Interaction of fungicide seed treatments and the *Fusarium*-maize (*Zea mays* L.) pathosystem

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ABSTRACT

Maize stand losses and seedling blights due to *Fusarium* spp. continue to be one of the most economically important diseases in maize production. Consequently, all maize seed is treated with a mixture of fungicides that improves seedling emergence and yield. Data on the interaction between *Fusarium* spp. and seed treatment fungicides are limited. The effectiveness of seven fungicide active ingredients against seed-borne *F. verticillioides* was assessed under controlled environmental conditions. In addition the effects of fungicide seed treatments on seedling blight development and early season growth and physiology of maize were evaluated in the field at two locations in Iowa. Under controlled environmental thiabendazole, captan, ipconazole and triticonazole significantly reduced (P=0.0306) the recovery of *F. verticillioides* from seeds and decreased infection of shoots and radicle tissues by the fungus in both experiments. In field experiments at V2, the incidence of *Fusarium* infection in plants grown from fungicide treated seed was lower than the control. The incidence of each *Fusarium* spp. isolated changed with time: at growth stage V2, *F. graminearum* was predominant while incidence of *F. subglutinans* and *F. verticillioides* increased as the growing season progressed at both locations. The distribution of each species among plant tissues also varied; *F. graminearum*, *F. oxysporum*, and *F. solani* were predominantly isolated from roots and mesocotyl tissues, whereas *F. proliferatum F. verticillioides* and *F. subglutinans* were more frequently isolated from crown tissues. Fungicide seed treatments significantly reduced the proportions of roots, mesocotyl and crown sections colonized by *F. graminearum*, *F. subglutinans* and *F. verticillioides.* A significant relationship between the severity of mesocotyl rot at V2 and severity of crown rot at V6 was observed and there was some evidence of a relationship between crown rot at V6

and stalk rot at R6 at one location. Photosynthetic performance, as measured by chlorophyll fluorescence, significantly decreased with increased incidence of *Fusarium* spp. at growth stages V2 and V4 and with greater root, mesocotyl, crown and stalk rot disease severity. This work provide more knowledge about the pathology of fungicide seed treatments against seed-borne *F. verticillioides* and against colonization of soil-borne *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans* and *F. verticillioides*. Furthermore, this study provides evidence of the benefits of fungicide seed treatments on mid-late season crown rot and late season stalk rots.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Maize (*Zea mays* L.) was discovered on November 5, 1492 by European explorers that landed in a land first thought to be India, but that later would become known as America (61). Currently, maize is grown throughout the world, and total production is approximately 23 billion bushels (575 million metric tons) on 335 million acres (135 million hectares) (48). The United States (U.S.) and the People's Republic of China (China) account for approximately 61% of global maize, production followed by Brazil, Mexico, France, Argentina, India and Italy (61). Maize is grown in all 50 states of the U.S., however approximately two thirds of U.S. total production comes from just 7 states, namely, Iowa (15.4%), Illinois (14.2%), Nebraska (9.8%), Minnesota (8.8%), Indiana (7.1%), South Dakota (5.4%), Ohio (4.3%) (48,61).

Since maize was first domesticated, significant improvements in grain yield potential have been accomplished. Averaged across all states, maize yield in the U.S. has increased from approximately 21 bu/acre (1300 kg ha^{-1}) in 1939 to 125 bu/acre (7800 kg ha^{-1}) in 2005, at a rate of approximately 4 bu/year (99 kg yr^{-1}) (16). This increase has been a result of various changes in crop management practices including increased plant density, use of nitrogen fertilizer, a shift to earlier planting dates, use of irrigation, enhanced weed and pest control, and improved efficiency of harvest equipment (29). In addition, maize breeding efforts have targeted heterotic vigor in commercial hybrids, increased yield potential, adaptability to different growing environments, abiotic/biotic stress resistance, and nutritional values for human/livestock feed. Crop management practices are responsible for

50% of the increase in maize yield in the last 67 years; the remaining 50% is a result of breeding improvements (16).

The recent introduction and use of biotechnology has transformed maize breeding efforts and further enhanced yield production. Many of today's hybrids contain genes that allow endogenous production of Delta-endotoxin derived from soil-borne *Bacillus thuringiensis* var. *kurstaki*, which is lethal to lepidopteran moths like *Obstrinia nubilalis*, *Spodoptera frugiperda*, *Helicoverpa zeae*, that are major pests of maize (34). Such hybrids are referred to as Bt-maize. Still other hybrid types contain transgenic genes that provide resistance to the herbicides glyphosate (*N*-(phosphonomethyl)glycine) and glufosinate (2-amino-4-(hydroxyl-methyl-phosphinyl)butanoic acid) (52). These modifications of the maize genome are identified as 'traits', and commercial hybrid denominations as 'stacks'.

As a result of these technological advances, maize seed had become a significant production cost for farmers. Agronomic practices in maize seed production fields are oriented to maximize seed quality and consequently increase seed germination and seedling vigor (2). Appropriate seed harvest, seed processing, and seed storage practices are used to reduce deterioration caused by mechanical damage, storage molds, and insects (19). However, seed is still susceptible to numerous pathogens during grain fill (41).

Environmental conditions during grain fill may favor the development of *Fusarium* ear rot (caused by *F. proliferatum*, *F. subglutinans,* and/or *F. verticillioides*) and *Gibberella* ear rot (caused by *F. graminearum*) (26). These pathogens are seed-borne and planting infected seed increases the risk of seedling blights that result in poor plant stand and reduced seedling vigor in the field. Seed-borne inoculum has been shown to be economically important in some crops. For example, seed-borne *Stagonospora nodorum* is the primary source of inoculum for Stagonospora blotch of wheat (40). Seed-borne *F. graminearum*

causes economic losses as a result of seedling blight in all wheat growing regions around the world (24).

The U.S. Corn Belt maize planting conditions are often stressful for maize seedling emergence; early growing conditions play an important part in disease development in the field. Seed transmission of seed-borne inoculum of *F. subglutinans,* and *F. verticillioides,* in maize has been documented (25,44,72). *Fusarium subglutinans* seed to plant transmission rate was estimated at 88% in artificially inoculated seed, and resulted in seedling blights in infected seedlings at 25^{o}C (25). Field studies assessing seed transmission of *F. verticillioides* demonstrated maize stalks were colonized before growth stage V10. Growth chamber studies have shown that *F. verticillioides* and *F. subglutinans* seed to plant transmission is not affected by temperatures comparable to those at planting time in the Midwest, that is between 10^{o}C to 15^{o}C (71,72).

All commercial maize seed grown in the U.S. Corn Belt is processed. Seed processing is the classification of seed in fractions according to size, weight, and quality (19). The significance of seed-borne *Fusarium* spp. as a source of inoculum for early season seedling blights and reduction on maize stand and vigor might be dependant on the amount of seed-borne *Fusarium* spp. present in seed for planting, however there has been little if any research concerning the importance of this source of inoculum and its contribution to seedling disease in the field. *Fusarium* spp. is commonly detected in commercial seed lots that are received at Iowa State University Seed Science Center (Dennis McGee, *personal communication),* however, the incidence of seed-borne *Fusarium* spp. in commercial maize seed available for the U.S. has not been evaluated. Furthermore, no disease thresholds for seed-borne *Fusarium* spp. have been established.

After seed processing, all commercial maize seed in the U.S. is treated with fungicide prior to planting. Seed treatment is known to increase emergence and yield, although the pathology behind this practice is not well understood (38). The effectiveness of fungicide seed treatments against *Fusarium* spp. is commonly evaluated in field experiments, and emergence and grain yield are used as indications of seed treatment efficacy (39). The specific action of the fungicide seed treatment on the target host-pathogen interaction is rarely determined, and data on the interaction between *Fusarium* and seed treatment fungicides are limited. The few studies that have been done have determined that most seed treatments are effective against soil-borne inoculum (42). However, preliminary data from growth chamber experiments suggests that fludioxonil, with which almost 100% of maize seed is treated, has no significant effect on seed-borne inoculum of *F. verticillioides* (Alison Robertson, *personal communication*). This suggests that seed treatment efficacy is affected by the source of *Fusarium* spp. inoculum. Since seed-borne inoculum may contribute to seedling blight and stalk rot (18), it is important to determine if seed treatment fungicides do effectively control seed-borne *Fusarium*. Fungicide seed treatments are effective at reducing vertical transmission of seed-borne *F. graminearum* (23) and *S. nodorum* in wheat (40), and at reducing soil-borne infection of *Fusarium* spp. Fungicide seed treatments are thought to form a protective zone around germinating seeds and thus reduce seed decay and seedling blights caused by soil-borne pathogens. It is hypothesized that the seed treatments applied to maize seed can kill or inhibit seed-borne pathogens upon germination. Support for this hypothesis was demonstrated by Galperin et al. (18) who showed that prochloraz completely suppressed seed-borne *F. moniliforme* (syn. *F. verticillioides*)

Apart from elucidating the effect of fungicides on seed-borne inoculum, an improved understanding of the effect of fungicide seed treatments against soil-borne *Fusarium* spp. is

also needed in order to optimize current management practices. Furthermore, since fungicide seed treatments positively impact early plant growth and development in other cereal crops such as wheat (11), barley (54), soybeans (9), the effect of this management practice on early season growth and development of maize could be investigated.

Thesis organization

This thesis is organized into four chapters. Chapter one is the general introduction. Chapter two is an article to be submitted to *Plant Disease* entitled "Effectiveness of fungicide active ingredients against seed-borne *Fusarium verticillioides* in maize (*Zea mays* L.)." It describes the efficacy of seven fungicide active ingredients against seed transmission of seed-borne *Fusarium verticillioides*. Chapter three is an article to be submitted to *Plant Disease* entitled "Colonization of maize (*Zea mays* L.) seedlings by *Fusarium* spp. in the field and its suppression by seed treatments" Chapter four is an article to be submitted to *Plant Disease* entitled "Seed treatments indirectly impact photosynthetic ability in maize (*Zea mays* L.) seedlings by reducing *Fusarium* spp. infection and seedling disease". Chapters three and four assess the effect of seed treatments on field performance of maize plants. Chapter three elucidates the pathology of fungicide seed treatments on infection and colonization of maize seedlings by *Fusarium* spp., and colonization distribution of *Fusarium* spp. within maize seedlings. In chapter four indirect effects of fungicide seed treatments on maize physiology are shown. Chapter five is the general conclusion of this research project.

Literature review

Taxonomy

The origin of the *Fusarium* genera is not very clear due to lack of fossils; however its worldwide diversity in species and teleomorphs suggests that it is an ancient genus (50,63). The most common species within this section include *Fusarium verticillioides* [(Saccardo)

Nirenberg], *F. subglutinans* [(Wollenweber and Reinking) Nelson, Toussoun & Marasas], and *F. proliferatum* [(Matsushima) Nirenberg] (30,50,63). The research discussed herein will focus mainly on *F. verticillioides*, however other *Fusarium* spp. will be mentioned and discussed as necessary.

Fusarium verticillioides (Saccardo) Nirenberg [syn. *F. moniliforme* (Sheldon)] teleomorph *Gibberella moniliformis* was originally named *Fusarium moniliforme* (Sheldon) in 1904, but since then the species has undergone several taxonomic reviews (7,73). Nirenberg (51) regrouped section Liseola and split *F. moniliforme* into *F. verticillioides*, and *F. proliferatum*, however Nirenberg's proposed classification of section Liseola was not accepted at that time. In 2003, the international society of plant pathology and the international committee on the taxonomy of fungi (ISPP/ICTF), subcommittee on *Fusarium* systematics agreed that *Fusarium moniliforme* represented a broad species concept, and formerly adopted Nirenberg proposed classification: thus *F. verticillioides* was used to designate mating population A of the *Gibberella fujikuroi* complex (59), and its teleomorph *G. moniliformis* was adopted.

Morphological characteristics

Fusarium verticillioides isolates rarely produce macroconidia, however when they do, they are formed in pale orange sporodochia and are slender, almost straight and usually 3 to 5 septate. Single celled microconidia are found grouped in long chains, and are oval to club shaped with a flattened base. They always form on monophialides. The morphological characteristics of *F. verticillioides* are very similar to *F. proliferatum*, but the microconidia chains form by *F. proliferatum* are usually shorter and form from polyphialides (31).

Maize-*F. verticillioides* pathosystem

Fusarium verticillioides is the most commonly reported fungus infecting maize (31). Infections can be endophytic (asymptomatic) or pathogenic resulting in disease symptoms, namely seedling blights, root rots, stalk rots, and ear rots.

Seedling diseases. Seedling blights arising from infection by *F. verticillioides* are characterized by soft or water-soaked mesocotyl that may be pinkish in color, and/or yellowish-brown lesions on the primary roots that later become black and necrotic. Seedling blights result in loss of stand and consequently reduced yield. The severity of early season disease is dependant of maize genotype, environment conditions that favor pathogen colonization (15), and pathogenicity of the *Fusarium* spp. (4,32). Mesocotyl and seminal roots are physiologically active and responsible for water and nutrient uptake by the seedling until growth stages V4 to V6, when adventitious roots become functional (21,57). Thus mesocotyl damage resulting from infection by *Fusarium* spp. interferes with the normal absorption of water and nutrients by the roots and negatively impacts photosynthesis (21,67).

Crown rot. Rot of the lower stalk that occurs four to six weeks before pollination is referred to as crown rot (69). Internally symptoms of crown rot may display nutrient deficiency symptoms or are discolored whitish-pink to salmon pith. Leaves on plants with severe crown rot between V4-R6 suddenly turn dull grayish-green and lower internodes become soft and tan to dark brown in color.

Stalk rot. Stalk rot appears after tasseling and results in stalk breakage and premature ripening (69). Infections by *F. verticillioides* become evident at various stages of growth, depending on the susceptibility of the inbred or hybrid (15). Maize stalk rot annually results in yield losses of 10 to 20% on susceptible hybrids in the U.S., however 100% yield losses had been reported (69). In Brazil, average losses reported as a result of stalk rot were 11

bu/ac (678 kg ha^{-1}) in 1997-1998 season, and 18.5 bu/ac (1,151 kg ha^{-1}) in the 1998-1999 growing season (13). Stalk rot disease incidence ranged between 11.2 to 79.3% in both growing seasons (13). Lodging resulting from stalk rot complicates harvesting (15).

Ear rot. *Fusarium* ear rot caused by *F. verticillioides* is described as white or light pink mold on random kernels, groups of kernels or physically injured kernels (41,58). As disease progresses, a cottony-pink mold growth develops on the infected kernels. Kernels infected late in the season develop whitish streaks on the pericarp. *Fusarium* ear rot is more common in warmer and drier areas, and is favored by warm, dry weather during the grain-filling period (35,41).

Mycotoxins. Ear rot cause by *F. verticillioides* can produce mycotoxins that have negative effects on swine, beef, dairy, poultry, horses among other livestock animals (53,70). Fumonisins, fusaric acid, trace levels of beauveracin and moniliformin are mycotoxins produced by *F. verticillioides* (14).

Source of inoculum

Fusarium verticillioides survives as mycelium in the soil/crop residue may remain infective for more than 630 days; although environmental fluctuations of temperature and moisture reduce survival of the mycelium (12). Infective hyphae penetrate the root epidermis of maize seedlings either directly or though ruptures made in the cortex by emerging roots, and then grow into the shoot (26). *Fusarium* spp. hyphae can also infect germinating seedlings through natural openings (45).

The most important source of inoculum for *Fusarium* ear rot and symptomless plant and kernel infection are microconidia of *F. verticillioides* that are produced on crop residue in high numbers (41). These spores accumulate on the maize stalk, leaf and silk tissues

during the growing season, and also are introduced into the plant by maize pests like European corn borer *Obstrinia nubilalis*, and ear worms *Helicoverpa zea* (41).

Seed-borne inoculum can also result in plant infection. Seed-to-seed transmission of *Fusarium verticillioides* has been demonstrated in the field (43). Thus symptomless colonization of maize kernels by *F. verticillioides* could be an important source of seed-borne inoculum for seedling and stalk diseases and mycotoxin accumulation. Still, the contribution of seed-borne *F. verticillioides* to disease and mycotoxin accumulation is not well understood. Incidence of seed-borne *F. verticillioides* in commercial maize seed grown in different regions of Brazil ranged from 8% to 57% (20). Thomas and Buddenhagen (64) isolated *F. verticillioides* from between 43 and 70% of symptomless maize kernels stored for 10 to 15 months and from 36 to 78% of freshly harvested kernels in white maize cultivars in Nigeria. The mean incidence of *F. moniliforme* (syn. *F. verticillioides*) in commercial maize seed samples from three different regions of India was 38.5% (46).

Favorable conditions for seedling disease caused by *F. verticillioides*

Cool (10^{o}C to 16^{o}C), wet soil planting conditions are conducive to seedling disease as a result of *F. verticillioides* infection (56). In the Corn Belt of the United States, maize is planted in early spring (beginning mid April in Iowa) as soon as soil temperatures are above 10^{o}C (27), although the optimum temperature for maize germination is 25^{o}C. Maize seed planted in cold soils imbibe cold water that can rupture cell membranes and embryo tissues (62). This damage results in the disruption of protein production and energy transformation in the activated cells (66), and exudation of carbon sources such as organic acids, sugars and amino acids, that create a nutrient rich environment which stimulates soil-borne pathogenic and non-pathogenic interactions between maize seedlings and soil-borne *Rhizoctonia* spp., *Pythium* spp., *Phytophthora* spp., and *Fusarium* spp. (33,69). Thus seed germination is

significantly slowed when planting in cold soils and germinating seedlings are susceptible to infection by numerous soil-borne pathogens (69).

Management of seedling disease

Conditioning of maize seed. Post-harvest seed quality can be improved by a variety of practices. Gravity tables remove undesirable seeds that are somewhat lighter as a result of fungal infection. Furthermore, seed is processed by size, weight and shape to reduce storage mold damage and increase seed lot uniformity, which is important for planter calibration and chemical seed treatment application. Generally maize seed from any one seed lot can be separated by shape into large flats (6.1%), large rounds (27.8%), medium flats (13.6%), medium rounds (29%), small flats (10.3%), small rounds (13.2%) (19).

The highest incidence of seed-borne *F. proliferatum, F. subglutinans,* and *F. verticillioides* has been detected on the lower density seed fraction, that is, small flats and small rounds, therefore, the removal of these fractions, can increase seed lot quality and reduce the chance of early season seedling blights, poor seedling vigor and stand loss due to the presence of seed-borne *Fusarium* spp. inoculum (19).

Chemical seed treatments. Fungicide seed treatments are widely used in grain crops for disease management. Seed treatment use dates back to the ancient Roman times, when wheat smut control was done by steeping the seed in wine or mixing bruised wheat leaves with seed. In the last century, significant improvements in seed treatments have occurred, for example, the use of organic mercurial fungicides for wheat in 1913 (61), chloranil, dichlone and thiram in 1940's, the "miracle" seed protectant, captan (1952), carboxin for management of loose smut of wheat, benomyl (1968), and tetracyclines for use against seed-borne bacteria (1970) (2,49).

Fungicide seed treatment control may be classified into three classes: disinfection, disinfestation and protection (2). Seed disinfection is the control of seed-borne inoculum established within a seed or seed coat tissue. Seed disinfestation is the control of seed-borne pathogens that are present on the seed surface either externally or passively. Seed protection protects seed from soil-borne facultative parasitic fungi that, under suitable environmental conditions, can cause seed rot and/or seedling blights, or symptomless systemic infection that may become pathogenic (49).

Maize fungicide seed treatments are applied as slurry, which improves coverage uniformity and helps overcome problems associated with dry powder application. Slurry treatments may include adhesives like dextran, gum arabic, methyl cellulose, or vegetable oils, however all slurries need to be water soluble. Desirable characteristics of fungicide seed treatment: (i) are effective under different agro-climatic conditions, (ii) are not phytotoxic, (iii) leave no harmful residues in plants or in the soil, and (iv) are compatible with other seed treatments (1).

In the U.S. Corn Belt, planting is initiated as soon as soil temperatures are above 12^{o}C. Such conditions favor infection and early season disease development by soil-borne pathogens, thus the use of seed treatments to manage early season diseases is common (6). Fungicide seed treatments form a protective barrier around the seed against soil-borne pathogens, although infection and colonization by soil-borne inoculum can still occur through the roots and result in systemic infection via the xylem (10). Thus, a systemic fungicide active ingredient that translocate to under/above seedling tissues could significantly enhance early season disease management. Movement of a fungicide depends on the transpiration rate of the plant, water solubility of fungicide seed treatment, and plant stress (17). Systemic active ingredients belonging to the following chemical groups methyl

benzimidazole carbamates (MBC) also known as benzimidazoles, quinone outside inhibitors (Qol) (or strobilurins), and demethylation inhibitors (DMI) (triazoles) are available. Active ingredients in these groups are able to penetrate the seed coat of the maize seed and translocate in the xylem to the endosperm, embryo, coleoptiles, and radicle (65). Some systemic fungicides (prochloraz, thiabendazole) have eradicant properties and are able stop the progress of existing infections (8,36).

Most of the research done to test the effectiveness of various maize fungicide seed treatments has been done in the field. Effectiveness is determined from assessments of stand count, plant height (assessment of plant vigor) and yield, and rarely disease assessments. Few studies are supported with lab and growth chamber evaluations of disease development that can provide more useful information and a better understanding of fungicide seed treatment effectiveness (6,22,42,68).

Currently used maize fungicide seed treatment active ingredients belong to the phthalimide, benzimidazole, strobilurin, phenylpyrrole chemical groups. New active ingredients belonging to benzimidazole and triazole chemical groups are being tested and are expected to be registered by 2010.

Characteristics of chemical groups

Phthalimides. Phthalimides have a multi-site mode of action, and it is thought they inhibit the synthesis of amino compounds and enzymes containing the –SH radical (68). Captan, the oldest, most widely use fungicide seed treatment in the history of maize production belongs to this group (55). Captan also has major effects on the nucleic acids of animals, humans and plants and results in inhibition of DNA synthesis (55).

Benzimidazoles. This group of fungicides was introduced into the market in the 1960s when thiabendazole 2-(4-thiazolyl) molecule was discovered. Benzimidazoles are

protective and systemic fungicides with activity against most ascomycetes and basidiomycetes. They affect spore germination (17) by interfering with spindle formation during mitosis by binding to and inactivating the fungal protein tubulin, which is the building block of the microtubules (65). Apart from disease control, benzimidazoles seed treatments have been shown to improve stand, improve plant vigor, increase photosynthetic performance, and positively affect yield responses in cereal crops (17). This group of fungicides is highly systemic. Some experiments have shown that active ingredients applied as a seed treatment are absorbed by the maize seed coat and uptaken by the maize roots (10,17,24).

Strobilurins. This group of fungicides is a variant of a natural antifungal compound, strobilurin A, that was isolated from the basidiomycete *Strobilurus tenacellus* and *Oudemansiella mucida* (60). Azoxystrobin, pyraclostrobin and trifloxystrobin are examples of active ingredients in this chemical group and all provide broad spectrum control against basidiomycetes and ascomycetes when applied as seed treatments (36). Strobilurins inhibit the mitochondrial respiratory chain at site III (17), by interfering with the function of the cytochrome bc1 complex, located in the inner mitochondrial membrane of fungi and other eukaryotes (60). As a result, inhibition of energy production metabolism results in a reduction of adenosine triphosphate (ATP) which slows down fungus growth and colonization (65). Certain physiological changes, for example, delayed senescence, altered amounts of phytohormones, increased antioxidative enzyme activity, and increased nitrate reductase activity in plants treated with strobilurin fungicides have been reported (47).

Phenylpyrroles. These fungicides are made from the antifungal compound pyrrolnitrin which is produced by the bacteria *Pseudomonas pyrrocinia* (1,60). Chemical modifications of pyrrolnitrin resulted in more stable and highly active compounds and were

first introduced as foliar fungicides (22). The most commonly used fungicide of this group is fludioxonil, which had been used successfully as fungicide seed treatment in cereals because it is active at low rates against seed-borne *F. graminearum, F. culmorum, Tilletia caries* and *Septoria nodorum* and soil-borne *F. verticillioides, F. graminearum, F. subglutinans, F. proliferatum, Aspergillus* spp., and *Penicillium* spp. Fungicides in the phenylpyrroles group affect the fungal lipophilic amino acid phenylalanine (60). Thus fludioxonil inhibits electron transport in the oxidative respiratory chain of fungi which is associated with the phosphorylation of glucose (17). Toxicity tests done to different *Fusarium* spp. revealed that phenylpyrroles are effective inhibitors of the mycelia growth; however they are more inhibitive to *Rhizoctonia* spp. (28,60).

Triazoles. This group of fungicides is effective against plant diseases caused by ascomycetes and basidiomycetes (65). Triazoles inhibit ergosterol biosynthesis (synonym=sterol biosynthesis) by blocking the conversion of lanosterol to ergosterol (17). Ergosterol is a cellular compound that plays a crucial role in the structure and function of the membranes of many fungi. Triazoles are highly systemic fungicides that penetrate cuticles and have a broad and systemic fungicide action (3,5). Ipconazole and triticonazole have been tested as fungicide seed treatments in maize, wheat, barley and other small grains. Triticonazole is systemic in maize plants and can decrease DNA content in mesocotyl tissues thus protecting against *Fusarium* spp. and other soil-borne fungi infection (6).

Effects of *Fusarium* spp. on aspects of maize physiology

Reduced photosynthetic performance has been associated with plant stress (37). Impaired photosynthesis in hosts infected with *Fusarium* spp. has also been demonstrated (57,67). Numerous technologies are available to measure photosynthetic performance. Chlorophyll fluorescence (CF) was found to be a suitable indicator of plant stress in the *F.*

oxysporum-tomato pathosystem (67). Tomato plants infected with *F. oxysporum* had lower photosynthetic performance. Similarly, in maize plants infected with *F. moniliforme* (syn. *F. verticillioides*) photosynthetic performance was shown to be reduced through CF measurements (57). Presumably impaired photosynthesis occurred in hosts that were infected by *Fusarium* spp. because of root and mesocotyl tissues damage associated with infection by *Fusarium* spp. as well as systemic colonization of the host that interfered with the normal absorption of water and nutrients by the roots and mesocotyl.

Research objectives

The objectives of this study are to:

(i) Determine the incidence of seed-borne *Fusarium* species in commercial maize hybrid seed produced in the U.S. Corn Belt.

(ii) Study the effectiveness of fungicide active ingredients against colonization of maize seedlings by seed-borne *F. verticillioides*.

(iii) Improve our understanding of the effect of seed treatment fungicides on infection and colonization of maize seedlings by soil-borne *F. graminearum*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides*.

(iv) Assess the effect of fungicide seed treatments on maize seedling physiology using chlorophyll fluorescence measurements as an indication of photosynthetic performance.

(v) Evaluate relationships between chlorophyll fluorescence and seedling vigor, disease severity and incidence of *Fusarium* spp. infection of maize seedlings.

(vi) Assess the contribution of early seedling disease management with seed treatment fungicides to management of late season crown and stalk rot.

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CHAPTER 2. EFFECTIVENESS OF FUNGICIDE ACTIVE INGREDIENTS AGAINST SEED-BORNE *FUSARIUM VERTICILLIOIDES* IN MAIZE (*ZEA MAYS* L.)

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Abstract

Fusarium verticillioides is one causal organism of maize ear rots that result in economic losses due to contamination of grain with mycotoxins and yield reduction. Futhermore *Fusarium verticillioides* is seed-borne and infected seed may have reduced seed vigor and also contribute to field disease. A survey was conducted in the 2006 maize growing season from the U.S. Corn Belt to estimate the incidence of seed-borne *F. verticillioides* in commercial hybrid maize seed. A total of 52 samples of conditioned seed lots that had not been treated with a fungicide were tested. Approximately 86.5 % of sampled seed lots were infected with seed-borne fungi. Within all maize seed assessed, isolated seed-borne pathogens present included: *Acremonium* spp. (1.4%), *Aspergillus* spp. (2.8%), *Fusarium* spp. (4.3 %), *Nigrospora oryzae* (0.1%) and *Penicillium* spp. (10.1%). *Fusarium verticillioides* was the most prevalent species of *Fusarium*. A controlled environment study was conducted to assess the effectiveness of fungicide seed treatments against seed-borne *F. verticillioides*. Maize seed was artificially inoculated with transgenic *F. verticillioides* strain TXI-79, labeled with genes for green fluorescent protein expression and Hygromycin B resistance. Inoculated seed was treated with azoxystrobin, captan, fludioxonil, ipconazole, thiabendazole, trifloxystrobin, or triticonazole. Experiments were conducted in growth

chamber conditions (14^{o}C, 65 % relative humidity and 12 hr day/12 hr night photoperiod). Plant vigor (radicle and shoot length) and seed transmission of TXI-79 to radicle and shoot were assessed at 7, 14 and 21 days after planting (dap). Thiabendazole, captan, ipconazole and triticonazole significantly reduced (P=0.0306) recovery of seed-borne TXI-79 and decreased infection of shoot and radicle tissues by the fungus. Fludioxonil and azoxystrobin did not significantly affect (P<0.05) seed-borne inoculum and seed-to-seedling transmission of TXI-79. No radicle or shoot rot was observed at any assessment date. Maize seedlings inoculated with TXI-79 were more vigorous than non-inoculated treated control plants at 21 dap. All fungicide treatments resulted in shorter radicles at 21 dap. Shoot lengths also were reduced at 21 dap for all fungicides except ipconazole and triticonazole. This study showed *F. verticillioides* is the most prevalent *Fusarium* spp. and that some seed treatment fungicides can reduce seed-borne *F. verticillioides* seed-to-seedling transmission in maize.

Introduction

Fusarium verticillioides [(Saccardo) Nirenberg (teleomorph, *Gibberella moniliformis* Wineland)] is common in maize plants and kernels in most production fields (34). Maize root rot and mesocotyl rot are early season diseases (seedling blight) that occur shortly after germination and have been attributed to infection of maize seedlings by fungi including *F. verticillioides*, *F. graminearum*, *F. proliferatum*, and *F. subglutinans* (55). Seedling blights result in poor emergence, non-uniform growth and development, gaps within rows and consequently reduced yields (27). Later in the growing season, infections of the crown, stalk and ear tissue by *F. verticillioides* can result in stalk rots and *Fusarium* ear rots (55). Stalk rot reduces yields by causing premature senescence and predisposes plants to lodging that increases harvesting time and ear lost (17). Mycotoxin contamination is commonly associated with ear rots (39).

Sources of inoculum for *F. verticillioides* include seed, crop residue and soil (38). *Fusarium verticillioides* is endemic to maize fields (34). It survives as mycelium in the soil/crop residue and may remain infective for more than 630 days; although environmental fluctuations of temperature and moisture reduce survival of the mycelium (12). Infective hyphae penetrate the root epidermis of maize seedlings either directly or though ruptures made in the cortex by emerging roots, and then grow into the shoot (31). *Fusarium* spp. hyphae can also infect germinating seedlings through natural openings (43).

Seed inoculum of *Fusarium* spp. also is common. In maize seed from Nigeria, the incidence of *Fusarium* spp. ranged from 43 to 70% in symptomless maize seed supplies that had been stored for 10 to 15 months and from 36 to 78% in freshly harvested kernels of white maize cultivars (52). The mean incidence of *F. moniliforme* infection was 38.5% in maize samples from three regions of India (44). In the U.S. Corn Belt, *F. moniliforme* (syn. *F. verticillioides*), *F. subglutinans*, *F. graminearum*, and *F. proliferatum* were isolated from 46% asymptomatic kernels associated with ear rot (42).

There have been several studies that have evaluated the contribution of seed-borne inoculum of *F. verticillioides* to the initiation of seedling blight epidemics with conflicting results (20,29). Gillette (21) showed the incidence of seed-borne *Fusarium* spp. was negatively correlated with emergence and germination of maize seed. Seed-borne *F. subglutinans* resulted in significant seedling leaf blight, stunting, and crown damage in maize (28).

Incidence of seed-borne *F. verticillioides* in commercial conditioned untreated seed lots from different regions of Brazil ranged from 8% to 57% (22). The incidence of *Fusarium* spp. in commercial maize seed in the U.S. Corn Belt has not been evaluated.

Fungicide seed treatments protect the germinating seed and seedling from infection by soil-borne pathogenic fungi (26), not much is known about their effects on seed-borne *F. verticillioides*. Active ingredients commonly used as seed treatments belong to several fungicide groups including the benzimidazoles, phthalimides, phenylpyrroles, strobilurins, and triazoles (8,35). Some fungicides (benzimidazoles, strobilurins, and triazoles) have systemic activity and are able to penetrate the seed coat and translocate to the endosperm, embryo, and/or be uptaken by the seedling's radicle and translocated to the growing points (19,35,53). Strobilurins have recently been registered for use on maize as a seed treatment (6), while active ingredients belonging to the benzimidazole and triazole groups are currently being evaluated on maize (11,54) and are expected to be registered in the foreseeable future. The effect of these active ingredients and those that have been registered on maize for many years, on seed-borne *Fusarium* spp. is not well understood, however laboratory studies on the effectiveness against soil-borne *Fusarium* spp. have been made (40).

The objectives of this study were to (i) determine the incidence of seed-borne *Fusarium* spp. in commercial maize hybrid seed produced in the U.S. Corn Belt, and (ii) to assess the effectiveness of commercial and experimental fungicide active ingredients against seed transmission of seed-borne *F. verticillioides*.

Materials and methods

Incidence of seed-borne *Fusarium* spp. in commercial hybrid seed

Fifty two samples of commercial hybrid maize seed produced in the U.S. Corn Belt in 2006 were provided by maize seed companies. Samples had been conditioned by each seed company but were not treated with either fungicide or insecticide. Incidence of seed-borne *Fusarium* spp. was assessed using the culture plate agar test (1,51). Briefly, one sub sample of 100 seeds from each seed lot was surface disinfested with a 10% bleach solution for 2

minutes, rinsed with sterile water and then blotted dry in sterile paper towels. All 100 surface sterilized seeds were plated onto potato dextrose agar (Difco®, 39 g/l) medium supplemented with pentachloronitrobenzene (Sigma®, 0.2 g/l), streptomycin sulfate solution (Sigma®, 40 mg/ml) and neomycin sulfate solution (Sigma®, 33 mg/ml) in 245mm x 245mm x 18mm Petri dishes (Fisherbrand®, Biodish XL) and incubated at 25°C, 12 hr day/ 12 hr night for one week (Appendix G).

Seed lot samples were assessed every 2 days for fungal growth. *Fusarium*-like mycelium was transferred to carnation leaf agar medium in 60 mm x 15 mm Petri dishes (Fisherbrand® Mediamiser) for identification based on morphological characters (5,24,33) (Appendix H). Other fungal growth was identified morphologically to genus (5). All identified *Fusarium* spp. isolates were purified and stored in silica gel (47).

The number of seed lots which had seed infected with seed-borne fungi, was determined as a percentage of the 52 seed lots assessed [# lots with at least one infected seed / 52 x 100)]. The prevalence of each fungal genus isolated was calculated as the percentage of seed lots from which that genus was isolated [#lots infected with a particular genus / 52 lots x 100)]. Incidence of each seed-borne fungal genus within a seed lot was calculated as the percentage of seeds infected with that genus. The mean incidence of seed infected with each fungal genus was calculated as a percentage of the number of seeds infected with a genus divided by the total number of seeds assessed [# seed infected with a specific genus / 5200 x 100)]. The incidence of seed-borne infection for each species of *Fusarium* isolated also was determined as a percentage of total number of seed infected with *Fusarium* spp. [# seeds infected with a particular species of *Fusarium* / total # seed infected with *Fusarium* spp. x 100)].

Fungicide efficacy

The effect of fungicide seed treatment fungicides on seed-borne *F. verticillioides*, seed-to-seedling transmission, and seedling vigor were assessed in growth chamber conditions until 21 days after planting (dap). Maize seed (Syngenta N67-W7) was disinfested by placing in water at 60^{o}C for 5 minutes (14). A modified agar seed health test (50) was done to determine the incidence of seed-borne *Fusarium* spp. in the seed lot after seed disinfestation. After cooling and drying, heat-treated seed was surfaced sterilized in 10% bleach solution for 2 minutes, followed by a 2 minutes rinse in sterile water, and finally blotted dry in sterile paper towels. *Fusarium verticillioides* strain TXI-79 (Pennsylvania State University code M-8114), transformed with genes for green fluorescent protein (GFP) and Hygromycin B resistance was used to inoculate maize seed (57). The protocol outline by Wilke et al. (57) was used to inoculate maize seed with TXI-79. Briefly, TXI-79 was grown on Nash Snyder medium (45) amended with 50 mg/ml of Hygromycin B (NSH) for 8 to 12 days, 12 hrs light/12 hrs dark, at 25^{o}C. A conidial spore suspension of 1x10^{8} CFU/ml was prepared with sterile distilled water. Dried, surface sterile maize seed was inoculated by placing 80 seeds inside a 125 ml sterile Erlenmeyer flask containing 50 ml of TXI-79 conidial spore suspension. Erlenmeyer flasks were sealed with aluminum foil and placed onto a rotary shaker (Lab-line®, instruments, INC, Junior Shaker) set at 110 rpm for 12 hours, after which inoculated maize seeds were removed, blotted dry onto sterile paper towels and then allowed to dry inside a fume hood for 5 days.

The success of seed inoculation with TXI-79 was assessed by sampling 10 inoculated maize seeds. Inoculated maize seeds were surface disinfested (as outlined above) and placed onto 95 mm x 15 mm Petri dishes (Fisherbrand®, Mediamiser) of NSH. To quantify average seed-borne inocula of TXI-79, a second sample of 50 g seed was ground with a cyclone

sample mill (UDY®, 3010-014). One gram of ground seed was suspended in 10 ml of sterile distilled water and well mixed. A 10 fold dilution series was done and 100 µl of each dilution was plated on NSH. In addition, seed moisture content was measured with a Dole® 400 moisture tester.

A total of seven fungicide active ingredients in sterile water-based slurries were evaluated (Table 2.1). Untreated TXI-79-inoculated seed and untreated, non-inoculated seed served as controls. Each fungicide seed treatment was applied as a water based slurry to a 50 g lot of maize seed (6 g slurry / kg seed) inoculated with TXI-79. Each fungicide seed treatment slurry was made following the manufacturer's recommended maize rate. The amount of fungicide needed to treat 10 kg of seed was weight with an analytical scale (Ohaus®, Analytical plus). Red seed colorant (Becker Underwood ®, 0.22 ml/1 kg seed) was added to each fungicide seed treatment slurry to monitor seed coverage. Each fungicide slurry was then filled with distilled water for a total of 60 g of slurry. For each treatment, the fungicide slurry and 50 g seed sample to be treated were weighed using an analytical scale (Ohaus®, Analytical plus), poured into a 125 ml Erlenmeyer flask that was sealed with a rubber cork, and hand shaken for 5 minutes until the fungicide seed treatment was evenly distributed on the maize seed coat. Treated maize seeds were stored at 4^{o} C.

Treated maize seed was planted in PVC cones (20 mm wide x 155 mm long) filled with sterile 50:50 silica sand-soil mixture. One week before planting, PVC cones were filled with 14 cm of sand-soil mixture, watered and placed inside a growth chamber (Percival®) set at 14^{o}C, 65 % relative humidity and 12 hr day/12 hr night photoperiod. One maize seed of each treatment was planted per cone; all seeds were laid at the same position and then each seed was covered with 5 cm of silica sand. The plants were kept inside growth chamber over the course of the experiment and watered once per week. Each cone was watered with

approximately 25 ml of sterile distilled water. Care was taken each time the experiment was watered to prevent cone overflow.

Maize seeds and seedlings were assessed at 7, 14, and 21 days after planting (dap). At each assessment date, twelve seeds and/or seedlings of each treatment were carefully removed from the PVC cones and washed with sterile water to remove soil and sand from the radicle. After washing, primary radicle and shoot lengths (mm) were measured with an electronic digital caliper (Aisle®), then the maize seedlings were surface sterilized for 2 minutes in 10% bleach solution, rinsed for 2 minutes in sterile water and blotted dry in sterile paper towels.

Surface sterilized maize seedlings from each treatment were dissected and pieces of tissue placed onto 95mm X 15mm (Fisherbrand®, Mediamiser) Petri dish with NSH. At 7 dap maize seeds were cut transversally with a sterile scalpel (Feather®, surgical blade N°22) and placed in the center of a Petri dish. The radicle and shoot from the same seedling also were dissected into segments, and placed on the same Petri dish. At 14 and 21 dap, only the radicle and shoot tissues were cultured. At each assessment date, plates were sealed with parafilm (Pechiney®, plastic packaging) and place on a 12h light / 12h dark bench at 25°C. The incidence of TXI-79 growth in seed, radicle and shoot tissues was estimated as a percentage of the number of infected dissections of each tissue divided by total of dissections made from the same tissue [(# infected dissections/#total dissections per plant tissue X 100)] for each treatment and assessment date. Samples of mycelium were placed on slides, and observed with a Leitz ® Fluovert inverted fluorescence microscope to visualize GFP expression and thus further confirm TXI-79-infection of the maize tissues.

The experiment was laid out as a randomized complete block design within the growth chamber with each 12 block (replications) containing level combinations of each

treatment and assessment date (7, 14, 21 dap) in a 7 X 3 factorial structure. Analysis of variance (ANOVA) was conducted on data generated from variables measured in each growth chamber experiment. Variables consisted of incidence of TXI-79 in seeds (7 dap only), root, and mesocotyl tissues; and radicle and shoots lengths at 7, 14 and 21 dap. The experiment was replicated twice under the same conditions. To assess treatment effects over the combined experimental replicates, an analysis of variance was conducted on the variable means measured by treatment, sampling date and experimental run (7 X 3 factorial analysis with experimental repeat as a block factor). Tukey's honest significant different (HSD) for multiple comparisons (49) was used to determine if the means from each treatment differed within each sampling date. Contrasts were made to compare the treatment effect of different fungicide groups on measured variables. Data analysis was conducted using the General Linear Model procedure (PROC GLM) of SAS version 9.1 (SAS Institute, 2002).

Results

Incidence of seed-borne *Fusarium* spp. in commercial hybrid seed lots.

Of the 52 seed lots assessed, 45 (86.5%) had some seed-borne fungal infection. The most prevalent genus in infected seed lots was *Fusarium* (86.5%) followed by *Penicillium* (80.8%) and *Aspergillus* (69.2%) (Table 2.2). The mean incidence of infections by *Fusarium* spp. was 4.3% (range 1-53%) (Table 2.2). Of the *Fusarium* spp., *F. verticillioides* was found in 50% of sampled seed lots followed by *F. graminearum* (32.7%), *F. proliferatum* (28.8%) and *F. subglutinans* (28.8%) (Table 2.3). Although *F. subglutinans* was not found as frequently as *F. verticillioides*, this species had the highest incidence of infection (35.1 %) within assessed seed lots (Table 2.3). It was common to find all four (or more than one) species of *Fusarium* in a single seed lot.

Fungicide efficacy

No seed-borne *Fusarium* spp. was detected after the heat treatment was applied to the maize seed lot and seed germination, which was 100%, was unaffected. Inoculation of maize seed with TXI-79 was successful and 100% seed-borne infection was achieved. The inoculum concentration of TXI-79 was estimated ≈ 6.4 X 10^3 CFU per gram of maize seed. Maize seed moisture content when fungicide seed treatments were applied was 11.6%. In all assessments, mycelium recovered from the tissue dissections placed on NSH medium fluoresced green and therefore was confirmation of infection by strain TXI-79.

Fungicide active ingredients captan, ipconazole, thiabendazole and triticonazole all significantly (P=0.0306) reduced the incidence of seed-borne TXI-79 in experiments 1 and 2 by 20 and 75%, 90 and 100%, 92 and 100%, and 92 and 91%, respectively (Table 2.6). The benzimidazoles (thiabendazole) and triazoles (ipconazole and triticonazole) tested were significantly (P=0.0088) more effective than captan and completely suppressed seed-borne inoculum (Table 2.5.) The active ingredients fludioxonil, azoxystrobin and trifloxystrobin did not have a significant (P>0.05) effect against seed-borne TXI-79 at the applied rates.

No symptoms of radicle rot, malformations or phytotoxicity were observed in any treatment. The incidence of TXI-79 in the radicle tissue of maize seedlings grown from inoculated, untreated seed at 7 dap was 75.0% and 91.7% in experiment 1 and 2, respectively and increased to 91.7% and 100% in each experiment by 21 dap, respectively (Table 2.7). Significant treatment effects (P<0.0001), non significant day effect (P=0.1651) and significant treatment by day interaction (P=0.0083) were recorded for radicle infection (Table 2.4). No TXI-79 was ever detected in the radicle of maize seedlings grown from seed treated with thiabendazole (Table 2.7). Fludioxonil had no significant effect (P>0.05) on TXI-79 colonization, and azoxystrobin only reduced the incidence of TXI-79 in radicle tissue at the

first two assessment dates in Experiment 1. All other active ingredients reduced the incidence of TXI-79 in radicle tissues. In general, captan, ipconazole and triticonazole were equally effective at reducing the incidence of TXI-79 in the radicle and were also more effective than trifloxystrobin.

No symptoms of shoot rot, malformations or phytotoxicity were observed in any of the treatments in both experiments. The incidence of TXI-79 in the shoot tissue of maize seedlings grown from inoculated, untreated seed was 100% at each assessment date in both experiments (Table 2.8). Significant treatment ($P<0.0001$), day ($P=0.0001$), and treatment by day interaction ($P=0.0034$) were recorded for this variable (Table 2.4). No TXI-79 was detected in the shoot of seedlings grown from seed treated with thiabendazole. A reduction in the colonization of shoot tissues in seedlings grown from seed treated with a strobilurin (azoxystrobin and trifloxystrobin) was detected in Experiment 1, although colonization of the mesocotyl tissue by TXI-79 increased at each assessment date. In experiment 2, neither strobilurin reduced colonization of shoots tissues by TXI-79. Fludioxonil was not effective at reducing TXI-79 colonization of the shoot (Table 2.8) in both experiments. All other seed treatments (captan, ipconazole and triticonazole) reduced ($P<0.0001$) TXI-79 colonization of the shoot tissue in each experiment and there was no difference in efficacy between the active ingredients (Table 2.5). Colonization of the shoot of seedlings treated with triazoles (ipconazole and triticonazole) decreased at each assessment date in both experiments.

Radicle length was significantly affected by treatment ($P<0.0001$), day ($P<0.0001$) and treatment by day interaction ($P<0.0001$) (Table 2.4). In general, maize seedlings grown from fungicide treated seed had shorter radicle lengths ($P=0.0109$) in both experiments compared with maize seedlings grown from TXI-79-inoculated, untreated seed (Table 2.9). An overall decrease in radicle length was observed in the second experiment.

Analysis of variance showed significant day ($P<0.0001$), treatment ($P<0.0005$) and treatment by day interaction effects on shoot length ($P<0.0164$) (Table 2.4). The mean length of the shoot was similar across all treatments at 7 and 14 dap in both experiments, however a significant ($P=0.0028$) increase in shoot growth was recorded at 21 dap in both experiments (Table 2.10). At this assessment date, the shoots of seedlings grown from seed treated with either ipconazole or triticonazole were longer than those of seedlings grown from other fungicide treatments but not different ($P=0.9230$) from seedlings grown from TXI-79 inoculated, untreated seed (Table 2.5).

Discussion

In our survey the incidence of seed-borne *Fusarium* spp. in commercial seed that had been conditioned but not treated with a fungicide was lower than that reported in other studies (44,52). However since previous studies did not use conditioned commercial hybrid seed, we suspect that incidence of seed-borne *Fusarium* spp. in unconditioned maize samples in U.S Corn Belt would be equal or greater to what we have reported.

We found that *Fusarium* spp. were the most common seed-borne fungi in maize seed lots from the U.S. Corn Belt, and that *F. verticillioides* was the most frequently isolated species. Previous studies have shown that *F. verticillioides* is the most frequently isolated pathogen in maize plants (34), and that it is common in seed-borne inoculum (13). We also found a high incidence of *F. subglutinans*, which suggests that environmental conditions in the growing regions of the U.S. Corn Belt where these seeds were produced favor infection by *F. subglutinans*. Munkvold and Stahr (42) also reported a predominance of *F. subglutinans* in kernels harvested from the field in Iowa. The transmission rate of *F. subglutinans* from seed-to-seedling had been determined between 75 to 100%, thus the prevalence of this seed-borne species is of as much concern as *F. verticillioides* since field

epidemics of seedling blights could occur when maize genotype and environmental conditions are favorable (28).

Other seed-borne fungal species also recovered from seed samples assessed in our study, included species that are commonly isolated from maize seed, namely *Acremonium* spp., *Aspergillus* spp., *Nigrospora oryzae*, and *Penicillium* spp. (58). Some studies have shown the latter three genera also contribute to maize seedling disease (23), while *Acremonium* spp. had been reported as antagonistic to kernel rotting and mycotoxin producing fungi like *A. flavus* and *F. verticillioides* (56).

Seed-to-seedling transmission of *Penicillium* spp. and field epidemics of seedling blight have been reported when the incidence of *Penicillium* spp. in seed planted was between 51 to 82% (23). Seedling blights caused by *Penicillium oxalicum* seed-borne inoculum are favored when growing condition temperature ranged from 15 ^{o}C to 20^{o}C (23). Since temperatures at planting in the U.S. Corn Belt can range between 10 ^{o}C to 15^{o}C, the risk of a field epidemic due to *Penicillium* spp. is high in some of the seed lots we tested.

The inoculation protocol used in our studies likely resulted in the seed coat and endosperm of maize seed being infested with TXI-79 (1). Colonization of maize seedlings from seed-borne TXI-79 inoculum was achieved, and although no foliar and underground symptoms of disease were observed in any of the experiments, others have reported asymptomatic colonization of maize by *F. verticillioides* in both laboratory and field studies (3,41,44,61). The low temperature used in the growth chamber studies simulated temperature at planting in the U.S. Corn Belt. Previous studies reported seed transmission of *F. verticillioides* occurred under a wide range of temperatures including 14^{o}C (57).

We found systemic colonization of maize with TXI-79 improved plant vigor, as measured by radicle and shoot length, which is similar to what Yates et al. (60) reported.

Fusarium moniliforme (syn. *F. verticillioides*) is capable of synthesizing plant hormones like abscisic and gibberellic acids that can cause different responses in plants (4,16,37). In maize, abscisic acid (ABA) had shown to increase tolerance to anoxia (25), and enhance embryo formation and germination (7). Gibberellic acid promotes germination, internodes and root elongation (2,59). However, despite these improved growth benefits, the current concern of mycotoxin contamination of foods means that *F. verticillioides* infection and colonization of maize is not considered beneficial.

We also report increased radicle and shoot growth and therefore enhanced plant vigor in triazole treatments. Triazoles have been shown to positively affect germination (10,18), and increase photosynthesis (30).

Captan is a multi-site preventive fungicide, that is non systemic and remains on the surface of the seed (32). This fungicide was first used as a seed treatment in 1953 and has been successfully used for the past 50 years (46). Seed companies have moved away from using captan for two main reasons; (i) large quantities of active ingredient were necessary to treat seed (71.1 g a.i. captan/100 kg seed vs. 2.5 g a.i. fludioxonil/100 kg seed); and (ii) the introduction of new chemical groups such as phenylpyrroles and strobilurins (36). Captan at a common commercial rate was more effective at suppressing seed-borne *F. verticillioides* than were azoxystrobin, fludioxonil, and trifloxystrobin at rates applied. However, since the captan seed treatment rate was higher in comparison with application to those of the other active ingredients, only a single isolate was used and no direct comparison can be made.

Thiabendazole, triticonazole and ipconazole were very effective at suppressing seed-borne inoculum of *F. verticillioides* TXI-79, possibly because these active ingredients are systemic and can penetrate the seed coat. Triticonazole and ipconazole are specifically used in the control of seed-borne diseases of wheat, small grains, and maize since they are able to

penetrate the seed during germination, as well as penetrate functional roots by mass flow absorption and translocate to other seedling tissues including the mesocotyl, and leaves (48). Unfortunately, no models have been published that explain seed treatment uptake of the other active ingredients we tested, but we hypothesize that the same mechanism is valid for thiabendazole since this active ingredient suppress mycelial growth of TXI-79 in the seed and consequently no radicle and shoot colonization occurred in plants from thiabendazole-treated seeds (15).

Thiabendazole, ipconazole and triticonazole fungicides are not currently labeled for use as seed treatments in maize in the US Corn Belt. This research has demonstrated that these active ingredients have excellent potential as complementary/alternative fungicide seed treatments to decrease the introduction of seed-borne *F. verticillioides* in maize fields. Moreover, *F. graminearum* isolates with reduced sensitivity to fludioxonil have been identified in field maize production areas from Ohio (9), and thus the maize industry requires new fungicide active ingredients for seed treatments to ensure effective seedling blight disease management.

This investigation has increased our knowledge about the effect of fungicide seed treatments on the interaction of seed-borne *F. verticillioides* with its host, maize. Fungicide seed treatments should be effective against seed-borne *F. verticillioides* since it is frequently isolated from maize seed, and epidemic risks are associated with this fungus (28).

We used artificially inoculated seed and controlled environmental conditions, to elucidate the effect of various fungicide active ingredients against seed-borne *F. verticillioides*. We have no reason to suspect that the effects of these active ingredients on naturally occurring seed-borne inoculum would differ. However, other *Fusarium* spp. are present in commercial seed, the effectiveness of these seed treatment active ingredients

against seed-borne *F. graminearum*, *F. proliferatum*, *F. subglutinans* should be determined. Finally, since maize is exposed to greater amounts of soil-borne inoculum throughout the growing season, via several infection pathways, it would be worthwhile to evaluate the effect of these fungicides against soil-borne *Fusarium* spp. and so further enhance our understanding of the effect of seed treatments on the maize-*Fusarium* spp. interaction.

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Table 2.1. Fungicide seed treatments used in controlled environment experiments.

Fungicide	Chemical group (activity)	Active ingredient (%)	Chemical name	Rate[z]
A10466C	benzimidazole (systemic)	thiabendazole (42.3%)	2-(thiazol-4-yl) benzimidazole	20.0
Captan® 400C	phthlamide (contact)	captan (37.4%)	N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide	71.1
Charter ® 25FS	triazole (systemic)	triticonazole (2.5%)	(+-)-(E)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	10.0
Dynasty®	strobilurin (systemic)	azoxystrobin (9.6%)	Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	1.0
Maxim® 4FS	phenylpyrrole (contact)	fludioxonil (40.3%)	4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile	2.5
Trilex® FL	strobilurin (translaminar)	trifloxystrobin (22%)	methyl (E)-methoxyimino-acetate	10.0
Vortex ®	triazole (systemic)	ipconazole (40.7%)	(1RS,2RS,5RS;1RS,2RS,5RS)-2-(4chlorobenzyl)-5-isopropyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	2.5

[z] g a.i./100 kg seed.

Table 2.2. Incidence (%) and prevalence (%) of seed-borne *Acremonium* spp., *Aspergillus* spp., *Fusarium* spp., *Nigrospora oryzae and Penicillium* spp. from 52 commercial maize hybrid seed samples collected from 2006 growing season. Maize seed samples were seed conditioned by seed companies but no pesticide seed treatments were yet applied.

Seed-borne fungi	Mean Incidence [z]	Minimum incidence[y]	Maximum incidence[y]	Prevalence[x] (standard error)
Acremonium spp.	1.4	1	44	30.8 (2.6)
Aspergillus spp.	2.8	1	25	69.2 (2.4)
Fusarium spp.	4.3	1	53	86.5 (3.4)
Nigrospora spp.	0.1	1	4	5.8 (1.0)
Penicillium spp.	10.1	1	84	80.8 (2.4)

[z] Mean incidence = percentage of infected seeds infected with each genus divided by the total number of seeds assessed from all seed lots (n=5200).
[y] Incidence = percentage of seeds infected with each genus within one seed lot (n=100).
[x] Prevalence = percentage of maize seed lots from which each genus was isolated (n=52 seed lots).

Table 2.3. *Fusarium* spp. composition isolated from 52 commercial maize hybrid seed samples collected from 2006 growing season. Maize seed samples were seed conditioned by seed companies but no pesticide seed treatments were yet applied.

***Fusarium* spp.**	**Mean Incidence[z]**	**Minimum incidence[y]**	**Maximum incidence[y]**	**Infected seed lots[x] (standard error)**
F. graminearum	11.7	1	5	32.7 (0.3)
F. proliferatum	19.4	1	12	28.8 (0.7)
F. subglutinans	35.1	1	53	28.8 (3.2)
F. verticillioides	33.8	1	25	50.0 (1.0)

[z] Mean incidence = percentage of seeds infected with a *Fusarium* spp. divided by the total number of *Fusarium* spp.-infected seeds (n=222).
[y] Incidence = percentage of seeds infected with each genus within one seed lot (n=100).
[x] Prevalence = percentage of seed lots from which each *Fusarium* spp. was isolated (n=52 seed lots).

Table 2.4. P-values of seed treatments effects on incidence of *F. verticillioides* strain TXI-79 in seed, radicle, and shoot of maize seedlings, and radicle and shoot length. The ANOVA of the sample means of treatment by date combinations (Tables 2.6-2.10) considering experiments as block factors (experimental replication).

	P-value				
	Incidence in seed	**Incidence radicle**	**Incidence shoot**	**Radicle length**	**Shoot length**
experiment (exp)	0.0240	0.6752	<0.0001	<0.0001	0.0004
treatment (trt)	<0.0001	<0.0001	<0.0001	<0.0001	0.0005
date (d)	No	0.1651	0.0001	<0.0001	<0.0001
trt x d	No	0.0083	0.0034	<0.0001	0.0164

Table 2.5. P-values of orthogonal contrasts for comparisons of fungicide seed treatment groups on incidence of *F. verticillioides* strain TXI-79 in seed, radicle, and shoot of maize seedlings, and radicle and shoot length.

	P-value				
	Incidence seed	**Incidence radicle**	**Incidence shoot**	**Radicle length**	**Shoot length**
un[z], inoc[y] vs. trt[x], inoc	<0.0001	0.0068	<0.0001	<0.0001	0.0016
un, inoc vs. (tbz[w], cap[v], ipc[u], trit[t])	0.0306	<0.0001	<0.0001	0.0575	0.0098
cap, inoc vs. (tbz, ipc, trit)	0.0088	0.0323	0.2546	0.4985	0.1424
un, inoc vs. (cap, ipc, trit)	<0.0001	0.0042	<0.0001	0.0416	0.0143
un, inoc vs. triazoles	<0.0001	<0.0001	<0.0001	<0.0001	0.9230
un, inoc vs. benzimidazoles	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
un, inoc vs. strobilurins	0.6299	0.6201	0.3928	<0.0001	0.0005
un, inoc vs. phenylpyrroles	0.8096	0.2458	0.6530	<0.0001	<0.0001

[z] untreated
[y] inoculated
[y] treated
[w] thiabendazole
[v] captan
[u] ipconazole
[t] triticonazole

Table 2.6. Recovery (%) from maize seeds artificially inoculated with *F. verticillioides* strain TXI-79 and treated with azoxystrobin, captan, fludioxonil, ipconazole, thiabendazole, trifloxystrobin, triticonazole or left untreated and planted in a growth chamber at 14^{o}C, 12 hr daylight cycle. Incidence was determined at 7 days after planting (dap) by plating 12 seeds per treatment on Nash Snyder medium amended with Hygromycin B (n=108 seeds).

Treatment	Experiment 1	Experiment 2
azoxystrobin	100 a[z]	83 a
captan	80 b	25 b
fludioxonil	92 a	92 a
ipconazole	10 c	0 c
thiabendazole	8 c	0 c
trifloxystrobin	92 a	100 a
triticonazole	8 c	9 c
untreated inoculated	100 a	92 a
untreated non-inoculated	0 c	0 c
HSD (P<0.05)	6	27

[z] Numbers from the same column followed with the same letter are not significantly different according to the Tukey's HSD test (P<0.05).

Table 2.7. Incidence (%) of *F. verticillioides* strain TXI-79 in radicle tissue of maize seedlings. Seedlings were grown from seed artificially inoculated with TXI-79 and treated with azoxystrobin, captan, fludioxonil, ipconazole, thiabendazole, trifloxystrobin, triticonazole or left untreated and planted in a growth chamber at 4°C, 12hr daylight cycle. To determine incidence, 12 radicles per treatment were dissected and assessed at each assessment date 7, 14 and 21 days after planting (dap) (n=324 radicles).

Treatment	**Experiment 1**			**Experiment 2**		
	7 dap	**14 dap**	**21 dap**	**7 dap**	**14 dap**	**21 dap**
azoxystrobin	33.0 d[z]	50.0 d	100.0a	83.3 ab	100.0 a	100.0 a
captan	66.7 b	60.2 cd	61.0 b	25.0 d	25.0 c	25.0 c
fludioxonil	71.7 ab	77.1 ab	100.0a	91.7 a	100.0 a	100.0 a
ipconazole	83.3 a	70.0 bc	33.3 c	90.0 a	37.5 c	10.0 d
thiabendazole	0.0 e	0.0 f	0.0 e	0.0 e	0.0 d	0.0 d
trifloxystrobin	25.0 d	66.7 bc	60.0 b	77.0 b	66.7 b	85.7 b
triticonazole	50.0 c	25.0 e	16.4 d	50.0 c	30.0 c	25.0 c
untreated, inoculated	75.0 ab	88.9 a	91.7 a	91.7 a	100.0 a	100.0 a
untreated, non-inoculated	0.0 e	0.0 f	0.0 e	0.0 e	0.0 d	0.0 d
HSD (P<0.05)	11.6	11.8	8.3	8.4	28.0	13.0

[z] Numbers from the same column followed with the same letter are not significantly different according to the Tukey's HSD test (P<0.05).

Table 2.8. Incidence (%) of *F. verticillioides* strain TXI-79 in shoot tissue of maize seedlings. Seedlings were grown from seed artificially inoculated with TXI-79 and treated with azoxystrobin, captan, fludioxonil, ipconazole, thiabendazole, trifloxystrobin, triticonazole or left untreated and planted in a growth chamber at 14°C, 12 hr daylight cycle. To determine incidence, 12 shoots per treatment were dissected and assessed at each assessment date, 7, 14 and 21 days after planting (dap) (n=324 shoots).

Treatment	Experiment 1			Experiment 2		
	7 dap	14 dap	21 dap	7 dap	14 dap	21 dap
azoxystrobin	50.0 c[z]	83.3 b	91.7 a	100.0 a	100.0 a	100.0 a
captan	66.7 b	25.0 e	75.0 b	75.0 b	66.0 b	75.0 b
fludioxonil	83.3 a	91.7 a	91.7 a	100.0 a	100.0 a	100.0 a
ipconazole	66.7 b	50.0 c	33.3 d	100.0 a	75.0 b	66.0 b
thiabendazole	0.0 e	0.0 f	0.0 e	0.0 c	0.0 c	0.0 c
trifloxystrobin	33.3 d	41.6 d	58.3 c	100.0 a	100.0 a	100.0 a
triticonazole	50.0 c	41.6 d	25.0 d	83.0 b	75.0 b	66.0 b
untreated, inoculated	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
untreated, non-inoculated	0.0 e	0.0 f	0.0 e	0.0 c	0.0 c	0.0 c
HSD (P<0.05)	16.7	8.3	8.3	24.0	19.0	21.0

[z] Numbers from the same column followed with the same letter are not significantly different according to the Tukey's HSD test (P<0.05).

Table 2.9. Mean length (mm) of primary roots of maize seedlings grown from seed inoculated with *F. verticillioides* strain TXI-79 and treated with azoxystrobin, captan, fludioxonil, ipconazole, thiabendazole, trifloxystrobin, triticonazole or left untreated and planted in a growth chamber at 14°C, 12 hr daylight cycle. Means are based on 12 plants per treatment and radicle length was assessed at 7, 14 and 21 days after planting (dap) (n=324 radicles).

Treatment	**Experiment 1**			**Experiment 2**		
	7 dap	**14 dap**	**21 dap**	**7 dap**	**14 dap**	**21 dap**
azoxystrobin	7.82 de[z]	25.60 b	80.61 c	5.06 c	18.22 c	29.50 cb
captan	12.77 bc	44.81 a	58.93 de	7.2 b	29.96 a	27.53 d
fludioxonil	5.58 e	53.17 a	66.28 d	7.28 b	30.25 a	53.96 b
ipconazole	10.19 cd	40.77 a	47.90 e	6.34 bc	28.67 b	36.23 c
thiabendazole	10.66 cd	40.08 a	83.91 bc	8.03 b	20.35 c	37.74 c
trifloxystrobin	10.57 cd	37.85 b	53.61 e	5.39 c	19.82 c	23.24 d
triticonazole	7.37 de	41.21 a	78.70 c	4.35 c	18.87 c	35.36 c
untreated, inoculated	16.57 a	56.85 a	102.07 a	15.27 a	36.07 a	62.40 a
untreated, non-inoculated	7.29 de	47.47 a	91.42 ab	7.07 bc	28.53 a	55.16 ab
HSD (P<0.05)	3.90	16.70	10.65	1.69	6.11	7.24

[z] Numbers from the same column followed with the same letter are not significantly different according to the Tukey's HSD test (P<0.05).

Table 2.10. Mean length (mm) of shoot of maize seedlings grown from seed inoculated with *F. verticillioides* strain TXI-79 and treated with azoxystrobin, captan, fludioxonil, ipconazole, thiabendazole, trifloxystrobin, triticonazole or left untreated and planted in a growth chamber at 14°C, 12 hr daylight cycle. Means are based on 12 plants per treatment and shoot length was assessed at 7, 14 and 21 days after planting (dap) (n=324 shoots).

Treatment	**Experiment 1**			**Experiment 2**		
	7 dap	**14 dap**	**21 dap**	**7 dap**	**14 dap**	**21 dap**
azoxystrobin	7.83 a[z]	19.91 a	24.62 cd	5.05 a	12.73 a	20.66 c
captan	6.11 a	21.81 a	23.56 de	5.27 a	16.53 a	20.07 c
fludioxonil	6.73 a	19.14 a	24.22 cde	5.06 a	16.07 a	24.03 b
ipconazole	6.79 a	18.83 a	35.59 a	5.82 a	15.61 a	32.26 a
thiabendazole	6.08 a	22.11 a	28.17 b	6.29 a	14.70 a	21.12 c
trifloxystrobin	7.45 a	19.06 a	21.04 e	7.96 a	11.17 a	21 22 c
triticonazole	5.44 a	17.39 a	39.10 a	4.44 a	17.96 a	33.88 a
untreated, inoculated	6.45 a	18.13 a	37. 89 a	6.67 a	16.41 a	34.61 a
untreated, non-inoculated	4.30 a	15.02 a	27.87 bc	7.51 a	16.20 a	20.40 c
HSD (P<0.05)	5.80	10.30	3.51	7.80	6.90	2.51

[z] Numbers from the same column followed with the same letter are not significantly different according to the Tukey's HSD test (P<0.05).

CHAPTER 3. COLONIZATION OF MAIZE (*ZEA MAYS* L.) SEEDLINGS BY *FUSARIUM* SPP. IN THE FIELD AND ITS SUPPRESION BY SEED TREATMENTS

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Abstract

Soil-borne *Fusarium* spp. are responsible for economic losses (in all maize production areas around the world) due to reduced plant stand, vigor, and grain yield as well as mycotoxin contamination. The effect of fungicide seed treatments on *Fusarium* spp. infection of maize was investigated at two locations where maize had been planted for the last two growing seasons in Iowa, in 2007. Maize seed was treated with either A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam) , or a premix of Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl). Destructive sampling was done at three growth stages, V2, V4 and V6, to assess colonization of roots, mesocotyl and crown tissues of maize by *Fusarium* spp. At V2, fewer plants grown from fungicide treated seed were infected with *Fusarium* spp. at both locations. At V4 and V6, all plants were colonized with *Fusarium* spp. at both locations. The predominant *Fusarium* spp. changed with time: at growth stage V2, *F. graminearum* was predominant but *F. subglutinans* and *F. verticillioides* incidence increased as the growing season progressed. The distribution of species among plant tissues also varied; *F. graminearum*, *F. oxysporum*, and *F. solani* were predominantly isolated from roots and mesocotyl tissues, while *F. proliferatum, F. verticillioides* and *F. subglutinans* were more frequently isolated from crown tissues.

Fungicide seed treatments did not affect plant tissue colonization by *Fusarium* spp. but did significantly reduce the number of root, mesocotyl and crown sections colonized with *F. graminearum*, *F. subglutinans* and *F. verticillioides.* All fungicide seed treatments reduced soil-borne *Fusarium* colonization of maize upto V6, and non significant differences between treatments were detected.

Introduction

In Iowa, maize (*Zea mays* L.) is planted as soon as soil conditions are favorable in order to optimize yields and allow longer-season hybrids to be grown in cool temperate regions (14). Most growers plant as soon as soil temperatures in the planting zone range from 10^{o}C to 14^{o}C (9), even though the optimum temperature for maize germination and emergence is 25^{o}C (33). At these low temperatures, emergence may take up to three weeks, and consequently, there is a prolonged opportunity for seedling disease caused by *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. (42). Maize seeds planted in cold soils imbibe cold water which results in rupture of cell membranes and embryonic tissues (39). Such damage results in exudation of carbon sources such as organic acids, sugars, and amino acids that create a nutrient rich environment which favors pathogenic and non-pathogenic interactions on germinating maize seedlings (17).

Early season maize seedling diseases can be caused by numerous species of *Fusarium* (42) namely *F. graminearum* [Schwabe (teleomorph, *Gibberella zeae* Schwein (Petch)], *F. oxysporum* (Schlechtendahl emend. Snyder & Hansen), *F. proliferatum* [(Matsushima) Nirenberg (teleomorph, *G. fujikuroi* var. *intermedia*)], *F. solani* [(Martius) Appel & Wollenweber emend. Snyder & Hansen (teleomorph *Haemanectria haematococca*)], *F. subglutinans* [(Wollenweber & Reinking) Nelson, Toussoun & Marasas (teleomorph, *G. subglutinans* Nelson, Toussoun & Marasas)], and *F. verticillioides* [(Saccardo) Nirenberg

(teleomorph, *G. moniliformis* Wineland)]. Mycelium of *F. graminearum, F. proliferatum, F. subglutinans*, and *F. verticillioides* can remain infective for more than 630 days under field conditions; although environmental fluctuations of temperature and moisture can reduce survival (7). Thus, buried maize residue colonized with these *Fusarium* spp. serves as an inoculum source for infection of maize seed, and seedling roots and mesocotyl tissues via epidermal cells (22).

Fusarium spp. are endemic to maize production fields within the United States, and can be isolated from any maize tissue (16). Infection occurs throughout the growing season (21). *Fusarium graminearum* was the most commonly found species of *Fusarium* on diseased maize seedlings during the 2004 and 2005 growing seasons in Ohio (6). Leslie et al. (16) reported that the most predominant *Fusarium* spp. in maize stalks between V10-V15 were *F. moniliforme* (syn. *F. verticillioides*), *F. proliferatum* or *F. subglutinans*. Similarly, Gatch and Munkvold (11) found that all mature maize stalks were colonized with *Fusarium* spp., and *Gibberella zeae* (anamorph *F. graminearum*) was the predominant species in the one year in which this survey was done.

Early disease symptoms caused by *Fusarium* spp. include seed, root, mesocotyl, and crown rots (42). When maize is planted in cold and wet soils, seedling blights can result in stand losses of up to 100%. Seedling disease is caused by a complex of soil-borne pathogens of which *Fusarium* spp. are usually predominant (35). In addition, this group of pathogens also causes late season crown, ear and stalks rots, while infection of grain by *F. graminearum*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides* also may result in mycotoxin contamination (8). Some *Fusarium* spp. may also colonize maize asymptomatically, namely *F. proliferatum* (26), *F. subglutinans* (13,43) and *F. verticillioides* (2). This endophytic-type infection can contribute to crown and stalk rot, and

also colonize maize ears and infect maize kernels (21), therefore contributing to seed-borne inoculum and mycotoxin accumulation.

To avoid stand loss due to seedling diseases, almost all maize seed planted in the US Corn Belt is treated with fungicides that are applied as a seed dressing (20,27). Seed treatment fungicides afford the seedling a zone of protection, and reduce rhizosphere colonization by *Fusarium* spp. and consequently reduce root infection and seedling disease (1). Fungicide seed treatments improve emergence and seedling establishment, presumably by protecting germinating seedlings from early season damping off diseases caused by *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp. (3). The effects of fungicide seed treatments on endophytic infections of maize by *Fusarium* spp. are not well understood.

Fungicide seed treatments are not equally effective against all soil-borne *Fusarium* spp. Captan seed treatment was shown to reduce rhizosphere colonization of maize seedlings by *F. graminearum* and *F. subglutinans*, however *F. oxysporum* and *F. solani* were unaffected by the fungicide (25). In the past two decades, numerous new active ingredients belonging to the benzimidazoles, strobilurins, phenylpyrroles and triazoles chemical groups have been introduced to the market. These chemical groups are relatively systemic compared with the phthalimide group to which captan belongs, and are able to penetrate the seed coat and translocate to the endosperm, embryo, coleoptiles, and radicle of maize seed via the xylem (18,37). Munkvold and O'Mara (20) also showed that efficacy of difenoconazole and fludioxonil against soil-borne *Fusarium* spp. were generally more effective than captan. This systemic ability should play an important role in reducing seed and seedling infection by soil-borne *Fusarium* spp. and also may prevent or reduce colonization of the germinating seedling (5,12,40). The exact pathology behind the effectiveness of seed treatment fungicides has yet to be fully evaluated (19).

The goal of this study was to enhance our understanding of the interaction of seed treatment fungicides and *Fusarium* spp. infection of maize under field conditions. Our objectives were to (i) elucidate the effect of seed treatment fungicides on infection and colonization of maize seedlings by soil-borne *F. graminearum*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides* and (ii) assess the colonization of maize roots, mesocotyl and crowns by *Fusarium* spp. in maize seedlings.

Materials and methods

Field trials were planted at Iowa State University Southeast Research Farm (SERF) (41°12'49.97"N 91°32'19.64"W), near Crawfordsville, Iowa on Mahaska silty clay loam soil type, pH 6.0, and Iowa State University Northeast Research Farm (NERF) at Nashua, Iowa (42°57'12.66"N 92°32'19.59"W), on Clyde silty clay loam soil type, pH 6.5. At both sites, the fields chosen had been continuously planted with maize for the previous four growing seasons. Each field was tilled prior to planting.

The trial at the SERF was planted April 19, 2007, and soil temperature at planting was 10°C. At NERF planting was done May 1, 2007, soil temperature at planting date was 15.5°C. A high incidence of *Fusarium* spp. seedling disease was expected, therefore no inoculum was applied to the fields. An Almaco® cone plot planter was set to plant at 80 000 plants /ha in each plot at 5 cm depth. Rain and temperature weather variables were recorded in situ at each location (Table 3.8). Plots dimensions were 5.3 m long by 5.25 m (8, 75 cm spaced rows) wide.

Standard agronomic practices were practiced at both locations. An application of starter fertilizer 196 kg/ha N was applied at SERF (Anhydrous Ammonia, 82.5-0-0) and NERF (UAN, 28-0-0) prior planting. Preemergence herbicide Bicep Lite Magnum II ®, (acetochlor, 2.5 kg a.i. /ha + atrazine, 3 kg a.i. /ha) was applied at SERF and Harness ® (S-

metolachlor, 0.25 kg a.i. /ha) at NERF. Secondary weed management was done with cultivation at 30 days after emergence (dae), approximate growth stage V4 (30) at both sites. Foliar insecticide was not applied at either site. A solution of Furadan 480® (carbofuran,1.75 kg a.i. /ha) was applied with side dressing equipment followed by cultivation to control root worm (*Diabrotica virgifera, D. barberi*) prior to planting and at 60 days after emergence (dae) when the crop was at growth stage V6 (30) at both locations.

Three fungicide seed treatments were evaluated at both sites (Table 3.1). Non-treated seed served as the control. Each fungicide was applied as a water based slurry (6 g slurry/kg seed) to 2 kg of maize seed (Garst®, 8545 hybrid). Each fungicide seed treatment slurry was made following the manufacturer's recommended maize rate. The amount of fungicide needed to treat 10 kg of seed was weight with an analytical scale (Ohaus®, Analytical plus). Red seed colorant (Becker Underwood ®, 0.22 ml/1 kg seed) was added to each fungicide seed treatment slurry to monitor seed coverage. Each fungicide slurry was then filled with distilled water for a total of 60 g of slurry. The moisture content of the seed at the time of treatment was 13.4%. A modified agar seed health test (32) was done to determine the incidence of seed-borne *Fusarium* spp. in the seed lot before applying the seed treatments. The fungicide seed treatment slurry was weighed using an analytical scale (Ohaus®, Analytical plus), poured into a Gustafson® (model BLT) seed treater, and thoroughly mixed for 5 minutes until fungicide seed treatment was evenly distributed on the maize seed coat. Treated maize seeds were kept in a cold room set at 4^{o} C.

Isolation of *Fusarium* spp. was done from tissues of the same seedlings that were assessed for seedling blight disease severity at growth stages V2, V4 and V6. The seedlings were surface sterilized with 10% bleach solution for 2 minutes, followed by one wash in sterile water for 2 minutes. To quantify colonization of the seedlings with systemic *F.*

graminearum, *F. proliferatum*, *F. subglutinans* and *F. verticillioides*, samples of crown and mesocotyl tissue and five 1 cm length sections of root tissue were selected at random (total of 7 tissue dissections per seedling) and plated on 95 mm x 15 mm Petri dishes (Fisherbrand® ,Mediamiser), filled with Nash-Snyder medium (23) supplemented with 0.1 g/l Rose Bengal (Sigma®, 0.1 g/l). The plates were kept at room temperature, 25°C, 12 hr day/ 12 hr night. Any white, septate mycelium that grew from a tissue segment was transferred to carnation leaf agar (CLA) medium (24) in 60 mm x 15 mm Petri dishes (Fisherbrand®, Mediamiser) for identification based on the conidiophores and conidia (morphological characteristics) (15,36). The CLA plates were kept at room temperature 25°C, 12 hr day/ 12 hr night (4).

In each treatment, the percentage of maize plants colonized with *Fusarium* spp. [(# of plants infected) / (total plants sampled) X 100] was determined at V2, V4, and V6. The tissue from which each *Fusarium* spp. was isolated was recorded to assess the effect of fungicide seed treatments on colonization of maize roots, mesocotyl, and crown tissues by *Fusarium* spp. Mean proportions of infected dissections with each species were estimated at each assessment date. The proportions of each *Fusarium* spp. in each tissue type assessed was estimated [proportions of specific *Fusarium* spp. in root/mesocotyl/crown tissue = (# of tissue sections from which each *Fusarium* spp. was isolated) / (total amount of *Fusarium* spp. isolated from at each assessment date)].

At each location the experiment was a randomized complete block design with 5 blocks in a split plot arrangement with seed treatment as the main plot effect and the sampling date (V2, V4, V6) as the split plot effect. Data analysis was conducted using the mixed procedure (PROC MIXED) of SAS version 9.1 (SAS Institute, 2002). By location analysis of variance was done to assess the effects of fungicide seed treatments on incidence of maize plants infected with *Fusarium* spp. at each assessment date. Analysis of variance

was also conducted for the mean proportion of each *Fusarium* spp. isolated from root, mesocotyl and crown tissues with mean separation was conducted with Tukey's honest significant difference (HSD) test for multiple comparisons test (28). The Simpson index was calculated for each treatment, and assessment date, and analysis of variance was done to determine if significant differences ($P<0.05$) occurred between treatments at each location using Tukey's HSD to separate the means within each assessment date. The Simpson diversity index $D = 1 - [\sum n_i(n_i\text{-}1)]/[N(N\text{-}1)]$, ($n_i$ = number of a organisms belonging to species, N = total number of individuals), measures the degree of concentration or diversity of a population when it is classified into groups (34). Thus the formula was used to assess *Fusarium* spp. richness, that is, the number of different *Fusarium* spp. isolated from each treatment, and *Fusarium* spp. evenness, which is a measure of the relative abundance of the different *Fusarium* spp. (11). The Simpson diversity index value range between 0 to 1, and the closest to 1 the value, greater the diversity and evenness of *Fusarium* spp. diversity.

To assess treatment effects over combined locations (experiment and replicates) an analysis of variance was conducted on variable means measured by treatment, sampling date and location in a split plot structure (fungicide seed treatments as the main subplot factors and location as a block factor).

Results

No seed-borne *Fusarium* spp. was detected in the assessed seed lot sample. Maize seed germination was 100% in laboratory conditions. Cool and wet planting conditions were observed at SERF from planting through growth stage V2 (Table 3.2). At NERF, conditions from planting through growth stage V6 were drier and warmer compared with SERF (Table 3.2).

Maize seedling sections were colonized with a number of *Fusarium* spp., including *F. graminearum, F. oxysporum, F. proliferatum, F. solani, F. subglutinans and F. verticillioides*. Fungicide seed treatments were very effective at reducing infection (total plants from which *Fusarium* spp. was isolated) of maize seedlings by *Fusarium* spp. at V2, however by V4, the incidence of *Fusarium* spp. infection was similar for all treatments plus the control (Table 3.4).

The mean proportion of tissue sections infected with *F. graminearum* was greatest at growth stage V2, and decreased through growth stage V6 (Fig. 3.1). Similarly, the mean proportions of tissues infected by *F. solani, F. oxysporum*, *F. proliferatum* decreased at each assessment date. In contrast, the mean proportions of tissue sections infected with either *F. verticillioides* or *F. subglutinans* (Fig. 3.1) increased at each growth stage, and these two species were the most frequently isolated species from crown tissues at growth stage V6 at both locations. Significant sampling date effects were recorded for *F. graminearum* (P=0.0001), *F. oxysporum* (P=0.0464), *F. solani* (P<0.0001), *F. subglutinans* (P<0.0001) *and F. verticillioides* (P<0.0001) (Table 3.3).

Fungicide seed treatments significantly reduced *F. graminearum* (P=0.0397) colonized dissections at growth stage V2 (Fig. 3.2) at both locations. A significant reduction of *F. subglutinans* (P=0.0267) and *F. verticillioides* (P=0.0235) at growth stages V4 and V6 (Figs.3.3 and 3.4) in plants grown from fungicide-treated seed also occurred at both locations. At growth stage V2, the reduction in the amount of proportions colonized with *F. graminearum* coincided with an increased of dissections colonized with *F. solani*, *F. oxysporum*, *F. proliferatum*, *F. subglutinans*, and *F. verticillioides* at SERF location and *F. proliferatum* and *F. subglutinans* at NERF in some of the treatments tested (Fig. 3.2). Similarly at growth stages V4 and V6, a reduction in the of dissections colonized with *F.*

subglutinans and *F. verticillioides* caused a slight increase in the amount of *F. graminearum*, *F. solani*, *F. oxysporum* and *F. proliferatum* colonized dissections at both locations (Fig. 3.3 and 3.4).

At both locations, *F. graminearum*, *F. solani* and *F. oxysporum* were more common in the root tissue of infected plants than in the mesocotyl and crowns (Figs 3.5, 3.6, 3.7 and 3.8). Although *F. proliferatum*, *F. subglutinans* and *F. verticillioides* also were isolated from the roots, these species were more common in the mesocotyl and crown tissues in both locations and all assessment dates (Figs 3.5, 3.6, 3.7 and 3.8). Fungicide seed treatments did not significantly affect tissue colonization patterns of the *Fusarium* spp.

Fungicide seed treatments significantly ($P<0.0001$) affected *Fusarium* spp. richness within maize seedling tissues at both locations; seven species of *Fusarium* were isolated from maize seedlings. Fungicide seed treatments affected the abundance of *F. graminearum* at V2, and *F. subglutinans* and *F. verticillioides* at V4 and V6, and thus resulted in more evenness in the diversity (Table 3.5). The Simpson index value was closer to 1 in all fungicide seed treated seedlings and differences were significant in all assessment dates and locations. Maize seedlings grown from fungicide treated seed had more diversity than untreated seedlings at both locations.

Discussion

In both locations, we found that *F. graminearum* was the predominant species infecting maize seedlings immediately after planting but as the growing season progressed, *F. subglutinans* and *F. verticillioides* became more common colonists of the plants. *Fusarium graminearum* is the most competent *Fusarium* spp. in colonization of the maize rhizosphere (25), and presumably this characteristic affords this species a competitive advantage over other *Fusarium* spp. present in the rhizosphere for infection prior to growth

stage V2. In addition, we suspect that environmental conditions during the first weeks of seedling development might favor *F. graminearum* inoculum production over other species. Since soil temperatures were greater at V4 and again at V6 at both locations, thus we suspect that soil temperatures might influence *Fusarium* spp. colonization.

Interactions between different *Fusarium* spp. in the colonization of maize have been reported. There is evidence suggesting that seed-borne *F. verticillioides* protects against *F. graminearum* infection (38). As the growing season progressed, conditions appear to favor *F. verticillioides* infection of maize more, and had consequently thus resulted in a reduction of *F. graminearum*. Endophytic infections (symptomless systemic infection) of maize plants by *F. verticillioides* (10) and *F. subglutinans* (43) have been demonstrated. Thus these two species may be more effective than *F. graminearum* at systemic colonization of maize stem tissues which might explain the increased number of dissections colonized with *F. verticillioides* and *F. subglutinans* at V4 and V6 and the reduction of *F. graminearum.* Infections by *F. proliferatum* can also lead to systemic colonization; however in our study we found a low incidence of this species in mesocotyl and crown. According to Olah et al. (26), infection by *F. proliferatum* occurs early in the growing season, and then infection suddenly decreases. Possibly *F. verticillioides* and *F. subglutinans* are better adapted for crown tissue colonization compared with *F. graminearum* and *F. proliferatum*.

Fusarium oxysporum and *F. solani* are opportunistic fungi that cause seedling disease when the host plant is stressed (41). Consequently, location of inoculum and inoculum concentration would be important for the invasion of root tissues when the seedling is stressed. We propose that since maize planting conditions in the U.S. Corn Belt are stressful due to cooler temperatures as a result of earlier planting (14), *F. oxysporum* and *F. solani* could contribute to field disease in some seasons. Since *F. graminearum*, *F. subglutinans* and

F. verticillioides were the predominant species in these studies, it could be argued that any effect of the seed treatment fungicides tested on *F. oxysporum*, *F. proliferatum* and *F. solani* could be masked. The effects of these seed treatments against those species should be evaluated further in the future.

Under cool, wet conditions, maize seed can take as long as 14 days to germinate and emerge (growth stage VE) (29). At SERF, VE occurred at 16 dap compared with NERF where conditions were warmer and VE occurred at 10 dap. We have shown that the effective period of fungicide seed treatments on maize is longer than reported by Wilson et al (44). In our work, reduced colonization of maize seedlings by *F. graminearum*, *F. subglutinans* and *F. verticillioides* at V2 (14 dae), V4 (28 dae) and V6 (52 dae), respectively, was clearly associated with the use of a seed treatment.

Seedling infection studies on individual active ingredients in controlled environmental conditions showed that thiabendazole, ipconazole and triticonazole were highly effective against seed-borne *F. verticillioides* (31). The A14918E premix contains thiabendazole and Vortex®-Trilex FL® – Allegiance FL® contains ipconazole. Both fungicides are known to have a residual effect in the soil, and also to have systemic properties (3). The residual period of thiabendazole and ipconazole in the soil may have increased the protection interval of these fungicide seed treatments against infection by *F. verticillioides* and *F. subglutinans*.

Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam) contains an insecticide seed treatment active ingredient. There are reports of enhanced seed protection by fungicides in the presence of an insecticide. Our data do not necessary support this since A14918E and Vortex®-Trilex FL®- Allegiance FL®, which did not contain seed treatment insecticide, fared just as well as the Cruiser Extreme 250 ® treatment. If the

insecticide protects against insect injury that provides an infection pathway for seedling blight pathogens, then the reason for our findings may be due to low insect pressure at each site; although this factor was not evaluated. Perhaps the synergistic effect, if any, of insecticide active ingredients on soil-borne *Fusarium* spp. colonization should be investigated in future work.

This is the first report of the effects of fungicide seed treatments on pathology of *Fusarium* spp. in the maize production system. Simpson's index showed that fungicide seed treatments affected the composition of *Fusarium* spp. colonizing maize plants. This is likely because the susceptibility of *Fusarium* spp. to different fungicide active ingredients varies.

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Table 3.1. Fungicide seed treatment rate used in field experiments.

Fungicide	Active ingredient	Chemical name	Rate[z]
A14918E	fludioxonil (40.3%)	4-(2,2-difluoro-1,3-bensodioxol-4-yl)-1H-pyrrole-3-carbonitrile	2.5
	mefenoxam (8.4%)	(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester	2.0
	azoxystrobin (9.6%)	methyl (E)-2--3-methoxyacrylate	1.0
	thiabendazole(42.9%)	(2-(thiazol-4-yl) benzimidazole	20.0
Cruiser Extreme 250®	fludioxonil (40.3%)	4-(2,2-difluoro-1,3-bensodioxol-4-yl)-1H-pyrrole-3-carbonitrile	2.5
	azoxystrobin (9.6%)	methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	1.0
	thiamethoxam	4H-1,2,5-oxadiazin-4-imine,3-[(2-chloro-5-thiazolyl) methyl]tetrahydro-5-methyl-N-nitro-	62.5
	mefenoxam (8.4%)	(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester	2.0
Vortex®-Trilex FL®-Allegiance FL®	trifloxystrobin (22%)	Methyl (E)-methoxyimino-{(E)-α-[1-(α, α, α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate	10.0
	ipconazole (40.7%)	(1RS,2RS,5RS;1RS,2RS,5RS)-2-(4chlorobenzyl)-5-isopropyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	2.5
	metalaxyl (28.35%)	N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)alanine, methyl ester	28.0

[z] g a.i. / 100 kg.

Table 3.2. Cumulative precipitation (mm) and average soil temperature (^{o}C) at growth stages V2, V4 and V6 at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

	V2		**V4**		**V6**		**Total**	
	NERF	SERF	NERF	SERF	NERF	SERF	NERF	SERF
Precipitation (mm)[z]	3.4	48.77	106.7	59.9	103.3	102.1	213.4	210.7
Temperature (^{o}C)[y]	17.1	18.3	19.3	20	22.8	22	19.3	19

[z] Cumulative precipitation from planting until respectively growth stage.
[y] Average temperature from planting until each growth stage.

Table 3.3. P-values of location (loc) treatment (trt), date (date) and treatment by date (trt x date) interactions, on occurrence of *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), *F. verticillioides* (F.vert), and Simpson diversity index (SI) on maize plants at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. The table represents analysis of variable means appearing in figures 3.1 to 3.8 combining SERF and NERF locations.

Source	**F.gram**	**F.sol**	**F.oxys**	**F.prol**	**F. sub**	**F. vert**	**SI**
loc	0.6202	0.1218	0.8992	0.7106	0.2822	0.4309	0.0827
trt	0.0397	0.4355	0.0757	0.1044	0.0267	0.0235	0.0235
date	0.0001	<0.0001	0.0464	0.1694	<0.0001	<0.0001	0.9349
trt x date	<0.0001	0.9005	0.9188	0.1220	0.0048	<0.0001	0.7629

Table 3.4. Percentage (%) of maize plants colonized with *Fusarium* spp. at V2, V4 and V6 growth stages grown from untreated or treated seed with A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam),Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl), and planted at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

Location	Treatment	V2	V4	V6
SERF	A14918E	20 c[z]	100 a	100 a
	Cruiser Extreme 250®	45 b	100 a	100 a
	Vortex®-Trilex FL®-Allegiance FL®	40 bc	100 a	100 a
	Untreated	80 a	100 a	100 a
	HSD (P<0.05)	24	NS[y]	NS
NERF	A14918E	65 bc	100 a	100 a
	Cruiser Extreme 250®	85 a	100 a	100 a
	Vortex®-Trilex FL®-Allegiance FL®	55 c	100 a	100 a
	Untreated	100 a	100 a	100 a
	HSD (P<0.05)	30	NS	NS

[z] Values from the same location followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.
[y] Non significant.

Table 3.5. Simpson diversity index values of *Fusarium* spp. isolated from dissections of root, mesocotyl and crown tissues made from seedlings grown from untreated seed or seed treated with either Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), or Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl), and planted at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

Location	Treatment	V2	V4	V6
SERF	A14918E	1.00[z] a	0.87 a	0.80 b
	Cruiser Extreme 250®	0.93 ab	0.82 ab	0.91 a
	Vortex®-Trilex FL®-Allegiance FL®	0.77 b	0.86 a	0.88 ab
	Untreated	0.71 b	0.79 b	0.73 b
	HSD (P<0.05)	0.17	0.05	0.1
NERF	A14918E	0.95 a	0.82 a	0.80 b
	Cruiser Extreme 250®	0.96 a	0.85 a	0.91 a
	Vortex®-Trilex FL®-Allegiance FL®	0.79 a	0.87 a	1.00 a
	Untreated	0.75 a	0.68 b	0.68 c
	HSD (P<0.05)	0.17	0.05	0.1

[z] Values from the same location and column followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.

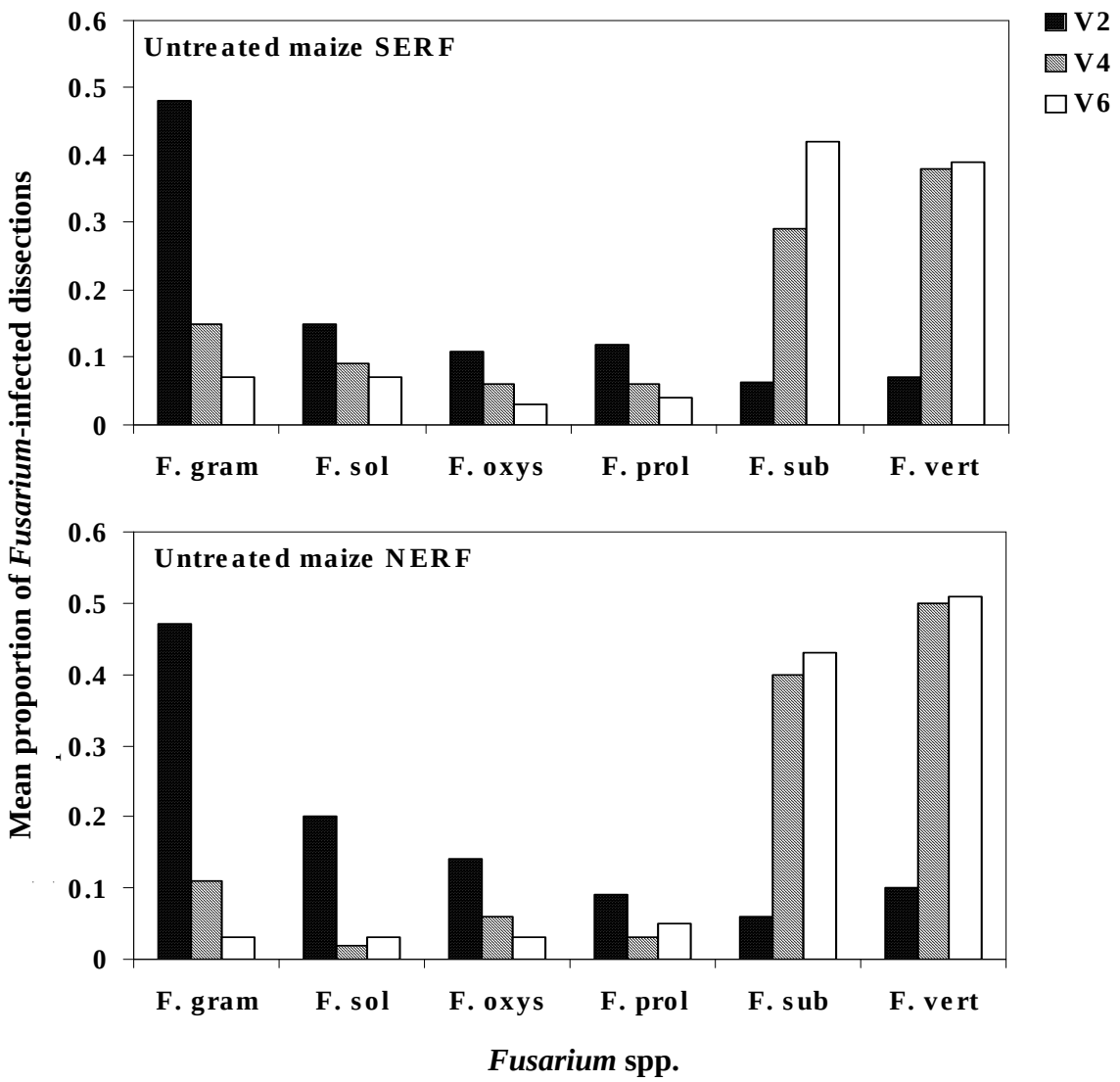

Figure 3.1. Mean proportion of dissections from roots, mesocotyl and crown tissues of maize seedlings grown from untreated seed colonized with *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) at growth stages V2, V4 and V6 (crowns only) at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

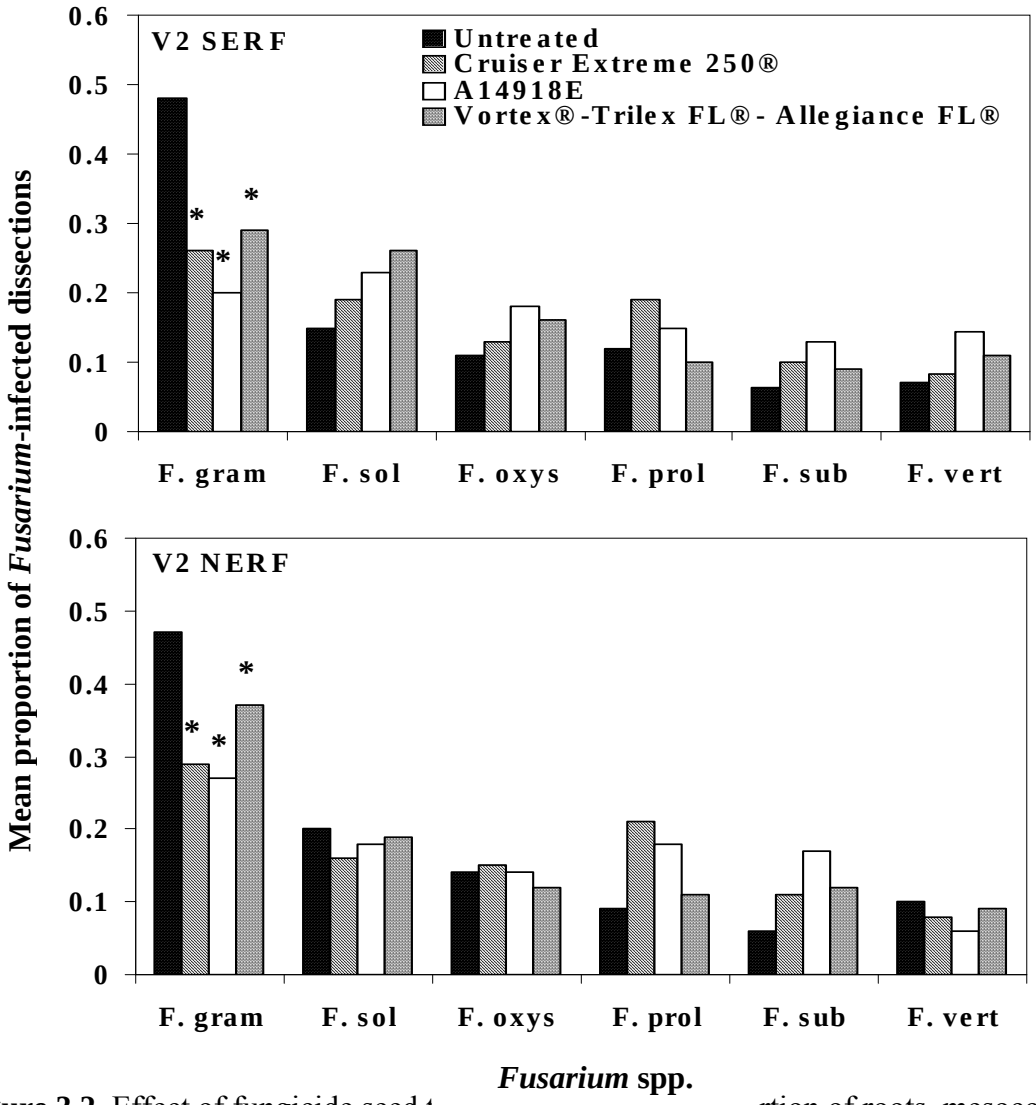

Figure 3.2. Effect of fungicide seed treatments on mean proportion of roots, mesocotyl and crown dissections of maize seedlings colonized by *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) at V2 growth stage. Maize was planted at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. Bars with asterisk were significantly reduced ($P<0.05$).

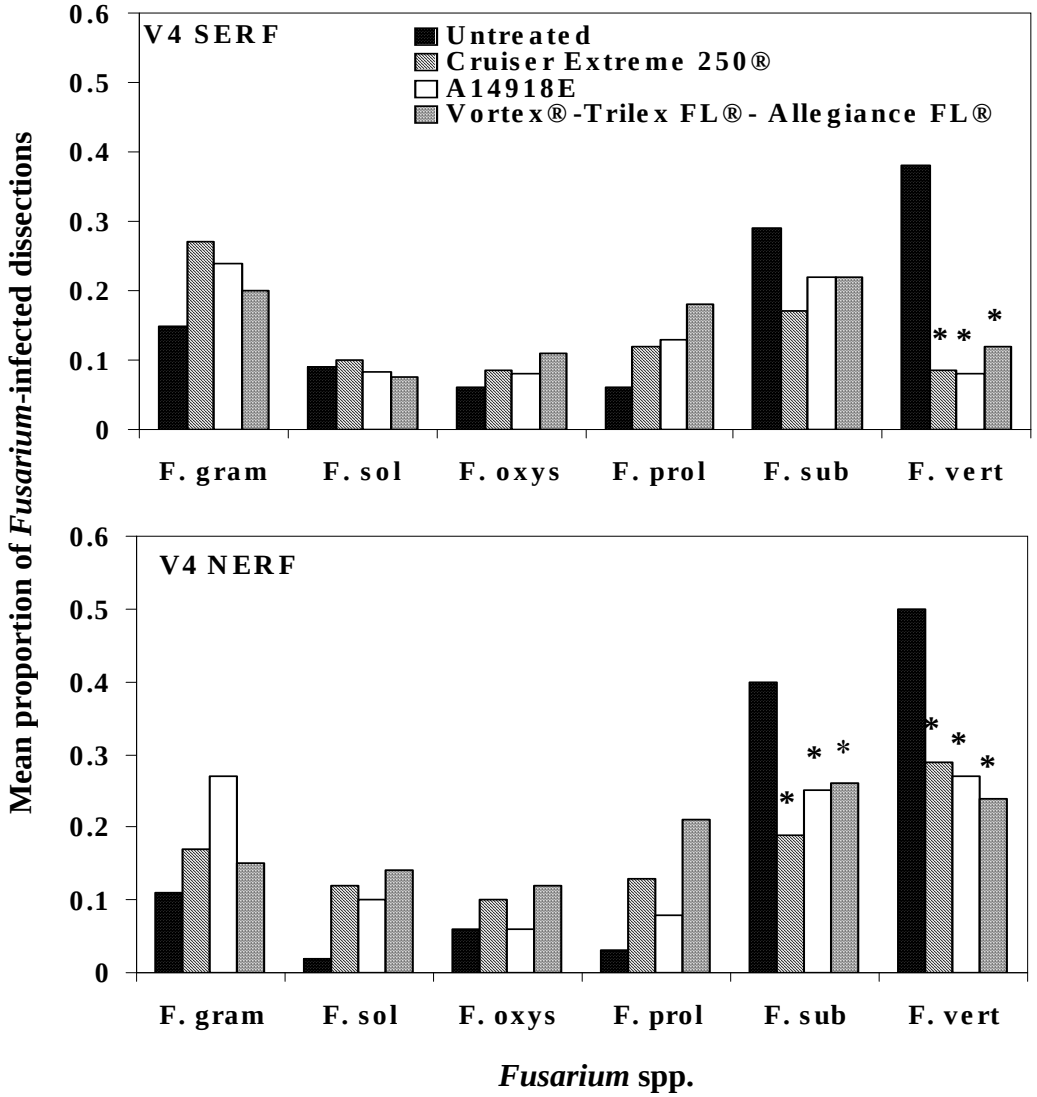

Figure 3.3. Effect of fungicide seed treatments on proportion of roots, mesocotyl and crown dissections of maize seedlings colonized with *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) at V4 growth stage, at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. Bars with asterisks were significantly different from the untreated control.

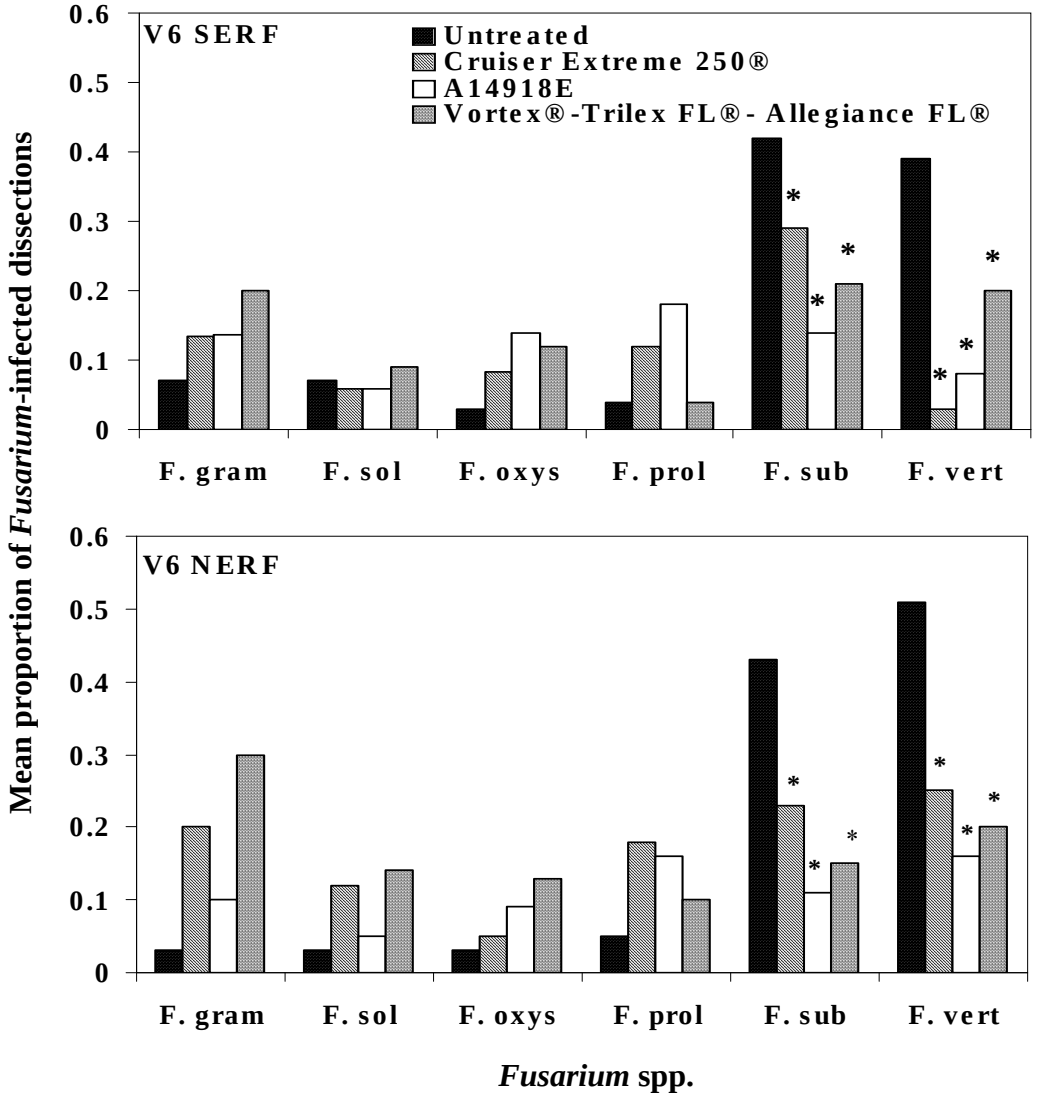

Figure 3.4. Effect of fungicide seed treatments on proportion of crown dissections of maize seedlings colonized by *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) at V6 growth stage, at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. Bars with asterisks were significantly different from the untreated control.

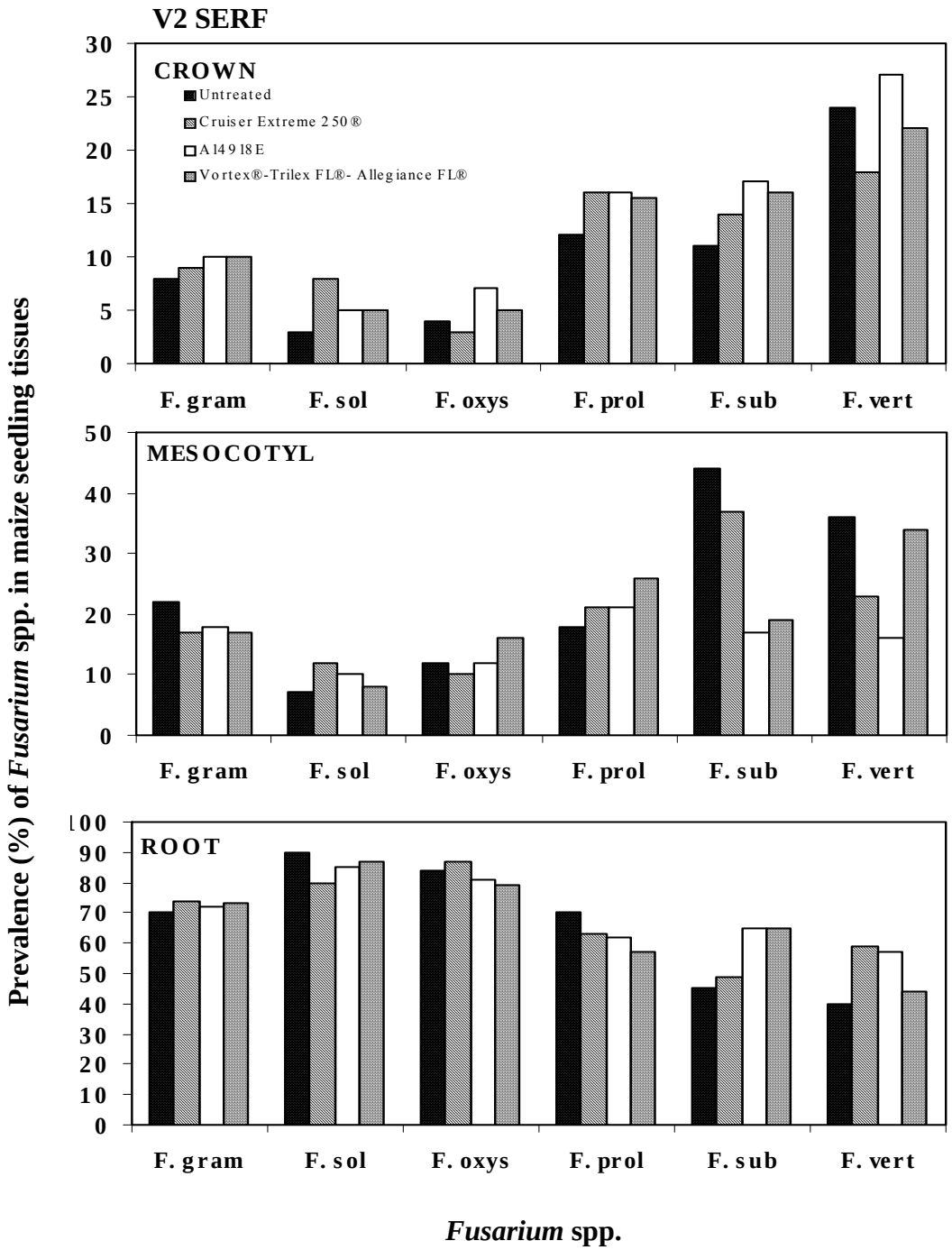

Figure 3.5. Prevalence (%) of *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) in root, mesocotyl and crown tissues of treated and untreated maize sampled at V2 at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, IA.

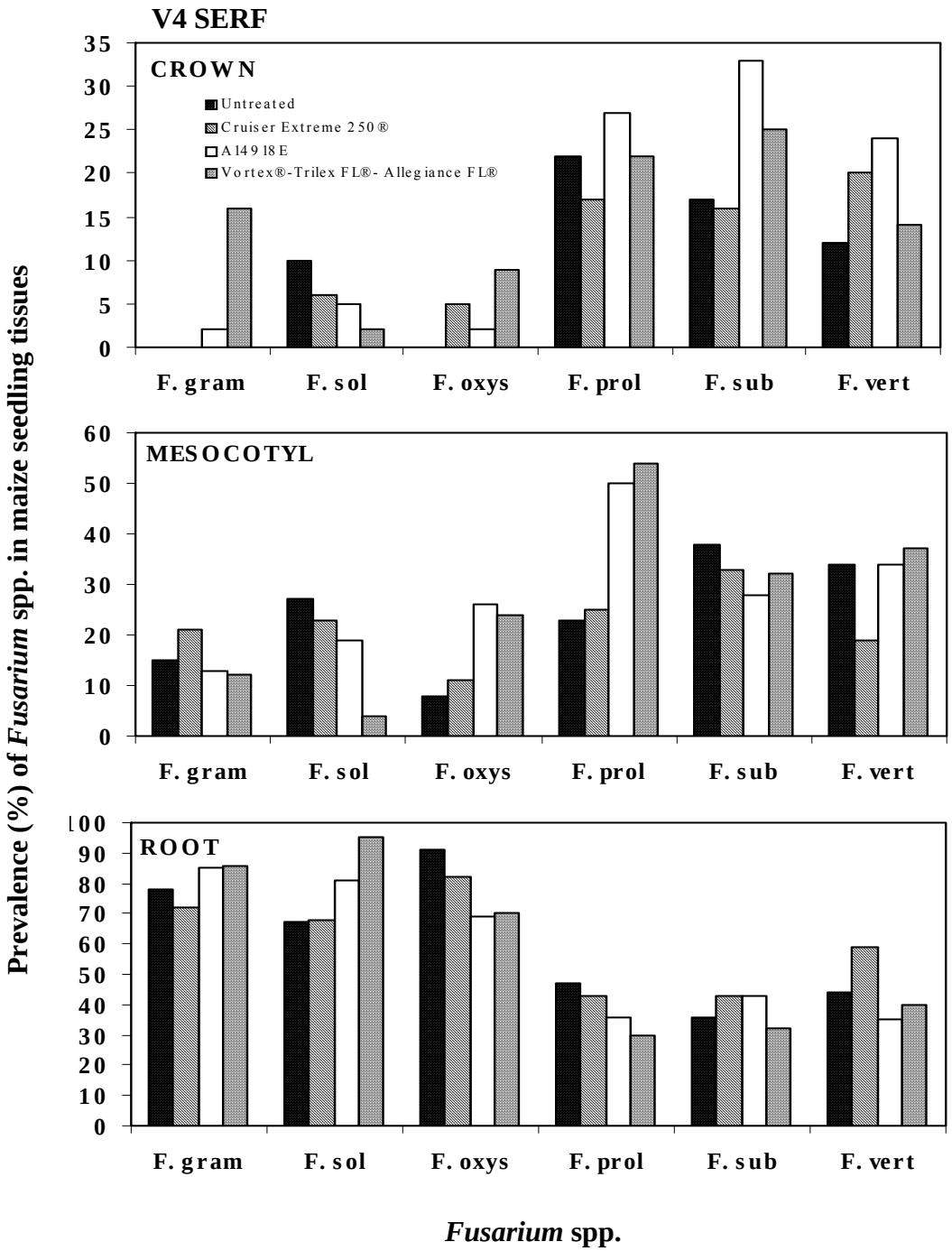

Figure 3.6. Prevalence (%) of *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) isolated from root, mesocotyl and crown tissues of treated and untreated maize sampled at V4 at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, IA.

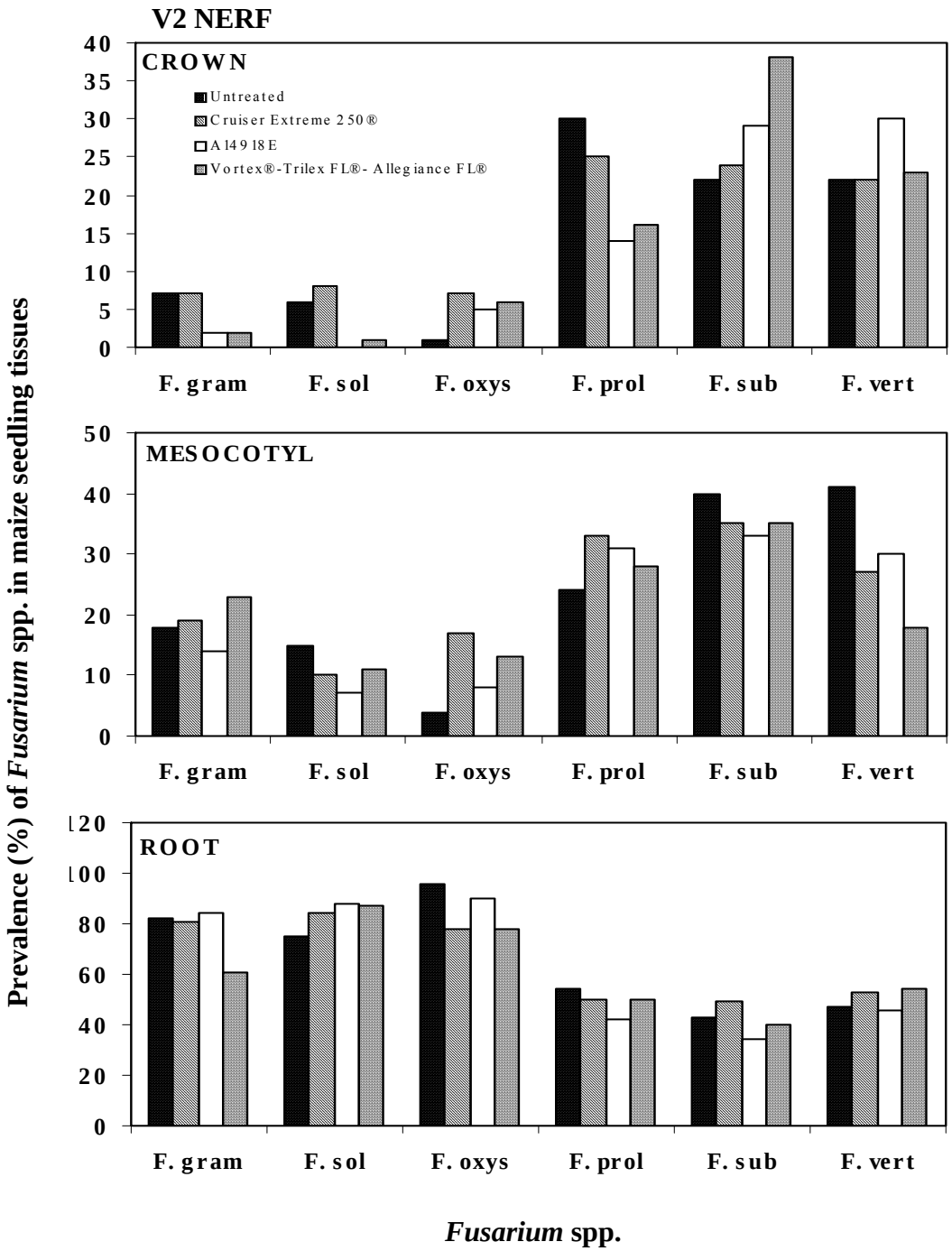

Figure 3.7. Prevalence (%) of *F. graminearum* (F.gram), *F. solani* (F.sol) , *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) isolated from root, mesocotyl and crown tissues of treated and untreated maize sampled at V2 at Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

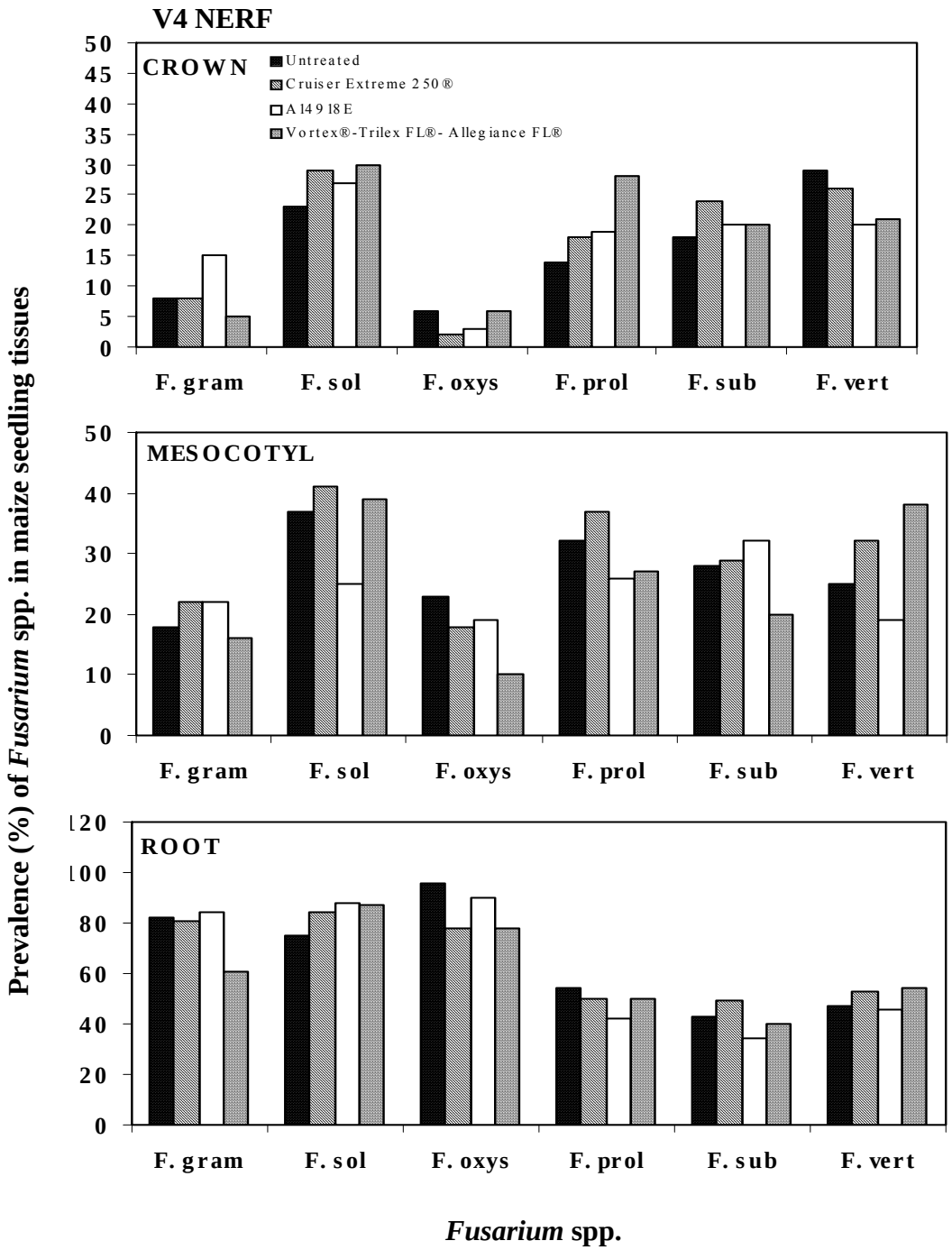

Figure 3.8. Prevalence (%) of *F. graminearum* (F.gram), *F. solani* (F.sol), *F. oxysporum* (F.oxys), *F. proliferatum* (F.prol), *F. subglutinans* (F.sub), and *F. verticillioides* (F.vert) isolated from root, mesocotyl and crown tissues of treated and untreated maize sampled at V4 at Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

CHAPTER 4. SEED TREATMENTS INDIRECTLY IMPACT PHOTOSYNTHETIC ABILITY IN MAIZE (*ZEA MAYS* L.) SEEDLINGS BY REDUCING *FUSARIUM* SPP. INFECTION AND SEEDLING DISEASE

A paper to be submitted to *Plant Disease*

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Abstract

Benefits of fungicide seed treatments on maize production are often measured in terms of emergence, plant height and grain yield at the end of the growing season. The effects of fungicide seed treatments on early season growth and physiology of maize were evaluated at two locations where maize have been planted for the last two growing seasons in Iowa, in 2007. Maize seed was treated with either A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), Cruiser Extreme 250 ® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam) or a premix of Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl). Root, mesocotyl and crown rot severity, incidence of *Fusarium* spp. colonization and chlorophyll fluorescence (CF) (estimate of photosynthetic performance) were assessed at growth stages V2, V4 and V6. Stalk rot severity was assessed at R6 growth stage. In addition, plant height was assessed at growth stages V4 and V6. Photosynthetic performance, as measured by CF, significantly decreased with increased incidence of *Fusarium* spp. at growth stages V2 and V4 at both locations. In general, CF values had a significant relationship with root, mesocotyl and crown rot severity at both locations. A significant relationship between the severity of mesocotyl rot at V2 and severity of crown rot at V6 was observed at SERF ($P<0.0001$) and NERF ($P=0.0022$) Also, some

evidence of a significant relationship between crown rot at V6 and stalk rot at R6 was observed at SERF ($P<0.0001$). Significant relationships between CF measurements and plant height measurements at V4 and V6 at both locations were detected. Maize plants treated with A14918E had higher CF readings and thus higher photosynthetic performance than other assessed fungicide seed treated plants.

Introduction

Maize is susceptible to infection by numerous species of *Fusarium* including: *F. graminearum* Schwabe [(teleomorph, *Gibberella zeae* Schwein (Petch))], *F. oxysporum* [(Schlechtendahl emend. Snyder & Hansen)], *F. proliferatum* [(Matsushima) Nirenberg (teleomorph, *Gibberella fujikuroi* var. *intermedia*)], *F. solani* [(Martius) Appel & Wollenweber emend. Snyder & Hansen (teleomorph *Haemanectria haematococca*)], *F. subglutinans* [(Wollenweber & Reinking) Nelson, Toussoun & Marasas (teleomorph, *Gibberella subglutinans* Nelson, Toussoun & Marasas)], and *F. verticillioides* [(Saccardo) Nirenberg (teleomorph, *Gibberella moniliformis* Wineland)]. Disease symptoms caused by *Fusarium* spp. include early season seedling blights (seed, root, and mesocotyl rot) and crown, stalk and ear rots in late season (30). Furthermore, infection of grain by *F. graminearum*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides* may result in mycotoxin contamination (6,17) that has a deleterious effects on human and livestock.

Since seedling blight can result in stand loss, almost all maize seed planted in the US Corn Belt is treated with fungicide that is applied as seed dressing (15,20). The effectiveness of seed treatment fungicides are usually evaluated in field trials and emergence, plant height (used as an indication of plant vigor) (1), and yield data are used to demonstrate product effectiveness (26). However, these data contribute very little information regarding disease ecology (13). In some instances, disease severity on roots and mesocotyl tissues between

growth stages VE and V6 have been evaluated (19). However collection of these data relies on destructive sampling and assessments that may be subjective. A more objective method of assessing the effectiveness of seed treatments would be beneficial.

Reduced photosynthetic performance is associated with plant stress (12). Chlorophyll fluorescence (CF) is a suitable indicator of plant stress in the *F. oxysporum*-tomato pathosystem (29). Similarly, in maize plants infected with *F. moniliforme,* photosynthetic performance, measured by CF was shown to be reduced (24). Photosynthesis was significantly reduced in hosts that were infected by *Fusarium* spp., presumably because root and mesocotyl tissues damage associated with infection by *Fusarium* spp. as well as systemic colonization of the host interfered with the normal absorption of water and nutrients by the roots and mesocotyl. Assessments of CF during early growth stages (V2-V6) may offer a non-destructive sampling alternative to assess plant vigor in fungicide seed treatment trials. Furthermore CF measurements also could serve as an indirect method to measure infection of germinating maize seedlings by soil-borne pathogens and/or disease severity and thus be used to assess the effectiveness of seed treatment fungicides on seedling disease.

The goal of this study was to if determine if CF could be used to evaluate the effectiveness of seed treatment fungicides against seedling disease and examine the effect of these products on maize physiology. Our objectives were (i) to evaluate if chlorophyll fluorescence measurements have a significant relationship with seedling vigor, disease severity and incidence of *Fusarium* spp. infection of maize seedlings and (ii) assess the contribution of early seedling disease management with seed treatment fungicides to management of late season crown and stalk rot.

Materials and methods

Field trials were planted at Iowa State University Southeast Research Farm (SERF) (41°12'49.97"N 91°32'19.64"W), near Crawfordsville, Iowa on Mahaska silty clay loam soil type, pH 6.0, and Iowa State University Northeast Farm (NERF) at Nashua, Iowa (42°57'12.66"N 92°32'19.59"W), on Clyde silty clay loam soil type, pH 6.5. At both sites, the fields chosen had been planted with maize following maize rotation for the last four growing seasons and each field was tilled prior planting.

No inoculum was applied to the fields since a high incidence of *Fusarium* spp. seedling disease was expected as a result of planting early into cool, wet soils. The trial at the SERF was planted April 19, 2007 and soil temperature at planting conditions was 10°C. At NERF planting was done May 1, 2007; soil temperature at planting date was 15.5°C. Rain and temperature variables were recorded in situ by each location weather station (Table 4.2). An Almaco® cone plot planter set to plant 80 000 plants /ha in each plot was used to plant the experiment at each location at 5 cm depth. Plots dimensions were 5.3 m long by 5.25 m (8-75 cm spaced rows) wide.

Standard agronomic practices were followed at both locations. Starter fertilizer, 196 kg/ha N was applied at SERF (Anhydrous Ammonia, 82.5-0-0) and NERF (UAN, 28-0-0). Preemergence herbicide, Dual II Magnum® (S-metolachlor, 0.25 kg a.i. /ha) was applied at SERF and Harness Xtra®, (acetochlor, 2.5 kg a.i. /ha + atrazine; 3 kg a.i. /ha) was applied at NERF. Secondary weed management at both sites was done with cultivation at 30 days after emergence (dae), growth stage V4 (22) both sites. Foliar insecticide was not applied at either site. A solution of Furadan 480® (carbofuran,1.75 kg a.i. /ha) was applied with side dressing

equipment followed by cultivation to control root worn (*Diabrotica virgifera, D. barberi*) when the crop was at 60 days after emergence (dae), V6 (22) growth stage, at both locations.

Three fungicide seed treatments were evaluated at both sites (Table 4.1). Non-treated seed served as the control. Each fungicide was applied as a water based slurry (6 g slurry / kg seed) to 2 kg of maize seed (Garst®, 8545 hybrid). Each fungicide seed treatment slurry was made following the manufacturer's recommended maize rate. The amount of fungicide needed to treat 10 kg of seed was weight with an analytical scale (Ohaus®, Analytical plus). Red seed colorant (Becker Underwood ®, 0.22 ml/1 kg seed) was added to each fungicide seed treatment slurry to monitor seed coverage. Each fungicide slurry was then filled with distilled water for a total of 60 g of slurry. The moisture content of the seed at the time of treatment was 13.4%. A modified agar seed health test (23) was done to determine the incidence of seed-borne *Fusarium* spp. in the seed lot before applying the seed treatments. The fungicide seed treatment slurry was weighed using an analytical scale (Ohaus®, Analytical plus), poured into a Gustafson® (model BLT) seed treater seed treater, and thoroughly mixed for 5 minutes until fungicide seed treatment was evenly distributed on the maize seed coat. Treated maize seeds were kept in a cold room set at 4^{o} C.

Plant vigor was estimated by measuring plant height of each of 10 plants taken arbitrarily from the central two rows of each plot at V4 and V6 growth stages respectively (22). The plant height was measured using a ruler from the soil to the tallest fully develop leave from each maize plant. Chlorophyll fluorescence measurements were done to assess photosynthetic performance on the same 10 seedlings that were sampled for plant height in the center two rows of each plot at V2, V4 and V6 growth stages respectively, using a pulse amplitude modulation fluorometer (PAM 2000, Walz®, Effeltrich, Germany) equipped with a fiber optic probe and leaf clip holder. Measurements were made on cloud free days and

readings were collected between approximately 1100 and 1200 hrs. Readings were made consistently on arbitrary chosen, apparently healthy maize plants, midway between the leaf tip and base and midway between the margin and the midrib of the leaf from the topmost fully expanded leaf in each plant. Care was taken not to change leaf orientation. CF values ranges from 0 – 1, and the closest to 1 the less stress the maize plant is.

Root and mesocotyl rot severity were assessed at growth stages V2 and V4. Crown rot severity was assessed at growth stages V2, V4 and V6. For each assessment, twenty seedlings from each treatment (4 per plot) were selected arbitrarily from anywhere in the plot, excluding the two middle rows. Measurements of CF were made on maize plants to be assessed for disease and then seedlings were dug up with a shovel. Most of the soil surrounding the roots was removed and each seedling placed in a 10" x 12" (254 mm x 304.8 mm) Ziploc® storage bag. The seedlings were placed in a cooler and taken back to the laboratory where they were carefully washed with distilled water to remove remaining soil debris on the root, mesocotyl and crown tissues. Root rot severity was assessed using the following scale (26): where 0 = apparently healthy roots, 1 = <25% of roots with disease rot symptoms, 2 = 25-49% of roots rotted, 3 = 50-74%, of the roots rotted 4 = 75 % or greater of the roots rotted, and 5 = wilted or dead seedlings (Appendix C). The same scale was adjusted to record rot symptoms from the mesocotyl (Appendix D) and crown (Appendix E) of each tissue, were 0 = no symptoms visible, 1 = <25% of the tissue showing disease rot symptoms, 2 = 25-49% of the tissue rotted, 3 = 50-74% of the tissues rotted, 4 = 75 % or greater of the tissue rotted, and 5 = completely rotted tissue.

Stalk rot was assessed at growth stage R6. Thirty maize plants from each 8-row plot (five maize plants from each row of the plot, excluding the two middle rows of the plot) were arbitrarily selected. Plants were removed with a shovel, and the lower third of the maize stalk

was cut in half longitudinally using a knife. Stalk rot severity (Appendix F) was assessed using the University of Illinois (0-5) stalk rot rating scale (8).

Isolation of *Fusarium* spp. was done from tissues of the same seedlings that were assessed for seedling blight disease severity at growth stages V2, V4 and V6. Immediately after disease severity assessments had been done, maize seedlings were surface disinfested with 10% bleach solution for 2 minutes, followed by one wash in sterile water for 2 minutes. Samples of crown and mesocotyl tissue and five 1 cm length sections of root tissue were selected at random (total of 7 tissue dissections per seedling) and placed on 95 mm x 15 mm Petri dishes (Fisherbrand®, Mediamiser), filled with Nash-Snyder (NS) medium (16) supplemented with 0.1 g/l Rose Bengal (Sigma®, 0.1 g/l) The plates were kept at room temperature 25^{o}C, 12 hr day/ 12 hr night. Any white, septate mycelium that grew from a tissue segment was transferred to carnation leaf agar (CLA) medium (18) in 60 mm x 15 mm Petri dishes (Fisherbrand®, Mediamiser) for identification based on conidiophores and conidia morphological characteristics (11,28). The CLA plates were kept at room temperature 25^{o}C, 12 hr day/ 12 hr night. Non-*Fusarium* fungi such as *Penicillium* spp., *Rhizoctonia* spp., and *Trichoderma* spp. were identified by morphological characters (2). For each treatment, the percentage (%) of root, mesocotyl and crown dissections that were colonized by *Fusarium* spp. was determined at V2, V4 and V6 [Percentage of dissections colonized = (# of dissections infected with *Fusarium* spp.) / (total dissections sampled) X100].

At each location the experiment was a randomized complete block design with 5 blocks in a split plot arrangement with seed treatment as the main plot effect and the sampling date (V2, V4, V6) as the split plot effect. By location, analysis of variance was done to assess the effects of fungicide seed treatments on plant height, mean root, mesocotyl

and crown rot severity, the proportion of root, mesocotyl and crown tissues from which *Fusarium* spp. were isolated, and CF. Data analysis was conducted using the General Mixed procedure (PROC MIXED) of SAS version 9.1 (SAS Institute, 2002). Mean separation was conducted with Tukey's honest significant difference (HSD) test for multiple comparisons test (21). Relationships between root rot severity, mesocotyl rot severity, crown rot severity, incidence of *Fusarium* spp. and CF were computed with PROC REG of SAS version 9.1 (SAS Institute, 2002) for each sampling date at each location.

To assess treatment effects over combined locations (experiment and replicates) an analysis of variance was conducted on variable means measured by treatment, sampling date and location in a split plot structure (fungicide seed treatments as the main subplot factors and location as a block factor).

Results

No seed-borne *Fusarium* spp. was detected in seed for this experiment, and seed germination was 100% under laboratory conditions.

Plants grown from treated seed were taller ($P<0.0001$) than control plants at SERF at growth stage V4 (Table 4.4), however by V6, there was no difference in height. At NERF, no differences in seedling height were evident. Seedling heights were generally more uniform in seedlings grown from seed treated with A14918E, Cruiser Extreme 250® and Vortex®-Trilex FL®-Allegiance FL® at both locations.

Fungicide seed treatments had a significant effect ($P<0.0001$) on maize CF (Table 4.3). Seedlings grown from untreated seed had lower CF values compared with those grown from treated seed. Among treatments, seedlings grown from seed treated with A14918E had significantly ($P<0.0001$) higher CF measurements at both locations at V4 and V6 growth stages when compared with Cruiser Extreme 250® and Vortex®-Trilex FL®-Allegiance FL®

treatments (Table 4.5). Significant seed treatment by day interaction (P<0.0001) for chlorophyll fluorescence was detected (Table 4.3).

Diseased seedlings had root lesions that were dispersed, variable in size and dark brown to reddish becoming necrotic (Fig. 4.9). All fungicide seed treatments significantly reduced (P<0.0001) mean root rot index at the V2 assessment (Table 4.6). Significantly less (P=0.0429) root rotting symptoms were observed in seedlings grown from seed treated with Vortex®-Trilex FL®-Allegiance FL® at SERF, however no difference between seed treatments was observed at NERF at V2 (Table 4.6). By growth stage V4, no significant differences (P=0.0663) in the amount of root rot between treatments and the control at either location were evident (Table 4.6).

Mesocotyl rot symptoms were brownish, sunken and varied in size (Fig. 4.10). Fungicide seed treatments significantly reduced (P=0.0298) mesocotyl rot at growth stage V2 at both locations (Table 4.7). Mesocotyl rot of the control seedlings was more severe at NERF compared with SERF (Table 4.7). By growth stage V4, mesocotyl rot had increased in all fungicide seed treatments evaluated and was comparative to mesocotyl rot observed in the control seedlings at both locations (Table 4.7).

Necrotic reddish to brown discoloration of the crown tissue was symptomatic of crown rot (Fig. 4.11). The crown rot started from the bottom (closest to the mesocotyl) and progressed upwards to the crown and internode tissues (Fig. 4.11). Fungicide seed treatments significantly (P=0.0003) reduced crown rot at all three growth stage assessments, V2, V4 and V6 (Table 4.8).

Late season stalk rot symptoms observed at growth stage R6 included internal discoloration and decay of the basal internodes, and crowns (Fig. 4.12). Maize plants grown from fungicide treated seed had greener stalks and had fewer signs or symptoms of pathogen

colonization at both locations (Table 4.9). Seed treatments significantly reduced stalk rot at SERF (P=0.0342), however at NERF, stalk rot only was reduced (P=0.0281) in Cruiser Extreme 250® treated maize plants (Table 4.9).

Fungicide seed treatments significantly reduced (P<0.0001) the number of root, mesocotyl and crown dissections infected with *Fusarium* spp. at both locations at V2, V4, and V6 growth stages(Figs 4.1, 4.2 and 4.3). At both locations, it was observed that in untreated maize plants, 50 -55 % of roots, mesocotyl and crown dissections were colonized with *Fusarium* spp. at V2 (Figs 4.1 and 4.2), and that colonization increased with time up to 75% at V4. Fungicide seed treatments reduced colonization by *Fusarium* spp. at both locations, however the proportion of seedling tissue dissections from treated seedlings that were colonized with *Fusarium* spp. also increased with time, from 20% at V2 to 40-50% at V4 at both locations (Figs. 4.1 and 4.2). At NERF, other fungal species that were isolated at V2 and V4 were predominantly *Rhizoctonia* spp., *Penicillium* spp. and *Trichoderma* spp. (Figs. 4.1 and 4.2).

At V6 growth stage, dissections were made only from the crown tissues. Fungicide seed treatments significantly reduced (P<0.05) colonization of crowns by *Fusarium* spp. at both locations (Figs 4.3), and *Fusarium* spp. was the predominant fungi in the crown tissues. Crowns of seedlings grown from seed treated with A14918E had the lowest colonization with *Fusarium* spp. at both locations, although this difference was not significant when compared with plants treated with Cruiser Extreme 250® or Vortex®-Trilex FL®-Allegiance FL® (Fig. 4.3).

Regression analysis showed significant relationships at SERF and NERF between maize photosynthetic performance (CF) and root rot severity at V2 and V4 (Figs 4.4 and 4.5). Similarly, at both locations, significant relationships between CF and mesocotyl rot severity

at V2 an SERF and NERF and at V4 in SERF and NERF were detected. A significant relationship was also detected between CF and crown rot index at V2 V4, and V6 (Figs 4.7, 4.8, and 4.9). Overall, trends showed higher disease severity was associated with lower photosynthetic performance.

At both locations, there was a relationship between severity of mesocotyl rot at growth stage V2, and severity of crown rot at V6 growth stage (Fig 4.10). At SERF, a significant relationship between stalk rot severity at growth stage R6 and crown rot severity at growth stage V6 was detected. Lower CF values indicated a higher incidence of *Fusarium* spp. At both locations, it was observed that plants with higher incidence of dissections colonized with *Fusarium* spp. had lower chlorophyll fluorescence values.

Discussion

Fungicide seed treatments improved plant vigor at one of two locations, where planting conditions were wet and cool (<12^{o}C). Baird et al. (1) also reported fungicide seed treatments enhanced maize growth when planted in unfavorable conditions that include low temperatures, since maize optimum planting temperature is between 20^{o}C to 25^{o}C (25).

We demonstrated that CF measurements are related to soil-borne infection of *Fusarium* spp. when no above ground symptoms are evident. Santos et al. (24) also showed a decrease in the photosynthetic rate of maize seedlings infected with *F. moniliforme* (syn. *F. verticillioides*) under controlled environmental conditions. This reduction in photosynthesis might be due to altered chloroplast location and orientation that occurs in maize plants endophytically infected with *F. moniliforme* (syn. *F. verticillioides*) (31). This is the first report, to our knowledge, indicating that *Fusarium* spp. reduce photosynthetic rates under field conditions.

Furthermore, we showed that CF assessments were effective at evaluating fungicide seed treatments and detecting differences between treatments under field conditions. Chlorophyll fluorescence indirectly estimates plants photosynthetic performance and thus can be used as an indicator of plant stress (12). Fungicide seed treatments indirectly benefit maize seedling growth by reducing plant stress associated with *Fusarium* spp. infection and disease development, and thereby maintaining photosynthetic performance.

Prevention of infection by *Fusarium* spp. might be reflected in an improvement of photosynthetic performance. We suspect that higher photosynthetic performance observed in maize plants treated with A14918E was due to thiabendazole, an active ingredient that is very systemic (4) and highly effective against ascomycetes like *Fusarium* spp. (5). However, breakdown compounds of benzimidazoles in plants (5) may contribute to an increase of photosynthesis rate. Maize seedling disease management practices should aim to protect mesocotyl health because this tissue transports water and nutrients to the developing seedling up until growth stage V6, when the nodal roots become physiologically active (9). Maize is susceptible to disease throughout the growing season (30). We have demonstrated that mesocotyl rot severity at V2 has a direct influence on crown rot severity at growth stage V6. Thus management of early season maize disease with fungicide seed treatments can be reflected in a reduction of crown rot.

A reduction in infection and colonization of crown tissues by *Fusarium* spp. by seed treatment fungicides also contributed to a lower incidence of stalk rot disease at R6 at one location. So, although fungicide active ingredients have a limited period of activity (14), their beneficial effects were still evident even at harvest in our trials. Nevertheless, to conclusively prove that seed treatment fungicides reduce stalk rot, additional years of research under varying growing conditions must be done since stalk rot development is complex and several

mitigating factors are known to play a role in disease development. Kommedahl et al. (10) tried unsuccessfully to show that infection of maize roots and mesocotyl with *Fusarium* spp. early in the season could lead to stalk rot. However higher levels of *Fusarium* spp. in the stalks versus the roots confounded the relationship. More recently, Munkvold et al. (7) showed maize pests such as the corn stalk borer (*Ostrinia nubilalis),* significantly contribute to *Fusarium* spp. colonization of maize stalks, and that *Bt* traits reduce *Fusarium* spp. colonization in maize stalks, presumably by reducing insect damage that favors infection by *Fusarium* spp.

In our trials, only one fungicide seed treatment, Cruiser Extreme 250®, contained an insecticide active ingredient thiamethoxam. This insecticide seed treatment offered little benefit, in terms of reduced disease severity or decreased infection by *Fusarium* spp., compared to the other fungicide only seed treatments tested. However, since we did not evaluate insect pressure at either site we are unable to establish if our data are a result of low insect pressure. There are reports that suggest a combination of fungicide and insecticide active ingredients in a seed treatment enhance the benefit of the seed treatments (27). Additional controlled studies are needed to further investigate these reports.

Overall, CF proved to be an objective and highly effective method of evaluating seed treatment fungicides in the field. However, there are some important factors of this technology that need to be considered to ensure accurate CF measurements are taken. The amount of light, time of day, and leaf position, and to a lesser extent, maize genotype and susceptibility to soil-borne pathogens can each affect assessments. Since most commercial maize seed treatments contain a combination of active ingredients, it is difficult to assess the benefit of each ingredient on *Fusarium* spp. infection and maize physiology. The effects of individual fungicide active ingredients on soil borne inoculum of *Fusarium* species that are

associated with seedling blights, such as *F. graminearum, F. subglutinans*, and *F. verticillioides* would greatly contribute to our knowledge on the pathology behind seed treatments and additional benefits associated with their use.

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Table 4.1. Fungicide seed treatments rate used in field experiment.

Fungicide	Active ingredient	Chemical name	Rate[z]
A14918E	fludioxonil (40.3%)	4-(2,2-difluoro-1,3-bensodioxol-4-yl)-1H-pyrrole-3-carbonitrile	2.5
	mefenoxam (8.4%)	(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester	2.0
	azoxystrobin (9.6%)	methyl (E)-2--3-methoxyacrylate	1.0
	thiabendazole(42.9%)	(2-(thiazol-4-yl) benzimidazole	20.0
Cruiser Extreme 250®	fludioxonil (40.3%)	4-(2,2-difluoro-1,3-bensodioxol-4-yl)-1H-pyrrole-3-carbonitrile	2.5
	azoxystrobin (9.6%)	methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	1.0
	thiamethoxam	4H-1,2,5-Oxadiazin-4-imine,3-[(2-chloro-5-thiazolyl) methyl]tetrahydro-5-methyl-N-nitro-	62.5
	mefenoxam (8.4%)	(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester	2.0
Vortex®-Trilex FL®-Allegiance FL®	trifloxystrobin (22%)	Methyl (E)-methoxyimino-{(E)-α-[1-(α, α, α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate	10.0
	ipconazole (40.7%)	(1RS,2RS,5RS;1RS,2RS,5RS)-2-(4chlorobenzyl)-5-isopropyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	2.5
	metalaxyl (28.35%)	N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)alanine, methyl ester	28.0

[z] g a.i. / 100 kg.

Table 4.2. Cumulative precipitation and average temperature from each assessment interval done at V1, V2, V4 and V6 growth stages at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

	V2		**V4**		**V6**		**Total**	
	NERF	SERF	NERF	SERF	NERF	SERF	NERF	SERF
Precipitation (mm)[z]	3.4	48.77	106.7	59.9	103.3	102.1	213.4	210.7
Temperature (^{o}C)[y]	17.1	18.3	19.3	20	22.8	22	19.3	19

[z] Cumulative precipitation from planting until respectively growth stage.
[y] Average temperature from planting until each growth stage.

Table 4.3. Table of fungicide seed treatments (trt), date, location (loc) and respective interactions on root disease index (RDI), mesocotyl disease index (MDI), crown disease index (CDI), stalk rot (SR), height (PH), chlorophyll fluorescence (CF), and occurrence of *Fusarium* spp. on sampled maize plants at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, IA.

Source	**RDI**	**MDI**	**CDI**	**SR**	**PH**	**CF**	***Fusarium* spp.**
loc	0.3833	0.9302	0.5219	0.1066	0.9924	0.6659	0.0279
trt	<0.0001	0.0298	0.0003	0.0182	<0.0001	<0.0001	<0.0001
date	0.0538	<0.0001	0.8944	---	<0.0001	<0.0001	<0.0001
date x trt	<0.0001	0.0095	0.8117	---	0.01	<0.0001	<0.0001

Table 4.4. Mean plant height (cm) of maize seedlings at V4 and V6 growth stages grown from untreated or seed treated with A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl), and planted at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

Location	Treatment	V4	V6
SERF	A14918E	11.9 a[z]	108.6 a
	Cruiser Extreme 250®	12.2 a	114.6 a
	Vortex®-Trilex FL®-Allegiance FL®	12.5 a	110.9 a
	Untreated	8.5 b	83.0 a
	HSD (P<0.05)	2.8	NS[y]
NERF	A14918E	13.9 a	103.4 a
	Cruiser Extreme 250®	13.9 a	104.9 a
	Vortex®-Trilex FL®-Allegiance FL®	14 .0a	108.9 a
	Untreated	12.1 a	99.8 a
	HSD (P<0.05)	NS	NS

[z] Values from the same location followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.
[y] Non significant.

Table 4.5. Chlorophyll fluorescence (CF) at V2, V4 and V6 growth stages of maize seedlings grown from untreated or seed treated with A14918E (fludioxonil + azoxystrobin + thiabendazole+ metalaxyl), Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), or Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + mefenoxam), and planted at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

Location	Treatment	V2	V4	V6
SERF	A14918E	0.370 a[z]	0.641 a	0.620 a
	Cruiser Extreme 250®	0.316 a	0.540 b	0.532 b
	Vortex®-Trilex FL®-Allegiance FL®	0.315 a	0.554 b	0.553 b
	Untreated	0.209 b	0.259 c	0.291 c
	HSD (P<0.05)	0.069	0.086	0.073
NERF	A14918E	0.613 a	0.640 a	0.637 a
	Cruiser Extreme 250®	0.513 b	0.489 b	0.544 b
	Vortex®-Trilex FL®-Allegiance FL®	0.504 b	0.529 b	0.537 b
	Untreated	0.223 c	0.246 c	0.229 c
	HSD (P<0.05)	0.063	0.126	0.081

[z] Values from the same location followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.

Table 4.6. Mean root rot index of maize seedlings grown from untreated seed or seed treated with either A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), or Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl). The trial was planted at two locations, Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. A total of 20 maize seedlings per treatment were sampled at growth stages V2 and V4.

Location	Treatment	V2	V4
SERF	A14918E	1.0 [z] b[y]	2.4 a
	Cruiser Extreme 250®	0.1 c	2.6 a
	Vortex®-Trilex FL®-Allegiance FL®	0.4 c	2.4 a
	Untreated	2.0 a	2.6 a
	HSD (P<0.05)	0.6	NS[x]
NERF	A14918E	1.1 b	1.7 a
	Cruiser Extreme 250®	1.5 b	2.0 a
	Vortex®-Trilex FL®-Allegiance FL®	1.4 b	2.0 a
	Untreated	2.2 a	1.6 a
	HSD (P<0.05)	0.8	NS

[z] Mean root disease index was estimated using the following scale (26) where 0 = apparently healthy roots, 1 = <25% of roots with disease rot symptoms, 2 = 25-49% of roots rotted, 3 = 50-74%, of the roots rotted 4 = 75 % or greater of the roots rotted, and 5 = wilted or dead seedlings.

[y] Values from the same location and column followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.

[x] Non significant.

Table 4.7. Mean mesocotyl rot index of maize seedlings grown from untreated seed or seed treated with A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), or Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl). The trial was planted at two locations, Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. A total of 20 maize seedlings per treatment were sampled at growth stages V2 and V4.

Location	Treatment	V2	V4
SERF	A14918E	0.3 [z] b[y]	2.0 a
	Cruiser Extreme 250®	0.3 b	2.1 a
	Vortex®-Trilex FL®-Allegiance FL®	0.4 b	2.0 a
	Untreated	1.1 a	2.7 a
	HSD (P<0.05)	0.4	NS[x]
NERF	A14918E	0.7 b	1.4 a
	Cruiser Extreme 250®	0.8 b	1.4 a
	Vortex®-Trilex FL®-Allegiance FL®	0.8 b	1.6 a
	Untreated	2.2 a	2.0 a
	HSD (P<0.05)	1.1	NS

[z] Mean mesocotyl disease index was estimated using a modified version of Soonthornpoct et al. (26) disease scale where 0 = no symptoms visible, 1 = <25% of the tissue showing disease rot symptoms, 2 = 25-49% of the tissue rotted, 3 = 50-74% of the tissues rotted, 4 = 75 % or greater of the tissue rotted, and 5 = completely rotted tissue.
[y] Values from the same location and column followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.
[x] Non significant.

Table 4.8. Mean crown rot index of maize seedlings grown from untreated seed or seed treated with A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), or Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl). The trial was planted at two locations, Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. A total of 20 maize seedlings per treatment were sampled at growth stages V2, V4 and V6.

Location	Treatment	V2	V4	V6
SERF	A14918E	1.2 [z] b[y]	1.2 b	1.1 b
	Cruiser Extreme 250®	1.3 b	1.3 b	1.2 b
	Vortex®-Trilex FL®-Allegiance FL®	1.2 b	1.1 b	1.1 b
	Untreated	1.5 a	2.0 a	2.6 a
	HSD (P<0.05)	0.2	0.5	0.7
NERF	A14918E	1.4 b	1.0 b	1.5 b
	Cruiser Extreme 250®	1.3 b	1.0 b	1.5 b
	Vortex®-Trilex FL®-Allegiance FL®	1.3 b	1.0 b	1.4 b
	Untreated	2.1 a	1.8 a	2.8 a
	HSD (P<0.05)	0.4	0.6	1.1

[z] Mean crown disease index was estimated using a modified version of Soonthornpoct et al. (26) disease scale where 0 = no symptoms visible, 1 = <25% of the tissue showing disease rot symptoms, 2 = 25-49% of the tissue rotted, 3 = 50-74% of the tissues rotted, 4 = 75 % or greater of the tissue rotted, and 5 = completely rotted tissue.
[y] Values from the same location and column followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.

Table 4.9. Mean stalk rot index in maize plants at R6 growth stage. Maize plants were grown from untreated seed or seed treated with A14918E (fludioxonil + azoxystrobin + thiabendazole + mefenoxam), Cruiser Extreme 250® (fludioxonil + azoxystrobin + thiamethoxam + mefenoxam), Vortex®-Trilex FL®-Allegiance FL® (ipconazole + trifloxystrobin + metalaxyl) and planted at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA. Results are based on visual assessments made to n=30 plants per treatment at each location.

Location	Treatment	R6
SERF	A14918E	2.0 [z] b[y]
	Cruiser Extreme 250®	2.0 b
	Vortex®-Trilex FL®-Allegiance FL®	2.3 b
	Untreated	3.6 a
	HSD (P<0.05)	1.2
NERF	A14918E	2.3 a
	Cruiser Extreme 250®	1.8 b
	Vortex®-Trilex FL®-Allegiance FL®	2.2 a
	Untreated	2.7 a
	HSD (P<0.05)	0.8

[z] Stalk rot disease severity was assessed using University of Illinois (0-5) stalk rot rating scale.
[y] Values from the same location and column followed by the same letter are not significantly different, according to the Tukey's HSD test (P< 0.05) performed by location.

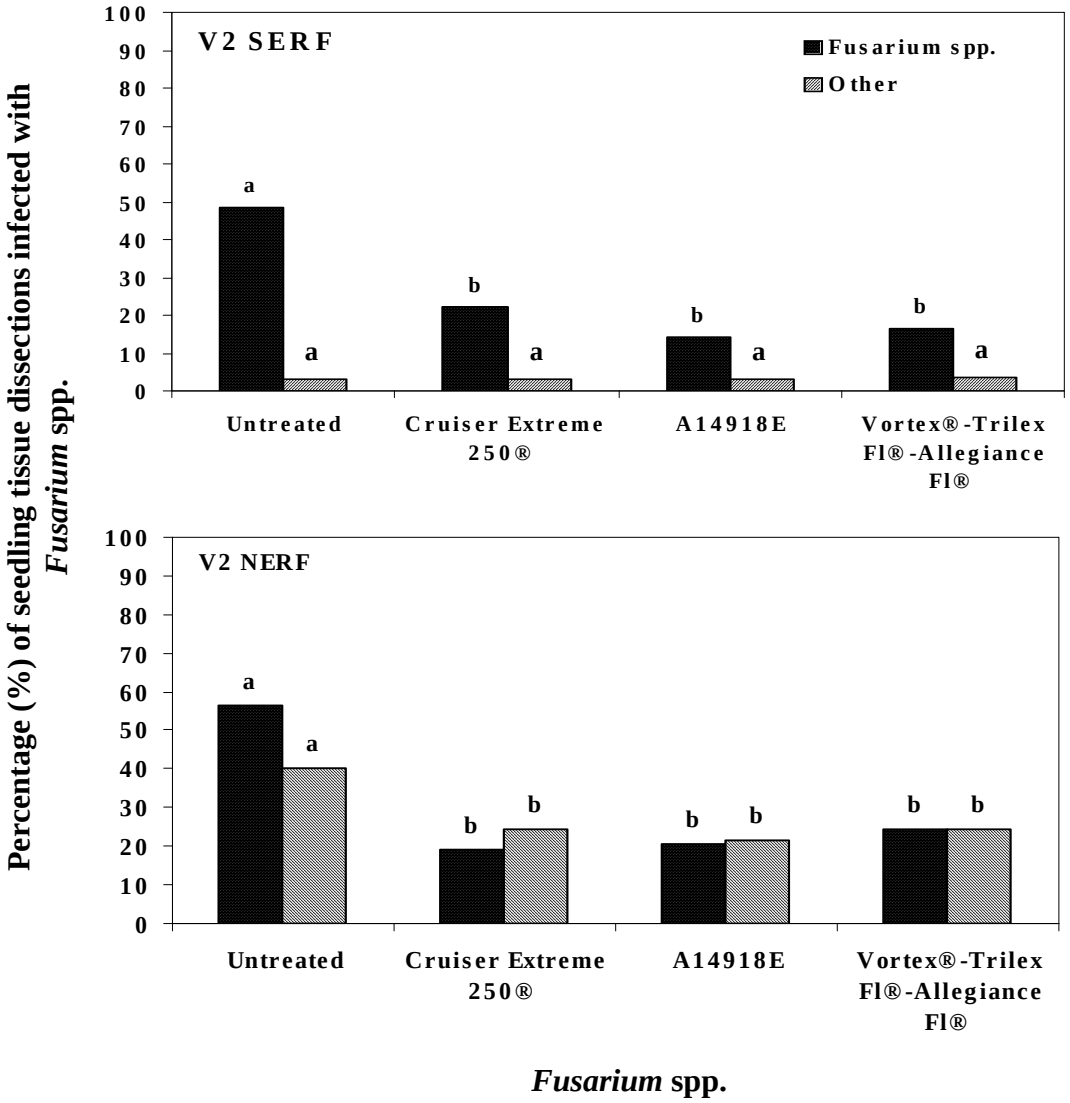

Figure 4.1. Percentage (%) of root, mesocotyl and crown tissue dissections that were infected with *Fusarium* spp., or infected with other fungi in maize seedlings sampled at growth stage V2 at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, IA. and at Iowa State University Northeast Research Farm (NERF) near Nashua, IA. For each fungal type, columns with the same letter are not significantly different, according to the Tukey's HSD test ($P< 0.05$).

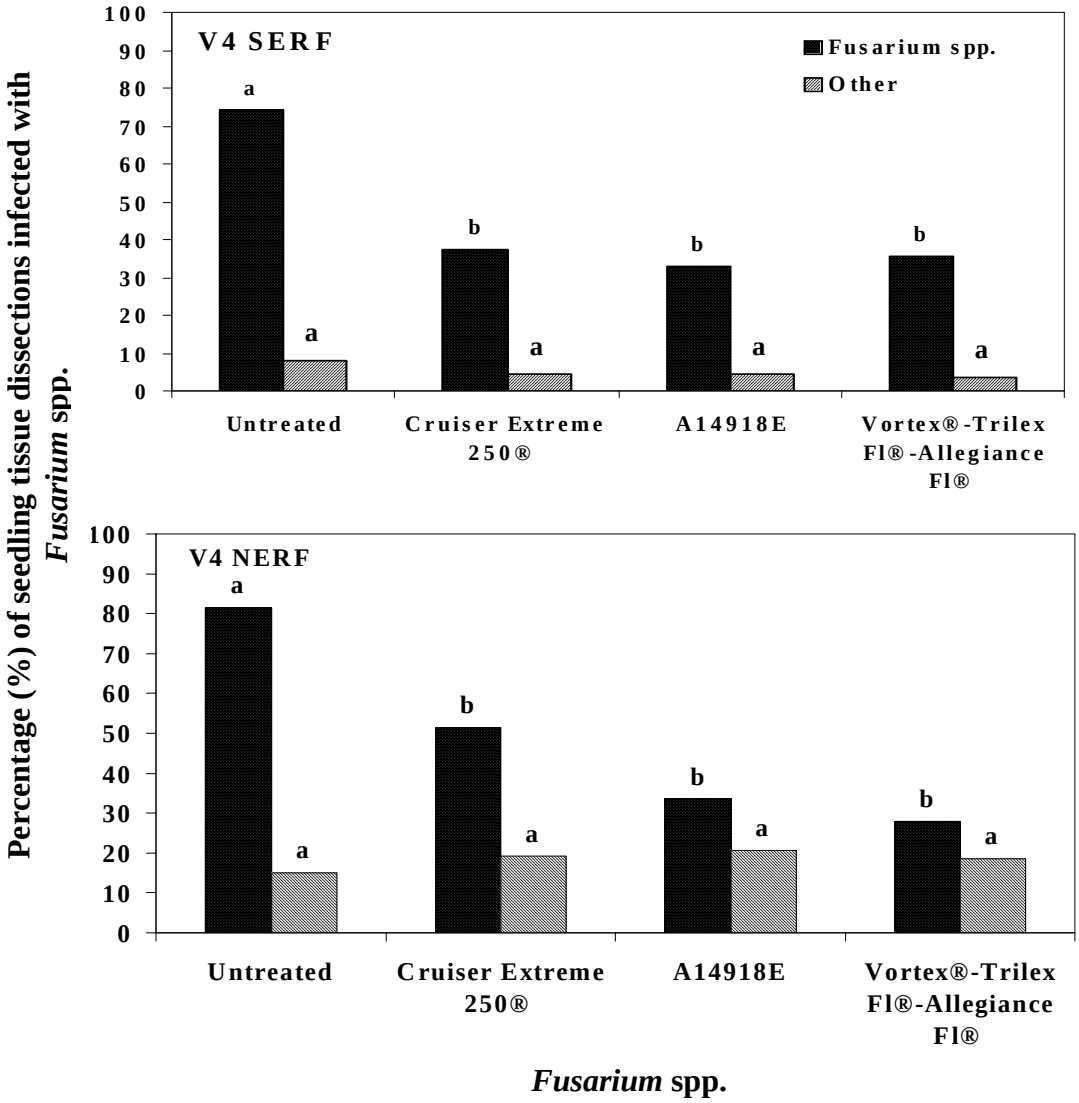

Figure 4.2. Percentage (%) of root, mesocotyl and crown tissue dissections that were infected with *Fusarium* spp., or infected with other fungi in maize seedlings sampled at growth stage V4 at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, IA and at Iowa State University Northeast Research Farm (NERF) near Nashua, IA. For each fungal type, columns with the same letter are not significantly different, according to the Tukey's HSD test ($P < 0.05$).

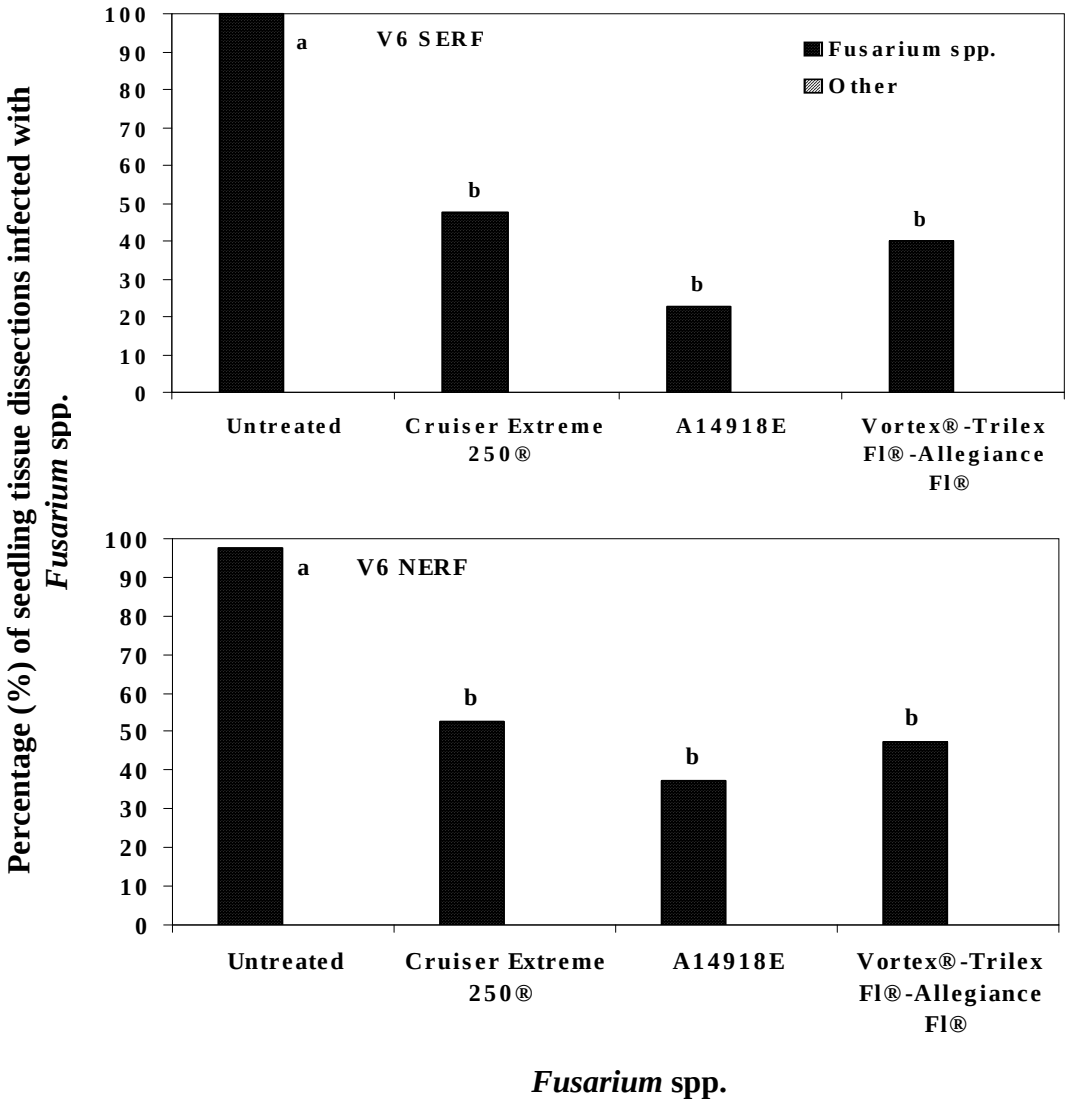

Figure 4.3. Percentage (%) of crown tissue dissections that were infected with *Fusarium* spp., or infected with other fungi in maize seedlings sampled at growth stage V6 at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, IA. and at Iowa State University Northeast Research Farm (NERF) near Nashua, IA. For each fungal type, columns with the same letter are not significantly different, according to the Tukey's HSD test ($P< 0.05$).

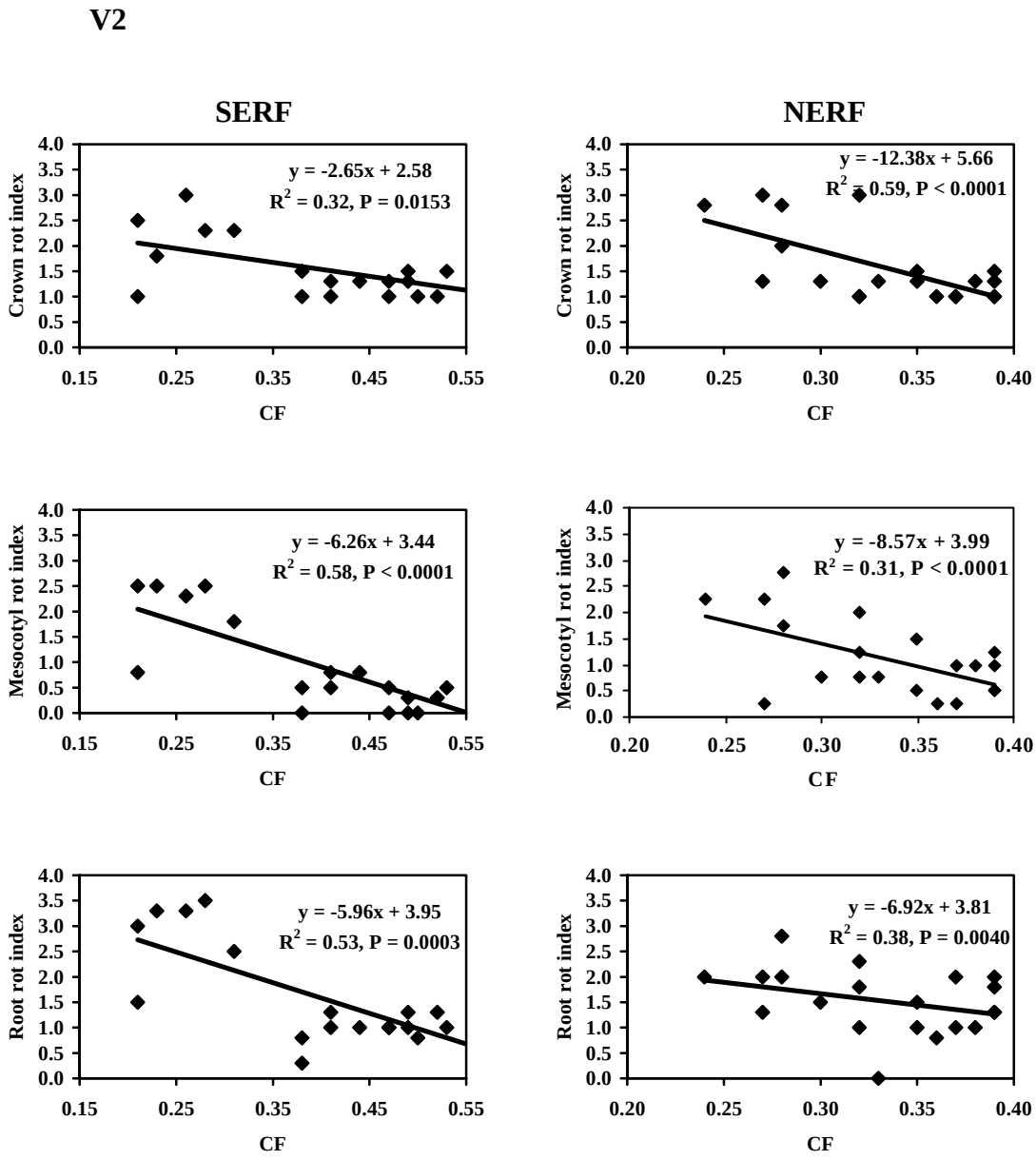

Figure 4.4. Relationship between chlorophyll fluorescence (CF), crown rot index, mesocotyl rot index, and root rot index at V2 growth stage at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

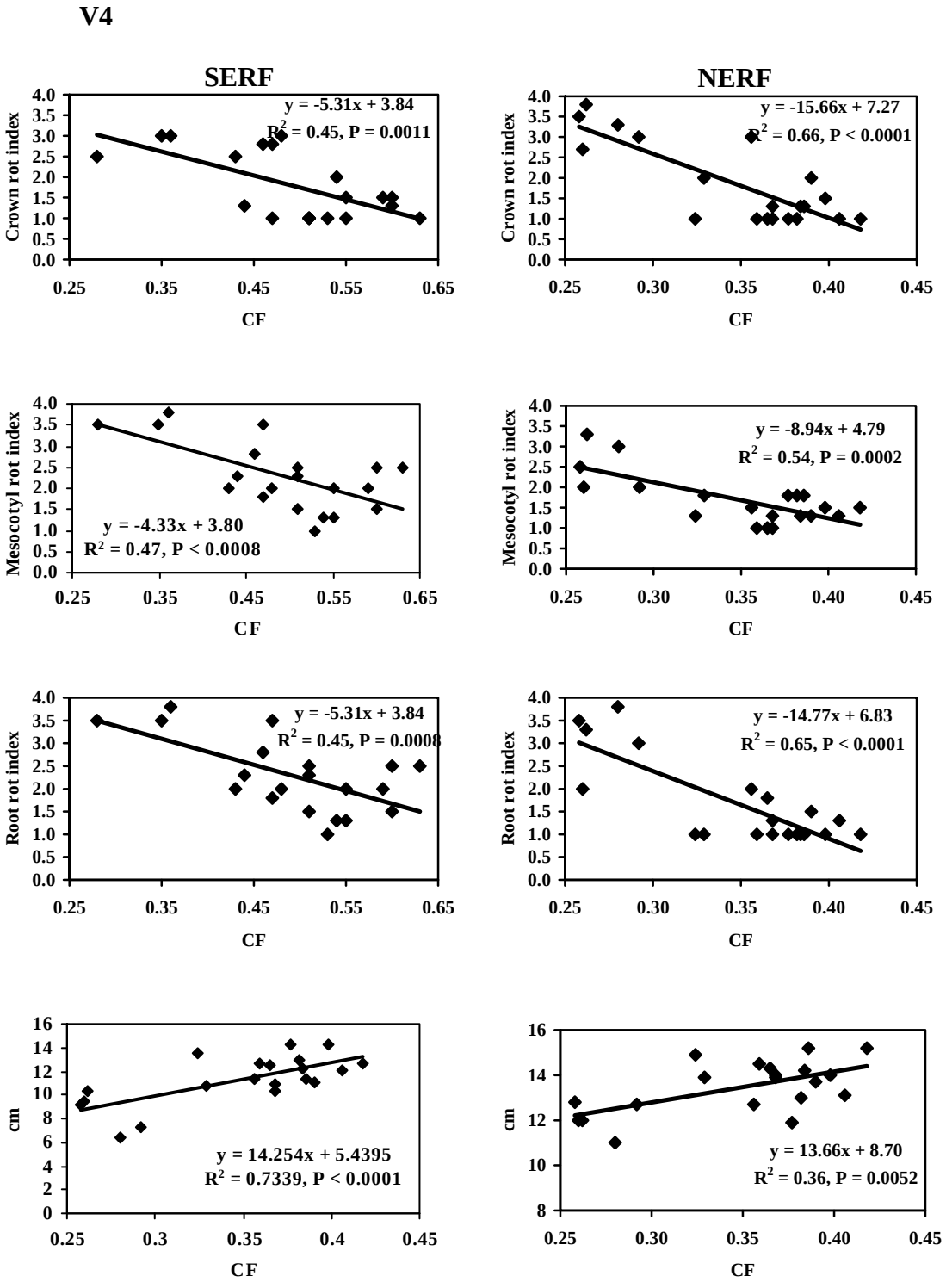

Figure 4.5. Relationship between CF, crown disease index, mesocotyl disease index, and crown disease index and plant height at V4 at SERF and NERF.

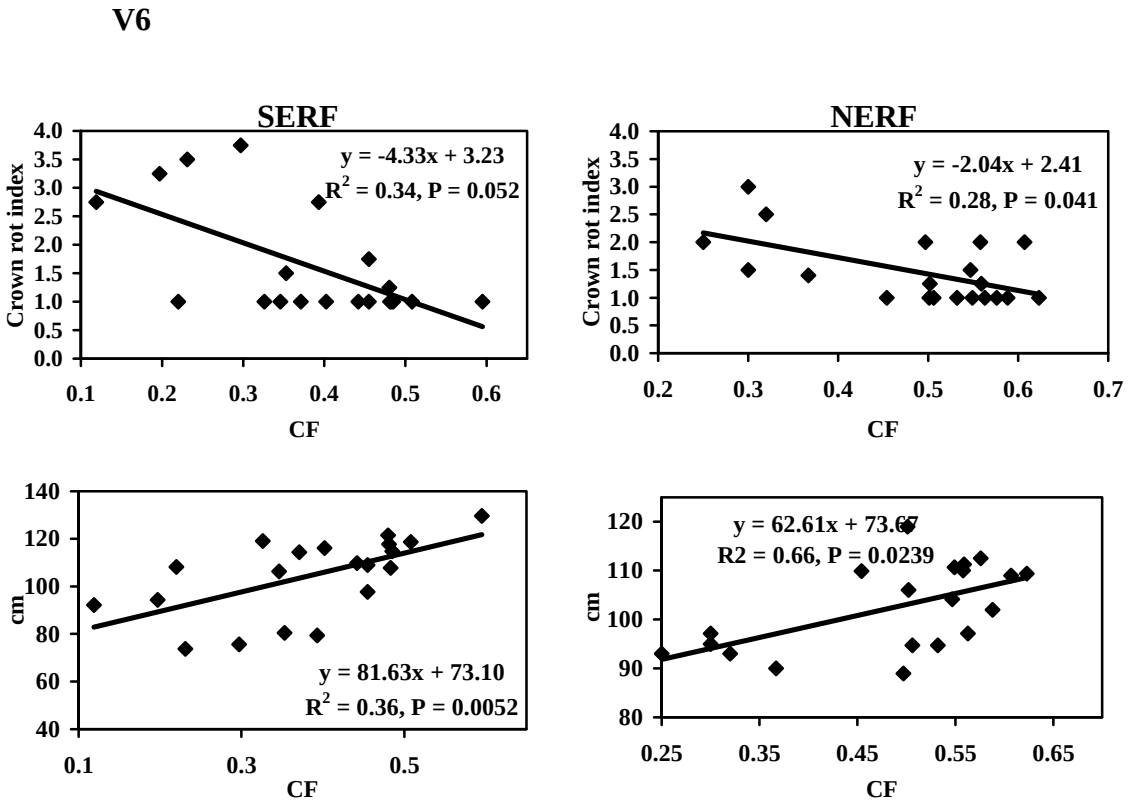

Figure 4.6. Relationship between chlorophyll fluorescence (CF), crown disease index, and plant height at V6 growth stage at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

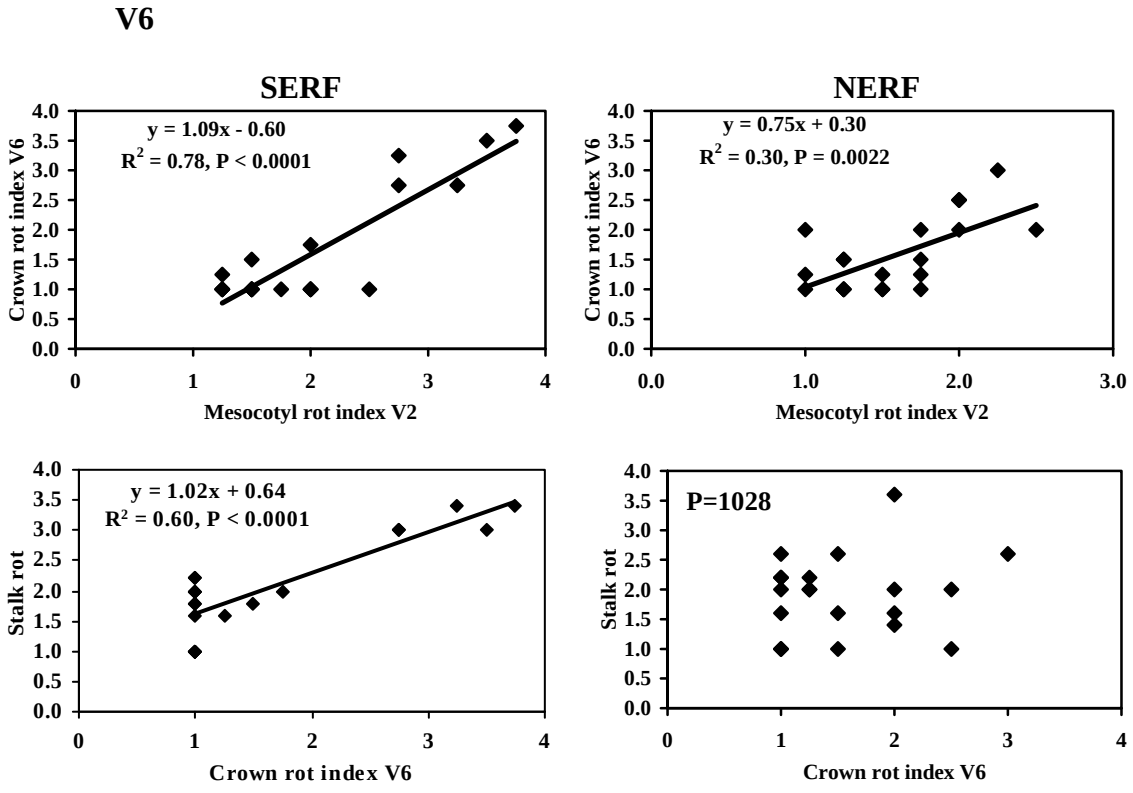

Figure 4.7. Relationship between mesocotyl disease severity at V2 growth stage and severity of crown rot at V6. Relationship between severity of crown rot at V6 growth stage and disease severity of stalk rot at R6 growth stage. Relationships were made from disease data obtained from the experiments planted at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

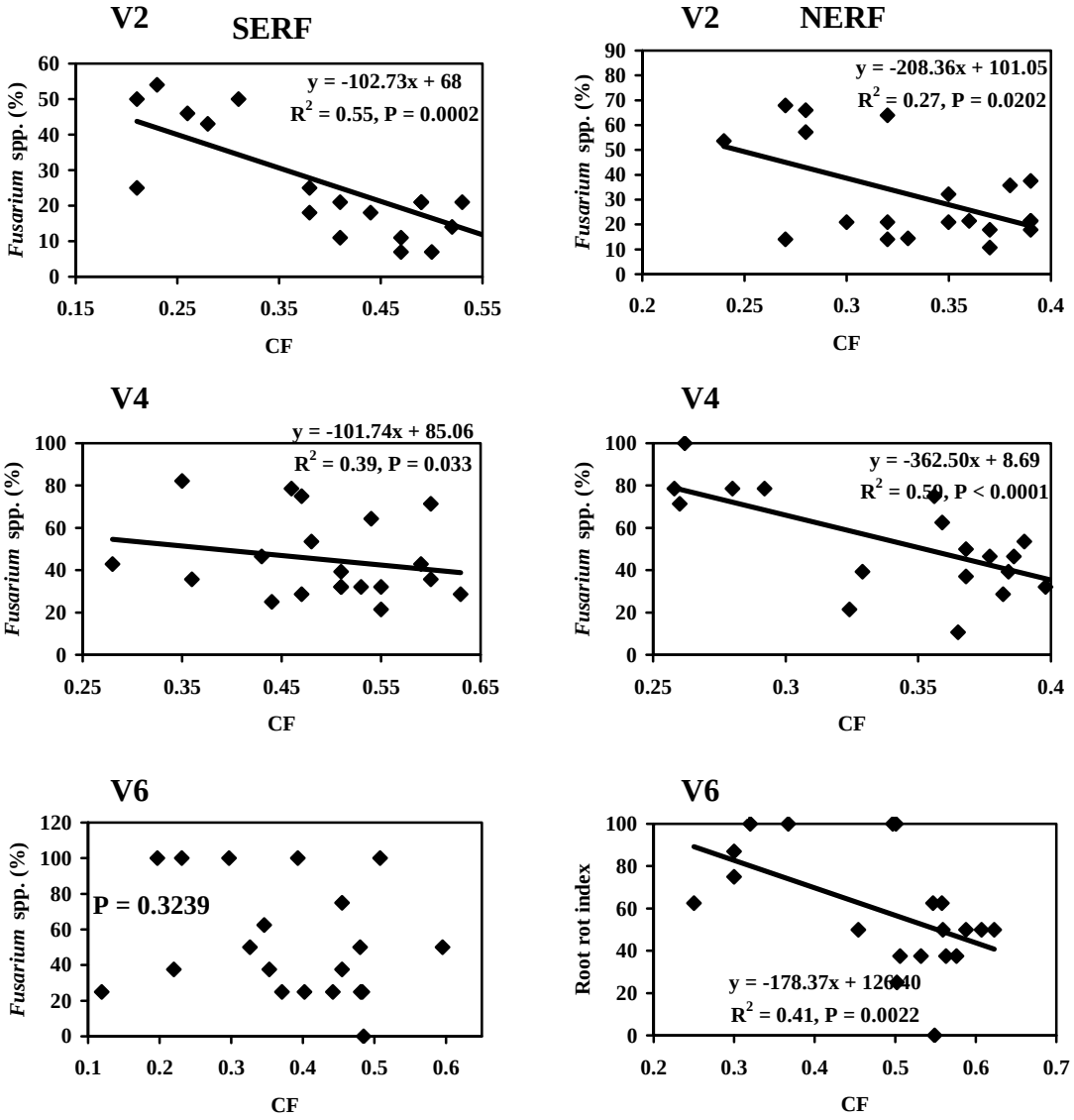

Figure 4.8. Relationship between chlorophyll fluorescence (CF) and incidence of *Fusarium* spp. at V2, V4 and V6 growth stage at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, and Iowa State University Northeast Research Farm (NERF) near Nashua, IA.

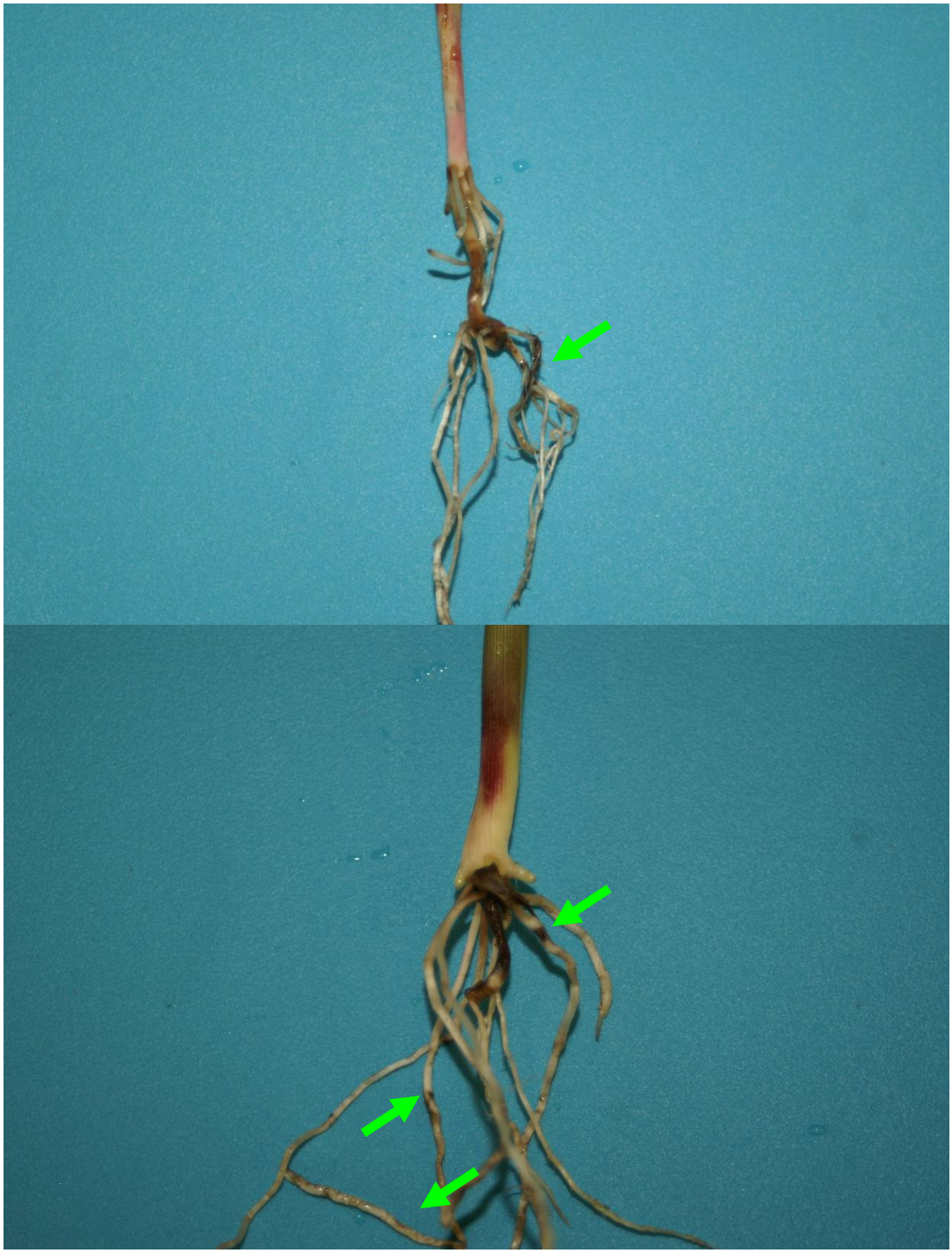

Figure 4.9. Root disease lesions observed on maize seedlings.

Figure 4.10. Mesocotyl disease lesions observed on maize seedlings.

Figure 4.11. Crown and pith disease lesions observed in maize seedlings.

Figure 4.12. Stalk rot lesions observed in maize plants grown from fungicide seed treated seed (a) and plants grown from non-treated seed (b). Maize plants were sampled at R6 growth stage at Iowa State University Southeast Research Farm (SERF) near Crawfordsville, IA.

CHAPTER 5. GENERAL CONCLUSIONS

Current fungicide seed treatments did not significantly affect seed-borne *Fusarium verticillioides* inoculum. However newer active ingredients that are expected to be included in seed treatment premixes by 2010, namely ipconazole, thiabendazole, and triticonazole, are able to significantly reduce and suppress seed-borne inoculum of *F. verticillioides*. The data we have generated will be of importance to seed companies, as it will enable them to select fungicide seed treatments that are more effective against *F. verticillioides*. Additional active ingredients with different modes of action in fungicide seed treatments will also prevent resistance build-up by *F. verticillioides* and other *Fusarium* spp.

Although our efforts were oriented in trying to suppress transmission of seed-borne *F. verticillioides* with fungicide seed treatments, we found that maize seedlings infected with *F. verticillioides* strain TXI-79 can show more vigor than non-infected, untreated seedlings. However, the contribution of *Fusarium* spp. mycotoxins in human and animal health are of significant importance, therefore management practices should be aimed at eradicating seed-borne *F. verticillioides*.

The contribution of this research to the pathology of fungicide seed treatments under field conditions is significant. In the scientific community, length of the effective period of fungicide seed treatments has always been questioned. In this study, we showed that the benefits of fungicide seed treatments in reducing infection and colonization of maize seedlings by *Fusarium* spp. are still evident at growth stage V6 in crown tissues. We have also shown that benefits of fungicide seed treatments go beyond just reducing seedling

blights and increasing stand and plant vigor, they also play some part in reducing later season crown and stalk rot. Still, crown and stalk rot are disease complexes and environmental conditions, pests, and maize genotype also contribute to disease development.

We also showed that infection by *F. graminearum* was more prevalent early in the growing season (up to V2), but then infection by *F. verticillioides* and *F. subglutinans* became predominant at our locations. This is an important contribution to the understanding of the epidemiology of soil-borne *Fusarium* spp. and how they interact with fungicide seed treatments.

We have also shown, for the first time, the benefits of fungicide seed treatments on maize physiology by the estimating photosynthetic performance using chlorophyll fluorescence. We found that due to application of fungicide seed treatments, plants grown from treated seed had higher photosynthetic rates than plants grown from untreated seed. Presumably higher rates of photosynthesis translate to less stressed plants and a greater chance of attaining yield potential. Significant relationships between plant height and disease severity prove that CF can be used as an alternative, non-destructive method to estimate disease severity cause by soil-borne diseases in maize.

Future research

Future research efforts for management of seed-borne inoculum should investigate the effectiveness of fungicide seed treatments against *F. graminearum*, *F. proliferatum* and *F. subglutinans*. Since we found a high incidence of seed-borne *Penicillium* spp. in commercial maize seed, it is recommended that the effectiveness of fungicides against this pathogen are also verified.

Further studies to increase our understanding of the *Fusarium*-maize interaction in presence and absence of fungicide seed treatments are recommended. This research showed

that fungicide seed treatments are effective against seed-borne *F. verticillioides*, but we did not investigate the importance of seed-borne inoculum to development of disease in the field.

Endophytic infection from seed-borne inoculum has been demonstrated by numerous researchers. In our field study we were able to show that apparently healthy maize plants can be infected with *Fusarium* spp. Future studies that address the endophytic/pathogenic interaction between maize and *Fusarium* spp. would contribute to our knowledge of this pathosystem. Modern molecular tools like Real Time Polymerase Chain Reaction (RT-PCR), would enable the quantification of systemic inoculum to be estimated and correlated with disease expression on seed inoculated with increasing concentrations of *Fusarium* spp.

Our quantitative technique to estimate *Fusarium* spp. colonization showed that infection of maize by *Fusarium* spp. varies with time and within tissues of maize. RT-PCR is a tool that could be used to further investigate this finding since it could be used to more accurately quantify each *Fusarium* specie in maize tissues.

In addition, the field studies done with chlorophyll fluorescence looked at the effect of fungicide seed treatment against all *Fusarium* spp., however it would be of interest to look at the effect of each species individually and determine how significantly each species reduces photosynthetic performance of maize. One approach to look at the effect of different species on photosynthetic performance could be planting maize seeds in pots inoculated with each *Fusarium* spp., and then make plant height, chlorophyll fluorescence assessments to determine plant vigor. Also, isolations could be useful to determine if colonization took place.

The effects of seed treatments on maize physiology need further investigation. Unfortunately, we were only able to conduct trials at two locations in one year. Questions and hypotheses remain that need to be verified and tested, for example, is enhanced

photosynthesis a result of decreased infection and colonization of maize seedlings by fungi, or breakdown products of active ingredients used as seed treatments? In these experiments, one treatment had an insecticide active ingredient, which can also contribute to a beneficial-synergism effect against pathogen colonization and probably in photosynthetic performance. A study to compare soil-borne fungi colonization in presence and absence of an insecticide seed treatment should be performed. Again, RT-PCR would provide more accurate information about systemic colonization with the target pathogen.

More importantly, modern maize crop production uses a wide diversity of genotypes, which probably vary in their susceptibility to infection by soil-borne pathogens. It is likely that some hybrids are more susceptible to *Fusarium* spp. colonization than others; therefore maize genotype should be another factor that needs to be considered for future research. An experiment with different maize genotypes, planted in soils inoculated with one or several soil-borne *Fusarium* spp. to compare colonization dynamics might provide a better insight of the role of genetics in soil-borne pathogen colonization.

Finally, this research showed some evidence that use of fungicide seed treatments may reduce late season crown and stalk rot reduction. The incidence and severity of stalk rot between treated and untreated maize plants would provide more knowledge of the real contribution of seed treatments to the prevention of stalk rot under controlled environmental conditions. Fungicide seed treated maize plants could be grown in greenhouse conditions, and then inoculated with pathogenic *Fusarium* spp. at R6. If there is still some fungicide seed treatment left in the crown or stalks tissues at V6 and R6 growth stages, incidence and severity of stalk rot should be lower in maize plants grown from fungicide treated seed.

APPENDIX A. BENEFITS OF FUNGICIDE SEED TREATMENT ON MAIZE ESTABLISHMENT AND YIELD AT CRAWFORDSVILLE IOWA, 2007

A paper submitted to Plant Disease Management Reports

MAIZE (*Zea mays* L.)
Seedling blights; *Fusarium* spp.

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Benefits of fungicide seed treatment on maize establishment and yield at Crawfordsville Iowa, 2007

Three maize seed treatments were evaluated for their effectiveness on stand, root, mesocotyl and crown disease severity, and yield of maize hybrid Garst 8545. Untreated seed served as a control. The trial was planted at Iowa State University Southeast Research and Demonstration Farm, near Crawfordsville, Iowa on 19 Apr and soil temperature at 4 in. depth was 50° F. Soil was chisel plowed in fall, 2006 and disk/field cultivated prior to planting. Preemergence herbicide (Dual II Magnum, 64 fl oz/A) and starter fertilizer (175 lbs/A N of anhydrous ammonia 82.5-0-0) were applied prior to planting. Cultivation was used for post emergence weed management. The experimental design was a randomized block design with 4 treatments and 5 blocks for a total of 20 plots. Each main plot was 8 rows wide (30-in. row spacing) by 17.4 ft long and planted with an Almaco 4 row planter calibrated to plant at 35 000 seeds/A. Stand counts on the two middle rows of each plot were done at V2, V4, V6 growth stages and data was extrapolated to maize plants /A. Disease assessments were made on 4 seedlings selected at random from each treatment at V6; root, mesocotyl and crown rot were evaluated. The inner two rows of each plot were harvested on 25 Sep at Crawfordsville with John Deere 4400 modified plot combine and yield was adjusted at 15.5% moisture.

Cold (50°F) and wet soil conditions at planting severely impacted maize establishment. Fungicide seed treatments significantly improved ($P<0.05$) stand establishment as indicated by significantly higher stand count numbers at V2, V4 and V6, but there were no significant differences ($P>0.05$) in the plant population between the three fungicide seed treatments. All fungicide seed treatments reduced crown rot severity at V4. Grain yields of maize grown from treated seed were statistically greater ($P<0.05$). No phytotoxicity was observed for any treatment.

Treatment, rate [z]	Plant Stand[y]			Disease severity[x] V4			Yield (bu/A)[w]
	V2	V4	V6	Root[w]	Mesocotyl	Crown	25 Sept
Untreated ……...……..	400 b[v]	13700 b	14600 b	2.4 a	2.7 a	2.6 a	112 b
Cruiser Extreme 250, 12.5 ……	4600 a	32000 a	31800 a	2.6 a	2.1 a	1.5 b	198 a
A14918E, 25.5 …….	4400 a	30800 a	31700 a	2.4 a	2.0 a	1.5 b	193 a
Vortex, 2.5 + Trilex FL, 10 + Allegiance FL, 28 ……...	2700 a	31500 a	31300 a	2.6 a	2.0 a	1.4 b	187 a

[z] g a.i./ 100 kg seed.
[y] plants /A.
[x] Disease severity scale: 0 = no symptoms visible, 1 = <25% of the tissue showing disease rot symptoms, 2 = 25-49% of the tissue rotted, 3 = 50-74% of the tissues rotted, 4 = 75 % or greater of the tissue rotted, and 5 = completely rotted tissue.
[w] bu/A @ 15.5% moisture.
[v] Column numbers followed by the same letter are not significantly different at $P<0.05$ as determined by Tukey's multiple comparison statistic test.

APPENDIX B. BENEFITS OF FUNGICIDE SEED TREATMENT ON MAIZE ESTABLISHMENT AND YIELD AT NASHUA IOWA, 2007

A paper submitted to Plant Disease Management Reports

MAIZE (*Zea mays* L.)
Seedling blights; *Fusarium* spp.

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Benefits of fungicide seed treatment on maize establishment and yield at Nashua Iowa, 2007

Three maize seed treatments were evaluated for their effectiveness on stand, root, mesocotyl and crown disease severity, and yield of maize hybrid Garst 8545. Untreated seed of the same hybrid was used as a check. The trial was planted at Iowa State University Northeast Research and Demonstration Farm at Nashua, Iowa. Planting was done 1 May, and soil temperature at 4 in. depth was 60° F. Soil was chisel plowed in fall, 2006 and disk/field cultivated before planting. Starter fertilizer (urea ammonium nitrate 28-0-0; 180 lbs/A N) was applied. Preemergence herbicide (Harness Xtra, 44 fl oz/A) cultivation was used for secondary weed management. The experimental design was a randomized block design with 4 treatments and 5 blocks for a total of 20 plots. Each main plot was 8 rows wide (30-in. row spacing) by 17.4 ft long and planted with an Almaco 4 row planter calibrated to plant at 35 000 seeds/A. Stand counts on the two middle rows of each plot were done at V2, V4, V6 growth stages and data was extrapolated to maize plants /A. Disease assessments were made on 4 seedlings selected at random from each treatment at V4; root, mesocotyl and crown rot were evaluated. The inner two rows of each plot were harvested on 10 Oct with John Deere 4400 modified plot combine and yield was adjusted at 15.5% moisture.

Cool (60°F) and wet soil conditions at planting impacted maize establishment. The plant population of maize grown from fungicide treated seed at V2, V4 and V6 was significantly greater ($P<0.05$) than the plant population of maize grown from untreated seed at each assessment date. There was no difference ($P>0.05$) in the plant population between the three fungicide seed treatments. Lower crown rot severity was recorded in plants grown from treated seed. Maize grown from untreated seed yielded less than treated maize seed but there were no significant differences in yield between treatments. None of the plants grown from treated seed showed symptoms of phytotoxicity.

Treatment, rate[z]	Plant Stand[y]			Disease severity[x] V4			Yield (bu/A)[w]
	V2	V4	V6	Root	Mesocotyl	Crown	10 Oct
Untreated ……..……..….	17900 b[v]	29000 b	30000 b	1.6 a	2.0 a	2.8 a	178 a
Cruiser Extreme 250, 12.5 ..……..	25100 a	31800 a	31900 a	2.0 a	1.4 a	1.5 b	188 a
A14918E, 25.5 …….....	23800a	30000 a	31400 a	1.7 a	1.4 a	1.5 b	187 a
Vortex, 2.5 + Trilex FL, 10 + Allegiance FL, 28 ……..…..	27600 a	31800 a	31900 a	2.0 a	1.6 a	1.4 b	180 a

[z] g a.i./ 100 kg seed.
[y] plants /A.
[x] Disease severity scale: 0 = no symptoms visible, 1 = <25% of the tissue showing disease rot symptoms, 2 = 25-49% of the tissue rotted, 3 = 50-74% of the tissues rotted, 4 = 75 % or greater of the tissue rotted, and 5 = completely rotted tissue.
[w] bu/A @ 15.5% moisture.
[v] Column numbers followed by the same letter are not significantly different at $P<0.05$ as determined by Tukey's multiple comparison statistic test.

APPENDIX C. ROOT ROT SEVERITY SCALE

0 1 2 3 4

APPENDIX D. MESOCOTYL ROT SEVERITY SCALE

APPENDIX E. CROWN ROT SEVERITY SCALE

APPENDIX F. STALK ROT SEVERITY SCALE

APPENDIX G. AGAR SEED HEALTH TEST

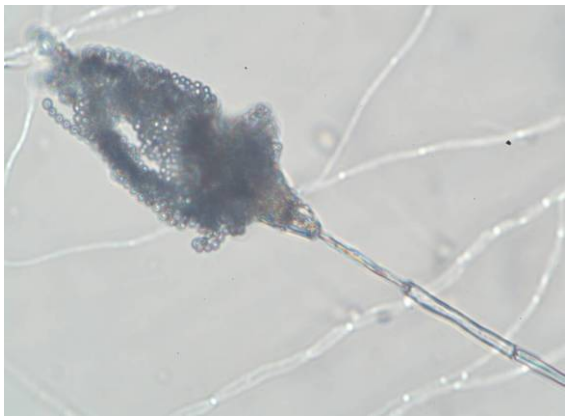
APPENDIX H. MAIZE SEED-BORNE FUNGI ISOLATED FROM SEED LOTS

F. graminearum

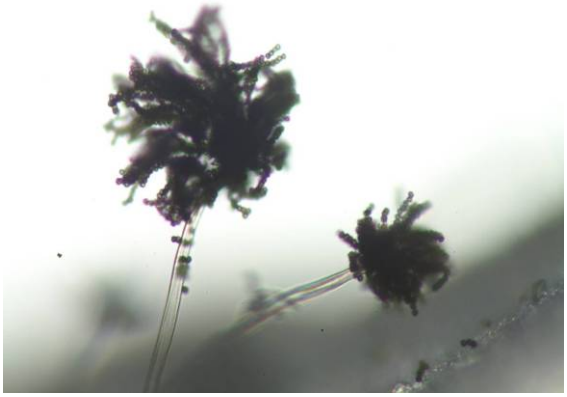
F. proliferatum

F. subglutinans

F. verticillioides



Penicillium spp.



Aspergillus spp.

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