THE IMPACT OF PLANT AGE, FUNGICIDE APPLICATION METHODOLOGY AND TIMING, AND DEPTH OF SOIL INOCULATION ON INFECTION BY *RHIZOCTONIA SOLANI* ON SUGARBEET

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Pathology

September 2010

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

ABSTRACT

Pooran-DeSouza, Somwattie; M.S.; Department of Plant Pathology, College of Agriculture, Food Systems, and Natural Resources; North Dakota State University, September 2010. The Impact of Plant Age, Fungicide Application Methodology and Timing, and Depth of Soil Inoculation on Infection by *Rhizoctonia solani* on Sugarbeet. Major Professor: Dr. Mohamed F. R. Khan.

Rhizoctonia root rot, caused by *Rhizoctonia solani* (Kühn), is the most important problem faced by sugarbeet (*Beta vulgaris* L.) growers in North Dakota and Minnesota. Research was conducted that may be used to manage the disease. Six cultivars from 2 to 8-leaf stage were evaluated for their ability to withstand infection after soil inoculation by *R. solani* AG 2-2 IIIB. All leaf stages of the cultivars evaluated were susceptible to *R. solani.* Sugarbeet plants at the 2-leaf stages were most susceptible and had significantly higher root rot severity than plants at the 4, 6 and 8-leaf stages. Cultivars Crystal 454 and Hillshog 3035 had the lowest root rot severity of the cultivars evaluated, but root rot severity was still greater than 50%. The cultural practice of planting early in soils when the temperature is about 10°C at the 10 cm depth may result in plants being older and more tolerant by the time the pathogen becomes infective at warmer soil temperatures. However, the fact that all plant stages were susceptible to *R. solani* may require additional protective measures in highly infested fields with a known history of severe Rhizoctonia root rot.

During greenhouse research, it was observed that azoxystrobin fungicide applied as a hypocotyl drench provided excellent control of Rhizoctonia root rot. It is recommended that growers use a foliar banded application of azoxystrobin at the 4-leaf stage to control Rhizoctonia root rot. Research was conducted to compare and evaluate the effect of foliar band and hypocotyl drench applications of azoxystrobin to control *R. solani.* The study

showed that foliar banded and hypocotyl drench applications of azoxystrobin provided significantly similar disease control under conditions that were ideal for disease development. However, disease from plants with a hypocotyl drench application was not significantly different than the non-inoculated control suggesting that further testing should be done to determine the utility of this application methodology in field conditions.

Research was conducted to determine the best time to apply azoxystrobin fungicide for effective *R. solani* control relative to timing of soil inoculation. Sugarbeet hypocotyls were drenched at the 4-leaf stage at 0, 7, 14, 21, and 28 days pre-inoculation and at 0, 3, 10, 14, and 21 days post-inoculation. Azoxystrobin applications prior to inoculation resulted in significantly lower root rot compared to fungicide applications at post inoculation. Among the post inoculation applications, treatments where the fungicide was applied within 2 hours provided the best disease control. Fungicide application at pre-inoculation provided effective control at all timings evaluated. This research reinforces the need for azoxystrobin application before infection to control the disease in field conditions.

The depth at which *R. solani* caused root rot infection of sugarbeet was studied after burying *R. solani* AG 2-2 IIIB inoculum at depths of 2.54, 7.62, and 12.7 cm. *R. solani* AG 2-2 IIIB infections occurred at all depths of inoculation. However, inoculum buried at 2.54 cm depth had significantly higher root rot severity than inoculum buried deeper. Root rot symptoms were prevalent on the upper portion of the sugarbeet root just below the soil line irrespective of the depth of inoculum placement. This suggests that the upper part of the root below the soil line is most vulnerable to *R. solani* infection. Consequently, in the soil fungicide application should target the root area just below the soil line for effective disease control.

ACKNOWLEDGEMENTS

Special thanks to the North Dakota State Board of Agricultural Research and Education and the Sugarbeet Research and Education Board for partially funding my research. I am heartily thankful to my major advisor Dr. Mohamed Khan for allowing me the opportunity to pursue my Masters here at North Dakota State University. His perpetual energy and enthusiasm for his research work has motivated me to do my best in accomplishing the tasks set before me. For his constant guidance, support, and encouragement from the start to the completion of my course, I am forever grateful.

I am grateful to my committee member, Dr. Melvin Bolton for allowing me to use the USDA/ARS facilities to conduct my research. Thank you for your time, encouragement, assistance, and suggestions in my research and thesis. Thanks to my committee members, Dr. Rubella Goswami and Dr. Laura Overstreet, for your encouragement, advice and valuable suggestions in my research and thesis. Thanks to Mr. Gary Neilson and Aaron Carlson who have assisted me with my research. I am truly grateful for all the technical knowledge you both have imparted to me. To my friends Kishore and Jaimin, faculty, staff, and students of the Department of Plant Pathology, thank you all for your tremendous support throughout my stay at NDSU. It was an enjoyable experience, one that I will always cherish.

To my husband and son whom have been my inspiration, encouragement and support I owe my deepest gratitude. Thanks to my in-laws, parents and siblings for all their support and encouragement.

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LITERATURE REVIEW

Sugar, past and present

Sugarcane (*Saccharum officinareum* L.) was the first plant from which sugar was commercially produced. Sugarcane originated from Papua Guinea but initial commercial sugar production and processing was done in India. Sugar became a commodity for trade and was exported to the Middle East and Europe. The growing need for this particular sweetener led to the introduction of sugarcane into the West Indies and South America. Despite a number of setbacks, sugar production in the West Indies flourished in the 1700s. Increase in international trade and profit from sugar at that time resulted in conflict over ownership of the West Indies sugar production, and a new alternative, sugarbeet, became the priority for continental Europe (Shoptaugh, 1997).

Sugarbeet (*Beta vulgaris* L.) is a biennial C_3 plant that belongs to the *Chenopodiaceae* family. This crop existed thousands of years ago in early Greek and Roman culture but was used as food and animal feed (Cooke and Scott, 1993). In 1744, Andreas Marggraf extracted sugar from white beetroot in Europe (Prussia) and by the 1800s sugarbeet production increased throughout Europe. Around this same period, migrant farmers from Germany began planting sugarbeet within the United States, and the first successful factory to produce white sugar was built in California in 1870 (American Crystal Sugar Company, 1998).

Large scale sugarbeet production in North Dakota and Minnesota commenced in 1926 with the construction of the East Grand Forks Factory in 1926 (American Crystal Sugar Company, 1998). Today, the estimated $3 billion sugarbeet industry in this region is controlled by three growers' cooperatives; the American Crystal Sugar Company, Minn-

Dak Farmers Cooperative and the Southern Minnesota Beet Sugar Cooperative that together produce about 60% of the US sugarbeet (Bangsund and Leistritz, 2004).

The United States is the second largest producer of sugarbeet worldwide with an annual production of 29.5 million tons valued at US $1.28 billion (USDA, 2010). There were ten sugarbeet producing states in the 2009 and 2010 cropping seasons. In 2009, Minnesota ranked the highest in production followed by Idaho, North Dakota, Michigan, Nebraska, Montana, Colorado, California, Wyoming and Oregon. Although sugarbeet makes up a small percentage of the agriculture production in Minnesota and North Dakota, it contributes significantly to the economies of the two states. In 2008, sugarbeet production by the two states was valued at US $751.7 million (USDA, 2010).

Although sugarbeet production in North Dakota and Minnesota is profitable, crop production is still threatened by diseases such as Rhizomania, Cercospora leaf spot, and root rots caused by *Fusarium, Aphanomyces,* and *Rhizoctonia.* Rhizoctonia root rot is the number one problem affecting sugarbeet production in North Dakota and Minnesota based on the 2009 crop survey (Stachler et al., 2009). As a result, it is necessary to conduct research to better understand the biology of the pathogen and develop effective, economical, and sustainable management strategies. Therefore, the objectives of this research were to determine at what growth stages of different cultivars are most susceptible to infection by *R. solani*; to compare and evaluate the effects of foliar band and hypocotyl drench applications for controlling Rhizoctonia root rot; to determine the best time to apply azoxystrobin relative to the time of inoculation for controlling root rot caused by *R. solani;* and to determine the depth at which *R. solani* causes infection of sugarbeet.

The pathogen - *Rhizoctonia solani*

Taxonomy

The *Rhizoctonia* genus was described by DeCandolle in 1815. *Rhizoctonia solani* described by Julius Kühn in 1858 is the most important species within this genus (Sneh et al., 1996). The sexual stage or teleomorph of *R. solani, Thanatephorus cucumeris* (Frank) Donk was identified in 1956 (Sneh et al., 1996) and belongs to the Domain Eukaryota; Kingdom: Fungi; Phylum: Basidiomycota; Order: Ceratobasidiales; Family: Ceratobasidiaceae (Agrios, 1997; Sneh et al., 1996).

R. solani was further classified into anastomosis groups (AGs) based on vegetative compatibility reactions that occur when hyphae of two similar isolates fuse and genetic material is exchanged. If a compatible reaction occurs between two isolates, they are placed within the same AG. If no fusion occurs, it is a vegetatively incompatible reaction and isolates are placed in different AGs (Agrios, 2005). Currently, there are fourteen (14) AGs ranging from AG 1-13 with a bridging group (AG-B1) that can fuse with different AGs (Stodart et al., 2007; Carling *et al.*, 2002). Anastomosis groups 1, 2, 3, 4, 6, 7, 8, and 9 were further subdivided into intra specific groups (ISGs) based on how often they fuse within an anastomosis group. The ISG isolates differ in their morphology, genetic makeup, nutritional requirements, host range and virulence (Carling et al., 2002; Vigalys and Cubeta, 1994). *R. solani* is a soil inhabiting fungus that infects a wide range of plants throughout the world.

Biology of the pathogen

R. solani is a sterile basidiomycete fungus that produces thread-like hyphae and no asexual spores (Agrios, 1997). The filamentous hyphae produced by *R. solani* have long

monilioid cells with each cell separated by a dolipore septum. The hyphae branch at a 90-degrees angle to the main hyphae and are constricted at the point of origin (Harveson et al., 2009; Whitney and Duffus, 1986). Mycelium produced by *R. solani* is initially colorless, and turns yellowish brown as they age. They are multinucleate with more than two nuclei per cell making them distinguishable from binucleate *Rhizoctonia* (Agrios, 1997). *R. solani* produces sexual spores under favorable environmental condition but this occurs rarely in nature. The four basidiospores are produced on a specialized structure called basidium. Basidiospores usually germinate under moist conditions and are wind dispersed (Agrios, 1997).

Distribution and host range

R. solani produces sclerotia of varying sizes and shape and it is pathogenic on a wide range of hosts (Sneh et al., 1996). *R. solani* is a diverse group that is common worldwide. In North Dakota and Minnesota, *R. solani* AG 2-1, 2-2, 3, 4 and 5 were isolated from field collected sugarbeet plants. AG 1, 2-2 and 4 were pathogenic to sugarbeet and caused pre-emergence damping off of seedlings whereas, AG 2-2 caused root rot of older sugarbeet plants (Windels and Nabben, 1989). AG 3 and 5 were mildly pathogenic to non-pathogenic on sugarbeet and AG3 produced sclerotia on sugarbeet (Windels et al., 1997).

The most pathogenic AG that attacks sugarbeet is AG 2. This AG 2 group comprises of six subsets 2-1, 2-2, 2-4, 2-BI, 2-2 LP and 2-3 with subsets 2-1, 2-2 and 2-4 being the more virulent groups (Carling et al., 2002). In the United States AG 2-2 IIIB and AG 2-2 IV are the most pathogenic on sugarbeet with AG 2-2 IIIB being the more

aggressive of the two sub groups (Bolton *et al.*, 2010; Brantner and Windels, 2007; Bolkan and Ribeiro, 1985).

AG 2-2 IIIB and IV are widely distributed in Minnesota and North Dakota. In terms of the prevalence of the two groups, out of 428 sugarbeet isolates of *Rhizoctonia* tested from North Dakota 66% were AG 2-2 IV and 25% were AG 2-2 IIIB. In Minnesota, out of 369 tested isolates, 56% were AG 2-2 IIIB and 23% AG 2-2 IV. Distribution of AGs within the North Dakota and Minnesota is due mainly to the crops used in rotation with sugarbeet. Wheat and small grains grown in North Dakota suppresses AG 2-2 IIIB populations since they are non-hosts of AG 2-2 IIIB. Rotation with soybean and corn, hosts of AG 2-2 IIIB, increases pathogen population in Minnesota (Brantner and Windels, 2007).

R. solani AG 2 has a broad host range and has been reported to infect barley, bean, corn, sorghum, muskmelon, red beet, sugarbeet, soybeans and wheat (Ohkura et al., 2009; Ruppel, 1985), and also weeds such as common lambsquarter, redroot pigweed and *Kochia* (Harveson et al., 2009). AG 2-2 infects table beet and snap beans in New York (Ohkura et al., 2009). In the Red River Valley of Minnesota and North Dakota AG 2-2 IIIB and AG 2-2 IV are pathogenic on broad bean, pinto bean, soybean, table beet, sugarbeet and corn. AG 2-2 IIIB is more pathogenic than AG 2-2 IV on susceptible and tolerant sugarbeet cultivars (Brantner et al., 2008; Engelkes and Windels, 1996). AG 2-2 IIIB caused basal stem rot in soybean and root lesions and root rot in corn. Both crops are used as rotation crops for sugarbeet in the North Dakota and Minnesota (Windels and Brantner, 2008).

Economic impact

Rhizoctonia root rot infections occur in patches in sugarbeet fields and it is prevalent under warm, wet conditions that enable pathogen development (Harveson et al.,

2009). *R. solani* causes major losses wherever sugarbeet is grown. In the United States, losses to *Rhizoctonia* average 2% annually and can reach 50% under favorable conditions (Whitney and Duffus, 1986, Harveson et al., 2009). Although the losses caused by *R. solani* have not been estimated in dollar value, it does affect the returns on 24% of acreages sown to sugarbeet in the United States (Harveson et al., 2009).

R. solani causes seedling damping off and crown and root rot. Poor plant stand due to damping off results in low yields. Major losses also occur when crowns and roots become infected by *R. solani* later in the growing season. Since most of the sucrose is stored in the larger portion of the root which consists of the vascular zones and the parenchymatous zone, damages to this part of the root may result in reduction in the stored sucrose (Cooke and Scott, 1993).

R. solani AG 2-2 IIIB infected roots develop cracks and fissures that causes breakage of the root during harvesting which leads to a reduction of harvestable root. Cracks can also serve as entry wounds for other microorganisms that can cause damage to the sugarbeet roots when placed into storage piles and can lead to hot spots that may result in storage losses (Gallian, 2001).

Symptoms

R. solani causes damping-off of seedlings and crown and root rot of older plants. Damping-off typically occurs after emergence. Brown to dark brown discoloration occurs on the hypocotyl starting just below the soil level and moves upwards. There is usually a clear distinction between healthy and infected tissues. Seedlings die when the hypocotyl is severely damaged (Whitney and Duffus, 1986).

R. solani AG 2-2 is primarily associated with crown and root rot of sugarbeet. Symptoms evident on the foliage are yellowing and wilting of the leaves with wilting common on infected plants during the hot period of the day. Crown infections occur when infested soil is deposited into the crown by cultivation practices such weed control prior to row closure or by wind or water. This causes a blackened necrotic area at the base of the leaf petiole and on the root surface. Lesions also develop that are circular to oval with a ladder like pattern. Cracks or splits in the crown may develop when infections are severe (Harveson et al., 2009; Whitney and Duffus, 1986).

Root rot infections occur on the taproot or main root and progress upward. This occurs in young 4-6 leaf stage plants under warm conditions early in the growing season. Lesions developed are localized, circular and coalesce to form larger lesions on root surfaces eventually causing rotting of root tissue (Harveson et al., 2009). Dry root canker also develops on the surface of sugarbeet roots and these consist of localized lesions that are circular measuring 1.5-2.5mm in diameter with dark and light concentric rings. Underneath the lesions are cankers with mycelium that are distinguishable from healthy tissue (Engelkes and Windels, 1996; Whitney and Duffus, 1986).

R. solani produces pectinase, pectin lyase, pectin methylesterase, cellulase, and phosphatase that aids in the breakdown of plant tissues (Sneh et al., 1996). The most abundant enzymes produced by *R. solani* AG 2-2 are exopolygalacturonase and pectin lyase (PNL) with the latter being produced in larger quantities. The PNL enzyme is responsible for causing decay of sugarbeet root tissue and is often associated with pathogenicity in sugarbeet (Bugbee, 1990). The PNL causes wilting in susceptible sugarbeet plants but not in resistant sugarbeet plants (Bugbee, 1990).

Disease cycle and epidemiology

R. solani overwinters in soil as sclerotia, bulbils, thickened hyphal mycelium and in crop debris (Harveson et al., 2009). It survives on living plant material for its food source but once the food source diminishes, its growth continues saprophytically (Sneh et al., 1996). Saprophytic survival is favored at temperatures of 10, 20 and 30°C but decreases at -10°C (Harikrishnan and Yang, 2004). *R. solani* survives on crop residues such as barley, bean and sorghum for eight weeks at temperatures of 20°C (Ruppel, 1985) and it is found in the upper 10 cm of field soils but is more predominant in the upper 5 cm (Papavizas et al., 1975).

Overwintering structures germinate under favorable conditions of moisture and temperature. *R. solani* growth requires stimulation by chemical signals from sugarbeet roots. Once the mycelia threads are produced, they grow over the plant surface (Sneh et al., 1996). *R. solani* AG 2-2 forms branched hyphae that differentiates into infection cushion and appressoria which produce infection pegs that allow the fungus to penetrate epidermal host tissues (Demirci and Döken, 1998; Sneh et al., 1996). In susceptible sugarbeet each individual hyphae form an infection cushion and penetrates directly infecting the periderm, outer secondary cortex, vascular rings, and xylem vessels resulting in necrosis and degeneration of plant tissues (Ruppel, 1963).

The pathogen infects sugarbeet at the crown, petiole or roots at optimal temperatures of 25-33°C (Whitney and Duffus, 1986; Harveson et al., 2009). AG 2-2 IIIB infects sugarbeet at temperatures 21.1-26.7°C within 6 days post inoculation under controlled environments and at lower temperatures of 15.6°C and 21.1°C disease occurs but much slower, with no disease at temperatures of 4.4°C to 15.6°C (Bolton et al., 2010; Khan

et al., 2008). Low temperatures and inadequate food source can reduce the inoculum density. At temperatures of 25°C the fungus grows and produces sclerotia (Harikrishnan and Yang, 2004).

R. solani AG 2-2 occurs in all kinds of soil but is favored in heavy, poorly drained soils and within low patches in fields (Whitney and Duffus, 1986).

Management

Rhizoctonia crown and root rot is controlled by an integrated approach that involves the use of host resistance, fungicides, and other agronomic practices that reduce the pathogen population to levels where it does not cause significant yield losses to the sugarbeet crop (Jacobsen et al., 2001).

Genetic resistance

The United States Department of Agriculture - Agriculture Research Service (USDA-ARS) located at Fort Collins began developing germplasm material with resistance to Rhizoctonia crown and root rot in the 1950s. Most germplasm material developed are derived from crosses made between original lines (mother lines) and good breeding lines of sugarbeet over a period of 8 to 15 years. Sugarbeet has a partially dominant resistance to *R. solani* with resistance governed by two or more genes along with several minor genes (Hecker and Ruppel, 1975). The inherited resistance in sugarbeet has quantitative disease resistance traits to *R. solani* AG 2-2 IIIB. The quantitative trait loci (QTLs) found on chromosomes 4, 5 and 7 in sugarbeet were responsible for 71% of phenotypic variations (Lein et al., 2008). The expressed sequence tags were similar to resistance genes found in

plants and bacterial artificial chromosomes that contain nucleotide-binding sites for disease resistance genes (Lein et al., 2008). Several germplasm (FC 720, FC722, FC722 CMS, FC723, FC723 CMS) with resistance to *R. solani* have been identified and registered (Panella and Hanson, 2007; 2006). Sugarbeet accession EL51 was identified for possible source of resistance to *R. solani* AG 2-2 and AG-4 (Nagendran et al., 2009).

Source of resistance to *R. solani* AG 2-2 were found in the *Beta* genus. Out of 697 accessions screened in field trials, 2% of fodder beet accessions were resistant to *R. solani* AG 2-2, 10% of garden beet and 12% of unspecified *B. vulgaris* spp. were resistant (Luterbacher et al., 2005). Currently, sugarbeet cultivars have partial resistance to *Rhizoctonia*. These partially resistant cultivars have a 10-15% lower yield potential (Jacobsen et al., 2001) than susceptible cultivars. The use of mixtures of resistant and susceptible sugarbeet cultivars in field trials reduced disease severity caused by *R. solani* but it is dependent on temperature, rainfall and inoculum density (Brantner and Windels, 2006). In Europe, the use of resistant cultivars improves sugarbeet yield at high disease levels and reduced the disease severity when used in rotation with other non-host crops (Buhre et al., 2009).

Sugarbeet plants produce high concentration of pectin lyases inhibitor protein (PNLIP) in defense to *R. solani* infection. The PNLIP retards pectin lyase and slows disease progress (Bugbee, 1993).

Biological control

Some promising biological agents that control *R. solani* AG 2-2 IIIB are Kodiak *(Bacillus subtilus)* a commercial preparation that reduces *Rhizoctonia* infection similar to low rates of azoxystrobin (Jacobsen et al., 1997), and *Bacillus* strain MSU-127 which

provides long-term protection of sugarbeet, equal to the low rates of azoxystrobin (76g ai/ha). Azoxystrobin and MSU-127 used as an in-furrow application increased sugarbeet yield by 15.9% and as crown applications at the 4-leaf stage increased root yield by 17% (Kiewnick et al., 2001). Yeasts *Candida valida, Rhodotorula glutinis* and *Trichosporon asahii* protected sugarbeet from damping off and crown and root rot (El-Tarabily, 2004). *Laetisaria arvalis* caused decline in *R. solani* populations in sugarbeet fields (Allen et al., 1985) and *Trichoderma harizanum* reduced *R. solani* populations and increased sugarbeet root weight (Abada, 1994). However, none of these products are used in commercial sugarbeet production since they do not perform well under field conditions.

Cultural practices

Crop rotation is a practice commonly used in sugarbeet production. This helps in controlling weeds, insects and diseases that affects the sugarbeet crop. A 3-year minimum rotation using non-host crops was recommended to decrease the *R. solani* population (Windels and Brantner, 2006; Windels, 1988). Crop used in sugarbeet rotation has a direct impact on the inoculum density of *R. solani*. In North Dakota, hard red spring wheat and small grains used as rotation crops reduce *R. solani* AG 2-2 IIIB populations whereas in southern Minnesota, soybean and corn rotation crops increases *R. solani* AG 2-2 IIIB populations (Sims, 2008; Windels and Brantner, 2008, 2006). In Europe, corn used in rotation with sugarbeet contributes significantly to the build-up of *R. solani* AG 2-2 IIIB and resulted in subsequent increase in root rot disease severity with decrease in sugar yields (Kluth and Varrelmann, 2010; Buhre et al., 2009).

Weeds adversely influence sugarbeet production since they compete with sugarbeet for nutrients, light and water. Yield losses in sugarbeet ranges from 1-61% depending on

the type and density of weeds (Stachler and Zollinger, 2009; Mesbah et al., 1994). Common weeds found in sugarbeet fields in Minnesota and North Dakota are: common lambsquarters (*Chenopodium album* L.), pigweed (*Amaranthus spp.*L.), *Kochia (Kochia scoparia* L.), common cocklebur (*Xanthium strumarium* L.), ragweed (*Ambrosia artemisiifolia L.*), smartweed (*Polygonum spp.* L.), waterhemp (*Amaranth spp.* L.), and biennial wormwood (*Artemisia biennis L.*) (Stachler et al., 2009). Common lambsquarter, pigweed and *Kochia* are hosts of *Rhizoctonia* (Harveson et al., 2009). Controlling weeds incurs a huge cost to growers since it requires three to four herbicide applications and one to two cultivation at either pre- or post-emergence of sugarbeet (Khan, 2010). Rapid adoption of Roundup ready sugarbeet has reduced the need for cultivation for weed control (Stachler et al., 2009).

Other practices include early spring planting of sugarbeet in cool soils before *R. solani* is active (Bolton et al., 2010; Khan et al., 2008) and to encourage good emergence and vigorous growth of sugarbeet seedlings (Windels, 1988). Tillage, fertilizer application, and sanitation help to reduce *R. solani* AG 2-2 IIIB inoculum density in field (Windels and Lamey, 1998).

Chemical control

Sugarbeet seeds were initially treated with Chloroneb and Pentachloronitrobenzene to provide control of damping-off. However, these fungicides are no longer used for sugarbeet seed treatments. In 2001, azoxystrobin (Quadris®Sygenta), a strobilurin fungicide, was registered for use on sugarbeet to control *R. solani* (Jacobsen et al., 2001). Prothioconazole (Proline 480SC® Bayer Crop Science), a triazole was registered in 2008 for use on sugarbeet (Khan, 2010). Azoxystrobin is widely used in Minnesota and North

Dakota for control of Rhizoctonia root rot; prothioconazole is used to a lesser extent. Azoxystrobin has protectant, curative, eradicant, translaminar and systemic properties with residual period of approximately 7-21 days (Muller and Bradley, 2008; Balba, 2007).

Foliar banded and in-furrow applications of fungicides reduced Rhizoctonia crown and root rot of sugarbeet (Kirk et al., 2008). Foliar applications of azoxystrobin at the 4 leaf and 8 leaf stages, and in older sugarbeet plants controlled *R. solani* AG 2-2 resulting in good extractable sugar yield, plant stand (Jacobsen et al., 2004b; Windels and Brantner, 2001) and reduced crown and root rot severity (Kiewnick et al., 2001) in resistant and susceptible cultivars (Kirk et al., 2008). Banded applications (18 cm) of azoxystrobin at the four and eight leaf stages reduced Rhizoctonia root rot of sugarbeet (Khan and Carlson, 2009; Windels and Brantner, 2009). In-furrow applications improved stands, reduced root rot and increased yield (Windels and Brantner, 2005). Single crown applications of azoxystrobin at the 4-leaf stage gave excellent protection than in-furrow applications (Kiewnick et al., 2001).

Multiple applications of azoxystrobin have also reduced crown and root rot at the 6 to 8 leaf stage (Kirk et al., 2008). Azoxystrobin reduced Rhizoctonia root and crown rot and improved sucrose yields compared to Proline, Inspire, Headline, Moncot, Carumba, BAS 556 F in field trials (Windels and Brantner, 2008). Azoxystrobin protects plants if applied prior to infection and it may trigger some other host responses that extend protection beyond fungicide decomposition under conditions favorable for infection (Windels and Brantner, 2005). Proline was effective in controlling Rhizoctonia crown and root rot in some field trials (Khan and Carlson, 2009) in both conventional and Roundup Ready sugarbeet systems (Windels and Brantner, 2009).

Azoxystrobin applied when soil temperatures were between 10-23°C reduced the disease severity and increased the sucrose recovered (Khan et al., 2010). In Michigan, azoxystrobin application at different soil temperatures did not improve disease control compared to applications based on planting dates, or growth stages (Kirk et al., 2008). Under controlled conditions, azoxystrobin and prothioconazole effectively controlled *R. solani* AG 2-2 IIIB at temperature of 26.7°C (Khan et al; 2008). Several pathogens have developed resistance to azoxystrobin and prothioconazole compounds (Balba, 2007); so far, there are no reports of *Rhizoctonia* developing resistance to these fungicides in sugarbeet.

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CHAPTER 1: THE EFFECT OF SUGARBEET CULTIVARS AND GROWTH STAGES ON INFECTION BY *RHIZOCTONIA SOLANI*

Introduction

Rhizoctonia solani AG 2-2 IIIB is a common pathogen found in sugarbeet fields in North Dakota and Minnesota. *R. solani* causes crown and root rot of sugarbeet that affecting the root quality and sucrose yield. In recent years, the acreage of rotation crops including corn, soybean, and edible beans have increased in North Dakota and Minnesota (USDA, 2010). Since *R. solani* is also a host of these crops (Windels and Brantner, 2009; Harveson et al., 2009), Rhizoctonia root disease has become more severe, probably because the pathogen's population has been increasing over the past decade (Brantner and Windels, 2007; Jacobsen et al., 2001). Root rot infections are favored by warm temperatures above 21oC and high moisture levels (Bolton et al., 2010; Khan et al., 2008). A high pathogen population, favorable wet and warm environmental conditions and numerous susceptible hosts have led to an increase prevalence of this pathogen within the region. In 2009, Rhizoctonia root rot was named as one of the most serious production problem affecting sugarbeet in North Dakota and Minnesota (Stachler et al., 2009).

In this region, Rhizoctonia crown and root rot is managed by using host resistance, agronomic practices, and fungicide applications (Khan and Carslon, 2009; Windels and Brantner 2006; Jacobsen et al., 2001). The use of host resistance for controlling *Rhizoctonia* is limited, since complete resistance is absent in commercial cultivars. Cultivars with partial resistance to *Rhizoctonia* are available but are not widely used since they have 10-15% less yield potential than susceptible cultivars (Jacobsen et al., 2001).

In Minnesota and North Dakota, sugarbeet accounts for 60% of the total United States planted acreage (USDA, 2010). The American Crystal Sugar Company (ACSC) must evaluate all potential sugarbeet cultivars for at least two years for yield and quality parameters and resistance level to specific pathogens (Niehaus, 2009). In 2010, ACSC approved sixty-four sugarbeet cultivars for sale. However, only two of the cultivars (Beta 1301R and Hillshog 3035 R) widely planted by growers had good tolerance to Rhizoctonia root rot (Niehaus, 2009).

Prior to 2008, only conventional sugarbeet cultivars were widely grown in North Dakota and Minnesota. The use of conventional cultivars required one or two cultivations along with herbicides for effective weed control (Khan, 2010). The deposit of *R. solani* infected soil into sugarbeet crown during cultivation typically resulted in Rhizoctonia crown rot (Stump et al., 2004). However, the introduction of Roundup Ready sugarbeet in 2008 has resulted in rapid adoption of this new technology. Currently, about 95% of US acreage is planted to Roundup Ready sugarbeet. The need for cultivation to control weeds was reduced or eliminated since two-herbicide applications of glyphosate provide excellent weed control in Roundup Ready sugarbeet (Khan, 2010). However, Rhizoctonia crown and root rot continues to be a major problem for sugarbeet growers (Stachler et al., 2009), including the 2010 crop (Khan, *personal communication*) although little or no cultivation was practiced. This study was conducted to better understand what growth stages of different cultivars are most susceptible to the pathogen so that protective measures may be taken to prevent infection.

Materials and method

Production of inoculum

R. solani AG 2-2 IIIB isolate number 87-36-4 was obtained from Dr. Melvin Bolton (USDA-ARS, Fargo, ND). Pure cultures of *R. solani* AG 2-2 IIIB isolate were grown on sterile petri plates of full strength potato dextrose agar (PDA) for six days prior to inoculating sterile barley grains.

Two pounds of measured barley were placed in a 2 liters conical flask to which 1liter of ¼-strength potato dextrose broth (PDB) was added and the flask was allowed to stand for 48 hours in a refrigerator at 4°C. The ¼ strength PDB was prepared using 6 grams of PDB per 1 liter of distilled water. Excess PDB was discarded and the barley was autoclaved twice for 1.5 hours at 121°C. Ten 1mm diameter plugs of six day old culture of *R. solani* AG 2-2 IIIB were used to inoculate the sterile barley under a hood. The inoculated flasks were then sealed and placed in an incubator at 25°C for three weeks or until mycelium completely covered the grains. Barley grains colonized with *R. solani* AG 2-2 IIIB were removed from flask and dried for 48-72 hours. Dried inoculum was used directly or stored at -20°C for long-term storage.

Greenhouse operations

Trials were conducted at the USDA greenhouse facilities located in Fargo, North Dakota. Six conventional sugarbeet cultivars Beta 1301, Beta 1305, Beta 4554, Crystal 454, Hilleshog 3035 and VanderHave 46519 were used in this experiment.

Sunshine Mix 1 peat soil (Sun Gro Horticulture Canada Ltd., Canada) was amended with Osmocote 14:14:14 (Scotts-Sierra Horticultural Products Company, Marysville, OH) fertilizer at 1 kg per 3.8 cubic feet bale prior to planting. Soils were placed into square pots

(T.O. Plastics Inc. Clearwater, MN) of 9.29 x 7.49 x 7.89 cm (2 and 4 leaf stages) and 10.66 x 8.68 x 12.47 cm (6 and 8-leaf stages) size. Sugarbeet cultivars were sown one week apart for five weeks to allow plants to attain 2, 4, 6, and 8 leaf stages. Seedlings were thinned one week after germination to allow one plant per pot. Plants were inoculated with two (~0.08 g) barley grains colonized with *R. solani* AG 2-2 IIIB (Bolton et al., 2010). Inoculum was placed ~2.0 cm below the soil surface on each side of the plant root. Greenhouse temperature during the experiment was 25±2°C during the day with light set to allow 12 hours photoperiod. Plants were watered as needed.

The experimental design was a Randomized Complete Block Design (RCBD) with a split plot arrangement. The main plot represents the different leaf stages and the subplots were the cultivars. Non-inoculated controls were included for each cultivar at each leaf stage. There were three plants per treatment, and each treatment had 12 replicates. The experiment was repeated twice.

Disease severity ratings

Fourteen days after inoculation, plants were removed from the containers, and roots were washed and rated for root rot disease severity (RDS) using a modified 0-7 disease rating scale (Ruppel et al., 1979). The rating scale indicates 0 = healthy roots with no lesions; 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; 7 = 75-100% root rot.

Statistical analysis

Each experiment was analyzed separately as a RCBD with a split plot arrangement. A folded F test was performed on the two experiments to test for homogeneity of variances. The experiments data were combined if no significant differences were observed at F=0.05 level of confidence. Analysis of variances (ANOVA) was use to analyze the data using SAS 9.1 software (Statistical Analysis System, Cary, NC). Least Significant Differences (LSD) was used to separate root rot severity means for leaf stage x cultivar interaction.

Results

There were significant differences in root rot severity for leaf stage x cultivar interactions at $P \leq 0.001$ level of confidence (Table 1.1). Across all cultivars, sugarbeet plants at the 2-leaf stage were the most susceptible with the highest root rot severity. Plants at the 4-leaf stage had significantly lower root rot disease severity compared to the 2-leaf stage but significantly more disease than older plants (Figure 1.1). Sugarbeet plants at the 6 and 8-leaf stages had similar levels of root rot disease severity which was the lowest when comparing all leaf stages. Older sugarbeet plants root rot ratings were > 5, which indicated that 50-75% of the root area was covered by symptoms. At the 2-leaf stage, symptoms were marked by a dark brown decay below the leaf petioles, wilting of leaves and collapse of the sugarbeet plants. At the time of disease assessment all 2-leaf sugarbeet plants were dead and roots rotted. Sugarbeet plants at the 4, 6 and 8-leaf stages had dark-brownish lesions or rotting on the upper portion of the plant root, and on the leaf petiole. The root rot did not extend to the lower portion of the roots (Appendix A). There was no chlorosis of leaves.

Table 1.1. Analysis of variance for mean root rot severity of six sugarbeet cultivars at four growth stages inoculated with *R. solani* AG 2-2 IIIB.

Sources of variation	**Degrees of freedom**	**Mean square**	**F-value**
Experiments	1	-	-
Replications/Experiments	6	-	-
Leaf stages	3	28.96	<0.0001**
Leaf stages x Experiments	3	9.61	<0.0001**
Pooled Error A	18	-	-
Cultivars	5	0.99	0.0010**
Cultivars x Experiments	5	0.70	0.0111*
Leaf stages x Cultivars	15	0.67	0.0004**
Leaf stages x Cultivars x Experiments	15	0.38	0.0601
Pooled Error B	120	-	-
Total	191	-	-

*indicates significance at P≤0.05 level of confidence
** indicate significance at P≤0.001 level of confidence

R. solani AG 2-2 IIIB was very effective at causing disease on all sugarbeet cultivars across all leaf stages. Crystal 454 had significantly lower root rot disease severity compared to all cultivars, with the exception of Hillshog 3035. Beta 4554 and Beta 1301 had the highest root rot disease severity but were not significantly different from VanderHave 46159, Beta 1301, nor Beta 1305. Although Crystal 454 had the lowest disease severity, it was still >5 on the 0-7 rating scale (Figure 1.2).

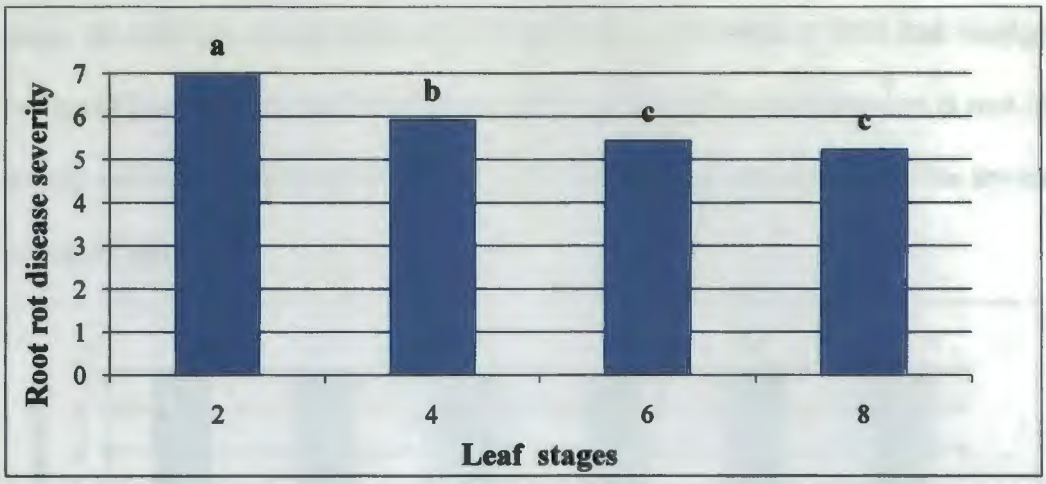

Figure 1.1. Mean root rot disease severity for leaf stages over all six cultivars. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' Protected Least Significant Differences (LSD=0.27).

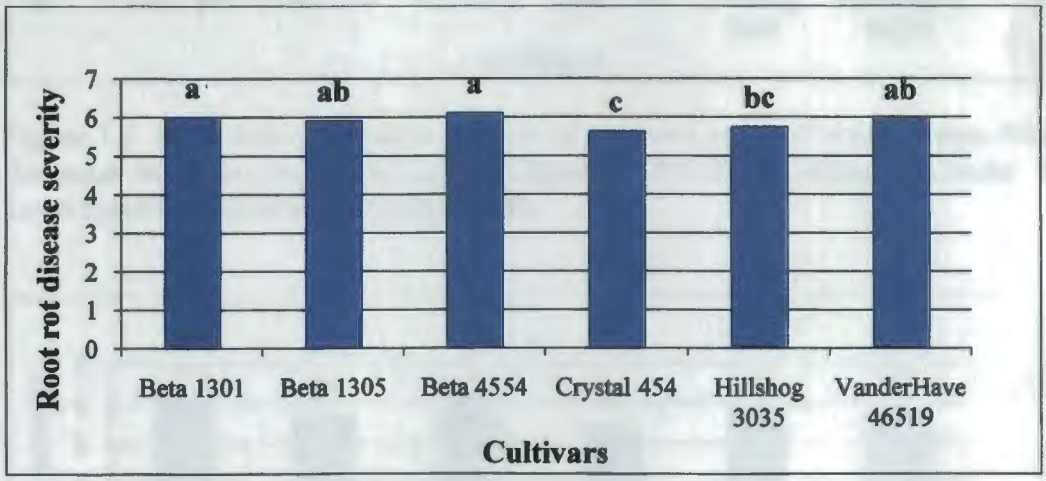

Figure 1.2. Mean root rot disease severity for six cultivars over all leaf stages. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' Protected Least Significant Differences (LSD=0.24).

There were no significant differences in root rot ratings among cultivars at the 2-leaf stage (Figure 1.3); all plants were dead at the time of evaluation. At the 4-leaf stage, there were significant differences in root rot severity among cultivars; Crystal 454, Hilleshog 3035 and Beta 1305 had the lowest root rot ratings (Figure 1.4). At the 6-leaf

stage, all cultivars except Beta 4554, Crystal 454 and Hillshog 3035 had similar root rot ratings (Figure 1.5). At the 8-leaf stage, there were significant differences in root rot ratings among cultivars and Crystal 454, Beta 1301 and VanderHave 46519 had the lowest root rot ratings (Figure 1.6).

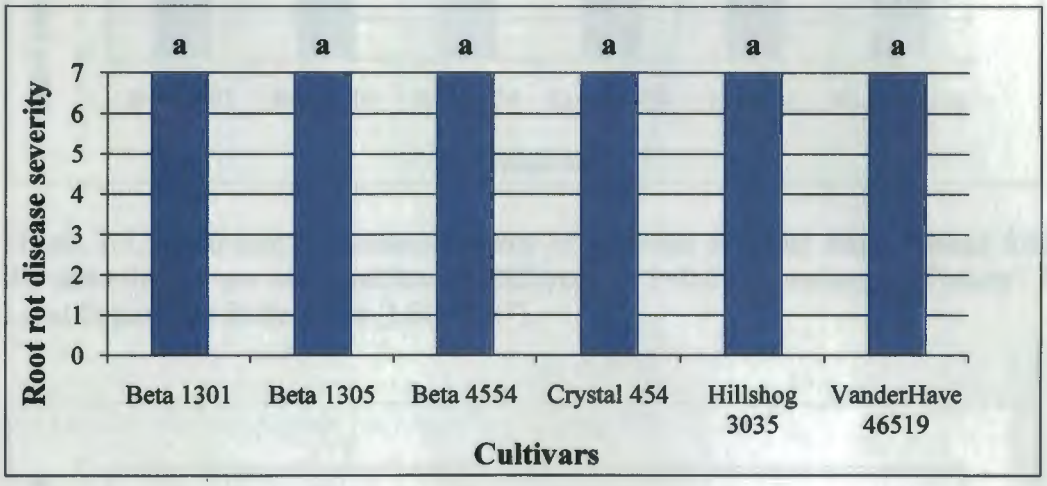

Figure 1.3. Mean root rot disease severity of cultivars at 2-leaf stage. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' Protected Least Significant Differences (LSD=0.47).

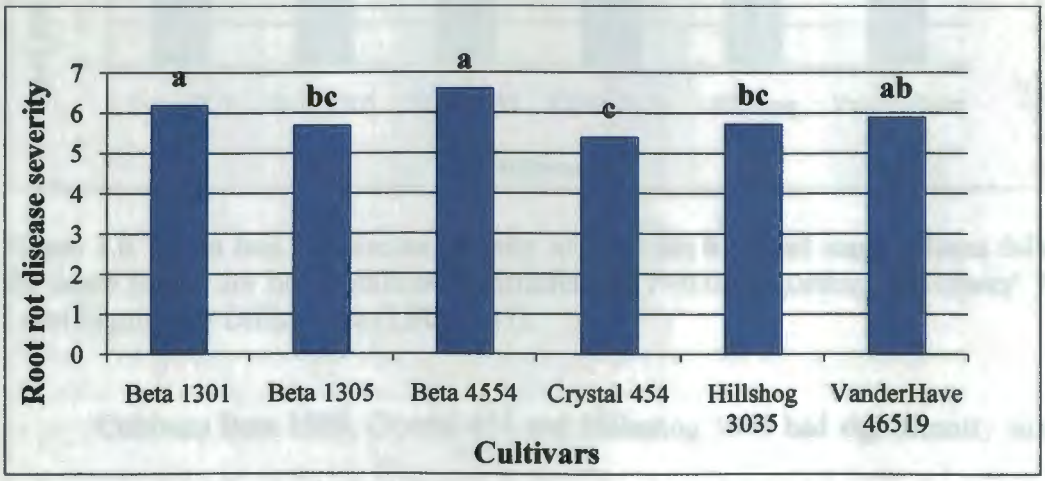

Figure 1.4. Mean root rot disease severity of cultivars at 4-leaf stage. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' Protected Least Significant Differences (LSD=0.47).

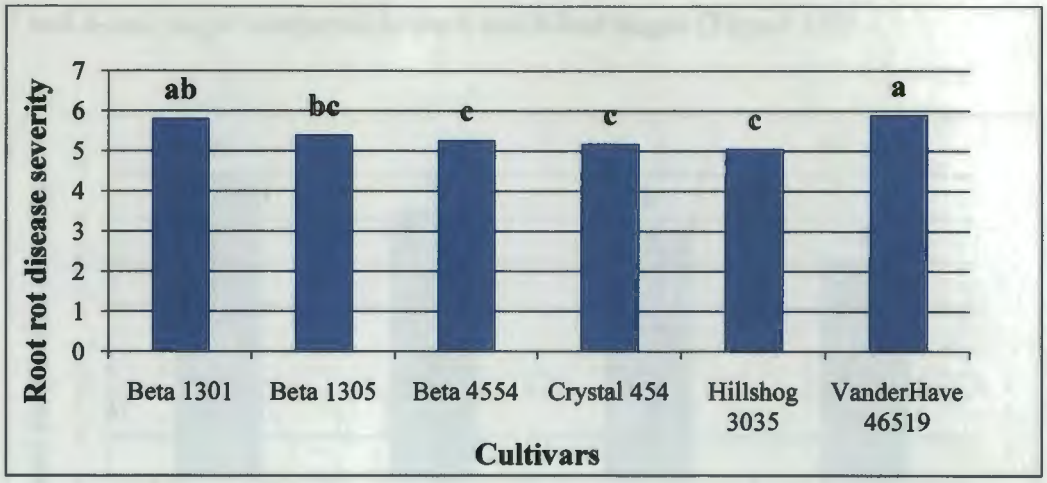

Figure 1.5. Mean root rot disease severity of cultivars at 6-leaf stage. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' Protected Least Significant Differences (LSD=0.47).

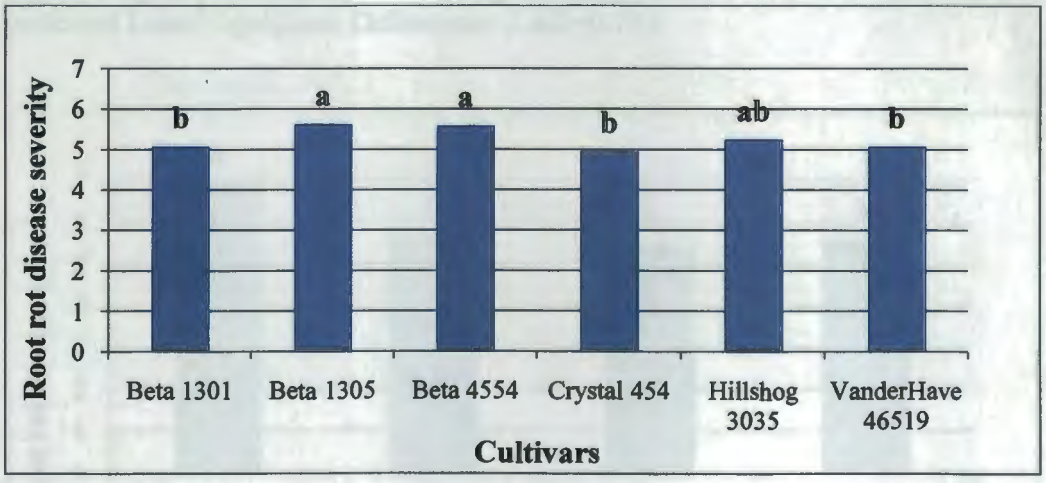

Figure 1.6. Mean root rot disease severity of cultivars at 8-leaf stage. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' Protected Least Significant Differences (LSD=0.47).

Cultivars Beta 1305, Crystal 454 and Hilleshog 3035 had significantly similar root rot severity at the 4, 6, and 8-leaf stages (Figures 1.8, 1.10, 1.11). Beta 1301 and Vanderhave 46519 were significantly similar at the 4 and 6- leaf stages, but not at the 2 and 8-leaf stages (Figures 1.7, 1.12). Beta 4554 had significantly higher root rot severity at the

2 and 4-leaf stages compared to the 6 and 8-leaf stages (Figure 1.9).

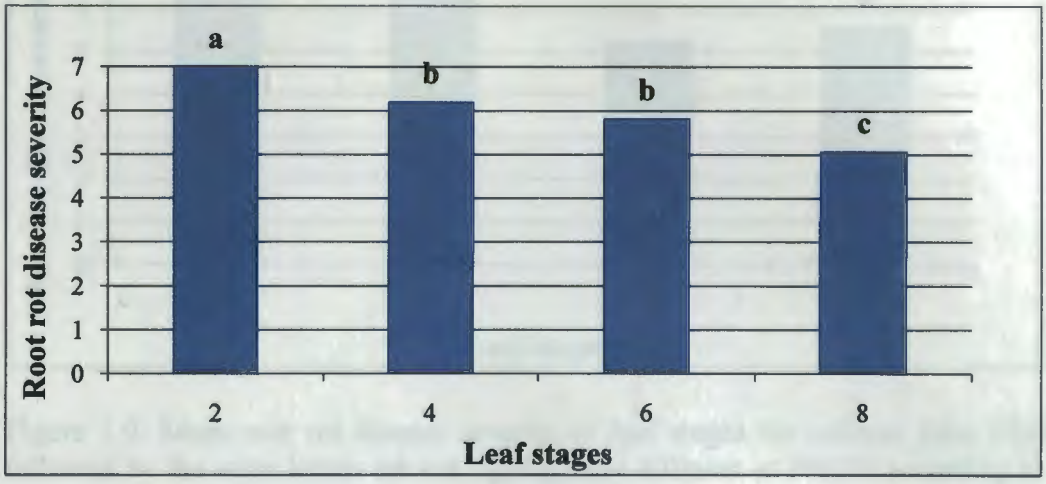

Figure 1.7. Mean root rot disease severity of leaf stages for cultivar Beta 1301. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' protected Least Significant Differences (LSD=0.74).

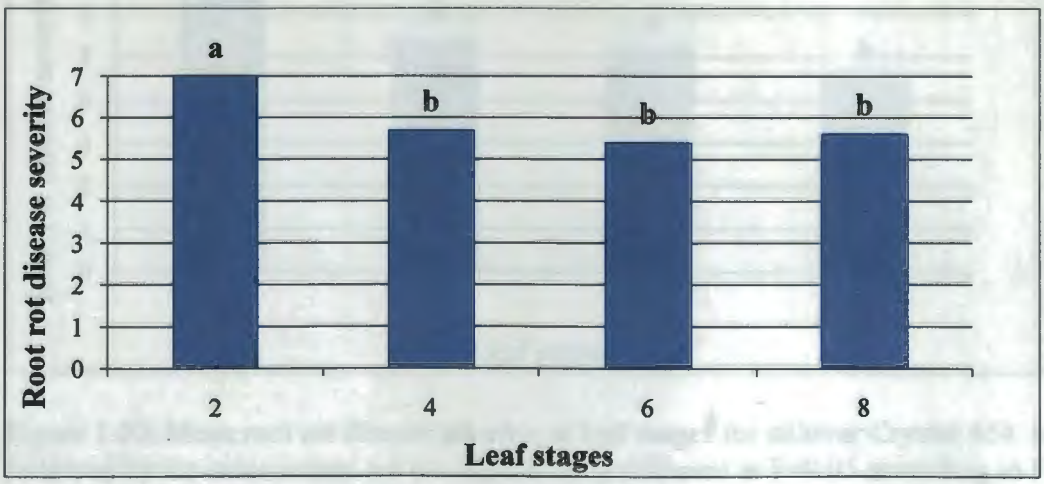

Figure 1.8. Mean root rot disease severity of leaf stages for cultivar Beta 1305. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' protected Least Significant Differences (LSD=0.74).

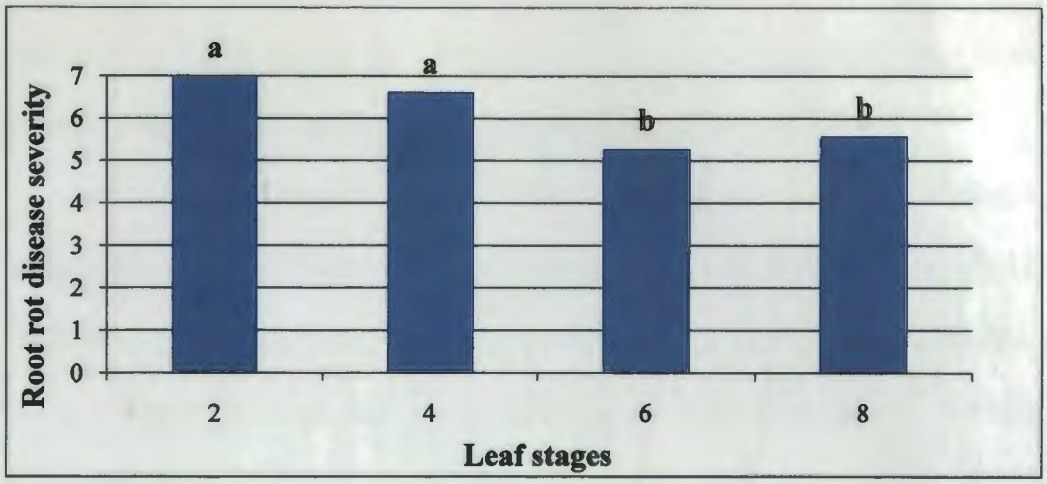

Figure 1.9. Mean root rot disease severity of leaf stages for cultivar Beta 4554. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' protected Least Significant Differences (LSD=0.74).

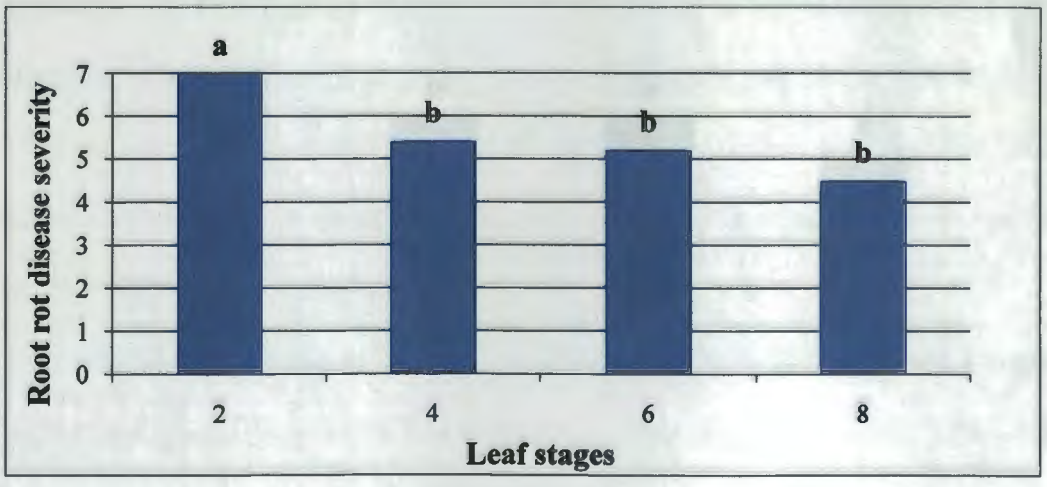

Figure 1.10. Mean root rot disease severity of leaf stages for cultivar Crystal 454. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' protected Least Significant Differences (LSD=0.74).

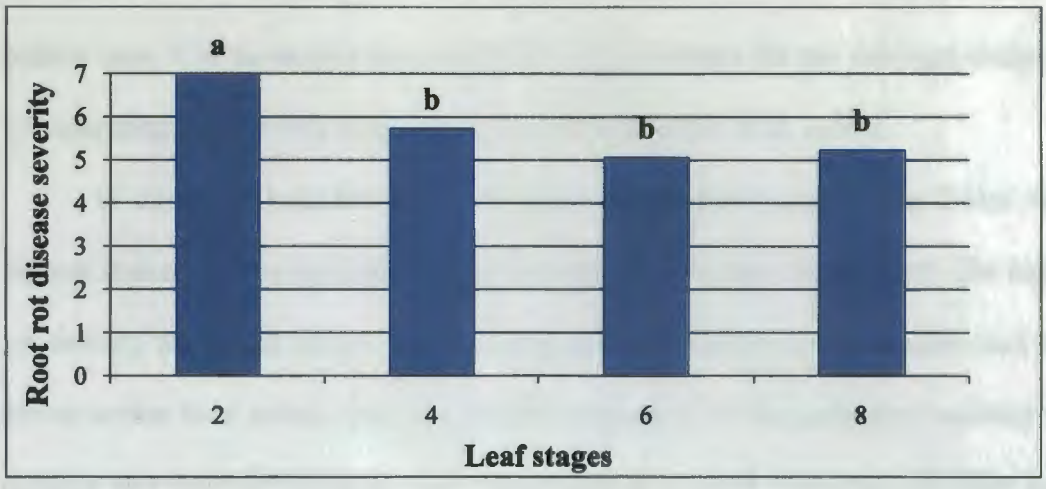

Figure 1.11. Mean root rot disease severity of leaf stages for cultivar Hillshog 3035. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' protected Least Significant Differences (LSD=0.74).

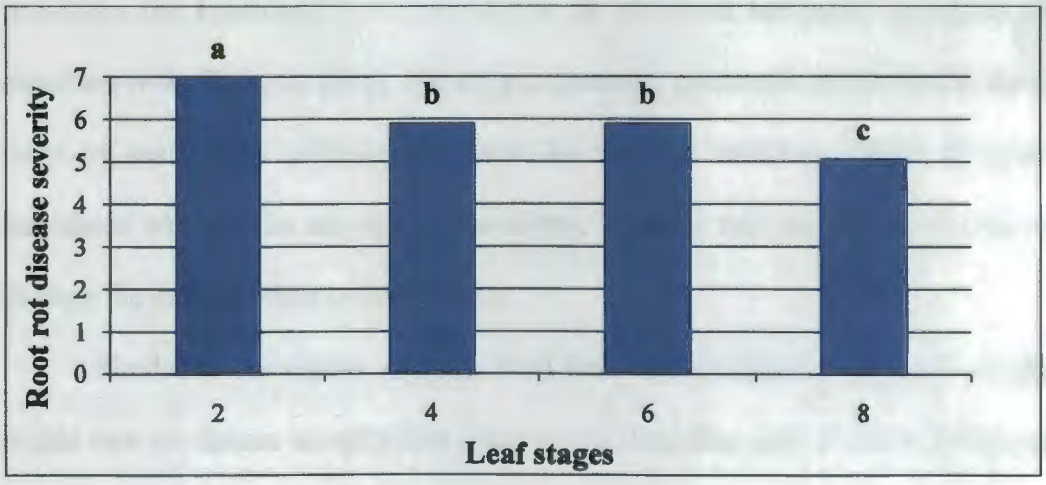

Figure 1.12. Mean root rot disease severity of leaf stages for cultivar VanderHave 46519. Means followed by the same letters are not significantly different at P=0.05 according to Fishers' Protected Least Significant Differences (LSD=0.74).

Discussion

R. solani AG 2-2 IIIB caused root rot infections at all growth stages in all the cultivars evaluated. Symptoms caused by *R. solani* AG 2-2- IIIB were wilting of leaves with no chlorosis and dark brown discoloration on the upper to mid portion of the roots and

petiole base. The mean root rot severity for all leaf stages for the cultivars evaluated was >5 indicating that 50-75% of the plant roots were affected by *R. solani.*

In this study, root rot disease severity for sugarbeet plants at the 2-leaf stage was highest indicating less resistance to Rhizoctonia root rot than older plants. The higher root rot severity and death of the 2-leaf stage plants may be due to the smaller root size that serves as the food source that was quickly exhausted by the pathogen resulting in more damage and death of younger plants compared to a larger food base in older sugarbeet plants. The root and/or hypocotyl of the two leaf stage beets are easily girdled after infection that results in the plants falling over and dying. Therefore, *R. solani* typically penetrates the epidermal layer and affects the periderm and outer secondary cortex in sugarbeet roots (Ruppel, 1963). The fungus generally penetrates deeper within the cortex of roots of susceptible cultivars compared to resistant cultivars. Since all plants were inoculated with similar amounts of inoculum, it would take more time for the fungus to damage the entire surface of larger roots.

Similar to our results, an inoculated field trial showed that 6 week old plants had higher root rot disease severity than older plants (Engelkes and Windels, 1994) suggesting that disease severity decreased with plant age.

Across all leaf stages, the evaluated cultivars demonstrated different levels of resistance to *R. solani* with Crystal 454 and Hilleshog exhibiting the lowest root rot ratings. In inoculated field trials, Hilleshog 3035 was approved and recommended to be used for managing Rhizoctonia root rot because it had lower root rot ratings (Neihaus, 2009). In this trial, none of the cultivars evaluated at any of the leaf stages had low levels of root rot. This

was probably because the isolate used was very aggressive in the greenhouse where temperature and moisture were ideal for disease development.

In Minnesota and North Dakota, early planting is recommended to allow for early emergence of sugarbeet in the growing season at the time when soil temperature is not conducive to pathogen development (Engelkes and Windels, 1994; Windels, 1988). Ideally, planting is recommended in mid-April to mid-May when the average daily soil temperature at the 10 cm soil depth ranged from 3-11°C (http://ndawn.ndsu.nodak.edu/). Since *R. solani* is most active during 25-33°C (Harveson et al., 2009, Bolton *et al.*, 2010), this means that most seedlings planted early will avoid infection. However, although older plants are more tolerant to the disease, preventative measures will still be required to prevent infection. Should planting be done later in the season or when the soil temperature is favorable for infection, protective measures will also be required for the seedlings as well as older plants.

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CHAPTER 2: EVALUATING APPLICATION METHODOLOGIES USING THE FUNGICIDE AZOXYSTROBIN FOR CONTROLLING RHIZOCTONIA ROOT ROT OF SUGARBEET CAUSED BY *RHIZOCTONIA SOLANI* AG 2-2 IIIB

Introduction

Rhizoctonia root and crown rot (RRCR) caused by *Rhizoctonia solani* Kühn is an important sugarbeet disease in the United States. Economic losses average 2% annually and can affect 25% of sown sugarbeet acreages (Whitney and Duffus, 1986; Harveson et al., 2009). In Minnesota and North Dakota, *R. solani* AG 2-2 IIIB and AG 2-2- IV are the most widely distributed anastomosis groups that affect sugarbeet (Brantner and Windels, 2007) with their distribution largely influenced by the rotational crops. Wheat and barley non-host crops used in rotation with sugarbeet reduce *R. solani* AG 2-2 IIIB inoculum density. However, when soybean and corn, which are hosts of the pathogen, are used in the rotation, they increase the pathogens' population (Windels and Brantner, 2007). *R. solani* AG 2-2 IIIB is most damaging to sugarbeet in warm soils that are wet or saturated (Bolton et al., 2010; Khan et al., 2008). The wet, warm conditions that have prevailed in North Dakota and Minnesota over the past decade, and the production of crops susceptible to *R. solani* in the rotation have resulted in an increase of Rhizoctonia root rot damage to sugarbeet. This disease has been named as one of the most serious problem that affects sugarbeet in the region (Stachler et al., 2009).

The primary means of controlling *R. solani* is the use of tolerant sugarbeet cultivars and fungicides. Sugarbeet cultivars that are tolerant to *R. solani* and produce high recoverable sucrose are limited. At American Crystal Sugar Company, 63 cultivars were

approved for growers use in 2009 (Nieuhaus, 2009). However, only two cultivars had good tolerance to *R .solani*, but their recoverable sucrose yields were lower than susceptible cultivars. It is recommended that growers who have fields with a history of severe Rhizoctonia root rot use tolerant cultivars and carry out fungicide applications for effective disease control. The fungicide azoxystrobin (Quadris ® Sygenta, USA) is most widely used for controlling Rhizoctonia root rot (Kirk et al., 2008:2007; McMullen and Markell, 2009). Azoxystrobin was approved for use on sugarbeet in 2000 to control *R. solani* in North Dakota and Minnesota (Khan, *personnel communication*). This strobilurin fungicide has protectant, curative, eradicant, translaminar and systemic properties. Azoxystrobin is a quinone outside inhibitor (QoI) fungicide that inhibits spore germination, mycelial growth and has some anti- sporulant activity (Balba, 2007).

Fungicide like azoxystrobin applied as either broadcast or banded are geared at targeting crown infections. These methods of application have provided some level of control of Rhizoctonia crown and root rot when used at the correct time and under favorable environmental conditions for infection (Stump et al., 2004; Windels and Brantner, 2001; Khan et al., 2009). In-furrow applications protect damping-off of seedlings and root rot early in the growing season but were not effective for late root rot (Stump *et al.*, 2004). Results for root rot infections were often inconsistent in field trials. This inconsistency in results may be due to timing of fungicide applications relative to the time of infection and/or the fungicides, applied to the foliage, were not able to reach the roots where they are needed to provide protection.

During other greenhouse trials where *R. solani* (Chapter 3) was used as a soil inoculum, it was observed that application of azoxystrobin as a hypocotyl drench resulted

in excellent disease control in sugarbeet, probably because the fungicide was providing better protection to the roots. Therefore, the objective of this research was to compare and evaluate the effect of foliar and hypocotyl drench applications of azoxystrobin for controlling Rhizoctonia root rot of sugarbeet caused by *R. solani.*

Materials and method

This experiment was conducted at the NDSU greenhouse facility located in Fargo, North Dakota. The sugarbeet cultivar Crystal 539 RR which is susceptible to *R. solani*, was used in this study (Niehaus, 2009). Three seeds were sown in Sunshine Mix 1 peat soils (Sun Gro Horticulture Canada Ltd., Canada) amended with Osmocote 14:14:14 fertilizer at 1 kg per 3.8 cubic feet bale prior to being placed into 9.29 x 7.49 x 7.89 cm pots. Plants were thinned at the two-leaf stage to allow one vigorous plant per pot. Greenhouse conditions were set to allow light for 12-h photoperiod and temperature averages were 25±2°C. Plants were watered daily to maintain adequate soil moisture that is essential for plant growth.

Plants at the 4-leaf stage were inoculated with two-barley grains colonized with *R. solani* AG 2-2 IIIB. Inoculum was placed 2.0 cm below the soil surface on each side of the plant root. Non-inoculated and inoculated controls were included in the experiment. Treatments consisted of applications of azoxystrobin (Quadris® Sygenta, USA) either as a hypocotyl drench or in an 18 cm foliar band to sugarbeet plants after inoculation. Azoxystrobin was applied at 0.672 L/ha using a spray volume of 121.6 L/ha. The spraying system (Spraying System Co., Wheaton, IL) was calibrated to deliver the fungicide at

275.8 kPal using a single flat fan nozzle 4001E. Hypocotyl drench applications were done using a micropipette and ~96 µl of the fungicide solution was applied per plant.

Plant pots were arranged in a Randomized Complete Block Design (RCBD) with six replicates and one plant per replicate. The experiment was repeated twice. Fourteen days after fungicide applications, plants were removed from containers, washed and roots were rated for root rot disease severity using a modified 0-7 rating scale (Ruppel et al., 1979). The rating scale indicates 0 = healthy roots with no lesions; 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 = 75-100% root rot.

Root rot disease severity data for each experiment was analyzed separately using analysis of variance. The Folded F-test F' = [(maximum variance/ minimum variance)] was used to test the variances for homogeneity of experiments. Experiments that were not significantly different at P=0.05 were combined. Experiments were considered as random effect and treatments as fixed effect. Fisher's Protected Least Significant Differences (LSD) was used to separate treatment means.

Results

The Folded F-test performed on the variances of the experiments was not significant at P=0.05 therefore the experiments were combined. There was significant differences in root rot severity for treatments at P≤0.05 level of confidence (Table 2.1).

Table 2.1. Analysis of variances for the combined experiments.

Sources of variation	Degrees of freedom	Mean Square	F-Value
Total	47	-	-
Experiments	1	-	-
Replication/Experiments	10	-	-
Treatments	3	71.67	0.0056*
Treatment x Experiment	3	1.64	0.5214
Error	30		

* indicates significance at P≤0.05 level of confidence.

Non-inoculated control plants did not show any root rot symptoms as expected. Inoculated plants with no fungicide application had significantly higher root rot than inoculated plants treated with fungicides (Figure 2.1).

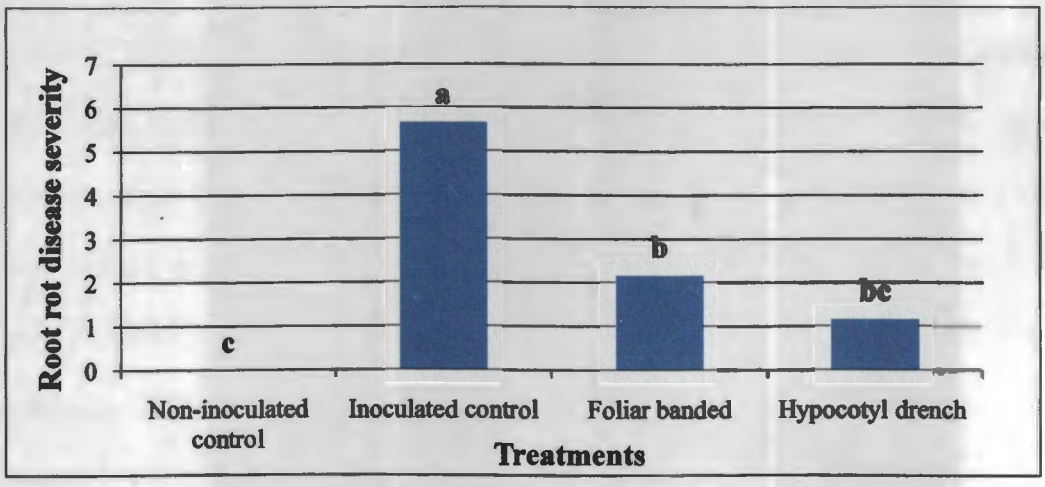

Figure 2.1. Mean root rot disease severity for azoxystrobin applied as a hypocotyl drench and foliar banded for controlling Rhizoctonia root rot. Means followed by the same letters were not significantly different at (P=0.05) level of confidence.

Inoculated plants without fungicides began showing foliar symptoms within one week of inoculation. No chlorosis was observed on leaves but dark brownish symptoms appeared at the base of the petioles around the soil-line that eventually resulted in necrosis of the leaves. At root evaluation, root symptoms were dark brown-blackish lesions that coalesced to form larger lesions (Figure 2.2).

Hypocotyl drench application had lower root rot severity (1.17) but it was not significantly different from the foliar band (2.17) application. Root rot severity was significantly higher for foliar band application compared to the non-inoculated control. However, root rot severity for the hypocotyl drench application was not significantly different from the non-inoculated control (Figure 2.1).

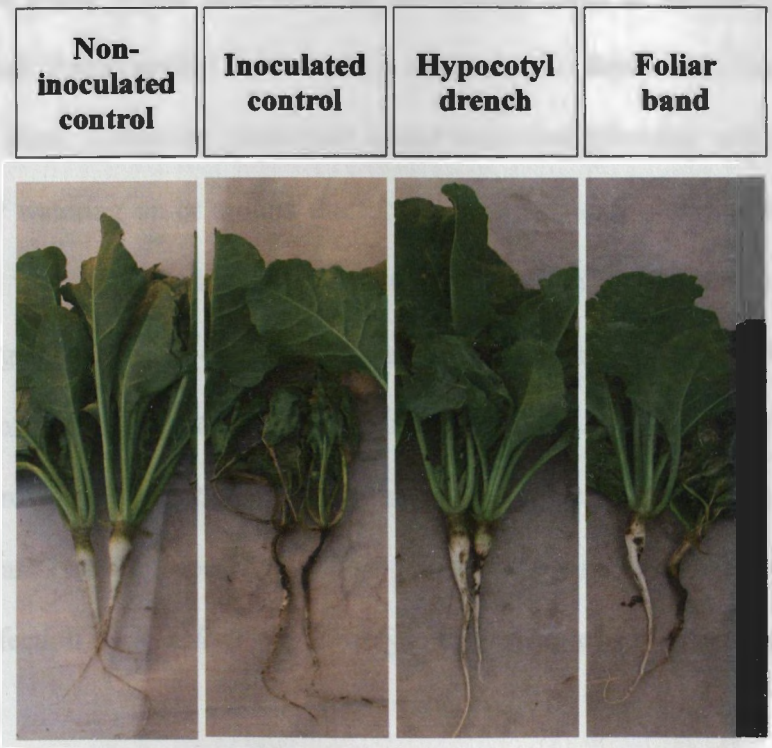

Figure 2.2. Root rot symptoms on sugarbeet treated with azoxystrobin using hypocotyl drench and foliar banded applications.

Discussion

In Minnesota and North Dakota, azoxystrobin is used for controlling Rhizoctonia root rot of sugarbeet caused by the pathogen *R. solani*. In this study, the hypocotyl drench and foliar band applications of azoxystrobin were both similarly effective in controlling Rhizoctonia root rot by reducing root rot severity. Foliar band applications of azoxystrobin were reported to reduce Rhizoctonia crown and root rot and increase sugarbeet yields in field trials (Khan and Carlson, 2009; Windels and Brantner, 2008; Franc and Stump, 2007; Jacobsen et al., 2004).

In the foliar-banded applications, the fungicide was sprayed on the leaves but also reached the soil directly since the foliage did not completely cover the soil. In addition, watering of the plants would have resulted in washing some of the fungicide from the leaves to the soil. Azoxystrobin is not known to translocate downwards from the point of application on plant. Therefore, protection to the roots was provided when the fungicide was washed by watering on or around the roots or the fungicide was washed into the soil where it killed the fungus and thus prevented soil infection by the fungus. Similarly, in field trials, fungicides sprayed on the foliage as well as on the soil, especially within rows, and irrigation or rainfall would have moved the fungicide on the roots and/or within the soil, thereby providing protection from the soil-borne fungus. In field trials, foliar band application of azoxystrobin does not always provide adequate coverage within rows and this leads to infection by *R. solani* and subsequent mortality of sugarbeet plants (Khan and Carlson, 2009).

Hypocotyl drench application provided effective control of Rhizoctonia root rot and was not significantly different from the non-inoculated control. This method of fungicide

application allows the fungicide to be in close contact with the plant where it can be easily absorbed by or cover the root and or hypocotyl that are infected by the pathogen. Targeting the hypocotyl and /or root for fungicide applications have the potential to provide better protection from the soil-borne pathogen. In field conditions, the root and /or hypocotyl are in close proximity to the *R. solani* in the soil. In the field, although cultivations have decreased with the advent of glyphosate tolerant sugarbeet (Stachler et al., 2009), *R. solani* infected soil can still get into crowns of plants especially during rainy weather from rain splash, or after flooding of fields from heavy rainfall events (Khan, *personal communication*). It may be useful to reconfigure the nozzle arrangement when applying fungicides for controlling *R. solani*, so that the most vulnerable plant parts such as the crown and the root and / or hypocotyl areas get covered with the fungicides.

In small grains, it has been shown that the use of side positioning of spray nozzles in fungicide application resulted in better Fusarium head blight control compared to the traditional forward spray pattern of fungicide (Halley et al., 2008). The nozzles used to apply these fungicides may be useful for field application of azoxystrobin. Further greenhouse and field research to determine the utility of targeting specific plant parts with fungicides should be conducted. It would be meaningful to know the means by which azoxystrobin provides protection from *R. solani* since protection may be obtained when the fungicide covers the root and prevent infection and/or it kills the fungus within the soil.

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CHAPTER 3: TIMING OF APPLICATION OF AZOXYSTROBIN RELATIVE TO INFECTION BY *R. SOLANI* FOR CONTROL OF RHIZOCTONIA ROOT ROT OF SUGARBEET

Introduction

Rhizoctonia solani described by Julius Kühn in 1858 is a serious soil-borne pathogen of sugarbeet worldwide (Harveson et al., 2009; Sneh et al., 1996). This pathogen was divided into several anastomosis groups, with AG 2-1, 2-2, 3, 4 and 5 reported to be pathogenic on sugarbeet and causing seedling damping off, and crown and root rot in older sugarbeet plants (Windels and Nabben, 1989). *R. solani* AG 2-2 is the most virulent group that was subdivided into intra specific groups AG 2-2 IIIB (the more aggressive) and AG 2-2 IV (Bolton et al., 2010). Both anastomosis groups are widely distributed in the Red River Valley (Brantner and Windels, 2007) and are the most damaging to sugarbeet.

In the US, Rhizoctonia affects 24% of planted sugarbeet acreage (Harveson et al., 2009). However, losses can reach up to 50% in some sugarbeet fields where pathogen population is high and conditions are favorable for disease development. Most of the losses incurred by *Rhizoctonia* in this region is a result of root rot damage. Typically, most plants infected with *R. solani* are killed and decomposed by October when the full harvest begins. Plants with lower levels of infection that survive become infected with other microorganisms that aids in the decay of stored beets thus reducing the tonnage and white sugar recovery (Jacobsen, 2006).

In Minnesota and North Dakota, Rhizoctonia crown and root rot is managed by using a combination of tolerant cultivars, agronomic practices, and fungicide application.

Since most of the cultivars available lack resistance to *R. solani*, growers must plant tolerant cultivars, plant as early as possible so that the most susceptible growth stages avoid the pathogen, and use fungicides when conditions become favorable for infection especially in fields with a known history of the disease. In North Dakota and Minnesota, azoxystrobin (Quadris ® Sygenta) is the most widely used fungicide for controlling Rhizoctonia root rot. This strobilurin fungicide has protectant, curative, eradicant, translaminar and systemic properties. It prevents spore germination, mycelial growth, penetration of the fungus and has anti-sporulant properties (Balba, 2007).

In most field trials done to determine the efficacy of azoxystrobin for controlling *R. solani*, the fungicide is applied first followed by inoculation in the crown. These treatments usually result in excellent disease control because the pathogen gets in direct contact with the fungicide protected plants and are killed.

Most infections by *R. solani* are believed to take place through the root or the upper part of the hypocotyl when infested soil is deposited within sugarbeet crown during weed control. In commercial sugarbeet production, Roundup Ready sugarbeet tolerant to the herbicide glyphosate (Roundup) is present on 95% of the US acreage. Since glyphosate provides excellent control of weeds, growers do not need to cultivate to assist in weed control resulting in a significant reduction in number of cultivations. However, many fields that were not cultivated were still affected by Rhizoctonia root rot.

Currently, azoxystrobin is recommended to be applied to sugarbeet in an 18 cm foliar band at 9.2 to 15.4 fl. oz/ac for controlling *R. solani* (Mueller and Bradley, 2008). Most growers use ground rig equipment to apply fungicides (Carlson et al., 2010). This means that wet field conditions, common in the Spring, may adversely impact timing of

fungicide application because the soil is too wet to drive across without creating soil compaction. Most growers start spraying for other foliar fungal diseases at first symptoms, and have good to excellent disease control (Carlson et al., 2010). However, when symptoms are observed for Rhizoctonia root rot, it is too late to apply fungicides for effective control (Windels and Brantner, 2002). Growers need to be educated on the importance of timing of azoxystrobin application relative to the time of infection for effective disease control. There is currently no published research which illustrates or addresses this issue. As such, the objective of this study was to determine the best time to apply azoxystrobin relative to the time of inoculation for controlling root rot caused by *R. solani* AG 2-2 IIIB.

Materials and method

Trials were conducted at the NDSU greenhouse facility located in Fargo, ND. Three sugarbeet seeds of a susceptible cultivar Crystal 539RR were sown in sunshine mix # 1 peat soil (Sun Gro Horticulture Canada Ltd., Canada) in 9.29 x 7.49 x 7.89 cm size pots. Plants were thinned at the two-leaf stage to allow one plant per pot. Plants were grown to the 4-leaf stage before treatments applications. Greenhouse conditions were set at 12 h photoperiod and temperature ranged from 27±2°C. Sugarbeet plants were watered daily to maintain the soil moisture essential for plant growth and pathogen development.

Inoculations were done using two (~ 0.08 g) barley grains colonized with *R. solani* AG 2-2 IIIB. Treatments included non-inoculated controls where no inoculum was applied to plants; an inoculated control where two grains of barley inoculum was placed in close proximity with plant roots at 2.0 cm below soil surface and no fungicide was applied. Fungicide application as a hypocotyl drench at 0, 3, 10, 14 and 21 days following

inoculation; and fungicide application as a hypocotyl drench followed by inoculations at 0, 7, 14, 21 and 28 days. The fungicide used was azoxystrobin, (Quadris® Sygenta), applied at the recommended rate of 0.67 L/ha. Approximately ~96 µl of fungicide solution was applied per plant hypocotyl.

The experiment layout was a Randomized Complete Block Design (RCBD) with twelve treatments appearing in each block. There were four replicates with one plant per replicate. The experiment was repeated three times. Fourteen days after final fungicide application, plants were removed from pots, washed and roots were rated for root rot disease severity using a modified 0-7 rating scale (Ruppel et al., 1979). The scale indicates 0 = healthy roots with no lesions, 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; 7 = 75-100% root rot.

The repeated experiments were analyzed separately using analysis of variances. Bartlett's chi-square test was performed on the variances to test for homogeneity among the repeated experiment. If no significant differences were observed among the repeats at $\chi^2 = 0.005$ level of confidence the repeated experiments was combined. The combined data was analyzed using a mixed effect. Experiment repeats were treated as a random effect and all treatments as fixed effects. The data was subjected to analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference was used to separate root rot severity means. SAS 9.1 (SAS 9.1; SAS Institute Inc., Cary, NC) software package was used to analyze all data.

Results

There were no significant differences among experiments at the $\chi^2 = 0.005$ level of confidence, therefore data from the three repeated experiments were combined. There were significant differences among the treatments at $P \leq 0.001$ levels of confidence (Table 3.1). All non-inoculated control plants were healthy and the inoculated control had the highest root rot severity which indicated that the *R. solani* inoculum was very effective. Sugarbeet plants treated first with azoxystrobin followed by inoculation had significantly lower root rot disease severity than the inoculated control (Table 3.2; Figure 3.4).

Table 3.1. Analysis of variance for the combined experiments.

Source of variation	**Degrees of freedom**	**F-value**
Experiments	2	-
Replications/Experiments	9	-
Treatments	11	<0.0001**
Treatments x Experiments	22	0.7270
Error	99	-
Total	143	-

** indicate significance at the $P \leq 0.001$ level of confidence.

Among the plants that were inoculated then treated with azoxystrobin, only those treatments applied at 0, 3 and 10 days after inoculation had significantly lower root rot severity compared to the inoculated control. Treatments done 3 and 10 days post inoculation had significantly more disease than the treatment applied on the day of

inoculation (Table 3.2). However, plants inoculated first then treated with azoxystrobin on the same day (within 2 hours after inoculation) had significantly higher root rot damage than plants first treated with azoxystrobin then inoculated (Figure 3.3).

Table 3.2. Mean root rot disease severity for sugarbeet treated with azoxystrobin at pre and post inoculations.

Treatments	**Mean root rot severity (0-7 rating scale)**
Non-inoculated (control)	0.00 d
Inoculated (control)	7.00 a
Inoculation followed by azoxystrobin at:	
0 days (2 hours)	1.83 c
3 days	4.92 b
10 days	4.83 b
14 days	6.75 a
21 days	6.42 a
Azoxystrobin followed by inoculation at:	
0 days (2 hours)	0.50 d
7 day	0.08 d
14 days	0.25 d
21 days	0.33 d
28 days	0.08 d

Means followed by the same letter are not significantly different according to Fisher's Protected Least Significant Differences (LSD=1.20) at P=0.05 confidence levels. (0= healthy roots and 7= roots completely rotted)

No significant differences in root rot disease severity were observed for plants treated with azoxystrobin followed by inoculation at 0, 7, 14, 21 and 28 days and the non-inoculated control. All treatment receiving azoxystrobin followed by inoculations had the lowest root rot severities with <1% of the root area with visible lesions. Sugarbeet plants treated with azoxystrobin before inoculations showed no above ground symptoms such as yellowing or wilting of leaves (Figure 3.1 and 3.2).

Figure 3.1. Sugarbeet plants inoculated with *R. solani* AG 2-2 IIIB followed by azoxystrobin application at different time.

Figure 3.2. Sugarbeet plants treatment with azoxystrobin followed by inoculation with *R. solani* AG 2-2 IIIB at different time.

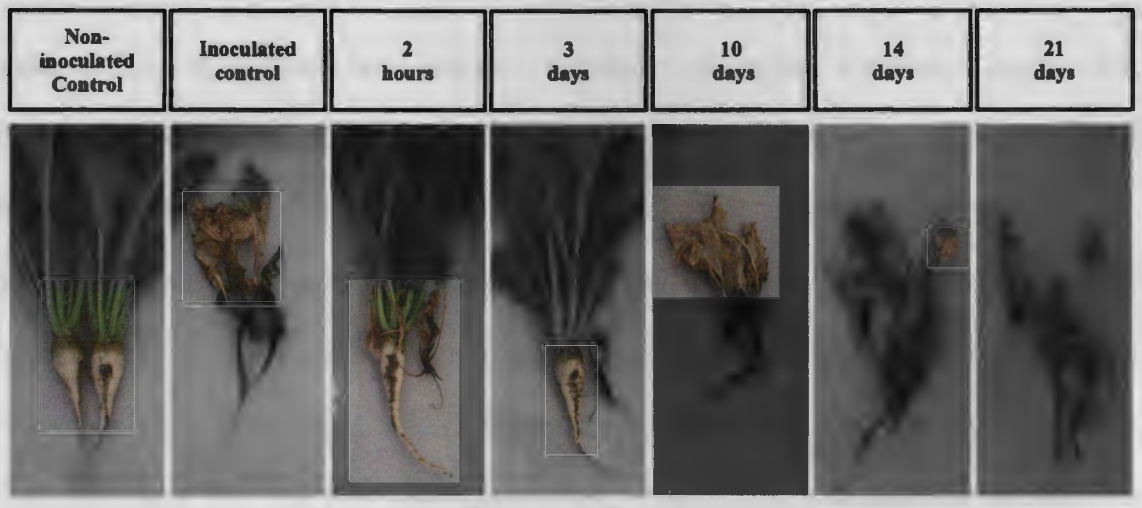

Figure 3.3. Root rot symptoms on sugarbeet plants inoculated with *R. solani* followed by azoxystrobin application at different time.

Figure 3.4. Root rot symptoms on sugarbeet plants with azoxystrobin application followed by inoculation with *R. solani* at different time.

Discussion

Timing of fungicide application is crucial for the control of *Rhizoctonia* root rot of sugarbeet under conditions favorable for infection by *R. solani*. The *R. solani* isolate used in the study is known to be very aggressive on sugarbeet (Bolton et al., 2010). This study demonstrated that it is difficult to control *R. solani* after infection takes place. Even when

azoxystrobin was applied a few hours after inoculation, it was not as effective as when the fungicide was first applied followed by inoculation and infection. Azoxystrobin prevented the fungus from quickly causing complete root damage when it was applied 3 and 10 days after inoculation. This was possibly due to the fungicide reducing mycelial growth (Blazier and Conway, 2000). Similar results were obtained in field trials conducted in Nebraska and Wyoming when foliar application of azoxystrobin was made at the time of crown inoculation and one week after inoculation (Stump et al., 2004). Azoxystrobin applied 2 to 3 weeks after inoculation did not protect sugarbeet plants since infection by *R. solani* AG 2-2 IIIB had already occurred. This suggests that azoxystrobin does not have curative effects for *R. solani* infections. (Windels and Brantner, 2005).

Azoxystrobin application prior to inoculation was more efficient in controlling root rot disease severity in this study. Azoxystrobin provided effective protection even when applied 28 days after inoculation. Strobilurin fungicides have systemic properties once taken up by plants has a residual period of 7-21 days (Mueller and Bradley, 2008). Protection lasting beyond 21 days could be due to the fungicide stimulating the plants defense mechanism to provide protection against the pathogen. Sugarbeet plants produce large amounts of pectin lyase inhibitor protein that inhibits pectin lyase produced by *R.* solani (Bugbee, 1993). The pectin lyase produced by *R. solani* was reported to be responsible for pathogencity in sugarbeet cultivars (Bugbee, 1990). Windels and Brantner (2005) postulated that sugarbeet plant defense responses to *R. solani* may have been triggered when plants are exposed to azoxystrobin and *R. solani* inoculum under favorable conditions suitable for infection.

In this research, azoxystrobin was applied as a hypocotyl drench to control Rhizoctonia root rot. This targeted method of fungicide application may have contributed to better protection of the vulnerable root and hypocotyl to the fungus. *R. solani* is known to cause infection when the soil temperature at the 10 cm depth is about (62 ^{o}F). Most soils in North Dakota and Minnesota would contain *R. solani* since the fungus is endemic. As such, most fields, but especially those with a history of Rhizoctonia root rot and planted to susceptible cultivars, should be sprayed with azoxystrobin before the soil temperature becomes favorable for disease development. Based on this study, it appears that azoxystrobin will provide protection against *R. solani* for 28 days. Further field research should be done to verify whether azoxystrobin, and prothioconazole, another fungicide that provides Rhizoctonia control in inoculated and field studies (Khan, 2010) would provide such prolonged period of protection when applied before the time of infection.

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CHAPTER 4: EFFECT OF DEPTH OF PLACEMENT OF *RHIZOCTONIA SOLANI* INOCULUM ON RHIZOCTONIA ROOT ROT INFECTION ON SUGARBEET

Introduction

Rhizoctonia root and crown rot of sugarbeet (*Beta vulgaris* L.) is caused by the soil-borne pathogen *Rhizoctonia solani* (Harveson et al., 2009). This soil borne pathogen survives saprophytically and parasitically in soils (Sneh et al., 1996) and is responsible for severe damage to the sugarbeet crop. In 2009, *Rhizoctonia* was the most serious production problem affecting sugarbeet in North Dakota and Minnesota according to a 2009 grower's survey conducted by NDSU (Stachler et al., 2009).

In North Dakota and Minnesota, non-genetically modified (non-GM) sugarbeet cultivars were widely planted by growers. The use of non-GM sugarbeet cultivars required growers to use three to four herbicide applications along with one to two cultivation practices to control weeds (Khan, 2010). During the cultivation practices, infested soils were thrown into sugarbeet crowns resulting in crown rot infections. The use of Roundup Ready sugarbeet in 2008 has resulted in fewer cultivation operations, since two applications of glyphosate is adequate for providing excellent weed control (Khan, 2010). The likelihood of infested soil being thrown into crowns is reduced and subsequently Rhizoctonia crown infections have declined. Thus, root rot infections will be more of a concern for growers in the future.

Harveson et al. (2009) have reported that *R. solani* AG 2-2 causes root rot, beginning at the root tip and progressing upwards in sugarbeet plant. If *R. solani* behaves in

this manner, targeting the pathogen or protecting the sugarbeet roots with the use of foliar fungicide may become more difficult.

Papivazas (1975) has reported that *R. solani* activity was limited to the upper 5-10 cm of soil in bean rotated with sugarbeet but there was no other information available on *R. solani* AG 2-2 activity below ground on sugarbeet. It is important to better understand the biology of *R. solani* so that management strategies may be developed that target the pathogen in its most vulnerable stages, or provide protection to plants where the pathogen is more likely to target.

Therefore, the objective of this study was to determine the depth at which *R. solani* AG 2-2 IIIB causes infection of sugarbeet.

Materials and method

Trials were conducted at North Dakota State University greenhouse facility located in Fargo, North Dakota. Three sugarbeet seeds of a susceptible cultivar Crystal 539RR were sown at 2 cm depth. Seeds were sown into plastic cone-tainers size 5 cm diameter x 21 cm deep (Stuewe & Sons, Inc., Corvallis, OR) filled with Sunshine Mix #1 peat soils (Sun Gro Horticulture Canada Ltd, Canada). Soil was amended with 1 kg of oscomcote 14:14:14 fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) per 3.8 cu ft bale of soil prior to being placed into cone-tainers. Plants were grown under average greenhouse temperatures of 27±2°C with 12 hours of photoperiod under fluorescent light. Plants were thinned upon seedling establishment to allow one vigorous plant to remain in the cone-tainers. At the four leaf stage (~ 4 weeks old), two grains of barley colonized with *R. solani* AG 2-2 IIIB inoculum was buried at 2.54, 7.62 and 12.7 cm depths below the soil

line. A metal forceps with the depths demarcated was used to place *R. solani* IIIB inoculum close to the sugarbeet roots. Non-inoculated controls were included.

Plant pots were arranged in a Randomized Complete Block Design (RCBD) with four replicates with one plant per replicate. This experiment was conducted three times.

Fourteen days after inoculations, plants were carefully removed from cone-tainers, washed, and the roots were rated for root rot disease severity using a 0-7 rating scale (Ruppel et al., 1979). The scale indicates 0 = healthy roots with no lesions, 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; 7 = 75-100% root rot.

Analysis of variances (ANOVA) was conducted on experiments separately and the variances were used to perform Bartlett's chi square test for homogeneity among experiments. Non-inoculated controls were included in the experiments but since all control plants were healthy and obtained a rating of zero on the rating scale, these data were not included in the analysis. The combined experiments were analyzed using analysis of variance. The experiments were considered as random effects and treatments as fixed effects. Fisher's Protected Least Significance Difference (LSD) was used to compare means at P=0.05. All data analysis was performed using SAS software version 9.1 (Statistical Analysis System, Cary, NC).

Results

The experiments were not significantly different at χ^2= 0.005 level of confidence; therefore all three experiments were combined. There were significant differences among treatments at P≤0.001 level of confidence (Table 4.1). *R. solani* AG 2-2 IIIB was capable of causing root rot infection on sugarbeet roots when inoculum was buried at 2.54, 7.62 and 12.7 cm depths. Root rot disease severity was significantly higher when inoculum was placed at the 2.54 cm depth compared to 7.62 cm and 12.7 cm depths. There were no significant differences between 7.62 cm and 12.7 cm depths (Table 4.2).

Table 4.1. Analysis of variance for the depth of inoculum burial on root rot severity of sugarbeet.

Sources of variation	**Degrees of freedom**	**F-value**
Experiments	2	-
Replicates/Experiments	9	-
Treatments	3	0.0006**
Treatments x Experiments	6	0.4497
Pooled Error	27	
Total	47	

**indicates significance at the P=0.001 level of confidence

No yellowing of leaves was evident on plants inoculated and infected by *R. solani* AG 2-2 IIIB. Infected plants became wilted as the disease progressed. Symptoms of *R. solani* AG2-2 IIIB infection was seen in inoculated sugarbeet plants in less than a week. This was evident by the dark grayish brown symptoms that were present below the crown

at the soil line in pots. Root rot symptoms were dark brown circular lesions that were more concentrated on the upper portion of the sugarbeet root (Figure 4.1).

Table 4.2. Rhizoctonia root rot disease severity at three depths of inoculation under greenhouse conditions.

Treatments	**Mean root rot severity ratings**
2.54 cm depth	3.16 a
7.62 cm depth	2.48 b
12.7 cm depth	1.97 b

Means followed by the same letters are not significantly different according to Fisher's Protected Least Significant Difference at P=0.05.

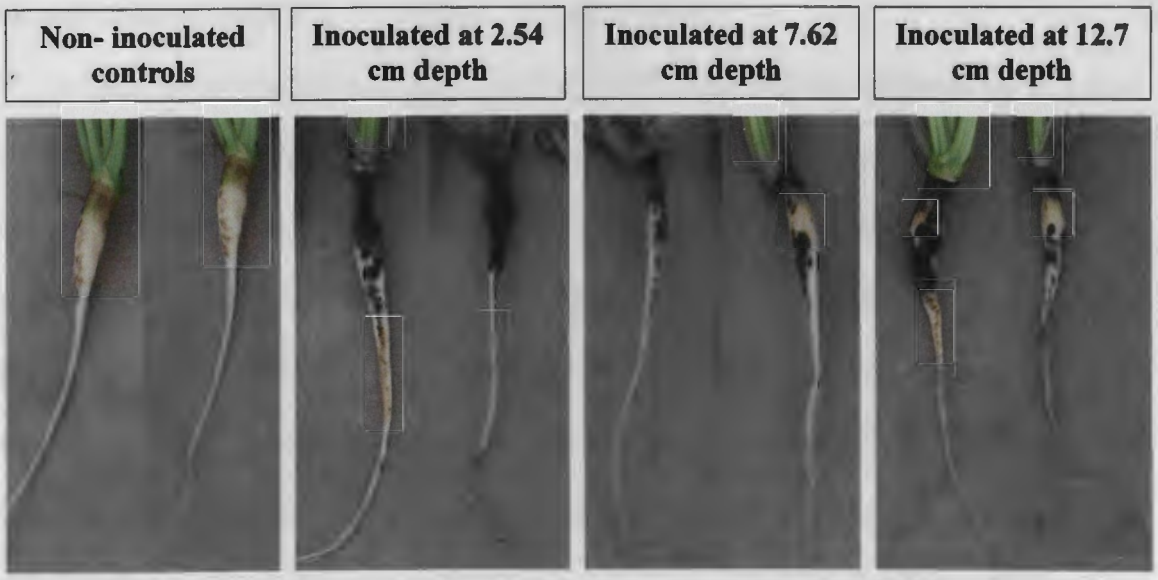

Figure 4.1 Root rot symptoms fourteen days after *R. solani* inoculum was buried at three depths.

Discussion

Papavizas et al., (1975) reported that *R. solani* isolated from bean fields rotated with sugarbeet was found to be confined to the upper 5-10 cm soil depth and very rarely evident

below this depth in soils. There is very little literature on the activity of the pathogen on the roots in the soil. This is the first report of the impact of *R. solani* on sugarbeet roots at different soil depths. This study showed that *R. solani* AG 2-2 caused Rhizoctonia root rot when buried at 2.54, 7.62 and 12.7 cm depths.

Root rot severity was highest at the 2.5 cm depth with symptoms apparent within one week of inoculation. This suggests that the pathogen may prefer the upper portion of the root at the soil line. *R. solani* AG 2-2 IIIB survives in field soil as a saprophyte. When a living nutrient source becomes available for the pathogen, it grows toward the nutrient source and begins its infection process (Sneh *et al.*, 2005). Young sugarbeet plants (two and four leaf stages) are ideal nutrient sources for *R. solani* since they tend to increase sucrose concentrations rapidly (Klotz and Finger, 2001).

In a crown inoculation study, Bugbee (1990) reported that infection by *R. solani* AG 2-2 occurred at the base of petioles and crowns rather than sugarbeet roots since crowns provided a more suitable carbon source for the pathogen. In the same study, *R. solani* AG 2-2 produced higher amounts of pectin lyase in crown cells than in root tissue; the pectin lyase was responsible for increasing susceptibility of sugarbeet crown to *R. solani.* Cook and Scott (1993) indicated that root symptoms begin at the crown and extended down the taproot. This usually occurs when sugarbeet crowns were inoculated with infested field soils but there was no mention of the point of inoculation. Harveson et al., (2009) reported that *R. solani* caused infection on the main root beginning at the root tip and progressed upward with most infection occurring when plants were in the 4-6 leaf stage under warm conditions in the growing season. Once inoculum is present and conditions are conducive for pathogen development, root infections can occur.

R. solani AG 2-2 IIIB infects sugarbeet at all depths and favors the crown/hypocotyl area close to the soil line even when buried at lower depths. Therefore, control of *R. solani* AG 2-2 IIIB should focus on protecting the root area closer to the soil line to prevent pathogen infections.

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APPENDIX A: SYMPTOMS ON SUGARBEET ROOTS OF SIX CULTIVARS AT FOUR GROWTH STAGES

Symptoms on sugarbeet cultivars inoculated with *R. solani* AG 2-2 IIIB at the 2-leaf stage.

Root rot symptoms on sugarbeet cultivars inoculated with *R. solani* AG 2-2 IIIB at the 4-leaf stage.

Root rot symptoms on sugarbeet cultivars inoculated with *R. solani* AG 2-2 IIIB at the 6-leaf stage.

Root rot symptoms on sugarbeet cultivars inoculated with *R. solani* AG 2-2 IIIB at the 8-leaf stage.