated controls and generally unsuccessful inoculations of that fungus, a sampling of McNeal seed was plated on V8 and water agar to detect the presence of *C. sativus* contamination in or on seed. On plates

TABLE 2.7: Effect of inoculation with *C. sativus* isolates on *Fusarium* spp. and *C. sativus* qPCR copy numbers in three greenhouse trials.

Isolate	Trial 1		Trial 2		Trial 3	
	Fusarium spp.	*C.sativus*	*Fusarium* spp.	*C.sativus*	*Fusarium* spp.	*C.sativus*
No *C. sativus*	9782_A	1426	1472	156_A	40523_A	168_A
Cs1033	7459_B	4436	4331	69_A	69760_A	200_A
Cs2016	21719_A	3692	75	172_A	76430_A	167_A
Cs2344	167_C	416	126	488_B	40_B	458_B

Response variables that share a letter indicate no significant difference at ($P \leq 0.05$)

sterilized in bleach for 3 and for 4 minutes, *C. sativus* grew out of the seed, indicating seed contamination by the fungus.

In two trials, *C. sativus* isolates had substantial effect on *Fusarium* spp. copy number ($P < 0.001$, trial 1; $P = 0.048$, trial 3) (see Table 2.7). The *C. sativus* isolates did not affect *Fusarium* spp. copy number in trial 2 ($P = 0.80$). Isolate Cs2344 reduced populations in both trials where *C. sativus* affected *Fusarium* spp. populations ($P < 0.001$, trial 1; $P = 0.015$, trial 3). Isolate Cs2344 reduced mean *Fusarium* spp. populations from 9,782 copies in pots not inoculated with *C. sativus* to 167 copies in trial 1, and from 40,523 copies in pots not inoculated with *C. sativus* to 40 copies in trial 3. Isolate Cs1033 significantly reduced mean *Fusarium* spp. populations in one trial ($P = 0.014$, trial 1), from 9,782 copies in pots not inoculated with *C. sativus* to 7,459 copies. The effect of Cs1033 was significantly less than the effect of Cs2344 ($P = 0.05$).

A statistical interaction effect occurred between *F. pseudograminearum* and *C. sativus* isolates, affecting *Fusarium* spp. copy number, in one greenhouse trial ($P = 0.035$, trial 1), and was close to significance in another ($P = 0.055$, trial 3). Because an interaction effect was present, *t* tests were performed between the individual isolate pairs of both species, and in trial 1, antagonisms were observed between Fp2317 and Cs1033 ($P = 0.008$), Fp2228 and Cs2344 ($P = 0.012$), and Fp2234 and Cs2016 ($P = 0.002$). In that trial, the antagonism between Fp2234 and Cs2016 was due to the *F. pseudograminearum* isolate being suppressed by an otherwise non-suppressive isolate Cs2016 (*data not shown*). The other two interactions were due to the *F. pseudograminearum* isolate being suppressed more by the suppressive isolate than the

other isolates were (see Figure 2.4). There may also have been an interaction between isolates Fp2317 and Cs2344 in trial 3 (P = 0.022), in which Fp2317 was suppressed more by the suppressive isolate Cs2344 than the other isolates were (*data not shown*).

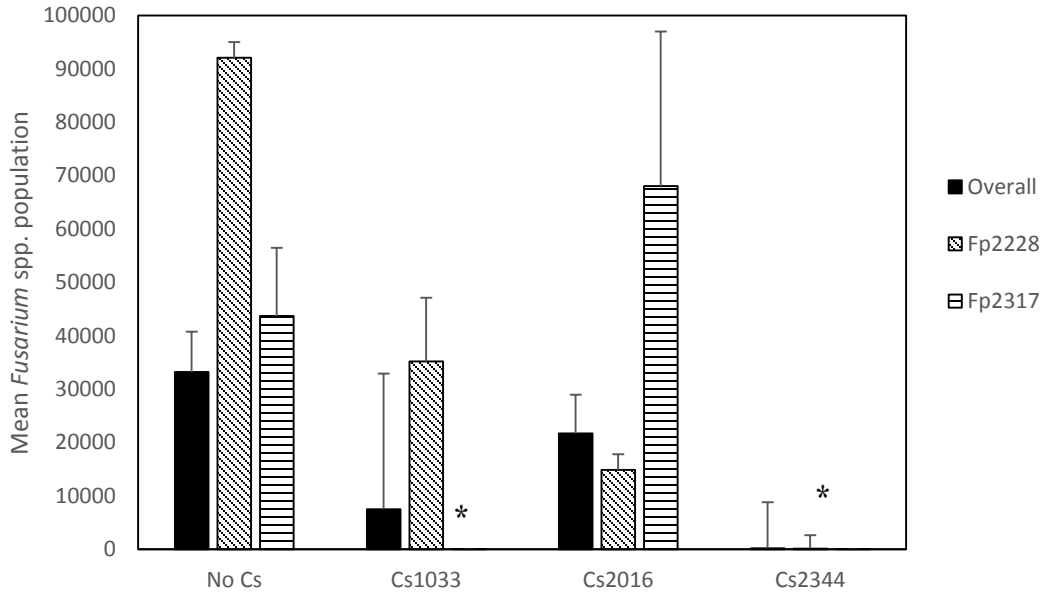

Fig. 2.4. Antagonistic interaction between isolates of *F. pseudograminearum* and *C. sativus*, which affected *Fusarium* spp. qPCR copy number in greenhouse trial 1. Significant (P < 0.05) interactions are starred (*). Significance was determined through use of contrasts.

Regression analysis of fungal populations in ARC provided no evidence that the fungal populations correlated significantly (R^2 = 0.011, P = 0.36, trial 1; R^2 = 0.010, P = 0.80, trial 2; R^2 = 0.021, P = 0.20, trial 3).

Discussion

This research is the first to demonstrate the ability of *C. sativus* to suppress *F. pseudograminearum* populations. This suppression was demonstrated both in greenhouse and field trials. This marks a stark contrast with previous research that had repeatedly

demonstrated no suppression by *C. sativus* (Tinline, 1977, Fernandez and Jefferson, 2004, Moya-Elizondo et al., 2011a). However, prior research has reported that co-inoculation with these pathogens results in less disease overall than is present with either pathogen inoculated alone (Scardaci and Webster, 1981). The inconsistency between studies may be explained by the fact that only one isolate of *C. sativus* was consistently suppressive (isolate Cs2344). Other isolates were either inconsistently suppressive or non-suppressive. In particular, *C. sativus* isolate Cs2344, which was used in prior research (Moya-Elizondo et al., 2011a), was found to be suppressive, and isolate Cs1033 was found to be somewhat suppressive in the greenhouse.

This study confirmed findings that inoculations with *F. pseudograminearum* isolate Fp2228 reduces *C. sativus* populations (Moya-Elizondo et al., 2011a). In addition, it identified significant variability among isolates of *F. pseudograminearum* in their ability to suppress. Specifically, isolate Fp2228 was significantly suppressive of *C. sativus,* and isolate Fp2317 demonstrated some capacity for isolate-specific suppression in the greenhouse, while isolates Fp2278 and Fp2234 did not. This isolate-specific suppression was more prominent in the greenhouse where trial conditions were well-controlled. The lack of strong support for a suppressive effect by *F. pseudograminearum* on *C. sativus* populations in the greenhouse can be explained by the fact that *C. sativus* isolates inconsistently increased populations of *C. sativus* with inoculation due to prior presence on seeds.

Significant variability in both the ability of isolates to suppress and to be suppressed were observed. For the latter in particular, in the greenhouse, isolate Cs2344

was only suppressed by the suppressive *F. pseudograminearum* isolate Fp2317. These results may explain much of the inconsistency in dynamics observed in the literature (Ledingham, 1942, Fernandez et al., 1985, Fernandez and Jefferson, 2004). The results may also provide prime opportunities for exploring the mechanisms of suppression, as both positive (suppressive) and negative (non-suppressive) controls are available. Given the variability in chemotypes for both species (A.T.Dyer, *unpublished data*), it is possible that antibiosis may be the mechanism of the observed antagonisms. Such a mechanism may provide an unexpected sources of control in the future.

The mechanisms by which *F. pseudograminearum* and *C. sativus* suppress one another are currently unknown. Some antibiosis was observed *in vitro* through the generation of inhibition zones by the suppressive *C. sativus* isolates, Cs1033 and Cs2344, which suppressed the growth of *F. pseudograminearum* isolates (*data not shown*). The non-suppressive Cs2016 did not generate a similar inhibition zone on corn meal agar. It is possible that antibiosis might not be observed between mycelium of *C. sativus* and *F. pseudograminearum* on other media, such as V8 media (*data not shown*) or on ¼ strength PDA (Singh et al., 2009). *Fusarium culmorum* has been observed *in vitro* to suppress the germination of conidia of *C. sativus* via unknown mechanisms (Ledingham, 1942*)*, and DON production by *Fusarium* spp. has also been associated with an antagonistic effect on chitinase gene expression (Lutz et al., 2003). In wheat, inoculation with *F. culmorum* or *F. graminearum* may prevent later infections by *C. sativus* (Tinline, 1977, Scardaci and Webster, 1981).

The lack of correlation between populations of the two pathogens suggests that zero sum dynamics may not play a significant role in the antagonisms described. This should be taken with some note of caution, as antagonisms were more pronounced for isolates that were also more aggressive pathogens (i.e., they produced larger populations in the host). Some evidence of resource competition has been reported (Fernandez and Jefferson, 2004). The lack of correlation, in addition to the observation on isolate aggressiveness, may indicate that resource limitation was not achieved across the plot as a whole, but may have been reached for individual plants within the bulked samples. To address this issue, examination of populations in individual plants, or even tillers, may be necessary. In the field there is little information about population densities. Previous studies within Montana have shown average infection rates for *C. sativus* at 15%, and 8% for *F. pseudograminearum* (Moya-Elizondo et al., 2011b), although this is undoubtedly significantly higher for some fields.

Because pathogen inoculations in the field were successful, a higher level of infection was observed in inoculated plots than in the uninoculated controls. This suggests that the competition between *C. sativus* and *F. pseudograminearum* in growers' fields may be less intense than those observed in inoculated fields, and it is important to note that these results must be interpreted with the typical grower's field in mind. However, based on a 2011 survey of the major wheat-growing areas of Montana, *Fusarium* spp. and *C. sativus* populations for the current trials were below levels recorded in growers' fields, even when the current trials were inoculated (Moya-Elizondo

et al., 2011b). Therefore, these dynamics may be representative of growers' fields that have high pathogen populations.

In the field, inoculation with all isolates of *F. pseudograminearum* and *C. sativus* reduced seedling emergence compared to uninoculated controls. Isolates of *C. sativus* had a slightly greater effect on emergence than isolates of *F. pseudograminearum*. No antagonistic interaction was observed in which emergence was greater in the presence of both fungi (*data not shown*), which agrees with the 2011 findings by Moya et al. and classic mixed-inoculation experiments dating back to the 1930s (Moya-Elizondo et al., 2011a, Hynes, 1935, Hynes, 1938). These published findings contradict those of others, in that co-inoculations with both *C. sativus* and *F. culmorum* reduced emergence less than the reduction in emergence from single inoculations (Ledingham, 1942). Only *C. sativus* reduced yield at both locations (9-11% yield loss to CRR); *F. pseudograminearum* only reduced yield at the Post Farm (8% yield loss to FCR). Yields at Lutz Farm did not show an inoculation effect due to elk damaging the crop just prior to harvest. In general, at the three locations in 2014, both *C. sativus* and *F. pseudograminearum* were effective as early- and late-season pathogens, but *C. sativus* had a greater effect both early and late in the season (*data not shown*). Crown rot severity was only significantly affected by *F. pseudograminearum* isolate Fp2228, which also generated the largest *Fusarium* populations in wheat tissues.

The findings presented here largely agree with the 2011 publication which this research builds upon (Moya-Elizondo et al., 2011a). In this study and the 2011 study, location had significant effect on yield, seedling emergence, and fungal qPCR copy

numbers. Inoculation with *F. pseudograminearum* and *C. sativus* significantly reduced seedling emergence in this study and the 2011 study, and no significant reduction in disease was observed in co-inoculated plots for either study. This study had more successful inoculations than the 2011 study, however, in that inoculation with both fungi increased their qPCR copy numbers in the field, and that inoculation with *F. pseudograminearum* reduced yields at one location, and *C. sativus* reduced yields at two locations. Conversely, *C. sativus* copy numbers were not significantly increased by inoculation in the 2011 study, and inoculation with *C. sativus* did not significantly affect yield in any of the field-years that study was conducted (Moya-Elizondo et al., 2011b).

The pathogen dynamics studied in this research were limited to the crown tissue of plants below the first node, and dynamics within different parts of the plant were not analyzed in this study or our previous work (Moya-Elizondo et al., 2011a). FCR symptoms may extend several internodes into the stem (Cook, 2010), and *Fusarium* spp. may dominate higher in the stem. In contrast, CRR is associated with infection through and discoloration of the subcrown internode (Piening, 1997, Tinline, 1977, Fernandez and Jefferson, 2004), and *C. sativus* may more effectively dominate in the subcrown internode compared to the crown. While sampling of the subcrown internode is notoriously difficult as the internode may not always be present, particularly if the internode is heavily diseased, an analysis of the fungal populations in the subcrown internode and above-crown tissue compared to the crown would illuminate the spatial dynamics of the pathogens more clearly.

In conclusion, the interaction between *C. sativus* and *F. pseudograminearum* in spring wheat is a more dynamic process than previously reported. This study specifically identifies isolate variability as a key factor in suppressiveness, or the lack thereof, between the species. Therefore, it could explain much of the inconsistencies in the literature (Moya-Elizondo et al., 2011a, Fernandez and Jefferson, 2004, Ledingham, 1942, Fernandez et al., 1985). Zero sum dynamics were not observed within this study, but this may be due to scale of focus and observations at a smaller scale might identify a resource limited effect. The variability across isolates provides exciting opportunities to explore the mechanisms of suppression. Our preliminary observations *in vitro* have shown there is variability among isolates in their ability to inhibit one another that may warrant further exploration. This study, along with previous research (Moya-Elizondo et al., 2011a), suggests that antagonism is a complicating factor that may need to be addressed in any crown rot management program in order to obtain effective control. Given that complex pathogen communities exist in the wheat rhizosphere, additional research involving other crown rot pathogens is warranted.

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CHAPTER 3

COMMUNITY DYNAMICS OF SPRING WHEAT RHIZOSPHERE PATHOGENS

Introduction

Examination of fungal pathogen dynamics in soil is a necessary, albeit messy, process. However, given that 25-30% of wheat crops worldwide are lost to disease and insect pests, with fungi being the most abundant plant pathogens (Bikram, 2010), the improvement of agriculture efficiency worldwide drives the need to understand how pathogens interact in their environment. In some fungal communities, interactions are fairly well-classified; for example, antagonistic interactions between wood-rotting fungi are frequent and well-documented (Boddy, 2000). However, this is not necessarily the case in soil environments. Fungal interactions may involve toxins or antibiotics, mycoparasitism, or hyphal interference in which hyphal death occurs upon contact with an antagonist. Synergism has been historically considered to be uncommon. Meta-analysis of published pathogen on pathogen interactions indicates that 74% are independent, 22% are antagonistic, and just 4% are synergistic (Stephens et al., 2013). Attack on the same plant part at the same increases the likelihood of interactions (Stephens et al., 2013). It is possible that the consequences of these interactions could be the overall suppression of pathogens in a disease complex. If antagonism occurs in a pathogen community, control of a single or multiple pathogens (e.g., via resistance) may have substantial, non-intuitive consequences on plant health. For instance, suppression of one pathogen might increase overall disease, if that particular pathogen is a significant

suppressor of other pathogens within the community. Conversely, if a community member works synergistically with other pathogens, its removal might substantially improve plant health more than one would expect. For these reasons, it could be important to elucidate the interactions among pathogens within a disease complex.

In Montana and the Pacific Northwest, the dryland root rot complex (DRRC) is a common biological constraint on small grain production (Smiley and Patterson, 1996, Moya-Elizondo et al., 2011b, Paulitz et al., 2002). It is caused by a complex of pathogens that are commonly co-isolated from affected plants. Five of the most common members of this complex are: *Fusarium pseudograminearum* (O'Donnel & T.Aoki; group I) (= *Gibberella coronicola*), the causal agent of Fusarium crown rot (FCR); *Cochliobolus sativus* (S.Ito & Kuribayashi) Drechsler ex Dastur (= *Bipolaris sorokiniana* (Sacc.) Shoemaker), the causal agent of common root rot (CRR); *Pythium ultimum*, the causal agent of Pythium root rot; *Rhizoctonia solani* (Kühn) AG-8 (=*Thanatephorus cucumeris*), the causal agent of Rhizoctonia root rot and bare patch; and *Penicillium* spp., the causal agent of dry seed decay. These five wheat pathogens can attack the same part of the plant and have been documented as interacting with each other and other microorganisms in the wheat rhizosphere; they have all been observed to be suppressed by or to suppress other organisms. In general, reports of suppression for *C. sativus* (Ledingham, 1942, Tinline, 1977, Fernandez and Jefferson, 2004), *Pythium* spp. (Fukui et al., 1994, Tedla and Stanghellini, 1992), and *R. solani* (MacNish, 1988, Lucas et al., 1993, Smiley and Wilkins, 1993, Smiley et al., 1996b) are common. Their suppression by other microbes has been shown to noticeably improve overall health and crop

performance. *Fusarium* spp. and *Penicillium* spp. are frequently documented as suppressing other wheat pathogens (Notz et al., 2002, Lutz et al., 2003, Yang et al., 2008). However, to date their effects on other pathogens have not been shown to improve overall yield. Because significant microbial interactions have been observed for all five diseases, further investigation into the interspecies dynamics of these varied community members is needed.

Wheat grown in Montana is dispersed over 2.29 million ha annually (the 3rd highest production in the United States in 2014) (http://www.nass.usda.gov). It is predominantly grown under no-till and conservation tillage farming practices that conserve moisture in the dry climate, conserve fuel and labor input, and reduce soil erosion (Dyer et al., 2009, Hogg et al., 2009). The adoption of these practices has had a noticeable effect on the five diseases proposed for this study. Increasing incidence of FCR has been associated with no-till farming (Burgess et al., 2001, Wildermuth et al., 1997). Higher populations of Pythium root rot (Cook et al., 1990), and Rhizoctonia root and crown rot (Roget et al., 1996, Weller et al., 1986, Pumphrey et al., 1987) have been reported in no-till soils than in conventionally tilled soils. By enhancing their ability to move through the soil profile, both *Pythium* species and *R. solani* benefit from the greater soil connectivity that characterizes no-till production (Glenn and Sivasithamparam, 1990, Otten et al., 2004). Conversely, CRR severity is reduced under no-till production (Conner et al., 1987, Tinline and Spurr, 1991). *Penicillium* species are ubiquitous contaminants and are an established problem in Montana for winter wheat planted into dry soil, although no research has been done concerning the effect of tillage on this

pathogen (Dyer et al., 2012). Given the impacts of the adoption no-till production has on these diseases, characterizing how individual pathogens in the complex interact will be invaluable in foretelling the overall impacts of no-till practices on wheat health.

Preliminary studies have been conducted in Montana to examine the interactions between wheat crown rot pathogens. One documents the significant suppression a single isolate of *F. pseudograminearum* had on a single isolate of *C. sativus* in the field (Moya-Elizondo et al., 2011a). That suppression was inconsistent across fields and years and did not take into consideration other pathogen species that interact within the system. The current work was conducted to develop a broader understanding of the interplay among species within the greater disease system. For this, dynamics within the dryland root and crown rot disease system were examined using inoculations with the five common pathogen members and assessing their effects on plant response variables alone and in competition with other pathogens.

Materials and Methods

Pathogen Isolates and Wheat Cultivar

Field trials were conducted to explore the community dynamics between pathogenic members of the rhizosphere. The DRRC-associated pathogens used were: *Fusarium pseudograminearum, Cochliobolus sativus, Penicillium sp.,* and *Pythium ultimum,* and *Rhizoctonia solani* AG-8. *Fusarium pseudograminearum* isolate 2228 was originally collected in Loma, MT (Liberty County) in 2002, and when tested for deoxynivalenol production, produced 0.55 PPM (A.T. Dyer, *Unpublished data*). The

isolate has been studied extensively (Hogg et al., 2007, Moya-Elizondo et al., 2011b, Moya-Elizondo et al., 2011a). The other pathogens were also sourced from Montana; *C. sativus* Cs2016 from Moccasin, MT (Judith Basin County, collected in 1997), *P. ultimum* Pu2408 from Huntley, MT (Yellowstone County, collected in 2014), and both *R. solani* AG-8 Rs2119 and *Penicillium* sp. from Bozeman, MT (Gallatin County, collected in 2002 and 1991, respectively). The field trials were conducted using the hard red spring wheat 'Choteau' (Montana Agricultural Experiment Station [Montana State University], 2004), because the cultivar has demonstrated moderate susceptibility to crown rots (Hogg et al., 2007), an important characteristic as response variables were based on plant response to disease.

Field Trials

In the summer of 2014, three trials were planted to evaluate the population dynamics in the rhizosphere pathogen community. The trials were conducted at three locations centered near Bozeman, Montana; the MSU-Arthur H. Post Research Farm, the Fort Ellis Research Farm, and the Lutz Research Farm. These locations were chosen because of historical differences in average annual precipitation, temperature, and soil type. The Post Farm receives 44 cm in annual precipitation, Fort Ellis 56 cm, and Lutz Farm 40 cm. High temperatures from May to August at the Post Farm averaged 23.7 °C with average lows of 8.4°C; temperatures at Fort Ellis averaged highs of 23.3°C and lows of 8.3°C. Lutz Farm averaged high temperatures of 24.4°C and lows of 7.1°C (www.ncdc.noaa.gov/cdo-web; all locations). Soil at the Post Farm is classified as an Amsterdam-Brodyk silt loam, 4-8% slopes. Fort Ellis soil is classified as a Blackmore

silt loam, 4-8% slopes, and the Lutz Farm soil as a Blackdog silt loam, 0-4% slopes (SoilWeb: UCDavis, NRCS-USDA, University of California). Four replicates were planted in a randomized block design at each of the three locations.

Two experimental designs were used to examine individual interactions using community members in paired trials (hereon referred to as the "paired trials"), and community effects utilizing the five four-pathogen combinations and one five-pathogen inoculation permutation with the five community members (hereon referred to as the "community inoculation trials"). All replicates of the paired trials consisted of all pairings of the five pathogens, single inoculations of each pathogen, and an uninoculated control for a total of 16 treatments replicated four times for 64 plots per field location. All replicates of the community inoculations trials consisted of a five-pathogen inoculation, all four-pathogen inoculation permutations, single inoculations of each pathogen, and an uninoculated control, for a total of 12 treatments replicated four times for 48 total plots per field location. Due to limitations in field space, the community inoculation trial was not planted at the Post Farm.

For all fungi, inoculum was produced and inoculated at a rate of 5 g/row per pathogen in-furrow at planting following the established procedure for oat kernel inoculum, with the exception that millet was substituted for oats (Mathre and Johnston, 1975). Plots inoculated with numerous pathogens were inoculated with 5 g of each pathogen (i.e., plots inoculated with all five pathogens were inoculated with 25 total grams of inoculum in that row, or 5 g/pathogen x 5 pathogens). Millet inoculum was used to increase the distribution of inoculum pieces within the row, as 5 g of millet

inoculum results in more individual pieces of inoculum than the same mass of oat inoculum. The experimental unit was a single-row plot 3 m (approximately 10 feet) long with 30-cm centers. Rows were seeded at a rate of 150 seeds per 3-m row and separated from each other by single uninoculated border rows. Plots were planted using a Marvin Berg seeder. All of the field locations were tilled in the fall of 2013. Post Farm was planted 14 May, 2014, Fort Ellis on 15 May 2014, and Lutz Farm was planted on 21 May 2014. No fertilizer was applied at any of the field locations due to the uncertain effect it would have on the wheat's expression of disease through the response variables measured. Weeds were controlled at all locations by hand hoeing and through the application of broadleaf herbicides as necessary; Post Farm was sprayed with a single application of "Huskie" (Bayer CropScience) on 2 June 2014 at a rate of 22 oz/acre, and Lutz Farm was sprayed with a single application of "Curtail" (Dow AgroSciences) on 34 June 2014 at a rate of 32 oz/acre. Herbicides were only applied to alleyways between plots for broadleaf weed control. No herbicides were applied at Fort Ellis.

Pre-Harvest Response Variables. Response variables measured at all 3 locations were: emergence, vigor, plant height, tiller count, and yield in bushels per acre. Plant emergence was recorded at the single-shoot growth stage (Feeke's growth stage 1) between 9 and 13 days after planting (DAP). Emergence was recorded on 27 May 2014 at Post Farm and Fort Ellis, and 30 May at Lutz Farm. Vigor ratings were taken when rows had begun to close and leaves began to overlap (during the tillering stage of development and before Feeke's stem extension stages had begun) 40 DAP on 23 June 2014 at Post Farm, 43 DAP on 27 June 2014 at Fort Ellis, and 37 DAP on 27 June 2014

at Lutz Farm. The rating takes into account overall mid-season plot health, fullness of the rows, gaps in rows from deceased plants, and poor stand. Vigor ratings were assigned on a scale of 1 to 5; a score of 0 was only assigned to plots with no plants, and a 5 to a plant that exhibited no gaps, no stunting, no reduction in stand, and whose rows had begun to overlap.

Plant heights were measured at the beginning of August when grain fill had begun, 15 to 28 days before harvest. Plant height was measured by selecting a representative handful of plants from the center of the plot and measuring the average height of the plants in centimeters. Height was measured at Post Farm and Fort Ellis on 1 August 2014, and on 11 August 2014 at Lutz Farm. Tiller counts were taken in August, 24 days before harvest at Post Farm, 21 days before harvest at Fort Ellis, and 12 days before harvest at Lutz Farm. All locations were at the end of flowering and the beginning of ripening when tiller counts were taken. To determine tiller count, all heads taller than 12 inches above soil were counted in 1 meter of row. Whiteheads, the empty white aborted seed heads that result from disease or drought stress, were not counted as a functional seed-bearing head.

Harvest. The single-row plots were harvested with a single-row Suzue rice binder (Mitsubishi Agricultural Machinery Co., Ltd., Tokyo) and threshed using a Vogel thresher (Bill's Welding, Pullman, WA). Harvest occurred at the Post Farm on 29 August 2014, on 26 August 2014 at Fort Ellis, and on 27 August 2014 at the Lutz Farm.

Statistical Analyses

Locations were analyzed separately for both the paired and community trials to analyze the disease dynamics individually and observe the frequency of specific pathogen interactions at several locations. In statistical analyses, non-independent interactions were defined as interactions in which the total effect of both pathogens inoculated together was different than the sum of the pathogens inoculated individually relative to the uninoculated control. If the effect, or plant response to disease, of pathogens inoculated together was significantly less than the sum of the individual inoculations, the cause was interpreted as antagonism; if the effect of pathogens inoculated together was significantly more than the sum of the individual effects, it was interpreted as synergism.

In the paired trials, the five fungal pathogens were treated as indicator variables, and all interaction effects in all possible two-way interactions were included in linear models in a multifactor analysis of variance. These analyses were performed in R Studio (version 0.98.932 [based on R i386 version 3.0.2]). The use of an interaction effect to test between four treatments (i.e., an uninoculated control, pathogen A, pathogen B, and both pathogens) is equivalent to utilizing contrasts to identify non-independent dynamics (Slinker, 1998, Maxwell and Delaney, 2004); therefore, contrasts were not utilized to analyze the paired trial data, as it would be redundant. Fisher's protected least significant difference (LSD) was utilized to compare response variable means when $p < 0.05$ for interaction effects.

For the community inoculation trials, the 16 treatments were analyzed as a single variable. Analysis was conducted in R studio using linear models in which the models

included treatment and block, and multifactor ANOVAs were generated. Individual contrasts were fitted for each pathogen using the 'gmodels' R package. The contrasts looked at the effect of each pathogen and generated a p-value for the likelihood that the difference in mean response variable between the single-inoculation and the uninoculated control was the same as the difference between the 5-pathogen inoculation and the 4-pathogen inoculation (see below for *F. pseudograminearum*). Contrasts were performed for all pathogens ($\alpha < 0.05$).

$$H_0: \quad \mu_{UTC} - \mu_{F.pseudo\ only} = \mu_{4\ pathogens,\ no\ F.pseudo} - \mu_{all\ pathogens}$$

$$H_A: \quad \mu_{UTC} - \mu_{F.pseudo\ only} \neq \mu_{4\ pathogens,\ no\ F.pseudo} - \mu_{all\ pathogens}$$

F. pseudograminearum had unexpectedly dominant effects on emergence and vigor, and this substantial effect made it difficult to compare means when *F. pseudograminearum* was present inconsistently. Because of *F. pseudograminearum* dominance, Thanks so contrasts were further refined to account for the effect of *F. pseudograminearum* in the community. These refined contrasts were performed for all pathogens except *F. pseudograminearum* (see below for *Penicillium* sp.). These contrasts assumed that the effects of the other pathogens as a community were constant.

$$H_0: \quad \mu_{UTC} - \mu_{Pen\ only} = \mu_{4\ pathogens,\ no\ Pen} - [(\mu_{4\ pathogens\ with\ Pen\ and\ Fp} + \mu_{4\ pathogens\ with\ Pen\ and\ Fp} + \mu_{4\ pathogens\ with\ Pen\ and\ Fp})/3]$$

$$H_A: \quad \mu_{UTC} - \mu_{Pen\ only} \neq \mu_{4\ pathogens,\ no\ Pen} - [(\mu_{4\ pathogens\ with\ Pen\ and\ Fp} + \mu_{4\ pathogens\ with\ Pen\ and\ Fp} + \mu_{4\ pathogens\ with\ Pen\ and\ Fp})/3]$$

Fisher's protected LSD was used to compare response variable means when ANOVA results indicated strong evidence of differences in means ($P < 0.05$).

Differences between yields at Lutz Farm, because yields were so small, were analyzed for significant differences between means using *t* tests in place of Fisher's LSD. Although use of treatment as a single variable in place of indicator variables for the pathogens is suboptimal (Slinker, 1998), the presence of so many pathogens inoculated together confounded that analysis, and there were insufficient degrees of freedom to test for interactions.

Results

Paired Trials

There were significant differences in all response variables among locations (all $P < 0.001$). Because of these substantial location effects, and because the goal of this research was to examine community dynamics in individual fields, locations were analyzed separately. At Post Farm, block significantly affected emergence, tiller count ($P < 0.001$, both), and yield ($P = 0.010$). Block was not consistently a significant factor at Fort Ellis; it only had a significant effect on plant height at that location ($P < 0.001$). At Lutz Farm, block significantly affected emergence ($P = 0.021$), tiller count, and yield

At the all three locations, inoculation with *F. pseudograminearum* had a significant effect on yield ($P = 0.005$, Post; $P < 0.001$, Ellis; $P = 0.037$, Lutz). Inoculation with *F. pseudograminearum* significantly reduced yield compared to the uninoculated controls; yield was reduced from 76.9 bushels/acre in uninoculated controls to 58.4 bushels/acre at Post Farm (24% reduction), 32.1 bushels/acre in uninoculated controls to 25.3 bushels/acre at Fort Ellis (21% reduction), and from 19.9 bushels/acre in uninoculated

controls to 15.8 bushels/acre at Lutz Farm (21% reduction) (see Appendix B: Figures 5.1-5.3 for yield graphs). In addition, inoculation with *F. pseudograminearum* had a significant effect on plant height at Post Farm and Fort Ellis ($P = 0.023$, $P = 0.039$, respectively), but not at Lutz Farm ($P = 0.78$). Inoculation with *F. pseudograminearum* reduced plant height to 58.4 cm from 78.5 cm in uninoculated controls at the Post Farm, and to 60.6 cm from 63.0 cm in uninoculated controls at Fort Ellis. Inoculation with *F. pseudograminearum* also significantly affected vigor at Post Farm and Fort Ellis ($P < 0.001$, $P = 0.041$, respectively), but not at Lutz Farm. Vigor at Post Farm was reduced from a score of 4.5 in uninoculated controls to 3.3 in inoculated plots, and at Lutz Farm it was reduced from 4.6 in uninoculated controls to 3.8 in inoculated plots. Inoculation with *F. pseudograminearum* significantly affected emergence ($P < 0.001$) and tiller count ($P = 0.040$) at the Post Farm as well, but not at Fort Ellis ($P = 0.083$, $P = 0.87$, respectively) or Lutz Farm ($P = 0.27$, $P = 0.26$, respectively). Inoculation reduced mean emergence ($x = 62.2$ seedlings/row) and tiller count ($x = 68.1$ tillers/m) compared to uninoculated controls ($x = 88.5$ seedlings/row, 80.5 tillers/m, respectively).

Inoculation with *P. ultimum* significantly affected yield at Post Farm and Fort Ellis ($P = 0.007$, $P = 0.009$, respectively), but not at Lutz Farm ($P = 0.28$). Yield at Post Farm was reduced from 76.9 bushels/acre in the uninoculated controls to 59.6 bushels/acre (22% reduction), and from 32.1 bushels/acre in the uninoculated controls to 30.2 bushels/acre (6% reduction) at Fort Ellis. Inoculation with *P. ultimum* also significantly affected vigor at Post Farm and Lutz Farm ($P < 0.001$, $P = 0.001$, respectively), but not at Fort Ellis. *P. ultimum* reduced vigor at Post Farm from a mean rating of 4.5 in uninoculated controls to

3.6 in inoculated plots, and from 4.9 in uninoculated controls to 3.8 in inoculated plots. Inoculation with *P. ultimum* significantly affected emergence ($P < 0.001$), plant height ($P = 0.005$), and tiller count ($P = 0.007$) at Post Farm as well, but not at Fort Ellis or Lutz Farm. Inoculation with *P. ultimum* reduced mean emergence ($x = 61.3$ seedlings/row), plant height ($x = 74.1$ cm), and tiller count ($x = 68.2$ tillers/m) compared to uninoculated controls ($x = 88.5$ seedlings/row, 78.5 cm, and 80.5 tillers/m, respectively).

At Post Farm, inoculation with *R. solani* significantly affected emergence ($P = 0.003$), vigor ($P = 0.010$), and plant height ($P = 0.017$). Inoculation with *R. solani* reduced mean emergence ($x = 64.5$ seedlings/row), vigor ($x = 3.5$), and plant height ($x = 74.4$ cm) compared to uninoculated controls ($x = 88.5$ seedlings/row, 4.5, and 78.5 cm, respectively).

Inoculation with *C. sativus* significantly affected vigor at Post Farm ($P = 0.013$), from a mean rating in the uninoculated controls of 4.5 to 3.6 in inoculated plots. Inoculation with *C. sativus* also significantly ($P < 0.001$) reduced mean emergence ($x = 64.3$ seedlings/row) compared to uninoculated controls ($x = 88.5$ seedlings/row).

Inoculation with *Penicillium* sp. was not associated with a significant effect on any of the response variables at any of the locations.

Among the paired trials, significant interactions were detected for five of the ten possible paired pathogen combinations. The rest showed no evidence of interactions. All of the interactions were antagonistic, where damage to the plant was less than the sum of the two pathogens inoculated individually, save for one weak synergistic relationship involving *F. pseudograminearum*.

Among the five pathogens tested, *C. sativus* interacted the most with other pathogens. *C. sativus* interacted significantly with *P. ultimum* at both Post Farm and Lutz Farm ($P = 0.021$, $P = 0.042$, respectively); at both locations, their interaction resulted in significant increases in emergence above the expected values (see Figures 3.1A-B). The

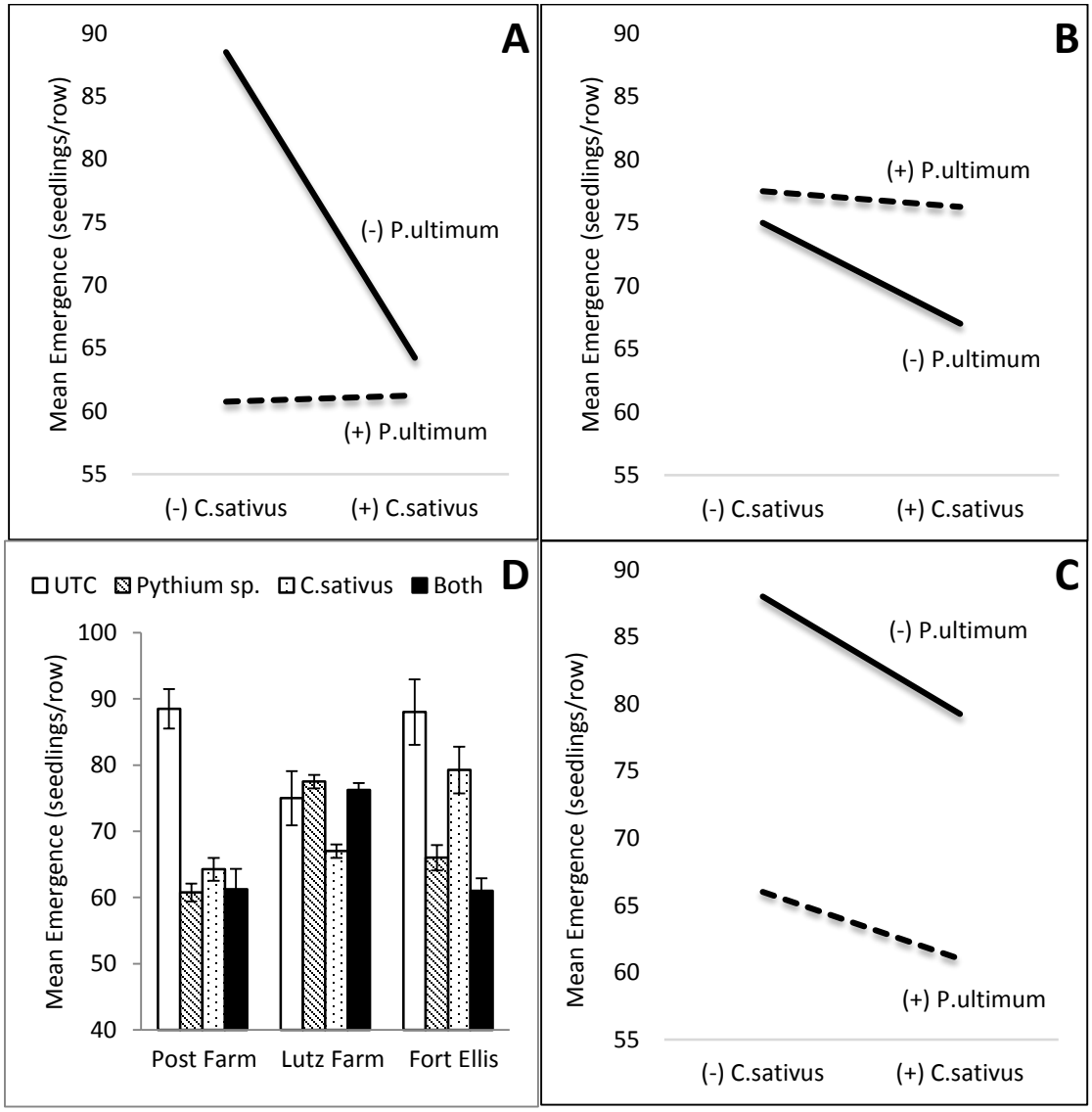

Fig. 3.1. Antagonistic effect of *Pythium ultimum* on emergence damage caused by *Cochliobolus sativus* at **A**, Post Farm ($P = 0.021$) and **B**, at Lutz Farm ($P = 0.042$), **C**, no interaction between *Cochliobolus sativus* and *P. ultimum* on emergence at Fort Ellis ($P = 0.50$), and **D,** bar graph detailing the same.

interaction was not observed at Fort Ellis (see Figure 3.1C). A significant interaction was also observed between *C. sativus* and *R. solani* at Fort Ellis, which resulted in significant increases in vigor (P = 0.043) above expected values (see Figure 3.2A or Appendix B: Figure 5.1A). *C. sativus* interacted significantly with *Penicillium* sp. at Lutz Farm (P = 0.017), which resulted in increased vigor compared to expected values (see Figure 2B or Appendix B: Figure 5.1B).

Among the two remaining interactions detected, *Penicillium* sp. interacted significantly with *P. ultimum* at Lutz Farm (P = 0.044), which resulted in increased vigor compared to the expected values (see Figure 3.2C or Appendix B: Figure 5.1C). According to LSD values calculated for the interaction effect (LSD = 0.85), vigor was significantly reduced by inoculation with *Penicillium* sp. (x = 3.6) and by inoculation with *P. ultimum* (x = 3.9), but vigor was not further reduced when both pathogens were present (x = 3.9).

The only synergistic interaction was observed between *F. pseudograminearum* and *R. solani* at the Lutz Farm (P = 0.019), and resulted in significant decreases to emergence compared to expected values (see Figure 3.2D or Appendix B: Figure 5.1D). Inoculation with *R. solani* or *F. pseudograminearum* alone did not result in significant decreases to emergence; however, inoculation with both pathogens did result in a significant decrease (x = 80.8 seedlings/row) compared to the uninoculated control (x = 88.0 seedlings/row). While not significant, the same pattern was observed at Post Farm (P = 0.33). The pattern was not observed at Fort Ellis.

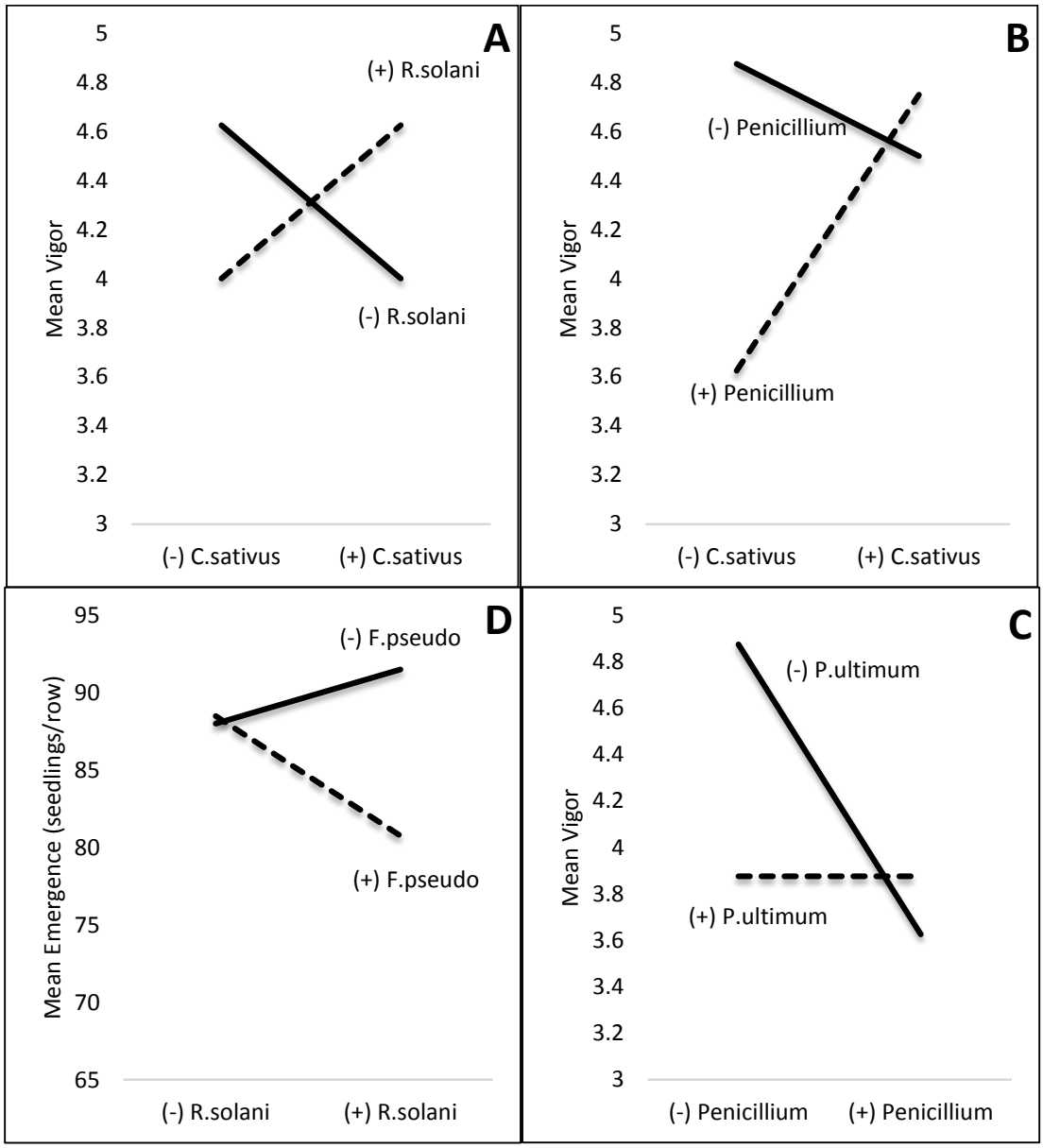

Fig. 3.2. Clockwise from top left. **A**, Mutually antagonistic effect of *Cochliobolus sativus* and *Rhizoctonia solani* affecting vigor at Fort Ellis, resulting in increased vigor in co-inoculated plots (P = 0.043); **B**, mutually antagonistic effect of *Cochliobolus sativus* and *Penicillium* sp. affecting vigor at Lutz Farm, resulting in increased vigor in co-inoculated plots (P = 0.002); **C**, antagonistic effect of *Pythium ultimum* on vigor damage caused by *Penicillium* sp. at Lutz Farm, resulting in increased vigor in co-inoculated plots; and **D**, synergistic effect of *Fusarium pseudograminearum* and *Rhizoctonia solani* affecting emergence at Lutz Farm, resulting in decreased emergence in co-inoculated plots (P = 0.002).

No significant interaction effects were observed affecting yield, tiller count, or plant height at any of the locations.

Community Inoculations

Location had a significant effect on all response variables ($P < 0.05$) except emergence ($P = 0.41$). Due to the presence of these location effects, and in order to meet the study's goal of examining community dynamics in individual fields, locations were further analyzed separately. Block had an inconsistent effect on some of the response variables at both locations. At Fort Ellis, block significantly affected vigor, plant height, tiller count, and yield ($P < 0.005$). At Lutz Farm, block significantly affected emergence, plant height, tiller count, and yield ($P < 0.001$).

There were significant differences between treatments in emergence ($P < 0.001$), vigor, ($P = 0.001$), and tiller count ($P = 0.007$) at Fort Ellis (see Table 3.1).

TABLE 3.1. Mean emergence (seedlings/3m), vigor (0-5), plant height (cm), tiller count, and yield (bushels/acre) for all treatments at Fort Ellis, with Fisher's protected LSD values. Treatments 8-12 are 4-pathogen combinations, designated by the pathogen eliminated

Treatment		Emergence (seedlings/3m)$_A$	Vigor Rating$_B$	Plant Ht (cm)	Tiller Count$_C$	Yield (bu/acre)
1	Uninoculated control	77.75	4.25	58.88	67.50	13.50
2	*F. pseudograminearum*	74.50	4.13	54.50	66.50	13.30
3	*R. solani*	78.75	4.50	55.25	63.13	12.63
4	*C. sativus*	63.75$_A$	4.38	54.88	62.88	12.58
5	*P. ultimum*	78.00	4.50	57.13	65.75	13.15
6	*Penicillium* sp.	86.00	4.75	55.88	63.25	12.65
7	All pathogens	48.25$_A$	3.38$_B$	55.25	55.88$_C$	11.18
8	No *Penicillium* sp.	57.00$_A$	3.50$_B$	54.13	57.38$_C$	11.48
9	No *F. pseudogram.*	74.25	4.38	55.50	66.75	13.35
10	No *R. solani*	49.25$_A$	3.63	55.50	55.88$_C$	11.18
11	No *C. sativus*	65.25	3.88	55.63	59.63$_C$	11.93
12	No *P. ultimum*	53.25$_A$	3.38$_B$	55.88	60.75	12.15
	Fisher's LSD ($\alpha = 0.05$)	13.10	0.71	-	7.34	-

$_{A, B, C}$ indicates significant difference from the uninoculated control based on Fisher's Protected LSD

TABLE 3.2. Mean emergence (seedlings/3m), vigor (0-5), plant height (cm), tiller count, and yield (bushels/acre) for all treatments at Lutz Farm, with Fisher's protected LSD values. Treatments 8-12 are 4-pathogen combinations, designated by the pathogen eliminated

Treatment		Emergence (seedlings/3m)$_A$	Vigor Rating$_B$	Plant Ht (cm)	Tiller Count	Yield (bu/acre)
1	Uninoculated control	86.25	4.13	73.00	84.25	16.85
2	*F. pseudograminearum*	83.00	4.00	68.00	75.50	15.10
3	*R. solani*	83.75	4.38	72.00	79.50	15.90
4	*C. sativus*	76.00	4.00	70.75	81.50	16.30
5	*P. ultimum*	69.00$_A$	4.00	72.75	85.75	17.15
6	*Penicillium* sp.	89.75	4.50	72.00	77.25	15.45
7	All pathogens	51.50$_A$	2.75$_B$	70.75	66.00	13.20^x
8	No *Penicillium* sp.	41.25$_A$	2.63$_B$	70.50	61.75	12.35^x
9	No *F. pseudogram.*	62.75$_A$	4.50	71.50	69.00	13.80
10	No *R. solani*	48.25$_A$	2.63$_B$	69.50	61.25	12.25^x
11	No *C. sativus*	62.50$_A$	3.75	71.50	69.75	13.95
12	No *P. ultimum*	73.00$_A$	3.63	69.50	82.00	16.40
	Fisher's LSD ($\alpha = 0.05$)	12.99	0.78	-	-	-

$_{A, B}$ indicates significant difference from the uninoculated control based on Fisher's Protected LSD
^{x} indicates significant difference from the uninoculated control ($P < 0.05$).

At Lutz Farm, there were significant differences between treatments in emergence ($P < 0.001$), vigor ($P < 0.001$), and yield ($P = 0.002$) (see Table 3.2).

The contrasts revealed significant ($\alpha < 0.05$) differences between the effect of *F. pseudograminearum* alone and within the community at Fort Ellis. The effect of *F. pseudograminearum* alone ($\mu_{\text{Fusarium only}} - \mu_{\text{UTC}}$) was not the same as the effect of *F. pseudograminearum* in the community ($\mu_{\text{all pathogens}} - \mu_{\text{4 pathogens, no Fusarium}}$), and the null hypothesis was rejected. The effect of *F. pseudograminearum* was greater in the community than alone ($P = 0.017$), and therefore the dynamic was identified as synergism. This resulted in a greater reduction in emergence compared to expected values. Inoculation with *F. pseudograminearum* alone reduced mean emergence by 3 plants compared to the uninoculated control (LSD = 13.10), whereas addition of *F. pseudograminearum* to the community significantly reduced emergence by 26 plants

compared to the 4-pathogen inoculation that excluded *F. pseudograminearum* (see Figure 3.3A). A small but similar trend of greater damage in the community was observed at Lutz arm, but it was not statistically significant (P = 0.38) (see Figure 3.3B).

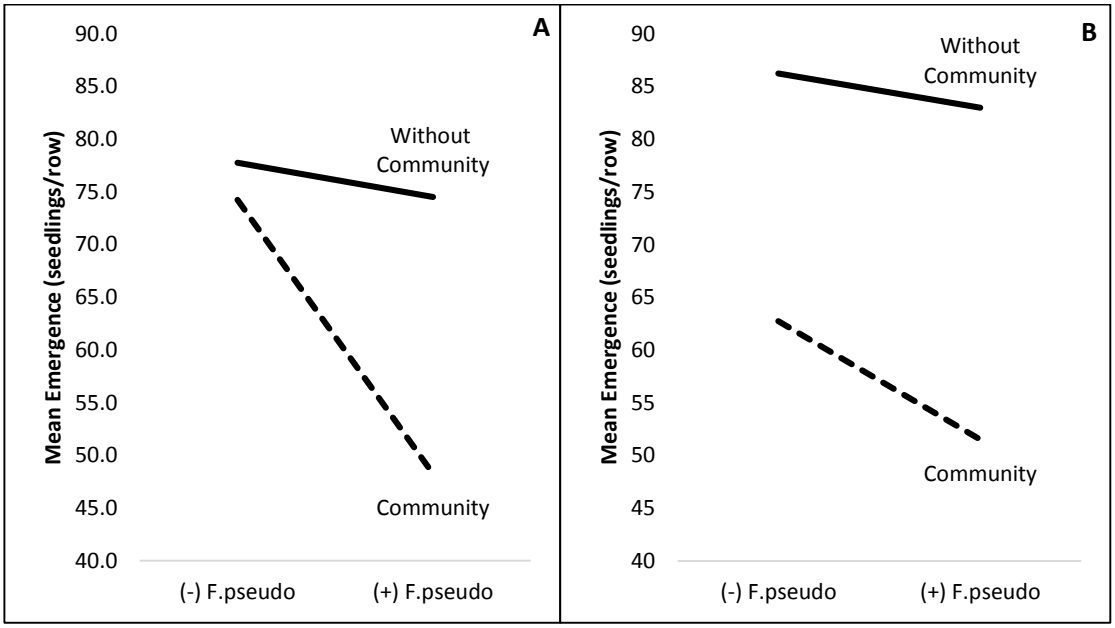

Fig. 3.3. A, Synergistic effect of *Fusarium pseudograminearum* inoculated with and without the community (P = 0.017) at Fort Ellis, resulting in increased damage to emergence in the community, and **B**, independent effect of *Fusarium pseudograminearum* inoculated with and without the community (P = 0.38) at Lutz Farm, demonstrating a similar pattern to the increased damage to

Inoculation with *F. pseudograminearum* also had a synergistic effect in the community at Lutz Farm, resulting in greater reductions to plant vigor than expected values predict (P = 0.005). Inoculation with *F. pseudograminearum* also had a synergistic effect in the community at Lutz Farm, resulting in greater reductions to plant vigor than expected values predict (P = 0.005). Inoculation with *F. pseudograminearum* alone reduced mean vigor rating by 0.125 compared to the uninoculated control (LSD = 0.78), whereas addition of *F. pseudograminearum* to the community significantly

reduced vigor rating by 1.75 compared to the 4-pathogen inoculation that excluded *F. pseudograminearum* (see Figure 3.4A). A similar pattern was observed at Fort Ellis, but was not statistically significant (P = 0.084) (see Figure 3.4B).

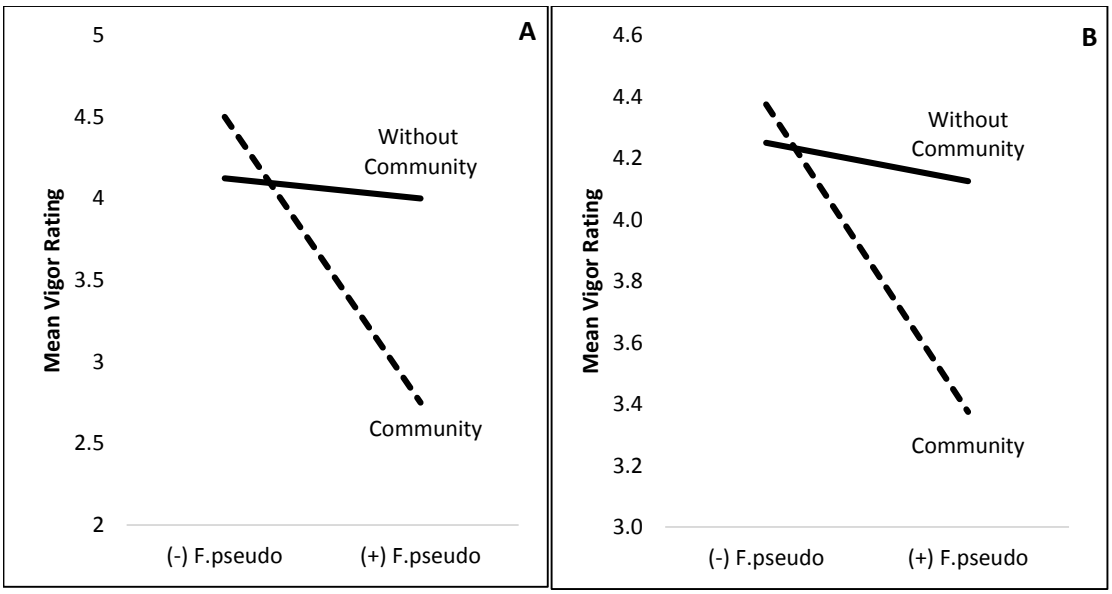

Fig. 3.4. **A**, Synergistic effect of *Fusarium pseudograminearum* inoculated with and without the community (P = 0.0050) at Lutz Farm, resulting in increased damage to vigor in the community, and **B**, independent effect of *Fusarium pseudograminearum* inoculated with and without the community (P = 0.084) at Fort Ellis, demonstrating a similar pattern to the increased damage to vigor observed at Lutz Farm.

The refined contrasts which accounted for the effect of *F. pseudograminearum* in the community further illuminated an antagonistic effect on the community by *Penicillium* sp. at Lutz Farm Inoculation with *Penicillium* sp. in the community was associated with significant increases in emergence compared to single inoculations of the pathogen (P = 0.051). Recalling the contrasts, the effect of *Penicillium* sp. alone ($\mu_{Penicillium\ only} - \mu_{UTC}$) was not the same as the effect of *Penicillium* sp. in the community ($[(\mu_{4\ pathogens\ with\ Penicillium\ and\ F.pseudo} + \mu_{4\ pathogens\ with\ Penicillium\ and\ F.pseudo} + \mu_{4\ pathogens\ with\ Penicillium\ and\ F.pseudo})/3] - \mu_{4\ pathogens,\ no\ Penicillium}$), and the null hypothesis was rejected. Inoculation

with *Penicillium* sp. alone was associated with a small increase in mean emergence of 3.5 plants compared to the uninoculated control, but addition of *Penicillium* sp. to the community increased emergence by 20.3 plants compared to plots inoculated with 4 pathogens, excluding *Penicillium* sp. (see Figure 3.5). This pattern was not observed for emergence at Fort Ellis (P = 0.27) (*data not shown*).

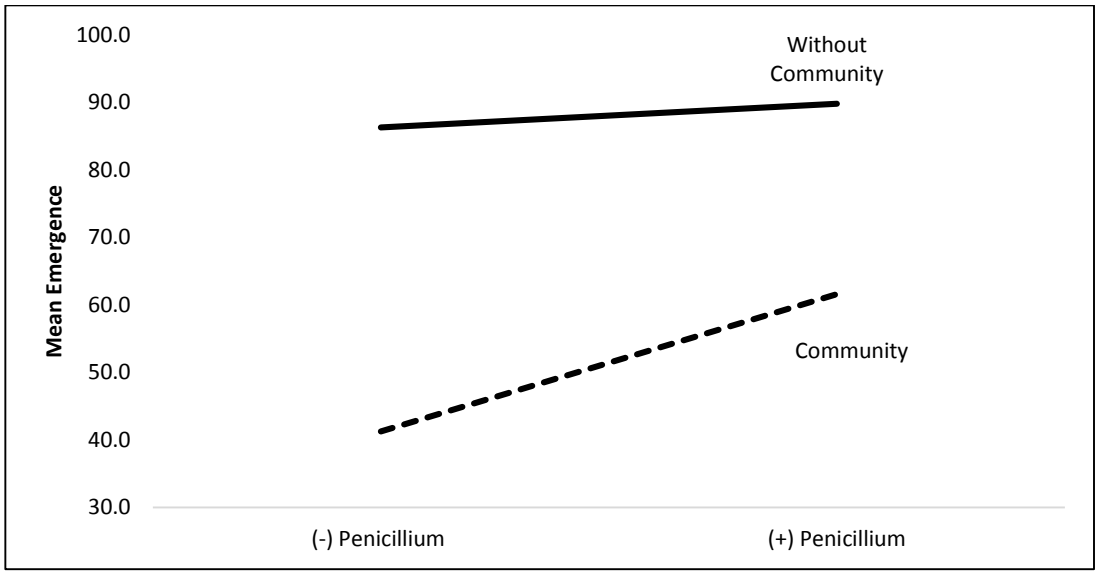

Fig. 3.5. Antagonistic effect of *Penicillium* sp. inoculated with and without the community (P = 0.051) at Lutz Farm, resulting in decreased damage to emergence in the community.

Discussion

In paired inoculation trials, half of all possible pathogen combinations resulted in some type of interaction (4 antagonisms, 1 synergism). In the community inoculation trials, 2/5 of all community pairings demonstrated interactions (1 antagonism and 1 synergism). Both findings closely match the ratios detected in a meta-analysis of pathogen dynamics conducted across 51 previously published studies (Stephens et al.,

2013). The preponderance of interactions for the current study argues for a combined response to root and crown pathogens as interactions suggest a multi-pathogen management approach would result in a greater benefit for the grower. For the more common antagonistic interactions, pathogen replacement appears a likely response to individual control approaches, i.e., control of an antagonistic pathogen would result in its replacement by other pathogens that were previously suppressed by the antagonist. It may explain the history of lackluster results obtained from control measures that are focused on a single pathogen species (Cook, 1980, Grosch et al., 2006). Conversely, methods of control which focus on synergistic interactions may potentially provide control that exceeds expectations. For instance, the results from the paired trials argue that targeting of either *F. pseudograminearum* or *R. solani* due to their synergistic effects would enhance the control of the other. In particular, *F. pseudograminearum* responds strongly to other pathogens within the community and partial control of this particularly troublesome pathogen may be achieved through focus on the other pathogens with which it interacts. The interactions effects identified in the current studies are modest but should not be underappreciated. All of the pathogens involved are endemic and as such are subject to compounding multi-year effects, where management practices with even a modest effect could precipitate effective control when performed over several years (Roget et al., 1996, Fernandez et al., 2007).

Among the paired trials, interactions involved *C. sativus, F. pseudograminearum, P. ultimum,* and *Penicillium* spp. The common root rot pathogen (*C. sativus*) was the most commonly suppressed pathogen, and some evidence suggests that *C. sativus* is

slower-growing than its competitors for crown tissue (Hynes, 1935). Given the frequency of its interactions and how common it is within Canadian and Montana fields (Moya-Elizondo et al., 2011b, Fernandez and Jefferson, 2004), it may be the largest barrier to achieving the benefits expected from controlling other root pathogens, as it is the pathogen most likely to replace other pathogen species when they are controlled through human intervention. In contrast, the effects of *F. pseudograminearum* were only found to be increased by other pathogens within the community, and therefore any control measures that are effective against it may enhance overall disease control. Surprisingly, the most suppressive pathogen species within these studies was *P. ultimum,* which appeared to have the most consistent suppressive effect on *C. sativus.* To our knowledge, no prior work has shown a similar effect of *P. ultimum* on other root pathogens. The findings suggest that the impacts of *P. ultimum* on wheat production may need to be reassessed to incorporate unrecognized benefits. Among the other pathogens, *Penicillium* sp. exhibited suppressive capacities, both in the community inoculation trials and in mutual suppression with *C. sativus* in the paired trials. Conditions were not conducive for this pathogen to effectively attack the wheat seed but apparently that does not mean that this organism is inactive in the rhizosphere during these times.

Antagonism as a competitive strategy makes sense, as the drive to capture and defend resources from competitors drives antagonistic strategies. This not only means more resources are held by the effective antagonist, but also results in less selection pressure on the host to resist the antagonist. The mechanisms of antagonism among the pathogens used in this study are unknown. Suppression of competitors may result from

direct antagonism of competitors, or indirect suppression. Indirect suppression is described as suppression that occurs as the result of eliciting a host defense response and is likely a rare event (Stephens et al., 2013). Direct suppression may occur through the production of antimicrobial compounds, through some form of hyperparasitism, or through resource competition. Although identifying the mechanism is beyond the scope of the current study, a preliminary *in vitro* screen suggests several of the species may produce inhibitory compounds (*data not shown*). While *in vitro* studies frequently do not translate into field performance, there are ample published studies that report antibiosis as a key factor in microbial interactions for species used in this study (Lutz et al., 2003, Yang et al., 2008).

While the characterization of interactions among pathogens may be an important first step in elucidating rhizosphere dynamics, it is important to note that these results describe the dynamics for limited numbers of isolates tested in the rhizosphere of a single spring wheat cultivar. This was necessary given the number of species involved and the limitations in terms of field space and other resources. As such, these results are not a statement on how any specific species interact with another. Fungal species, and isolates of those species, are highly variable as demonstrated by numerous studies specific to the five species used here (Tunali et al., 2012, Mudge et al., 2006) and closely-related species (Scherm et al., 2013, Magan and Olsen, 2004, Yang et al., 2008), and this variability needs to be further explored to establish general trends with regards to pathogen on pathogen interactions. With that said, the findings presented here provide important data for focusing future research on particular pathogen interactions, which will allow for

more in depth assessments. For *P. ultimum,* the ability to suppress other pathogens is a novel finding, one that may open new directions of study.

This study measured interaction solely through the effects they have on plant response variables. Previous studies have shown these plant responses to be less sensitive to the underlying dynamics at hand than direct measures of pathogen populations by real-time quantitative PCR (Moya-Elizondo et al., 2011a). Therefore it is entirely possible that significant interactions occurred that were not detectable in the current study. Population analyses would also increase our understanding of what is happening to species within the system. This is particularly true with regards to the community trials, where pathogen interactions may involve only a couple of the species involved. Population analyses would not only provide information on those species involved, but also would provide insight into potential zero sum dynamics that often characterize resource limitations, a largely unexplored mechanism of interaction.

The exploratory nature of this research necessitated refining the community inoculation contrasts as results were formulated. The substantial effect of *F. pseudograminearum* in the community on early-season response variables meant that other pathogens could not reasonably be explored through the contrasts without accommodating for the *F. pseudograminearum* effect. The practical issue with this *F. pseudograminearum* dominance is due to the substantial effect that removing the pathogen from the community had on the 4-pathogen plots. The 4-pathogen inoculations that excluded *F. pseudograminearum* had substantially higher emergence and vigor than the 4-pathogen plots which included *F. pseudograminearum,* and including the healthier

plots in the contrasts had the potential to greatly influence the community side of the contrasts. Because of this undue influence that the community plots without *F. pseudograminearum* had, they were removed from analysis for the other root pathogens. The refined contrasts were not utilized for response variables that had not already demonstrated significant effect by *F. pseudograminearum*, meaning that the refined contrasts were only used when appropriate.

In conclusion, interactions in the root and crown rot community occur with some frequency, with antagonism being a more frequent occurrence than synergism. This may be due to the fact that they attack the same plant parts, a condition that increases the likelihood of non-independent pathogen dynamics. Suppression of *C. sativus* by several DRRC pathogens is of particular interest due to how common *C. sativus* is in Montana and the Canada. This work is also the first to report *P. ultimum* suppressing other pathogens. The antagonistic strategies used by suppressive pathogens are of practical interest as they may result in the identification of compounds useful in disease control. There is precedent for this application of naturally-occurring fungal antagonisms; the strobilurin bio-rational fungicides were developed from naturally occurring antagonistic activity (Anke et al., 1977, Anke, 1995). Analysis of chemical exudates from the suppressive pathogens would do much to develop a greater understanding of the mechanisms of suppression. Future DNA analysis of fungal biomass in root and crown tissue would also illuminate more specifically the dynamics between these root and crown rot community members.

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APPENDICES

APPENDIX A

CHAPTER 2 SUPPLEMENTAL INFORMATION

TABLE 4.1: Greenhouse and field treatments using all possible pairs of *F. pseudograminearum* and *C. sativus* isolates, including single inoculations of each isolate.

C. sativus isolate	*F. pseudo.* isolate
Cs1033*	Fp2228
Cs1033*	Fp2234
Cs1033*	Fp2278
Cs1033*	Fp2317
Cs1033*	none
Cs2016	Fp2228
Cs2016	Fp2234
Cs2016	Fp2278
Cs2016	Fp2317
Cs2016	none
Cs2344	Fp2228
Cs2344	Fp2234
Cs2344	Fp2278
Cs2344	Fp2317
Cs2344	none
none	Fp2228
none	Fp2234
none	Fp2278
none	Fp2317
none	none

*Isolate Cs1033 was not used in the field trials.

TABLE 4.2: Analysis of variance for three greenhouse trials involving inoculations with *F. pseudograminearum* and *C. sativus*

	Fusarium spp.		*C. sativus*	
Greenhouse Trial 1	*F*	*p*	*F*	*p*
F.pseudo isolate	11.75	<0.001	1.13	0.35
C.sativus isolate	8.00	0.0002	0.90	0.14
Interactions:				
F.pseudo x *C.sativus*	2.07	0.035	1.34	0.22
Greenhouse Trial 2	*F*	*p*	*F*	*p*
F.pseudo isolate	5.19	0.0018	0.91	0.47
C.sativus isolate	0.34	0.80	7.00	0.0007
Interactions:				
F.pseudo x *C.sativus*	1.22	0.30	2.50	0.015
Greenhouse Trial 3	*F*	*p*	*F*	*p*
F.pseudo isolate	9.16	< 0.001	1.11	0.36
C.sativus isolate	2.80	0.048	4.21	0.009
Interactions:				
F.pseudo x *C.sativus*	1.88	0.055	1.32	0.23

APPENDIX B

CHAPTER 3 SUPPLEMENTAL INFORMATION

TABLE 5.1: Treatment numbers assigned to paired trials, for interpretation of subsequent graphs

	Pathogen 1	Pathogen 2		Pathogen 1	Pathogen 2
1	*F.pseudograminearum*	*C.sativus*	9	*C.sativus*	--
2	*F.pseudograminearum*	*P.ultimum*	10	*P.ultimum*	*Penicillium* sp.
3	*F.pseudograminearum*	*Penicillium* sp.	11	*P.ultimum*	*R.solani*
4	*F.pseudograminearum*	*R.solani*	12	*P.ultimum*	--
5	*F.pseudograminearum*	--	13	*Penicillium* sp.	*R.solani*
6	*C.sativus*	*P.ultimum*	14	*Penicillium* sp	--
7	*C.sativus*	*Penicillium* sp.	15	*R.solani*	--
8	*C.sativus*	*R.solani*	16	Uninoculated	control

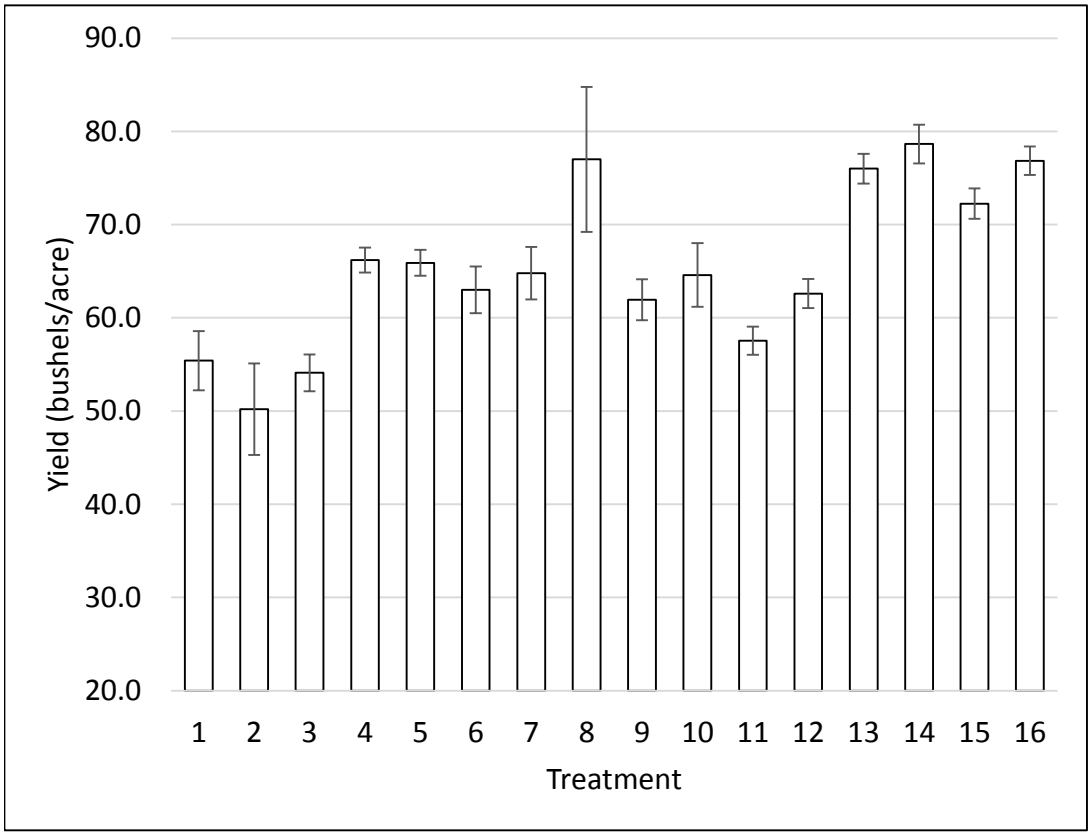

Fig. 5.1. Yields for all treatments in the paired trials at the Post Farm. At Post Farm, inoculation with *F. pseudograminearum* and *P. ultimum* significantly reduced yields. Bars indicate standard error. See Appendix B: TABLE 5.1 for treatment legend.

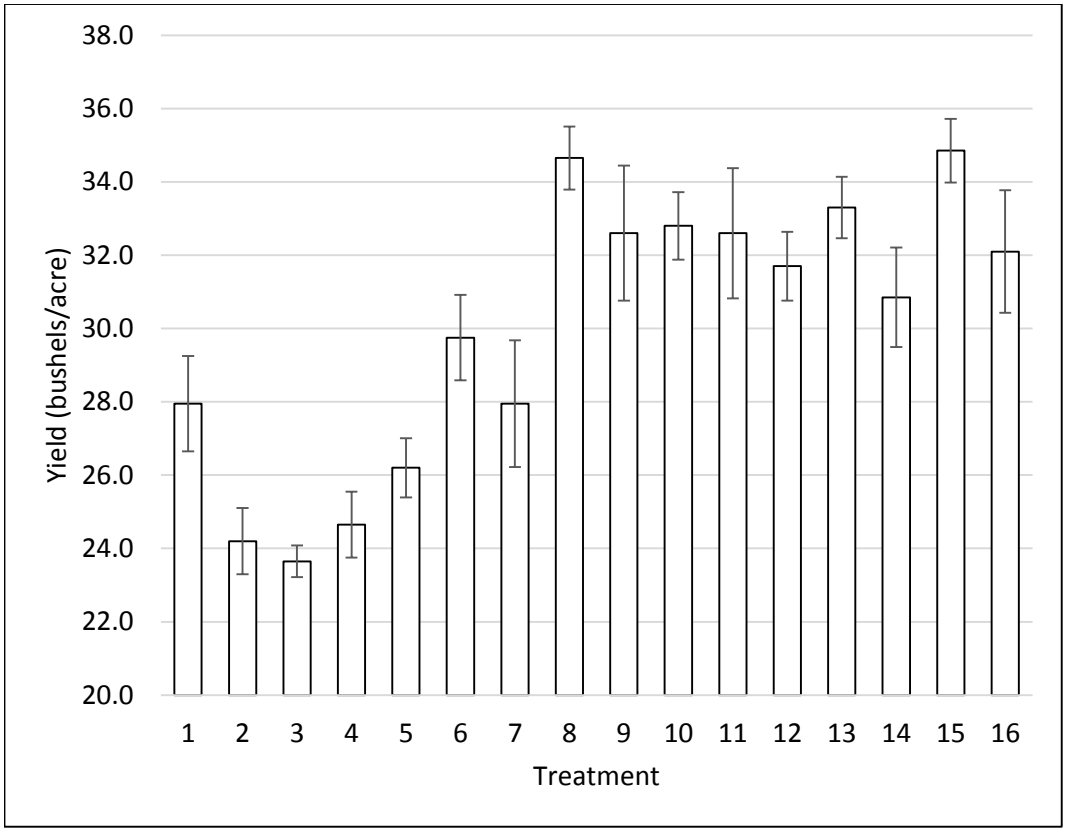

Fig. 5.2. Yields for all treatments in the paired trials at Fort Ellis. At Fort Ellis, inoculation with *F. pseudograminearum*, *C. sativus*, and *P. ultimum* significantly reduced yields. Bars indicate standard error. See Appendix B: TABLE 5.1 for treatment legend.

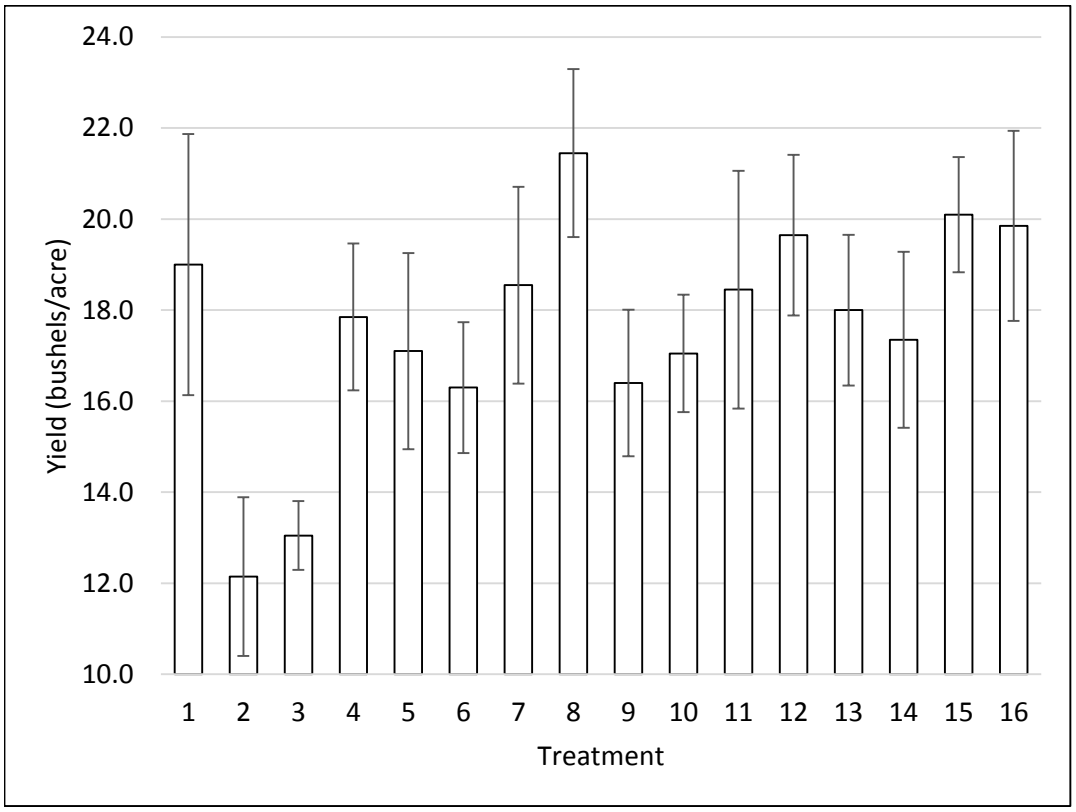

Fig. 5.3 Yields for all treatments in the paired trials at Lutz Farm. At Lutz Farm, inoculation with *F. pseudograminearum* significantly reduced yields. Bars indicate standard error. See Appendix B: TABLE 5.1 for treatment legend.

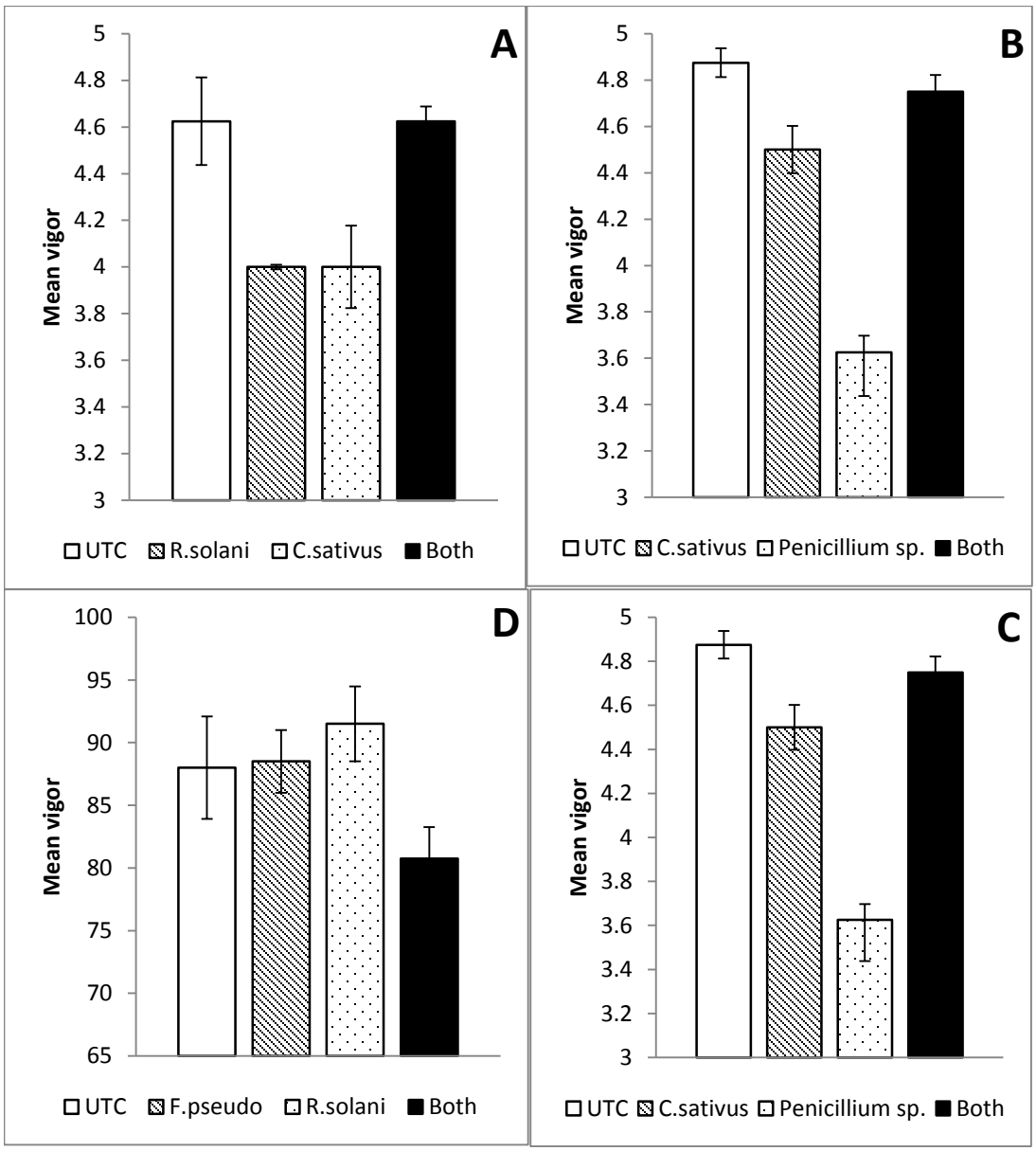

Fig. 4. Clockwise from top left. **A**, Mutually antagonistic effect of *Cochliobolus sativus* and *Rhizoctonia solani* affecting vigor at Fort Ellis, resulting in increased vigor in co-inoculated plots (P = 0.043); **B**, mutually antagonistic effect of *Cochliobolus sativus* and *Penicillium* sp. affecting vigor at Lutz Farm, resulting in increased vigor in co-inoculated plots (P = 0.002); **C**, antagonistic effect of *Pythium ultimum* on vigor damage caused by *Penicillium* sp. at Lutz Farm, resulting in increased vigor in co-inoculated plots; and **D**, synergistic effect of *Fusarium pseudograminearum* and *Rhizoctonia solani* affecting emergence at Lutz Farm, resulting in decreased emergence in co-inoculated plots (P = 0.002).