EVALUATING CHEMICAL SEED TREATMENTS FOR FUSARIUM ROOT ROT CONTROL IN DRY BEANS AND FIELD PEAS

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MASTER OF SCIENCE

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ABSTRACT

This study evaluated commercially available seed treatment products for their ability to control *Fusarium solani* and *F. avenaceum*, causal agents of root rot in dry edible bean and field peas, respectively, through *in-vitro*, growth chamber and field trials. Disease severity was assessed using a 0 to 5 scale, and root health parameters were recorded. The *in-vitro* tests conducted were not considered good predictors of fungicide performance in growth chamber or field trials in case of dry beans for management of *F. solani*. In case of field peas, *in-vitro* and growth chamber studies provided consistent results and allowed the identification of fludioxonil, trifloxystrobin and pyraclostrobin as the most effective products to manage *F. avenaceum*. Overall, integration of chemical seed treatments along with cultural practices; crop varieties partially resistant to root rot, and drench application is necessary to effectively manage Fusarium root rot of dry beans and field peas in field conditions.

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LITERATURE REVIEW

Introduction

A legume is a plant or the fruit or seed of the plant in the family Fabaceae or Leguminosae. Legumes include beans, peas, lentils, peanuts, and other podded plants that are used as food. They have been cultivated for thousands of years, although many of the varieties of beans and peas that are common today were unknown until relatively recent times (29). Legumes have played an important role in the traditional diets of many regions throughout the world. It is difficult to picture the cuisines of Asia, India, South America, the Middle East, and Mexico without soybeans, lentils, black beans, chickpeas and pinto beans, respectively (29). In contrast in western countries beans tend to play only a minor dietary role despite the fact that they are low in fat and are excellent sources of protein, dietary fiber, and a variety of micronutrients and phytochemicals. Intake of bean has actually declined during the past century in many European countries (21).

Pulses are the dry edible seeds of leguminous plants. According to the FAO definition, the term "pulses" excludes legumes used for oil extraction (soybean, peanut) and those harvested green for food (green pea, green bean). Pulses include dry bean, dry peas, lentils and chickpeas. They are used as food for humans and other animals around the world. They are a rich source of protein, dietary fiber, vitamins and minerals. They also contain phytochemicals which reduce the risk of certain types of cancer and other diseases (4). Beans are included in the same group as nuts, meat, poultry, fish, and seeds in the US Department of Agriculture food guide pyramid. The 2005 U.S. Dietary Guidelines for Americans recommend more frequent consumption of lentils, dry peas and beans because of their nutritional properties (15). Pulses, like many leguminous

crops play an important role in crop rotation due to their nitrogen fixation ability. They are planted in rotation with other crops, generally cereal grains such as wheat and barley, and are able to convert atmospheric nitrogen into nitrogen usable for plant growth, reducing the need for additional fertilization of following crops (45).

Dry bean

Dry bean, *Phaseolus vulgaris* L. belongs to the family Fabaceae, subfamily Papilionoideae and tribe Phaseoleae. The genus *Phaseolus* comprises over 30 species, of which only 5 species have been domesticated. The domesticated species include *P. acutifolius* A. Gray (tepary bean), *P. coccineus* L. (scarlet runner bean), *P. lunatus* L. (lima bean), *P. polyanthus* Greenman (year-long bean) and *P. vulgaris* (common bean) (43). Dry beans are legumes harvested for the seed within pods once the plant matures.

Dry beans are known to be one of the earliest crops of the New World and were used as a staple food in the low to mid-altitudes of the Americas for thousands of years (5). They were grown by Native Americans in the US even before the Europeans arrived (44). *P. vulgaris* has two centers of domestication: Mesoamerican (Central America or Middle America) and Andean (South America). Mesoamerican beans are characterized by small and medium sized seeds and Andean beans are characterized by large sized seeds (43). Both gene pools can be distinguished at morphological and molecular levels.

Dry beans are widely grown in North, Central, and South America, Africa, Asia and Europe. Important dry bean producing nations of the world include India followed by Brazil, Myanmar, the United States, China, Mexico, United Republic of Tanzania, Uganda and Kenya. The United States is the fifth largest producer of dry edible beans in the world (13). The U.S.

commercial dry bean industry began in New York during the mid-1800s (2). North Dakota (25%) is currently the leading producer of dry beans in the U.S. followed by, Michigan (17%), Minnesota (11.5%), Nebraska (10.6%) and Idaho (9.4%) (33). Dry beans are nutritionally rich, essentially composed of protein, carbohydrates, vitamins and minerals, and are low in fat. They are a major source of dietary protein containing between 21-25% protein by weight (3). They act as a good source of protein at minimal cost, relative to the animal protein sources.

Fusarium root rot of dry edible beans

Root rot of dry edible bean is caused primarily by *F. solani* f. sp. *phaseoli* in a complex with *Rhizoctonia solani* and *Fusarium oxysporum* in Minnesota (11). In other parts of the USA, *Aphanomyces euteiches* f. sp. *phaseoli* (39) and *Pythium* spp. (17) have also been associated with the disease (16, 40). Fusarium root rot of dry bean, a soil-borne disease has been reported to cause severe yield losses in all bean growing regions including California, Colorado, Wisconsin, Washington, Nebraska, North Dakota, New York, Minnesota, and Michigan (6). Yield losses due to the disease approach 50% in severely infested areas (12). In Nebraska, 40-50% yield reductions due to *Fusarium* root rot were estimated in great northern and pinto beans (48) and 89% yield losses were reported in Colorado due to *Fusarium* root rot in pinto beans (50). In New York, yield losses up to 80% were observed in severely affected fields due to root rot caused by *Fusarium, Rhizoctonia, Thielaviopsis*, and *Pythium* (1). In addition to *Fusarium*, several other fungal pathogens have also been associated with this disease including *Rhizoctonia*, *Thielaviopsis*, *Pythium*, *Aphanomyces* and *Phymatotrichum* (19).

Signs and symptoms

Initial symptoms of *Fusarium* root rot appear as longitudinal narrow, brick-red colored lesions or streaks on the hypocotyl and tap root. Later, these streaks become numerous, coalesce, and the entire cortex of hypocotyls and older portions of root systems may become necrotic. Necrosis is confined largely to the cortex. Severely infected plants exhibit stunting and premature defoliation. Numerous adventitious roots are produced by the diseased plants as a survival mechanism in response to infection by *Fusarium* spp. (6).

Field pea

Pea, *Pisum sativum* L. belongs to the family Fabaceae and genus *Pisum*. The currently accepted taxonomic classification according to Kosterin and Bogdanova (24) recognizes three species: *Pisum abyssinicum* A. Br.; *Pisum sativum* L., and *Pisum fulvum*.

Peas are believed to have possibly originated in southwestern Asia, i.e., northwestern India, Pakistan or adjacent areas of former USSR and Afghanistan and later spread to temperate parts of Europe (30). Four centers of origin namely, Central Asia, the Near East, Abyssinia and Mediterranean have been identified based on genetic diversity (30). According to morphological similarities and cytological clues it is believed that the Near East *humile* peas were the primary wild stock for cultivated peas (51).

Findings of carbonized remains of pea in archaeological sites, suggest that pea plants were domesticated in the Near East arc, also known as Fertile Crescent of Southwest Asia (51). Wild and primitive forms were found in ecologically diverse sites stretching from the Mediterranean to Afghanistan and into the highlands of Ethiopia (26). Peas were first seen in Neolithic farming villages of the Near East which dates back to 7000-6000 B.C. Peas are also

found in Neolithic settlements in Europe (51). After domestication, pea was disseminated to other regions including Russia to the north, Europe to the west, the Indian subcontinent and China to the east. Pea was introduced into the Americas soon after Columbus discovered the country. Winter type pea was introduced from Austria in 1922 (30).

Dry pea, or field pea, is an important cool season legume crop grown in the U.S. Major field pea producing areas in the world include Canada, Russian Federation, China, India, the United States of America, France, Ukraine, Australia, Ethiopia and Germany. The area under this crop has rapidly increased from 337,500 acres in 2003 to 649,000 acres in 2012. The United States is the ninth largest producer of dry edible peas in the world (13). The leading dry edible pea producing states during 2010 included North Dakota (57%), Montana (29%) and Washington (9%). In North Dakota 235,000 acres were planted to pea in 2012, contributing to more than 41% of production in the U.S. (32). Dry pea is composed mainly of carbohydrate (60%), protein (25%), fiber (25.5%), sugars (8%), lipid (1.2%) and moisture (11%) (47).

Fusarium root rot of field peas

Fusarium root rot on pea was first reported in Montana in 1918 and Wisconsin in 1923, around the same time it also was reported in Europe (26). Plots infested with *F. solani* f. sp. *pisi* in eastern Washington recorded up to 30% yield loss (26). Several *Fusarium* species have previously been associated with root rot in pea, apart from the primary pathogen, *F. solani* f. sp. *pisi,* these include *F. avenaceum, F. culmorum, F. graminearum, F. sambucinum* var. *coeruleum, F. equiseti, F. poae, F. sporotrichioides* and *F. tabacinum* (9). However, *F. avenaceum* was reported to be the most common *Fusarium* spp. isolated from discolored roots of pea and lentils grown in the eastern part of Saskatchewan, Canada (14). Field pea surveys conducted across

North Dakota in the past three years also have shown that *F. avenaceum* is the most prevalent pathogen of pea root rot in the state (8).

Signs and symptoms

The pathogen generally affects the cotyledonary attachment area, epicotyls and hypocotyls. Initial symptoms appear as reddish brown streaks on primary and secondary roots that coalesce later in the season. The root color becomes dark reddish brown in the seed zone and at the ground line and a red discoloration of the primary vascular system is observed around the area of cotyledon attachment. However, the discoloration does not progress above the soil line and the pathogen is rarely isolated from above ground plant tissue. Severely infected plants may exhibit above ground symptoms which include graying, yellowing and necrosis of the lower foliage and stunted plant growth (26).

Factors affecting disease severity

The severity of root rot not only depends on the virulence of the pathogen causing the disease, but also on several other factors and conditions. For example, a study conducted by Kraft *et al*. (25) revealed that soil compaction increases the severity of *Fusarium* root rot on pea. In that study, reductions of root lengths of susceptible pea lines were twice as much of that of resistant lines. Results of a study conducted by Ortiz-Ribbing *et al*. (38) with Sudden Death Syndrome in soybean revealed that the effect of the location of the initial infection site, whether tap root or lateral roots, on disease severity vary with the cultivars used. Some cultivars had significantly greater reductions in several root characteristics and significantly higher AUDPC values when infected in the tap root compared to the infection of lateral root.

Reductions in root length have been reported after infection of roots with *F. solani* on citrus (10), pea (25), and soybean (31) root systems. These results support the idea that root systems change after infection by a pathogen, and that this change may play a role in development of disease. *F. solani* f. sp. *glycines* is able to spread from infected lateral roots inward to the taproot. This indicates that infection of the lateral roots may play a greater role in SDS development than anticipated.

Fusarium root rot is spread within and between fields via contaminated seeds, infected host tissue, infested soil, wind, drainage, irrigation water and mechanically through tillage and equipment. The fungus can survive in soil for over 30 years and cause root rot (19, 23, 46). The optimum temperature for the disease development ranges between 16°C to 24°C. The disease severity was reported to be greatest at 21°C and less at 14°C and 28°C (19). Soil compaction, low soil oxygen, excess soil moisture, low temperatures, water stress, high planting density, herbicide toxicity and toxic substances favor the development of disease (6). Short periods of flooding may also increase the disease severity (18).

Current management strategies

Host resistance

No dry bean cultivars are known to be completely resistant to Fusarium root rot. However, recently cultivars with some levels of resistance or tolerance to the disease have been identified (41). Resistance to Fusarium root rot in both dry bean and pea is quantitatively inherited (22). Currently there are no commercial cultivars that are completely resistant to root rot. However, the commercial cultivars that are reported to be tolerant to root rot caused by *F.*

solani f. sp. *pisi* have been released (26). But, there is no information regarding resistance to root rot caused by *F. avenaceum*.

Cultural practices

Since soil conditions influence the development of dry bean root rot, cultural practices can be utilized to minimize yield loss due to disease. Mounding soil around the plant increases adventitious root formation and thereby minimizes damaging effects of disease on productivity of plants. Increasing the spacing between plants within the row reduces disease spread. However, plant populations that provide complete ground cover often gives greater seed yields (6). The most effective means of root rot management is by minimizing soil compaction which can be achieved by loosening sub-layers with chisels before or at the time of planting (7). Rotating beans with crops such as alfalfa and small grains also reduces the compaction and increases the water holding capacity of the soil (34).

The cultural practice for reducing root rot in field peas mainly emphasize on good tillage practices that reduce soil compaction and promote favorable soil moisture. Rotating crops such that peas are not planted in the same field for more than once in five years helps in delaying the onset and reduces disease severity. Addition of fertilizers to the soil also contributes to reduction of disease (26).

Bio-control

Treating the seeds with Rhizobium leguminosarum and the vesicular-arbuscular mycorrhiza Glomus mosseae have shown promising results in reducing disease severity and increasing nutrient uptake and plant growth in dry beans (20). Bacillus subtilis and Bacillus pumilus are recommended for use as seed treatment against Fusarium (27).

Chemical control

Seed treatments and soil fumigation are the best way to control Fusarium root rot. Fumigating the soil with chemical such as chloropicrin helps in managing the disease in dry bean (6). Azoxystrobin, fludioxonil and trifloxystrobin containing fungicides are recommended for use as seed treatments against *Fusarium* sp. (27).

Although studies pertaining to chemical control of Fusarium root rot in beans are minimal, seed treatments have been tested against *Fusarium* sp. in other host plants. One such study on Fusarium root rot of tomato shows that biological control agents can sometimes be far more effective when combined with chemical treatment or other management strategies rather than using them alone. A combination of *Burkholderia cepacia* with a low concentration of fungicide carbendazim, 1µg mL^{-1} resulted in a reduction of disease symptoms by 46% as compared to 20% symptom reduction obtained with bacterium alone and no control when fungicide alone was used at this concentration (36).

Sedaxane is a new broad-spectrum seed treatment fungicide whose physicochemical properties and activity spectrum have been optimized for use as a seed treatment providing both local and systemic protection of the seed and roots of target crops. Sedaxane when integrated with existing seed care products expands the broad spectrum and sustained impact of seed applied fungicides (42). Sedaxane offers a unique advantage in preserving root health against constant challenge of seed and soil borne pathogens as suggested by greenhouse and field studies (42). Sedaxane showed high levels and consistent protection against *Ustilago nuda*, *Pyrenophora graminea* and *Rhizoctonia* spp, under greenhouse conditions (37, 42, 49). Its efficacy against *Rhizoctonia* spp. resulted in increased yield compared with the untreated check, under field

conditions. Sedaxane has been found to be effective against snow mould under very high disease pressure conditions.

Sedaxane can be used as a seed treatment in a wide variety of crops due to its broad spectrum and high level of activity in combination with excellent crop tolerance. It is a potential tool for precautionary resistance management when combined with other fungicides, especially against pathogens showing a potential for resistance development, such as *Monographella nivalis* (49). Sedaxane resulted in control of *R. solani* on corn and soybean, and also improved yield and root health of plants over several growing seasons (35).

However, in the available literature, there has been no comprehensive study evaluating chemical seed treatments for their ability to control Fusarium root rot of dry bean and field pea, and to enhance root growth at laboratory, greenhouse and field conditions. Therefore, an attempt was made to screen a collection of chemical seed treatment products through *in vitro*, growth chamber and field assessments for their ability to control Fusarium root rot of dry bean and field pea caused by *Fusarium solani* f. sp. *phaseoli* and *Fusarium avenaceum*, respectively, and also to study their effect on root growth.

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CHAPTER 1: EVALUATING EFFICACY OF SEED TREATMENTS FOR FUSARIUM ROOT ROT CONTROL IN DRY BEANS

Abstract

Dry edible bean (*Phaseolus vulgaris* L.) is a major legume crop grown in the United States and North Dakota has been the leading producer in the US since 2009. In recent years, *Fusarium* species have been identified as the major causal agents of root rots in dry bean in North Dakota. Since complete resistance to Fusarium root rot currently is not available in commercial cultivars of dry beans, integration of chemical control is essential for effective disease management. This study focuses on evaluating commercially available seed treatment fungicides for control of root rot caused by *Fusarium solani* the most common *Fusarium* species associated with root rot in dry beans. Seed treatment products evaluated in this study include thiabendazole (methyl benzimidazole carbamate), ipconazole (demethylation inhibitor), mefenoxam, metalaxyl (phenyl amide), sedaxane (succinate dehydrogenase inhibitor), azoxystrobin, pyraclostrobin, trifloxystrobin (quinone outside inhibitor), and fludioxonil (phenyl pyrrole), which were assessed both individually, and in combination, through *in-vitro*, growth chamber, and field trials. The i*n vitro* tests conducted were not considered good predictors of fungicide performance in growth chamber or field trials. Fungicides that were very effective in lab trials did not do as good in growth chamber and vice versa. Under field conditions, none of the fungicides evaluated whether alone or in mixtures provided consistent reductions in disease severity or improved plant stands. The lack of efficacy in protecting plants against root rots may not necessarily be a reflection of the lack of efficacy of the compounds evaluated but the results of not applying the proper approach. Protecting the seeds may give a boost to germination but may not be enough to protect the roots through-out the growing period and multiple fungicide

applications may be required to supplement the effect. Overall, integration of chemical seed treatments along with cultural practices; crop varieties partially resistant to root rot, and drench application are likely to be required to effectively manage Fusarium root rot. Findings from this study provide preliminary information regarding the potential of some of the chemicals used in the seed treatments to control *Fusarium* species associated with root rot in dry bean.

Introduction

Dry edible bean (*Phaseolus vulgaris* L.) is a major legume crop grown in the United States where North Dakota is the largest producer with an area of 800,000 acres planted to dry edible bean in 2010. The state contributes to 36% of the nation's production of dry bean (14). Fusarium root rot of dry bean, a soil-borne disease has been reported to cause severe yield losses in all bean growing regions including California, Colorado, Wisconsin, Washington, Nebraska, North Dakota, New York, Minnesota, and Michigan (5).Yield losses due to Fusarium root rot approach 50% in severely infested areas (6). In Nebraska, 40-50% yield reductions were estimated in great northern and pinto beans (22) and 89% yield losses were reported in pinto beans (24). In New York, yield losses of up to 80% were observed in severely affected fields due to root rot caused by *Fusarium, Rhizoctonia, Thielaviopsis*, and *Pythium* (1). Root rot is a growing concern for dry bean production in the state as per the field surveys conducted in the state (8). Dry beans are susceptible to many root rot pathogens and fungal species commonly associated with this disease on dry beans include: *Fusarium solani* f. sp. *phaseoli*, *Rhizoctonia solani* and *Pythium* spp. (8, 15). Among these, *Fusarium* spp. have been identified as the major root rot causing pathogen of dry beans in North Dakota in recent years (8). Since root rot is a soil borne disease, there is constant risk of roots getting exposed to the pathogens present in soil throughout the growing season. No commercial cultivars grown in this region have complete

resistance to dry bean root rot at present, although a few sources of partial resistance are available (4). Hence, integration of chemical control is essential for effective disease management. Seed treatments can protect the plant from pathogen in its initial growing stages (2-3 weeks), thereby allowing the plant to establish itself in the ground. So, a seed treatment protects the genetic potential of the plant during the initial two to three weeks after planting. This project focuses on the evaluation of chemical seed treatment as a potential disease management tool for the management of Fusarium root rot at the laboratory, growth chamber (18) and field levels for dry beans.

Seed treatments are recommended for controlling most seed-borne and soil-borne diseases like Fusarium root rot (10). Such treatments can be of various types includig resistance inducers, microorganisms, plant extracts, bio fertilizers and chemicals (6, 7, 9, 12, 13, 20, 23, 24). Some antagonistic bacterial seed treatments are effective against *F. verticillioides* in maize in greenhouse conditions (17). Applications of *B. subtilis* in mixtures with *Rhizobium* were found to be promising for bean root rot control in Minnesota (6). Novel strains of *B. subtilis* that can be used as seed and soil treatments were identified to effectively control Fusarium root rot of soybean caused by *F. oxysporum* and *F. graminearum* at greenhouse level (24).

Some of the fungicides most commonly used on dry beans as seed treatments are listed in Tables 1.1 and 1.2. Of these compounds, mefenoxam and metalaxyl are systemic fungicides with protective and curative action against Oomycetous fungi that cause damping-off (3) and are commercially applied in mixtures with other fungicides. These fungicides belong to the phenyl-amide group and inhibit rRNA biosynthesis in fungi (2). Thiabendazole is a benzimidazole compound that interrupts mitosis and cell division by binding to β-tubulin (2). Thiabendazole

controls fungi other than Oomycetes and is registered for use against Fusarium tuber rot (11). Thiabendazole is not registered for use in dry beans (11). Sedaxane is a relatively new compound that inhibits mitochondrial respiration chain by binding to succinate dehydrogenase (2). It is considered to have good control of *Rhizoctonia solani* and some species of *Fusarium* although its activity against *F. solani* f. sp. *phaseoli* has not been determined. Previous studies have demonstrated that sedaxane is a broad spectrum fungicide that has a physiological effect on root growth when used against various seed-borne and soil-borne pathogens other than *Fusarium* spp. on corn, soybean and small grains (16, 19, 21). Azoxystrobin, pyraclostrobin and trifloxystrobin inhibit mitochondrial respiration by binding to cytochrome bc1 at QoI site (2). These three fungicides are registered in North Dakota for use in dry bean as seed treatments (11). Azoxystrobin and trifloxystrobin provide excellent control of *Rhizoctonia solani* and good control of some species of *Fusarium* according to respective fungicide labels. Pyraclostrobin is effective against *Fusarium graminearum* (fungicide label). Fludioxonil is a non-systemic fungicide which inhibits transport-associated phosphorylation of glucose, reducing mycelial growth (2). Fludioxonil is very effective against *F. graminearum* and provides good control of other *Fusarium* species. Ipconazole inhibits sterol biosynthesis in fungi while thiram has a multi-site contact activity (2). These two fungicides have been in the market for long time; ipconazole is effective against a number of fungal species including *Fusarium* as per the fungicide label, thiram is effective against multiple seed-borne and soil-borne fungi according to treatment label. Most of these fungicides (Table1.1 and Table 1.2) are registered for use as seed treatments on dry beans except thiabendazole and ipconazole. Thiabendazole (Mertect 340 F), ipconazole (Rancona), fludioxonil (Maxim 4 FS; Apron Maxx and Cruiser Maxx), azoxystrobin (Dynasty 100 FS), pyraclostrobin (Stamina) and trifloxystrobin (Trilex) are intended for control of

Fusarium spp. along with other soil-borne and seed-borne pathogens according to fungicide labels. Ipconazole is recommended for protection against seed rot caused by *Fusarium* spp. in both dry beans and field peas. However, none of these have been specifically evaluated against *Fusarium solani* f. sp. *phaseoli*. The objective of this study was to screen a collection of chemical seed treatment products through *in vitro*, growth chamber and field assessments for their ability to control Fusarium root rot of dry bean caused by *Fusarium solani* f. sp. *phaseoli* (Fsp).

Materials and methods

The seeds used in all experiments described below were from the root rot susceptible dry bean var. Red Hawk. These seeds were treated with individual compounds (single active ingredient-ai) or their combinations (Tables 1.1 and 1.2) at different rates by Syngenta Seedcare at their facilities in Stanton, MN. The efficacy of these treatments was evaluated in the laboratory (*in vitro* trials) using a simple petri-dish assay, in growth chambers using sand-cornmeal inoculum, and in field trials using infested wheat inoculum and using natural soil infestations. All compounds used in these studies were supplied by Syngenta Seedcare.

Table 1.1. Single active ingredient compounds evaluated as seed treatments for their efficacy to control Fusarium root rot of dry bean in controlled environment trials.

Treatment code	Commercial name	Active ingredients	g ai/ 100 kg seed
Non-treated control	Non-inoculated, non-treated control	None	---
Fludioxonil (2.5 g)	Maxim 4 FS	Fludioxonil	2.5
Fludioxonil (5 g)	Maxim 4 FS	Fludioxonil	5
Azoxystrobin	Dynasty 100 FS	Azoxystrobin	2.5
Ipconazole	Rancona	Ipconazole	1.5
Thiabendazole	Mertect 340 F	Thiabendazole	50
Sedaxane	Sedaxane	Sedaxane	2.5
Pyraclostrobin	Stamina	Pyraclostrobin	5.0
Trifloxystrobin	Trilex	Trifloxystrobin	5.0

Table 1.2. Seed treatment containing multiple active ingredients screened for their efficacy to control Fusarium root rot of dry bean in controlled environment and in field conditions.

Treatment code	Commercial name	Active ingredients	g ai/ 100 kg seed
NI_NTC	Non-inoculated, non-treated control	None	
I_NTC or NTC	Inoculated, Non-treated control	None	
Mef/Flu/Azo	Apron XL	Mefenoxam	15
	Maxim 4 FS	Fludioxonil	2.5
	Dynasty 100 FS	Azoxystrobin	2.5
Mef/Flu	Apron XL	Mefenoxam	15
	Maxim 4 FS	Fludioxonil	5
Mef/Flu/Sed	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Sedaxane	Sedaxane	2.5
Mef/Ipc	Apron XL	Mefenoxam	7.5
	Rancona 3.8	Ipconazole	1.5
Mef/Thia	Apron XL	Mefenoxam	7.5
	Mertect 340 F	Thiabendazole	50
Mef/Flu/Azo/Ipc	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Dynasty 100 FS	Azoxystrobin	2.5
	Rancona	Ipconazole	1.5
Mef/Flu/Azo/Thia	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Dynasty 100 FS	Azoxystrobin	2.5
	Mertect 340 F	Thiabendazole	50
Mef/Flu/Azo/Thia/Ipc	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Dynasty 100 FS	Azoxystrobin	2.5
	Mertect 340 F	Thiabendazole	50
	Rancona	Ipconazole	1.5
Mef-flu/Flu/Sed	Apron Maxx RTA	Mefenoxam + Fludioxonil	7.5 + 5
	Maxim 4 FS	Fludioxonil	5
	Sedaxane	Sedaxane	2.5
Mef-flu/Ipc/Sed	Apron Maxx RTA	Mefenoxam + Fludioxonil	7.5 + 5
	Rancona	Ipconazole	1.5
	Sedaxane	Sedaxane	2.5
Mef-flu/Mef/Flu/Sed	Apron Maxx RFC	Mefenoxam + Fludioxonil	6.0
	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	2.5
	Sedaxane	Sedaxane	2.7
Tmx-mef-flu/Flu/Sed	CruiserMaxx Beans	Thiamethoxam + Mefenoxam + Fludioxonil	56.7*
	Maxim 4 FS	Fludioxonil	2.5
	Sedaxane	Sedaxane	3.4
Met/Tri	Allegiance	Metalaxyl	15.5
	Trilex	Trifloxystrobin	5.0
Met/Th/Pyr	Acquire	Metalaxyl	6.2
	Thiram 42 S	Thiram	62.5
	Stamina	Pyraclostrobin	5.0

*calculated based on CruiserMaxx label.

In-vitro **evaluation of single active ingredient (ai) and combination seed treatments**

Petri-dish trials

In this method a 5 mm diameter agar plug was collected from the growing edge of a 7 day old colony of *Fusarium solani* f. sp. *phaseoli* (Fsp), isolate 08/RG/BF/28, cultured on full-strength potato dextrose agar (PDA). The medium was made by mixing 24 g of potato dextrose broth (PDB, BD Difco, Franklin Lakes, NJ) with 15 g agar (BD Difco, Franklin Lakes, NJ) in 1000 ml distilled water and autoclaving it at 121^{o} C for 20 minutes at 103.42 kPa. The agar plug was placed at the center of a large plastic BD Falcon Petri-dish (150X15 mm, BD Difco, Franklin Lakes, NJ) containing 1/8th strength PDA with the mycelium facing the agar surface and incubated at 21^{o} C (room temperature) with 14 h light daily for seven days. The medium was prepared by mixing 3 g PDB and 10 g agar in 1000 ml distilled water and autoclaving it as described above. On the eighth day of incubation, eight seeds from a single treatment were placed in each dish along the circumference of the growing isolate leaving approximately 1 cm space between the growing tip of isolate and the seed (Figure 1.1). Once all treatments had been plated the dishes were incubated for six additional days as described.

After incubation area of the petri-dish covered by fungal colony, and the number of seeds overgrown/colonized by the fungus were recorded. The experiments for each group of compounds (single and in mixtures) were laid out in a completely randomized design (CRD) with three replications per treatment where each dish represented a replication. The experiments were repeated once.

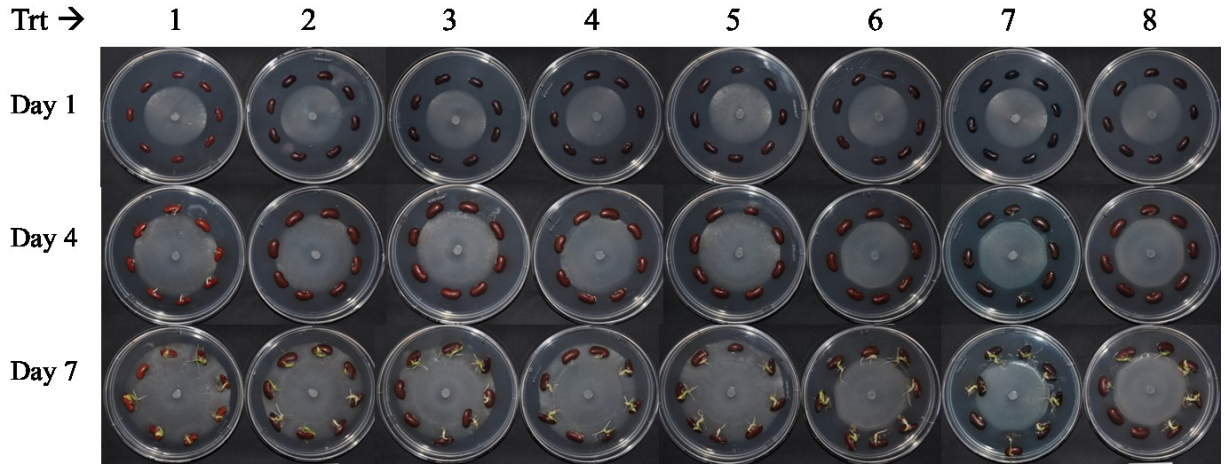


Figure 1.1. Response of fungal growth to different seed treatments at 1, 4 and 7 days after inoculation: a pictorial representation from preliminary trials.

Growth chamber evaluation of single ai and combination seed treatments

Sand cornmeal inoculum layer method

Treatments were compared under growth chamber conditions along with inoculated non-treated (positive) and non-inoculated non-treated (negative) controls using the modified sand-cornmeal inoculum layer method described by Bilgi et al. (4). Fsp isolate 08/RG/BF/28 previously obtained from a commercial dry bean field in North Dakota in 2008 was used to establish the efficiency of the range of seed treatments. Inoculum was prepared by placing eight 5 mm plugs of the isolate grown for 7 days on half-strength PDA into autoclavable bags containing sterilized (121°C, 103.42 kPa for 45 min under dry setting) sand-cornmeal mixture (54 g of regular play sand, 6 g of Quaker yellow cornmeal and 12 ml of distilled water).

Inoculated bags were incubated at room temperature for seven days and shaken daily by hand to ensure uniform growth of fungus throughout the bag. Once the mixture was colonized, 266 ml plastic drinking cups with holes at the bottom for water drainage were layered with 15 g of sterilized (121°C, 103.42 kPa for 45 min under dry setting) vermiculite(premium grade, Sun Gro Horticulture Distribution Inc. Washington, U.S.A) followed by 15 g of inoculum and 8 g of

vermiculite. Two seeds per treatment were placed on the upper vermiculite layer and covered with another 8 g of vermiculite. Three cups of each treatment were placed in trays in a growth chamber maintained at 14 h light daily with day and night temperatures of 21 and 18°C, respectively. Cups were watered daily. Severity of root rot was evaluated 21 days after planting using a 0-5 scale where 0 = no visible symptoms; 1 = 1-20% discoloration with individual localized lesions; 2 = 21-40% discoloration with coalesced lesions but tissues are firm with some reduction in root mass; 3 = 41-60% discoloration and root tissue lesions combined with considerable softening, rotting and reduction in root mass; 4 = 61-80% discoloration and internal pith tissues of roots affected; 5 = 81-100% discoloration, root softening and rotting along with heavy reduction in root mass (Figure 1.2).

The roots were scanned using a root scanner (Epson Expression 10000XL) after rating and the WinRHIZO software (Regent Instruments, Quebec, Canada) was employed to analyze scanned images of roots to estimate root surface area, total root length and number of root tips. The experiment was laid out in a completely randomized design (CRD) with non-inoculated and inoculated controls and three replications per treatment. The experiment was performed three times.

Figure 1.2. Pictures of roots with differing levels of discoloration and root mass depicting the root rot rating scale used for disease evaluations.

Field evaluation of combination products

Inoculated field trials

A plot located at the North Dakota State University's Carrington Research Extension Center in Carrington, North Dakota was used for these trials. The site was inoculated with an equal mixture of three aggressive Fsp isolates, 08/RG/BF/28, 08/RG/BF/100 and Fsp NDSU, grown separately on autoclaved wheat for 10 days in aluminum trays and covered with aluminum foil. After incubation under dark conditions, the wheat grains were sun-dried at room temperature in the greenhouse. This inoculum was incorporated into the soil at the rate of 2 g/ linear foot of row at the time of planting in 2011 and 4 g /foot before planting in 2012. The experiment was set-up in a randomized complete block design (RCBD) consisting of four replications and sixteen treatments (Table 1.2) including an inoculated non-treated control and a non-inoculated non-treated control. The experiment was conducted in 2011 and repeated in 2012.

During the first year (2011) the trial was planted on June 2 with a seeding rate of 236,294 pure live seeds per hectare. Plant stand count was taken 15 and 28 days after planting (DAP), June 17 and June 30, respectively. This trial consisted of 4-row plots with 38.1 cm row spacing and plot size of 1.52 m x 7.62 m. Due to unexpected herbicide damage, the root rot severity ratings had to be recorded at late vegetative stages, approximately 1 week prior to bloom initiation (Jul 12, 2011) instead of pre-flowering stage. In the second year (2012), the trial was planted on May 30 with a seeding rate of 227,213 pure live seeds per hectare and plant stand counts were taken at two time points, June 11 (12 DAP) and June 18 (19 DAP). This trial consisted of 2-row plots with 76.2 cm row spacing and plot size of 1.52 m x 7.62 m. The root rot severity was assessed at pre-flowering stage (Jul 11, 2012).

Field trials under natural disease pressure

This experiment was conducted in fields with history of Fusarium root rot problems (Juan Osorno, personal communication). The trials were located at Staples and Perham in 2011 and 2012 respectively. The experimental design was an RCBD consisting of four replications and 15 treatments (Table 1.2) which included a non-treated negative control.

During the first year (2011) the trial was planted on May 26 at Staples with a seeding rate of 172,222 seeds per hectare and stand count was taken at one time point, June 29 (34 DAP). This trial consisted of 2-row plots with 76.2 cm row spacing and plot size of 1.52 m x 6.10 m. The root rot severity was assessed at pre-flowering stage (July 13, 2011). In the second year (2012), the trial was planted on May 17 at Perham with a seeding rate of 172,222 seeds per hectare and stand count was taken at two time points, June 7 (21 DAP) and June 15 (29 DAP). This trial consisted of 2-row plots with 76.2 cm row spacing and plot size of 1.52 m x 6.10 m. The root rot severity was assessed at pre-flowering stage (July 2, 2012).

Sampling method

Root samples were collected from the experimental fields in Carrington and Staples in 2011 and Carrington and Perham in 2012, respectively. In addition to root samples, soil samples were collected for inoculated trial conducted in Carrington during both years. Soil samples were collected from all four corners and the center of the field in an "X" – pattern with the help of a shovel. They were brought back to the laboratory in Ziploc bags and the samples were ground before using for analysis. During both years, roots were arbitrarily sampled from each plot in the field when plants were at pre-flowering stage. These roots were pooled by plot in Ziploc bags and brought to the laboratory in ice boxes to prevent drying of plant tissue. They were stored in

coolers until analyzed. Foliar tissue was also sampled, transported and stored in the same method. The root samples were used for root rot rating, calculating root mass and pathogen isolation. Ten roots were sampled during each year for the inoculated trial at Carrington. Six roots were sampled from each plot in Staples in 2011 while in 2012 ten roots were sampled from each plot in Perham. Root rot rating was done using the same scale used in the growth chamber experiments after the roots were washed under running tap water. The foliar mass and root mass was assessed by cutting the tops off at the node where the cotyledons were attached, drying the foliage and root separately for 2-3 days at room temperature until completely dried, and then weighing the biomass. Total number of roots collected per plot were weighed on a weighing scale and mean root mass per plant was calculated and used for analysis. Mean foliar mass per plant was calculated in the same way.

Pathogen isolation and identification

A few symptomatic roots from each plot were plated to identify the pathogens present. Infected roots were washed and surface disinfested by immersing them in 10% bleach (NaOCl) for two minutes followed by a dip in 70% ethanol for 30 seconds and three subsequent washes in sterile distilled water. The samples were then air-dried, plated onto potato dextrose agar (PDA) amended with 0.3 mg/ml of streptomycin, an antibiotic to avoid growth of bacteria in petri-dish. These plates were incubated at room temperature (~21^{o} C) with a 12 hour photoperiod. Soil samples were plated onto PDA amended with 0.3 mg/ml of streptomycin using the serial dilution technique and incubated at room temperature with a 12 hour photoperiod. Serial dilution was conducted by placing 4-5 test tubes containing 9 ml of distilled water in a row. Soil samples were then serially diluted by adding 1g of the ground soil sample to the first test tube followed by adding 1ml of the soil solution from first test tube to the second and so on. 1ml of soil solution

from each of these serially diluted test tubes was plated onto PDA amended with antibiotic. The concentration of soil solution was chosen in such a way that the different colonies present in the soil could be easily separated using single-spore isolation. After 7-8 days, mixed cultures of fungal species were obtained. Fungal colonies were separated through serial sub-cultures on the same media and pure cultures established through single-spore isolation. Species were identified based on morphological characteristics. Morphological characteristics evaluated included fungal growth pattern, color and texture of the colony, mycelia type and spore structure.

Statistical analyses

Statistical analyses for the above studies were performed using SAS version 9.2 (SAS Institute, Cary, NC). Data was analyzed using either the analysis of variance (PROC ANOVA) or generalized linear model (PROC GLM) procedures. Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05.

In-vitro evaluation of single ai and combination seed treatments

Data collected for *in-vitro* evaluation included the area of petri-dish covered by fungal colony and percent of seeds colonized by the fungus after six days of incubation. Levene's test was performed on the collected data to verify homogeneity of variances as the experiment was conducted more than once. Upon acceptance of the null hypothesis that variances were homogeneous, a combined analysis was performed. Data was analyzed using analysis of variance procedure (PROC ANOVA). Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05.

Growth chamber evaluation of single ai and combination seed treatments

Root rot severity was estimated using a categorical scale. The median values of severity were calculated for each experimental unit and used for the analysis. The median data was analyzed using non-parametric ANOVA-type statistics. First, medians were ranked using PROC RANK and the mean ranks for each treatment was calculated using PROC MEANS. Then ranked medians were analyzed using PROC MIXED to determine whether there were differences between treatments. The SAS macro LD_CI.SAS developed by E. Brunner (University of Gottingen, Germany) was used to calculate the treatment relative effects and their confidence intervals to determine which treatments were different. The estimated relative effect for severity is inversely proportional to the efficacy of seed treatment (smaller relative effects indicate more effective control).

Root growth parameters like root length, root surface area and number of root tips also were analyzed. Levene's test was performed on the collected data to verify homogeneity of variances as the experiment was conducted more than once. If the null hypothesis, variances were homogeneous, was accepted then a combined analysis was performed; otherwise data from each experiment were analyzed separately. Data were analyzed using PROC GLM. Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05. Pearson correlation analysis was performed using PROC CORR in SAS to determine whether root growth parameters were associated to root rot severity.

Field evaluation of combination products

Root rot severity was estimated using the 0-5 scale used in the growth chamber experiments. Since more than one root was uprooted from each plot (replication), median disease

severity for each plot was calculated. This median value was used for further analysis. The severity data was analyzed using non-parametric statistics as described above. Data collected for other traits, like plant stand, average root mass, average foliar mass and yield excluding disease severity, were analyzed using PROC ANOVA. Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05.

Results

In-vitro **evaluation of single ai and combination seed treatments**

In-vitro assessment of single ai seed treatments showed that fungal colony growth and seed colonization were reduced significantly with the use of ipconazole and thiabendazole (Figure 1.3). The other compounds were not as effective and allowed the pathogen to grow in a similar way to that of the non-treated control. Overall, ipconazole and thiabendazole provided maximum protection against the pathogen.

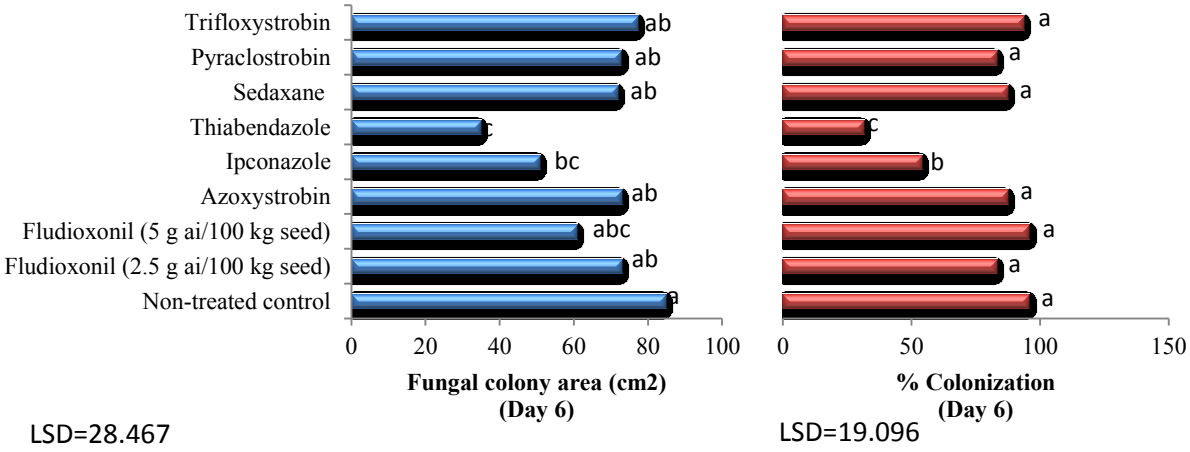


Figure 1.3. Response of *F. solani* f. sp. *phaseoli* colony area (cm^2) and percent seed colonization to single ai seed treatments six days after placing seeds. * Bars with the same letter are statistically not different at P = 0.05.

In-vitro assessment of seed treatments with multiple ai used in combination showed that fungal colony growth and seed colonization were reduced significantly with all ipconazole and thiabendazole containing seed treatments (Figure 1.4). However, the mixtures containing mefenoxam/ipconazole, mefenoxam/fludioxonil/ipconazole/sedaxane and mefenoxam/fludioxonil/azoxystrobin/thiabendazole did not perform as well as some of the other ipconazole and thiabendazole containing seed treatments even though the amount of active ingredient for these two compounds was the same in all the mixtures (Figure 1.4). Overall, treatments including ipconazole and/or thiabendazole were most effective inhibitors of fungal growth and seed colonization (Figure 1.4).

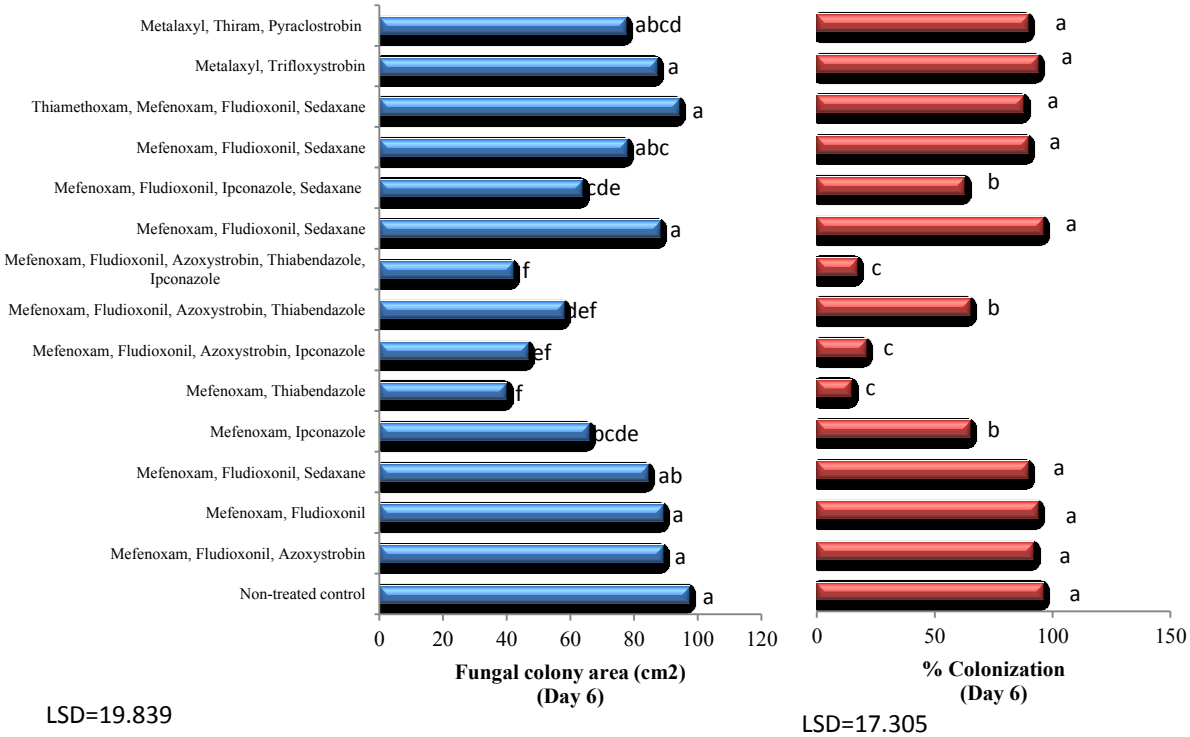


Figure 1.4. Response of *F. solani* f. sp. *phaseoli* colony area (cm^2) and percent seed colonization by pathogen to multiple ai seed treatments six days after placing seeds. * Bars with the same letter are statistically not different at P = 0.05.

Growth chamber evaluation of single ai and combination seed treatments

Reduction in root rot severity as a result of single ai seed-treatments evaluated in this study was observed with azoxystrobin, sedaxane, pyraclostrobin and trifloxystrobin (Figure 1.5, Appendix Table B1). Seedlings produced by seeds treated with thiabendazole or ipconazole had root rot severities similar to those of the non-treated control. None of the treatments evaluated produced a significant increase in root length, root surface area or number of root tips compared to the positive control (Figure 1.6). Values for root length ranged from 335 cm to 473 cm with an average of 403 cm, root surface area ranged from 161 cm^2 to 266 cm^2 with an average of 205 cm^2 and number of root tips ranged from 472 to 679 with an average of 544 root tips. All root growth parameters assessed, using the WinRHIZO software (Regent Instruments, Quebec, Canada) were positively correlated to each other (Appendix Table A1).

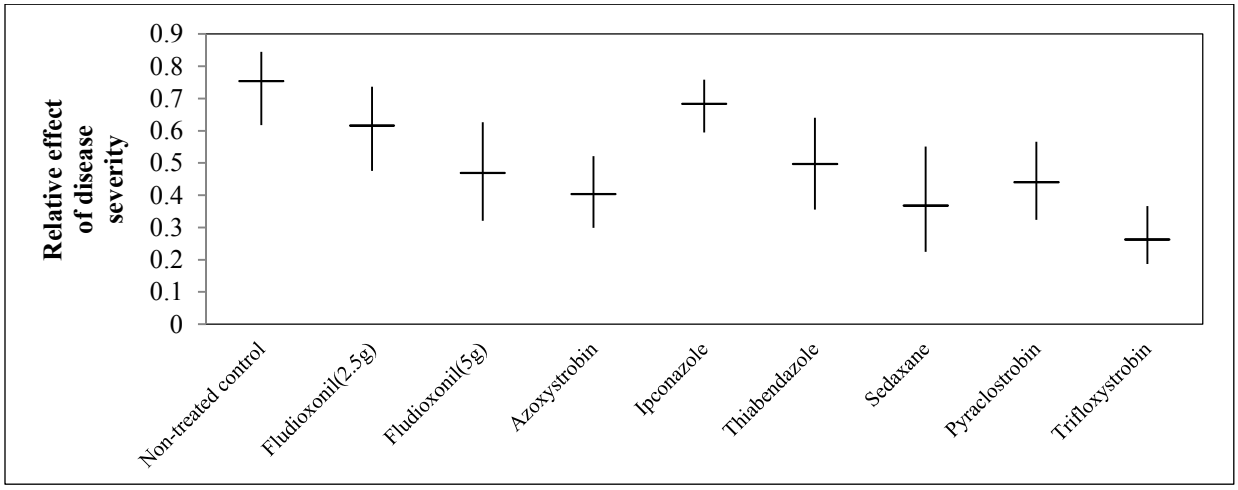


Figure 1.5. Relative effects of single fungicide seed treatments on severity of dry bean root rot caused by *Fusarium solani* f. sp. *phaseoli*. Vertical lines represent the 95% confidence interval.

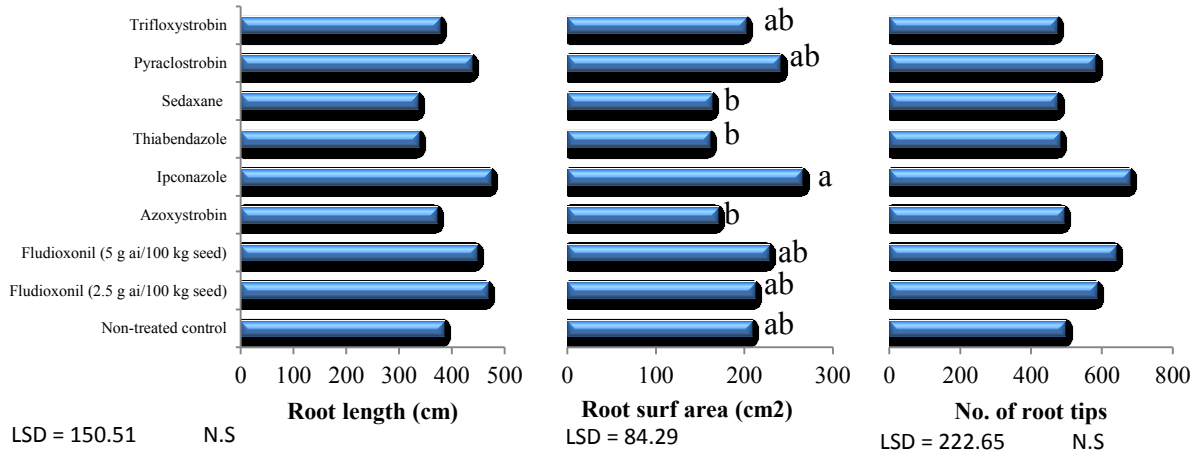


Figure 1.6. Effects of single fungicide seed treatments on length, surface area and root tip number of dry bean roots. * Bars with the same letter are statistically not different at P = 0.05 and N.S = Not significantly different at P = 0.05.

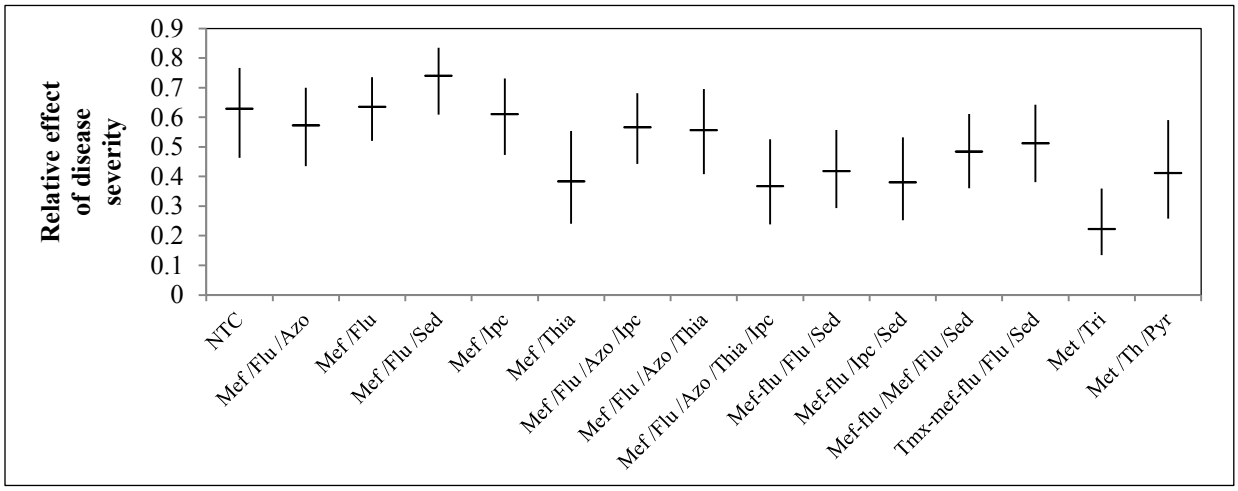
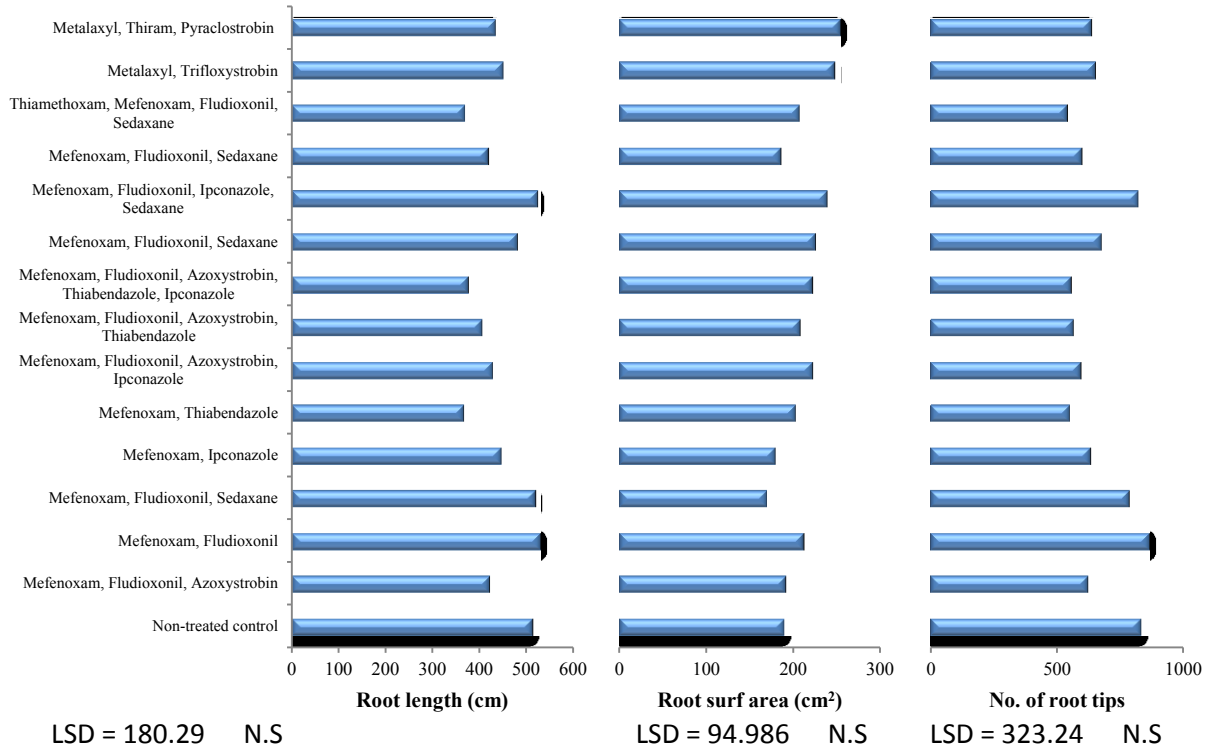


Figure 1.7. Relative effects of multiple ai seed treatments on severity of dry bean root rot caused by *Fusarium solani* f. sp. *phaseoli*. Vertical lines represent the 95% confidence interval.



LSD = 180.29 N.S LSD = 94.986 N.S LSD = 323.24 N.S

Figure 1.8. Effects of multiple ai seed treatments on length, surface area and root tip number of dry bean roots. * N.S = Not significantly different at P = 0.05.

The combination of metalaxyl and trifloxystrobin was the only treatment that significantly reduced ($P < 0.05$) root rot severities compared to the inoculated but not protected control (Figure 1.7, Appendix Table B2). However, neither this combination nor any other evaluated treatments produced greater root length, root surface area or number of root tips per plant compared to the non-protected seeds (Figure 1.8). Values for root length ranged from 366 cm to 529 cm with an average of 445 cm, root surface area ranged from 170 cm^2 to 254 cm^2 with an average of 210 cm^2 and number of root tips ranged from 539 to 862 with an average of 659 root tips. All the root growth parameters were not always positively correlated unlike in the case of single active ingredient treatments.

Field evaluation of combination products

Field trials that included an inoculated trial and a trial under natural disease pressure were performed for two successive years 2011 and 2012.

Inoculated field trials

During 2011, disease severity was recorded when plants were just entering the flowering stage 41 days after planting, a little earlier than planned due to unintended herbicide damage. Plant standings ranged between 63,566 and 113,462 and an average of 91,878 plants per ha 15 days after planting; 13 days later, plant stands ranged between 66,984 and 121,664 with an average of 96,267 plants per ha (Figure 1.9). The seed treatment combinations tested did not show any significant effect (P = 0.05) on plant stands during the period assessed (Figure 1.9).

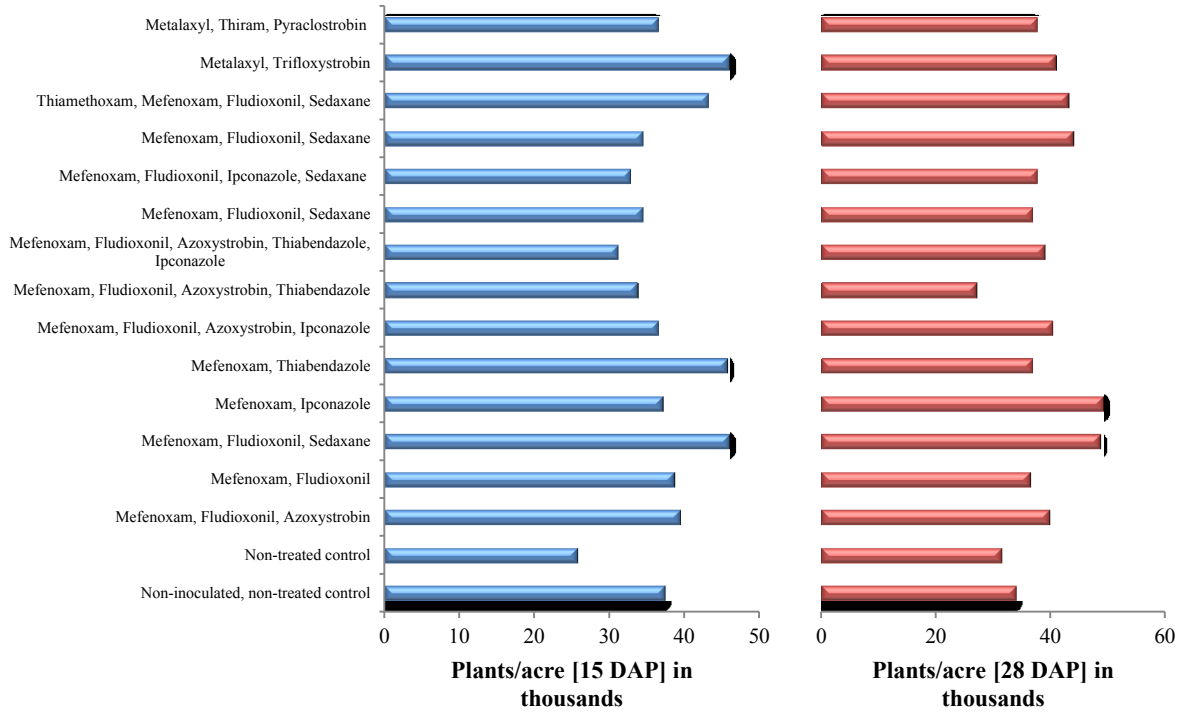


Figure 1.9. Effect of multiple ai seed treatments on plant stand 15 and 28 days after planting (DAP) in an inoculated field trial conducted at Carrington during 2011.

The median root rot severities for the non-inoculated-non-treated and inoculated-but-not-treated controls were 1.25 and 2, respectively (Appendix Table B3). Eight fungicide combinations had median severities of 1 (Appendix Table B3). However, the combinations of mefenoxam, fludioxonil, and azoxystrobin; mefenoxam, fludioxonil, azoxystrobin, and ipconazole; and metalaxyl and trifloxystrobin had greater median root rot severity than the non-inoculated-non-treated control. However, when the estimated relative effect of these treatments for root rot severity were analyzed, no differences could be detected (P = 0.05) (Figure 1.10).

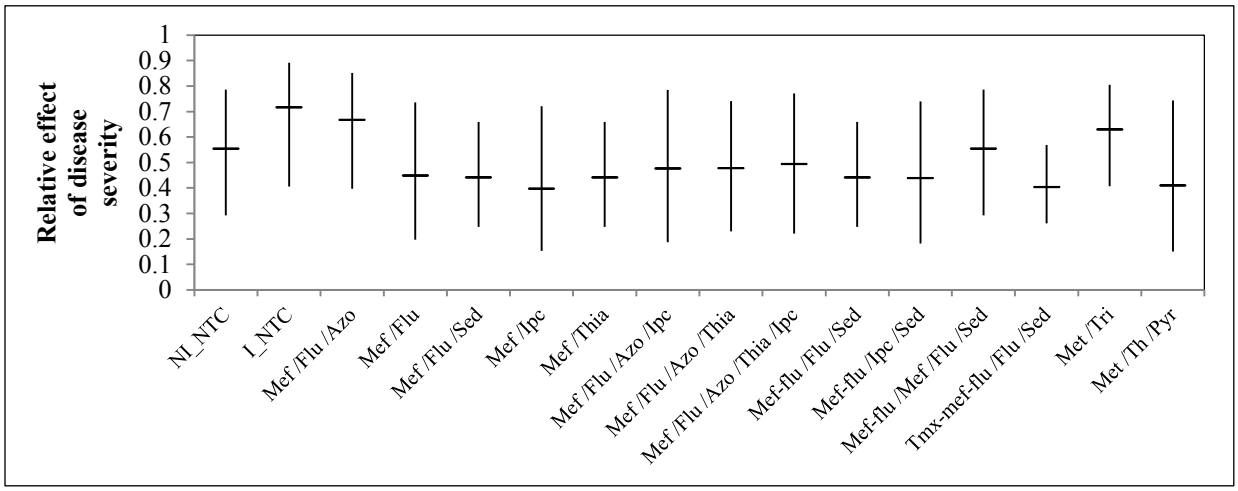


Figure 1.10. Relative effects of multiple ai seed treatments on severity of dry bean root rot in an inoculated field trial conducted at Carrington during 2011. Vertical lines represent the 95% confidence interval.

Significant differences in average root and foliar masses were observed among treatments (Figure 1.11). Values for average root mass ranged from 0.21 to 0.33 grams per plant and average foliar mass ranged from 1.60 to 2.86 grams per plant. The mixture of thiamethoxam, mefenoxam, fludioxonil, and sedaxane provided significantly higher foliar mass compared to inoculated, non-treated control which is 2.86 grams per plant. *Fusarium* species isolated from infected roots include *F. oxysporum, F. graminearum, F. solani* and *F. equiseti*. Percentage of plant pathogens was not estimated during 2011.

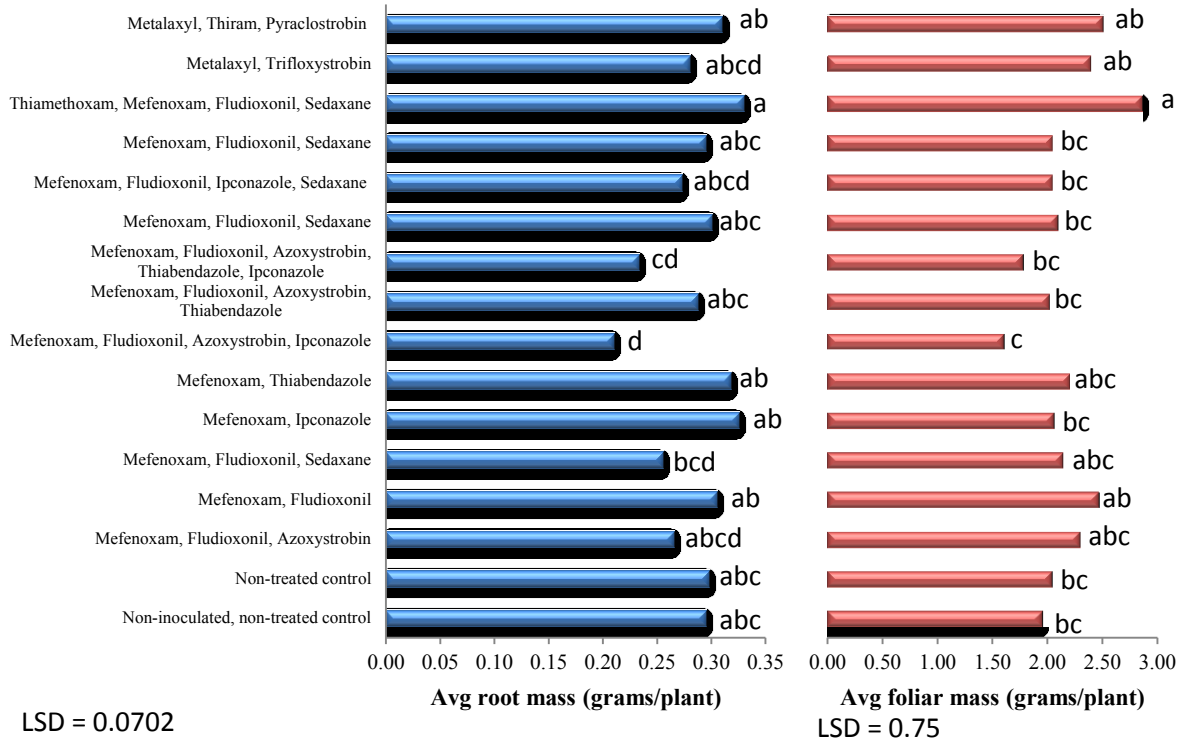


Figure 1.11. Effect of multiple ai seed treatments on average root mass and average foliar mass in an inoculated field trial conducted at Carrington during 2011. * Bars with the same letter are statistically not different at P = 0.05.

During 2012, inoculated dry bean trial at Carrington indicated that the seed treatment combinations tested did not show any significant effect on root rot control, average root mass, average foliar mass and yield (Appendix Table B4, Figure 1.13 and Figure 1.14). They did not have any positive effect on plant stand either (Figure 1.12). Instead, the non-inoculated non-treated control showed lower plant stand as compared to the inoculated non-treated control which exhibited the highest plant stand. Also, plant stand was significantly lower in plots where seeds treatment combinations like mefenoxam/fludioxonil/azoxystrobin/thiabendazole and mefenoxam/fludioxonil/azoxystrobin/thiabendazole/ipconazole were used. *Fusarium* species isolated from infected roots collected from this study included *F. oxysporum, F. avenaceum, F. equiseti, F. solani* and *F. redolens*. The most prevalent species among these were *F. avenaceum* and *F. oxysporum* which were retrieved from 52 and 28.4% of the samples, respectively; while

F. solani, the species inoculated, comprised only 0.4% of the total pathogen population isolated from symptomatic roots.

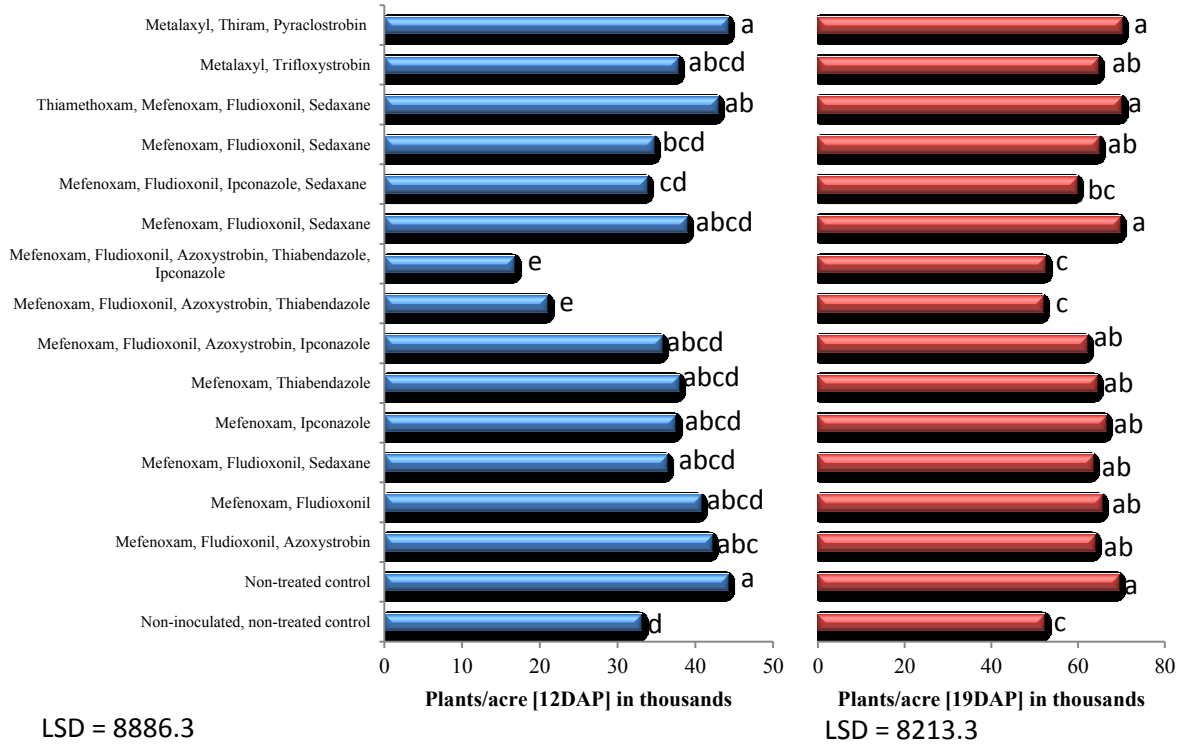


Figure 1.12. Effect of multiple ai seed treatments on plant stand 12 and 19 days after planting (DAP) in an inoculated field trial conducted at Carrington during 2012. * Bars with the same letter are statistically not different at P = 0.05.

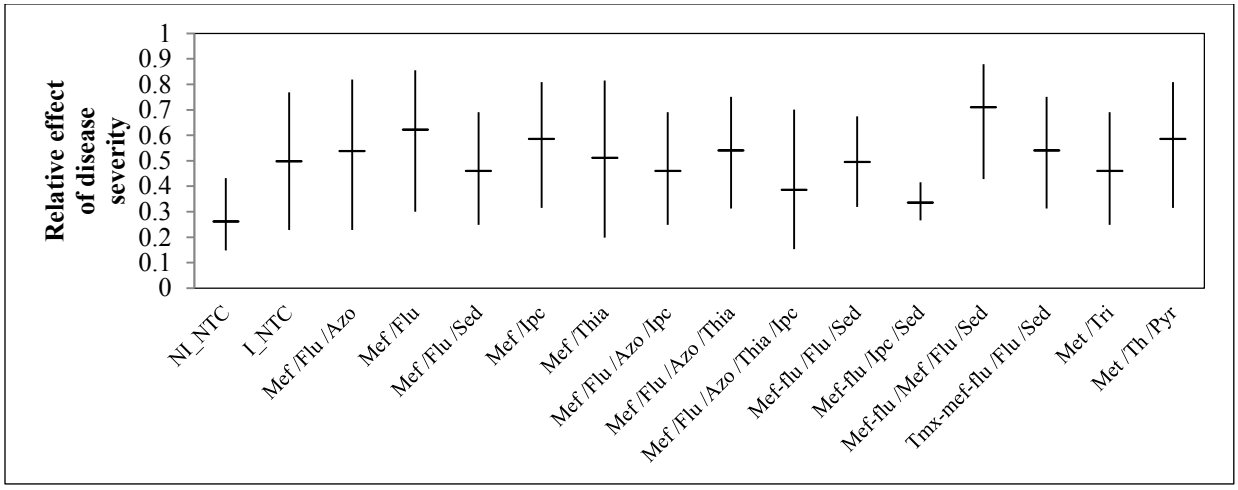


Figure 1.13. Relative effects of multiple ai seed treatments on severity of dry bean root rot in an inoculated field trial conducted at Carrington during 2012. Vertical lines represent the 95% confidence interval.

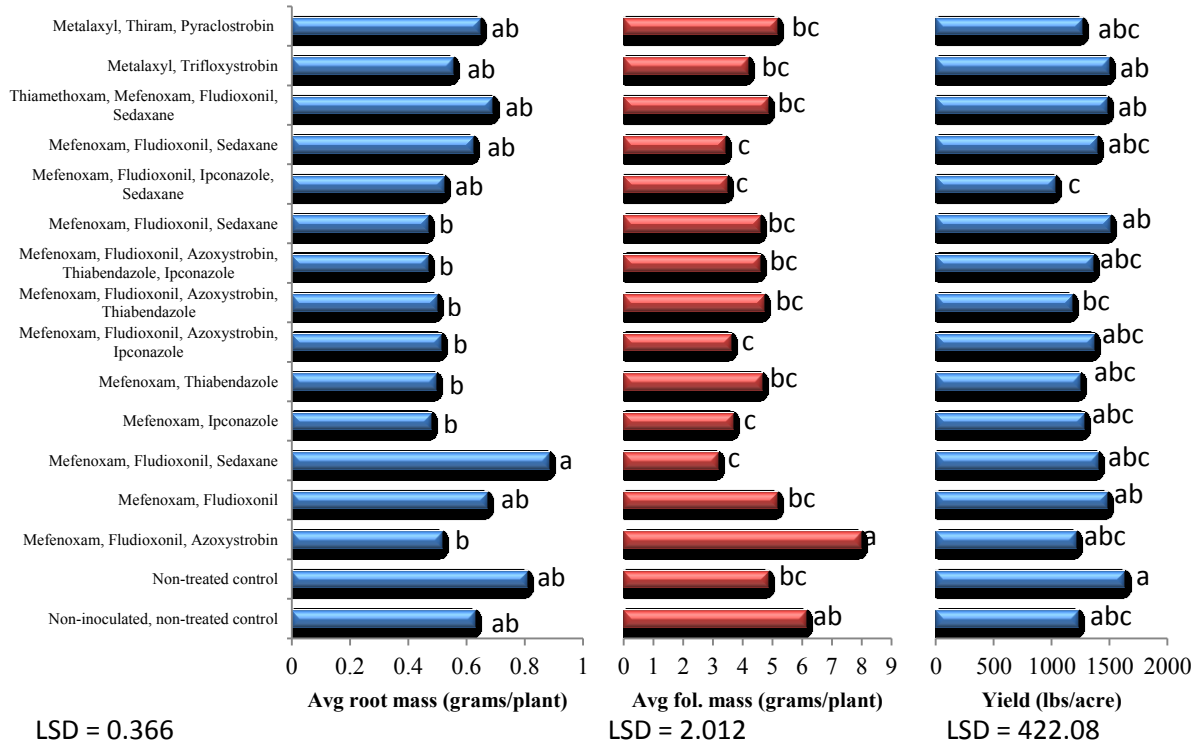
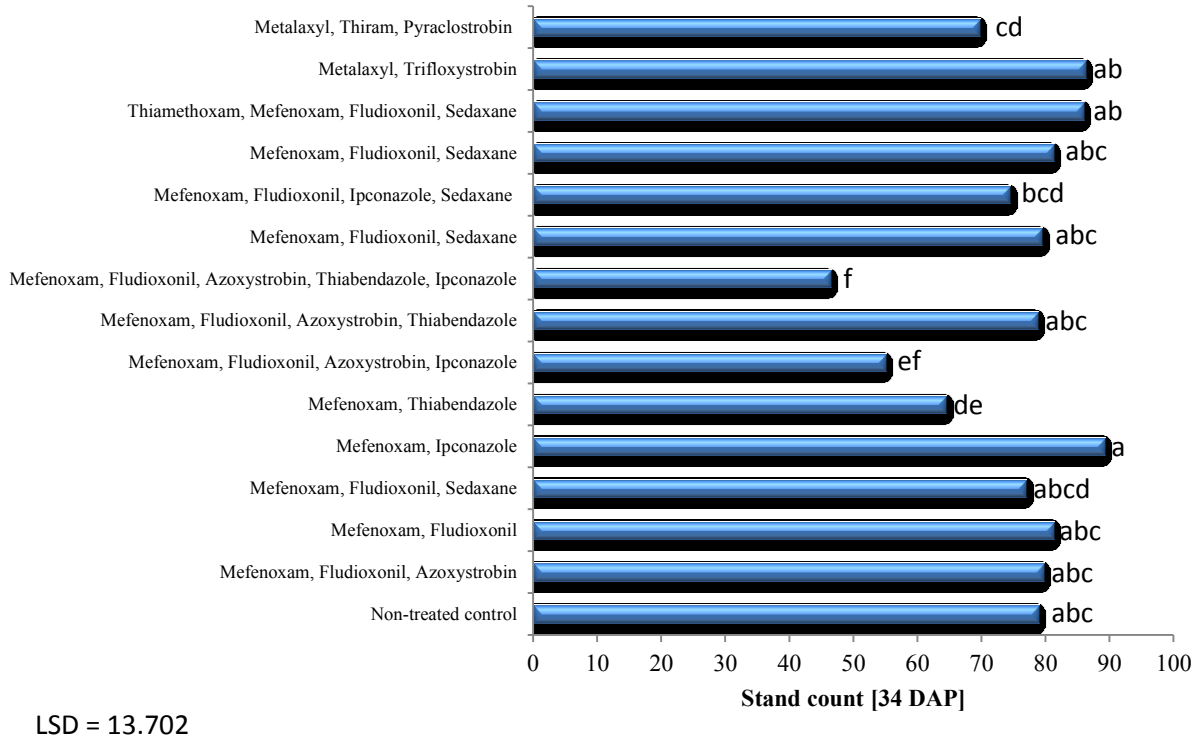


Figure 1.14. Effect of multiple ai seed treatments on average root mass, average foliar mass and yield in an inoculated field trial conducted at Carrington during 2012. * Bars with the same letter are statistically not different at P = 0.05.

Field trials under natural disease pressure

During 2011, dry bean trial under natural disease pressure at Staples was also harvested early to keep data points consistent with inoculated trial at Carrington. Data indicates that the treatments did not significantly increase root rot control and growth parameters that included average root mass and plant stand as compared to non-treated control (Appendix Table B5, Figures 1.15, 1.16 and 1.17). Only, thiamethoxam/mefenoxam/fludioxonil/sedaxane combination showed significant increase in average foliar mass compared to non-treated control (Figure 1.17). Mefenoxam/thiabendazole, mefenoxam/fludioxonil/azoxystrobin/ipconazole and mefenoxam/fludioxonil/azoxystrobin/thiabendazole/ipconazole combinations provided a significantly lower plant stand compared to non-treated control which could be caused due to incompatibilities among the different chemicals. *Fusarium* species isolated from infected roots

include *F. oxysporum, F. solani* and *F. redolens*. Percentage of plant pathogens was not estimated during 2011.



LSD = 13.702

Figure 1.15. Effect of multiple ai seed treatments on plant stand 34DAP in a field trial conducted at Staples under natural disease pressure during 2011. * Bars with the same letter are statistically not different at P = 0.05.

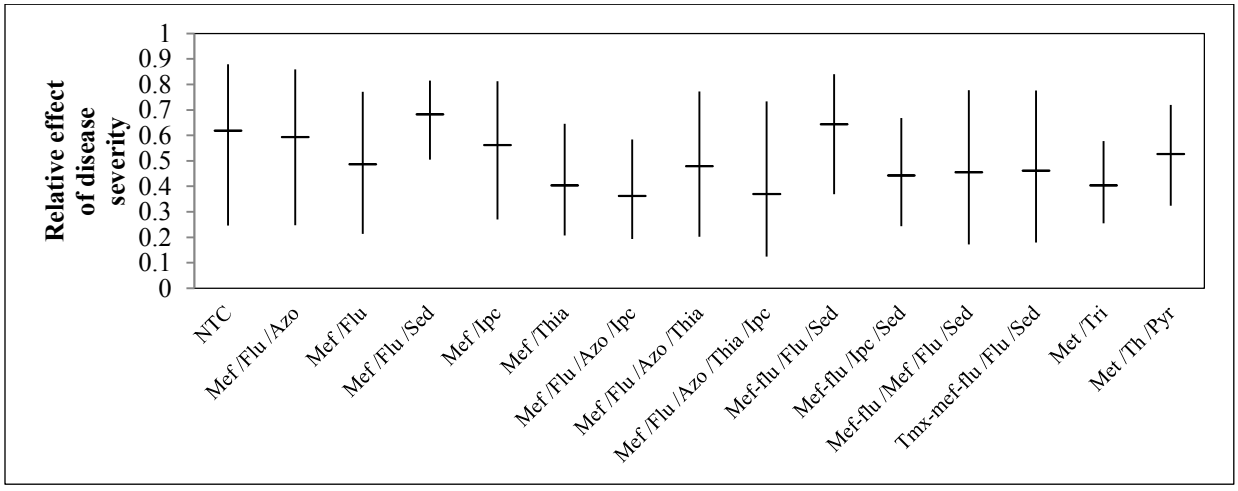
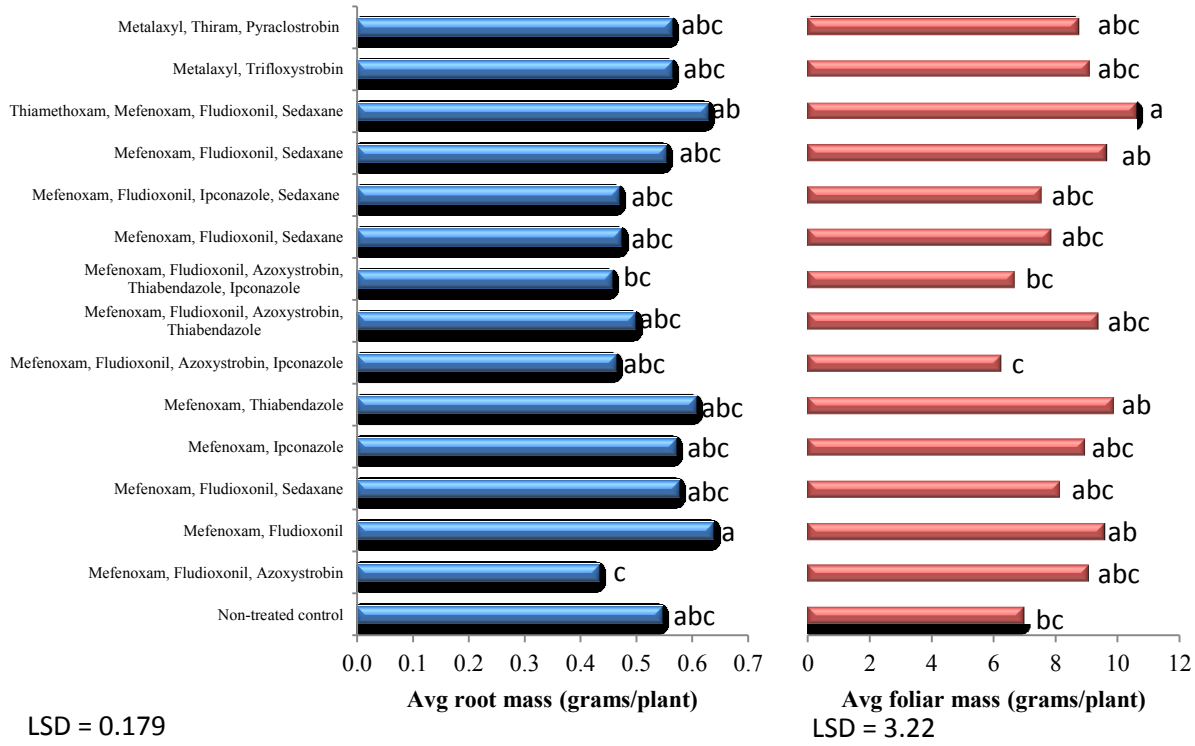


Figure 1.16. Relative effects of multiple ai seed treatments on severity of dry bean root rot in a field trial conducted at Staples under natural disease pressure during 2011. Vertical lines represent the 95% confidence interval.



LSD = 0.179

LSD = 3.22

Figure 1.17. Effect of multiple ai seed treatments on average root mass and average foliar mass in a field trial conducted at Staples under natural disease pressure during 2011. * Bars with the same letter are statistically not different at P = 0.05.

During 2012, at Perham the thiamethoxam/mefenoxam/fludioxonil/sedaxane combination provided better plant stand (Figure 1.18) and metalaxyl/trifloxystrobin provided higher root mass of 1.34 grams per plant as compared to non-treated control (Figure 1.20). Overall, findings from these studies do not suggest that seed treatments reduce root rot severities or increase growth significantly compared to the non-treated control except in one case of slight increase in root mass with the metalaxyl/trifloxystrobin treatment (Figure 1.20). *Fusarium* species isolated from infected roots include *F. oxysporum, F. avenaceum, F. equiseti, F. solani* and *F. redolens*. *F. oxysporum* was the most prevalent species isolated among them comprising of 34% of total pathogen population while *F. solani* comprised of only 3% of total pathogen population retrieved from symptomatic roots.

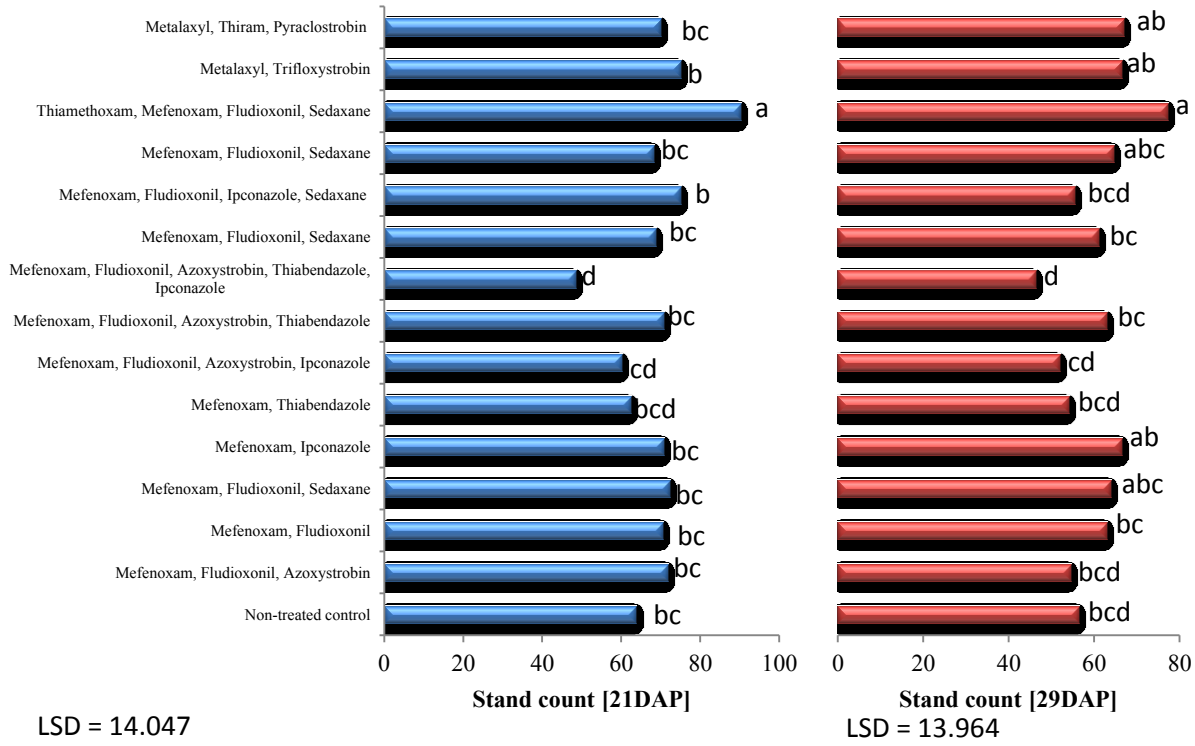


Figure 1.18. Effect of multiple ai seed treatments on plant stand 21 and 29 days after planting (DAP) in a field trial conducted at Perham under natural disease pressure during 2012. * Bars with the same letter are statistically not different at P = 0.05.

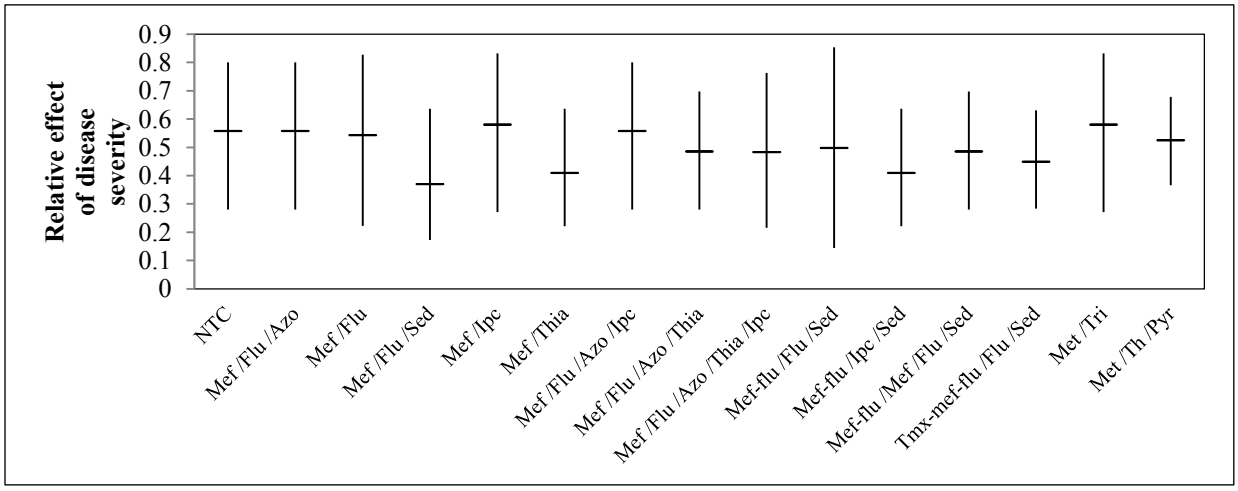


Figure 1.19. Relative effects of multiple ai seed treatments on severity of dry bean root rot in a field trial conducted at Perham under natural disease pressure during 2012. Vertical lines represent the 95% confidence interval.

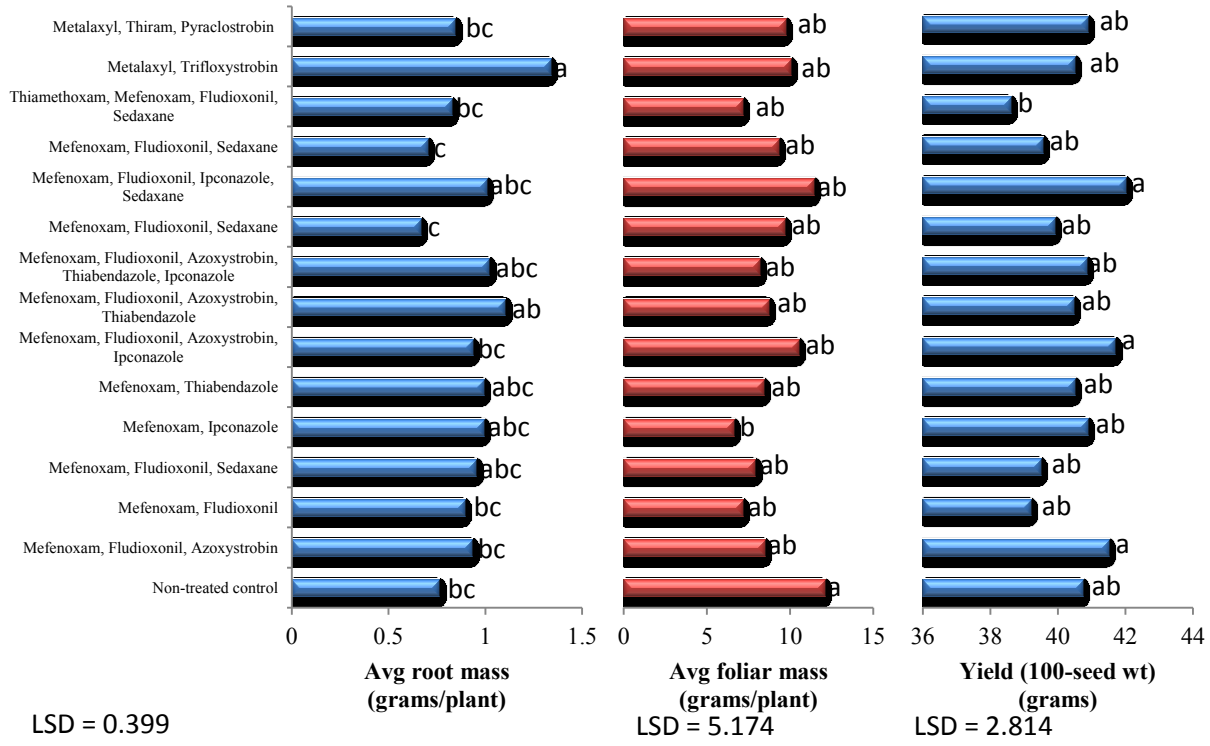


Figure 1.20. Effect of multiple ai seed treatments on average root mass, average foliar mass and yield in a field trial conducted at Perham under natural disease pressure during 2012. * Bars with the same letter are statistically not different at P = 0.05.

Discussion

According to fungicide labels, thiabendazole, ipconazole, fludioxonil, azoxystrobin, pyraclostrobin and trifloxystrobin are targeted towards control of *Fusarium* spp. along with other soil-borne and seed-borne pathogens. However, no recommendations have been made for use of these fungicides as seed treatment against Fusarium root rot except ipconazole which is recommended for use against seed rot caused by *Fusarium* spp. in both dry beans and field peas. The studies reported in this thesis provide information regarding the efficacy of these seed treatments against Fusarium root rot in dry beans when they are used alone or in combination with one other.

The results of *in-vitro* studies were not consistent with those observed in growth chamber conditions for all compounds. For example, ipconazole and thiabendazole individually or in combination with other chemicals result in significant inhibition of fungal growth and seed colonization. However, when these compounds were evaluated in growth chambers alone or in combination with other fungicides, they were not as effective. The opposite was observed for compounds such as sedaxane, azoxystrobin, pyraclostrobin and trifloxystrobin which had little effect in controlling growth of mycelial colonies or preventing seed colonization *in vitro* but were significantly better than non-treated control in reducing root rot severity when used individually in growth chamber trials. Interestingly, however, these four compounds were not as effective when used in combinations with other fungicides. The reasons for this are not clear. Since all chemistries were prepared and applied at the facilities of Syngenta, issues with potential chemical incompatibilities were not tested at our end although no phytotoxicity was observed. Trifloxystrobin was found to be most effective in controlling root rot both individually and when in combination with metalaxyl based on growth chamber assessments but it did not have any effect on other physiological parameters. It is very likely that the efficacy of the mixture of trifloxystrobin and metalaxyl reflects the efficacy of trifloxystrobin rather than that of metalaxyl since the latter does not have any effect on *Fusarium* according to fungicide label. All combination treatments that included thiabendazole were marginally better than the non-treated controls; they had a median disease rating of 2 compared to 3 of the control and a mean rank that was smaller too. While these differences were not statistically significant at $P = 0.05$, they were at $P = 0.1$. This is likely so because of the natural variability associated with the experimental units and the way the experiments were conducted (experimental error) that are preventing us from detecting these differences at P=0.05.

Results from inoculated field trials had in general low disease pressure thus the results may not be as conclusive as we wanted them to be; however, an interesting observation was obtained. Root samples collected from the Carrington studies revealed that roots were colonized mostly by *F. avenaceum* (52%) and *F. oxysporum* (28.4%), while *F. solani*, the species used as inoculum in these studies was recovered from less than 1% of the samples processed. Two conclusions could be drawn from this; 1) these two *Fusarium* species are more prevalent than *F. solani* in Carrington soils and were able to enter the roots when the roots reached areas in the soil where the fungicides were no longer effective; and 2) the low rate of recovery of *F. solani* suggests the seed treatments may have been effective in preventing the inoculated pathogen from infecting roots. It is also possible however; that the substrate used to carry the inoculum into the soil may have been colonized by saprophytes which affected in a negative way the ability of the inoculum to attack the roots.

In contrast to inoculated trials, trials conducted in Perham and Staples in fields with history of high disease pressure did have good disease pressure and produced interesting results. Seed treatments did not increase plant stands but some treatments actually ended with plant densities that were lower than those observed in the non-protected controls. The lack of positive impact on plant stand was somewhat expected since Fusarium root rots are better known for causing chronic root rots rather than pre-emerging damping off (4); however, a reduction in plant stand due to seed treatments was unexpected. The combination of mefenoxam, fludioxonil, azoxystrobin and ipconazole produced the lowest plant stands although no plant toxicity symptoms were observed. The negative effect on plant stand might be due to incompatibilities among the different active ingredients used within a single combination treatment. Besides their effect on plant stand, none of the seed treatments reduced the severity of root rots. This result is

also somewhat expected. Fusarium root rot is a chronic disease that damages the roots throughout the growing season. The efficacy of seed treatments in trials conducted in controlled environments was detected because the roots of plants were exposed to the inoculum for a much shorter period of time than in the fields and because the inoculum was placed closer to the seeds, position at which they were more exposed to the toxic effect of the fungicides. In contrast, under field conditions, root growth exposes tissues to the pathogen in areas where the fungicides are no longer present or at times when the compounds have already been degraded or metabolized by soil microbes.

When considering individual compounds, sedaxane did not show any physiological effect on root growth parameters in dry beans as opposed to its positive effect on root growth in other crops like corn, soybean and small grains as per previous reports (11, 13, 16, 18, 20). Improvement in root health related to fungicidal seed treatment has been reported previously in food legumes. In a study by Muthomi et al.,(2007), it was demonstrated that adding fungicide(s) along with rhizobial inoculants is not only useful in root rot management, but also enhances nodulation in food legumes (9) suggesting that apart from controlling the disease, seed treatments can have an impact on other physiological parameters related to plant health and growth.

In conclusion, the single ai seed treatments ipconazole and thiabendazole that inhibited fungal growth and seed colonization in the *in-vitro* studies were also effective when used in combination with other compounds. Thiabendazole was not significantly different than the non-treated control in growth chamber studies at $P = 0.05$. However, the confidence interval for the relative treatment effect of the non-treated control ranged from 0.62 to 0.84 while thiabendazole

ranged from 0.36 to 0.64. So, it is possible that at P values between 0.06 and 0.1, thiabendazole would be significantly different from non-treated control which implies that thiabendazole was the best in both lab and growth chamber. Sedaxane, azoxystrobin, pyraclostrobin and trifloxystrobin significantly reduced disease severity as compared to non-treated control when used individually under growth chamber conditions. Among combinations, metalaxyl/trifloxystrobin was effective in reducing disease severity under growth chamber conditions. Apart from reducing disease severity, seed treatments can also have an effect on other root growth parameters. Fludioxonil (5 g ai/100 kg seed), ipconazole and pyraclostrobin, each when used alone appear to have a positive impact although not statistically significant on root growth parameters like root surface area, root length and number of root tips under growth chamber conditions. However, thiamethoxam/mefenoxam/fludioxonil/sedaxane combination showed significant increase in foliar mass during inoculated field trial in 2011 and this combination also increased root mass although it wasn't statistically significant. It showed significant increase in root mass during 2011 field trial under natural disease pressure. During 2012 field trial under natural disease pressure, thiamethoxam/mefenoxam/fludioxonil/sedaxane exhibited a significant increase in plant stand and metalaxyl/trifloxystrobin significantly increased root mass compared to non-treated control. The findings from the petri-dish and growth chamber trials provide information regarding the potential efficacy of the compounds against *F. solani*. However the findings from the inoculated trials and field trials appear to have been inconclusive. We believe that further investigation regarding the dominant *Fusarium* species associated with root rot in dry beans need to be conducted to be able to set up appropriate parameters to determine efficacy of the compounds under field conditions in North Dakota.

Chemical seed treatments only protect the seed from pathogens during the initial stages of plant growth, for about two weeks from the planting date in most cases. In order to obtain continued protection throughout the life of the crop, it is essential to integrate seed treatments with cultural practices and other methods of root rot management. Also, a drench application at a later time may be necessary until more effective levels of resistance become available. However, further experiments need to be performed in order to determine the most effective management strategy.

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CHAPTER 2: EVALUATING EFFICACY OF SEED TREATMENTS FOR FUSARIUM ROOT ROT CONTROL IN FIELD PEAS

Abstract

Field pea (*Pisum sativum* L.) is an important cool season legume crop grown in the United States and North Dakota is the leading producer with 174,000 ha planted in 2010. Field peas are susceptible to many root rot pathogens and in recent years *Fusarium avenaceum* has been identified as a major causal agent in North Dakota. Complete resistance to Fusarium root rot is not available in commercial cultivars of peas making integration of chemical control essential for effective disease management. This study focuses on evaluating commercially available seed treatment fungicides for control of root rot caused by *F. avenaceum* on field peas. Seed treatment products evaluated in this study include thiabendazole, ipconazole, mefenoxam, metalaxyl, sedaxane, azoxystrobin, pyraclostrobin, trifloxystrobin, and fludioxonil. These compounds were assessed individually or in combinations under *in-vitro*, growth chamber, and field trial conditions. All trials in controlled environments were conducted using a completely randomized design with three replications and each trial was conducted twice. Field trials were conducted in 2011 and 2012 at single locations each year using a randomized complete block design with four replications. Results of *in vitro* and growth chamber trials indicated that fludioxonil, pyraclostrobin and trifloxystrobin or mixtures that included them inhibited fungal growth and seed colonization *in vitro* and they reduced root rot severity in growth chambers when used alone. However, none of the seed treatments showed significant reduction in root rot severities under field conditions. This study provided information regarding the potential efficacy of several common seed treatment compounds against *F. avenaceum*. However, further evaluation would be required to assess their efficacy under field conditions. Overall, integration

of chemical seed treatments along with use of cultural practices; field pea varieties partially resistant to root rot, and drench application are likely to be required to effectively manage Fusarium root rot.

Introduction

Field pea (*Pisum sativum* L.) is an important cool season legume crop grown in the United States. North Dakota is the largest producer of the crop with an area of 95,101 ha (235,000 acres) planted to field pea in 2012 (16). The state contributed to 41% of the nation's production of field pea in 2012 (16). Root rot is a growing concern for field pea production in the state as per the field surveys conducted in the state. Field peas are susceptible to a wide range of fungal root rot pathogens which include *Fusarium solani* f. sp. *pisi*, *Rhizoctonia solani*, *Pythium* and *Aphanomyces* (20). *Fusarium* spp. has been identified as the major causal agent of field pea root rot in North Dakota in recent years (7, 9) with *F. avenaceum* being the most predominant and aggressive (5). No commercial cultivars currently grown in this region have complete resistance to field pea root rot caused by *F. avenaceum* (8), although a few sources of partial resistance are available (4). Hence, it is likely that integration of chemical control will be required for effective disease management.

Seed treatments are one of the most common tools used for the management of seed-borne and soil-borne diseases like Fusarium root rot (11) . Resistance inducers, microorganisms, plant extracts, bio fertilizers and chemicals can be used in the form of seed treatments (6, 10, 13, 14, 22, 24). In maize, antagonistic bacterial seed treatments were found to be effective against *F. verticillioides* under greenhouse conditions (19). Novel strains of *B. subtilis* were identified that can be used as seed and soil treatments to effectively control Fusarium root rot of soybean

caused by *F. oxysporum* and *F. graminearum* at greenhouse level (26). A strain of *Clonostachys rosea* , was identified as a mycoparasite of organisms associated with the pea root rot complex (PRRC) like *Alternaria alternata*, *Aphanomyces euteiches*, *Fusarium oxysporum pisi*, *F. solani pisi*, *Mycosphaerella pinodes*, *Pythium* spp., *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* and was effectively used as a seed treatment to control the disease (24). This strain significantly reduced root rot severity, increased *in vitro* seed germination and seedling emergence and the results were greater or statistically equivalent to those achieved with Thiram (24).

Table 2.1. The active ingredient, mode of action (1) and primary target of seed treatments currently registered for use on field peas in North Dakota are listed in table below.

Active Ingredient	Mode of Action	Group Name	Target Pathogen	Contact/ Systemic
Mefenoxam Metalaxyl	A: nucleic acid synthesis	Phenylamides	*Pythium*, *Phytophthora* and downy mildews	Systemic (curative and protective)
Thiabendazole	B: mitosis and cell division	MBC – fungicides (Methyl Benzimidazole Carbamates)	*Aspergillus, Botrytis, Cladosporium* and *Fusarium*	Systemic (curative and protective)
Sedaxane	C. respiration	SDHI (Succinate dehydrogenase inhibitors)	Broad spectrum	Contact and systemic
Azoxystrobin Pyraclostrobin Trifloxystrobin	C. respiration	QoI-fungicides (Quinone outside Inhibitors)	Broad spectrum	Systemic (curative and protective)
Fludioxonil	E: signal transduction	Phenylpyrroles	*Fusarium, Rhizoctonia* and *Alternaria*	Non-systemic
Ipconazole	G: sterol biosynthesis in membranes	DMI-fungicides (DeMethylation Inhibitors)	Broad spectrum	Systemic
Thiram	Multi-site contact activity	Dithiocarbamate	*Pythium*	Contact

*table based on FRAC list and seed treatment labels.

Chemical seed treatments form an integral component of the management strategy for root rots in peas. Most of the fungicides listed (in Table2.2 and Table 2.3) are recommended for use as seed treatment on field peas (12). Thiabendazole (Mertect 340 F), ipconazole (Rancona), fludioxonil (Maxim 4 FS, Apron Maxx and Cruiser Maxx), azoxystrobin (Dynasty 100 FS),

pyraclostrobin (Stamina) and trifloxystrobin (Trilex) are targeted towards control of *Fusarium* spp. along with other soil-borne and seed-borne pathogens according to fungicide labels. Ipconazole is recommended for protection against seed rot caused by *Fusarium* spp. in both dry beans and field peas. Previous studies have demonstrated that sedaxane is a broad spectrum fungicide that has a physiological effect on root growth when used against various seed-borne and soil-borne pathogens other than *Fusarium* spp. on corn, soybean and small grains (17, 18, 21, 23, 25).

However, in the available literature, there has been no comprehensive study evaluating these chemical seed treatments for their ability to control Fusarium root rot of field pea, and enhance root growth in laboratory, growth chamber and field conditions. Therefore, an attempt was made to screen a collection of chemical seed treatment products through *in vitro*, growth chamber and field assessments for their ability to control Fusarium root rot of field pea caused by *Fusarium avenaceum* and also to study their physiological effect on root growth.

Materials and methods

The seeds used in all experiments described below were from the root rot susceptible field pea variety DS Admiral. These seeds were treated with individual compounds (single active ingredient-ai) or their combinations (Tables 2.2 and 2.3) at different rates by Syngenta Seedcare at their facilities in Stanton, MN. The efficacy of these treatments was evaluated in the laboratory (*in vitro* trials) using a simple petri-dish assay, in growth chambers using sand-cornmeal inoculum, and in field trials using natural soil infestations. All compounds used in these studies were supplied by Syngenta Seedcare.

Table 2.2. Single active ingredient compounds evaluated as seed treatments for their efficacy to control Fusarium root rot of field pea in controlled environment trials.

Treatment code	Commercial name	Active ingredients	g ai/ 100 kg seed
Non-treated control	Non-inoculated, non-treated control	None	---
Fludioxonil (2.5 g)	Maxim 4 FS	Fludioxonil	2.5
Fludioxonil (5 g)	Maxim 4 FS	Fludioxonil	5
Azoxystrobin	Dynasty 100 FS	Azoxystrobin	2.5
Ipconazole	Rancona	Ipconazole	1.5
Thiabendazole	Mertect 340 F	Thiabendazole	50
Sedaxane	Sedaxane	Sedaxane	2.5
Pyraclostrobin	Stamina	Pyraclostrobin	5.0
Trifloxystrobin	Trilex	Trifloxystrobin	5.0

Table 2.3. Seed treatment containing multiple active ingredients screened for their efficacy to control Fusarium root rot of field pea in controlled environment and in field conditions.

Treatment code	Commercial name	Active ingredients	g ai/ 100 kg seed
Non-treated control	Non-inoculated, non-treated control	None	
Mef/Flu/Azo	Apron XL	Mefenoxam	15
	Maxim 4 FS	Fludioxonil	2.5
	Dynasty 100 FS	Azoxystrobin	2.5
Mef/Flu	Apron XL	Mefenoxam	15
	Maxim 4 FS	Fludioxonil	5
Mef/Flu/Sed	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Sedaxane	Sedaxane	2.5
Mef/Ipc	Apron XL	Mefenoxam	7.5
	Rancona 3.8	Ipconazole	1.5
Mef/Thia	Apron XL	Mefenoxam	7.5
	Mertect 340 F	Thiabendazole	50
Mef/Flu/Azo/Ipc	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Dynasty 100 FS	Azoxystrobin	2.5
	Rancona	Ipconazole	1.5
Mef/Flu/Azo/Thia	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Dynasty 100 FS	Azoxystrobin	2.5
	Mertect 340 F	Thiabendazole	50
Mef/Flu/Azo/Thia/Ipc	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	5
	Dynasty 100 FS	Azoxystrobin	2.5
	Mertect 340 F	Thiabendazole	50
	Rancona	Ipconazole	1.5
Mef-flu/Flu/Sed	Apron Maxx RTA	Mefenoxam + Fludioxonil	7.5 + 5
	Maxim 4 FS	Fludioxonil	5
	Sedaxane	Sedaxane	2.5

Table 2.3. Seed treatment containing multiple active ingredients screened for their efficacy to control Fusarium root rot of field pea in controlled environment and in field conditions (continued).

Treatment code	Commercial name	Active ingredients	g ai/ 100 kg seed
Mef-flu/Ipc/Sed	Apron Maxx RTA	Mefenoxam + Fludioxonil	7.5 + 5
	Rancona	Ipconazole	1.5
	Sedaxane	Sedaxane	2.5
Mef-flu/Mef/Flu/Sed	Apron Maxx RFC	Mefenoxam + Fludioxonil	6.0
	Apron XL	Mefenoxam	7.5
	Maxim 4 FS	Fludioxonil	2.5
	Sedaxane	Sedaxane	2.7
Tmx-mef-flu/Flu/Sed	CruiserMaxx Beans	Thiamethoxam + Mefenoxam + Fludioxonil	56.7*
	Maxim 4 FS	Fludioxonil	2.5
	Sedaxane	Sedaxane	3.4
Met/Tri	Allegiance	Metalaxyl	15.5
	Trilex	Trifloxystrobin	5.0
Met/Th/Pyr	Acquire	Metalaxyl	6.2
	Thiram 42 S	Thiram	62.5
	Stamina	Pyraclostrobin	5.0

*calculated based on CruiserMaxx label.

In-vitro **evaluation of single active ingredient (ai) and combination seed treatments**

Petri-dish trials

In this method a 3mm diameter agar plug was collected from the growing edge of a 7 day old colony of *Fusarium avenaceum* isolate Pea 41, cultured on full strength potato dextrose agar (PDA). The medium was made by mixing 24 g of potato dextrose broth (PDB, BD Difco, Franklin Lakes, NJ) with 15 g agar (BD Difco, Franklin Lakes, NJ) in 1000 ml distilled water and autoclaving it at 121^{o} C for 20 minutes at 103.42 kPa. The agar plug was placed at the center of a regular plastic BD Falcon petri-dish (100X15 mm, BD Difco, Franklin Lakes, NJ) containing 1/8th strength PDA with mycelium facing the agar surface and incubated at 21^{o} C (room temperature) with 14 h light and 10 h dark cycles for 3-4 days. The medium was prepared by mixing 3 g PDB and 10 g agar in 1000 ml distilled water and autoclaving it as described. On the fourth day of incubation, eight seeds from a single treatment were placed in each dish along the circumference of the growing isolate leaving approximately 1 cm space between the growing

tip of isolate and the seed (Figure 2.1). Once all treatments had been plated the dishes were incubated for seven additional days as described.

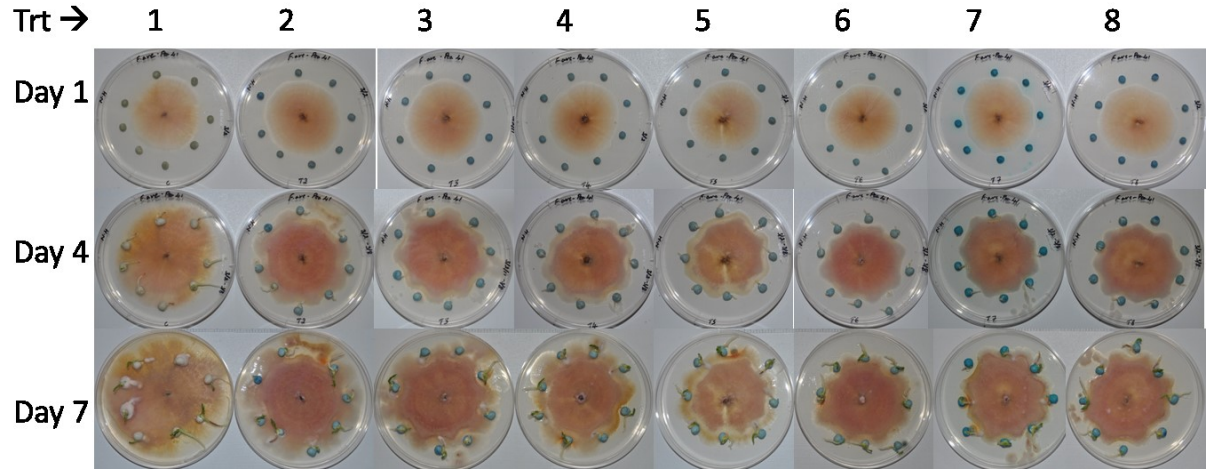


Figure 2.1. Response of fungal growth to different treatments at 1, 4 and 7 days after inoculation: a pictorial representation from preliminary trials.

After incubation area of the petri-dish covered by fungal colony and the number of seeds overgrown/colonized by the fungus were recorded. The experiments for each group of compounds (single and in mixtures) were laid out in a completely randomized design (CRD) with three replications per treatment where each dish represented a replication. The experiments were repeated once.

Growth chamber evaluation of single ai and combination seed treatments

Sand cornmeal inoculum layer method

Treatments were compared under growth chamber conditions along with inoculated non-treated (positive) and non-inoculated non-treated (negative) controls using the modified sand-cornmeal inoculum layer method described by Bilgi et al. (2). A highly aggressive isolate of *F. avenaceum*, Pea 41 obtained from a root rot affected grower's field in North Dakota was used to establish the efficiency of the range of seed treatments. Inoculum was prepared by placing eight 5 mm plugs of the isolate grown for 7 days on half-strength PDA into autoclavable bags

containing sterilized (121°C, 103.42 kPa for 45 min under dry setting) sand-cornmeal mixture (54 g of regular play sand, 6 g of Quaker yellow cornmeal and 12 ml of distilled water).

Inoculated bags were incubated at room temperature for seven days and shaken daily by hand to ensure uniform growth of fungus throughout the bag. Once the mixture was colonized, 266 ml plastic drinking cups with holes at the bottom for water drainage were layered with 15 g of sterilized (121°C, 103.42 kPa for 45 min under dry setting) vermiculite(premium grade, Sun Gro Horticulture Distribution Inc. Washington, U.S.A) followed by 15 g of inoculum and 8 g of vermiculite. Three seeds per treatment were placed on the upper vermiculite layer and covered with another 8 g of vermiculite. Three cups of each treatment were placed in trays in a growth chamber maintained at 14 h light and 10 h dark cycles with day and night temperatures of 21 and 18°C, respectively. Cups were watered daily. Severity of root rot was evaluated 14 days after planting of pea seeds. A 0-5 scale was developed for assessment of disease severity where 0 = no symptoms; 1 = fine light brown lesions on the taproot or few lateral roots or dark brown lesions restricted to a length < 1cm; 2 = 1.5-2.5cm long brown lesions which may or may not be spreading to lateral roots or flecks of brown lesions that coalesce to form longer lesion or dark brown lesions restricted to a length < 2cm; 3 = reddish brown to black lesions > 2.5cm long and spreading to lateral roots with a reduction in root mass; 4 = severe reddish brown to black lesions with roots starting to decay at the point of lesion or reddish brown to black lesion extending up to 3-4cm in length or higher with a reduction in root mass; 5 = severe reddish brown to black lesions and the tap root is pinched off/decayed (Figure 2.2).

The roots were scanned using a root scanner (Epson Expression 10000XL) after rating and the WinRHIZO software (Regent Instruments, Quebec, Canada) was employed to analyze

scanned images of roots to estimate root surface area, total root length and number of root tips. The experiment was laid out in a completely randomized design (CRD) with non-inoculated and inoculated controls with three replications. The experiment was performed three times.

Figure 2.2. Pictures of roots with differing levels of lesion length/intensity and root mass depicting the root rot rating scale used for disease evaluations.

Field trials under natural disease pressure

This experiment was conducted in fields with history of Fusarium root rot in North Dakota (Michael Wunsch, personal communication). The trials were located at Newburg and Carrington in 2011 and 2012, respectively. The experimental design was an RCBD consisting of four replications and 15 treatments (Table 2.3) which included a non-treated control.

During the first year (2011), the trial was planted on June 6 at Newburg with a seeding rate of 858,366 seeds per hectare. This trial consisted of plots sized 1.52 m x 7.62 m. Stand count could not be taken as the trial suffered from very poor emergence due to excessive moisture and flooding. The root rot severity was assessed on July 18 (42 DAP). In the second year (2012), the trial was planted on April 23 at Carrington with a seeding rate of 858,366 seeds per hectare and stand count was taken at two time points, May 14 (21 DAP) and May 22 (29 DAP). This trial consisted of plots sized 1.37 m x 7.62 m. The root rot severity was assessed at bloom stage on June 11 (49 DAP).

Sampling method

Root samples were collected from the experimental fields in Newburg in 2011 and Carrington in 2012, respectively. During each year, ten roots were arbitrarily sampled from each plot in the field at both the locations. These roots were pooled by plot in Ziploc bags and brought to the laboratory in ice boxes to prevent drying of plant tissue. They were stored in coolers until analyzed. Foliar tissue was also sampled, transported and stored in the same method. The root samples were used for root rot rating, calculating root mass and pathogen isolation. Root rot rating was done using the same scale used in the growth chamber experiments after the roots were washed under running tap water. The foliar mass and root mass was assessed by cutting the tops off at the node where the cotyledons were attached, drying the foliage and root separately for 2-3 days at room temperature until completely dried, and then weighing the biomass. Total number of roots collected per plot were weighed on a weighing scale and mean root mass per plant was calculated and used for analysis. Mean foliar mass per plant was calculated in the same way.

Pathogen isolation and identification

A few symptomatic roots from each plot were plated to identify the pathogens present. Infected roots were washed and surface disinfested by immersing them in 10% bleach (NaOCl) for two minutes followed by a dip in 70% ethanol for 30 seconds and three subsequent washes in sterile distilled water. The samples were then air-dried, plated onto potato dextrose agar (PDA) amended with 0.3 mg/ml of streptomycin, an antibiotic to avoid growth of bacteria in petri-dish. These plates were incubated at room temperature (~21^{o} C) with a 12 hour photoperiod. Species were identified based on morphological characteristics. Morphological characteristics evaluated included fungal growth pattern, color and texture of the colony, mycelia type and spore structure.

Statistical analyses

Statistical analyses for the above studies were performed using SAS version 9.2 (SAS Institute, Cary, NC). Data was analyzed using either the analysis of variance (PROC ANOVA) or generalized linear model (PROC GLM) procedures. Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05.

In-vitro evaluation of single ai and combination seed treatments

Data collected for *in-vitro* evaluation included the area of petri-dish covered by fungal colony and percent of seeds colonized by the fungus after seven days of incubation. Levene's test was performed on the collected data to verify homogeneity of variances as the experiment was conducted more than once. Upon acceptance of the null hypothesis that variances were homogeneous, a combined analysis was performed. Data was analyzed using analysis of variance procedure (PROC ANOVA). Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05.

Growth chamber evaluation of single ai and combination seed treatments

Root rot severity was estimated using a 0-5 scale depicted in Figure 2.2. The median values of severity were calculated for each experimental unit and used for the analysis. The median data was analyzed using non-parametric ANOVA-type statistics. First medians were ranked using PROC RANK and the mean ranks for each treatment was calculated using PROC MEANS. Then ranked medians were analyzed using PROC MIXED to determine whether there were differences between treatments. The SAS macro LD_CI.SAS developed by E. Brunner (University of Gottingen, Germany) was used to calculate the treatment relative effects and their confidence intervals to determine which treatments were different. The estimated relative effect

for severity is inversely proportional to the efficacy of seed treatment (smaller relative effects indicate more effective control).

Root growth parameters like root length, root surface area and number of root tips also were analyzed. Levene's test was performed on the collected data to verify homogeneity of variances as the experiment was conducted more than once. If the null hypothesis that variances were homogeneous was accepted, then a combined analysis was performed, otherwise the data were analyzed separately. Data were analyzed using PROC GLM. Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05. Pearson correlation analysis was performed using PROC CORR in SAS to determine whether root growth parameters were associated to root rot severity.

Field evaluation of combination products

Root rot severity was estimated using a 0-5 scale depicted in Figure 2.2. Since more than one root was uprooted from each plot (replication), median disease severity for each plot was calculated. This median value was used for further analysis. The severity data was analyzed non-parametrically as described above. Data collected for other traits, like plant stand, average root mass, average foliar mass and yield excluding disease severity, were analyzed using PROC ANOVA. Comparison of means was performed using Fisher's protected least significant difference (LSD) test with P = 0.05.

Results

<u>*In-vitro* evaluation of single ai and fungicide combinations</u>

In-vitro assessment of single ai seed treatments indicated that all chemical treatments reduced fungal colony growth significantly (Figure 2.3). Fludioxonil, thiabendazole,

pyraclostrobin and trifloxystrobin reduced percent seed colonization as compared to the non-treated check (Figure 2.3). Overall, fludioxonil at both rates tested (2.5 g ai /100 kg seed and 5 g ai /100 kg seed), thiabendazole and pyraclostrobin appeared to be most effective inhibitors of fungal growth and seed colonization (Figure 2.3).

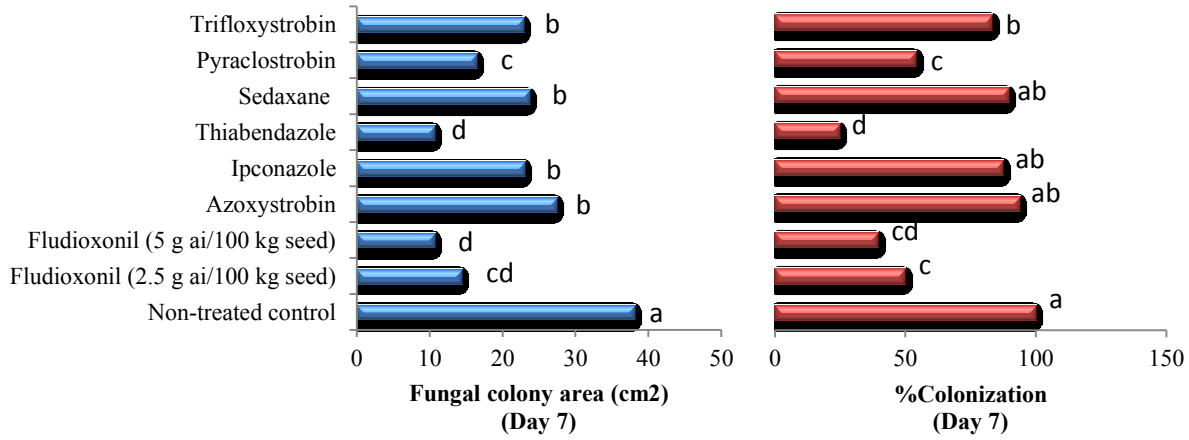


Figure 2.3. Response of *F. avenaceum* colony area (cm^2) and percent seed colonization to single ai seed treatments on seventh day after placing seed. * Bars with the same letter are statistically not different at P = 0.05.

In-vitro assessment of seed treatments with multiple ai used in combination showed that fungal colony growth was reduced significantly with all seed treatments as compared to non-treated check (Figure 2.4). Seed colonization was reduced significantly with all treatments except mefenoxam/ipconazole combination (Figure 2.4). Overall, treatments including thiabendazole and/or fludioxonil were most effective inhibitors of fungal growth and seed colonization (Figure 2.4).

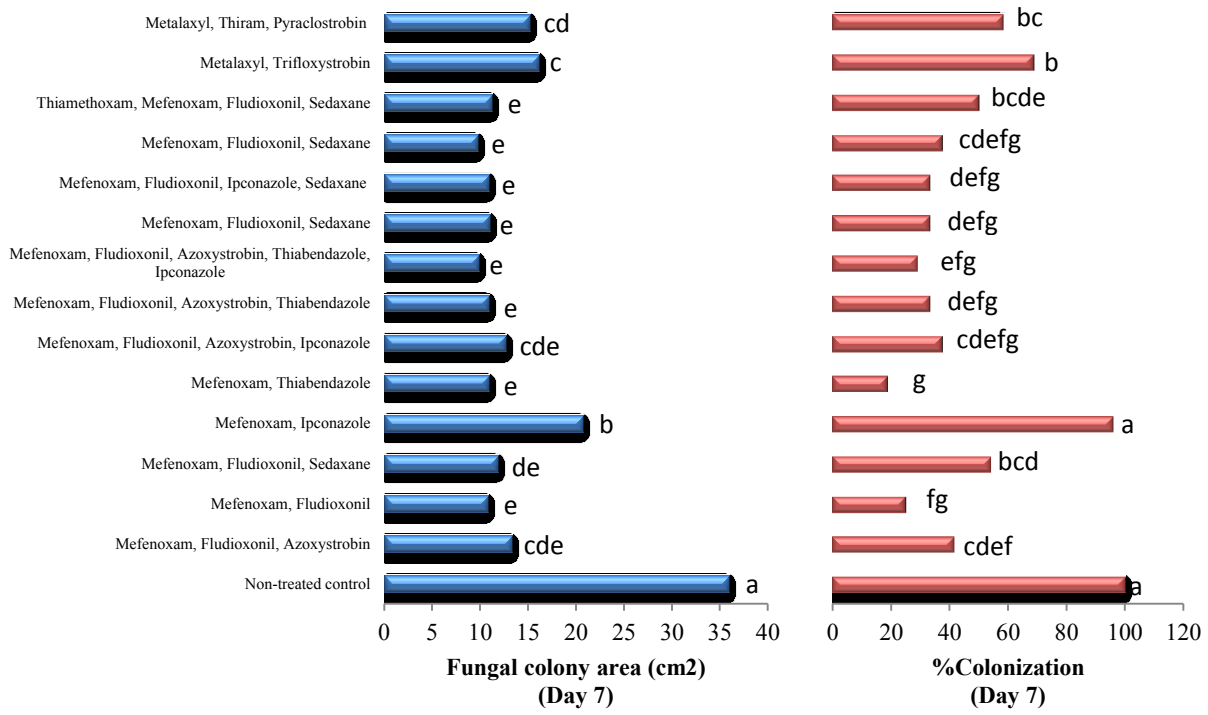


Figure 2.4. Response of *F. avenaceum* colony area (cm^2) and percent seed colonization by pathogen to multiple ai seed treatments on seventh day after placing seed. * Bars with the same letter are statistically not different at P = 0.05.

Growth chamber evaluation of single ai and combinations

Reductions in root rot severities as a result of single ai seed-treatments evaluated in this study were observed with fludioxonil, ipconazole, pyraclostrobin and trifloxystrobin (Appendix Table B7 and Figure 2.5). Median disease rating for fludioxonil at rates 2.5g ai/100kg seed and 5g ai/100kg seed, pyraclostrobin and trifloxystrobin was 3.0 and for ipconazole was 2.0 compared to a median disease rating of 5.0 for non-treated control. Fludioxonil at the higher rate of 5g ai /100kg seed and ipconazole showed significant increase in the root growth parameters like root length, root surface area and number of root tips as compared to non-treated control and other treatments (Figure 2.6). Values for root length ranged from 22 cm to 61 cm with an average of 41 cm, root surface area ranged from 11 cm^2 to 25 cm^2 with an average of 17 cm^2 and number of root tips ranged from 47 to 168 with an average of 109 root tips. Root growth

parameters like root length, root surface area and number of root tips assessed using the WinRHIZO software (Regent Instruments, Quebec, Canada) were found to be positively correlated with each other, while root rot severity was negatively correlated to all the root growth parameters (Appendix Table A3).

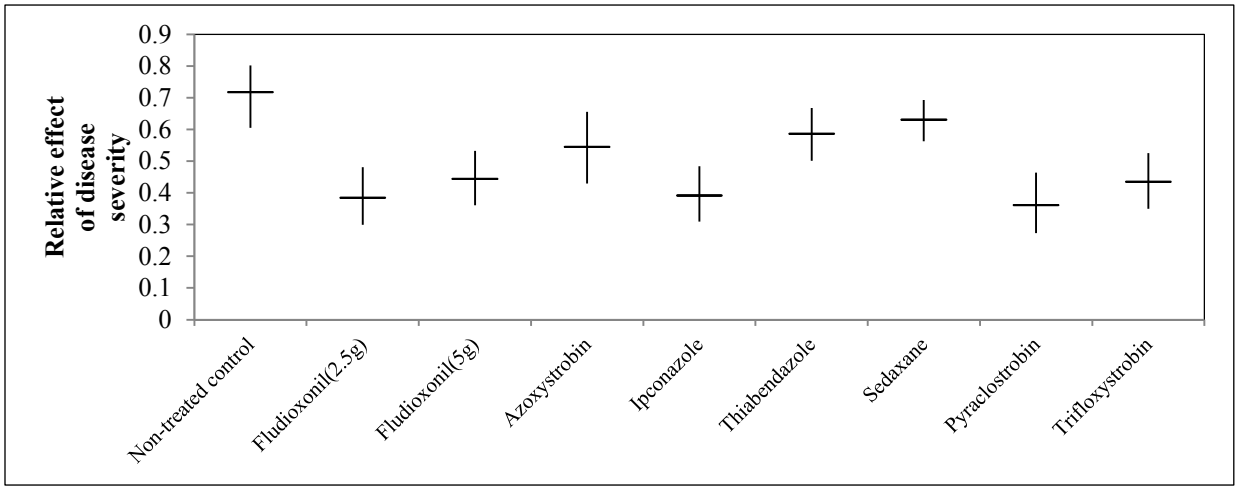


Figure 2.5. Relative effects of single fungicide seed treatments on severity of field pea root rot caused by *Fusarium avenaceum* under growth chamber conditions. Vertical lines represent the 95% confidence interval.

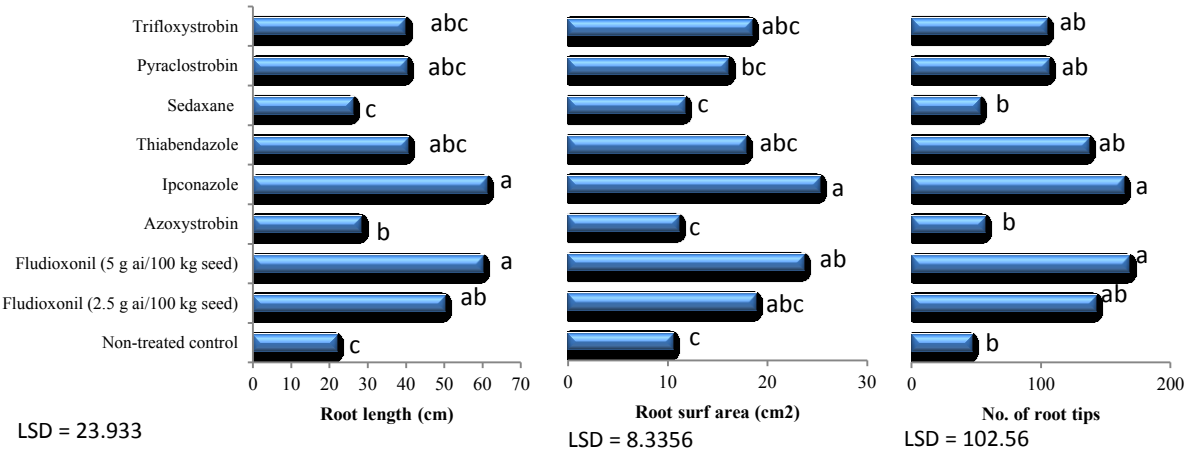


Figure 2.6. Effects of single fungicide seed treatments on length, surface area and root tip number of field pea roots. * Bars with the same letter are statistically not different at P = 0.05.

The data obtained from the repeated experiments of growth chamber trials with seed treatments containing multiple ais (Table 2.3) could not be combined for root growth parameters (Appendix Table A4). Root rot control results indicate that mefenoxam/fludioxonil/sedaxane, metalaxyl/trifloxystrobin and metalaxyl/thiram/pyraclostrobin combinations significantly reduced root rot rating as compared to non-treated control (Appendix Table B8 and Figure 2.7).

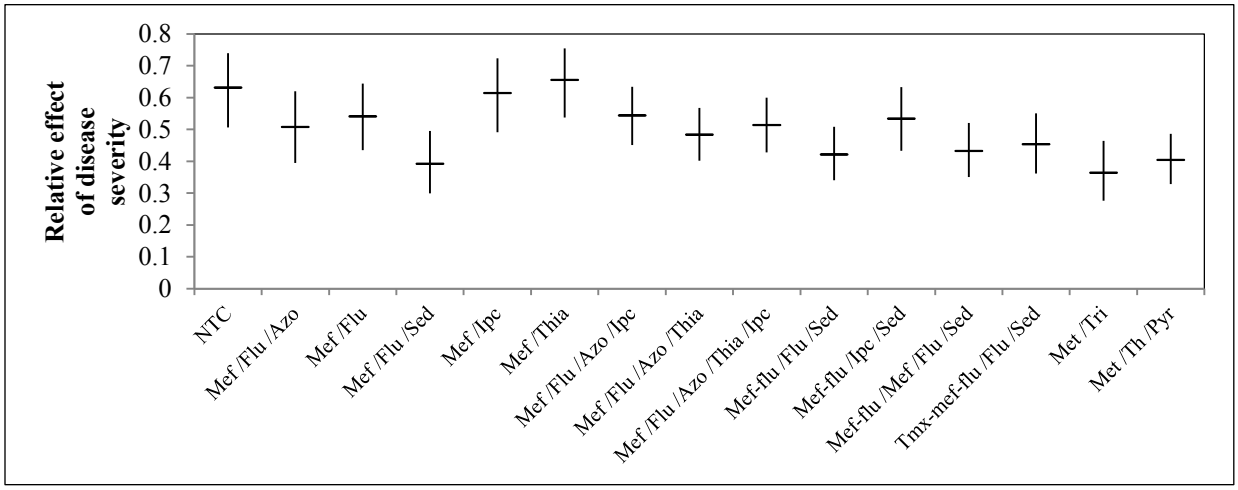


Figure 2.7. Relative effects of multiple ai seed treatments on severity of field pea root rot caused by *Fusarium avenaceum* under growth chamber conditions. Vertical lines represent the 95% confidence interval.

Field trials under natural disease pressure

Field assessment that included a trial under natural disease pressure was performed for two successive years 2011 and 2012. During 2011, the field pea trial at Newburg was affected by flooding and thus yield data could not be collected. Data indicates that the treatments were not significantly different from non-treated control for all parameters including root rot control, average root mass and average foliar mass (Appendix Table B9, Figures 2.8 and 2.9). *Fusarium* species isolated from pea roots include *F. oxysporum, F. solani, F. redolens* and *F. acuminatum*. The most prevalent species among these were *F.oxysporum* and *F. solani* which comprised 58% and 19%, respectively, of total pathogen population isolated from symptomatic roots.

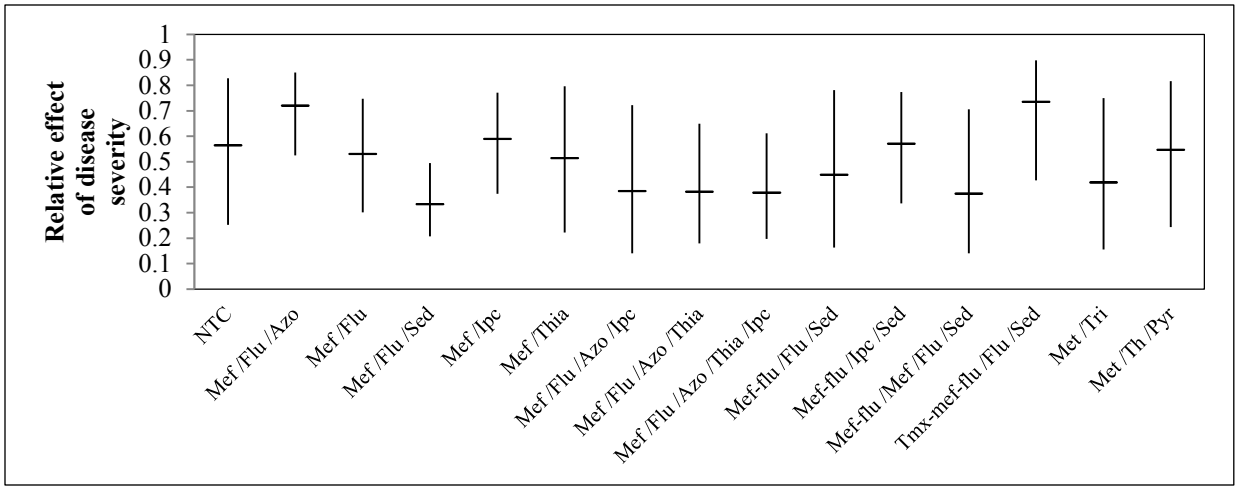
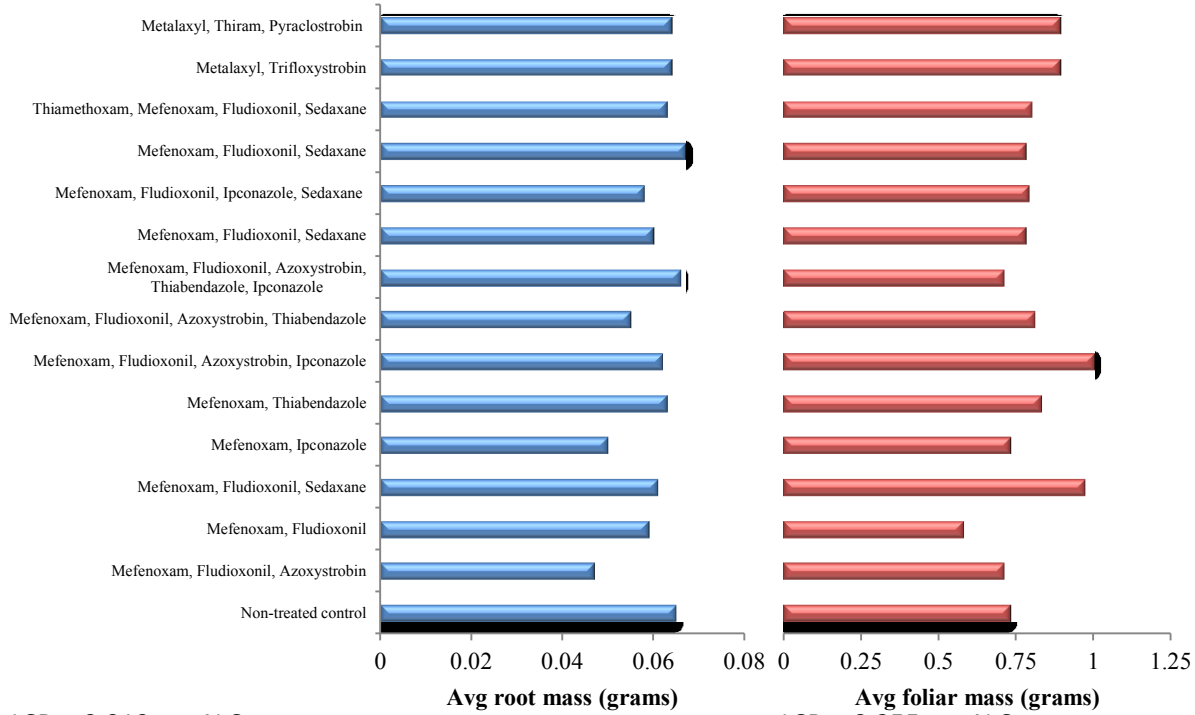


Figure 2.8. Relative effects of multiple ai seed treatments on severity of field pea root rot in a field trial conducted at Newburg under natural disease pressure during 2011. Vertical lines represent the 95% confidence interval.

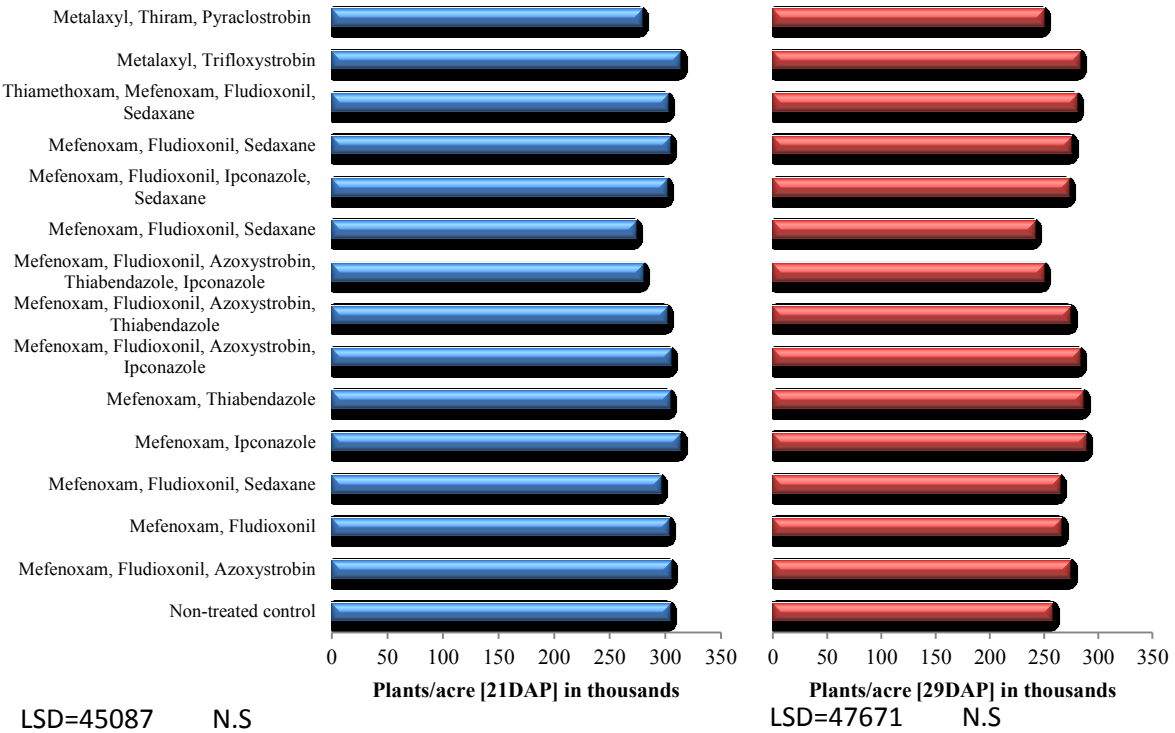


LSD = 0.019 N.S LSD = 0.355 N.S

Figure 2.9. Effect of multiple ai seed treatments on average root mass and average foliar mass in a field trial conducted at Newburg under natural disease pressure during 2011. * N.S = Not significantly different at P = 0.05.

Seed treatments in the 2012 field pea trial at Carrington did not have a significant effect on plant stand and root mass compared to the non-treated control (Figure 2.10 and Figure 2.12).

Similarly, their effect on disease severity (Figure 2.11; Appendix Table B10) was not statistically different (P > 0.05), although the combination of mefenoxam, fludioxonil, azoxystrobin and ipconazole may have provided a significantly better protection at P=0.1. When comparing the effect of fungicide mixtures on the average foliar weight, seeds protected with the mixture of mefenoxam, fludioxonil, azoxystrobin and ipconazole had higher foliar weight (2.84 grams/plant) compared to the non-protected control and the mixtures of metalaxyl with trifloxystrobin, mefenoxam with fludioxonil and sedaxane, and metalaxyl with thiram and pyraclostrobin (Figure 2.12). The latter three mixtures had provided significantly better control of root rot in growth chamber experiments but in this field trial they did not; and the foliar mass of plants protected by them were not different than that of the non-protected control.



LSD=45087 N.S LSD=47671 N.S

Figure 2.10. Effect of multiple ai seed treatments on plant stand 21 and 29 days after planting (DAP) in a field trial conducted at Carrington under natural disease pressure during 2012. * N.S = Not significantly different at P = 0.05.

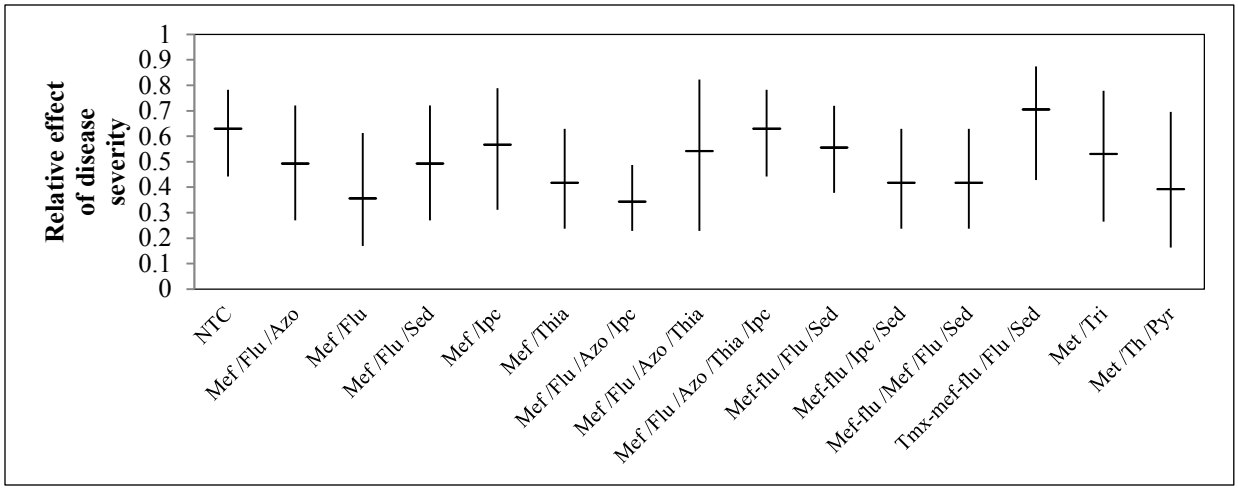


Figure 2.11. Relative effects of multiple ai seed treatments on severity of field pea root rot in a field trial conducted at Carrington under natural disease pressure during 2012. Vertical lines represent the 95% confidence interval.

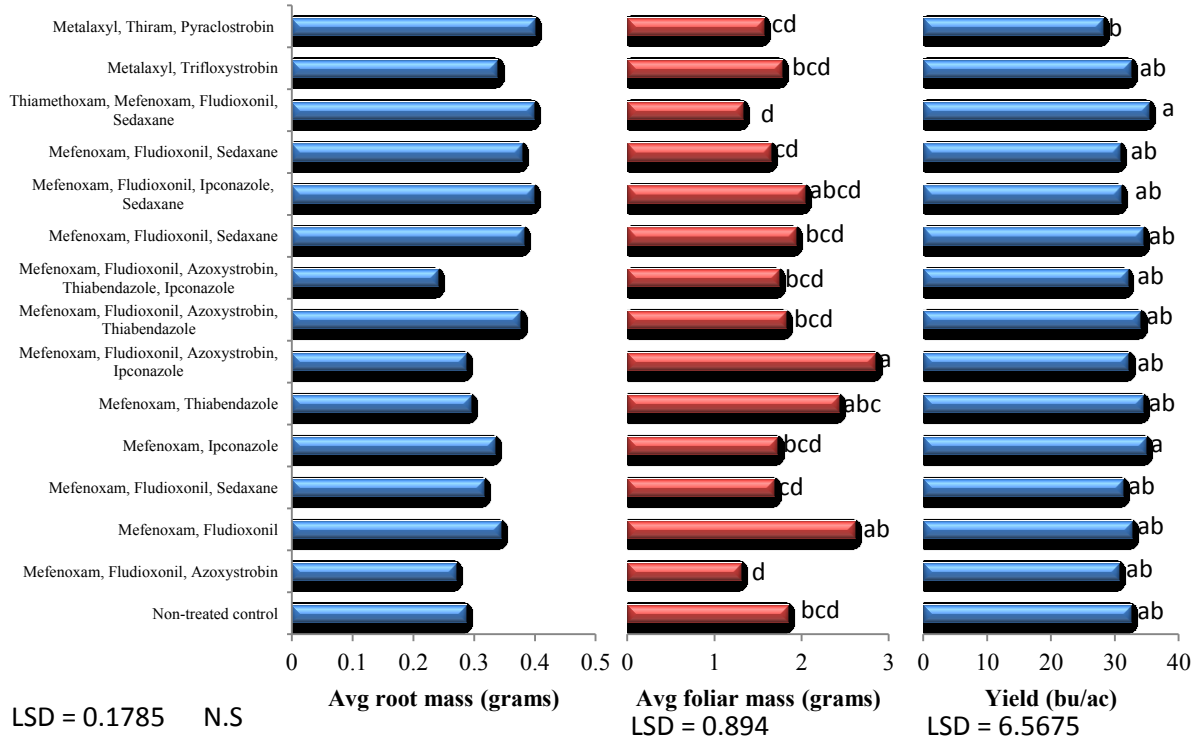


Figure 2.12. Effect of multiple ai seed treatments on average root mass, average foliar mass and yield in a field trial conducted at Carrington under natural disease pressure during 2012. * Bars with the same letter are statistically not different at P = 0.05 and N.S = Not significantly different at P = 0.05.

Discussion

According to fungicide labels, thiabendazole, ipconazole, fludioxonil, azoxystrobin, pyraclostrobin and trifloxystrobin are targeted towards control of *Fusarium* spp. along with other soil-borne and seed-borne pathogens. However, no recommendations have been made for use of these fungicides as seed treatments against Fusarium root rot except ipconazole which is recommended for use against seed rot caused by *Fusarium* spp. in both dry beans and field peas and literature in this area is limited. The studies reported in this thesis provide information regarding the efficacy of some commonly available seed treatments against Fusarium root rot in field pea when they are used alone or in combination with one other.

Results observed in laboratory conditions were also reflected in growth chamber trials. The *in-vitro* studies suggest that fludioxonil, pyraclostrobin, trifloxystrobin and thiabendazole, individually or in combination with other chemicals significantly inhibited fungal growth and seed colonization. The first three compounds and ipconazole also reduced root rot significantly as single ai treatments in growth chamber studies while the mixtures of fludioxonil with mefenoxam and sedaxane; and the mixtures of trifloxystrobin with metalaxyl and of pyraclostrobin with metalaxyl and thiram significantly reduced root rot as compared to non-treated control. Trifloxystrobin alone reduces disease severity to some extent; but when combined with metalaxyl it provided relatively higher degree of root rot control. Thiabendazole was the only compound whose activity was not consistent; it proved effective in laboratory trials but not in the growth chamber studies alone or in combinations with other compounds.

The activity observed in growth chamber trials did not translate into better protection under field conditions. Of the two years when trials were conducted, the 2011 trial had higher

disease pressure. Root rot severity for the non-protected controls in 2011 had a median of 4 in the 0 to 5 scale while the median for the 2012 trial was 3. In spite of this difference, none of the seed treatments reduced root rot severity significantly compared to the non-protected controls in any of the two years the trials were conducted. However at low disease pressure, in the 2012 trial, the combination of mefenoxam with fludioxonil, azoxystrobin and ipconazole increased foliar mass significantly compared to the non-treated check. These results are in agreement with a recent report (3) where seed treatment with Apron Maxx (Mefenoxam+Fludioxonil) improved emergence, nodulation and yield of field peas inoculated with *F. avenaceum* under both greenhouse and field conditions.

Sedaxane, one of the new chemistries, had limited activity against *F. avenaceum* in all trials, laboratory, growth chamber and field as expected based on its primary targets. However, it did not show any physiological effect on root growth parameters in field pea as opposed to its positive effect on root growth in other crops like corn, soybean and small grains (17, 18, 21, 23, 25).

In conclusion, the single ai seed treatments fludioxonil (at both rates), thiabendazole, pyraclostrobin and trifloxystrobin that inhibited fungal growth and seed colonization when used individually in the *in-vitro* studies were also effective when used in combination with other compounds. Among these compounds, fludioxonil, pyraclostrobin and trifloxystrobin were also effective in reducing disease severity under growth chamber conditions when used individually. Trifloxystrobin and pyraclostrobin were effective in reducing disease severity even when used in combination with other compounds. Apart from reducing disease severity, seed treatments can also have an effect on other root growth parameters. Ipconazole and fludioxonil (5 g ai/100 kg

seed) each when used alone significantly increased root surface area, root length and number of root tips in comparison to non-treated control under growth chamber conditions. The findings from the petri-dish and growth chamber trials provide information regarding the potential efficacy of the compounds against *F. solani*. However the findings from the field trials appear to have been inconclusive. We believe that further investigation regarding the dominant *Fusarium* species associated with root rot in field peas need to be conducted to be able to set up appropriate parameters to determine efficacy of the compounds under field conditions in North Dakota.

Chemical seed treatments only protect the seed from pathogens during the initial stages of plant growth, for about two weeks from the planting date in most cases. In order to obtain continued protection throughout the life of the crop, it is essential to integrate seed treatments with cultural practices and other methods of root rot management. Also, a drench application at a later time may be necessary until more effective levels of resistance become available. However, further experiments need to be performed in order to determine the most effective management strategy.

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APPENDIX A: CORRELATION COOEFFICIENT OF ROOT GROWTH PARAMETERS AND ROOT ROT SEVERITY

Table A1. List of correlation coefficients of root growth parameters like surface area, root tips, root length and root rot severity with respect to each other for dry bean single ai treatments.

Pearson Correlation Coefficients, N = 9
Prob > |r| under H0: Rho=0

	SA	RT	RL	RR
SA	1.00000	0.86243 0.0028	0.88802 0.0014	0.34100 0.3692
RT	0.86243 0.0028	1.00000	0.91522 0.0005	0.38136 0.3112
RL	0.88802 0.0014	0.91522 0.0005	1.00000	0.35590 0.3472
RR	0.34100 0.3692	0.38136 0.3112	0.35590 0.3472	1.00000

*SA = Surface area; RT = No. of root tips; RL = Root length; RR = Root rot severity

Table A2. List of correlation coefficients of root growth parameters like surface area, root tips, root length and root rot severity with respect to each other for dry bean combination treatments.

Pearson Correlation Coefficients, N = 15
Prob > |r| under H0: Rho=0

	SA	RL	RT	RR
SA	1.00000	-0.03805 0.8929	-0.05880 0.8351	-0.73600 0.0018
RL	-0.03805 0.8929	1.00000	0.96179 <.0001	0.42907 0.1105
RT	-0.05880 0.8351	0.96179 <.0001	1.00000	0.41858 0.1205
RR	-0.73600 0.0018	0.42907 0.1105	0.41858 0.1205	1.00000

*SA = Surface area; RT = No. of root tips; RL = Root length; RR = Root rot severity

Table A3. List of correlation coefficients of root growth parameters like surface area, root tips, root length and root rot severity with respect to each other for field pea single ai treatments.

Pearson Correlation Coefficients, N = 9
Prob > |r| under H0: Rho=0

	SA	RT	RL	RR
SA	1.00000	0.95891 <.0001	0.97736 <.0001	-0.73903 0.0229
RT	0.95891 <.0001	1.00000	0.96778 <.0001	-0.72760 0.0263
RL	0.97736 <.0001	0.96778 <.0001	1.00000	-0.80115 0.0094
RR	-0.73903 0.0229	-0.72760 0.0263	-0.80115 0.0094	1.00000

*SA = Surface area; RT = No. of root tips; RL = Root length; RR = Root rot severity

Table A4. List of mean and standard deviation of root growth parameters like surface area, root tips, root length and root rot severity for field pea combination treatments.

Level of EXP	N	SA		RT		RL		RR	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
1	114	15.24	10.18	121.22	96.79	72.33	55.42	2.99	1.13
2	116	41.20	23.46	478.01	346.20	117.24	75.75	2.47	1.03
3	115	97.50	42.91	582.00	255.31	244.41	86.49	1.60	0.99

APPENDIX B: MEDIAN, MEAN RANK AND ESTIMATED RELATIVE EFFECT OF ROOT ROT SEVERITY

Additional tables for chapter 1

Table B1. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on dry bean plants produced from seeds treated with single fungicides. Severity was measured using a 0-5 scale.

Treatment code	Median Disease rating	Mean rank	Estimated relative Effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
Non-treated control	3.00	82.04	0.76	0.62	0.84
Fludioxonil (2.5 g)	2.00	67.13	0.62	0.48	0.74
Fludioxonil (5 g)	1.00	51.25	0.47	0.32	0.63
Azoxystrobin	1.00	44.17	0.40	0.30	0.52
Ipconazole	2.00	74.38	0.68	0.59	0.76
Thiabendazole	2.00	54.25	0.50	0.36	0.64
Sedaxane	1.00	40.29	0.37	0.22	0.55
Pyraclostrobin	2.00	48.08	0.44	0.32	0.57
Trifloxystrobin	1.00	28.92	0.26	0.19	0.37

Table B2. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on dry bean plants produced from seeds treated with multiple ai combinations. Severity was measured using a 0-5 scale.

Treatment Code	Median Disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
I_NTC or NTC	3.00	113.83	0.63	0.46	0.77
Mef/Flu/Azo	2.00	103.71	0.57	0.43	0.70
Mef/Flu	2.50	114.96	0.64	0.52	0.74
Mef/Flu/Sed	3.00	133.75	0.74	0.61	0.83
Mef/Ipc	2.00	110.54	0.61	0.47	0.73
Mef/Thia	2.00	69.58	0.38	0.24	0.55
Mef/Flu/Azo/Ipc	2.50	102.46	0.57	0.44	0.68
Mef/Flu/Azo/Thia	2.00	100.79	0.56	0.41	0.70
Mef/Flu/Azo/Thia/Ipc	2.00	66.83	0.37	0.24	0.53
Mef-flu/Flu/Sed	2.00	75.92	0.42	0.29	0.56
Mef-flu/Ipc/Sed	2.00	69.04	0.38	0.25	0.53
Mef-flu/Mef/Flu/Sed	2.00	87.75	0.48	0.36	0.61
Tmx-mef-flu/Flu/Sed	2.00	92.83	0.51	0.38	0.64
Met/Tri	1.00	40.71	0.22	0.13	0.36
Met/Th/Pyr	2.00	74.79	0.41	0.26	0.59

Table B3. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on dry bean plants produced from seeds treated with multiple ai combinations in an inoculated field trial conducted at Carrington during 2011. Severity was measured using a 0-5 scale.

Treatment Code	Median disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
NI_NTC	1.25	36.00	0.55	0.29	0.79
I_NTC or NTC	2.00	46.38	0.72	0.41	0.89
Mef/Flu/Azo	1.75	43.25	0.67	0.40	0.85
Mef/Flu	1.25	29.25	0.45	0.20	0.74
Mef/Flu/Sed	1.00	28.75	0.44	0.25	0.66
Mef/Ipc	1.00	26.00	0.40	0.15	0.72
Mef/Thia	1.00	28.75	0.44	0.25	0.66
Mef/Flu/Azo/Ipc	1.50	31.00	0.48	0.19	0.78
Mef/Flu/Azo/Thia	1.00	31.13	0.48	0.23	0.74
Mef/Flu/Azo/Thia/Ipc	1.00	32.13	0.49	0.22	0.77
Mef-flu/Flu/Sed	1.00	28.75	0.44	0.25	0.66
Mef-flu/Ipc/Sed	1.25	28.63	0.44	0.18	0.74
Mef-flu/Mef/Flu/Sed	1.25	36.00	0.55	0.29	0.79
Tmx-mef-flu/Flu/Sed	1.00	26.38	0.40	0.26	0.57
Met/Tri	1.75	40.88	0.63	0.41	0.80
Met/Th/Pyr	1.00	26.75	0.41	0.15	0.74

Table B4. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on dry bean plants produced from seeds treated with multiple ai combinations in an inoculated field trial conducted at Carrington during 2012. Severity was measured using a 0-5 scale.

Treatment Code	Median disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
NI_NTC	1.00	17.25	0.26	0.15	0.43
I_NTC or NTC	1.00	32.38	0.50	0.23	0.77
Mef/Flu/Azo	1.50	35.00	0.54	0.23	0.82
Mef/Flu	1.50	40.38	0.62	0.30	0.86
Mef/Flu/Sed	1.00	30.00	0.46	0.25	0.69
Mef/Ipc	1.50	38.00	0.59	0.32	0.81
Mef/Thia	1.50	33.25	0.51	0.20	0.82
Mef/Flu/Azo/Ipc	1.00	30.00	0.46	0.25	0.69
Mef/Flu/Azo/Thia	1.25	35.13	0.54	0.31	0.75
Mef/Flu/Azo/Thia/Ipc	1.00	25.25	0.39	0.15	0.70
Mef-flu/Flu/Sed	1.25	32.25	0.50	0.32	0.67
Mef-flu/Ipc/Sed	1.00	22.00	0.34	0.27	0.42
Mef-flu/Mef/Flu/Sed	2.00	46.00	0.71	0.43	0.88
Tmx-mef-flu/Flu/Sed	1.25	35.13	0.54	0.31	0.75
Met/Tri	1.00	30.00	0.46	0.25	0.69
Met/Th/Pyr	1.50	38.00	0.59	0.32	0.81

Table B5. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on dry bean plants produced from seeds treated with multiple ai combinations in a field trial conducted at Staples under natural disease pressure during 2011. Severity was measured using a 0-5 scale.

Treatment Code	Median disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
NI_NTC	4.50	37.63	0.62	0.25	0.88
Mef/Flu/Azo	4.25	36.13	0.59	0.25	0.86
Mef/Flu	3.75	29.75	0.49	0.21	0.77
Mef/Flu/Sed	4.00	41.50	0.68	0.50	0.82
Mef/Ipc	3.75	34.25	0.56	0.27	0.81
Mef/Thia	3.50	24.75	0.40	0.21	0.65
Mef/Flu/Azo/Ipc	3.25	22.25	0.36	0.19	0.58
Mef/Flu/Azo/Thia	3.50	29.25	0.48	0.20	0.77
Mef/Flu/Azo/Thia/Ipc	3.00	22.75	0.37	0.12	0.73
Mef-flu/Flu/Sed	4.25	39.13	0.64	0.37	0.84
Mef-flu/Ipc/Sed	3.75	27.13	0.44	0.24	0.67
Mef-flu/Mef/Flu/Sed	3.50	27.88	0.46	0.17	0.78
Tmx-mef-flu/Flu/Sed	3.50	28.25	0.46	0.18	0.78
Met/Tri	3.25	24.75	0.40	0.25	0.58
Met/Th/Pyr	3.75	32.13	0.53	0.32	0.72

Table B6. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on dry bean plants produced from seeds treated with multiple ai combinations in a field trial conducted at Perham under natural disease pressure during 2012. Severity was measured using a 0-5 scale.

Treatment Code	Median disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
NI_NTC	3.00	34.00	0.56	0.28	0.80
Mef/Flu/Azo	3.00	34.00	0.56	0.28	0.80
Mef/Flu	3.00	33.13	0.54	0.22	0.83
Mef/Flu/Sed	2.50	22.75	0.37	0.17	0.64
Mef/Ipc	3.00	35.38	0.58	0.27	0.83
Mef/Thia	2.75	25.13	0.41	0.22	0.64
Mef/Flu/Azo/Ipc	3.00	34.00	0.56	0.28	0.80
Mef/Flu/Azo/Thia	3.00	29.63	0.49	0.28	0.70
Mef/Flu/Azo/Thia/Ipc	2.75	29.50	0.48	0.22	0.76
Mef-flu/Flu/Sed	2.75	30.38	0.50	0.14	0.85
Mef-flu/Ipc/Sed	2.75	25.13	0.41	0.22	0.64
Mef-flu/Mef/Flu/Sed	3.00	29.63	0.49	0.28	0.70
Tmx-mef-flu/Flu/Sed	2.75	27.50	0.45	0.28	0.63
Met/Tri	3.00	35.38	0.58	0.27	0.83
Met/Th/Pyr	3.00	32.00	0.53	0.37	0.68

Additional tables for chapter 2

Table B7. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on field pea plants produced from seeds treated with single fungicides. Severity was measured using a 0-5 scale.

Treatment code	Median Disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
Non-treated control	5.00	174.87	0.72	0.61	0.80
Fludioxonil (2.5 g)	3.00	94.07	0.39	0.30	0.48
Fludioxonil (5 g)	3.00	108.52	0.44	0.36	0.53
Azoxystrobin	4.50	133.02	0.55	0.43	0.66
Ipconazole	2.00	95.89	0.39	0.31	0.48
Thiabendazole	4.00	143.20	0.59	0.50	0.67
Sedaxane	4.00	153.78	0.63	0.56	0.69
Pyraclostrobin	3.00	88.39	0.36	0.27	0.46
Trifloxystrobin	3.00	106.26	0.44	0.35	0.53

Table B8. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on field pea plants produced from seeds treated with multiple ai combinations. Severity was measured using a 0-5 scale.

Treatment Code	Median disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
Non-treated control	4.00	256.28	0.63	0.51	0.74
Mef/Flu/Azo	3.00	206.22	0.51	0.40	0.62
Mef/Flu	3.00	219.85	0.54	0.44	0.64
Mef/Flu/Sed	2.00	159.44	0.39	0.30	0.50
Mef/Ipc	4.00	249.50	0.61	0.49	0.72
Mef/Thia	4.00	266.06	0.66	0.54	0.75
Mef/Flu/Azo/Ipc	3.00	220.93	0.54	0.45	0.63
Mef/Flu/Azo/Thia	2.50	196.74	0.48	0.40	0.57
Mef/Flu/Azo/Thia/Ipc	2.00	208.83	0.51	0.43	0.60
Mef-flu/Flu/Sed	2.00	171.26	0.42	0.34	0.51
Mef-flu/Ipc/Sed	2.50	216.91	0.53	0.43	0.63
Mef-flu/Mef/Flu/Sed	2.50	176.02	0.43	0.35	0.52
Tmx-mef-flu/Flu/Sed	2.00	184.54	0.45	0.36	0.55
Met/Tri	2.00	148.17	0.36	0.28	0.46
Met/Th/Pyr	2.00	164.26	0.40	0.33	0.49

Table B9. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on field pea plants produced from seeds treated with multiple ai combinations in a field trial conducted at Newburg under natural disease pressure during 2011. Severity was measured using a 0-5 scale.

Treatment Code	Median disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
Non-treated control	4.00	34.38	0.56	0.25	0.83
Mef/Flu/Azo	4.50	43.75	0.72	0.52	0.85
Mef/Flu	3.50	32.38	0.53	0.30	0.75
Mef/Flu/Sed	3.00	20.50	0.33	0.21	0.49
Mef/Ipc	4.00	35.88	0.59	0.37	0.77
Mef/Thia	4.00	31.38	0.51	0.22	0.80
Mef/Flu/Azo/Ipc	2.75	23.63	0.39	0.14	0.72
Mef/Flu/Azo/Thia	3.25	23.50	0.38	0.18	0.65
Mef/Flu/Azo/Thia/Ipc	3.00	23.25	0.38	0.20	0.61
Mef-flu/Flu/Sed	3.25	27.50	0.45	0.16	0.78
Mef-flu/Ipc/Sed	4.00	34.75	0.57	0.34	0.77
Mef-flu/Mef/Flu/Sed	2.50	23.00	0.38	0.14	0.71
Tmx-mef-flu/Flu/Sed	5.00	44.63	0.74	0.43	0.90
Met/Tri	3.00	25.63	0.42	0.16	0.75
Met/Th/Pyr	4.25	33.38	0.55	0.24	0.82

Table B10. Median, mean rank, and estimated relative treatment effects for Fusarium root rot severity on field pea plants produced from seeds treated with multiple ai combinations in a field trial conducted at Carrington under natural disease pressure during 2012. Severity was measured using a 0-5 scale.

Treatment Code	Median disease rating	Mean rank	Estimated relative effect	Confidence interval (95%) for relative treatment effect	
				Lower limit	Upper limit
Non-treated control	3.00	47.80	0.63	0.44	0.78
Mef/Flu/Azo	2.00	37.50	0.49	0.27	0.72
Mef/Flu	2.00	27.20	0.36	0.17	0.61
Mef/Flu/Sed	2.00	37.50	0.49	0.27	0.72
Mef/Ipc	3.00	43.10	0.57	0.31	0.79
Mef/Thia	2.00	31.90	0.42	0.24	0.63
Mef/Flu/Azo/Ipc	2.00	26.30	0.34	0.23	0.49
Mef/Flu/Azo/Thia	3.00	41.20	0.54	0.23	0.82
Mef/Flu/Azo/Thia/Ipc	3.00	47.80	0.63	0.44	0.78
Mef-flu/Flu/Sed	2.00	42.20	0.56	0.38	0.72
Mef-flu/Ipc/Sed	1.50	31.90	0.42	0.24	0.63
Mef-flu/Mef/Flu/Sed	2.00	31.90	0.42	0.24	0.63
Tmx-mef-flu/Flu/Sed	2.50	53.40	0.71	0.43	0.87
Met/Tri	2.00	40.30	0.53	0.26	0.78
Met/Th/Pyr	2.00	30.00	0.39	0.16	0.70