UNDERSTANDING AND MANAGING *RHIZOCTONIA SOLANI* IN SUGARBEET

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ABSTRACT

Rhizoctonia crown and root rot of sugarbeet (*Beta vulgaris* L.) caused by *Rhizoctonia solani* Kühn is one of the most important production problems in Minnesota and North Dakota. Greenhouse studies were conducted to determine the efficacy of azoxystrobin to control *R. solani* at seed, cotyledonary, 2-leaf and 4-leaf stages of sugarbeet; compatibility, safety, and efficacy of mixing azoxystrobin with starter fertilizers to control *R. solani*; and the effect of placement of azoxystrobin in control of *R. solani*. Results demonstrated that azoxystrobin provided effective control applied in-furrow or band applications before infection at all sugarbeet growth stages evaluated; mixtures of azoxystrobin and starter fertilizers were compatible, safe, and provided control of *R. solani*; and azoxystrobin provided effective control against *R. solani* when placed in contact over the sugarbeet root or into soil close to the roots.

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LITERATURE REVIEW

Sugarbeet History and Development

Sugarbeet (*Beta vulgaris* L.) belongs to the Chenopodiaceae family and is an important source of sugar in most temperate parts of the world. Sugarbeet roots contain a high concentration of sucrose that renders its value increasingly important around the world to cultivate commercially, besides another important sugar crop, sugarcane (*Saccharum officinarum*). Before the introduction of sugarbeet, sugarcane was the only source of sugar worldwide and was cultivated only in tropical parts of the globe. The unique capacity of sugarbeet to produce a large amount of sucrose that can be extracted and crystallized as well as having a short growing season led sugarbeet to become a popular crop for sugar production in many countries with temperate climates within a short period of time.

The first milestone in the sugarbeet industry occurred in 1947 when the German chemist, Andreas Sigismund Marggraf, showed that sugar crystals could be extracted from beet and the sugar was identical to that from sugarcane (Francis, 2006). Fifty years later, his student Franz Carl Achard, now universally recognized as 'the father of the beet sugar industry,' selected high sugar producing varieties and developed a sugar extraction process (Francis, 2006). Achard's research resulted in the Prussian government funding the construction of the first sugarbeet factory in Lower Silesia in 1801 (Francis, 2006). The sugarbeet industry was given a boost in 1811 when Napoleon I became interested in Achard's discovery and decreed that sugarbeet factories be built to process French-grown sugarbeet to minimize the effect of the British blockade (Francis, 2006). With that development, France processed some 35,000 tonnes of beets by the end of 1813 (Shoptaugh, 1997). After Napoleon's fall from power, cane sugar reappeared on the European market (Francis, 2006). The sugarbeet industry in France slowly increased

between 1820 and 1839 after advances in sugar processing (Francis, 2006). Sugarbeet production also increased in Germany in the 1830s (Francis, 2006). Gradually production of sugar from beets expanded to other parts of the world, including Europe, Asia and to the western hemisphere, particularly the United State of America, Argentina, Canada, and Chile (Whitney and Duffus, 1986) with much improvement and invention on better technology for cultivation.

The first sugarbeet factory in the United States was established in 1838 in Northampton, MA, whereas the first to successfully produce white sugar was built in 1870 in Alvarado, CA (Francis, 2006; Whitney and Duffus, 1986). Currently sugarbeet is cultivated in 10 states of USA which includes California, Colorado, Idaho, Michigan, Minnesota, Montana, Nebraska, North Dakota, Oregon, and Wyoming (Harveson et al., 2002; USDA-ERS, 2013). In the Red River Valley of Minnesota and North Dakota, the first sugarbeet factory was constructed in 1926 in East Grand Forks (Shoptaugh, 1997). Today there are three sugarbeet cooperatives, Minn-Dak Farmers Cooperative, American Crystal Sugar Company, and Southern Minnesota Beet Sugar Cooperative located in Minnesota and North Dakota. These cooperatives produce just under 60% of the US sugarbeet crop (Bangsund et al., 2012; USDA-ERS, 2013) and the total economic activities of the industry in this region was estimated at over $3 billion (Bangsund and Leistritz, 2004).

In 2009, France, USA, Germany, Russia, and Turkey were the world's five largest sugarbeet producers with 228.2 million metric tons of sugarbeet (FAOSTAT, 2009). The United States became the second largest producer with around 29.78 million metric tons at that time (FAO, 2009).

Biotic disorders caused by pathogens are always major limiting factors for any domesticated crop. Sugarbeet has several diseases caused by fungi, bacteria, viruses and nematodes. Among the diseases Rhizoctonia crown and root rot caused by *Rhizoctonia solani*, Fusarium yellows caused by *Fusarium oxysporum* f. sp. *betae*, Aphanomyces root rot caused by *Aphanomyces cochlioides*, Cercospora leaf spot caused by *Cercospora beticola*, and Rhizomania caused by Beet necrotic yellow vein virus which is transmitted by the vector *Polymyxa betae* are prevalent in the Red River Valley of North Dakota and Minnesota. Rhizoctonia crown and root rot caused by the fungus *Rhizoctonia solani* is one of the most important diseases of sugarbeet in the USA. In 2009 Rhizoctonia crown and root rot was ranked as the number one production problem of sugarbeet in Minnesota and North Dakota according to an annual crop survey (Stachler et al., 2009).

Rhizoctonia Crown and Root Rot

Rhizoctonia crown and root rot (RCRR) caused by the soil-borne fungi *R. solani* has been one of the most important disease of sugarbeet in the United States for many years (Edson, 1915; Schneider and Whitney, 1986; Franc et al., 2001) and is increasingly becoming a problem in Europe (Buddemeyer and Märländer, 2004; Buhre et al., 2009). The fungus is composed of different populations called anastomosis groups or AGs, which attack certain crops and plant parts (Leach, 1986; Sneh et al., 1991). The AG that causes Rhizoctonia crown and root rot of sugarbeet is *R. solani* AG 2-2 which is further divided into subpopulations (called intraspecific groups or ISGs) designated AG 2-2 III B and AG 2-2 IV. These ISGs are differentiated by their growth at 35 ˚C; the AG 2-2 IIIB populations grow and survive at 35 ˚C, whereas AG 2-2 IV does not (Sneh et al., 1991). AG 2-2 IIIB is generally more aggressive in attacking sugarbeet than AG 2-2 IV (Ogoshi, 1987; Windels and Brantner, 2007; Bolton et al., 2010). The disease is

considered as threatening or affecting economic returns of 24% (Windels et al., 2009) of the sugarbeet acres grown in the United States and 5 to 10% in Europe (Büttner et al., 2003). Yield losses of up to 50% may occur (Allen et al., 1985; Herr, 1996). In the Red River Valley and Southern Minnesota, Rhizoctonia crown and root rot has become more prevalent and severe during the last decade. Infections might occur from 12 to 35˚ C but optimal temperature for infection ranges between 18 to 30˚ C, whereas infections occur rarely below 15˚ C (Leach, 1986; Bolton et al., 2010). One important aspect behind high disease pressure in this region is the increased production of soybean and edible bean crops which are susceptible to stem and root rot caused by *R. solani* AG 2-2 IIIB (Engelkes and Windels, 1994; Ogoshi, 1987). Traditionally, growers rotated sugarbeet with barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum*) which are non-hosts of *R. solani*. Over the past two decades significant acreage of wheat and barley has been replaced with soybean (*Glycine max*) and corn (*Zea mays*), both of which are host of *R. solani* (Brantner and Windels, 2007). The sugarbeet growing areas in North Dakota and Minnesota have been in a wet cycle for the past 15 years (Khan and Bolton, 2010). Continuous wet weather, short interval between sugarbeet crops and frequent planting of the pathogen's host crop in rotation provides a favorable environment for increased incidence and severity of Rhizoctonia crown and root rot of sugarbeet.

Description of the Pathogen: *Rhizoctonia solani*

Taxonomy

Rhizoctonia solani Kühn originally described by Julius Kühn on potato in 1858 (teleomorph, *Thanatephorus cucumeris* (Frank) Donk) is a basidiomycete in the family Ceratobasidiaceae (Asher and Hanson, 2006). Within the genus *Rhizoctonia, R. solani* is the most studied species (González García et al., 2006). The genus concept in *Rhizoctonia* was first

established by De Candolle in 1815 (Ogoshi, 1987). *Rhizoctonia solani* is a species complex which is composed of divergent populations called anastomosis groups (AGs). Fusion of isolates belonging to like AG isolates results in hyphal fusion and they are regarded as genetically related or otherwise unrelated if showing somatic incompatibility (Rauf et al., 2007; Sneh et al., 1991). Fourteen anastomosis groups have been identified and described in *R. solani* (Carling, 1996; Carling et al., 2002a; and González Garcìa et al., 2006). Based on cultural morphology, nutritional requirements, temperature effect on growth, host specificity, frequency of hyphal fusion and pathogenicity, several AGs are subdivided into intraspecific groups or ISGs (Sneh et al., 1991). Subdivision of AG2 is based on hyphal fusion frequency, and effect of temperature on growth and virulence (Carling et al., 2002b; González Garcìa et al., 2006). The subset of AG-2 includes AG 2-1 (Ogoshi, 1976), AG 2-2 IIIB (Ogoshi, 1987; Ogoshi and Ui, 1979;Watanabe and Matsuda, 1966), AG 2-2 IV (Ogoshi and Ui, 1979; Oniki, 1977), AG 2-2 LP (Hyakumachi et al., 1998), AG 2-2 WB (Godoy-Lutz et al., 2008), AG 2-3 (Kanematsu and Natio, 1995; Natio and Kanematsu, 1994), AG 2-4, and AG 2 BI (Carling et al., 2002). *Rhizoctonia solani* cause several diseases on sugarbeet. Isolates of AG 2-2 IIIB cause crown and root rot (Buhre et al., 2009; Engelkes and Windels, 1996) while isolates of AG 2-2 IV cause crown and root rot and foliar blight of sugarbeet (Engelkes and Windels, 1996; Matsumoto and Matsuyama, 1999).

Biology of the pathogen *R. solani*

The genus *Rhizoctonia* represents a large, diverse and complex group of fungi. All species of *Rhizoctonia* initially exist as sclerotia with no internal tissue differentiation and germinate as a hyaline sterile mycelium. The most important species of *R. solani* contain several nuclei (multinucleate *Rhizoctonia*), whereas mycelial cell of several other species contain two nuclei (binucleate *Rhizoctonia*) (Agrios, 2005). The mycelia which is initially colorless becomes

yellowish or light brown to dark brown in color gradually with age (Agrios, 2005). Mycelium consists of long cell and branches of mycelium are initiated from the right angle of the main hyphae, there is a slight constriction present at the junction of the main hyphae and its branches with a cross wall near attachment, are the main identifying characteristics of *R. solani* (Agrios, 2005). The fungus produces bulbils (a rounded mass of fungal cell), which are dry, hard, resembling sclerotium, and measure 0.1-10 mm in diameter (Agrios, 2005). There is significant evidence that *R. solani* and several other species are "collective" species consisting of several more or less unrelated strains (Agrios, 2005). As mentioned earlier, strains of *Rhizoctonia* are distinguishable from one another through hyphal anastomosis reaction which represents an expression of somatic or vegetative incompatibility (Anderson, 1982). From a biological point of view, hyphal anstomosis is one of several mechanisms involved in compatibility and sexual recognition processes that ultimately allow the preservation of unique heterokaryons in fungi (González Garcìa et al., 2006). Pairing of isolates belonging to the same AG results in hyphal fusion (anastomosis) leading to either acceptance (self-pairing) or rejection (somatic incompatibility). When pairing does not occur between AGs, they are considered to be genetically different (Ceresini, 1999).) During the anastomosis period, five to six cells on either side of fusion cells become vacuolated and die, appearing as a clear zone at the junction of the paired colonies. This "killing reaction" between isolates of the same anastomosis group is the expression of somatic or vegetative incompatibility (Agrios, 2005). These anastomosis groups represent the genetically different populations within one single species (Agrios, 2005). The genetic mechanism of *R. solani* that controls the recognition process during anastomosis is not well understood (Ceresini, 1999).

The teleomorphic stage of various AGs of *R. solani* (*Thanatephorus cucumeris*) occasionally develops during period of high relative humidity, on the abaxial side of infected petioles as a powdery, grayish-white, pellicle-like hymenium composed of barrel-shaped to subcylindrical basidia (6-12 × 1.5-3.5 µm) (Kotila, 1947; Herr, 1981). Up to four sterigmata form on each basidium, each of them bearing a smooth, thin-walled, apiculate, ovate, hyaline basidiospore (4.8-8.0 × 8.0-12.9 µm) (Asher and Hanson, 2006). In 1978, a report from Japan described foliar blight in sugarbeet caused by *R. solani* AG 2-2 of *T. cucumeris* (Naito and Sugimoto, 1978). Hymenium of *T. cucumeris* was reported in Ohio in 1979 and 1980 (Herr, 1982). Sugarbeet plants bearing basidiospores of *T. cucumeris* were observed in a field near East Grand Forks, Minnesota in 1993 (Windels et al., 1997).

Distribution and host range

Rhizoctonia solani is a ubiquitous pathogen and distributed around the crop growing region in the world. Different AGs have different host range and distribution. Cereal crops such as wheat, barley, and corn are considered non-host for the isolate of *R. solani* AG 2-2 IIIB and AG IV (Windels and Brantner, 2008). In Europe, however, the situation is different. *Rhizoctonia solani* AG 2-2 IIIB caused root and stalk rot of corn in Europe, which is also primarily responsible for Rhizoctonia crown and root rot of sugarbeet (Ithurrart et al., 2004). The anastomosis group *R. solani* AG 2-2 IIIB and AG IV has a wide host range including rice (*Oryza sativa* L.), mat rush (*Lomandra longifolia*), ginger (*Zingiber officinale*), turfgrass (*Cynodon dactylon*), corn, sugarbeet, and *Chrysanthemum* spp. (González Garcìa et al., 2006). The ISGs of *R. solani* AG 2-2 (IV and IIIB) are distributed throughout the sugarbeet-growing areas of Minnesota and North Dakota, while AG 2-2 IV predominates in the Red River Valley and AG 2-2 IIIB in southern Minnesota (Brantner and Windels, 2007). Those differences in population

distribution were probably due to changes in the crop rotation system which has shifted from small grains, primarily spring wheat to increased production of soybean, edible bean (*Phaseolus sp*.) and corn (Brantner and Windels, 2007).

Symptoms and disease development

According to Edson (1915), the first seedling disease on sugarbeet was reported in 1888 by Eidam. Seedling diseases of sugarbeet are widespread. In addition to *R. solani*, other soilborne pathogens, especially *Aphanomyces cochlioides* Drechs, *Pythium aphanidermatum,* (Edson) Fitzp.and *P. ultimum* Trow, commonly cause similar seedling disease symptoms in sugarbeet (Herr, 1996). Seedling diseases initiated by seed decay, pre-emergence and post-emergence damping off primarily injure seedlings root or hypocotyl resulting in deformed, stunted, and poorly developed older plants (Windels and Jones, 1989). Post-emergence damping off is typically initiated by *R. solani* which infects the hypocotyl region below the soil line, and severely infected seedlings collapse and die (Whitney and Duffus, 1986). Prior to the development of Rhizoctonia anastomosis grouping (AG) system, it was reported that *R. solani* strains causing seedling diseases of sugarbeet differed in pathogenicity/virulence compared with those that caused root rot on older beets (Houston, 1945). Recently AG 2-2 was reported as the major anastomosis group in sugarbeet associated with Rhizoctonia crown and root rot whereas AG 4 was the most frequent anastomosis group related to sugarbeet damping-off (Nagendran et al., 2009). Rhizoctonia seedling diseases are exacerbated by conditions which retard seed germination, emergence and post-emergence growth (Whitney and Duffus, 1986).

The first above ground symptoms of crown and root rot is a sudden wilting and chlorosis of foliage and necrosis of petioles near the crown (Asher and Hanson, 2006). Wilted leaves subsequently die, forming a dry, brown or black rosette which persists throughout the growing

season (Whitney and Duffus, 1986; Asher and Hanson, 2006). These symptoms usually occur on older plants, and generally associated with the developing canopy (Herr, 1996). Duggar (1899) reported sugarbeet root rot to be caused by *R. betae* later relegated to be synonymous with *R. solani* (Duggar, 1915).

Another important Rhizoctonia root rot designated by Richard as dry rot canker (Richard, 1921) has been reported from California, Colorado, Minnesota, Montana, Nebraska, North Dakota, Utah and Wyoming (Whitney and Duffus, 1986). Dry rot canker disease symptoms occur on the sugarbeet root surface and consists of many, defined, localized, circular, sunken lesions varying in size from 2 to 25 mm in diameter, with an alternating dark and light color concentric ring pattern (Whitney and Duffus, 1986; Herr, 1996). Beneath the lesions are deep cankers filled with mycelium and dry remains of host tissue which is sharply delimited from adjacent healthy tissue (Whitney and Duffus, 1986).

Disease cycle and epidemiology

Rhizoctonia solani overwinters in soil as bulbils, or thickened hyphae, monilioid cells (specialized hyphae composed of compact cells), and sclerotia or in plant debris (Whitney and Duffus, 1986; Boosalis and Scharen, 1959; Roberts and Herr, 1979). The dormant stage of *R. solani* becomes active with warm temperature (Whitney and Duffus, 1986). Optimum temperature is 15 to 18˚C for most strains (AG and ISG group) of the fungus, but some strains are most active at much higher temperature, up to 35˚C (Sneh et al., 1991; Agrios, 2005). Infection may occur in petioles, crowns or roots of older plants when soil temperature becomes favorable for infection (Asher and Hanson, 2006). Disease is more severe in soils that are moderately wet, than soils that are waterlogged or dry (Agrios, 2005). Root disease severity is positively correlated with increasing temperature for both of the pathogenic ISGs of sugarbeet,

and maximum disease symptoms were evident at 22˚C; no disease symptoms were observed at 11˚C (Bolton et al., 2010). Rhizoctonia crown and root rot symptoms and severity was highest with 75 to 100% moisture holding capacity, but disease was evident even at 25% moisture holding capacity (Bolton et al., 2010). The fungus grows over the surface of the host, hyphae attach to the plant surface and repeatedly forms 'T-shaped branches' which consists of compact masses of hyphae (infection cushion) from which an infection peg directly penetrates the host tissue by hydrostatic pressure with the aid of cell wall-degrading enzymes, or penetrates through wounds (Herr, 1996). The fungus grows inter- and intra-cellularly within sugarbeet root tissue (Ruppel, 1973). Younger plants are more prone to infection than older plants (Pierson and Gaskill, 1961).

The highest inoculum density of *R. solani* was observed in the upper 10 cm soil and no activity was recorded below 10 cm soil (Papavizas et al., 1975). *Rhizoctonia solani* is highly dependent on plant tissue and almost disappears when the latter is exhausted, the pathogen *R. solani* grows rapidly from a suitable energy base, but survives poorly in competition once the living host is dead (Papavizas et al., 1975).

Management of *R. solani*

Cultural control

Cultural control that could be helpful in controlling the disease include organic farming, using crop rotation, maintaining proper soil fertility by using organic amendments, using different cultivation practices that reduces the inoculum density in soil and using a balanced fertilization system (Ariena et al., 1996).

Organic farming

Soil inoculum density was reduced with organic farming compared with conventional farming as these different farming systems resulted in qualitatively different soil environment (Ariena et al., 1996). Those qualitative differences include thicker top soil layer, lower soil bulk density, greater water holding capacity, cation exchange capacities, and higher organic matter associated with microbial biomass and enzymatic activities (Doran et al., 1987). Some studies found that populations of beneficial fungi and bacteria are higher in organically rich soil relative to conventional field (Sivapalan et al., 1993). Increased diversity of soil fauna in organic or low-input farming systems was also demonstrated in some studies (El Titi and Ipach 1989; El Titi and Landes, 1990; El Titi and Richter, 1987). Another study on the effect of farming system on soil borne pathogen and root diseases revealed lower *R. solani* population in tomato (*Solanum lycopersicum*) field which was cultivated organically compared with conventional and high-input plots (Scow et al., 1994).

Organic amendments

Organic amendment such as manure, compost or cover crop can be implemented in low input farming system, which might have an effect on *Rhizoctonia* disease depending on material that have been use with its decomposition state (Ariena et al., 1996). Some greenhouse experiments have successfully used cellulase-based material for suppression of disease (Papavizas, 1970). Addition of neem (*Azadirachta indica*) oil cake and *Gliricidia* leaves reduced *R. solani* on rice both in greenhouse and field experiment (Baby and Manibhushanrao, 1993). Another study indicated that amendments with cellulose, rice stubble or water hyacinth (*Eichhorinia crassipes*) reduced saprophytic growth of *R. solani* and damping off of cauliflower (*Brassica oleracea*) in potted soil (Kundu and Nandi, 1985). Addition of ammonium nitrate to

these organic amendments increased saprophytic growth and infection by the pathogen as the C: N ratio decreased from about 60 to 100% to 20% (Kundu and Nandi, 1985). Disease severity decreased by high C: N ratio as within this environment antagonistic actinomycete and bacterial populations increased and competed for nitrogen (Ariena et al., 1996). The state of decomposition is also very important in determining the effect of organic amendments on saprophytic growth and infection by *R. solani* (Ariena et al., 1996). Fresh amendments generally increased inoculum density and disease severity (Wall, 1984). On the other hand, partially decomposed or composted organic matter often suppressed the pathogen (Chung et al., 1988a). Studies on *R. solani* diseases from dry bean (*Phaseolus vulgaris*), cotton (*Gossypium spp*) and radish (*Raphanus sativus* L.) showed suppression of disease by composted sewage sludge when added 2 to 4 weeks before planting, but the disease on cotton was not affected by the amendment when it was applied one week before planting (Lumsden et al., 1983).

Crop rotation

Crop rotation with non-host crops is an important cultural control practice. Although *Rhizoctonia* species have a wide host range, individual anastomosis groups (AGs) or their sub-groups often have much more limited host range (Butler, 1993). For example, AG 2-2 and AG 2-1 and have a narrower host range (Anderson, 1982). Sugarbeet, potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), sweet clover (*Melilotus alba*) and dry bean are considered host crop of *R. solani* (Maxon, 1938). Cereal crops including wheat, barley and corn are considered non-host for *R. solani* AG 2-2, and thus recommended for rotation with broadleaf crops such as sugarbeet, soybean, and sunflower (*Helianthus annuus*) in the upper Midwest (Windels and Brantner, 2008). However, studies from Europe revealed that *R. solani* AG 2-2 IIIB caused root and stalk root of corn which also causes Rhizoctonia crown and root rot on sugarbeet (Ithurrart et al.,

2004). Root and brace rot of corn caused by *R. solani* AG 2-2 IIIB was also reported from south-eastern USA (Sumner et al., 1999; Sumner et al., 1982) but not from Midwest before 2006. Recent trials in Midwest on *R. solani* AG 2-2 IIIB population in rotation studies using wheat, soybean and corn indicated that *R. solani* caused an increased lesion only on corn, and this was the first report in the Midwest on susceptibility of corn to *R. solani* (Windels and Brantner, 2008). So, cultivation of corn as a preceding crop of sugarbeet may increase inoculum level of *R. solani*. For designing cropping sequence to avoid high inoculum pressure in addition to the susceptibility of preceding crops to *Rhizoctonia* isolates, some additional factors need to be considered (Ruppel, 1985; Rush and Winter, 1990). One of these factors is the residual nitrate in soil from the previous crop, as they might strongly correlate with *Rhizoctonia* disease severity in the following crop (Rush and Winter, 1990). Another factor could be the amount of residual organic debris that might have positive effect on *Rhizoctonia* disease development (Rush and Winter, 1990). Shorter crop rotation induces an increase disease severity in sugarbeet (Buhre at al., 2009). If a higher proportion of host plants are cultivated in the crop sequence then inoculum potential will increase over time, apparently inoculum potential will decrease with less host plants in the sequence (Buhre et al., 2009).

Cultivation method

Disease incidence can be varied by biological and physical properties of soil which are influenced by tillage operations (Buhre et al., 2009). Largest differences in parasitic and saprophytic activity of *Rhizoctonia* species can be seen in the effects of till versus no-till systems (Weller et al., 1986; Cook, 1994). Negative effects of soil compaction on Rhizoctonia disease severity have been observed in sugarbeet (Buddemeyer and Märländer, 2004) and bean (Tu and Tan., 1991). The timing of tillage after the previous crop, as well as, the time allowed for

residues to decompose, may also affect disease pressure in the following crop (Spech and Leach, 1987). Although soil properties benefited from reduced tillage (Frede et al., 1994), high infestation of *R. solani* have been documented from reduced tillage compared with plowing in cereals (Paulitz, 2006; Rovira, 1986). In potato and soybean these negative effects were not confirmed when cultivated in the soil with reduced soil tillage (Sturz et al., 1997).

Fertilizers

Manipulation of fertilizers will be an important strategy in managing disease (Elmer, 1997) as the nutritional status of the plant can influence its susceptibility to disease (Afanasiev and Carlson, 1942; Elmer and Ferrandino, 1994; Elmer and LaMondia, 1995; Huber and Watson, 1974). It is well known that fertilizers can have a profound effect on disease severity of *Rhizoctonia* (Engelhard, 1989; Parmeter, 1970). All essential elements can have significant influence on disease severity (Engelhard, 1989; Huber, 1978, 1980). Response to a particular nutrient might have different aspects, for example some might be useful for a deficiency, some could be adequate supply, excess amount could create toxicity, or salinity (Ariena et al., 1996). Deficiencies of potassium, nitrogen, or calcium increase *Rhizoctonia* incidence or severity (Baker and Martinson, 1970), whereas disease potential could increase if excessive nitrogen fertilizer is applied (Baker and Martinson, 1970). Also greenhouse studies have demonstrated that sugarbeet seedlings were more susceptible to disease caused by *R. solani* when sugarbeets were treated with NH_4-N as opposed to NO_3-N (Afanasiev and Carlson, 1942). Lower incidence of infection was reported when compared with a non-fertilized treatments in a naturally *Rhizoctonia* infested field (Hills and Axtell, 1950). Another study from Nebraska based on natural infestation did not find any nitrogen effect on 3-, 4-, and 6- year sequences, but they noticed significantly more Rhizoctonia root rot in a 2-year rotations in the non-fertilized

treatments (Schuster and Harris, 1960). However, rates and timing of fertilizer application is also critical for tolerating or avoiding *Rhizoctonia* infection to some degree (Hecker and Ruppel, 1980). No beneficial effect was noticed by increasing preplant application of N and side-dressed N from deficient to excess amount (Hecker and Ruppel., 1978). So, in order to obtain better control against *R. solani* balanced fertilization with proper timing of application should be considered carefully.

Biological control

Research on biological control of soilborne pathogens has been advanced and accelerated at a rapid rate (Lewis et al., 1995). The reason behind this phenomenon is partly due to increased knowledge in the production, formulation, and delivery of different kinds of biocontrol agents, including fungi, bacteria, and actinomycetes (Baker and Dunn, 1990; Hornby, 1990; Tjamos et al., 1992; Lumsden and Vaughn, 1993).

Soil bacteria may affect the formation and survival of sclerotia, breakdown of their dormancy and mycelial saprophytic growth in soil (Leach and Garber, 1970). Rhizobacteria has a significant effect on the reduction of inoculum density of *R. solani* as well as suppress its saprophytic and pathogenic activity (Homma, 1996). The defense mechanism of rhizobacteria is to colonize roots and defend the infection courts (any parts of plant tissue that have food base, e.g. seed coat, endosperm, embryo, the emerging radicle, cotyledons, hypocotyl etc.) against attack by pathogens (Homma, 1996). Laboratory experiments show rapid and substantial reduction of sclerotia and hyphal inoculum of *R. solani* as a consequence of attack by the mycoparasite *Verticillium biguttatum* (Velvis et al., 1989). Potato tuber-borne sclerotia of *R. solani* were frequently infected with hyperparasite (most common was *Verticillium biguttatum*) which led to the idea that local accumulations of *V. biguttatum* might have a significant role on

R. solani inoculum reduction (Velvis et al., 1989). Experiments with this hyperparasite indicated that *V. biguttatum* is the main antagonist which caused a rapid reduction of the viability of sclerotia and hyphal material of *R. solani*, when artificially added to the soil (Velvis et al., 1989).

Binucleate *Rhizoctonia* (BNR) which is morphologically similar to *R. solani*, but contains binucleate cells, belongs to the genus *Ceratobasidium* (Sneh et al., 1991). It has shown potentiality as a biocontrol agent on a variety of crops including dry bean, sugarbeet, potato, tomato, turf grass, and poinsettias (Burpee and Goulty, 1984; Cardoso and Echandi, 1987; Escande and Ecandi, 1991; Harris et al., 1993; Hwang and Benson, 2002; Muslim et al., 2003). Greenhouse experiment showed positive result when interaction of seven soybean cultivars and isolates of BNR were studied in the presence of *R. solani* AG 2-2 and AG-4 (Khan et al., 2005). Where soybean was inoculated with AG-4 group, BNR significantly increased soybean emergence and survival with reduction in disease severity (Khan et al., 2005). The BNR also reduced disease severity in cultivars that were inoculated with AG 2-2 group (Khan et al, 2005). The mechanism of control by BNR were probably the colonization of soybean tissue before the pathogenic *Rhizoctonia* isolates initiated infection as BNR were consistently isolated from hypocotyls and roots of soybean (Khan et al., 2005).

Trichoderma isolates were used as a biocontrol agent against soilborne phytopathogen *R. solani* by using dual culture and bioassay method on dry bean plants to study the effect on controlling disease severity (Barakat et al., 2007). The study identified that the biological control agent used as a conidial suspension greatly reduced the disease index of dry bean plant caused by *R. solani* in different rates and the most effective isolates was *Trichoderma harzianum* that reduced the disease by 65% (Barakat et al., 2007). In vitro results showed many antagonistic fungi are effective on soilborne plant pathogens, are also active in agricultural ecosystems

depending upon the method of their application into the soil (Lewis and Papavizas, 1991). For example when conidia of *Trichoderma hamatum* (TRI-4) and *Gliocladium virens* (Gl-21) were added to soil they were ineffective in controlling damping-off of ornamentals, beans, cotton, sugarbeets or radish; whereas bran/germling (actively growing hyphae on bran) preparations were very effective (Lews and Papavizas, 1985; Lumsden and Locke, 1989; Papavizas and Lewis, 1989). A four year field study using biocontrol agent TRI-4 and Gl-21 with different preparations methods such as bran/germlings, a powder (pyrax/biomass), and alginate pellets containing milled fermentor biomass of the fungi showed similar results described earlier (Lewis and Papavizas, 1991). Among all three preparation methods, bran/germlings consistently prevented disease, reduced pathogen saprophytic activity and stimulated proliferation of population of the biocontrol fungi (Lewis and Papavizas, 1991). Biological control of damping-off in sugarbeet caused by *R. solani* was studied in the greenhouse using *Trichoderma* species (Hanson, 2003). In this experiment, *Trichoderma* strains were grown on ground wheat bran and peat moss, air dried, and then applied on sugarbeet seed with a latex sticker (Hanson, 2003). Sugarbeet seeds were planted and inoculated with *R. solani* colonized barley grain and survival of sugarbeet under disease pressure was compared (Hanson, 2003). Isolates of *Trichoderma virens* showed biocontrol activity against sugarbeet seedling damping-off caused by *R. solani*, all isolates colonized roots well, and some biocontrol-effective isolates showed antibiosis against *R. solani* and peroxidase activity was significantly higher in those isolates (Hanson, 2003). Besides *Trichoderma* and *Gliocladium* other fungi have the potential as a biocontrol agent (Lewis and Papavizas, 1992). *Laetisaria arvalis* Burds, a soil-inhabiting basidiomycete which was originally isolated by Dr. M G. Boosalis, was suggested as a potential biocontrol fungus (Burdsall et al., 1980). Reports from field studies documented that isolates of *L. arvalis* prevented damping-off

of sugarbeet (Odvody et al., 1980), peppers (*Capsicum annuum*) (Conway, 1986), and fruit root of cucumbers (*Cucumis sativus* L.) (Lewis and Papavizas, 1980) caused by *R. solani*. Further evaluation was done with *L. arvalis* as a potential biocontrol agent for possible commercial development of the antagonist (Lewis and Papavizas, 1992). Six isolates of *L. arvalis* were grown on sterile wheat bran moistened with water and incubated for 5-15 days before addition to soil, were very effective in reducing *R. solani* inoculum (Lewis and Papavizas, 1992). *Laetisaria arvalis* isolates added to pathogen-infested loamy sand soil at a rate of 0.5% (w/w) prevented post-emergence damping-off of cotton, sugarbeet, radish and lettuce (*Lactuca sativa* L.) (Lewis and Papavizas, 1992).

Beneficial bacteria have been intensively studied as biocontrol agents against soilborne diseases (Handelsman and Stabb, 1996; Weller, 1988). *Pseudomonas* CMR12a is a well-known biocontrol strain that can produce phenazines and cyclic lipopeptides (CLPs) (D'aes et al., 2011). *Pseudomonas* CMR12a (involvement of both phenazines and CLPs) in controlling two aggressive anastomosis groups (AG 2-2 and AG-4) of *R. solani* on dry bean showed that the wild-type strain CMR12a dramatically reduced disease severity caused by both AGs of *R. solani* (D'aes et al., 2011). A CLP-deficient and a phenazine-deficient mutant of CMR12a still protected against disease to a lesser extent, while two mutant deficient in both phenazine and CLP completely lost their ability as a biocontrol agent (D'aes et al., 2011). Positive field results have been documented while fungicides and antagonistic bacteria *Bacillus* spp. have been used for their potential to reduce disease pressure and improve sucrose concentration in trials inoculated with *R. solani* AG 2-2 (Kiewnick et al., 2001). The combination of azoxystrobin applied at 76 g a.i.ha^{-1} and the *Bacillus* isolate MSU-127 resulted in effective disease control with highest sucrose yield (Kiewnick et al., 2001).

Use of germplasm resistant to *R. solani*

Cultivars resistance to *R. solani* is an effective strategy of controlling disease, as it is the most effective and environmentally safe way to manage plant disease (Sherf and MacNab, 1986). Genetic control for resistance to *R. solani*, causal organism of Rhizoctonia crown and root rot in sugarbeet have been studied for a long time (Panella and Ruppel., 1996). Sugarbeet resistance breeding program began in 1950 and released the first two resistant cultivars in 1966 (Gaskill, 1968). Those were selected based on mass selection or recurrent field selection using artificial epiphytotic (sudden destructive outbreak of plant disease) (Gaskill, 1968; Panella, 1998). Resistance to *R. solani* in sugarbeet is polygenic, having at least two loci, two or three alleles, and modifying genes in some populations (Hecker and Ruppel, 1975). A total of 15 germplasm line tolerant to *R. solani* were released within 10 years from 1986 to 1996 (Panella and Ruppel, 1996). Germplasm enhancement effort have been continued till 1977 (Hecker and Ruppel, 1977) and several improved germplasm have been released or registered for use as pollinators in commercial hybrids (Hecker and Ruppel, 1985, 1988, 1991). It is believed that almost all tolerant to RCRR breeding lines originated from the open pollinated variety GWS 359-52R, which was produced by the Great Western Sugar Co. (Panella, 1998). Some concerns related to yield potentiality of *R. solani* resistant cultivars compared with susceptible cultivars have been raised among sugarbeet breeders (Ruppel and Hecker, 1994). In this regard, research focused on relationships between disease severity and yield parameters have been compared between susceptible and resistant cultivars in a three year (1989, 1990 and 1991) field experiment (Ruppel and Hecker, 1994). From the 1981 data, it was found that there was no loss in root yield in the most resistant germplasm (either FCHY or FC 709), even they produced higher root yields than non-inoculated controls (Ruppel and Hecker, 1994). Also percent lost in recoverable

sucrose in the commercial hybrids was four to 26 times greater than in the resistant experimental germplasm (Ruppel and Hecker, 1994). Similar results were found from both 1990 and 1991 trials; susceptible HM55 had the highest disease indices difference and percent decrease in root yield, recoverable sucrose and % sucrose, in both year. The commercial cultivar ACH184 showed the best performance and was comparable to resistant cultivars (Ruppel and Hecker, 1994). The highly-resistant breeding line FC 709 was superior over all commercial cultivars, and the resistant FCHY was comparable to ACH184, also no hidden losses were found in the experimental germplasm due to *R. solani* (Ruppel and Hecker, 1994). Other studies described resistance was ineffective when infection occurred during early seedling stages and the resistance level was higher in developed plants (Engelkes and Windels, 1994). However in a study, the accession EL51 sugarbeet seedlings survived when inoculated with *R. solani* AG-2-2, and its disease development pattern was documented (Nagendran et al., 2008). In a seedling nursery of fields plot seedling survival was higher in EL51 compared with the susceptible hybrid, when artificially inoculated with *R. solani* AG-2-2, indicates EL51 as a source of resistance to Rhizocotnia damping-off which might open new window in the sugarbeet breeding program (Nagendran et al., 2008). One report by Ruppel (1973) identified that crown and root rot resistance to R. *solani* is not due to the mechanical barrier of mature sugarbeet roots (Ruppel, 1973). To compare the penetration mechanism of *R. solani* in both resistant and susceptible cultivars it was observed that, fungal hyphae could penetrate both of the cultivars, and the pathogen was restricted to the periderm or outer secondary cortex in resistant germplasm, whereas *R. solani* invaded within several vascular rings in susceptible germplasm (Nagendran et al., 2008). Evidence also indicated that *R. solani* AG-2-2 R-1 was unable to colonize beyond the endodermis of EL51 seedlings (Nagendran, 2006). The genetic basis for *Rhizoctonia* resistance

in sugarbeet is considerably narrow (Lein et al., 2008). A genetic map was developed from the corresponding F_2 parents by crossing between a resistant and a susceptible parent (Lein et al., 2008). The map comprised 38 expressed sequence tags (ESTs) with high similarity to genes that are involved in resistant reactions of plants (R-ESTs) and 25 bacterial artificial chromosomes (BACs) containing nucleotide binding site (NBS)-motifs typical for disease resistant genes (Lien et al., 2008). Also three quantitative trait loci (QTL) for Rhizoctonia crown and root rot have been identified on chromosomes 4, 5, and 7 respectively and altogether, the QTL explained 71% of the phenotypic variance (Lein et al., 2008). This indicates marker assisted selection could be an alternative for selecting resistant offspring from segregating populations (Lien et al., 2008).

Chemical control

Chemical control has achieved the best acceptance over all other control measures against *R. solani* in the Red River Valley. Certain seed treatment fungicide (e.g., thiram and maneb) provides some control of seed rot and seedling diseases caused by *R. solani*. In 2001, azoxystrobin (Quadris® Syngenta, Greensboro, NC, USA) was registered in the strobilurin fungicide group, to provide control against *R. solani* (Jacobsen et al., 2001). Azoxystrobin and Prothioconazole (Proline 480SC, Bayer Crop Sciences) provides effective disease control, but they must be applied before infection takes place (Brantner and Windels, 2002; Jacobsen at al., 2002; Khan et al., 2010). Azoxystrobin applied at planting can delay early infection and enhance establishment of vigorous stands (Jacobsen et al., 1999; Karaoglanidis and Karadimos, 2006; Kiewnick et al., 2001), but does not completely prevent infections and development of crown and root rot later in the season (Kiewnick et al., 2001). Economic constraints have led to practices that attempt to control both phases of this disease with a single fungicide application made in the early portion of the season, either at planting or when plants are immature (up to

growth stage [GS] 6 to 8) (Karaoglanidis and Karadimos, 2006; Whitney and Duffus, 1986). Including the methods of fungicide application foliar, banded and in-furrow application reduced amount of diseases (Kirk et al., 2008). Azoxystrobin is effective for reducing sugarbeet seedling losses due to Rhizoctonia damping-off if applied as an in-furrow application (Brantner and Windels, 1999 and 2003). The timing of fungicide application is a critical factor in Rhizoctonia crown and root rot management. Timing application prior to infection can offer long-term disease protection (Kiewnick et al, 2001; Stump et al, 2004; Windels and Brantner, 2002). In this regard, temperature and moisture parameters have been estimated to find out the potential threshold that limits the infection (Bolton et al, 2010). Study showed that *R. solani* infects sugarbeet when the average daily soil temperature at 10 cm depth reached 18˚C (Khan et al., 2010).

Research should be conducted to better understand the infection processes and the mechanism by which protection is provided, so fungicide could be better targeted for more effective disease control. Previous research suggests that fungicides should be applied before the soil temperature at the 10 cm soil depth reaches 18˚C (Khan et al., 2010). Further research is necessary to determine whether one time fungicide application would be adequate in seasons when conditions (warm and wet soil) are favorable for disease development starting early in the season.

Azoxystrobin is most widely used for disease control. The mode of action of azoxystrobin is the inhibition of fungal respiration system. Azoxystrobin binds in the Q_o sites (or ubiquinol site) of chytochrome b in the electron transport chain of mitochondria and stops electron transport from chytochrome b to c (Brandt et al., 1993; Von Jagow and Becker, 1982). This disruption in electron transport system reduces nicotinamide adenine dinucleotide (NADH)

oxidation and adenosine triphosphate (ATP) synthesis. Previous research shows that when the inoculum is placed at different soil depths, *R. solani* caused more infection when placed nearby the upper part of the root (Desouza, 2010). Greenhouse research also showed that when azoxystrobin was applied to the leaves and prevented from getting onto the soil covered with aluminum foil, the fungicide did not provided effective Rhizoctonia root rot control indicating that the fungicides was not translocated downwards to the root (Desouza, 2010). These results suggest that fungicides should target the upper part of the root for best disease control.

Since completely resistant variety is not available and varieties with partial resistant tend to have yield penalty; thus fungicides are most widely used to control the pathogen. More research is needed to better understand how to improve disease control using fungicides. Consequently, the objectives of this research were to determine i) whether azoxystrobin protects sugarbeet seeds and seedlings younger than the four leaf stages against *R. solani* infection in a favorable environment, ii) the compatibility and safety of mixing azoxystrobin with starter fertilizers in controlling *R. solani* of sugarbeet and iii) placement of azoxystrobin in controlling *R. solani* in sugarbeet.

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CHAPTER I: EFFICACY OF AZOXYSTROBIN IN CONTROLLING *RHIZOCTONIA SOLANI* INFECTION AT DIFFERENT GROWTH STAGES OF SUGARBEET

Abstract

Sugarbeet (*Beta vulgaris* L.) is one of the most profitable crops grown in Minnesota and North Dakota and makes a significant economic contribution to these States. Rhizoctonia crown and root rot (RCRR) caused by *Rhizoctonia solani* Kühn anastomosis group (AG) 2-2 is one of the most important, prevalent and widespread soil borne diseases of sugarbeet in this region. A control strategy against this disease is through timely application of effective fungicides before infection takes place. Azoxystrobin consistently provides effective control against RCRR when applied before infection. For many years, azoxystrobin was applied at or just prior to cultivation when sugarbeets were about the 4-leaf stage to protect against RCRR. Since 2009, warmer conditions in spring resulted in early planting and increased reports of seedling mortality and root rot caused by *R. solani*. The research objective was to determine whether azoxystrobin protects sugarbeet seeds and seedlings younger than the four leaf stages against *R. solani* infection in a favorable environment. Sugarbeet at the seed, cotyledonary, 2-leaf and 4-leaf stages were sprayed with azoxystrobin and then inoculated with *R. solani* grown on barley (*Hordeum vulgare*) grain. Azoxystrobin was applied directly on the seed as an in-furrow, in an 18 cm band to soil covered seeds, and in 18 cm bands to cotyledonary, 2-leaf, and 4-leaf stage sugarbeet followed by inoculation. Inoculated and non-inoculated controls were included for all the sugarbeet growth stages. There was no significant difference between survivals in the non-inoculated control compared to the different sugarbeet plant growth stages treated with azoxystrobin indicating that the fungicide provided effective control against *R. solani*,

irrespective of the sugarbeet growth stage. Results indicated that sugarbeet seeds and seedlings are highly susceptible to infection by *R. solani* in favorable environmental conditions and fungicide protection should be provided before infection takes place.

Introduction

Rhizoctonia crown and root rot (RCRR) of sugarbeet (*Beta vulgaris* L.) is one of the major production problems for growers worldwide including North Dakota and Minnesota in the United States. The causal organism is *Rhizoctonia solani* Kühn which is a soilborne fungus that cause pre- and post-emergence damping off, crown and root rot, and infrequently, foliar blight (Schneider and Whitney, 1986; Windels and Lamey, 1998; Kotila, 1947). Rhizoctonia crown and root rot may result in 50% or more reduction in yield also adversely impacting sucrose extraction (Allen et al., 1985; Kiewnick et al., 2001; Whitney and Duffus, 1986). Several factors have been reported that contribute to disease development by providing favorable conditions for the pathogen to invade and colonize host tissue. These factors include improper nutrient balance (Yoshida et al., 1979), cultivation of crops before sugarbeet that are susceptible to *R. solani* (Ruppel, 1985), and planting sugarbeet in poorly drained soil (Franc et al., 2001). *Rhizoctonia solani* is a species complex composed of divergent populations (Gonzàlez et al., 2006). The species complex has been divided into various homogenous groups (AGs) based on hyphal anastomosis (Taheri and Tarighi, 2012). Fourteen AGs have been described for *R. solani* (Carling, 1996; Carling et al., 2002; Gonzàlez et al., 2006). Several AGs are further subdivided into intraspecific groups (ISGs) to reflect differences observed in characteristics such as cultural morphology, nutritional requirements, temperature effect on growth, host specificity, frequency of hyphal fusion, and pathogenicity (Sneh et al., 1991). These AGs attack certain crops and plant parts (Leach, 1986; Sneh et al. 1991). On sugarbeet, isolates of AG 2-2 IIIB are known to cause

crown and root rot (Buhre et al., 2009; Engelkes and Windels, 1996) and isolates of AG 2-2 IV cause crown and root rot as well as foliar blight (Engelkes and Windels, 1996; Matsumoto and Matsuyama, 1999). AG 2-2 IIIB and AG 2-2 IV are differentiated by their ability to grow at 35˚ C (Sneh et al., 1991); AG 2-2 IIIB populations grow and survive at 35˚ C while AG 2-2 IV does not (Sneh et al., 1991). AG2-2 IIIB is considered more aggressive and damaging than AG 2-2IV (Leach, 1986; Bolton et al., 2010). Infections by *R. solani* may occur from 13 to 35˚ C, but optimal temperature for infection ranges from 18 to 30˚ C (Leach, 1986; Bolton et al., 2010). *Rhizoctonia solani* is ubiquitous and is most damaging in wet and warm conditions, especially in fields where sugarbeet follows soybean, edible bean, and corn (Engelkes and Windels, 1994; Ogoshi, 1987). Since there is no sugarbeet variety available which is completely resistant to *R. solani* and varieties with partial resistance typically yield 10 to 20% less compared to high-yielding susceptible varieties (Panella and Ruppel, 1996), effective fungicides are necessary to help provide control against Rhizoctonia root and crown. Among the fungicides azoxystrobin (Quadris ® Syngenta, Greensboro, NC, USA) provides effective control against RCRR, but the recommendations for its use indicate that it must be applied before infection takes place (Stump et al 2004; Kienwick et al. 2001; Jacobsen et al., 2002; Bolton et al., 2010; Khan and Bradley, 2010). Also another study on fungicide timing for controlling RCRR of sugarbeet indicated that fungicide timing based on soil temperature cannot be effective over timing of application based on leaf stages alone (Kirk et al., 2008). Because in this growth stage 2 and 4 several agricultural practices conducive to deposition of infested soil in the sugarbeet crown causing crown rot are common (Kirk et al., 2008). The mechanism by which protection is afforded by azoxystrobin to immature sugarbeet plans remain unclear (Kiewnick et al., 2001). Since azoxystrobin application was conducted at 4 to 6 leaf stages in early to mid-June when the soil temperature become

favorable for *R. solani* infection, but infection by *R. solani* starting at the seedling stage has been increasing since 2009 (Stachler et al., 2009) as soil temperature has been increasing at planting during mid-late May. In favorable temperature and moisture conditions, *R. solani* initiates infection (Bolton et al., 2010). Azoxystrobin typically prevents disease if applied before infection takes place, but is ineffective at controlling *R. solani* after infection (Desouza, 2010). So, it's an increasing concern for the growers that when they should apply azoxystrobin, whether they need to apply the fungicide based on soil temperature or they should wait up to 4 to 6 leaf stage of sugarbeet. Since 2009, soil temperature at the 10 cm depth is increasing gradually at planting, sugarbeet in mid to late May and it is well known that *R. solani* AG 2-2 IIIB is very effective above 18˚ C, so soil that are naturally inhabited by *R. solani* will have no control against the pathogen, if fungicide application is delayed to the 4 to 6 leaf stages. The objective of this study was to determine whether azoxystrobin provides protection to sugarbeet seeds and seedlings at the cotyledonary, 2- and 4-leaf stages against *R. solani* in an environment favorable for infection and disease development.

Materials and Methods

Inoculum preparation

R. solani AG 2-2 IIIB (isolate obtained from Dr. Carol Windels, University of Minnesota, Northwest Research and Outreach Center, Crookston, MN) was grown on PDA (Potato Dextrose Agar) plate for 14 days and inoculum was prepared according to the method described by Pierson and Gaskill (Pierson and Gaskill, 1961). About 4.5 Kg untreated barley grain were placed in aluminum foil tray. One fourth strength of PDB (Potato Dextrose Broth) was prepared by using 2,500-2,800 ml of de-ionized water per tray. At first 18 g of PDB were mixed with 2,500-2,800 ml of de-ionized water in solution. Then, that PDB solution was poured in the

aluminum foil steam tray. After that foil tray was tightly covered with aluminum foil and stored at 4.5-7˚ C for 72 hours. Trays were removed from the cold storage; water was drained and the tray was sealed with paper tape and autoclaved for 2 hour at 121˚ C using liquid setting. Autoclaved barley grains were cooled for 24 hours, then inoculated using the *R. solani* AG 2-2 IIIB isolates grown on a PDA plate under the laminar flow bench hood to avoid any potential contamination. One PDA plate isolates was used per tray. After inoculation trays were resealed again with paper tape and then stored at room temperature for about 3 to 4 weeks. After that time, barley grain was dried for 4 days in the greenhouse, and then stored at -20˚ C for 2-3 months.

Experimental design and greenhouse trial

Greenhouse trial was conducted at North Dakota State University located in Fargo, ND. The sugarbeet cultivar Crystal 539RR susceptible to *R. solani* was used for this experiment. Trays measuring 53 x 28 x 6 cm (T.O. Plastics Inc. Clearwater, MN) were filled with Sunshine Mix 1 peat soil (Sun Gro Horticulture Canada Ltd., Canada). A 3-cm deep furrow was made in the middle of each tray into which 10 evenly spaced seeds were planted. Greenhouse conditions was set to allow light for 12-hour photoperiod and temperature was maintained at 20 ± 2˚ C. Trays were watered daily to provide adequate conditions for favorable plant and pathogen growth. Barley grains colonized with *R. solani* were buried 2.5 cm below the soil surface and close to the hypocotyl region of sugarbeet. Sugarbeet was planted one week after ward to have all the plant stages ready for spray in the same day, i.e. for four leaf stages sugarbeets were planted at the first week, and in the following two weeks planting was done for 2-leaf and cotyledonary stage. In-furrow fungicide application and 18 cm band application on soil surface was done in the same day after planting, along with all other sugarbeets that were planted

successively for a three weeks. The growth stages of sugarbeet that have been used in this experiment were seed stage i.e, just planting sugarbeet seeds in the furrow by exposing the furrow until fungicide application and inoculation; seed stages i.e. planting seed in the furrow followed by covering the furrow with soil before fungicide application and inoculation; cotyledonary stage, two-leaf stage and four-leaf stage of crop. Sugarbeet seeds, and sugarbeet at cotyledonary stage, 2- and 4-leaf stages treated with azoxystrobin at 167 g a.i. ha^{-1} (Quadris, ® Syngenta, Greensboro, NC, USA) followed by inoculation. Azoxystrobin was applied in-furrow directly over sugarbeet seeds followed by inoculation and coverage with soil. Azoxystrobin was also applied in an 18 cm long band directly over soil covered seeds, and cotyledonary, 2-leaf and 4-leaf sugarbeet followed by soil inoculation. Azoxystrobin was applied using a spraying system (Spraying System Co., Wheaton, IL) calibrated to deliver the spray solution at 276 kPa with a speed of 1.3 miles per hour through a single flat fan nozzle 4001E. Soil inoculation was done to simulate field conditions. For each growth stages of sugarbeet one inoculated and one non-inoculated control was included. The experimental design was a Randomized Complete Block Design (RCBD) with four replicates. The experiment was repeated twice.

Disease evaluation by stand count

Four weeks after the treatment were applied when the inoculated controls were all dead, data was taken for surviving healthy plants. Since sugarbeets treated with fungicide didn't show any Rhizoctonia root and crown root symptoms, so root rating was disregarded.

Data analysis

Each experiment was analyzed separately and a folded F-test was used to determine homogeneity of the data. Experimental data were combined as no significant differences were observed at F=0.025 level of confidence. Analysis of variance was done using the SAS general

linear models (Proc GLM) procedure (Version 9.3, SAS Institute Inc., Cary, NC). When ANOVA indicated a significant difference among the treatments, the treatment means were separated using Tukey's mean separation test ($P \leq 0.05$)). Before doing mean separation test original data was transformed using $\sqrt{(X + 0.5)}$ where X=data. Tukey's mean separation test was represented by letters a and b, i.e. means followed by the same letter are not significantly from each other but they are significantly different than other letters. Runs and treatments were considered as fixed effect.

Table 1.1. Analysis of variance for impact of azoxystrobin on growth stages of sugarbeet for controlling *R. solani*

Source of variation	DF	Mean Square	F Value	Pr > F
Run	1	40.83	1.25	0.27
Rep(Run)	6	28.61	0.87	0.52
Treatment	14	15187.86	463.36***	<.0001
Run × Treatment	14	21.19	0.65	0.82
Error	84	32.78	_	_
Total	119	_	_	_

***Indicates significant at $P \leq 0.001$ level of confidence

Results

The analysis of variance (Table 1.1) indicated that there were significant differences among the treatments at $P \leq .001$ level of confidence. At 28 days after treatment, all azoxystrobin applications resulted in significantly greater number of surviving plants compared to the inoculated control where *R. solani* caused death of most or all the plants, irrespective of the physiological stage of the seed or plant at the time of treatment (Table 1.2).

Table 1.2. Impact of azoxystrobin on growth stages of sugarbeet for controlling *R. solani*

Treatments	MFA[†]	MSP[‡] (%)
Inoculated control for in-furrow	No fungicide	0b
Non-inoculated control for in-furrow	No fungicide	88a
Azoxystrobin 167 g a.i. ha^{-1} in-furrow + Inoculated	In-furrow	86a
Inoculated control for band application on soil surface	No fungicide	0b
Non-inoculated control for band application on soil surface	No fungicide	90a
Azoxystrobin 167 g a.i. ha^{-1} on soil suface + Inoculated	18 cm band	90a
Inoculated control at cotyledonary stage	No fungicide	0b
Non-inoculated control at cotyledonary stage	No fungicide	90a
Azoxystrobin 167 g a.i. ha^{-1} at cotyledonary stage + Inoculated	18 cm band	93a
Inoculated control at 2-leaf stage	No fungicide	0b
Non-inoculated control at 2-leaf stage	No fungicide	90a
Azoxystrobin 167 g a.i. ha^{-1} at 2-leaf stage + Inoculated	18 cm band	89a
Inoculated control at 4-leaf stage	No fungicide	1b
Non-inoculated control at 4-leaf stage	No fungicide	90a
Azoxystrobin 167 g a.i. ha^{-1} at 4-leaf stage + Inoculated	18 cm band	90a
LSD (0.05)		6

[†], Method of fungicide application, [‡], Mean survived plants, Treatment means followed by the same letter are not significantly different from each other based on Tukey's mean separation test ($P \leq 0.05$)

There were no significant differences in the percentage of surviving healthy plants between the non-inoculated control and azoxystrobin treatments which indicated that the fungicide was effective in preventing *R. solani* from causing disease and mortality.

There were no significant differences among the band applied fungicides to soil covered seeds and cotyledonary, 2- and 4- leaf stage sugarbeet (Table 1.2). Also, the in-furrow applied azoxystrobin provided significantly greater control than the inoculated control and similar control to the non-inoculated control. The number of surviving plants in the in-furrow fungicide application was not significantly different than the band applied azoxystrobin applied to seed stages, cotyledonary stage, 2-leaf stage and 4-leaf stage of sugarbeet. All the growth stages of sugarbeet where fungicide was applied either as an in-furrow application or as an 18 cm band gives similar control like non-inoculated control, and they are statistically non-significant.

Discussion

In conventional sugarbeet, cultivation was necessary to complement herbicide applications to provide acceptable levels of weed control and 99.7% of respondents indicated using this practice in North Dakota and Minnesota according to a survey (Carlson et al., 2007). Cultivation was typically done at the 4 to 6-leaf stage and the movement of *R. solani* infested soil into the crown of plants resulted in crown rot (Schneider et al., 1982). Most of the research conducted to evaluate fungicide efficacy in sugarbeet applied the fungicides to the plants just prior to artificial inoculation of the crown with *R. solani*. So that, sugar cooperatives typically recommended that azoxystrobin be applied to 4- to 6-leaf stage sugarbeet for effective control of Rhizoctonia crown rot (Source: Ag Note-553). In 2007, the availability of glyphosate tolerant seeds became available and was rapidly adopted by growers with over 95% of sugarbeet acreage planted using this technology by 2010 (Khan et al., 2010; Kniss, 2010). Glyphosate tolerant

sugarbeet facilitates the use of glyphosate which provides excellent weed control and thereby no longer requires the need for cultivation as a method of weed control. In 2010, only 10% of the sugarbeet acres were cultivated in North Dakota and Minnesota (Carlson et al., 2011). This significant reduction in cultivation would have reduced the possibility of sugarbeet fields becoming infected with *R. solani* through throwing of infested soils in crowns. Although the production practice of cultivation had changed, the recommendation for *R. solani* control with azoxystrobin remained the same. During 2009 through 2011, growers in North Dakota and Minnesota reported that Rhizoctonia root rot was their most important problem (Carlson et al., 2010; 2012). During this period, especially in 2010 and 2011, growers were planting sugarbeet when average daily soil temperature at the 10 cm depth was already at 18˚C and moisture was adequate for emergence and infection. This would imply that earlier planted seeds in different seedling stages or seeds will be in an environment favorable for infection by *R. solani*. If growers wait until plants are at the 4- to 6-leaf stage, azoxystrobin application will not be effective since infection could have already taken place. Our research is consistent with other research (Bolton et al., 2010, Stump et al., 2004, Kirk et al., 2008; Kiewnick et al., 2001) which showed that sugarbeet seeds and seedlings are susceptible to infection by *R. solani* in favorable conditions. Our results indicated that seeds and seedlings can be effectively protected from *R. solani* infection once azoxystrobin is applied preventatively. This would suggest that fields with a history of *R. solani* should be protected with an effective fungicide such as azoxystrobin before the daily average soil temperature threshold at which infection takes place is reached.

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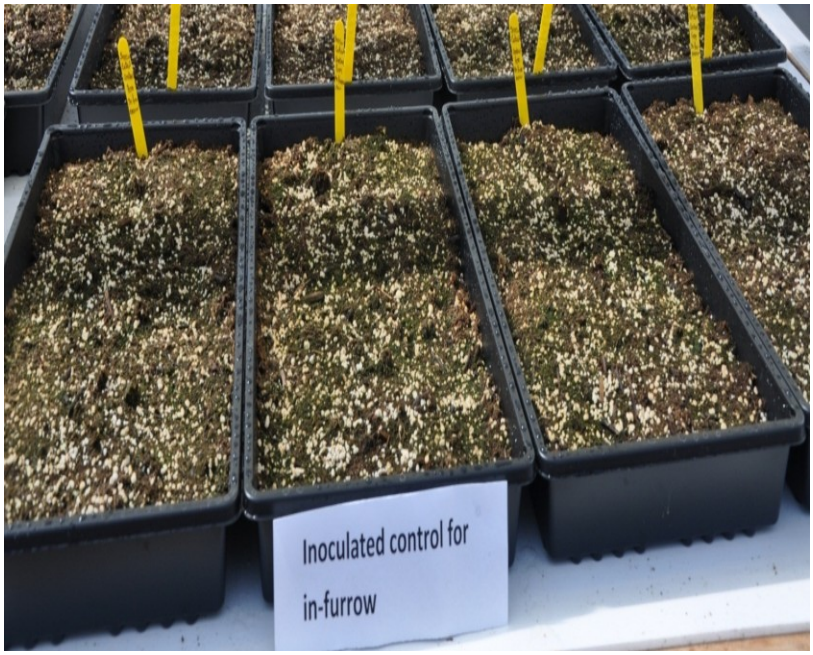
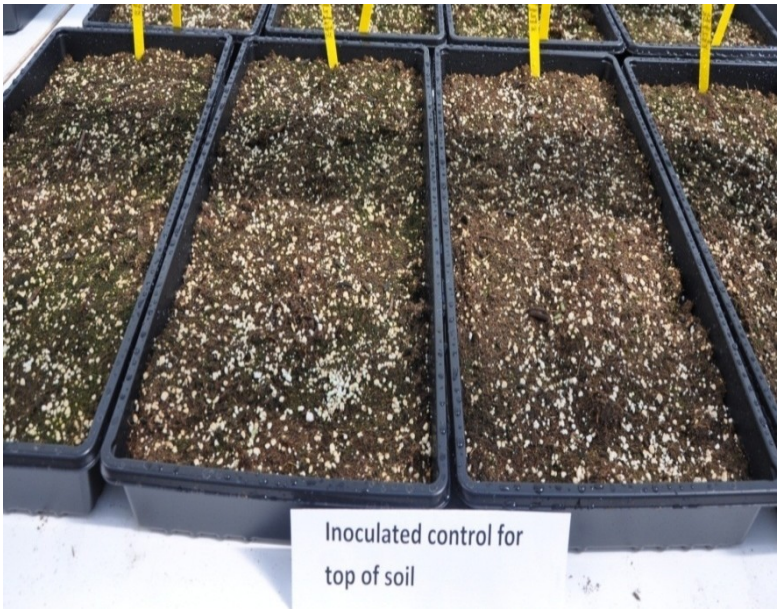
A



B

Fig. 1.1. Mean number of survived plants when azoxystrobin was applied as an in-furrow application followed by inoculation (A) and inoculated control without any fungicide application (B)

C



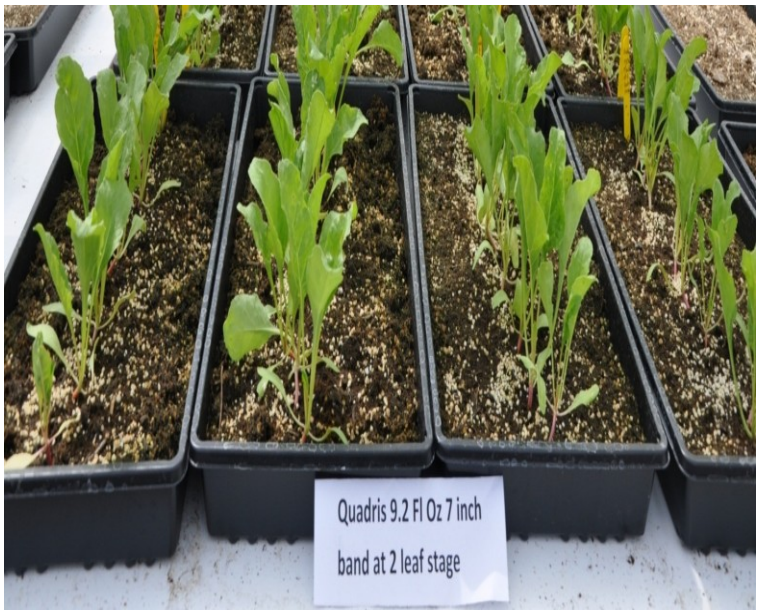
D

Fig. 1.2. Mean number of survived plants when azoxystrobin was applied as a 18 cm band on soil surface followed by inoculation (C) and inoculated control without any fungicide application (D)

E

F

Fig. 1.3. Mean number of survived plants when azoxystrobin was applied as a 18 cm band at cotyledonary stage of sugarbeet followed by inoculation (E) and inoculated control at cotyledonary stage without any fungicide application (F)



G

H

Fig. 1.4. Mean number of survived plants when azoxystrobin was applied as a 18 cm band at 2-lf stage of sugarbeet followed by inoculation (G) and inoculated control at 2-lf stage without any fungicide application (H)

I

J

Fig. 1.5. Mean number of survived plants when azoxystrobin was applied as a 18 cm band at 4-lf stage of sugarbeet followed by inoculation (I) and inoculated control at 4-lf stage without any fungicide application (J)

CHAPTER II: COMPATIBILITY AND SAFETY F MIXING AZOXYSTROBIN AND STARTER FERTILIZERS FOR CONTROLLING *RHIZOCTONIA SOLANI* IN SUGARBEET

Abstract

Rhizoctonia crown and root rot (RCRR) of sugarbeet caused by *Rhizoctonia solani* is one of the most important production problems in Minnesota and North Dakota. Azoxystrobin provides effective disease control when applied before infection takes place. Application of starter fertilizers at planting is a common practice for sugarbeet growers in this region. The objective of this research was to determine the compatibility and safety of mixing azoxystrobin with starter fertilizers in controlling *R. solani* of sugarbeet. Research was conducted in a greenhouse at North Dakota State University, Fargo, North Dakota. The greenhouse was maintained at a temperature of 20 ± 2˚ C. Three different starter fertilizers 10-34-0, 6-24-6 and Redline at 28.05 L ha^{-1}plus azoxystrobin was used at 167 g a.i. ha^{-1}. *Rhizoctonia solani* grown on barley was the inoculum and used at one barley grain placed closed to each sugarbeet seed. Ten seeds of sugarbeet cultivar Crystal 539RR susceptible to *R. solani* were planted in a furrow made in each tray. Treatments were applied in-furrow over the seeds followed by inoculation and then covering the furrows with soil. There were 12 treatments including a non-inoculated and an inoculated control; starter fertilizers used without and with inoculation; azoxystrobin use alone followed by inoculation compared with azoxystrobin mixed with each of the different starter fertilizers followed by inoculation. Plants were watered daily and evaluated 28 days after the inoculation when the controls were all dead. The non-inoculated control and treatments where only starter fertilizers were applied resulted in healthy plants. Starter fertilizers did not have any fungicidal activity; all inoculated plants treated or not with starter fertilizers died. Azoxystrobin

provided similar effective control of *R. solani* when used alone or mixed with starter fertilizers; there was no phytotoxicity. This study suggests that mixtures of azoxystrobin with different starter fertilizers evaluated under greenhouse conditions were compatible, resulted in no phytotoxicity and effectively controlled *R. solani*.

Introduction

Sugarbeet (*Beta vulgaris* L.) is considered a globally important food crop considering its importance as a food and fodder crop with a high source of sugar and for providing 25% of the world's sucrose (Draycott, 2006). The United States of America (USA) is the largest global consumer of sweeteners and one of the largest importers of sugar (Harveson et al., 2002). In 2009, the USA became the second largest sugarbeet producer with 29.78 million metric ton sugarbeet per year (FAOSTAT, 2009). Sugarbeet is cultivated in 10 states of the USA including California, Colorado, Idaho, Michigan, Minnesota, Montana, Nebraska, North Dakota, Oregon, and Wyoming (Harveson et al., 2002; USDA-ERS, 2013). Among these 10 states, Minnesota and North Dakota are the dominant producers with around 57% production of the nation (Bangsund et al., 2012).

Rhizoctonia solani Kühn (Teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk) is a destructive fungus with a wide host range and can cause damping-off of seedlings; root, crown and stem rot (Anderson et al., 1982; Ogoshi et al., 1987). The *R. solani* complex is a taxonomic entity composed of morphologically similar groups that share the following characteristics: multinucleate cells with dolipores (composed of a pore cap surrounding a septal swelling and septal pore), production of selerotia, and lack of conidia (Parmer et al., 1970). Hyphal anastomosis is an important taxonomic tool for identifying isolates of *R. solani* in solid media.

Three different anastomosis reactions can occur: complete, intermediate and no reaction (Carling and Kuninaga, 1990). On the basis of these anastomosis reactions, 14 anastomosis groups (AG) have been described (Carling, 1996; Carling et al., 2002; Gonzàlez et al., 2006). These AG groups are distinct according to their physiological and genetic makeup. On sugarbeet, *R. solani* AG 2-2 group with intraspecific groups (ISGs) IIIB and IV is most devastating and aggressive. The ISGs of *R. solani* (IV and IIB) are found throughout the sugarbeet growing areas of Minnesota and North Dakota, with AG 2-2 IV predominating in the Red River Valley and AG 2-2 IIIB in Southern Minnesota (Brantner and Windels, 2007). Since crop rotation has shifted with increased production of soybean, edible bean and corn; there appears to be a concurrent change in the prevalence of *R. solani* AG 2-2 IV to AG 2-2 IIIB in this region (Brantner and Windels, 2007). *R. solani* AG 2-2 IIIB is more aggressive and damaging compared with AG 2-2 IV (Engelkes and Windels, 1994; Ogoshi., 1987). *R. solani* AG 2-2 IIIB is a heat tolerant isolate and can survive and continue growth up to 35˚ C while AG 2-2 IV does not tolerate high temperature (Sneh et al., 1991). Optimum temperature for infection by *R. solani* ranges from 18 to 30˚ C and moisture level of 75 to 100%, although infection takes place with 25% moisture conditions (Bolton et al., 2010). This disease adversely impacts 24% of the acres sown to sugarbeet in the United States and 5-10% in Europe (Büttner et al., 2003). Disease severity varies from field to field but yield losses can be significant and losses higher than 50% have been reported (Windels and Brantner, 2009; Herr, 1996; Allen et al., 1985, Büttner et al., 2002). Rhizoctonia crown and root rot may significantly reduce yield (Khan et al., 2010).

In the Red River Valley of North Dakota and Minnesota and in Southern Minnesota, Rhizoctonia crown and root rot has become prevalent and severe during the last decade. In 2009, Rhizoctonia root rot was named as one of the most serious production problem affecting

sugarbeet in Minnesota and North Dakota (Stachler et al., 2009). Diseases caused by soilborne pathogens are more difficult to control than those caused by foliar pathogens, and usually cause more devastating losses to producers because they are difficult to detect before significant damage can occur (Rush, 1990). Genetic resistance has some possibility in disease reduction but breeding for resistance to soilborne pathogens including *R. solani* is difficult because the inheritance of resistance is multigenic and the heritabilities are lower than with single gene resistance (Afanasiev and Sharp, 1961). Consequently, highly resistant variety is rare as the presence of minor genes increases the difficulty of identifying and isolating major resistance genes (Hecker and Ruppel, 1975; Hecker and Ruppel, 1977).

Research shows that some fungicides provided effective control against *R. solani* (Franc et al., 2001). Azoxystrobin a strobilurin fungicide within FRAC group 11 consistently provides effective control of Rhizoctonia crown and root rot when applied prior to infection (Franc and Stump, 2007; Bolton et al., 2010, Jacobson et al., 2002; Kiewnick et al., 2001; Windels and Brantner, 2002).

Application of starter fertilizers at planting is a common practice for sugarbeet growers in North Dakota and Minnesota. Starter fertilizers are a condensed form of fertilizer generally found in a liquid form and is applied to soil in close proximity to the seedling root system (Source: http://www.crystalsugar.com/agronomy/gold/fact/ag_starter_fertilizer_lr.pdf). Starter fertilizers are effectively used to apply phosphorus fertilizer to the sugarbeet crop, and the direct application over seeds consequently promotes germination of plant (Source: http://www.crystalsugar.com/agronomy/gold/fact/ag_starter_fertilizer_lr.pdf). The application of starter fertilizers and azoxystrobin will save time and fuel, reduce the number of passes over a field, and reduce production cost. Moreover, along with protection against *R. solani* by

azoxystrobin, sugarbeet will obtain essential nutrient from liquid starter fertilizer that will ultimately contribute to yield. There is no report on the safety of plants when treated with a mixture of azoxystrobin and starter fertilizers and the effect of these mixtures on controlling *R. solani*. The objective of this study was to evaluate the efficacy of azoxystrobin applied in mixtures with starter fertilizers in controlling *R. solani* and the safety of the mixtures to sugarbeet seeds and seedlings.

Materials and Methods

Research was conducted in a greenhouse at North Dakota State University in Fargo, ND. Sugarbeet cultivar Crystal 539RR was used as a Rhizoctonia susceptible variety (Niehaus, 2011). Sunshine mix 1 peat soil (Sun Gro Horticulture Ltd., Canada) was filled in a tray measuring 25x14x13 cm (T.O. Plastics Inc. Clearwater, MN). One furrow measuring 2.5 cm deep was made in the middle of the tray into which 10 sugarbeet seeds were planted. *R. solani* AG 2-2 IIIB infested barley grain was used as inoculum (Pierson and Gaskill, 1961). Fungicide used for this experiment was azoxystrobin at 167 g a.i. ha^{-1}. Three different types of starter fertilizers - 10-34-0, 6-24-6 (Neutra-Flo Company, Grand Sioux City, IA) and Redline (West Central Inc., Willmar, MN) were used at 28.05 L ha^{-1} as an in-furrow application alone or mixed with azoxystrobin. When individually mixtures were used, each starter fertilizers of 28.05 L ha^{-1} were added to a mixture of 46.7 L ha^{-1} water and 167 g a.i. ha^{-1} fungicide. All treatments were applied in-furrow using a spraying system (De Veries Manufacturing, Hollandale, MN) calibrated to deliver the solution at 138 kPal with a speed of 3.91 mile per hour through a single flat fan nozzle 4001E. There were 12 treatments: each of the three starter fertilizers were applied without any inoculation; each of the three starter fertilizers were applied and followed with inoculation; azoxystrobin was applied alone followed by inoculation; azoxystrobin was mixed with each of

the three starter fertilizers separately and applied followed by inoculation; non-inoculated control; inoculated control. Trays were watered daily and data collection was done 28 days after spraying. Stand counts of surviving sugarbeet plants were taken and sugarbeet roots were washed carefully and evaluated for any root rot symptoms present on the tap root. The experimental design was a randomized complete block design (RCBD) with 3 replicates and the experiment repeated two times in the same environment.

Data analysis

Each experiment was analyzed separately and a folded F-test was used to determine whether the data were homogeneous. Experimental data were combined as no significant differences were observed at F=0.025 level of confidence. Analysis of variance was done using the SAS general linear models (Proc GLM) procedure (Version 9.3, SAS Institute Inc., Cary, NC). Fisher's protected least significant difference of means at $\alpha = 0.05$ was calculated to compare treatment means. Runs and treatments were considered as fixed effect.

Results

The analysis of variance (Table 2.1) indicated that there were significant differences among the treatments at $P \leq .001$ level of confidence. As expected, the non-inoculated control resulted in 88% survivability of healthy plants with no disease symptoms which was significantly greater than the 2% of surviving plants in the inoculated control confirming that the *R. solani* inoculum was effective (Table 2.2). The starter fertilizers used alone with artificial inoculation were ineffective in controlling *R. solani* and resulted in percentages of surviving plants similar to the inoculated control (Table 2.2).

Table 2.1. Analysis of variance showing the effect of applying azoxystrobin and starter fertilizers alone and as a mixture on sugarbeet for controlling *R. solani*

Source of variation	DF	Mean Square	F Value	Pr > F
Run	1	148.21	1.74	0.19
Rep(Run)	4	76.12	0.89	0.4757
Treatment	11	9596.95	112.68***	<.0001
Run × Treatment	11	146.29	1.72	0.1008
Error	44	85.16	_	_
Total	71	_	_	_

***Indicates significant at $P \leq 0.001$ level of confidence

The use of starter fertilizers alone in-furrow did not impact the survivability since the percentage of plants that survived when treated with starter fertilizers alone were not significantly different from the non-inoculated control which did not receive any starter fertilizers. Azoxystrobin applied in-furrow alone provided effective control of *R. solani* resulting in a similar percentage of survivors as the non-inoculated control which was significantly higher than the inoculated control. The percent survivals of sugarbeet stands with the addition of starter fertilizers along with azoxystrobin was significantly higher than the inoculated control.

The addition of starter fertilizers with azoxystrobin did not adversely impact the efficacy of the fungicide since the percentages of survivors treated with the fungicide and starter fertilizers mixtures were not significantly different from the percentages of survivors when azoxystrobin was used alone or the non-inoculated control. None of the treatments where starter

fertilizers were used alone or in a mixture with azoxystrobin resulted in poor emergence or phytotoxicity of seedlings.

Table 2.2. Effect of azoxystrobin and starter fertilizers used alone and in mixtures on controlling *R. solani* in sugarbeet

Treatments	MFA[†]	MSP[‡] (%)
Non-Inoculated control	No application	88a
Inoculated control	No application	2b
10-34-0 at 28.05 L ha^{-1}	In-furrow	85a
10-34-0 at 28.05 L ha^{-1}+ inoculated	In-furrow	8b
6-24-6 at 28.05 L ha^{-1}	In-furrow	88a
6-24-6 at 28.05 L ha^{-1} + inoculated	In-furrow	10b
Redline at 28.05 L ha^{-1}	In-furrow	88a
Redline at 28.05 L ha^{-1}+ Inoculated	In-furrow	2b
Azoxystrobin167 g a.i. ha^{-1} + Inoculated	In-furrow	88a
Azoxystrobin167 g a.i. ha^{-1} + 10-34-0 at 28.05 L ha^{-1}a + Inoculated	In-furrow	83a
Azoxystrobin167 g a.i. ha^{-1} + 6-24-6 at 28.05 L ha^{-1}+ Inoculated	In-furrow	82a
Azoxystrobin167 g a.i. ha^{-1} + Redline at 28.05 L ha^{-1}+ Inoculated	In-furrow	88a
LSD[§] (0.05)		11

[†], Method of fungicide application, [‡], Mean survived plants, [§], Treatment means followed by the same letter are not significantly different from each other based on Fisher's protected LSD ($P \leq 0.05$)

Discussion

Sugarbeet growers in North Dakota and Minnesota typically apply starter fertilizers high in phosphorus as an in-furrow application directly over the seed at planting. This practice was initiated in early 2000s to reduce production costs when Sims and Smith (2004) showed that the use of 28 L ha^{-1} of 10-34-0 applied directly over seeds at planting resulted in similar yield as applying 112 kg ha^{-1} of diammonium phosphate (DAP) fertilizer as a broadcast application in soils with low phosphorus levels (Sims and Smith, 2004).

Disease caused by *Rhizoctonia solani* has become the most important problem affecting sugarbeet production in North Dakota and Minnesota starting in 2009 (Carlson et al., 2010). Research shows that in the presence of adequate moisture, *R. solani* initiates infection at 18˚C (Khan et al., 2010). In 2010 through 2012, sugarbeet planting in North Dakota and Minnesota was done when average daily soil temperature at the 10 cm depth was at 18˚C or higher (http://ndawn.ndsu.nodak.edu/). Consequently, growers who have fields with a history of Rhizoctonia damping off and/or root rot need to take preventive measures such as applying an effective fungicide at planting or before the threshold soil temperature at which infection takes place is reached to control the disease. The use of resistant cultivars is not an option since most commercial sugarbeet cultivars have low to intermediate levels of resistance to *R. solani*, and even more resistant cultivars are susceptible at cotyledonary and seedling stages (Jacobsen at al., 2001).

Our research was consistent with other studies (Franc and Stump, 2007; Bolton et al., 2010, Jacobson et al., 2002; Kiewnick et al., 2001; Khan et al., 2010; Windels and Brantner, 2002), which showed that azoxystrobin effectively controlled *R. solani* when applied before infection took place. In addition, disease control was maintained when azoxystrobin was mixed

with starter fertilizers and there were no adverse effects on emergence of seedlings nor was there any phytotoxicity. Seedling emergence in the non-inoculated check was 88% (Table 2.2) which was not significantly different from any of the inoculated plants treated with azoxystrobin. Emergence in greenhouse conditions is typically higher than field conditions where emergence may range from 54% (Niehaus, 2011) to 77% (Niehaus, 2012) depending on soil and environmental conditions. This study suggests that starter fertilizers may be mixed with azoxystrobin to provide control of *R. solani* in field conditions similar to the greenhouse. However, field research should also be conducted to determine the safety and efficacy of these mixtures in cooler conditions since planting may take place in years when soils are 5 or 10˚ C. Other options available to avoid mixing fertilizers with a fungicide include applying azoxystrobin at planting to provide protection against *R. solani* to get a good plant density and post-apply a starter fertilizer; or broadcast phosphorus in the fall and apply azoxystrobin at planting for disease control.

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A

B

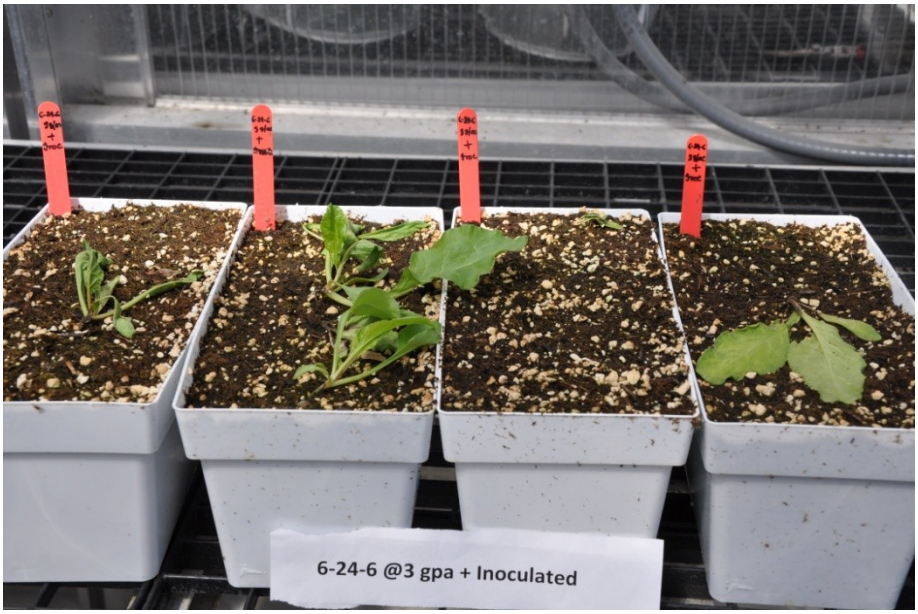
Fig. 2.1. Symptoms on sugarbeet foliage when non-inoculated (A) and inoculated (B)

C

D

Fig. 2.2. Symptoms on sugarbeet plants when 10-34-0 was applied as an in-furrow application without inoculation (C) and 10-34-0 applied as an in-furrow followed by inoculation (D)

E



F

Fig. 2.3. Symptoms on sugarbeet plants when 6-24-6 was applied as an in-furrow application without inoculation (E) and 6-24-6 applied as an in-furrow followed by inoculation (F)

G

H

Fig. 2.4. Symptoms on sugarbeet plants when Redline was applied as an in-furrow application without inoculation (G) and Redline applied as an in-furrow followed by inoculation (H)

Fig. 2.5. Symptoms on sugarbeets when azoxystrobin was applied as an in-furrow application followed by inoculation (I), azoxystrobin applied as a mixture with 10-34-0 followed by inoculation (J), azoxystrobin applied as a mixture with 6-24-6 followed by inoculation (K), and azoxystrobin applied as a mixture with Redline followed by inoculation (L)

Fig. 2.6. Symptoms on sugarbeet root when 10-34-0 was applied as an in-furrow application without any inoculation (A), 6-24-6 was applied as an in-furrow application without any inoculation (B), and Redline was applied as an in-furrow application without any inoculation (C)

Fig. 2.7. Symptoms on sugarbeet roots when azoxystrobin was applied as an in-furrow application followed by inoculation (A), azoxystrobin applied as a mixture with 10-34-0 followed by inoculation (B), azoxystrobin applied as a mixture with 6-24-6 followed by inoculation (C), and azoxystrobin applied as a mixture with Redline followed by inoculation (D)

CHAPTER III: EVALUATION OF PLACEMENT OF AZOXYSTROBIN IN CONTROLLING *RHIZOCTONIA SOLANI* OF SUGARBEET

Abstract

Rhizoctonia crown and root rot of sugarbeet caused by *Rhizoctonia solani* Kühn is the most common and serious root diseases of sugarbeet in the USA. Among the fungicides azoxystrobin provides effective disease control but mechanism of control is unknown. The objective of this study was to find out how azoxystrobin placement influences *R. solani* infection. The experimental was conducted in the greenhouse with a temperature of 20±2˚ C and 12 hours photoperiod. Sugarbeets susceptible to *R. solani* were planted and grown to four leaf stage. The fungicide azoxystrobin was applied at 167 g a.i. ha^{-1} either in a band or as a root dip. Barley grain colonized with *R. solani* was used as inoculum (2 grains /plant or pot). Azoxystrobin at 167 g a.i. ha^{-1} was applied in a band at the four leaf stage followed by inoculation with *R. solani* infested barley grain. Also azoxystrobin was band-applied on soil surface that had been inoculated before spraying and was left three and seven days without watering. Three and seven days later sugarbeet plants of four-leaf stage were transplanted on those pots followed by watering daily. As a root dip purpose, four-leaf stage sugarbeet roots were dipped in azoxystrobin and then transplanted into a fresh pot of soil followed by inoculation. An inoculated and non-inoculated check was also included for this experiment. After 21 days of azoxystrobin application, data was collected by rating the sugarbeet root using Ruppels 0 to 7 scale (Ruppel et al., 1979). Results demonstrated that azoxystrobin provides control against *R. solani* when applied either in a band or as a root-dip compared with inoculated control. There was a slight difference between azoxystrobin in band application on 4-leaf stage and the azoxystrobin applied in band on inoculated soil surface and 3 days later transplant. No

significant difference was prominent among azoxystrobin root dip treatment and azoxystrobin applied in band on previously inoculated soil surface and 7 days later transplant.

Introduction

The soil-borne pathogen *Rhizoctonia solani* Kühn is indigenous in most sugarbeet (*Beta vulgaris* L.) production areas in the United States (Hecker and Ruppel, 1974). This pathogen has a wide host range; including sugarbeet. *Rhizoctonia solani* can cause different disease symptoms based on the growth stage of the sugarbeet plant at infection. For example, *R. solani* cause damping off of sugarbeet seedlings, crown and root rot of older plants, and infrequently foliar blight of older plants. Damping-off by *R. solani* initiated by attacking the hypocotyl below ground and severely diseased seedlings collapse and die (Whitney and Duffus, 1986). The aboveground symptoms of crown rot are sudden wilting and chlorosis of foliage with black necrosis of petioles near the crown, wilted leaves collapse and form a brown and black rosette which persists throughout the growing season (Whitney and Duffus, 1986). Crown and root rot symptoms might lead sugarbeet to be partially or fully rotted, infected root areas are dark brown to black and within that infected tissue light to dark brown dry rot develop (Whitney and Duffus, 1986).

Among these diseases, crown and root rot is very important because it occurs more often and may lead to huge economic losses. It is estimated that 5-10% of the total sugarbeet area in Europe is affected by Rhizoctoniaroot rot (Büttner et al., 2003), even economic losses could reach above 50% in a very favorable environmental condition (Allen et al., 1985; Büttner et al., 2002). The fungus commonly invades sugarbeet roots near the soil-root interface i.e. hypocotyl region or at the base of the petioles (crown region). Progression of the disease leads to partial or total rot of the crown and root, death of the leaves and plants (Hecker and Ruppel, 1975; Scolten

et al., 2001). The basidiomycete *R. solani* (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is a widespread and highly biodiverse plant pathogen (Führer Ithurrart et al., 2004). This biodiversity manifest itself in different isolates or anastomosis groups (AG) with different host susceptibility (Führer Ithurrart et al., 2004). Currently 14 anastomosis groups have been identified for *R. solani* (Carling, 1996; Carling et al., 2002; Gonzàlez et al., 2006). For sugarbeet, AG 2-2IIIB, AG 2-2IV and AG 4 are relevant as pathogens that cause infection (Engelkes and Windels, 1996; Rush et al., 1994; Führer Ithurrart, 2003). Among these AG 4 cause damping off on younger plants or seedlings, isolates of AG 2-2 mainly infect mature sugarbeet plants causing Rhizoctonia crown and root rot (Herr 1996). Pathogenicity test confirm *R. solani* AG 2-2 IIIB is more aggressive in attacking soybean, edible bean and sugarbeet compared to AG 2-2 IV, which cause moderate root rot on these crop (Windels and Brantner, 2007). The pathogen can tolerate a wide range of temperature from 12 to 35˚ C, with an optimum temperature between 20-30˚ C (Leach, 1986). Pathogen activity is also favored by wet areas or fields with poorly drainage condition (Franc et al., 2001). The population of *R. solani* has been increasing in prevalence and severity from the last decade (Windels and Brantner, 2005). Several factors might work behind in building up *R. solani* inoculum density like, wet and warm summer, cultivation of susceptible other host crop such as soybean, edible bean, corn, previous year crop residues, as well as deposition of infested soil on sugarbeet crown during cultivation.

Control of *R. solani* can be achieved through seed treatment, early planting, cultivation to hasten drying of soil, slow cultivation to avoid “hilling” soil in beet crowns, rotation with non-host crops, removal of weed host, and using host resistant (Windels and Lamy, 1998). None of this method can give protection against Rhizoctonia crown and root rot totally. Seed treatment fungicide although works for a short time but in the long run they are no longer effective. Also,

there is no complete resistant cultivar and partial resistant has significant yield reduction. Azoxystrobin (Quadris®Syngenta, Greensboro, NC, USA) being registered and received full – label approval to control *R. solani* of sugarbeet. It was the first synthetic strobilurin product to be announced at the Briton crop Protection Conference of 1992 as a commercial fungicide by Syngenta (Balba, 2007). The mode of action of strobilurin fungicide was novel and target specific. They bind to one specific site in the mitochondria, the quinol oxidation (Q_o) site (or ubiquinol site) of chytochrome b and thereby stop electron transfer between chytochrome b and chytochrome c, which halts reduced nicotinamide adenine dinucleotide (NADH) oxidation and adenosine triphosphate (ATP) synthesis (Brandt et al., 1993; Von Jagow, 1982). Fungicides are classified according to where in the disease cycle fungicides are active, based on these Strobilurins are categorized in protective or protectant fungicides group. Because Strobilurin containing product azoxystrobin is effective prior to infection and the initiation of the disease cycle of *R. solani* (Balba, 2007). Several other study also recommended to apply azoxystrobin prior to the initiation of *R. solani* infection and progression of disease cycle in order to attain good control against Rhizoctonia crown and root rot (Brantner and Windels, 2002; Jacobsen et al., 2002; Bolton et al., 2010; Khan et al., 2010). Strobilurin fungicides are also weakly systemic, so used mainly as protectant, curative and translaminar fungicides (Balba, 2007). As strobilurin are weakly systemic fungicides, a good coverage of the entire plant canopy is essential for the fungicide to be effective (Balba, 2007). Several studies have been done on azoxystrobin action in controlling crown rot of sugarbeet caused by *R. solani* at four or older leaf stages since agricultural practices leads to deposit soil on sugarbeet crown initiates crown infection and rot (Kirk et al, 2008; Kiewnick et al., 2001). There is no report on how azoxystrobin obtains control against Rhizoctonia root rot along with crown rot, because root rot infection is also very severe

and since azoxystrobin is weakly systemic so how the fungicide obtain control in sugarbeet root also along with crown. The objective of this study was to find out how placement of azoxystrobin provides protection against *R. solani* infection.

Materials and Methods

The study was conducted at North Dakota State University greenhouse facility located in Fargo, ND. Sugarbeet cultivar Crystal 539RR (Niehaus, 2011) was used as a *R. solani* susceptible variety. Three sugarbeet seeds were sown 2.5 cm deep in Sunshine Mix 1 peat soil (Sun Gro Horticulture Ltd. Canada) per pot measuring 9.3 × 7.5 cm (T.O. Plastics Inc. Clearwater, MN) in measurement. Plants were thinned at cotyledonary stage to allow one vigorous plant per pot. Fungicide was applied at 4-leaf stage followed by inoculation. Azoxystrobin (Quadris®Syngenta, Greensboro, NC, USA) fungicide was used in this experiment. *Rhizoctonia solani* AG 2-2 IIIB isolate was colonized on barley grain and used for inoculation (Pierson and Gaskill, 1961). Banded fungicide application was done using a spraying system (De Veries Manufacturing, Hollandale, MN) calibrated to deliver the spray solution at 138 kPa with a speed of 3.91 miles per hour through a single flat fan nozzle 4001E. The experiment was setup as a Complete Randomized Design (CRD) with 8 replicates. Experiments were repeated twice in the same environment (runs).

Evaluation by disease scoring and data collection

Disease data was collected after 21 days post fungicide application. Sugarbeets root was washed carefully and roots were rated according to Ruppels 0-7 scale (Ruppel et al., 1979). Where, 0 = healthy plants with no lesion, 1 = <1% with visual lesions, 2 = 1-5% of root surface with visible lesions, 3 = 5-25% of root surface with dry root canker, 4 = 25-50% of root surface with dry root canker, 5 =50-75% of the root surface with dry root canker, 6 = 75% of the root

surface with dry root canker and 7 = plant is completely dead with fully rotted roots and wilted dry leaves.

Fungicide application

Table 3.1. Placement of azoxystrobin at 4-leaf stage of sugarbeet using different methods

Treatments	† MFA
Non-Inoculated check	No fungicide
Inoculated check	No fungicide
Azoxystrobin was applied at 4-leaf stage followed by inoculation	18 cm band
Two *R. solani* infested barley grain were placed 1.5 cm deep in the center of soil-filled pots, which were treated with azoxystrobin. Three days later, 4-leaf stage healthy sugarbeets were removed carefully from another pot and transplanted into the pot of soil that was inoculated and sprayed 3 days before.	18 cm band
Two *R. solani* infested barley grain were placed 1.5 cm deep in the center of soil-filled pots, which were treated with azoxystrobin. Seven days later, 4-leaf stage healthy sugarbeets were removed carefully from another pot and transplanted into the pot of soil that was inoculated and sprayed seven days before.	18 cm band
Sugarbeet plants were taken out from the pots and roots were washed carefully followed by dipping the roots into azoxystrobin. Roots were air-dried for three minutes then transplanted into fresh pot of soil followed by inoculation.	Root dipping

†, Method of fungicide application

Data analysis

Data was analyzed with non-parametric analysis of the MIXED procedure of SAS by using LSMEANS option (version 9.2, SAS Institute Inc., Cary, NC) (Brunner and Puri, 2001; Shah and Madden, 2004). Also the estimated treatment relative effects were compared using LD_CI macro (Brunner and Puri, 2001).

Results

Results demonstrated that all the fungicide treatments had significantly lower disease severity compared to the inoculated nonprotected control (Table 3.2). The 18 cm band application to 4-leaf stage sugarbeet, typically applied by growers, provided effective disease control. When azoxystrobin was used as a root dip, or when applied to the soil surface of inoculated pots followed by transplanting 3 or 7 days later, disease control was similar to that obtained by the recommended application. The highest mean disease rank found in the inoculated control and lowest mean disease rank was observed in the non-inoculated control (Table.3.2). Other treatments where fungicides were applied either as an 18 cm band or in a root dip gives good control against *R. solani*, since mean disease rank is significantly lowered than the inoculated control (Table. 3.2).

There are also significant differences predominant between treatments where azoxystrobin was applied as an 18 cm band on foliage followed by inoculation with treatments where azoxystrobin was applied on soil surface as an18 cm band and sugarbeet was transplanted 3 days later (Fig. 3.1). Although foliar band application of azoxystrobin treatment is not significantly different than the root dipping treatment and where fungicide was applied on inoculated soil surface followed by transplanting sugarbeet 7 days later (Fig. 3.1).

Table 3.2. Effect of placement of azoxystrobin for controlling *R. solani* on sugarbeet

Treatment	Mean rank	Estimated relative effect	Confidence interval (95%) for treatment's relative effect	
			Lower limit	Upper limit
Non-Inoculated control	28	0.2865	0.2508	0.3269
Inoculated Control	88.47	0.9163	0.9115	0.9166
Azoxystrobin 18 cm band on foliage+Inoculated	54.5	0.5625	0.475	0.6447
Azoxystrobin 18 cm band on inoculated soil surface followed by transplanting sugarbeet 3 days later	35.44	0.3639	0.2937	0.444
Azoxystrobin 18 cm band on inoculated soil surface followed by transplanting sugarbeet 7 days later	42.28	0.4352	0.3436	0.5337
Azoxystrobin as a root dip+Inoculated	42.31	0.4355	0.353	0.5235

The results demonstrated that the fungicide provided control when it is directly over the root through which the pathogen has to penetrate, and when it is in the soil close to the roots and the pathogen.

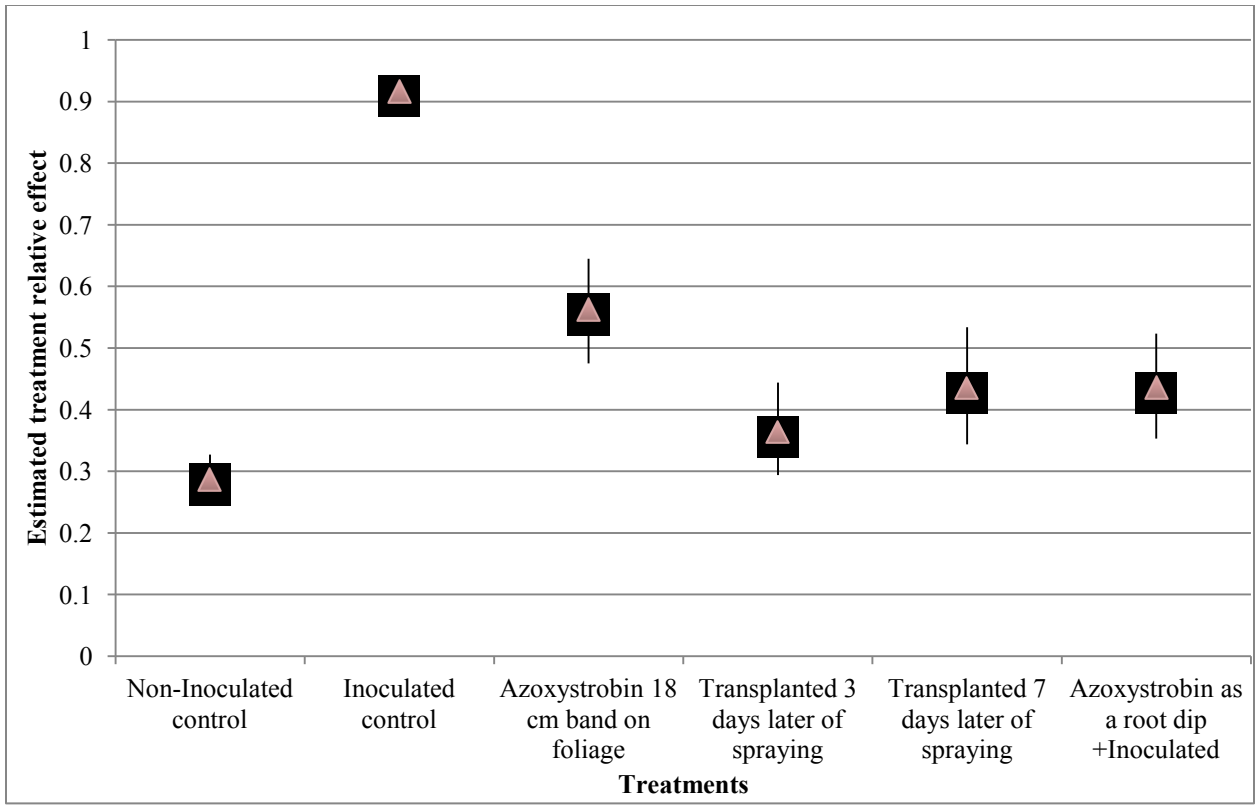


Figure 3.1. Estimated relative effect and confidence interval (Lower and Upper limit) for the treatments.

Discussion

In Minnesota and North Dakota, sugarbeet growers typically use foliar application of azoxystrobin to manage *R. solani* before soil temperatures reaching 18˚ C, the threshold at which infection would take place. In this study, azoxystrobin applied foliarly on four-leaf stage sugarbeets provided a significantly better control of *R. solani* than the inoculated nonprotected control. Reports on foliar application of azoxystrobin on four-leaf stage sugarbeet plants showed it could significantly improve plant stands compared to the non-treated control (No fungicide applied) in the field (Stump et al., 2004), which was also confirmed by other authors (Khan and Carlson, 2009; Windels and Brantner, 2008). Azoxystrobin is a systemic fungicide that is redistributed by the xylem vessels to upper parts of the plant but its movement toward the root is limited (Balba, 2007). In the foliar application, the fungicide was sprayed onto the soil directly

since the canopy did not cover the soil completely. Furthermore, during watering the fungicide was probably washed off from leaves and got into the soil where the pathogen was killed due to contact with the fungicide.

This study also showed that azoxystrobin can protect the plants from infection by *R. solani* even if the fungicide was previously applied onto soil in dry conditions up to 7 days. It appears that the azoxystrobin provides control once it is present in the soil in close proximity to the roots or on the root surface. Similarly, another study found that application methods, such as soil drench and drip chemigation, which allowed more fungicide to reach into the soil or get into more contact with the plant root provided better protection against soilborne disease than foliar applications (Meyer and Hausbeck, 2013).

Some authors believe that root exudates stimulate *R. solani* to colonize and later infect the sugarbeet plant (Kerr and Flentje, 1957; Flentje et al., 1963; De Silva and Wood, 1964). This study suggests that the fungicide on the root or in the soil inhibits germination or prevent mycelial growth of the pathogen and thus stops or reduces infection. Root dipping, which was effective in this study will not be practical. However, this study suggest that fungicide such as azoxystrobin should target the plant parts, in this case the root that is focus of the fungus, and/or the soil around the roots where the fungus lives to prevent infection from taking place.

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A

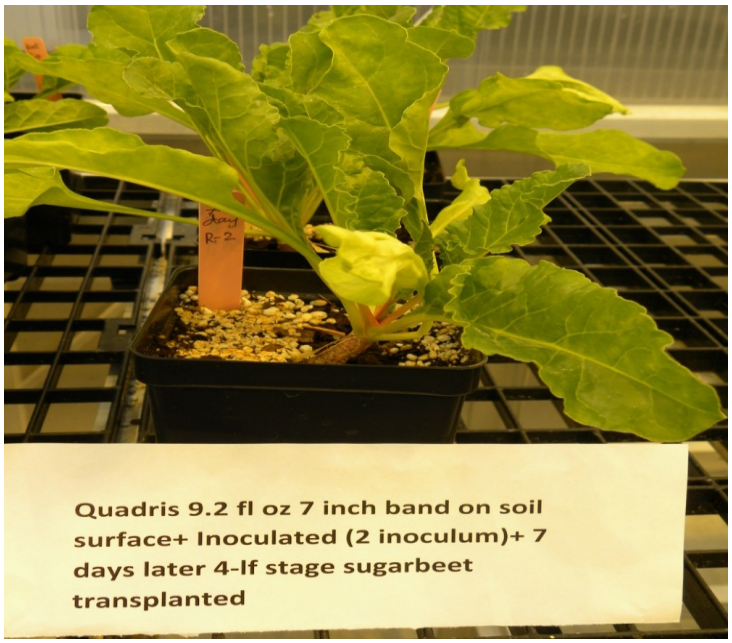
B

Fig.3.2. Symptoms on sugarbeet plants when non-inoculated at 4-lf stage (A), and inoculated control (B)

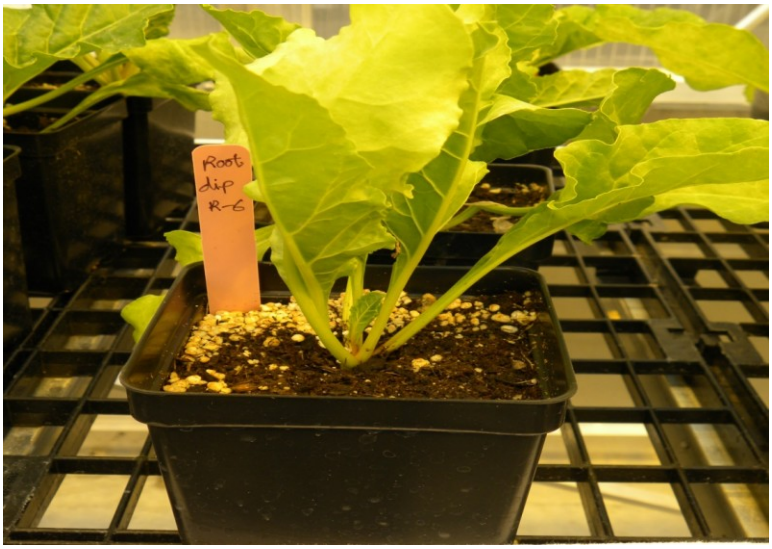
C

D

Fig. 3.3. Symptoms on sugarbeets when azoxystrobin applied as 18 cm band followed by inoculation (C) and 4-lf stage sugarbeet transplanted into a soil filled pot that had been inoculated and had its soil surface being sprayed with azoxystrobin from 18 cm band 3 days before (D)



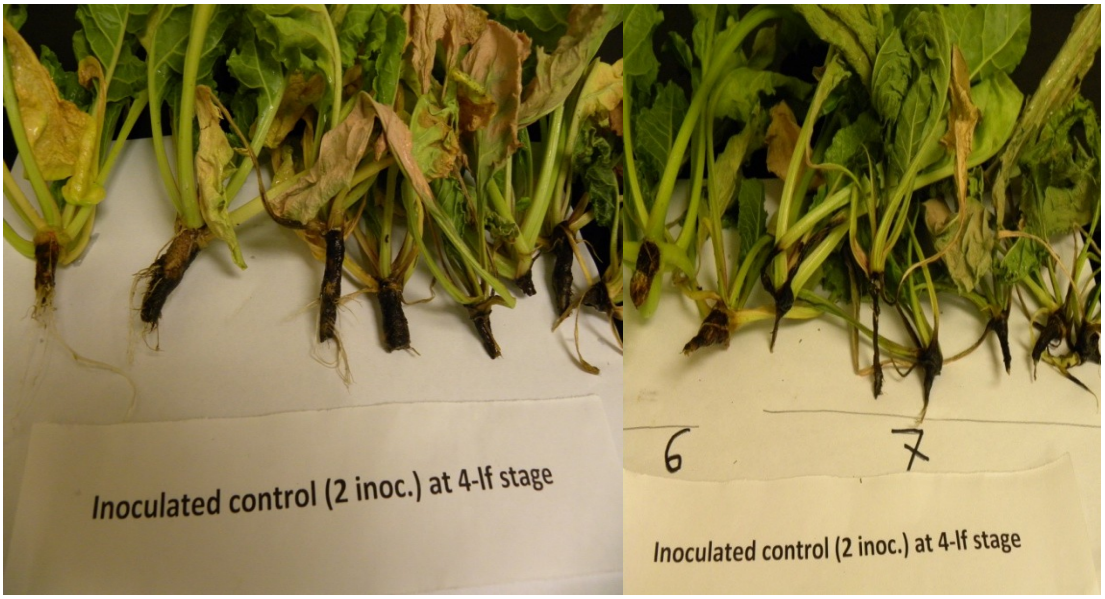
E



F

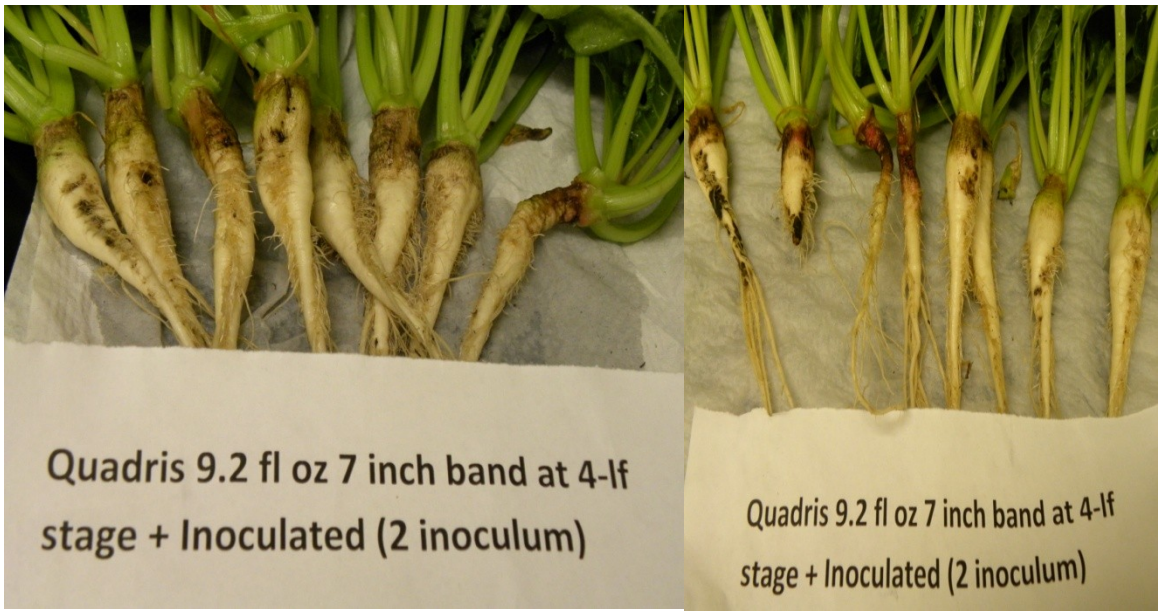
Fig. 3.4. Symptoms on sugarbeets when 4-lf stage sugarbeet transplanted into a soil filled pot that had been inoculated and had its soil surface being sprayed with azoxystrobin from 18 cm band 7 days before (E) and azoxystrobin applied as a root dip followed by inoculation (F)

A



B

Fig. 3.5. Root rot symptoms on sugarbeet roots when no inoculation was done (A) and inoculated at 4-lf stages (B)



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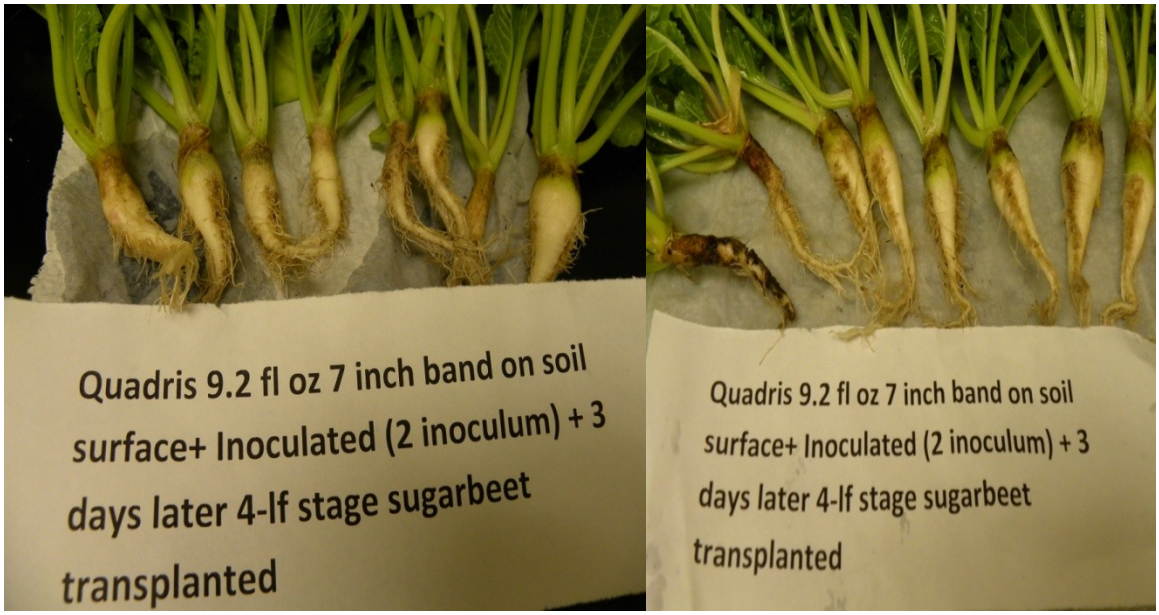
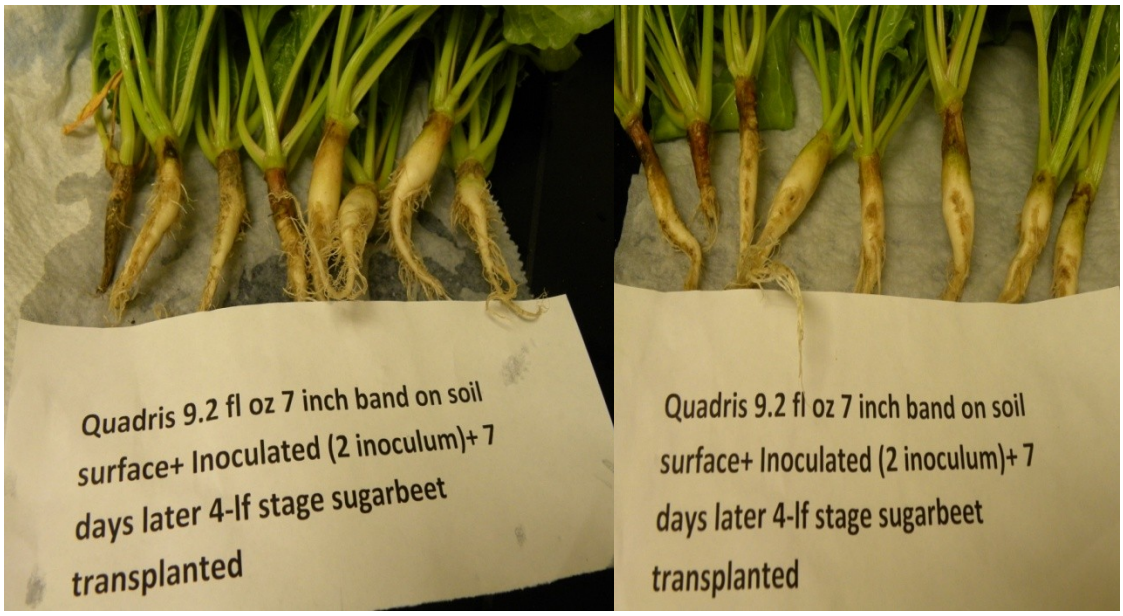


D

Fig. 3.6. Root rot symptoms on sugarbeet when azoxystrobin applied as a 18 cm band followed by inoculation (C) and 4-lf stage sugarbeet transplanted into a soil filled pot that had been inoculated and had its soil surface being sprayed with azoxystrobin from 18 cm band 3 days before (D)



E

F

Fig. 3.7. Root rot symptoms on sugarbeet when 4-lf stage sugarbeet transplanted into a soil filled pot that had been inoculated and had its soil surface being sprayed with azoxystrobin from 18 cm band 7days before (E) and azoxystrobin applied as a root dip followed by inoculation (F)