SENSITIVITY OF *RHIZOCTONIA SOLANI* AND *APHANOMYCES COCHLIOIDES* TO FUNGICIDES, AND FITNESS OF TETRACONAZOLE-RESISTANT ISOLATES OF *CERCOSPORA BETICOLA* AFTER EXPOSURE TO DIFFERENT TEMPERATURE REGIMES

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

North Dakota and Minnesota produce 55% of USA sugarbeet production. Diseases caused by *Rhizoctonia solani*, *Aphanomyces cochlioides*, and *Cercospora beticola* are the major diseases affecting sugarbeet production in North Dakota and Minnesota. Growers mainly use partial resistant varieties and fungicides to manage diseases of sugarbeet. Sensitivity of *R. solani* and *A. cochlioides* to fungicides were evaluated in vitro using mycelium radial growth assay and by evaluating disease severity on inoculated plants treated with fungicides in the greenhouse. Phenotypic stability of tetraconazole-resistant isolates of *C. beticola* after exposure to different temperature regimes was evaluated. For *R. solani*, mean EC_{50} values for baseline isolates were 49.7, 97.1, 0.3, 0.2, and 0.9 µg ml^{-1} and for non-baseline isolates were 296.1, 341.7, 0.9, 0.2, and 0.6 µg ml^{-1} for azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole, respectively. The mean EC_{50} values of azoxystrobin, trifloxystrobin, and pyraclostrobin increased with a change factor of 6.0, 3.5, and 2.7, respectively. All fungicides at labeled rates effectively controlled *R. solani* in vivo. For *A. cochlioides*, tetraconazole, prothioconazole, and pyraclostrobin reduced mycelium radial growth in vitro with mean EC_{50} values of 3.5, 2.4, and 0.8 µg ml^{-1}, respectively. However, these fungicides were not effective at controlling *A. cochlioides* in vivo. Sugarbeet plants up to three weeks old were found susceptible to *A. cochlioides*. Resistant isolates of *C. beticola* had no fitness penalty as measured by spore production, spore germination, mycelium radial growth, and disease severity after exposure to different temperature regimes. However, isolate 09-347, resistant to tetraconazole, reverted to a moderate resistance level after exposure to -20ºC, and -20ºC to 4ºC to -20ºC to 4ºC with a factor of change of 38.6 and 32.8, respectively. This research indicated that *R. solani* sensitivity to the evaluated QoIs had decreased, but they were still effective at labeled rates under greenhouse

conditions, and rotation of different fungicide classes could be a useful strategy to manage fungicide resistance. No fitness penalty was found after exposure of *C. beticola* isolates to cold treatments. However, *C. beticola* isolates resistant to tetraconazole became more sensitive to this fungicide after exposure to cold treatments.

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CHAPTER ONE. LITERATURE REVIEW

Sugarbeet Industry

As world population increased the demand for sucrose increased. Sucrose can be extracted from sugarbeet (*Beta vulgaris* L.) and sugarcane. Sugarbeet is a relatively new crop that provides 25% of the world's sucrose requirement (Draycott, 2006). Andreas Maorggraf in 1747 obtained sucrose crystals from sugarbeet that was identical to sugarcane crystals. Forty years later, his student Franz Carl Achard demonstrated that sucrose can be commercially extracted from the White Silesian beet he bred. He built the first beet sugar factory in 1801. The sugarbeet industry expanded to other counties including France, Russia, Austria, Britain, Japan, Turkey, China, Pakistan, Iraq, Iran, Syria, Egypt, Algeria, Morocco, Tunisia, Canada, and the USA (Draycott, 2006).

In the USA, sugarbeet production started in 1838 when the first beet sugar factory was built in Massachusetts. Unfortunately, this factory was closed soon after because of low sucrose extraction. In 1870, the first successful beet sugar factory was built in California and by 1900 there were 34 factories in the USA (Francis, 2005). Today, sugarbeet is produced in 10 states including Michigan, North Dakota, Minnesota, Colorado, Montana, Nebraska, Wyoming, California, Idaho, and Oregon (USDA-ERS, 2014).

Sugarbeet planting in North Dakota and Minnesota began 1890. In 1926, the first beet sugar factory located in East Grand Forks was established by American Beet Company renamed American Crystal Sugar Company (ACSC). Today, ACSC owns five factories in Drayton, Hillsboro, East Grand Forks, Crookston, and Moorhead. Other factories found in North Dakota and Minnesota are owned by Minn-Dak Farmers Cooperative and the Southern Minnesota Beet Sugar Cooperative (Strand, 1998).

World sugarbeet production reached 269.1 million tons in 2013, with U.S. production contributing 12% (32.8 million metric tons) harvested from 484,813 hectares (USDA- ERS, 2014). North Dakota and Minnesota were the largest production area in the USA with 264,154 hectares which contributed 55% of the U.S. sugarbeet production, and $3.2 billion of total economic activity (Bangsund et al., 2012).

Sugarbeet production faces several problems, including weeds, insects, and diseases. Several foliar and root diseases can limit sugarbeet production. Cercospora leaf spot (CLS) is the most important foliar disease while damping-off, Rhizoctonia crown and root rot (RCRR), Aphanomyces root rot, Fusarium yellows/decline and Rhizominia are the most important root diseases (Asher and Hanson, 2006).

Damping-off and Rhizoctonia Crown and Root Rot (RCRR)

Rhizoctonia solani. The *Rhizoctonia* genus was first described by DeCandolle in 1815 (Ogoshi, 1996). After 43 years, *R. solani*, the most important species of this genus, was described by Kühn in 1858 on potato (Ogoshi, 1996). The characteristic features of *R. solani* are vegetative growth, a multinucleate pale to dark brown mycelium, a hyphae branched at a right angle, the presence of constriction at the base of the branches, young branches with a septum, formation of sclerotia, the absence of conidia, the absence of clamp connection, the absence of clamydospores, and the absence of spermatia (Anderson, 1982; Brown and McCarter, 1976; Parmeter, 1970). *Rhizoctonia solani* Kühn is a soil-borne pathogen (Blazier and Conway, 2004) and it is found in the soil as mycelium or sclerotia (Parmeter, 1970). The teleomorph stage of *R. solani* is *Thanatephorus cucumeris* (Frank) Donk (Anderson, 1982; Franc et al., 2001; Parmeter, 1970; Windels et al., 1994), which appears as white mycelium at the base of sugarbeet leaves (Windels et al., 1994) and is rarely seen.

Rhizoctonia solani is distributed worldwide (Blazier and Conway, 2004; Franc et. al., 2001) and has a wide host range including soybean (*Glycine max* (L) Merr; Liu and Sinclair, 1991), cotton (*Gossypium hirsutum* L.; Brown and McCarter, 1976), canola (*Brassica napus* L.; Yitbarek et al., 1987), wheat (*Triticum aestivum* L.; Wiseman et al., 1996), potato (*Solanum tuberosum* L.; Escande and Echandi, 1991), rosemary (*Rosmarinus officinalis* L.; Conway et al., 1997), turfgrass species (Couch, 1995), corn (*Zea mays* L.; Ithurrart et al., 2004), and beet (Carling et al., 1987). The types of diseases that *R. solani* can cause include seedling damping-off, root rot, collar rot, stem canker, crown rot, bud and fruit rots, and foliage blight. In sugarbeet, *R. solani* causes damping-off as well as *Rhizoctonia* crown and root rot.

Rhizoctonia solani was classified into 13 anastomosis groups (AG) based on hyphal fusion: AG-1 to AG-13 (Gonzales Gracia et al., 2006; Yang and Li, 2012). Five AGs were recorded on sugarbeet: AG-1 IB, AG-1 IC, AG-2-1, AG-2-2, and AG-4 (Yang and Li, 2012). The most destructive AG for sugarbeet was AG-2-2 with two subgroups, AG-2-2 IIIB and AG-2-2 IV. The two subgroups were found to have different aggressiveness levels on sugarbeet; Panella (2005) and Bolton et al. (2010) found that AG-2-2 IIIB was more aggressive than AG-2-2 IV. However, Windels and Brantner (2011) found that some isolates of AG-2-2 IV were more aggressive than AG-2-2 IIIB. The distribution of AG 2-2 subgroups varied in Southern Minnesota and the Red River Valley in southern Minnesota, AG-2-2 IIIB comprised 56%, AG-2-2 IV 23%, and intermediate 21%, while in the Red River Valley, the highest percentage was AG-2-2 IV (66%), followed by AG-2-2 IIIB 27% and intermediate 7% (Brantner and Windels, 2007).

In sugarbeet, *R. solani* is considered an economically important pathogen. It causes annual yield losses of 2%, but the losses could reach up to 30-60% (Neher and Gallian, 2011;

Franc et al., 2001). The damage caused by *R. solani* varies from field to field, ranging from 0-50% (Leach, 1986).

Symptoms and the infection process. The favorable conditions for infection by *R. solani* are soil moisture from 25-100%, but the disease is more severe with a higher moisture level (Bolton et al., 2010) and optimal temperatures between 20 and 30ºC, but infection can occur at any temperature between 13 and 35ºC (Leach, 1986). *Rhizoctonia solani* produces different types of symptoms on sugarbeet: damping-off in the seedling stage and crown rot and root rot in older plants. Root infection starts as black lesions that grow to cover the entire root. The infection remains on the root surface until the advanced stages of the disease when it moves interiorly. Sugarbeet roots show cracks on the root surface and severe rot. The observed symptoms of RCRR on the upper-plant parts are wilting leaves; black necrosis on the petioles; stunting; plant death; and formation of a black dry rosette (Franc et al., 2001; Neher and Gallian, 2011).

Rhizoctonia solani is considered a necrotrophic to hemibiotrophic fungus, and it is found in soil as sclerotia or mycelia. Root exudates from host plants lead to sclerotia germination or mycelia growth. Exudates from the seedling stage stimulate fungus growth more than the exudates from older plant (Gonzales Gracia et al., 2006). The fungus can penetrate plants through direct penetration, through natural openings, or through wounds. Direct penetration can be achieved by the formation of a cushion structure from which a penetration peg or hyphae penetrate the plant epidermis or cuticle, and by the formation of appressoria (Gonzales Gracia et al., 2006). Wounds formed during lateral root development act as place for *R. solani* penetration (Gonzales Gracia et al., 2006; Parmeter, 1970). During penetration, *R. solani* secretes several enzymes, such as pectin lyase and cellulase, for host-tissue degradation (Lisker et al., 1975).

After penetration, colonization occurs, and several hydrolytic enzymes are secreted followed by plasmolysis and cytoplasm collapse leading to severe damage and host-tissue killing (Gonzales Gracia et al., 2006).

Disease management. Damping-off and RCRR in sugarbeet can be managed using crop rotation, resistant cultivars, and fungicides. Sugarbeet should be rotated with crops such as wheat that are not a host for the AGs of *R. solani* that infect sugarbeet. There is no known sugarbeet cultivar that is immune to *R. solani* and also has good yield and high quality. Cultivars with partial resistance are sometimes grown, but most producers use susceptible cultivars because of their potential for high yield quantity and quality (Brantner and Windels, 2007).

Applying fungicides is one of the most important methods to control *R. solani*. Several fungicides can now (in 2014) be used to manage damping-off and RCRR.These fungicides could be applied as a seed treatment, soil treatment, or foliar treatment (Markell and Khan, 2012). Fungicides should be applied before the daily average soil temperature at the 10-cm soil depth reaches 18°C (Khan and Bolton, 2010; Khan et al., 2005). Chloroneb, fludioxonil, hymexazol, mefenoxam, metalaxyl, metconazole, and thiram are used as seed-treatment fungicides (Brantner et al., 2012; Khan, 2012; Markell and Khan, 2012). Some fungicides, such as azoxystrobin and pyraclostrobin are used for both soil and foliar application (Brantner et al., 2012; Markell and Khan, 2012; Windels and Brantner, 2005). Other fungicides, such as prothioconazole, are used as a foliar fungicide (Markell and Khan, 2012).

Azoxystrobin, pyraclostrobin, and penthiopyrad were found to increase sugar yield by 44% if they were applied in-furrow (Brantner et al., 2012). Penthiopyrad was also effective if used as a seed treatment (Brantner et al., 2012; Khan, 2012). Treating sugarbeet seeds with

penthiopyrad and later applying azoxystrobin were effective for controlling damping-off and RCRR disease (Khan, 2012).

Sensitivity of *R. solani* to fungicides. Sensitivity of fungi to fungicides is measured by calculating the effective concentration that kills 50% of the population (EC_{50}; Russell, 2004). Resistance development depends on the fungicide mode of action, the number of fungicide applications, and the fungal biology (Brent and Holloman, 2007). It is uncommon for *R. solani* to develop resistance to fungicides because of the fungus biology. Fungicide sensitivity was evaluated for several *R. solani* AGs from different crops including cotton, tobacco (*Nicotiana tabacum* L.), rice (*Oryza sativa* L.), potato, soybean and turfgrass.

Some authors have reported that azoxystrobin (QoI) was effective against *R. solani* (Blazier and Conway, 2004; Jin et al., 2009; Sundravadana et al., 2007) while some have reported that azoxystrobin was not effective (Blazier and Conway, 2004; LaMondia, 2012; Olaya et al., 2012). Sensitivity of *R. solani* was evaluated for several fungicides belonging to the SDHI group. Thifluzamide, boscalid, penflufen, sedaxane, flutolanil, and carboxin were found effective at reducing mycelium radial growth of *R. solani* (Ajayi and Bradley, 2014; Campion et al., 2003; Chen et al., 2012; Csinos and Stephenson, 1999; Kataria et al., 1991). Martin et al. (1984) found that carboxin was not effective against *R. solani* and the EC_{50} value was 38.8 µg ml^{-1}. Most DMI fungicides were effective against *R. solani*, except fenarimol and imazalil, which failed to reduce the mycelium growth of *R. solani* (EC_{50} > 500 µg ml^{-1}) (Kataria et al., 1991). *Rhizoctonia solani* was sensitive to prothioconazole, cyproconazole, triadimefon, hexaconazole, prochloraz, ipoconazole, and triflumizole (Ajayi and Bradley, 2014; Carling et al., 1990; Csinos and Stephenson, 1999; Kataria et al., 1991; Martin et al., 1984). For the dicarboximide group, *R. solani* was sensitive to iprodione fungicide (Campion et al., 2003; Carling et al., 1990; Csinos

and Stephenson, 1999; Kataria et al., 1991; Martin et al., 1984). Ten *R. solani* AGs showed a wide variation of EC_{50} values for vinclozolin, with the EC_{50} range from 7.5-49 µg ml^{-1} (Kataria et al., 1991). Also *R. solani* from different AGs showed a variation in their sensitivity to Pentachloronitrobenzene (PCNB) (Carling et al., 1990; Csinos and Stephenson, 1999; Martin et al., 1984). For benomyl (benzimidazole), all tested *R. solani* isolates were sensitive (Carling et al., 1990; Martin et al., 1984). For mancozeb, AG-3 and AG-4 had high EC_{50} values (Csinos and Stephenson, 1999). *Rhizoctonia solani* AGs showed wide variation for their sensitivity to fenpropimorph (amines), furmecyclox (methyl benzimidazole carbamates), thiabendazole (methyl benzimidazole carbamates), and pencycuron (phenylureas group) (Campion et al., 2003; Kataria et al., 1991).

Damping-Off and Root Rot

 Aphanomyces cochlioides. *Aphanomyces cochlioides* Drechs. belongs to the kingdom Chromista, phylum Oomycota, class Oomycetes, and order Saprolegniales (Agrios, 2005). *Aphanomyces cochlioides* was first described by Drechsler from Michigan in 1929 (Drechsler, 1929). It has non-septate hyphae and produces different spore types: asexual zoospores which include primary zoospores (which are pear-shaped and biflagellate); secondary zoospores (which are produced from encysted primary zoospores); and sexual oospores which have hyaline to yellow color, 16-24 µm diameter, and a thick wall (1.5-2 µm) (Harveson et al., 2007).

 The environmental conditions which favor infection and disease development are high soil moisture and warm temperature from 20 to 30ºC, but infection can occur at a lower temperature 13ºC (Papavizas and Ayers, 1974). *Aphanomyces cochlioides* is distributed worldwide wherever sugarbeet is grown. It was reported in Sweden (Amein, 2006), Australia (Martin, 2003), Canada (McKeen, 1949), Poland (Moliszewska and Piszczek, 2008), Britain

(Payne et al., 1994), and the USA (Harveson, 2000a, 2000b; Harveson et al., 2002). The *A. cochlioides* distribution was uniform in fields with a high level of inoculum, but it was aggregated in fields with low and moderate levels of inoculum (Dyer et al., 2004).

Aphanomyces cochlioides infects different crops within *B. vulgaris* including wild species of *Beta* (*B. maritima* L. and *B. patellaris* Moq); spinach (*Spinacia oleracea* L.) (Larsson, 1994; Papavizas and Ayers, 1974); and weeds such as pigweed (*Amaranthus palmeri* S. Wats.), lambsquarters (*Chenopodium berlandieri* Moq.), and Kochia (*Neokochia americana* (S.Wats.) G.L. Chu and S.C. Sand; Franc et al., 2001). Aphanomyces root rot is an economically important disease which reduces plant stand and yield in the fields and adversely impacts storage. In Montana, Colorado, Wyoming, and Nebraska, the percentage of infested sugarbeet hectares was 35%; in Michigan, it was 11% (Harveson et al., 2007); and in North Dakota and Minnesota, 50% of sugarbeet fields were reported as infested (Beale et al., 2002). In storage, Aphanomyces root rot affects extractable sucrose with the percentage of loss depending on disease severity and storage duration. At high root rot (index of ≥80), the loss percentage was 43% (Campbell and Klotz, 2006; Klotz and Campbell, 2009).

Symptoms and infection process. *Aphanomyces cochlioides* does not cause infection before plant emergence; the symptoms on the roots are yellow-to-brown lesions, water-soaked black lesions, root constriction, and root disintegration in severe infection. The above-ground symptoms are thread-like hypocotyls and stunted plants; the leaves become yellow, wilted, and brittle (Harveson and Rush, 1993; Harveson et al., 2002; Franc et al., 2001; Papavizas and Ayers, 1974; Windels, 2000). Symptoms caused by *A. cochlioides* were classified into two types, acute and chronic, depending on the stage of the infected sugarbeet. Acute symptoms occur in the seedling stage while chronic symptoms occur in older plants (Franc et al., 2001).

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Under favorable environmental conditions and the presence of root exudates, oospores (the overwintering stage of *A. cochlioides*) germinate and colonize sugarbeet plants. Oospores may cause infection directly or by producing sporangia. The sporangia produce a lot of encysted primary zoospores that, in turn, convert to biflagellate secondary zoospores. The fungus penetrates the host tissue using the appresoria; the mycelia grow intracellularly, producing sporangia and zoospores and at the end of the season, oogonia are formed and fertilized by antheridia, again producing oospores (Franc et al., 2001; Islam and Tahara, 2001).

Disease management. Aphanomyces root rot can be managed using early planting, cultivation, tillage, elimination of alternate hosts, rotation, and hymexazol-treated (Tachigaren 70WP, Sankyo Co., Ltd., Tokyo, Japan) seeds (Windels and Brantner, 2000). Other promising methods to control *A. cochlioides* are using the biological agents such as *Pseudomonas jessenii* (Deora et al., 2010) and applying spent lime (calcium carbonate), a byproduct from sugar production (Brantner et al., 2013; Olsson et al., 2011).

In the years between 1974 and 1984, Aphanomyces root rot was managed using fenaminosulf as a seed treatment which was the only available treatment for *A. cochlioides*. After 1984, fenaminosulf production was halted, leaving sugarbeet production with no treatment for *A. cochlioides* (Harveson et al., 2007). Since 1995, Tachigaren was the only registered fungicide for *A. cochlioides* management to be used as a seed treatment to prevent early season infection (Harveson et al., 2007).

Very few studies were conducted to evaluate the efficacy of fungicides to manage *A. cochlioides*. The most widely used fungicide for *A. cochlioides* control in sugarbeet is Tachigaren, which interfere with RNA and DNA synthesis (FRAC 2014). Cyazofamid, a quinine inside inhibitor (QiI) fungicide, was only effective against Oomycetes including *A. cochlioides*.

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The EC$_{50}$ value of cyazofamid was 0.2 µg ml^{-1} (Mitani et al., 2001). Gaulin et al. (2010) found that *A. euteiches* has a cyp51 gene which encodes for the DMI target enzyme, sterol P450 14α-demethylase. Therefore, it will be useful to determine if triazoles have the potential to control *A. cochlioides* in sugarbeet.

Cercospora Leaf Spot

Cercospora beticola. In 1876, Cercospora leaf spot (CLS) was first reported by Saccardo, and the causal agent was first described as *C. beticola* in 1953 (Chupp, 1953). Cercospora leaf spot disease originated in central Europe and the Mediterranean area as its host sugarbeet (Groenewald et al., 2005).

Cercospora beticola is a hemibiotrophic fungus belonging to the phylum ascomycota, class hyphomycetes, and order Hyphales. It has no known sexual stage although other *Cercospora* species have a teleomorph stage that belongs to the *Mycospherella* genus (Crous et al., 2001; Wieland and Koch, 2004). Bolton et al. (2012c) found strong evidence for potential sexual reproduction of *C. beticola* in the USA. The fungus reproduces asexually by producing conidia and overwinters as stromata (Pseudostromata) in infected crop residues (Asher and Hanson, 2006; Khan and Khan, 2010; Khan et al., 2008). *Cercospora beticola* hyphae are septate, hyaline, and are 2 to 4 µm in diameter. Conidiophores are hyaline at the tip and pale brown at the base, septate, unbranched, and are 10-100 x 3-5.5 µm in size. The conidia are hyaline, septate, straight to slightly curved, and have a size of 20-200 x 2.5-4 µm (Asher and Hanson, 2006; Weiland and Koch, 2004).

Cercospora beticola is distributed worldwide and has been reported in North America, South America, Europe, Africa, and Asia (Holtschulte, 2000; Asher and Hanson, 2006). *Cercospora beticola* is not a host-specific fungus (Groenewald et al., 2006). The host range

includes wild and cultivated species of *Beta*, species belonging to different genera of Chenopodiaceae (Asher and Hanson, 2006; Weiland and Koch, 2004), *Amaranthus* (Weiland and Koch, 2004), *Carthamus* (Lartey et al., 2005), *Chrysanthemum*, *Malva*, *Limonium*, and *Apium* sp (Groenewald et al., 2006).

Cercospora leaf spot is the most destructive foliar disease that affects sugarbeet (Asher and Hanson, 2006; Jacobsen and Franc, 2009; Skaracis et al., 2010; Weiland and Koch, 2004; Wolf and Verreet, 2002). It was first reported as a destructive disease in Europe in 1878 and USA in 1895 (Halsted, 1895). In North Dakota and Minnesota, an outbreak of CLS occurred in 1980 (Windels et al., 1998). American Crystal Sugar Company estimated the loss to CLS in an epidemic in 1998 at $40 million (Ellington et al., 2001). Jacobsen and Franc (2009) reported that losses due to CLS under favorable conditions could reach 40% or greater, and under moderated disease conditions, 30% losses in recoverable sucrose due to CLS are common (Khan et al., 2001). A CLS outbreak can result in the complete loss of a sugarbeet crop (Rossi et al., 2000b). Cercospora leaf spot causes a reduction in the sugar percentage because sugarbeet plants regenerate new leaves which divert photosynthate from roots to new leaves (Franc, 2010).

The favorable conditions for *C. beticola* sporulation, germination, and infection are high temperatures from 25 to 35 ºC, with night temperatures above 18 ºC, and high relative humidity from 90 to 95% for 5 to 8 hours (Franc, 2010; Khan and Khan, 2010; Khan et al., 2008). Pool and McKay (1916) found that conidial production and infection can occur at 60% relative humidity if the humidity lasts for at least 15 to 18 hours.

Symptoms and the infection process. The spots caused by *C. beticola* are circular, 2-5 mm in diameter, and have a grey center and red-to-purple margins. The grey centers have black pseudostromata which are the overwintering stage (Skaracis et al., 2010; Weiland and Koch,

2004). Spots coalesce as the disease progresses, and the entire leaf becomes necrotic and collapses, but remains attached to the plant (Asher and Hanson, 2006). Symptoms may also occur on the petioles (Franc, 2010). Signs of CLS are the black pseudostromata which, under humid conditions, germinate and produce conidiophores as well as conidia (Ruppel, 1986).

Cercospora leaf spot is a polycyclic disease. The sources of primary inoculum are pseudostroma, alternative hosts, and seeds (Asher and Hanson, 2006). Pseudostroma produces conidiophores which bear conidia through the stomata. The conidia disperse by wind, rain, water splash, and insects (Asher and Hanson, 2006; Khan et. al., 2008; McKay and Pool, 1918). Once the conidia reach the surface of sugarbeet leaves and under favorable conditions they germinate and penetrate the leaf surface through stomata. After penetration, the hyphae grow intercellularly, and during infection, the fungus produces toxins such as cercosporin and beticolin, and as a result of the infection, the tissue is killed. At the end of the season and with unfavorable conditions, pseudostromata develop. Symptoms take 5 to 7 days to appear as a small chlorotic lesion, and after 10 to 13 days, necrotic lesions enlarge (Steinkamp et al., 1979). Necrotic lesions produce conidia after 3 days (Rossi et al., 2000a), and the maximum number of conidia produced by necrotic lesions occurs after 10 days (Franc, 2010).

Disease management. Managing CLS relies on crop rotation, resistant cultivars, and the application of fungicides (Jacobsen, 2010; Secor et al., 2010a; Skaracis et al., 2010; Upchurch and Kuykendall, 2010). Crop rotation with non-host crops for three years is recommended to reduce the initial inoculum (Pundhir and Mukhopadhyay, 1987). Several fungicides are registered in sugarbeet for CLS control, including dithiocarbamate, benzimidazole, triphenyltin hydroxide (TPTH), triazole (DMI), and quinone outside inhibitor (QoI). The number of fungicide applications varies according to environmental conditions and disease pressure. In the

USA, three to four applications are needed during the growing season to reduce the CLS disease's effect on yield (Secor et al., 2010a). Fungicide-resistant management is critical to keep fungicides effective and available for a prolonged period. For fungicide-resistant management, fungicides from different Fungicide Registrattion Action Committee (FRAC) groups should be rotated, mixed, or applied based on prediction models such as the Shane-Teng model and the BeetCast model (Windels, 2010).

Sensitivity of *C. beticola* to fungicides. Due to several fungicide applications during the growing season, *C. beticola* developed resistance to several fungicide groups including benzimidazole (Briere et al., 2001; Campbell et al., 1998, Davidson et al., 2006; Giannopolitis and Chrysayi-Tokoudbalides, 1980), triphenyltin hydroxide (Briere et al., 2001; Bugbee, 1995, 1996; Giannopolitis and Chrysayi-Tokoudbalides, 1980), triazole (demethylase inhibitors; DMIs) (Bolton et al., 2012a; Karaoglanidis et al., 2000, 2002, 2003; Secor et al., 2010b), and quinone outside inhibitors (QoI) (Birla et al., 2012; Bolton et al., 2013; Kirk et al., 2012).

In 1999, triazole fungicides (FRAC 3) (which inhibit sterol biosynthesis in the fungal membrane) were first used on sugarbeet in the USA and resistant isolates was reported several years later, similar resistance was already reported for *C. beticola* on sugarbeet in Greece where triazoles were in use earlier (Karaoglandis et al., 2000, 2002, 2003; Secor et al., 2010b). Resistance was due to the over expression of the cyp51 gene which encodes for the DMI target enzyme, sterol P450 14α-demethylase in *C. beticola* (Bolton et al., 2012a).

The fitness of resistant isolates plays an important role in developing resistance to fungicides for any fungal population (Peever and Milgroom, 1994). Several studies were conducted to study the fitness of DMI-resistant *C. beticola* isolates. The fitness of *C. beticola* isolates was found to be negatively affected by DMI resistance. Resistant isolates were found to

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have less virulence, spore production (Karaoglanidis et al., 2001; Moretti et al., 2003), and mycelium radial growth (Moretti et al., 2003; Nikou et al., 2009). In other studies, resistant and sensitive isolates were similar in spore germination (Moretti et al., 2003; Karaoglanidis et al., 2001), mycelium growth, competitive ability, incubation period, germ tube length (Karaoglanidis et al., 2001), spore production (Nikou et al., 2009; Moretti et al., 2003), virulence (Nikou et al., 2009), and disease severity (Bolton et al., 2012b).

The stability of resistance to DMIs was found to be negatively influenced by environmental conditions and successive transfers. Overwintering adversely affected DMI-resistant isolates. *Cersospora beticola* isolates that were resistant to DMIs showed an increased sensitivity after exposure to cold conditions (Karaoglanidis andThanassoulopoulos, 2002). Also, resistant isolates were found to be less frequent than sensitive isolates at the beginning of one growing season compared with the end of the previous growing season, indicating that resistant isolates had less ability to survive the overwintering period or that they were weak competitors (Karaoglanidis et al., 2002). Other pathosystems showed similar increased sensitivity for DMI-resistant isolates after exposure to cold conditions *Monilinia fructicola* isolates from peach (*Prunus persica* (L.) Stokes) showed increased sensitivity to DMI after exposure to 4ºC, 5ºC, and -20ºC (Cox et al., 2007; Zhu et al., 2012), and *Venturia inaequalis* isolates from peach showed an increase in sensitivity after they were stored at 2ºC for 7 months (Koller et al., 1991). Successive transfer was found to have no effect on the stability of resistance to DMI for *C. beticola* (Karaoglanidis and Thanassoulopoulos, 2002), but with other fungi such as *V. inaequalis* and *M. fructicola*, successive transfer made resistant isolates reverted back sensitive to DMI fungicides (Cox et al., 2007; Koller et al., 1991). It is not know what makes resistance to DMI fungicides unstable (Zhu et al., 2012).

This research was conducted to understand how to better manage *R. solani*, *A. cochlioides*, and *C. beticola* of sugarbeet using fungicides in North Dakota and Minnesota. For *R. solani*, the objectives were to develop baseline sensitivity of *R. solani* for QoI (azoxystrobin, trifloxystrobin, and pyraclostrobin), SDHI (penthiopyrad), and DMI (prothioconazole) fungicides; to determine if a shift in sensitivity to azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole has occurred; to determine if cross sensitivity existed among the tested fungicides; to evaluate the efficacy of the tested fungicides against *R. solani* isolates in the greenhouse; and to evaluate if there was variation in the rate of mycelium radial growth between *R. solani* isolates with high and low EC_{50} values for azoxystrobin and trifloxystrobin. For *A. cochlioides*, the objectives were to determine the efficacy of hymexazol, tetraconazole, prothioconazole, and pyraclostrobin in reducing mycelium radial growth of *A. cochlioides* in vitro; to test the efficacy of those fungicides in the greenhouse, and to determine the most susceptible stages of sugarbeet plants to *A. cochlioides*. For *C. beticola*, the objectives were to determine if there was a variation in spore production, spore germination, radial growth, sensitivity to tetraconazole, and disease severity of *C. beticola* isolates resistant to tetraconazole after exposure to different temperature regimes: -20ºC (4 weeks); 4ºC (4 weeks); 20ºC (4 weeks); -20ºC (2 weeks) to 4ºC (2 weeks); -20ºC (1 week) to 4ºC (1 week) to -20ºC (1 week) to 4ºC (1 week); and -20ºC (1 week) to 20ºC (1 week) to -20ºC (1 week) to 20ºC (1 week).

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CHAPTER TWO. SENSITIVITY OF *RHIZOCTONIA SOLANI* TO AZOXYSTROBIN, TRIFLOXYSTROBIN, PYRACLOSTROBIN, PENTHIOPYRAD, AND PROTHIOCONAZOLE

Introduction

Rhizoctonia solani Kühn (Basidiomycetes) is a soil-borne pathogen which is found as mycelium or sclerotia in the soil (Menzies, 1970). *Thanatephorus cucumeris* (Frank) Donk is the teleomorph stage of *R. solani*, and it was first described in sugarbeet (*Beta vulgaris* L.) in 1947 by Kotila (Herr and Roberts, 1980; Kotila, 1947; Windels and Kuznia, 1993; Windels et. al., 1997). In sugarbeet, *R. solani* causes damping-off as well as Rhizoctonia crown and root rot (RCRR) (Asher and Hanson, 2006). *Rhizoctonia solani* is an economically important pathogen that causes annual yield losses of 2%, but the losses could reach up to 30 to 60% (Neher and Gallian, 2011). Management of sugar cooperatives and growers' representatives from factory districts have listed diseases caused by *R. solani* as the most important problem found by growers in North Dakota and Minnesota (Khan M. F. R. personal communication). In North Dakota and Minnesota, damping-off and RCRR caused by anastomosis group AG-2-2 are increasing in prevalence (Brantner and Nielsen, 2013).

Rhizoctonia solani has 13 anastomosis groups, AG-1 to AG-13 (Carling et al., 2002; Yang and Li, 2012). AG-1 IB, AG-1 IC, AG-2-1, AG-2-2, and AG-4 have been recorded on sugarbeet (Yang and Li, 2012). The most destructive AG on sugarbeet is AG-2-2; it has two subgroups, AG-2-2 IIIB and AG-2-2 IV (Bolton et al., 2010; Panella, 2005; Windels and Brantner, 2011). There are variations in aggressiveness between and within AG-2-2 subgroups (Panella, 2005; Windels and Brantner, 2011).

Crop rotation, use of partially resistant cultivars, planting early, and fungicides can be used to manage *R. solani* in sugarbeet (Khan, 2012; Khan and Bolton, 2010; Rush and Winter, 1990; Windels and Brantner, 2007; Windels and Lamey, 1998). Growers typically use cultivars which tend to be more susceptible and apply fungicides because of the high yield potential of the susceptible cultivars (Bolton et al., 2010). Fungicide application is an important method to control *R. solani* and several fungicides are labeled to manage *R. solani* in sugarbeet including Quadris® (azoxystrobin, active ingredient (a.i.), 22.9%; Syngenta, Greensboro, NC, USA) which was registered in 1999, Gem® (trifloxystrobin, a.i., 42.6%; Bayer, Research Triangle Park, NC, USA) which was registered in 2002, Headline® (pyraclostrobin, a.i., 23.6%; BASF, Research Triangle Park, NC, USA) which was registered in 2002, Proline® (prothioconazole, a.i., 41%; Bayer, Research Triangle Park, NC, USA) which was registered in 2008, and Vertisan® (penthiopyrad, a.i., 20.6%; DuPont Crop Protection, Wilmington, DE, USA) which was registered in 2012 but is not available commercially (Friskop et al., 2014, Secor et al., 2010). In 2013, the quinine outside inhibitor (QoI) fungicides especially azoxystrobin (Quadris) and pyraclostrobin (Headline), and to a lesser extent prothioconazole (Proline) were used to control *R. solani* (Carlson et al., 2013).

Fungicides registered for controlling *R. solani* have a specific active-site mode of action and were considered, according to the Fungicide Registration Action Committee (FRAC), as medium or high risk based on the ability of the targeted fungi to develop resistance to these fungicides. The QoI fungicides (FRAC 11) which include azoxystrobin, pyraclostrobin, and trifloxystrobin, inhibit complex III (cytochrome bc1) in the mitochondria (FRAC, 2014). Resistance to the QoI group was reported in *R. solani* AG-1-1A from rice in 2012, and the source of the resistance was F129L mutation where phenylalanine (F) at position 129 was replaced by

leucine (L) (Olaya et al., 2012). Penthiopyrad, which belongs to the succinate dehydrogenase inhibitors (SDHI; FRAC 7), inhibits mitochondrial respiration by affecting the succinate dehydrogenase enzyme (complex II) (FRAC, 2014). Prothioconazole is a demethylation inhibitor fungicide (DMI; FRAC 3) which affects sterol biosynthesis in fungal cells.

Sensitivity of fungi to fungicides is measured by calculating the effective concentration that kills 50% of the population (EC$_{50}$; Russell, 2004). Fungicide sensitivity was evaluated for several *R. solani* AGs from different crops including cotton (*Gossypium hirsutum* L.), tobacco (*Nicotiana tabacum* L.), rice (*Oryza sativa* L.), potato (*Solanum tuberosum* L.), soybean (*Glycine max* (L) Merr) and turfgrass. The fungicides evaluated were QoIs (Blazier and Conway, 2004; Jin et al., 2009; LaMondia, 2012; Olaya et al., 2012; Sundravadana et al., 2007), SDHIs (Ajayi and Bradley, 2014; Campion et al., 2003; Chen et al., 2012; Csinos and Stephenson, 1999; Kataria et al., 1991; Martin et al.,1984), and DMIs (Ajayi and Bradley, 2014; Carling et al., 1990; Csinos and Stephenson, 1999; Kataria et al., 1991; Martin et al., 1984). *Rhizoctonia solani* from rice, showed shift in sensitivity to azoxystrobin were the pathogen became insensitive to azoxystrobin (Olaya et al., 2012). No shift in sensitivity was found for thje fungicides belong to SDHI (Ajayi and Bradley, 2014).

Azoxystrobin was labeled for use on sugarbeet in 1999, and since that time it has been the most widely used product to control *R. solani*. *Rhizoctonia solani* from sugarbeet in North Dakota and Minnesota was not evaluated for sensitivity to azoxystrobin or to other fungicides. The ability of *R. solani* to develop resistance to a single-site, active fungicide became a great concern after *R. solani* AG-1-1A from rice developed resistance to azoxystrobin (Olaya et al., 2012). It would be useful to determine sensitivity of *R. solani* to fungicides so that the pathogen

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sensitivity could be monitored over time to help decide how best to manage the fungus while preserving the utility of fungicides.

The objectives of this research were 1) to develop baseline sensitivity of *R. solani* for QoI (azoxystrobin, trifloxystrobin, and pyraclostrobin), SDHI (penthiopyrad), and DMI (prothioconazole) fungicides and to determine if a shift in sensitivity to azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole has occurred, 2) determine if cross sensitivity existed among the tested fungicides, 3) evaluate the efficacy of the tested fungicides against *R. solani* isolates in the greenhouse, and 4) evaluate if there was variation in the rate of mycelium radial growth between *R. solani* isolates with high and low EC_{50} values for azoxystrobin and trifloxystrobin.

Materials and Methods

Source of *R. solani* isolates. *Rhizoctonia solani* Kühn isolates were obtained from the Northwest Research and Outreach Center, University of Minnesota, USA (Carol Windels and Jason Brantner). These isolates were collected from sugarbeet fields in Minnesota and North Dakota. One hundred and five *R. solani* isolates were used in this study; 27 isolates were collected before 1999 (prior to registration for any fungicides currently used in sugarbeet) and were used for the baseline sensitivity study and 78 isolates collected between 2005 and 2012 (after exposure to fungicides) were used to evaluate if any shift in sensitivity occurred in *R. solani* over time (Table 2.1).

For long-term storage, the isolates were transferred to half-strength potato dextrose agar media (PDA; potato dextrose broth, 12 g; agar, 15 g; and distilled water, 1 L) amended with 50 mg ml^{-1} ampicillin and left at room temperature (20±2ºC) for 4 days. The plates were then kept in a refrigerator (4ºC) and transferred every month to keep the isolates active (Harveson, 2006).

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Determination of AG-2-2 subgroups. AG-2-2 IIIB and AG-2-2 IV subgroups were

determined following Sneh et al. (1991) and Brantner and Windels' (2007) methods which work

on the principle that subgroup AG-2-2 IIIB grows at 35ºC while AG-2-2 IV does not. A 3-mm

mycelium plug of each *R. solani* isolate was transferred to a 9-cm diameter Petri dish containing

15 ml half-strength PDA. One known isolate for each subgroup (AG-2-2 IIIB, 890; and AG-2-2

IV, 40) was included in the experiment as a control; those known isolates were determined by

Brantner and Windels (2007). Four plates were prepared for each isolate; two plates were

incubated at 25ºC, and two plates were incubated at 35ºC (Model 50036; Percival Scientific,

Boone, IA, USA). On the surface of the plate, a line was drawn at the culture margin after 24

hours (baseline); then, after 48 hours, mycelium radial growth was measured between the culture

margin and the baseline. The percentage of growth was calculated [(growth at 35ºC / growth at

25ºC) x 100]. If the growth percentage was equal to or more than the percentage of the AG-2-2

IIIB control isolate (890), the isolates were considered as AG-2-2 IIIB. If the percentage of

growth was very low or if there was no growth, the isolates were considered as AG-2-2 IV. If the

percentage of growth was less than the growth of the AG-2-2 IIIB, the isolates were considered

as intermediate (Brantner and Windels, 2007; Sneh et al., 1991). The experiment was conducted

as a complete randomized design (CRD) with two replicates. The experiment was repeated once,

and the data were analyzed using SAS (PROC GLM) version 9.3 (SAS Institute, Inc.; Cary, NC,

USA).

In vitro sensitivity of *R. solani* to quinone outside inhibitors (azoxystrobin,

trifloxystrobin, and pyraclostrobin), succinate dehydrogenase inhibitors (penthiopyrad),

and demethylase inhibitors (prothioconazole). Since *R. solani* produces no asexual spores, the

mycelium radial-growth assay was used to evaluate *R. solani* sensitivity to the fungicides as

described by Kataria et al. (1991) with slight modifications. A cork borer was used to cut 3-mm

diameter mycelium plugs from 4-day-old cultures of *R. solani*. The plugs were inverted onto the

fungicide-amended and non-amended plates and kept at room temperature ($22 \pm 2ºC$) in the dark

for 72 hours. Two perpendicular diameters were measured for each plate and averaged. The

percentage of mycelium growth reduction relative to the growth in the non-amended media was

calculated [100 - (growth diameter in amended media / growth diameter in non-amended media)

x 100)], and regressed against the fungicide concentrations logarithm, the concentration that

causes 50% mycelium inhibition was determined by interpolation of the 50% intercept (Russell,

2004) using SAS version 9.3.

Table 2.1. Year of collection, state of origin, and number of *Rhizoctonia solani* isolates used for mycelium radial-growth assay and in greenhouse studies.

Year	State	Number of isolates
	Baseline isolates before 1999	
1986	Minnesota	5
1987	Minnesota	2
	North Dakota	4
1988	Minnesota	3
1987-1988	Minnesota	3
	North Dakota	4
1989	Minnesota	1
1993	Minnesota	5
Sub-total		**27**
	Non-baseline isolates	
2005	Minnesota	1
	North Dakota	3
2006	Minnesota	14
	North Dakota	6
2007	Minnesota	12
2008	Minnesota	9
	North Dakota	2
2012	Minnesota	30
	North Dakota	1
Sub-total		**78**
Total		**105**

Technical grades of azoxystrobin (96% active ingredient (a.i.); Syngenta, Greensboro,

NC, USA), trifloxystrobin (98.8% a.i.; Bayer, Research Triangle Park, NC, USA), pyraclostrobin

(98% a.i.; BASF, Research Triangle Park, NC, USA), penthiopyrad (95% a.i.; Vertisan®, DuPont, Wilmington, DE, USA), and prothioconazole (99.4% a.i.; Bayer, Research Triangle Park, NC, USA) were used to prepare 100-mg ml^{-1} stock solutions in acetone (EM Science, Gibbstown, NJ, USA). Tenfold serial dilutions were prepared to have 0-, 0.01-, 0.1-, 1-, and 10-mg ml^{-1} fungicide concentrations. Salicylhydroxamic acid (SHAM; Sigma Chemical Co., St. Louis, MO, USA) was dissolved in methanol (Sigma Chemical Company Co., St. Louis, MO, USA) to obtain a 100- mg ml^{-1} stock solution. One liter of a half-strength PDA media was amended with 1 ml of one of the fungicide concentrations and 1 ml of SHAM, which was used to prevent an alternative oxidation respiration pathway (Wood and Hollomon, 2003). SHAM was not used with prothioconazole (DMI) fungicide because this fungicide does not affect mitochondrial respiration (FRAC, 2014).

Isolates that showed an EC$_{50}$ value >100 µg ml^{-1}, as was the case for azoxystrobin and trifloxystrobin, were tested again using higher fungicide concentrations of 0, 1, 10, 100, 500, and 1000 µg ml^{-1}. Due to low solubility of the technical grades in water (azoxystrobin 6 µg ml^{-1} and trifloxystrobin 0.6 µg ml^{-1}), formulated products of azoxystrobin (Quadris) and trifloxystrobin (Gem; Table 2.2) were used to prepare fungicide concentrations. Using formulated products to calculate EC$_{50}$ values was reported by Kataria et al. (1991) and Sundravadana et al. (2007).

Table 2.2. Properties of the fungicides used to evaluate sensitivity of *Rhizoctonia solani* in vitro and in the greenhouse studies.

Fungicide	MOA[a]	Active ingredient	Active ingredient %	Application rate range (ml/ha)
Quadris	QoI	Azoxystrobin	22.9	453-1111
Gem	QoI	Trifloxystrobin	42.6	212-263
Headline	QoI	Pyraclostrobin	23.6	658-877
Vertisan	SDHI	Penthiopyrad	20.6	1023-2192
Proline	DMI	Prothioconazole	41.0	365-417

[a] MOA, mode of action; QoI, quinone outside inhibitors; SDHI, succinate dehydrogenase inhibitors; DMI, demethylase inhibitors.

A reproducibility test was done as described by Wong and Wilcox (2002). One isolate (393) was chosen randomly as a control isolate. This isolate was tested 5 times in different experiments, the EC_{50} values were calculated, and then, the 95% confidence intervals (CI) of the EC_{50} mean were calculated. Isolate 393 was included in each experiment and if the mean EC_{50} of the control isolate did not fall within the 95% CI, the experiment was dropped and repeated another time (Table 2.3).

Table 2.3. Means and confidence intervals for the EC_{50} values of *Rhizoctonia solani* isolate, 393 used as the control.

Fungicide	Mean EC_{50} ($\mu g\ ml^{-1}$)	Confidence interval 95%
Azoxystrobin	533.5	334.0-899.9
Trifloxystrobin	483.0	222.4-928.0
Pyraclostrobin	0.3	0.1-0.5
Penthiopyrad	0.2	0.1-0.3
Prothioconazole	0.4	0.1-1.0

Alternative oxidation respiration pathway. This experiment was conducted to determine if *R. solani* uses the alternative oxidation respiration pathway. Five isolates of *R. solani* were randomly chosen. The EC_{50} values for azoxystrobin with and without SHAM were determined as previously described. The experiment was repeated once. The student's t-test was used to compare between with and without SHAM treatments for each isolate, and the F-test was used to compare the combined means for all isolates between with and without SHAM treatments using SAS version 9.3.

Efficacy of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole in controlling *R. solani*. The efficacy of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole for controlling *R. solani* was evaluated in the Agricultural Experiment Station greenhouse at North Dakota State University in Fargo, ND, USA. Eight *R. solani* isolates were chosen based on the subgroups and EC_{50} values for

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azoxystrobin and trifloxystrobin. Four isolates of AG-2-2 IIIB (two isolates with a high EC_{50} value and two isolates with a low EC_{50} value) and four isolates of AG-2-2 IV (two isolates with a high EC_{50} value and two isolates with a low EC_{50} value) were randomly chosen. Because of space limitation and the time required to prepare and inoculate the high number of treatments (4 isolates x 5 fungicides x 8 fungicide concentrations x 3 replicates = 480 treatments), the isolates were divided and evaluated in two experiments (Table 2.4).

Table 2.4. Subgroups, isolates, and azoxystrobin and trifloxystrobin EC_{50} values for *Rhizoctonia solani* isolates that were used in the greenhouse study.

Experiment	Subgroup	Isolate	EC_{50} (μg ml^{-1})	
			Azoxystrobin	Trifloxystrobin
1	IIIB	850	3.5	5.9
1	IIIB	22-1	868.1	589.8
1	IV	60	0.4	2.7
1	IV	393	707.3	450.2
2	IIIB	946	4.2	3.4
2	IIIB	571	876.6	876.6
2	IV	31-1	0.3	0.5
2	IV	40-2	830.4	888.4

The *Rhizoctonia solani* inoculum was prepared following Stump et al. (2004) with some modifications. Barley (*Hordeum vulgare* L.) grains were used instead of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.). Barley grains were mixed with water and soaked for 30 minutes instead of overnight, and the grains were transferred to spawn microsac bags with a filter (50 cm x 20 cm x 13 cm; Mycelia, Veldeken, Belgium) and autoclaved for 20 minutes instead of 1.5 hours at 121ºC. The bags were left to dry in the fume hood overnight. One plate of *Rhizoctonia solani* was mixed with 100 ml of sterilized, distilled water in a blender for 1 minute at 5,000 rpm. The suspension was added to the barley grains in the bags, and then sealed (Plastic Film Sealer, FR-300L, China) and incubated for 4 weeks at 25ºC in the dark. The bags were shaken daily, and after 4 weeks, the bags were opened and kept in the fume hood to dry.

Seeds of Crystal 539RR, a sugarbeet cultivar susceptible to *R. solani* (Niehaus, 2011),

was used. Sunshine Mix LC1 (73 to 83% Canadian sphagnum peat moss, perlite, and dolomite

lime) (Sun Gro Horticulture Distribution, Inc.; Agawam, MA, USA) was used to fill 25x14x13-

cm plastic trays (T. O. Plastic, Inc.; Clearwater, MN, USA). Ten seeds were planted in a furrow

(2 cm deep) made in the middle of the trays. Serial fungicide dilutions (0, 0.1, 1, 10, 100, 1,000,

and 10,000 µg ml^{-1}) as well as the field application rates of azoxystrobin 672 ml/ha,

trifloxystrobin 256 ml ha^{-1}, pyraclostrobin 672 ml ha^{-1}, penthiopyrad 2,192 ml/ha, and

prothioconazole 417 ml/ha were prepared (Table 2.2). Fungicides were applied as an in-furrow

application using a Generation III Research Sprayer (De Veries Manufacturing; Hollandale, MN,

USA) through a 4001E flat-fan nozzle calibrated to deliver the solutions at 138 kPal and 6.3

km/hr. The order of treatments was started with the control (distilled water) and then with the

fungicides from the lowest to highest concentrations. Distilled water was used to rinse the

sprayer between fungicides. After applying the fungicides, one *R. solani* inoculated barley grain

was placed 1 cm to the side of each sugarbeet seed.

After inoculation, seeds and inocula were covered with LC1 mix and trays were placed

under greenhouse conditions at 20±2ºC (Argus Control Systems, Ltd.; British Columbia,

Canada), and irrigated as needed. The roots were washed carefully under tap water and evaluated

after 3 weeks using a 0 to 7 scale: 0 (no disease), 1 (crown area slightly scurfy), 2 (<5%

infection), 3 (6-25% infection), 4 (26-50% infection), 5 (51-75% infection), 6 (>75% infection),

and 7 (the root completely deteriorated) (Windels and Nabben-Schindler, 1996). To confirm that

the symptoms were caused by *R. solani* the fungus was re-isolated from infected plants by

plating small pieces of the infected roots on WA media. A three-way factorial randomized

complete block design (RCBD) with three replicates was used. Isolates, fungicides, and

concentrations were the factors. The non-parametric Kruskal-Wallis test was used to analyze the data as described by Shah and Madden (2004). The median value was calculated for each tray, and the respective mean rank for all isolates, fungicides, and concentrations was calculated using Proc Rank in SAS. Using the ranked disease severities relative effects, standard errors, and the confidence intervals were calculated for each treatment (isolates x fungicides x concentration) using longitudinal data- confidence interval (LD-CI) macro to compare between different treatments (Shah and Madden, 2004).

Rhizoctonia solani **fitness**. To determine if there was a fitness penalty for isolates with high EC_{50} values for azoxystrobin and trifloxystrobin fungicides, the radial growth rate was compared between *R. solani* isolates with high EC_{50} values and low EC_{50} values. Six isolates of each AG-2-2 subgroup were chosen of which 3 isolates had high EC_{50} values and 3 isolates had low EC_{50} values. Three millimeter diameter plugs were transferred from 4-day-old cultures to a half-strength PDA, and the plates were kept in the dark at room temperature ($22 \pm 2°C$) for 3 days. The daily radial growth rate was measured. Four replicates were used for each isolate, and the experiment was repeated once. Fitness experiments were done as a complete randomized design (CRD). The data were analyzed using repeated measures analysis of variance (Proc GLM) using SAS version 9.3. Because sphericity test was significant the adjusted univeriate test degree of freedom (Greenhouse-Geisser Epsilon) was used.

Results

Subgroups of *R. solani* AG-2-2. The two experiments were combined because the variances of the experiments were homogenous based on F-test, and because there was no significant interaction between the experiment and isolate. Before 1999, the majority (82%) of *R. solani* isolates was AG-2-2 IV, 14% were AG-2-2 IIIB, and 4% were AG-2-2 intermediate. In

the isolates collected from 2005 to 2012, the percentage of AG-2-2 IV isolates was reduced to 51%; and the percentage of AG-2-2 IIIB increased to 45%, and the percentage of intermediate (4%) remained the same.

In-vitro sensitivity of *R. solani* to azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole. The two experiments for each fungicide were combined based on the lack of significance for the experiment and the lack of significant interaction between the experiment and the isolate. Baseline isolates showed low EC_{50} values for pyraclostrobin, penthiopyrad, and prothioconazole and a wide range of EC_{50} values for azoxystrobin and trifloxystrobin. The mean EC_{50} values were 49.7, 97.1, 0.32, 0.2, and 0.9 µg ml^{-1}, and the ranges of EC_{50} values were 0.43-597.43, 0.14-823.54, 0.04-2.70, 0.04-2.27, and 0.11-2.40 µg ml^{-1} for azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole, respectively (Figure 2.1a). Although azoxystrobin, trifloxystrobin, and pyraclostrobin belong to the same FRAC fungicide group (QoI), isolates typically exhibited low EC_{50} values for pyraclostrobin, but showed a wide range of EC_{50} values for azoxystrobin and trifloxystrobin.

Isolates collected between 2005 and 2012 showed a similar trend in EC_{50} values as the baseline isolates. There was a wide range of EC_{50} values for azoxystrobin and trifloxystrobin, with means of 269.1 µg ml^{-1} and 341.7 µg ml^{-1}, respectively. Low EC_{50} values of 0.9, 0.2, and 0.6 µg ml^{-1} were found for pyraclostrobin, penthiopyrad, and prothioconazole, respectively. The ranges of EC_{50} values were, 0.18-876.58, 0.09-888.41, 0.02-6.43, 0.02-0.61, and 0.03-0.56 µg ml^{-1} for azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole, respectively (Figure 2.1b). No shift in mean EC_{50} values was observed for *R. solani* isolates tested for penthiopyrad and prothioconazole, but azoxystrobin, trifloxystrobin, and

pyraclostrobin resulted in increases in the mean EC_{50} values with a resistant factor of 6.0, 3.5, and 2.7, respectively. The frequency of isolates with EC_{50} values >10 µg ml^{-1} for azoxystrobin and trifloxystrobin increased in the non-baseline isolates by 30% (Figure 2.1).
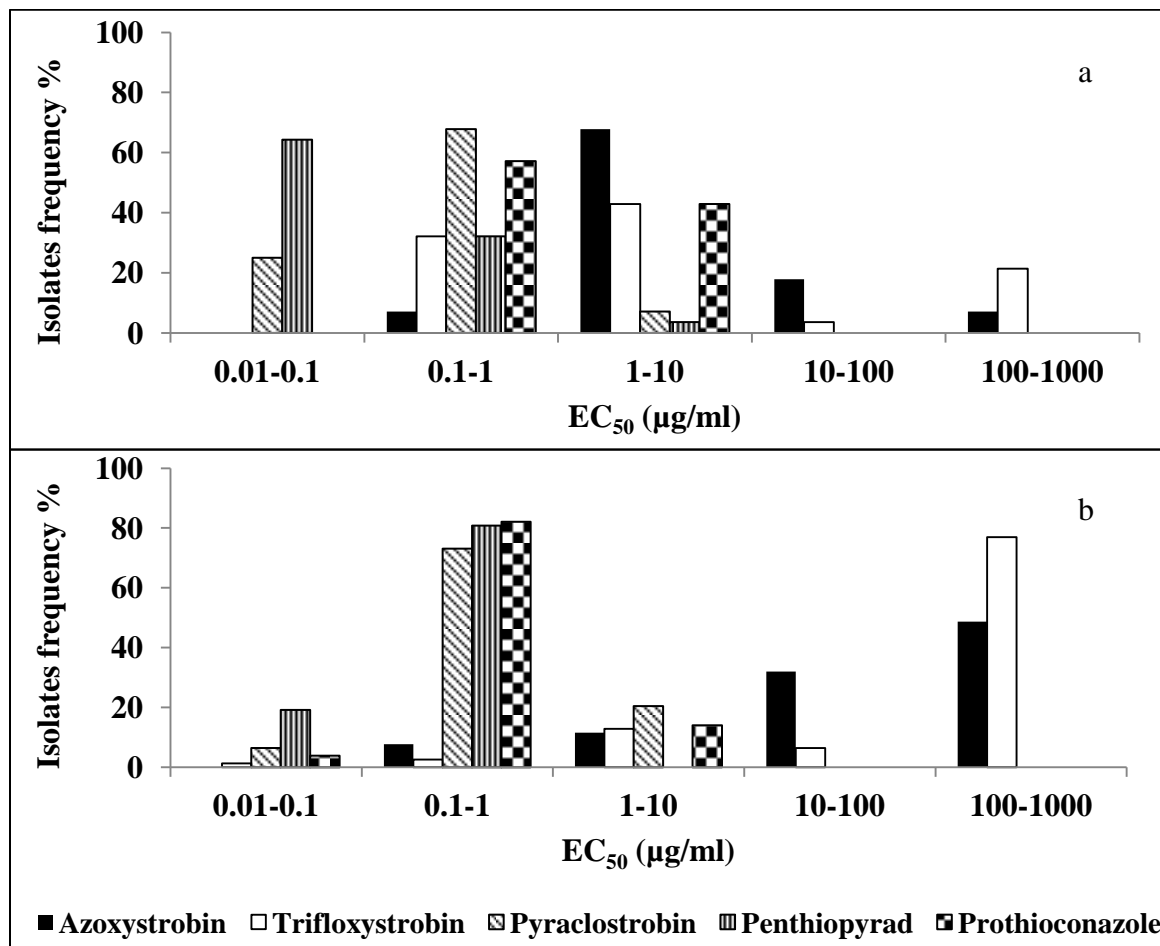
Figure 2.1. Frequency distribution of EC_{50} values of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole for a) 27 baseline isolates before 1999 and b) 78 non-baseline isolates from 2005 to 2012.

There was a significant positive correlation in baseline isolates between pyraclostrobin and both trifloxystrobin and penthiopyrad, and between trifloxystrobin and penthiopyrad. A negative correlation was found between prothioconazole and trifloxystrobin, prothioconazole and pyraclostrobin, prothioconazole and penthiopyrad, and between azoxystrobin and penthiopyrad (Table 2.5). In non-baseline isolates a sifnificant posistive correlation was between azoxystrobin

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and trifloxystrobin, azoxystrobin and pyraclostrobin, trifloxystrobin and pyraclostrobin, and

penthiopyrad and pyraclostrobin. Significant negative correlation was found between

pyraclostrobin and prothioconazole (Table 2.6). There was negative cross sensitivity in non-

baseline isolates between all fungicides that affect mitochondrial respiration and prothioconazole

which inhibits demethylase enzyme (DMI).

Table 2.5. Pearson correlation coefficient of 27 baseline isolates between EC_{50} values of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole. Numbers in parentheses refer to *P* value.

	Azoxystrobin	Trifloxystrobin	Pyraclostrobin	Penthiopyrad
Azoxystrobin				
Trifloxystrobin	0.31 (0.11)			
Pyraclostrobin	0.03 (0.87)	0.80 (<0.0001)[*]		
Penthiopyrad	-0.02 (0.90)	0.43 (0.02)[*]	0.71 (<0.0001)[*]	
Prothioconazole	0.06 (0.76)	-0.32 (0.09)	-0.35 (0.07)	-0.19 (0.31)

* Significant at $P \leq 0.05$

Table 2.6. Pearson correlation coefficient of 78 non-baseline isolates between EC_{50} values of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole. Numbers in parentheses refer to *P* value.

	Azoxystrobin	Trifloxystrobin	Pyraclostrobin	Penthiopyrad
Azoxystrobin				
Trifloxystrobin	0.65 (<0.0001)[*]			
Pyraclostrobin	0.30 (0.01)[*]	0.38 (<0.0006)[*]		
Penthiopyrad	0.06 (0.65)	0.15 (0.21)	0.35 (<0.002)[*]	
Prothioconazole	-0.07 (0.51)	-0.16 (0.17)	-0.24 (0.03)[*]	-0.05 (0.06)

* Significant at $P \leq 0.05$

Alternative oxidation respiration pathway. No significant difference was found

between the two experiments based on F-test. All five isolates showed lower azoxystrobin EC_{50}

values when SHAM was added to the media. Two isolates showed no significant difference for

the EC_{50} values between with SHAM and without SHAM, while three isolates showed a

significant difference for the EC_{50} values between with SHAM and without SHAM treatments.

The mean EC_{50} values for SHAM was significantly lower than the mean EC_{50} value without

SHAM (*P* 0.0001; Table 2.7).

Table 2.7. Comparison of azoxystrobin EC_{50} (effective concentration that inhibits mycelium growth by 50%) values of *Rhizoctonia solani* isolates without and with salicylhydroxamic acid (SHAM).

	EC_{50} (µg ml^{-1})		
Isolates	With SHAM	Without SHAM	p [a]
22-1	482.0	842.4	0.0010*
31-1	362.1	645.2	0.0631
393	453.9	864.1	0.0152*
68	568.7	857.1	0.0116*
946	444.4	604.0	0.2755
Mean	462.2	762.6	<0.0001*

[a] *P* value from the t-test was used for mean comparison for the individual isolates; the *P* value from an F-test was used for comparison of overall isolates EC_{50} means.
* Significantly different at $P \leq 0.05$.

Efficacy of different concentrations of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole at controlling *Rhizoctonia solani* in the greenhouse. To confirm the causal agent *R. solani* was re-isolated from the infected sugarbeet plants. In the two experiments, the main factors (isolates, fungicides, and fungicide concentrations) and all interactions were significant. In the control treatment (fungicide concentration 0 µg ml^{-1}), the *R. solani* isolates with high EC_{50} values showed higher disease severity compared with the isolates with low EC_{50} values. In the first experiment disease severity was significantly higher for the isolate with high EC_{50} value from AG-2-2 IV subgroup (Table 2.8), and in second experiment disease severity was significantly higher for the isolate with high EC_{50} value from AG-2-2 IIIB subgroup (Table 2.9).

All *R. solani* isolates were controlled by 10,000 µg ml^{-1}, and the REs were not significantly different from the non-inoculated control. At recommended labeled rates used (Table 2.2), *R. solani* isolates were controlled by all fungicides, and the disease severity was not significantly different from the non-inoculated control (Table 2.8, 2.9).

The low concentrations ≤10 µg ml^{-1} were not effective at controlling *R. solani* and the REs were significantly different from the non-inoculated control, except for isolate 31(AG-2-2

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IV; low EC_{50} value) which was controlled by azoxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole at concentrations ≤ 10 µg ml^{-1}.

Rhizoctonia solani **fitness**. The two experiments were combined because the experiment was not significant and because there was no significant interaction among the experiment and the subgroup, EC_{50}, and day. The main factors (subgroup, EC_{50}) were significant, and the two-way interactions were significant, too. The rate of mycelium radial growth was variable. AG-2-2 IIIB isolates with high EC50 values showed significantly higher rate of radial groth in day two. AG-2-2 IV isolates with high EC50 values showed significantly lower rate of growth in the day one and three. The mean growth rates overall all days were not significantly different between high and low EC_{50} values for the AG-2-2 IIIB subgroup while AG-2-2 IV isolates with high EC_{50} values had a significantly lower mean growth rate (Table 2.10).

Discussion

An alternative oxidation pathway helps fungi to overcome the inhibitory effect of QoI and SDHI fungicides in vitro, and to stop the alternative respiration pathway SHAM should be used (Ziogas et al., 1997). In this research, it was found that *R. solani* (AG-2-2 IIIB and AG-2-2 IV) uses the alternative respiration pathway to overcome the effect of QoI fungicide in vitro. These results were supported by other studies which also showed that *R. solani* uses alternative respiration pathway (LaMondia, 2012; Jin et al., 2009). Therefore, SHAM should be added to the media when evaluating *R. solani* sensitivity to QoI and SDHI fungicides in order to eliminate false high EC_{50} values.

Table 2.8. Efficacy of fungicides at recommended application rate at controlling *R. solani* isolates with low and high EC_{50} values for azoxystrobin and trifloxystrobin using sugarbeet susceptible cultivar crystal 539RR in the greenhouse.

Subgroup	Azoxystrobin EC_{50}	Trifloxystrobin EC_{50}	Isolate	Fungicide	Concentration µg ml^{-1}	Disease severity	Upper limit	Lower limit
AG-2-2 IIIB	3.52	5.85	850		0	0.77	0.86	0.64
AG-2-2 IIIB	3.52	5.85	850	Azoxystrobin	3000	0.19	0.20	0.18
AG-2-2 IIIB	3.52	5.85	850	Trifloxystrobin	2000	0.32	0.50	0.18
AG-2-2 IIIB	3.52	5.85	850	Pyraclostrobin	3000	0.19	0.20	0.18
AG-2-2 IIIB	3.52	5.85	850	Penthiopyrad	9000	0.19	0.20	0.18
AG-2-2 IIIB	3.52	5.85	850	Prothioconazole	3000	0.19	0.20	0.18
AG-2-2 IIIB	868.11	589.79	22-1		0	0.82	0.87	0.75
AG-2-2 IIIB	868.11	589.79	22-1	Azoxystrobin	3000	0.33	0.47	0.22
AG-2-2 IIIB	868.11	589.79	22-1	Trifloxystrobin	2000	0.58	0.76	0.38
AG-2-2 IIIB	868.11	589.79	22-1	Pyraclostrobin	3000	0.36	0.60	0.18
AG-2-2 IIIB	868.11	589.79	22-1	Penthiopyrad	9000	0.24	0.34	0.16
AG-2-2 IIIB	868.11	589.79	22-1	Prothioconazole	3000	0.29	0.44	0.18
AG-2-2 IV	0.43	2.70	60		0	0.52	0.65	0.38
AG-2-2 IV	0.43	2.70	60	Azoxystrobin	3000	0.23	0.31	0.16
AG-2-2 IV	0.43	2.70	60	Trifloxystrobin	2000	0.23	0.31	0.16
AG-2-2 IV	0.43	2.70	60	Pyraclostrobin	3000	0.19	0.20	0.18
AG-2-2 IV	0.43	2.70	60	Penthiopyrad	9000	0.19	0.20	0.18
AG-2-2 IV	0.43	2.70	60	Prothioconazole	3000	0.19	0.20	0.18
AG-2-2 IV	707.26	450.20	393		0	0.85	0.86	0.84
AG-2-2 IV	707.26	450.20	393	Azoxystrobin	3000	0.23	0.31	0.16
AG-2-2 IV	707.26	450.20	393	Trifloxystrobin	2000	0.30	0.45	0.18
AG-2-2 IV	707.26	450.20	393	Pyraclostrobin	3000	0.24	0.33	0.16
AG-2-2 IV	707.26	450.20	393	Penthiopyrad	9000	0.37	0.49	0.26
AG-2-2 IV	707.26	450.20	393	Prothioconazole	3000	0.23	0.31	0.16

Table 2.9. Efficacy of fungicides at recommended application rate at controlling *R. solani* isolates with low and high EC_{50} values for azoxystrobin and trifloxystrobin using a sugarbeet susceptible cultivar crystal 539RR in the greenhouse.

Subgroup	Azoxystrobin EC_{50}	Trifloxystrobin EC_{50}	Isolate	Fungicide	Concentration $\mu g\ ml^{-1}$	Disease severity	Upper limit	Lower limit
AG-2-2 IIIB	4.21	3.36	946		0	0.79	0.85	0.73
AG-2-2 IIIB	4.21	3.36	946	Azoxystrobin	3000	0.30	0.31	0.30
AG-2-2 IIIB	4.21	3.36	946	Trifloxystrobin	2000	0.30	0.31	0.30
AG-2-2 IIIB	4.21	3.36	946	Pyraclostrobin	3000	0.30	0.31	0.30
AG-2-2 IIIB	4.21	3.36	946	Penthiopyrad	9000	0.30	0.31	0.30
AG-2-2 IIIB	4.21	3.36	946	Prothioconazole	3000	0.30	0.31	0.30
AG-2-2 IIIB	876.58	876.63	571		0	0.92	0.93	0.91
AG-2-2 IIIB	876.58	876.63	571	Azoxystrobin	3000	0.30	0.31	0.30
AG-2-2 IIIB	876.58	876.63	571	Trifloxystrobin	2000	0.39	0.57	0.24
AG-2-2 IIIB	876.58	876.63	571	Pyraclostrobin	3000	0.45	0.63	0.28
AG-2-2 IIIB	876.58	876.63	571	Penthiopyrad	9000	0.30	0.31	0.30
AG-2-2 IIIB	876.58	876.63	571	Prothioconazole	3000	0.68	0.86	0.42
AG-2-2 IV	0.28	0.45	31-1		0	0.67	0.70	0.63
AG-2-2 IV	0.28	0.45	31-1	Azoxystrobin	3000	0.30	0.31	0.30
AG-2-2 IV	0.28	0.45	31-1	Trifloxystrobin	2000	0.30	0.31	0.30
AG-2-2 IV	0.28	0.45	31-1	Pyraclostrobin	3000	0.30	0.31	0.30
AG-2-2 IV	0.28	0.45	31-1	Penthiopyrad	9000	0.30	0.31	0.30
AG-2-2 IV	0.28	0.45	31-1	Prothioconazole	3000	0.30	0.31	0.30
AG-2-2 IV	830.42	888.41	40-2		0	0.69	0.87	0.42
AG-2-2 IV	830.42	888.41	40-2	Azoxystrobin	3000	0.30	0.31	0.30
AG-2-2 IV	830.42	888.41	40-2	Trifloxystrobin	2000	0.30	0.31	0.30
AG-2-2 IV	830.42	888.41	40-2	Pyraclostrobin	3000	0.30	0.31	0.30
AG-2-2 IV	830.42	888.41	40-2	Penthiopyrad	9000	0.30	0.31	0.30
AG-2-2 IV	830.42	888.41	40-2	Prothioconazole	3000	0.30	0.31	0.30

Table 2.10. Growth rate of *Rhizoctonia solani* AG-2-2 IIIB and AG-2-2 IV with high and low EC_{50} values of azoxystrobin and trifloxystrobin at days 1, 2, and 3.

EC_{50}	Growth Rate (mm/day)							
	Day 1		Day 2		Day 3		Mean growth rate	
	AG-2-2 IIIB	AG-2-2 IV	AG-2-2 IIIB	AG-2-2 IV	AG-2-2 IIIB	AG-2-2 IV	AG-2-2 IIIB	AG-2-2 IV
High	0.96	0.78	3.64	2.16	3.22	2.34	2.60	1.76
Low	1.22	1.36	2.92	2.19	3.38	3.51	2.50	2.35
P	0.06	0.01*	0.01*	0.92	0.17	0.004*	0.28	0.04

* Significantly different at $P \leq 0.05$.

Although pyraclostrobin belongs to QoI fungicides, all *R. solani* isolates showed low EC_{50} values for this fungicide compared to azoxystrobin and trifloxystrobin. *Rhizoctonia solani* isolates showed high mean EC_{50} values for azoxystrobin and trifloxystrobin which was consistent with other studies (Blazier and Conway, 2004; LaMondia, 2012). In contrast Jin et al. (2009) and Sundravadana et al. (2007) found that *R. solani* had low EC_{50} values, which could be because they evaluated one isolate whereas 105 isolates which were a good representation from the different growing areas were used in this study. The high EC_{50} values of azoxystrobin and trifloxystrobin can be explained by four theories 1) Azoxystrobin inhibits mycelium respiration at an early stage of mycelium growth; with time, expression of the cytochrome bc1 gene becomes stronger, and SHAM can not reduce oxygen consumption which makes *R. solani* insensitive to these fungicides as reported by Jin et al. (2009). 2) Mycelium growth of *R. solani* hardley depends on respiration (Jin et al., 2009). 3) Azoxystrobin may affect other metabolismic pathways in the fungi (Jin et al., 2009). 4) *Rhizoctonia solani* may use additional mechanism of alternate oxidation besides the alternative respiration pathway which is inhibited by SHAM (LaMondia, 2012). *Rhizoctonia solani* isolates showed shift in mean EC_{50} value for the QoI fungicides (azoxystrobin, trifloxystrobin, and pyraclostrobin). Azoxystrobin followed by pyraclostrobin are the most widely used fungicides to control *R. solani* in sugarbeet since 1999

with little or no rotation with other fungicide groups (Carlson et al., 2013), and thus high selection pressure on *R. solani* isolates have occurred. Trifloxystrobin was not used as the other QoIs for controlling *R. solani* (Khan M. F. R. personal communication) and the increase in its mean EC_{50} value could be due to the high positive cross sensitivity with azoxystrobin.

Although *R. solani* isolates had high EC_{50} for azoxystrobin and trifloxystrobin they were effectively controlled by these fungicides in the greenhouse indicating that high EC_{50} did not translate into resistance, as was reported by LaMondia (2012) and Jin et al. (2009). In contrast Olaya et al. (2012) found that *R. solani* from rice (AG-1-1A) developed resistance to azoxystrobin and the source of resistance was the F129L mutation. This could be due to the nature of *R. solani* growth in rice where the fungus can spread by growing from plant to plant across the surface of the water or by aerial hyphae which allowed for more hyphal fusions and more chance for isolates to develop resistance (Groth et al., 2014). Efficacy of azoxystrobin and trifloxystrobin in vivo but not in vitro could be explained by the fact that azoxystrobin and trifloxystrobin inhibit mycelium respiration at an early stage of mycelium growth and with time expression of the cytochrome bc1 gene becomes stronger, and SHAM can not reduce oxygen consumption which makes *R. solani* insensitive to these fungicides but in vivo the alternative oxidation pathway was not induced (Jin et al., 2009). For azoxystrobin to be effective, it should be used before the infection takes place (Stump et al., 2004) and before the soil temperature at a 10-cm depth reaches 18°C (Khan et al., 2005) which suggests that azoxystrobin may prevents sclerotia germination or early mycelium radial growth. The laboratory mycelium radial growth bioassay indicated that *R. solani* isolates with high QoI EC_{50} values were able to survive high rates of fungicides. Based on the greenhouse study, having high EC_{50} values did not translate into survival after exposure to fungicides at labeled rates. As such, isolates determined to have

high EC_{50} values should not be considered as resistant but should be evaluated in vivo using one of the recommended labeled rate as was recommended by Mitkowski et al. (2009).

Rhizoctonia solani isolates showed low EC_{50} values for penthiopyrad as reported for other members of the same chemical group (pyrzole-4-carboxamides) including penflufen, and sedaxane (Ajayi and Bradley, 2014). Penthiopyrad was first used on sugarbeet commercially in 2014 as a seed treatment (Kabina, Mitsui Chemical, Japan) for R. solani management. As such, since R. solani was not exposed to penthiopyrad, there was no shift in its mean EC_{50} value. In the greenhouse, penthiopyrad was effective at one of the recommended application rates for controlling R. solani. This study provides a baseline data for penthiopyrad which will be useful for future monitoring of R. solani so that strategies can be implemented to maintain sensitivity of the fungus to this fungicide.

Rhizoctonia solani isolates showed low EC_{50} values for prothioconazole similar to that reported by Ajayi and Bradley (2014). Other triazoles were found effective at reducing mycelium radial growth of R. solani including cyproconazole (Kataria et al., 1991), ipconazole (Ajayi and Bradley, 2014), triadimefon (Martin et al., 1984), and hexaconazole (Carling et al., 1990). It was not surprising that there was no increase in prothioconazole mean EC_{50} value. Although this product became available in 2006, it was not widely used for control of R. solani and was used only on 9% of the planted area in 2013 (Carlson et al., 2013). Low usage meant low selection pressure and coupled with its negative cross sensitivity with the widely used QoI fungicides were likely responsible for its low EC_{50} value. Recommended application rate of prothioconazole was effective at controlling R. solani in the greenhouse. Prothioconazole negative cross sensitivity with penthiopyrad and QoI fungicides makes it an excellent choice to be used for R. solani management to prevent or delay resistance development to QoI fungicides.

Pyraclostrobin, penthiopyrad, and prothioconazole were effective at low concentrations in vitro where the EC_{50} values were less than10 µg ml^{-1}, but in vivo, those concentrations were not effective. It is possible that these fungicides need to be in direct or close contact with *R. solani* mycelium to be effective. In the laboratory, the fungicides are well distributed in the media and in close contact with the fungus. In the greenhouse, pyraclostrobin, penthiopyrad, and prothioconazole at lower labeled rates could be tied-up the organic potting media and thus become ineffective.

There was no change in the rate of mycelium radial growth of AG-2-2 IIIB isolates with high EC_{50} values. AG-2-2 IV isolates with high EC_{50} values showed a decrease in the rate of mycelium radial growth compared with isolates with low EC_{50} values. It appears that mycelium growth of AG-2-2 IV was more sensitive than AG-2-2 IIIB, because at high temperature (35ºC) mycelium growth of AG-2-2 IV was completely stopped (Brantner and Windels, 2007; Sneh et al., 1991). In the greenhouse, *R. solani* isolates with high EC_{50} values for azoxystrobin and trifloxystrobin showed higher disease severity than isolates with low EC_{50} values. This increase in aggressiveness of *R. solani* isolates with high EC_{50} value is important because there was an increase in the frequency of those isolates through the years. With time, this fungus could develop resistance to QoI fungicides unless fungicide-resistant management strategies are applied. Fungicides from different FRAC groups such as SDHI or DMI fungicides should be rotated, or mixed, to delay or prevent QoI resistance development in *R. solani*.

In conclusion, *R. solani* isolates shifted to high EC_{50} values for QoI fungicides. However, in vivo, all *R. solani* isolates were controlled by all tested fungicides at one of the labeled rates. In areas where *R. solani* isolates had high EC_{50} values to QoI fungicides, the strategy of avoiding

use of QoI for a season or two, and using other modes of action to reduce QoI less sensitive

isolates can be effective.

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CHAPTER THREE. SENSITIVITY OF *APHANOMYCES COCHLIOIDES* TO TETRACONAZOLE, PROTHIOCONAZOLE, PYRACLOSTROBIN, AND HYMEXAZOL

Introduction

Aphanomyces cochlioides Drechsler, a fungal-like organism which belongs to Oomycetes. It is a soil borne pathogen which causes root rot and damping-off in sugarbeet and survives unfavorable conditions as oospores in the soil (Windels and Brantner, 2000; Windels and Nabben-Schindler, 1996). *Aphanomyces cochlioides* reproduces sexually by producing oospores and asexually by producing motile zoospores (Asher and Hanson, 2006). Warm temperature (16 to 35ºC) and wet soils are conducive for the development of Aphanomyces root rot disease (Windels and Engeleks, 1995; Windels and Nabben-Schindler, 1996). The typical symptoms for Aphanomyces damping-off are threadlike appearance of cotyledons and blackening of the roots, usually starting from the root tip moving upwards. Aphanomyces root rot symptoms are water-soaked black lesions on the root and on the stem near the soil surface, wilting of plants during warm and dry conditions and collapse of sugarbeet plants during severe infection when the tap roots are destroyed (Franc et al., 2001). Infection by *A. cochlioides* depends on sugarbeet cultivar, developmental stage of sugarbeet, zoospore concentration, and hymexazol treatment (Windels and Bratner 2000).

Aphanomyces root rot can be managed using Tachigaren® (Hymexazol 70% active ingredient (a.i.), Mitsui Chemicals Agro) treated seeds; early planting, improved drainage; and elimination of alternate hosts (Windels and Brantner, 2000; Windels and Nabben-Schindler, 1996). Use of spent lime (Calcium carbonate) was found to be effective against *A. cochlioides* by significantly reducing infection and increasing yield (Brantner et al., 2013; Olsson et al., 2011).

In the laboratory, biological control was found promising for some bacteria such as *Pseudomonas jessenii* (Deora et al., 2010) and *Lysobacter* sp. strain SB-K88 (Islam et al., 2005).

Few fungicides were found effective in vivo for controlling Oomycete pathogens because the fungicide targets are absent from these pathogens (Lee et al., 2008).The only fungicide used to control *A. cochlioides* is Tachigaren which had been used as a seed treatment since 1995 (Harveson et al., 2007). There are few studies conducted to evaluate the efficacy of fungicides to manage *A. cochlioides*. Cyazofamid fungicide affects respiration by inhibiting quinine inside inhibitor (QiI) (FRAC 21). This fungicide was found effective against Oomycetes, including *A. cochlioides*, and the EC_{50} value of cyazofamid was 0.2 µg ml^{-1} (Mitani et al., 2001).

Madoui et al. (2009) and Gaulin et al. (2010) found that *A. euteiches* has a cytochrome P450 sterol 14alpha-demethylase (*cyp51*) enzyme which is the target site for DMI fungicides. Further, in vitro study showed two such fungicides (DMI) were effective at reducing mycelium growth of *A. euteiches*. Pyraclostrobin was found effective in vitro and in field for members of Oomycetes including Phytophthora and Pythium (Kerns et al., 2009; Rebollar-Alviter et al., 2005; Rebollar-Alviter et al., 2007). These reports indicate the potential for DMIs and pyraclostrobin fungicides for controlling *A. cochlioides*.

The objectives of this study were 1) to determine the efficacy of tetraconazole, prothioconazole, and pyraclostrobin in reducing mycelium radial growth of *A. cochlioides* in vitro and to test the efficacy of these fungicides in the greenhouse, and 2) to determine the susceptible stages of sugarbeet plants to *A. cochlioides* for both hymexazol treated and non-treated seeds using seeds and 1 to 7 week old plants.

Materials and Methods

Aphanomyces cochlioides **isolates**. *Aphanomyces cochlioides* isolates were obtained

from University of Minnesota, Northwest Research and Outreach Center, Crookston, Minnesota

(Jason Brantner). These isolates were collected from Minnesota, North Dakota, and Texas. Fifty-

six isolates of *A. cochlioides* from sugarbeet fields were used in this study (Table 3.1).

Table 3.1. Year of collection, state of origin, and number of *Aphanomyces cochlioides* isolates
used in mycelium radial growth assay and in greenhouse studies.

Year	State	Number of isolates
1994	Minnesota	6
1997	North Dakota	14
1997	Texas	10
1997	Minnesota	12
2010	Minnesota	3
2011	Minnesota	7
2012	Minnesota	4
Total		56

Long term storage. For long term storage, *A. cochlioides* cultures free of contamination

were used. Fungal plugs were transferred to one edge of 10% PDA plates amended with

penicillin (50 mg/l). Before the mycelium reached the opposite edge of the plate, plugs from the

growing mycelium were transferred to the center of 20% water agar media (WA) plates. These

plates were kept at $20 \pm 2°C$. After growth of the mycelium, the cultures were cut to plugs and

transferred to two vials containing sterilized distilled water. Vials were kept in the dark at room

temperature (Windels, 2000).

In vitro sensitivity to fungicides. Mycelium radial growth assay was done according to

Mitani et al. (2001) with some modification. Using a cork borer, 5 mm mycelium plugs were cut

from 4-day old cultures. The plugs were placed inverted in fungicide amended and non-amended

10% PDA media. Plates were kept at room temperature ($20 \pm 2°C$) in the dark for 72 hours, and

then the average of two perpendicular diameters were calculated for each plate. The percentage

of mycelium growth reduction relative to the growth in the non-amended media was calculated [100 - (growth diameter in amended media / growth diameter in non-amended media) x 100)], and regressed against the fungicide concentrations logarithm, the concentration that causes 50% mycelium inhibition was determined by interpolation of the 50% intercept (Russell, 2004) using SAS version 9.3. (SAS Institute Inc.; Cary, NC, USA). The experiment was done twice with two replicates for each isolate and fungicide concentration.

Technical grades of prothioconazole (99.4% a.i., Bayer, Research Triangle Park, NC, USA), tetraconazole (98% a.i., Sipcam Agro USA Inc., GA, USA), pyraclostrobin (98% a.i., BASF, Research Triangle Park, NC, USA), and hymexazol (70% a.i., Mitsui Chemicals Agro, China) were used to prepare 100 mg ml^{-1} stock solution in acetone (EM Science, NJ, USA). Ten-fold serial dilutions were prepared to have 0, 0.01, 0.1, 1, and 10 mg ml^{-1} fungicide concentrations. One liter of 10% PDA media was amended with 1 ml of one of the fungicide concentrations to get final concentrations of 0, 0.01, 0.1, 1, and 10 µg ml^{-1}. Acetone concentration in media did not exceed 0.1% (Burrell and Corke, 1980). For reproducibility, WL405 isolate was used as a control and was tested for all fungicides in each experiment. If the mean EC$_{50}$ value of WL405 isolate did not fall within the confidence interval the experiment was repeated again (Wong and Wilcox, 2002). The experimental design was a complete randomized design (CRD) with two replicates for each fungicide concentration. The experiment was repeated once and the two experiments were tested for homogeneity of variance using F-test.

Susceptible stages of sugarbeet to *A. cochlioides*. The experiment was conducted in the Agricultural Experiment Station greenhouse at North Dakota State University in Fargo, ND, USA. Sugarbeet seeds, Crystal 539RR, susceptible to *A. cochlioides* (Niehaus 2011) treated and non-treated with hymexazol were used. The rate of hymexazol was 45g active ingredient

(a.i.)/100,000 seeds. Plastic trays 25x14x13 cm (T. O. Plastic Inc.; Clearwater, MN, USA) were filled with Sunshine Mix LC1 (73 to 83% Canadian sphagnum peat moss, perlite, and dolomite lime; Sun Gro Horticulture Distribution Inc.; Agawam, MA, USA). Fifteen sugarbeet seeds were planted per tray 2-cm deep at weekly intervals for seven weeks to have 1- to 7-week old plants and thinned to have 10 plants per tray. One to seven week old plants and seeds (10/tray) were inoculated using 500 µl of 100,000 zoospores/ml for each plant (Windels and Brantner, 1999). The zoospore suspension was placed in the soil near the hypocotyls of plants using a micropipette. After inoculation, trays with sugarbeet plants and seeds were placed in the greenhouse at 20±2ºC (Argus Control Systems, Ltd.; British Columbia, Canada) and were watered as needed. Sugarbeet plants were evaluated two weeks after inoculation using a 0 to 7 scale, where (0) was no disease, (1) crown area slightly scurfy, (2) <5% infection, (3) <25% infection, (4) 26 to 50% infection, (5) 51-75 % infection, (6) >75% infection, and (7) the root completely deteriorated (Windels and Nabben-Schindler, 1996). To confirm the causal agent of the symptoms in sugarbeet plants, the pathogen was re-isolated from infected plants by plating small pieces of infected roots on WA media.

Spores from WL405 isolate were prepared following the method published by Islam et al. (2007). The media that was used for zoospore production consisted of 17 g corn meal agar (CMA) (Sigma Aldrich, USA) and 4 g yeast extract (YE) (Becton, Dickinson and Company, USA) dissolved in 1 L of 50 mM phosphate buffer. *Aphanomyces cochlioides* plugs were transferred to the center of (CMA-YE) media and kept in the dark. After six days the media with mycelium was cut into 8 pieces, washed with distilled water three times and left in 40 ml autoclaved distilled water for 16 hrs. Zoospore suspension was filtered through sheet cloths, and spore concentration was determined using a hemacytometer (Islam et al., 2007). To prepare 1 L

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of 50 mM sodium phosphate puffer (7 PH), 30 ml of disodium phosphate (Na_2HPO_4; 1 M) and 19.5 ml of monosodium phosphate (NaH_2PO_4; 1 M) were used.

The experimental design was a complete randomized design with two factors sugarbeet stage (seed and 1- to 7-week old plants) and hymexazol (with and without hymexazol). The experiment was repeated once with 3 replicates per treatment. The data were analyzed using the non-parametric Kruskal-Wallis test. Disease severity median was calculated for each tray, and mean rank was calculated using Proc Rank with SAS. Using the ranked disease severities standard errors and the confidence intervals were calculated for each treatment using longitudinal data- confidence interval (LD-CI) macro to compare between different treatments (Shah and Madden, 2004).

Efficacy of tetraconazole, prothioconazole, pyraclostrobin, and hymexazol at controlling *A. cochlioides*. This experiment was conducted to determine the efficacy of tetraconazole 949.9 ml/ha (11.6% a.i., Eminent®, SIPCAM Agro USA Inc., GA, USA), prothioconazole 416.5 ml /ha (41% a.i.; Proline®, Bayer, Research Triangle Park, NC, USA), pyraclostrobin 672.3 ml/ha (23.6 % a.i.; Headline®, BASF, Research Triangle Park, NC, USA), and hymexazol (70% a.i. Tachigaren®, Mitsui Chemicals Agro, China) at controlling *A. cochlioides* under greenhouse conditions. The rates of tetraconazole, and prothioconazole were chosen based on the labeled rate for *Cercospora beticola* and *Erysiphe polygoni*, and pyraclostrobin were chosen based on the labeled rate for *Rhizoctonia solani* management in sugarbeet (Friskop et al., 2014).Tetraconazole, prothioconazole, and pyraclostrobin were applied before inoculation as an in-furrow application using a Generation III Research Sprayer (Devries Manufacturing Hollandale, MN), and hymexazol was applied as a seed treatment at 45 g a.i./100,000 seeds.

Efficacy of the fungicides was tested on sugarbeet plants inoculated at the seed and at 2-week old. Crystal 539RR, a sugarbeet cultivar susceptible to *A. cochlioides*, was used. For inoculating the seed stage, 10 seeds were planted in each tray and sprayed with the fungicides as described previously. Inoculation was done using 500 µl of zoospore concentration (100,000 zoospores/ml) placed on soil near each seed, and then the seeds were covered with LC1 mix. The trays were placed in the greenhouse at 20±2ºC (Argus Control Systems, Ltd.; British Columbia, Canada), and watered as needed. After 3 weeks, severity was evaluated using a scale from 0 to 7 (Windels and Nabben-Schindler, 1996). For the older plants, fifteen seeds were planted, and after germination were thinned to 10 plants per tray. Inoculation was done at the 2-week stage using the inoculation method described previously. Disease severity was evaluated after three weeks using a scale from 0 to 7 (Windels and Nabben-Schindler, 1996). The experimental design was a complete randomized design (CRD) with fungicides as treatment; the experiment was repeated once and three replicates for each treatment were used. The data was analyzed using the non-parametric analysis as previously described for the susceptible stage experiment. To confirm the causal agent of the symptoms in sugarbeet plants, the pathogen was re-isolated from infected plants by plating small pieces of infected roots on WA media.

Results

For sensitivity of *A. cochlioides* to fungicides, the two experiments were combined based on lack of significant effect of experiment and interaction between the experiment and the isolate. All tested fungicides inhibited mycelium radial growth in vitro. The mean EC_{50} values were 3.5, 2.4, 0.8, and 0.5 µg ml^{-1} for tetraconazole, prothioconazole, pyraclostrobin, and hymexazol, respectively. Frequency of isolates with EC_{50} values between 0.1 and 1 µg ml^{-1} were 66 % and 82 % for pyraclostrobin and hymexazol, respectively. Frequency of isolates with EC_{50}

values between 1 and 10 µg ml^{-1} were 98% for both tetraconazole and prothioconazole (Figure 3.1). Asignificant positive correlation was found between prothioconazole and both tetraconazole and pyraclostrobin (Table 3.2).

In greenhouse experiments, *Aphanomyces cochlioides* was re-isolated from the infected plants. For efficacy of tetraconazole, prothioconazole, pyraclostrobin, and hymexazol at controlling *A. cochlioides*, all tested fungicides were found significantly different from the non-inoculated control when sugarbeet plants were inoculated at the 2-week stage. Prothioconazole, pyraclostrobin, and hymexazol were not significantly different from each other, and the disease severities were 0.4, 0.4 and 0.5, respectively.
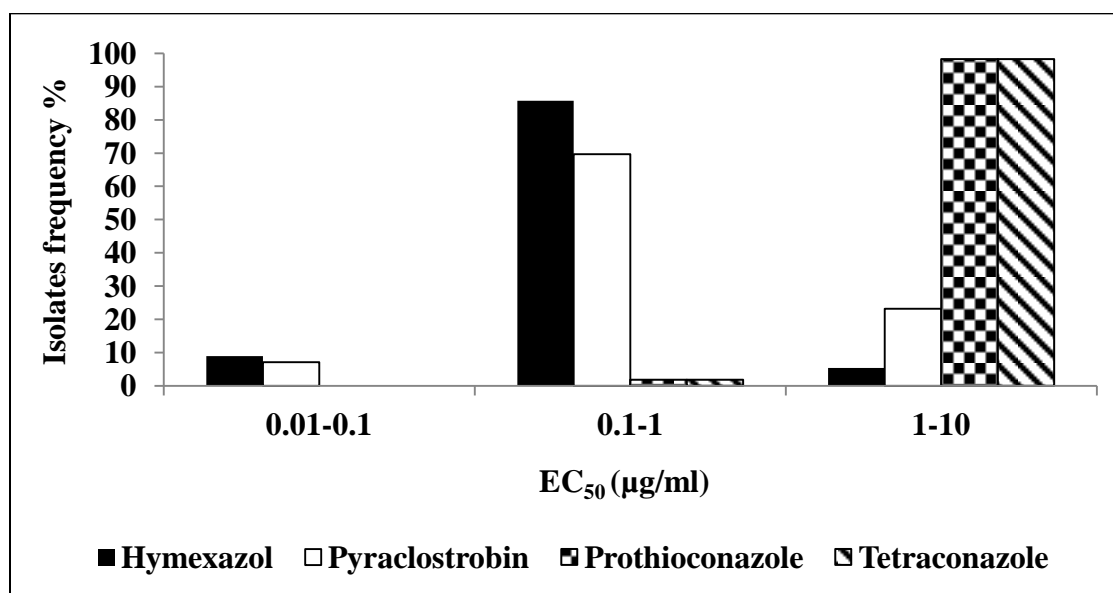
Figure 3.1 Frequency distribution of EC$_{50}$ of *Aphanomyces cochlioides* isolates of hymexazol, pyraclostrobin, prothioconazole, and tetraconazole using mycelium radial growth assay.

Table 3.2. Pearson correlation coefficient of *Aphanomyces cochlioides* isolates between EC_{50} values of hymexazol, tetraconazole, prothioconazole, and pyraclostrobin. Numbers in parentheses refer to *P* value.

	Hymexazol	Tetraconazole	Prothioconazole
Hymexazol			
Tetraconazole	0.003 (0.979)		
Prothioconazole	- 0.16 (0.25)	0.44 (<0.0008)[*]	
Pyraclostrobin	0.15 (0.26)	0.02 (0.86)	0.40 (0.002)[*]

[*] Significant at $P \leq 0.05$

Tetraconazole was not significantly different from the inoculated control (Figure 3.2a). When sugarbeet plants were inoculated at the seed stage, all tested fungicides were significantly different from the non-inoculated control. Sugarbeet plants showed the lowest disease severity when the plants were treated with hymexazol compared with other fungicides (Figure 3.2b). Tetraconazole, prothioconazole, and pyraclostrobin were not significantly different from each other (Figure 3.2b).

Seed stage was found susceptible to *A. cochlioides* when seeds were not treated with hymexazol (Figure 3.3). Seed stage treated with hymexazol was not infected by *A. cochlioides* after 2 weeks of inoculation, and the disease severity was not significantly different from the non-inoculated check (Figure 3.3). Sugarbeet plants inoculated at 1, 2, and 3 weeks were found susceptible to *A. cochlioides* for both hymexazol treated and non-treated seeds. Sugarbeet at 4-week and older stages were healthy and the disease severities were not significantly different from non-inoculated control (Figure 3.3).
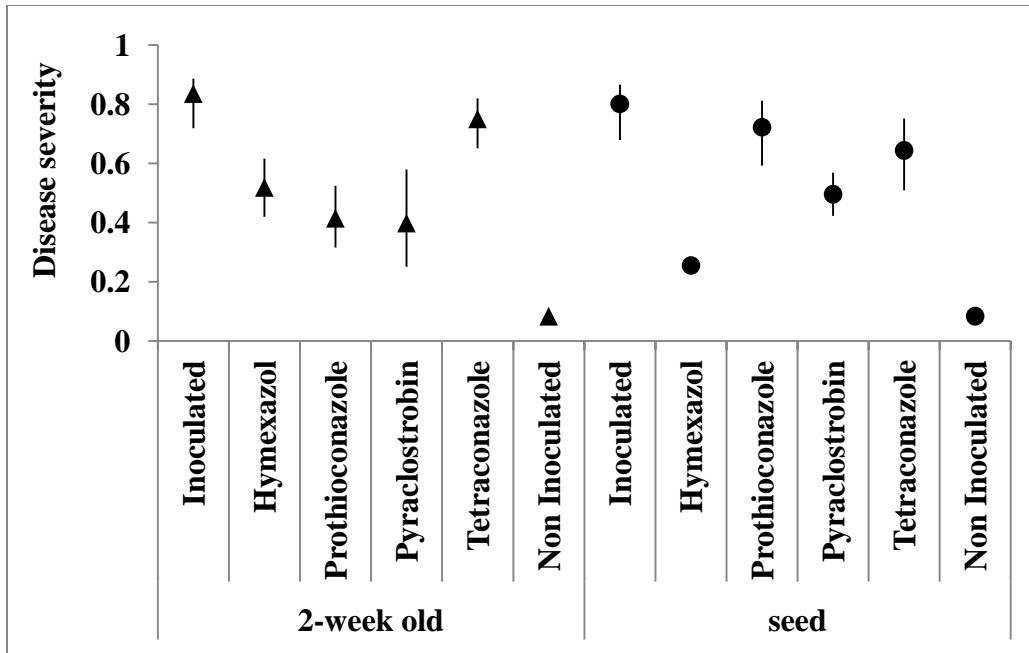
Figure 3.2. Efficacy of pyrclosttrobin (672.3 ml/ha), hymexazol, prothioconazole (416.5ml /ha), and tetraconazole (949.9ml/ha) in controlling *Aphanomyces cochlioides*. Sugarbeet plants (Crystal 539RR) were inoculated at a) 2 weeks old b) seed stage. The plants were inoculated with 500µl of 100,000 spores ml^{-1} zoospore concentration.
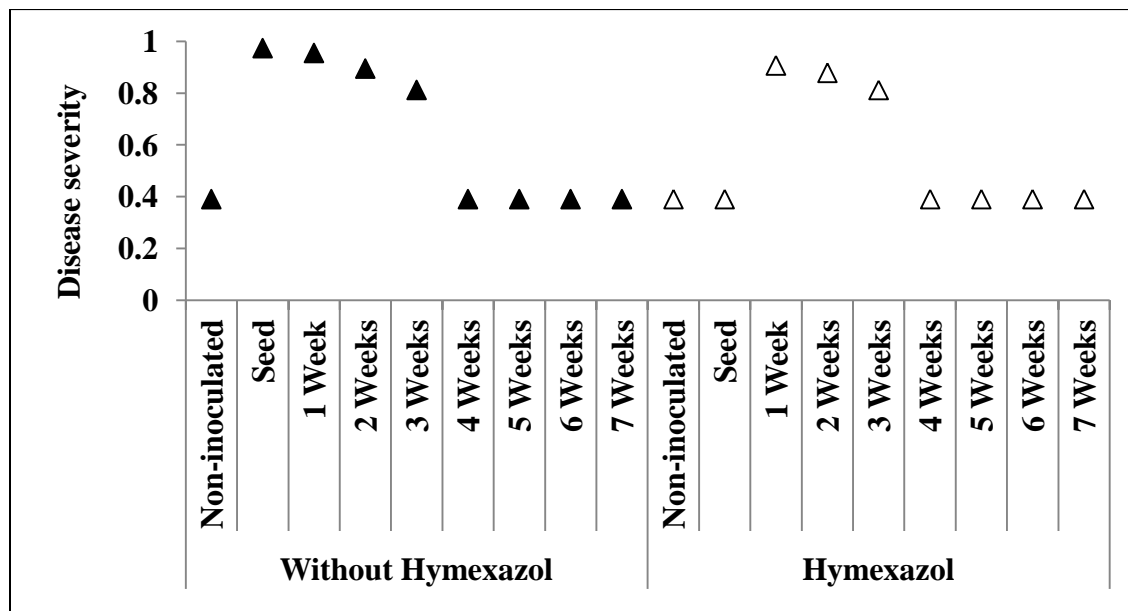
Figure 3.3. Susceptibility of sugarbeet plants (Crystal 539RR) at seed, 1, 2, 3, 4, 5, 6, and 7 weeks old with and without hymexazol to *Aphanomyces cochlioides*. The plants were inoculated with 500 µl of 100,000 spores ml^{-1} zoospore concentration.

Discussion

Tetraconazole, prothioconazole, pyraclostrobin, and hymexazol fungicides were able to reduce mycelium radial growth in vitro, but in greenhouse tetraconazole, prothioconazole, and pyraclostrobin, were not effective regardless if the inoculation was done at the seed stage or at the 2 week stage. Hymexazol was found ineffective at controlling *A. cochlioides* when sugarbeet plants were inoculated at 2 weeks old, but when the plants were inoculated at the seed stage, the relative effect was low and hymexazol was the most effective fungicide. Loss of hymexazol efficacy could be due to fungicide degradation or wash off during watering as reported in previous studies (Windels and Brantner, 2000). Harveson et al. (2007) found that hymexazol degraded with time and the percentage of degradation depended on soil temperature and moisture. After 7 days the percentage of degradation was found to be 3.3, 7.8, 15, and 25% at soil temperature of 15, 20, 25, and 30ºC, respectively (Harverson et al., 2007). We are not aware of any other studies that have evaluated tetraconazole, prothioconazole, and pyraclostrobin for controlling *A. cochlioides*.

Aphanomyces euteiches was found to have *cyp51* (Madoui et al., 2009; Gualin et al., 2010), and the product of this gene is the target site for triazoles. Triazoles were found effective in reducing mycelium radial growth of *A. eutichus* in vitro; there are no reports of in vivo testing. In this study, triazoles were also found to be effective at reducing mycelium radial growth in vitro. However, in the greenhouse, the triazoles were not effective, probably because of binding of these fungicides to organic potting materials used in this study or the high zoospores concentration used, or it could be the ability of this organism to metabolize exogenous sterol (Madoui et al., 2009).

Sugarbeet seeds non-treated with hymexazol were found susceptible to *A. cochlioides* at seed, 1, 2, and 3 week after planting; this result was supported by Windels and Brantner (2000). Sugarbeet seeds treated with hymexazol were found susceptible at 1-3 weeks old stage which could be due to the high spore concentration used or the fungicide washed off during watering. Windels and Brantner (2000) reported that under favorable environmental conditions and high zoospore concentration, sugarbeet plants will die within 2 weeks even if partial resistant sugarbeet seeds treated with hymexazol were used. In contrast, Haverson et al. (2007) found that under favorable conditions, hymexazol delayed the infection by *A. cochlioides* for 2 weeks after planting. At 4 week and older stages, sugarbeet plants became resistant and hymexazol had no role in protecting plants against *A. cochlioides.* This is consistent across all reports (Huijbregts et al., 1995). It is important that greenhouse condition, sugarbeet plant stage, and zoospores concentration be consistent when evaluating fungicide efficacy for *A. cochlioides*.

Since hymexazol provided control against *A. cochlioides* at the early stage of sugarbeet growth, and no other fungicides was found effective against *A. cochlioides* in the greenhouse to provide protection against late infection, partial resistant cultivars, planting early in the season, and using spent lime should be used to protect sugarbeet plants against *A. cochlioides*. Efforts should continue to evaluate other products for controlling *A. cochlioides* so that they can be used in rotation with hymexazol.

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CHAPTER FOUR. FITNESS OF TETRACONAZOLE-RESISTANT ISOLATES OF *CERCOSPORA BETICOLA* AFTER EXPOSURE TO DIFFERENT TEMPERATURE REGIMES

Introduction

Cercospora leaf spot (CLS) is one of the most destructive foliar diseases affecting sugarbeet (*Beta vulgaris* L.) (Skaracis et al., 2010; Weiland and Koch, 2004). It is caused by the hemibiotrophic fungus, *Cercospora beticola* Sacc. (Crous et al., 2001) which has no known sexual stage (Bolton et al., 2012c). *Cercospora beticola* overwinters as stromata and reproduces asexually by producing conidia throughout the growing season (Asher and Hanson, 2006). The favorable conditions for disease development are high temperatures from 25ºC to 35ºC during the day and above 18ºC during the night, as well as high relative humidity from 85 to 95% (Khan and Khan, 2010; Khan et al., 2008). Cercospora leaf spot is a polycyclic disease, and under favorable conditions, significant crop losses will occur. American Crystal Sugar Company estimated a loss to CLS in 1998 at $40 million (Ellington et al., 2001).

Crop rotation, use of resistant cultivars, and applying fungicides are the main practices used to manage CLS (Jacobsen, 2010; Secor et al., 2010a; Skaracis et al., 2010). Because CLS is a polycyclic disease, several fungicide applications are needed during the growing season to control the pathogen. In the USA, three to four applications may be needed during the growing season (Secor et al., 2010a). Several fungicides belonging to different Fungicide Resistant Action Committee (FRAC) groups have been registered to be used with sugarbeet for CLS management, including members of the dithiocarbamate (FRAC M3), benzimidazole (FRAC 1), triphenyltin hydroxide (TPTH; FRAC 30), demethylase inhibitor (DMI; FRAC 3), and quinone outside inhibitor (QoI; FRAC 11) groups (Friskop et al., 2014). Using fungicides from the same

FRAC group increases the risk of resistance development. FRAC recommends rotating or mixing fungicides from different FRAC groups to manage fungicide resistance (Brent and Hollomon, 2007).

Through the years, *C. beticola* developed resistance to several fungicides, including benzimidazole (Briere et al., 2001; Campbell et al., 1998; Davidson et al., 2006; Giannopolitis and Chrysayi-Tokousbalides, 1980; Rupel and Scott, 1974), TPTH (Briere et al., 2001; Bugbee, 1995, 1996; Giannopolitis and Chrysayi-Tokousbalides, 1980), triazoles (DMIs) (Karaoglanidis et al., 2000, 2002; Secor et al., 2010b), and QoI (Bolton et al., 2013; Kirk et al., 2012).

Resistance to triazoles could be due to single-site mutations (Wyand and Brown, 2005), overexpression of the *cyp51* gene (Bolton et al., 2012a; Schnabel and Jones, 2001) and energy-dependent drug efflux mechanisms (Nakaune et al., 1998; Palani and Lalithakumari, 1999). In *C. beticola* the source of resistance was the overexpression of cyp51 gene (Bolton et al., 2012a).

The fitness of resistant isolates plays an important role in developing resistance to fungicides for any fungal population (Peever and Milgroom, 1994). Several studies were conducted to study the fitness of DMI-resistant *C. beticola* isolates; some studies showed variations in *C. beticola* fitness between resistant and sensitive isolates while other research showed no variations. Fitness of *C. beticola* isolates was negatively affected by DMI resistance. Resistant *C. beticola* isolates were found to have less virulence, spore production (Karaoglanidis et al., 2001; Moretti et al., 2003), and mycelium radial growth (Moretti et al., 2003; Nikou et al., 2009). In other studies, resistant and sensitive isolates were similar in spore germination (Moretti et al., 2003; Karaoglanidis et al., 2001), mycelium growth, competitive ability, incubation period, germ tube length (Karaoglanidis et al., 2001), spore production (Nikou et al., 2009; Moretti et al., 2003), virulence (Nikou et al., 2009), and disease severity (Bolton et al., 2012b).

The stability of resistance to DMIs was found to be negatively influenced by environmental conditions and successive transfer. Overwintering adversely affected DMI-resistant isolates. *Cersospora beticola* isolates that were resistant to DMIs showed an increase in sensitivity after exposure to cold conditions (Karaoglanidis and Thanassoulopoulos, 2002). Also, DMI-resistant isolates were found to be less frequent than sensitive isolates at the beginning of one growing season compared to the end of the previous growing season, indicating that resistant isolates had less ability to survive the overwintering period or that they were weak competitors (Karaoglanidis et al., 2002). Other pathogens showed similar increased sensitivity for DMI-resistant isolates after exposure to cold conditions; *Monilinia fructicola* isolates showed increased sensitivity to DMI after exposure to 4ºC, 5ºC, and -20ºC (Cox et al., 2007; Zhu et al., 2012), and *Venturia inaequalis* isolates showed an increase in sensitivity after they were stored at 2ºC for 7 months (Koller et al., 1991). Successive transfer was found to have no effect on the stability of resistance to DMI for *C. beticola* (Karaoglanidis and Thanassoulopoulos, 2002), but with other fungi such as *V. inaequalis* and *M. fructicola*, successive transfer resulted in reverting resistant isolates back sensitive to DMI fungicides (Cox et al., 2007; Koller et al., 1991). It is not known what causes DMI resistant isolates to become unstable (Zhu et al., 2012).

Because of the long, severe cold season in North Dakota and Minnesota, this research was conducted to determine if there was a variation in spore production, spore germination, radial growth, sensitivity to tetraconazole, and disease severity of *C. beticola* isolates resistant to tetraconazole after exposure to different temperature regimes: -20ºC (4 weeks); 4ºC (4 weeks); 20ºC (4 weeks); -20ºC (2 weeks) to 4ºC (2 weeks); -20ºC (1 week) to 4ºC (1 week) to -20ºC (1 week) to 4ºC (1 week); and -20ºC (1 week) to 20ºC (1 week) to -20ºC (1 week) to 20ºC (1 week).

Information obtained from this study will be useful for management of fungicides used for *C. beticola* control.

Materials and Methods

To test if there was a fitness penalty for tetraconazole-resistant and sensitive *C. beticola* isolates after exposure to different temperature regimes, four isolates were chosen based on sensitivity to tetraconazole (Bolton et al., 2012a). Two isolates had very low EC_{50} values, and two isolates had high EC_{50} values (Table 4.1).

Table 4.1. Tetraconazole-resistant and -sensitive isolates of *Cercospora beticola* isolates that were used in fitness, sensitivity to tetraconazole, and greenhouse study after exposure to different temperature regimes.

Group[a]	Isolate	EC_{50} ($\mu g\ ml^{-1}$)
Very low EC_{50}	07-230	0.006
Very low EC_{50}	08-640	0.008
High EC_{50}	07-981	>1
High EC_{50}	09-347	>1

[a] Bolton et al., 2012a

Preparation of *C. beticola* inoculum. Spores of *C. beticola* were produced following the method reported by Secor and Rivera (2012). The isolates were transferred to clarified V8 medium (CV8) (15 g Agar, 100 ml CV8, and 900 ml dH_2o). After incubation at room temperature for 14 days two ml of tween-sterilized distilled water (1 L dH_2O, 20 µl Tween 20, and 200 mg Ampicillin) were added to the culture surface and scraped using a microscopic slide, then 500 µl of the scraped mycelium were transferred and spread on a fresh CV8 plate. The plates were left to dry in the fume hood and then placed under fluorescent light for 6 days. Then five ml of tween-sterilized distilled water was added to the culture surface and shaken gently to dislodge the spores. Spore concentration10,000 spores/ml was prepared using a hemacytometer. The percentage of spore germination was tested by taking 100 µl of spore concentration and placing it in water-agar media (WA) (15 g agar; 1 L dH_2O). The plates were incubated at room temperature under florescent light for 24 hours, and then, a total of 100 spores were counted

(germinated and nongerminated). The germination percentage was calculated using [%

germination=((germinated spores)/(germinated spores+nongerminated spores))x 100].

Sugarbeet plants. Three seeds of *C. beticola*-susceptible sugarbeet variety

(BTS89RR10) (Niehaus, 2011) were planted in 15-cm diameter plastic pots (T. O. Plastic, Inc.;

Clearwater, MN, USA) that were filled with sunshine potting mix LC1 (Sun Gro Horticulture

Distribution, Inc.; Agawam, MA, USA). After emergence, only one plant was kept per pot, and

the plants were fertilized using Osmocote 15-9-12. The pots were placed in the greenhouse with

a 16-hour photoperiod and an average day and night temperature of 24ºC and 16ºC, respectively.

The plants were watered as needed.

Inoculation. Sugarbeet plants were inoculated at the 4-leaf stage using a preval spray gun

(Preval, Coal City, IL, USA). The first three true leaves were sprayed with spores until runoff.

After inoculation, the pots were placed in the humid chambers with a misting controller (1626D,

Phytotronics, Inc.; Earth City, MO, USA) for 10 days. The plants were misted for 20 seconds

every 2 minutes in the first day and then for 10 seconds every 2 minutes for the rest 9 days, after

which they were moved to the greenhouse. For each isolate 18 plants were inoculated.

Temperature regimes. One month after plant inoculation, the three inoculated leaves

from each plant were excised and placed at different temperature regimes: -20ºC (4 weeks); 4ºC

(4 weeks); 20ºC (4 weeks); -20ºC (2 weeks) to 4ºC (2 weeks); -20ºC (1 week) to 4ºC (1 week) to

-20ºC (1 week) to 4ºC (1 week); and -20ºC (1 week) to 20ºC (1 week) to -20ºC (1 week) to 20ºC

(1 week). Three replicates were used for each regime. After one month, the leaves were placed in

humid chambers for 24 hours to induce sporulation. Spores were then collected by adding tween-

distilled water and pipetting the lesion on the leaves. Spores were cultured on WA media, and

after 24 hours, 3 germinated spores for each temperature regime were transferred to CV8 media and kept at 20±2ºC for 14 days (Secor and Rivera, 2012).

Fitness and sensitivity of *C. beticola* to tetraconazole. Agar plugs (5 mm) from 14-day-old cultures were transferred to CV8 plates and kept in the dark at room temperature (20±2ºC) for 14 days. Radial growth was then measured. For spore production, agar plugs (5 mm) from 14-day-old cultures were transferred to CV8 media and kept under fluorescent light at room temperature (20±2ºC). After 14 days, spores were dislodged from the culture surface by adding 2 ml of tween-distilled water. Spore concentrations were determined using a hemacytometer. The germination percentage was determined by placing 100 µl of spore suspension on WA media. After 24 hours, the number of germinated spores per 100 spores was recorded, and the germination percentage was calculated as previously described.

The sensitivity of *C. beticola* isolates to tetraconazole from different temperature regimes in addition to the original isolates was tested following the mycelium radial growth assay method of Secor and Rivera (2012). Technical grade of tetraconazole (98% active ingredient; Sipcam Agro USA Inc., GA, USA) was used to prepare 100 mg ml^{-1} stock solution in acetone (EM Science; Gibbstown, NJ, USA), a 10-fold serial dilution was used to have 0.01, 0.1, 1, and 10 mg ml^{-1} solutions. One liter of CV8 media was amended with 1 ml of those concentrations to have final concentrations of 0.01, 0.1, 1, 10, and 100 µg ml^{-1}. Only acetone was added to nonamended media (0 µg ml^{-1}). A 5-mm plug diameter from a 14-day-old culture was inverted in the middle of the CV8 plate and kept in the dark for 14 days at room temperature. The mean diameter for each plate was then calculated. The percentage of mycelium growth reduction relative to the growth in the non-amended media was calculated [100 - (growth diameter in amended media / growth diameter in non-amended media) x 100)], and regressed against the fungicide

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concentrations logarithm, the concentration that causes 50% mycelium inhibition was determined by interpolation of the 50% intercept (Russell, 2004) using SAS version 9.3 (SAS Institute, Inc.; Cary, NC, USA). The design for all experiments was a two-way factorial design within the isolate; the factors were temperature-regime replicates and the temperature regimes. Two replicates were used for each treatment. The experiment was repeated once. The F-test was used to test the homogeneity of variance for the two experiments and Tukey was used to separate between means at significant level of 0.05 using SAS version 9.3.

To measure disease severity, sugarbeet plants were inoculated and kept in humid chambers as previously described, then moved to the greenhouse. Disease severity was evaluated by counting the number of lesions on the inoculated leaves after 4 weeks. The number of lesions was transformed to a category from 1 to 10 using the rating scale published by Jones and Windels (1991) and Bolton et al. (2012b): category 1 (1-5 spots/leaf), category 2 (6-12 spots/leaf), category 3 (13-25 spots/leaf), category 4 (26-50 spots/leaf), category 5 (51-75 spots/leaf), category 6 (76-99 spots/leaf), category 7 (100-124 spots/leaf), category 8 (125-49 spots/leaf), category 9 (150-200 spots/leaf), and category 10 (> 200 spots/leaf). To confirm the causal agent of the symptoms in sugarbeet plants *C. beticola* was re-isolated from infected plants by collecting spores from the lesions and culturing on CV8 media. The non-parametric Kruskal-Wallis test was used to analyze the data. The median for each pot was calculated, and Proc Rank was used to calculate mean rank using SAS 9.3. Using the ranked disease severities standard errors and the confidence intervals were calculated for each treatment using longitudinal data-confidence interval (LD-CI) macro to compare between different treatments (Shah and Madden, 2004).

Results

For spore production, the two experiments were combined based on the F-test for homogeneity of variance and the lack of two- and three-way interactions among the experiment, temperature replicates, and temperature regimes. The temperature replicates and temperature-regime factors were not significant. There was no significant difference in spore production for all *C. beticola* isolates between the original isolate and all temperature regimes. No significant differences were found between the means of sensitive and resistant isolates for the untreated original isolates (Table 4.2).

Table 4.2. The number of spores produced by *Cercospora beticola* isolates from different temperature regimes after 14 days of incubation under florescent light at room temperature (20±2°C).

Temperature regimes °C[*]	Spore production (spores/ml)			
	Sensitive isolates		Resistant isolates	
	07-230	08-640	09-347	07-981
4	27833	28333	28417	29167
20	28750	29917	29250	30000
-20	28250	29167	29250	28333
-20 to 20 to -20 to 20	28667	28500	29167	28333
-20 to 4	28417	27917	28750	28500
-20 to 4 to -20 to 4	28583	28833	29667	28917
Original	27500	28667	28750	29417
Original mean	28084		29084	

No significant difference at $P \leq 0.05$.
[*] -20°C (4 weeks), 4°C (4 weeks), 20°C (4 weeks), -20°C (2 weeks) to 4°C (2 weeks), -20°C (1 week) to 4°C (1 week) to -20°C (1 week) to 4°C (1 week), and -20°C (1 week) to 20°C (1 week) to -20°C (1 week) to 20°C (1 week).

For spore germination, the two experiments were combined based on the F-test for variance homogeneity and the lack of two- and three-way interactions among the experiment, temperature replicates, and temperature regimes. The temperature replicates and temperature-regime factors were not significant. Spore germination was not significantly different between all treatments for each isolate (Table 4.3).

Table 4.3. Percentage of germinated spores of *Cercospora beticola* isolates from different temperature regimes after 24 hours of incubation under florescent light at room temperature (20±2°C).

Temperature regimes °C[*]	Spore germination (%)			
	Sensitive isolates		Resistant isolates	
	07-230	08-640	09-347	07-981
4	99.75	99.50	99.50	99.58
20	99.25	99.50	99.33	99.58
-20	99.58	99.75	99.50	99.83
-20 to 20 to -20 to 20	99.50	99.75	99.33	99.83
-20 to 4	99.50	99.42	99.50	99.83
-20 to 4 to -20 to 4	99.67	99.50	99.92	99.75
Original	100.00	99.92	99.92	99.92
Original mean	99.96		99.92	

No significant difference at $P \leq 0.05$.

[*] -20°C (4 weeks), 4°C (4 weeks), 20°C (4 weeks), -20°C (2 weeks) to 4°C (2 weeks), -20°C (1 week) to 4°C (1 week) to -20°C (1 week) to 4°C (1 week), and -20°C (1 week) to 20°C (1 week) to -20°C (1 week) to 20°C (1 week).

For all isolates, the two experiments were combined for the radial-growth experiment based on the lack of significance for the F-test for the homogeneity of variance and the lack of two- and three-way interactions among the experiment, temperature replicate, and temperature regimes. There were no significant differences among the three temperature replicates, and the temperature-regime factor was significant for all isolates. The radial growth varied among *C. beticola* isolates exposed to different temperature regimes and there was no fitness penalty for the resistant isolates after exposure to different temperature regimes. The original sensitive isolate 07-230 (no temperature treatment) showed significantly lower radial growth (3.65 cm) than those exposed to other temperature regimes. For the other sensitive isolate (08-640), the radial growth was 5.20 cm from the original culture, and it was significantly higher compared with isolates exposed to all temperature treatments. For isolate 07-230, the radial growth from the -20°C to 4°C regime was significantly higher than the other temperature regimes while, for the other sensitive isolate (08-640) (Table 4.4). Resistant isolates showed more variations in

mycelium radial growth after exposure to different temperature regimes. For resistant isolate 09-347, two treatments, -20ºC and -20ºC to 4ºC to -20ºC to 4ºC, resulted in significantly higher radial growth: 4.33 cm and 4.43 cm, respectively compared to all other treatments. For the other temperature regimes, the radial growth was not significantly different from each other and from the original (Table 4.4). For the 07-981 isolate, the highest radial growth (3.88 cm) was for the -20ºC to 4ºC regime which was not significantly different from original isolate growth, and growth at -20ºC to 4ºC to -20ºC to 4ºC (3.82 cm), 4ºC (3.76 cm), and -20ºC (3.75 cm) regimes (Table 4.4). The original resistant and sensitive isolates were compared to see if there was variation between the tetraconazole-resistant and sensitive isolates for mycelium radial growth. The mean mycelium growth of the resistant isolates was 3.45 cm which was significantly lower than the mean mycelium-growth of sensitive isolates which was 4.41 cm (Table 4.4).

Table 4.4. Mycelium radial growth of tetraconazole-sensitive and -resistant isolates of *Cercospora beticola* before and after exposure to different temperature regimes for one month.

| Temperature regimes ºC[*] | Radial growth (cm) | | | |
| | Sensitive isolates | | Resistant isolates | |
	07-230	08-640	09-347	07-981
20	$4.18^{bc¥}$	4.71^{b}	3.27^{b}	3.53^{bc}
4	4.14^{bc}	4.63^{bc}	3.19^{b}	3.76^{ab}
-20	4.10^{c}	4.80^{b}	4.33^{a}	3.75^{ab}
-20 to 20 to -20 to 20	4.25^{bc}	4.58^{bc}	3.22^{b}	3.49^{c}
-20 to 4	4.47^{a}	4.43^{c}	3.13^{b}	3.88^{a}
-20 to 4 to -20 to 4	4.28^{b}	4.63^{bc}	4.43^{a}	3.82^{a}
Original	3.65^{d}	5.18^{a}	3.24^{b}	3.65^{abc}
Original mean	$4.41^{A§}$		3.45^{B}	

¥ Numbers followed by same lowercase letter are not significantly different within the column at $P \leq 0.05$.

§ The mean for original isolates followed by the uppercase letter are not significantly different at $P \leq 0.05$.

* -20ºC (4 weeks), 4ºC (4 weeks), 20ºC (4 weeks), -20ºC (2 weeks) to 4ºC (2 weeks), -20ºC (1 week) to 4ºC (1 week) to -20ºC (1 week) to 4ºC (1 week), and -20ºC (1 week) to 20ºC (1 week) to -20ºC (1 week) to 20ºC (1 week).

For sensitivity of *C. beticola* to tetraconazole, the two experiments were combined for all isolates based on the lack of significance of the F-test for homogeneity of variance and the lack of two- and three-way interactions among the experiment, temperature replicate, and temperature regimes. The temperature-replicate factor was not significant for all isolates, and the temperature regime factor was significant for three isolates 08-640, 09-347, and 07-981. *Cercospora beticola* isolates sensitive to tetraconazole (07-230 and 08-640) remained sensitive after all temperature regimes, and the EC_{50} values of all temperature regimes were not significantly different from the original EC_{50} values (Table 4.5). For resistant isolate 09-347, the EC_{50} value of the original isolate was 8.72 µg ml^{-1}. The EC_{50} values decreased significantly at two temperature regimes, the -20ºC and -20ºC to 4ºC to -20ºC to 4ºC, which had EC_{50} values of 0.22 µg ml^{-1} and 0.26 µg ml^{-1}, respectively. The FC was 38.6 for the -20ºC regime and 32.8 for the -20ºC to 4ºC to -20ºC to 4ºC regime. For the other temperature regimes, the EC_{50} values remained high; even the isolates from those regimes showed significant differences from the original isolate. The original resistant isolate, 07-981, showed an EC_{50} value of 16.22 µg ml^{-1}. All isolates from all temperature regimes had an FC of 1-1.3, except for the isolate from the -20ºC to 20ºC to -20ºC to 20ºC regime which had an FC of 0.85. The lowest EC_{50} values were 12.2 µg ml^{-1} for -20ºC to 4ºC to -20ºC to 4ºC and 13.56 µg ml^{-1} for -20ºC to 4ºC, which were significantly different from the EC_{50} value of the original isolate. The -20ºC to 20ºC to -20ºC to 20ºC regime showed a significantly higher EC_{50} value compared with the original EC_{50} value (Table 4.5).

Table 4.5. EC$_{50}$ values of *Cercospora beticola* isolates that were resistant and sensitive to tetraconazole before and after exposure to different temperature regimes.

Temperature regimes °C[#]	EC$_{50}$ (µg ml^{-1})							
	Sensitive isolates				Resistant isolates			
	07-230	FC[*]	08-640	FC	09-347	FC	07-981	FC
20	0.008$^{a¥}$	0.86	0.007^{ab}	1.11	10.26^{a}	0.84	15.36^{bc}	1.06
4	0.008^{a}	0.87	0.007^{b}	1.15	9.10^{b}	0.95	14.04^{bcd}	1.16
-20	0.008^{a}	0.83	0.009^{a}	0.94	0.22^{d}	38.64	15.43^{bc}	1.05
-20 to 20 to -20 to 20	0.008^{a}	0.86	0.008^{ab}	1.04	8.61^{bc}	1.00	19.15^{a}	0.85
-20 to 4	0.008^{a}	0.84	0.008^{ab}	0.96	7.83^{c}	1.10	13.59^{cd}	1.19
-20 to 4 to -20 to 4	0.008^{a}	0.89	0.007^{ab}	1.11	0.26^{d}	32.84	12.20^{d}	1.33
Original	0.007^{a}		0.008^{ab}		8.64^{bc}		16.22^{b}	

$^{¥}$ Numbers followed by same letter are not significantly different within a column at $P \leq 0.05$.
* Factor of change= EC$_{50}$ value of original isolate / EC$_{50}$ value of isolates from different temperature regime.
$^{#}$ -20ºC (4 weeks), 4ºC (4 weeks), 20ºC (4 weeks), -20ºC (2 weeks) to 4ºC (2 weeks), -20ºC (1 week) to 4ºC (1 week) to -20ºC (1 week) to 4ºC (1 week), and -20ºC (1 week) to 20ºC (1 week) to -20ºC (1 week) to 20ºC (1 week).

To confirm that the symptoms on sugarbeet plant were caused by *C. beticola*, the fungus was re-isolated from infected plants. For disease severity experiments, the temperature-regime factor was significant for the 07-230, 08-640, and 07-347 isolates, but not for 07-981 isolate. The temperature replicate and the interaction between the temperature regimes and the temperature replicates were not significant. In general, there were variations among disease severities for the isolates exposed to different temperature regimes. Sensitive isolates (07-230 and 08-640) exposed to the -20ºC to 4ºC to -20ºC to 4ºC regime caused a significant increase in disease severity compared to the original disease severity (Table 4.6). For sensitive isolate 07-230, -20ºC to 4ºC regimes had disease severity significantly higher than the original isolate with RE of 0.56 (Table 4.6). For the 09-347 resistant isolate, all temperature regimes had REs that were not significantly different than the original isolate, except for 20ºC which had an RE that was significantly lower than the original isolate (0.32; Table 4.6). For 07-981, the REs for all treatments were not significantly different than the original isolate (Table 4.6).

Discussion

The fitness of resistant isolates is the main factor for the development and evolution of pathogen resistance to fungicides (Peever and Milgroom, 1994). If the resistant isolates are more fit than the sensitive isolates in the absence of a fungicide, then the frequency of resistant isolates will increase, and with time, the fungicide may become ineffective. The fitness of resistant isolates is not only affected by genetic traits, but also by environmental conditions (Antonovics and Alexander, 1989). Cold temperatures have adversely affected DMI-resistant isolates of several fungi, such as *C. beticola* (Karaoglanidis and Thanassoulopoulos, 2002; Karaoglanidis et al., 2002), *M. fructicola* (Cox et al., 2007; Zhu et al., 2012), and *V. inaequalis* (Koller et al., 1991).

After exposure to different temperature regimes, the sensitivity of *C. beticola* to tetraconazole was stable for sensitive isolates. Although there were significant variations among the temperature regimes, the EC_{50} values were classified as very low, and the isolates were considered to be sensitive to tetraconazole. Similar stability of sensitive isolates was found in other studies. Karaoglanidis and Thanassoulopoulos (2002) found that sensitive isolates of *C. betcola* were not affected by cold temperature, and the factors of change ranged from 0.9 to 1.1 for isolates from the mycelium that were exposed to 3ºC for 5 and 10 months and from 0.8 to 1.4 for the isolates from the conidia that were exposed to 3ºC for 3 and 6 months. Koller et al. (1991) also found that sensitive isolates of *V. inaequalis* maintained their sensitivity to flusilazole after they were stored at 2ºC for 7 months.

Table 4.6. Effect of temperature regimes on disease severity caused by four known *Cercopsora beticola* isolates

Isolate	Temperature regimes (ºC)[*]	Median Disease rank	Disease severity	95% CI of the disease severity[a]	
				Lower limit	Upper limit
07-230	Original	4.0	0.32	0.21	0.45
	4	4.7	0.52	0.40	0.64
	20	4.8	0.50	0.40	0.61
	-20 to 20 to-20 to 20	4.4	0.41	0.30	0.54
	-20 to4	4.9	0.56	0.47	0.64
	-20 to 4 to -20 to 4	5.0	0.67	0.58	0.75
	-20	4.8	0.52	0.39	0.64
08-640	Original	4.0	0.48	0.39	0.57
	4	4.5	0.38	0.32	0.44
	20	5.0	0.62	0.50	0.72
	-20 to 20 to-20 to 20	4.0	0.49	0.38	0.60
	-20 to4	5.0	0.47	0.34	0.61
	-20 to 4 to -20 to 4	5.0	0.73	0.63	0.80
	-20	5.0	0.34	0.27	0.41
07-981	Original	6.0	0.52	0.42	0.62
	4	5.5	0.39	0.28	0.52
	20	6.0	0.52	0.38	0.66
	-20 to 20 to-20 to 20	6.0	0.47	0.37	0.58
	-20 to4	6.0	0.47	0.37	0.58
	-20 to 4 to -20 to 4	6.0	0.57	0.48	0.66
	-20	6.0	0.55	0.45	0.64
09-347	Original	4.5	0.59	0.47	0.70
	4	5.0	0.66	0.51	0.77
	20	4.0	0.33	0.23	0.46
	-20 to 20 to-20 to 20	4.0	0.52	0.43	0.61
	-20 to4	4.0	0.38	0.29	0.49
	-20 to 4 to -20 to 4	4.5	0.54	0.45	0.63
	-20	4.0	0.47	0.39	0.57

[a] 95% confidence intervals of disease severity
[#] -20ºC (4 weeks), 4ºC (4 weeks), 20ºC (4 weeks), -20ºC (2 weeks) to 4ºC (2 weeks), -20ºC (1 week) to 4ºC (1 week) to -20ºC (1 week) to 4ºC (1 week), and -20ºC (1 week) to 20ºC (1 week) to -20ºC (1 week) to 20ºC (1 week).

Resistance to DMIs was unstable after exposing resistant isolates to cold treatments in *C. beticola* (Karaoglanidis et al., 2002; Karaoglanidis and Thanassoulopoulos, 2002), *V. ineaqualis* (Koller et al., 1991), and *M. fructicola* (Cox et al., 2007, Zhu et al., 2012). The instability of resistant isolates was also found in this study where two treatments (-20ºC and -20ºC to 4ºC to -

20ºC to 4ºC) adversely affected the 09-347 isolate and resulted in an increase in sensitivity to tetraconazole. For the other resistant isolate (07-981), there was a decrease in the EC_{50} values for five of the six regimes, and the highest FC was 1.3 for the -20ºC to 4ºC to -20ºC to 4ºC regime. The 07-981 isolate had an original EC_{50} value that was higher than the EC_{50} value for 09-347 which may have contributed to the variation in their responses to different temperature regimes. The variations in the instability among resistant isolates were also reported by Koller et al. (1991) who found that isolates with higher EC_{50} values were more stable than isolates with lower EC_{50} values. In this study, the isolates were kept just for one month at different temperature regimes which might not have been enough to cause a pronounced decrease in the EC_{50} values as occurred in other studies where different fungal pathogens were incubated for 3 months and longer (Karaoglanidis and Thanassoulopoulos, 2002; Koller et al., 1991). Cox et al. (2007) found that in *M. fructicola* the percentage of growth inhibition at the discriminatory dose of 0.3 µg ml^{-1} increased by 165% after 8 months of incubation at 5ºC and by 273% after 34 months at the same temperature.

The instability of resistant isolates was reported for other fungicides. Resistance of *C. beticola* to TPTH was found unstable in North Dakota and Minnesota, and the resistant isolates reverted to sensitive again. The instability was explained by the reduction in the TPTH use, exposure to different mode of action including QoI and DMI which were used in most areas instead of TPTH, the lack of fitness of resistant isolate, and the inability of resistant isolates to survive the adverse winter conditions (Secor et al., 2010b), but no study was done to determine if the reversion back to sensitivity to TPTH was as a result of cold conditions. Metalaxyl-resistant isolates of *Phytophthora infestans* were found less frequently than sensitive isolates at the beginning of the growing season, which was explained by the adverse effect of overwintering on

the survival of fungus. This adverse effect of overwintering on the *P. infestans* survival was confirmed in the laboratory by exposing resistant and sensitive isolates to cold temperature and looking to fitness parameters which showed that resistant isolates did not survive the cold temperature (Kadish and Cohen, 1992). In contrast, our experiment showed that *C. beticola* resistant isolates had the same level of survivability as sensitive isolates and that the cold treatment had adverse effects on the stability of sensitivity to tetraconazole.

The mechanism by which DMI-resistant isolates revert to sensitive again after cold treatments is unknown (Zhu et al., 2012). In propiconazole-resistant isolates of *M. fructicola*, Mona element (a unique sequence found upstream of cyp51 gene and triggers the overexpression of this gene in resistant isolates) was also found from those resistant isolates that reverted back to sensitive again after exposure to cold treatments (Zhu et al., 2012). In *C. beticola*, overexpression of *cyp51* gene resulted in resistance to DMI fungicides, so to know what caused the increase in sensitivity of resistant isolates the overexpression level of *C. beticola* isolates before and after exposure to cold treatment.

The instability of sensitivity to tetraconazole after cold treatments in *C. beticola* is important because in North Dakota and Minnesota the long, cold winter season could have adverse effects on the resistant isolates. It will be useful to sample sugarbeet fields for *C. beticola* early and late in the season to determine if the cold winter impacts the frequency of tetraconazole-resistant isolates.

There were no significant differences in spore production and spore germination between the original "non-treated" resistant and sensitive isolates. Similar results were reported for *C. beticola* (Moretti et al., 2003; Nikou et al., 2009) and other pathogens including *M. fructicola* (Cox et al., 2007) and *Pyrenophora teres* (Peever and Milgroom, 1994). However, Karaoglanidis

et al. (2001) found that sensitive *C. beticola* isolates had significantly higher spore production compared to resistant isolates which could be due to their evaluation of sporulation in vivo and not in vitro as was done in this study. Mycelium radial growth varied between individual isolates; resistant isolates had the same or lower mycelium radial growth compared to sensitive isolates. Karaoglanidis et al. (2001) found similar variation in mycelium radial growth of *C. beticola*. However, for the mean mycelium radial growth of the original isolates, the resistant isolates had significantly lower radial growth compared to sensitive isolates, which was also found by Moretti et al. (2003). In contrast Nikou et al. (2009) and Karaoglanidis et al. (2001) found that mean mycelium radial growth was not significantly different between resistant and sensitive isolates which could be due to the difference in research methodologies.

All temperature regimes had no effect on spore production and spore germination of both tetraconazole-resistant and -sensitive isolates. However, temperature regimes had different effect on mycelium radial growth of tetraconazole-resistant and -sensitive isolates. The most pronounced effect was on the resistant 09-346 isolate where two regimes (-20ºC and -20ºC to 4ºC to -20ºC to 4ºC) resulted in significantly higher radial growth than the original isolate, and after exposure to those two regimes resistant isolate reverted to moderately resistant level.

In the greenhouse, all isolates after exposure to different temperature regimes were able to cause disease symptoms on sugarbeet plants. The temperature regimes effects varied among the isolates, and no fitness penalty was found in resistant isolates after exposure to different temperature regimes. No previous studies were done to compare the fitness of DMI-resistant isolates before and after exposing them to different temperature regimes for any fungal pathogen.

Cercospora beticola isolates resistant to tetraconazole had no fitness penalty for mycelium radial growth, spore production, spore germination, and disease severity after

exposure to cold temperatures. However, resistance to tetraconazole was unstable, and the cold winter in North Dakota and Minnesota may have adverse effects on DMI-resistant isolates which could have an important role in fungicide resistance management. Even though isolates with resistance to DMIs are adversely affected by cold temperatures, some resistant isolates may still survive. Based on these results, if this phenomenon occurs in the field, it may be prudent to not use DMI fungicides early in the disease season, and use other chemistries with the aim of significantly reducing the population of DMI-resistant isolates so as to prolong the usefulness of DMI fungicides for controlling *C. beticola*.

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Isolate	Year	State	Subgroup	EC_{50} ($\mu g\ ml^{-1}$)				
				Azoxystrobin	Trifloxystrobin	Pyraclostrobin	Penthiopyrad	Prothioconazole
7	1988	ND	IV	72.51	6.52	0.06	0.04	1.31
8	1988	ND	IV	4.09	3.29	0.13	0.06	0.45
9	1988	ND	IV	7.04	0.62	0.10	0.07	0.35
10	1988	ND	IV	5.35	2.31	0.11	0.15	0.57
11	1988	MN	IV	3.54	7.89	0.15	0.05	0.49
12	1988	MN	IV	2.34	0.33	0.06	0.09	0.66
18	1988	MN	IV	42.02	0.72	0.16	0.09	2.03
23	1986	MN	IV	2.50	0.21	0.07	0.04	0.43
24	1986	MN	INT	75.33	823.54	2.07	0.29	0.25
25	1986	MN	IV	5.07	0.75	0.31	0.14	0.24
26	1986	MN	IV	5.14	332.21	0.31	0.10	1.11
27	1986	MN	IV	0.47	171.08	0.60	0.13	0.47
29	1987	MN	IV	1.73	0.14	0.04	0.13	1.65
30	1987	MN	IV	1.08	4.10	0.05	0.05	1.80
31	1987	ND	IV	5.20	3.81	0.21	0.05	0.44
35	1987	ND	IIIB	8.88	461.00	1.92	2.27	1.78
39	1987	ND	IV	9.47	3.88	0.17	0.06	0.51
40	1987	ND	IV	6.03	4.06	0.17	0.12	1.42
41	1988	MN	IV	4.55	0.76	0.08	0.09	1.08
49	1989	MN	IV	597.43	459.21	0.19	0.13	0.05
59	1988	MN	IV	1.09	0.52	0.44	0.06	0.91
60	1988	MN	IV	0.43	2.70	0.09	0.07	0.99
68	1983	MN	IIIB	7.46	13.52	0.26	0.08	0.34
69	1993	MN	IIIB	58.97	399.11	0.34	0.09	0.11
70	1993	MN	IV	3.76	0.60	0.12	0.05	1.26
71	1993	MN	IV	15.20	6.16	0.18	0.04	0.75

Isolate	Year	State	Subgroup	EC$_{50}$ (µg ml^{-1})				
				Azoxystrobin	Trifloxystrobin	Pyraclostrobin	Penthiopyrad	Prothioconazole
72	1993	MN	IV	4.50	4.38	0.12	0.07	1.44
106	2005	ND	IV	316.73	685.77	0.13	0.08	0.42
186	2005	MN	IV	30.73	323.13	0.07	0.17	0.52
195	2005	ND	IV	72.08	508.66	0.56	0.04	1.10
200	2005	ND	IV	310.83	427.43	0.30	0.04	0.43
253	2006	ND	IV	29.57	351.55	0.15	0.06	0.29
255	2006	ND	IIIB	806.79	605.36	0.15	0.17	0.47
258	2006	MN	IV	647.17	599.08	0.66	0.23	0.17
286	2006	MN	IIIB	368.83	688.11	0.75	0.13	0.80
296	2006	MN	IV	22.75	54.68	0.14	0.18	1.10
300	2006	MN	IV	666.84	303.72	0.39	0.02	0.53
315	2006	MN	IV	674.96	372.09	0.35	0.26	0.35
331	2006	MN	IIIB	564.65	612.28	0.29	0.61	0.50
385	2006	MN	IIIB	706.02	386.70	2.52	0.17	0.43
393	2006	ND	IV	707.26	450.20	0.29	0.17	0.40
407	2006	MN	IV	286.85	318.95	4.42	0.55	0.26
413	2006	MN	IV	619.48	446.07	0.10	0.03	2.22
424	2006	MN	IIIB	637.28	734.65	4.12	0.17	0.24
470	2006	ND	IIIB	141.29	341.18	4.98	0.45	0.22
481	2006	ND	IV	2.13	379.37	1.37	0.12	1.07
496	2006	MN	IV	153.13	167.63	0.46	0.07	1.07
542	2006	ND	IV	600.30	365.88	0.79	0.10	0.91
571	2006	MN	IIIB	876.58	876.63	5.28	0.34	0.18
588	2006	MN	IV	678.59	462.21	2.51	0.21	0.56
599	2006	MN	IV	176.84	4.74	0.17	0.22	0.70
776	2007	MN	IIIB	68.05	632.04	0.80	0.33	0.35
780	2007	MN	IIIB	115.21	500.82	1.49	0.21	0.15

Isolate	Year	State	Subgroup	EC$_{50}$ (µg ml^{-1})				
				Azoxystrobin	Trifloxystrobin	Pyraclostrobin	Penthiopyrad	Prothioconazole
790	2007	MN	IIIB	563.92	549.36	0.69	0.14	0.51
801	2007	MN	IV	56.78	268.61	0.15	0.06	0.37
823	2007	MN	IV	42.16	248.97	0.52	0.11	0.18
839	2007	MN	IIIB	433.16	695.96	6.43	0.33	0.17
850	2007	MN	IIIB	3.52	5.85	1.01	0.31	0.10
866	2007	MN	IIIB	142.04	1.26	0.29	0.36	0.19
874	2007	MN	IV	870.77	591.77	0.22	0.08	0.66
890	2007	MN	IIIB	29.42	264.41	0.40	0.16	0.09
906	2007	MN	IIIB	394.17	284.07	1.00	0.28	0.36
946	2007	MN	IIIB	4.21	3.37	2.39	0.44	0.35
1005	2008	ND	IIIB	528.36	407.67	0.24	0.13	0.71
1012	2008	MN	IIIB	11.53	563.29	0.27	0.21	0.40
1051	2008	MN	IV	502.91	306.59	0.05	0.16	0.10
1058	2008	MN	IIIB	295.10	550.76	0.71	0.18	0.44
1076	2008	MN	IIIB	536.68	351.30	0.81	0.21	1.87
1090	2008	ND	IV	582.13	517.78	0.12	0.06	0.64
1103	2008	MN	IV	0.37	0.09	0.10	0.08	0.29
1112	2008	MN	IIIB	521.06	394.23	1.07	0.50	0.35
1146	2008	MN	IIIB	537.18	649.84	0.63	0.10	0.44
1174	2008	MN	IIIB	551.23	357.00	0.67	0.17	0.27
1177	2008	MN	IV	528.57	459.35	4.02	0.13	0.23
100-2	2012	MN	IIIB	0.95	106.48	0.36	0.24	2.26
101-2	2012	MN	IV	51.19	628.75	0.18	0.08	0.47
102-1	2012	MN	IIIB	36.71	269.34	0.68	0.24	0.37
13-1	2012	MN	IV	0.53	0.36	0.07	0.10	0.22
17B-1	2012	MN	IV	31.33	1.32	0.26	0.11	1.63
22-1	2012	MN	IIIB	868.11	589.79	0.86	0.36	0.16

Isolate	Year	State	Subgroup	EC$_{50}$ (μg ml^{-1})				
				Azoxystrobin	Trifloxystrobin	Pyraclostrobin	Penthiopyrad	Prothioconazole
23-2	2012	MN	IV	13.14	6.69	0.19	0.34	0.34
24-1	2012	MN	IV	41.37	131.86	0.22	0.14	0.43
25-1	2012	MN	IIIB	24.45	476.74	0.73	0.58	0.34
26-8	2012	MN	IV	52.15	1.69	0.62	0.18	0.71
27-4	2012	MN	IIIB	68.53	93.30	0.19	0.29	0.65
28-4	2012	MN	IIIB	30.09	485.98	0.41	0.23	0.43
29-1	2012	MN	IV	0.67	6.25	0.39	0.27	0.68
30-3	2012	MN	IIIB	88.14	232.96	0.26	0.22	0.37
31-1	2012	MN	IV	0.28	0.45	0.23	0.10	0.03
39-5	2012	MN	INT	504.75	530.63	1.05	0.44	0.30
40-2	2012	MN	IV	830.42	888.41	1.74	0.36	0.69
41-2	2012	MN	INT	30.66	2.42	0.17	0.22	0.69
42-3	2012	MN	IIIB	1.11	554.27	0.11	0.12	0.75
43A-4	2012	MN	IIIB	706.17	777.18	0.41	0.34	0.27
43B-2	2012	MN	IV	50.62	154.45	0.37	0.14	0.40
46-1	2012	ND	IV	1.00	131.64	0.08	0.30	1.06
47-1	2012	MN	IIIB	2.88	31.51	0.13	0.10	0.08
48-1	2012	MN	IV	0.18	1.05	0.02	0.08	1.25
49-1	2012	MN	IV	2.38	9.07	0.13	0.06	0.33
54-2	2012	MN	IV	23.17	140.58	0.23	0.10	1.48
61-1	2012	MN	IIIB	47.60	365.62	0.29	0.37	0.70
80-1	2012	MN	IIIB	2.17	33.63	0.34	0.36	0.84
94-3	2012	MN	IV	3.95	3.44	0.16	0.08	0.26
96-1	2012	MN	IV	31.17	267.16	0.17	0.41	0.60
97-2	2012	MN	INT	24.90	315.02	0.32	0.32	0.43

APPENDIX B. STATISTICAL ANALYSIS FOR DETERMINATION OF *RHIZOCTONIA*

***SOLANI* AG-2-2 SUBGROUPS**

Source of variation	DF	Mean square	*P*
Trial	1	2.59	0.7729
Isolate	104	5228.45	<0.0001
Trial x Isolate	104	15.97	0.9999
Error	314	31.05	

APPENDIX C. DETERMINATION OF *RHIZOCTONIA SOLANI AG-2-2* SUBGROUPS

Isolates	Growth % (Growth 35˚C/ Growth 25˚C)	AG-2-2 Subgroup
7	0	IV
8	0	IV
9	0	IV
10	0	IV
11	0	IV
12	0	IV
18	0	IV
23	0	IV
24	22	Intermediate
25	0	IV
26	0	IV
27	0	IV
29	0	IV
30	0	IV
31	0	IV
35	117	IIIB
39	0	IV
40	0	IV
41	0	IV
49	0	IV
59	0	IV
60	0	IV
68	129	IIIB
69	75	IIIB
70	0	IV
71	0	IV
72	0	IV
106	0	IV
186	0	IV
195	0	IV
200	6	IV
253	0	IV
255	31	IIIB
258	0	IV
286	112	IIIB
296	0	IV
300	0	IV

Isolates	Growth % (Growth 35˚C/ Growth 25˚C)	AG-2-2 Subgroup
315	5	IV
331	96	IIIB
385	78	IIIB
393	0	IV
407	0	IV
413	0	IV
424	60	IIIB
470	58	IIIB
481	0	IV
496	0	IV
542	0	IV
571	53	IIIB
588	0	IV
599	0	IV
776	52	IIIB
780	91	IIIB
790	90	IIIB
801	0	IV
823	0	IV
839	60	IIIB
850	57	IIIB
866	69	IIIB
874	0	IV
890	30	IIIB
906	29	IIIB
946	76	IIIB
1005	81	IIIB
1012	95	IIIB
1051	0	IV
1058	30	IIIB
1076	90	IIIB
1090	0	IV
1103	0	IV
1112	98	IIIB
1146	94	IIIB
1174	88	IIIB
1177	0	IV
100-2	79	IIIB
101-2	0	IV

Isolates	Growth % (Growth 35˚C/ Growth 25˚C)	AG-2-2 Subgroup
102-1	65	IIIB
13-1	7	IV
17B-1	0	IV
22-1	118	IIIB
23-2	0	IV
24-1	4	IV
25-1	59	IIIB
26-8	1	IV
27-4	85	IIIB
28-4	29	IIIB
29-1	1	IV
30-3	31	IIIB
31-1	0	IV
39-5	24	Intermediate
40-2	1	IV
41-2	17	Intermediate
42-3	42	IIIB
43A-4	54	IIIB
43B-2	0	IV
46-1	0	IV
47-1	52	IIIB
48-1	8	IV
49-1	1	IV
54-2	0	IV
61-1	76	IIIB
80-1	104	IIIB
94-3	0	IV
96-1	0	IV
97-2	20	Intermediate

APPENDIX D. STATISTICAL ANALYSIS SUMMARY FOR SENSITIVITY OF

RHIZOCTONIA SOLANI TO FUNGICIDES

Table D.1. Combined analysis of variance for sensitivity of *Rhizoctonia solani* to azoxystrobin in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	40402.53	0.0819
Isolate	104	309016.37	<0.0001
Experiment x Isolate	104	12644.27	0.5966
Error	210	7482.00	

Table D.2. Combined analysis of variance for sensitivity of *Rhizoctonia solani* to trifloxystrobin in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	1125.35	0.6985
Isolate	104	262574.22	<0.0001
Experiment x Isolate	104	4674.19	0.9962
Error	210	7482.00	

Table D.3. Combined analysis of variance for sensitivity of *Rhizoctonia solani* to pyraclostrobin in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	0.35	0.5666
Isolate	104	5.49	<0.0001
Experiment x Isolate	104	0.40	1.0000
Error	210	1.07	

Table D.4. Combined analysis of variance for sensitivity of *Rhizoctonia solani* to penthiopyrad in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	0.004	0.6821
Isolate	104	0.235	<0.0001
Experiment x Isolate	104	0.016	0.9576
Error	210	0.022	

Table D.5. Combined analysis of variance for sensitivity of *Rhizoctonia solani* to prothioconazole in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	0.07	0.3475
Isolate	104	0.99	<0.0001
Experiment x Isolate	104	0.05	0.9986
Error	210	0.08	

APPENDIX E. STATISTICAL ANALYSIS SUMMARY FOR SENSITIVITY OF

RHIZOCTONIA SOLANI TO AZOXYSTROBIN WITH AND WITHOUT

SALICYLHYDROXAMIC ACID

Source of variation	DF	Mean square	P
Trial	1	84311.87	0.0806
Isolate	4	68710.24	0.0562
Trial x Isolate	4	6286.86	0.9047
SHAM	1	901881.98	<0.0001
Trial x SHAM	1	65701.56	0.1199
Isolate x SHAM	4	17957.90	0.5875
Trial x Isolate x SHAM	4	6423.31	0.9013
Error	20	24895.54	

APPENDIX F. STATISTICAL ANALYSIS SUMMARY FOR EFFICACY OF

FUNGICIDES AT CONTROLLING *RHIZOCTONIA SOLANI* IN VIVO

Table F.1. Test statistic for the effects of isolate (850, 22-1, 571, and 946), fungicide, and fungicide concentration at controlling *Rhizoctonia solani* in vivo.

Effect	df_N[a]	df_D[b]	*F*	*P*
Isolate	3	900	317.39	<0.0001
Fungicide	4	900	5.70	0.0315
Isolate x Fungicide	12	900	8.89	0.4863
Concentration	8	900	241.72	<0.0001
Isolate x Concentration	24	900	20.54	<0.0001
Fungicide x Concentration	32	900	2.26	0.0028
Isolate x Fungicide x Concentration	96	900	2.58	0.2300

[a] Degree freedom of numerator
[b] Degree freedom of denominator

Table F.2. Test statistic for the effects of isolate (393, 60, 40-2, and 31-1), fungicide, and fungicide concentration at controlling *Rhizoctonia solani* in vivo.

Effect	df_N[a]	df_D[b]	*F*	*P*
Isolate	3	900	186.96	<0.0001
Fungicide	4	900	2.66	0.0002
Isolate x Fungicide	12	900	0.96	<0.0001
Concentration	8	900	267.20	<0.0001
Isolate x Concentration	24	900	9.50	<0.0001
Fungicide x Concentration	32	900	1.86	<0.0001
Isolate x Fungicide x Concentration	96	900	1.11	<0.0001

[a] Degree freedom of numerator
[b] Degree freedom of denominator

Table G.1. Non-parametric analysis for efficacy of fungicides at controlling of *Rhizoctonia solani* isolates (22-1, 393, 60, and 850) under greenhouse conditions.

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	0	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	0.1	0.86	0.70	0.79	1.63
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	1	0.86	0.70	0.79	1.63
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	10	0.86	0.70	0.79	1.63
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	100	0.82	0.59	0.72	4.10
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	1000	0.71	0.36	0.54	9.24
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	672.3 ml/ha	0.47	0.22	0.33	4.76
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	10000	0.37	0.18	0.27	2.51
AG-2-2 IIIB	868.11	589.79	22-1	Quadris	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IIIB	868.11	589.79	22-1	Gem	0	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Gem	0.1	0.88	0.70	0.81	2.32
AG-2-2 IIIB	868.11	589.79	22-1	Gem	1	0.86	0.70	0.79	1.63
AG-2-2 IIIB	868.11	589.79	22-1	Gem	10	0.83	0.65	0.75	2.29
AG-2-2 IIIB	868.11	589.79	22-1	Gem	100	0.79	0.60	0.70	2.58
AG-2-2 IIIB	868.11	589.79	22-1	Gem	1000	0.83	0.65	0.75	2.29
AG-2-2 IIIB	868.11	589.79	22-1	Gem	255.9 ml/ha	0.76	0.38	0.58	11.03
AG-2-2 IIIB	868.11	589.79	22-1	Gem	10000	0.48	0.22	0.34	4.91
AG-2-2 IIIB	868.11	589.79	22-1	Gem	NonInoculated	0.40	0.18	0.28	3.21
AG-2-2 IIIB	868.11	589.79	22-1	Headline	0	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Headline	0.1	0.88	0.70	0.81	2.32
AG-2-2 IIIB	868.11	589.79	22-1	Headline	1	0.88	0.73	0.82	1.46
AG-2-2 IIIB	868.11	589.79	22-1	Headline	10	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Headline	100	0.86	0.70	0.79	1.63
AG-2-2 IIIB	868.11	589.79	22-1	Headline	1000	0.82	0.57	0.71	4.45
AG-2-2 IIIB	868.11	589.79	22-1	Headline	672.3ml/ha	0.60	0.18	0.36	14.38
AG-2-2 IIIB	868.11	589.79	22-1	Headline	10000	0.31	0.16	0.23	1.57
AG-2-2 IIIB	868.11	589.79	22-1	Headline	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	0	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	0.1	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	1	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	10	0.86	0.70	0.79	1.63

Table G.1. Non-parametric analysis for efficacy of fungicides at controlling of *Rhizoctonia solani* isolates (22-1, 393, 60, and 850) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration μg ml^{-1}	Upper limit	Lowerli mit	Disease severity	Variance
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	100	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	1000	0.72	0.49	0.61	3.79
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	2192.3ml/ha	0.34	0.16	0.24	2.43
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	10000	0.31	0.16	0.23	1.57
AG-2-2 IIIB	868.11	589.79	22-1	Vertisan	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IIIB	868.11	589.79	22-1	Proline	0	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Proline	0.1	0.86	0.70	0.79	1.63
AG-2-2 IIIB	868.11	589.79	22-1	Proline	1	0.86	0.70	0.79	1.63
AG-2-2 IIIB	868.11	589.79	22-1	Proline	10	0.87	0.75	0.82	1.03
AG-2-2 IIIB	868.11	589.79	22-1	Proline	100	0.88	0.73	0.82	1.46
AG-2-2 IIIB	868.11	589.79	22-1	Proline	1000	0.73	0.50	0.62	3.76
AG-2-2 IIIB	868.11	589.79	22-1	Proline	416.5ml/ha	0.44	0.18	0.29	4.59
AG-2-2 IIIB	868.11	589.79	22-1	Proline	10000	0.46	0.22	0.33	4.13
AG-2-2 IIIB	868.11	589.79	22-1	Proline	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IV	707.26	450.2	393	Quadris	0	0.86	0.84	0.85	0.03
AG-2-2 IV	707.26	450.2	393	Quadris	0.1	0.87	0.75	0.82	1.03
AG-2-2 IV	707.26	450.2	393	Quadris	1	0.87	0.75	0.82	1.03
AG-2-2 IV	707.26	450.2	393	Quadris	10	0.88	0.73	0.82	1.46
AG-2-2 IV	707.26	450.2	393	Quadris	100	0.74	0.41	0.58	8.40
AG-2-2 IV	707.26	450.2	393	Quadris	1000	0.40	0.18	0.28	3.21
AG-2-2 IV	707.26	450.2	393	Quadris	672.3 ml/ha	0.31	0.16	0.23	1.57
AG-2-2 IV	707.26	450.2	393	Quadris	10000	0.31	0.16	0.23	1.57
AG-2-2 IV	707.26	450.2	393	Quadris	NonInoculated	0.20	0.18	0.19	0.02
AG-2-2 IV	707.26	450.2	393	Gem	0	0.86	0.84	0.85	0.03
AG-2-2 IV	707.26	450.2	393	Gem	0.1	0.86	0.64	0.77	3.23
AG-2-2 IV	707.26	450.2	393	Gem	1	0.83	0.61	0.73	3.37
AG-2-2 IV	707.26	450.2	393	Gem	10	0.89	0.64	0.79	4.20
AG-2-2 IV	707.26	450.2	393	Gem	100	0.82	0.59	0.72	3.72
AG-2-2 IV	707.26	450.2	393	Gem	1000	0.76	0.31	0.55	16.26
AG-2-2 IV	707.26	450.2	393	Gem	255.9 ml/ha	0.45	0.18	0.30	5.29
AG-2-2 IV	707.26	450.2	393	Gem	10000	0.36	0.16	0.24	2.81
AG-2-2 IV	707.26	450.2	393	Gem	NonInoculated	0.31	0.16	0.23	1.57

Table G.1. Non-parametric analysis for efficacy of fungicides at controlling of *Rhizoctonia solani* isolates (22-1, 393, 60, and 850) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IV	707.26	450.2	393	Headline	0	0.86	0.84	0.85	0.03
AG-2-2 IV	707.26	450.2	393	Headline	0.1	0.88	0.72	0.81	1.73
AG-2-2 IV	707.26	450.2	393	Headline	1	0.78	0.57	0.69	3.13
AG-2-2 IV	707.26	450.2	393	Headline	10	0.82	0.54	0.70	5.82
AG-2-2 IV	707.26	450.2	393	Headline	100	0.83	0.62	0.74	3.10
AG-2-2 IV	707.26	450.2	393	Headline	1000	0.82	0.34	0.60	18.97
AG-2-2 IV	707.26	450.2	393	Headline	672.3ml/ha	0.33	0.16	0.24	2.11
AG-2-2 IV	707.26	450.2	393	Headline	10000	0.31	0.16	0.23	1.57
AG-2-2 IV	707.26	450.2	393	Headline	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IV	707.26	450.2	393	Vertisan	0	0.86	0.84	0.85	0.03
AG-2-2 IV	707.26	450.2	393	Vertisan	0.1	0.87	0.75	0.82	1.03
AG-2-2 IV	707.26	450.2	393	Vertisan	1	0.88	0.72	0.81	1.73
AG-2-2 IV	707.26	450.2	393	Vertisan	10	0.87	0.75	0.82	1.03
AG-2-2 IV	707.26	450.2	393	Vertisan	100	0.76	0.50	0.64	5.06
AG-2-2 IV	707.26	450.2	393	Vertisan	1000	0.56	0.28	0.41	5.79
AG-2-2 IV	707.26	450.2	393	Vertisan	2192.3ml/ha	0.49	0.26	0.37	3.58
AG-2-2 IV	707.26	450.2	393	Vertisan	10000	0.20	0.18	0.19	0.02
AG-2-2 IV	707.26	450.2	393	Vertisan	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IV	707.26	450.2	393	Proline	0	0.86	0.84	0.85	0.03
AG-2-2 IV	707.26	450.2	393	Proline	0.1	0.83	0.64	0.75	2.56
AG-2-2 IV	707.26	450.2	393	Proline	1	0.86	0.64	0.77	3.49
AG-2-2 IV	707.26	450.2	393	Proline	10	0.83	0.63	0.75	2.86
AG-2-2 IV	707.26	450.2	393	Proline	100	0.86	0.65	0.77	2.97
AG-2-2 IV	707.26	450.2	393	Proline	1000	0.86	0.66	0.78	2.71
AG-2-2 IV	707.26	450.2	393	Proline	416.5ml/ha	0.31	0.16	0.23	1.57
AG-2-2 IV	707.26	450.2	393	Proline	10000	0.20	0.18	0.19	0.02
AG-2-2 IV	707.26	450.2	393	Proline	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Quadris	0	0.65	0.38	0.52	5.19
AG-2-2 IV	0.43	2.7	60	Quadris	0.1	0.58	0.53	0.56	0.24
AG-2-2 IV	0.43	2.7	60	Quadris	1	0.52	0.48	0.50	0.13
AG-2-2 IV	0.43	2.7	60	Quadris	10	0.50	0.42	0.46	0.41
AG-2-2 IV	0.43	2.7	60	Quadris	100	0.39	0.21	0.29	2.30

Table G.1. Non-parametric analysis for efficacy of fungicides at controlling of *Rhizoctonia solani* isolates (22-1, 393, 60, and 850) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IV	0.43	2.7	60	Quadris	1000	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Quadris	672.3 ml/ha	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Quadris	10000	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Quadris	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Gem	0	0.65	0.38	0.52	5.19
AG-2-2 IV	0.43	2.7	60	Gem	0.1	0.57	0.35	0.46	3.59
AG-2-2 IV	0.43	2.7	60	Gem	1	0.58	0.29	0.43	6.25
AG-2-2 IV	0.43	2.7	60	Gem	10	0.50	0.32	0.41	2.36
AG-2-2 IV	0.43	2.7	60	Gem	100	0.45	0.40	0.42	0.21
AG-2-2 IV	0.43	2.7	60	Gem	1000	0.39	0.18	0.27	2.94
AG-2-2 IV	0.43	2.7	60	Gem	255.9 ml/ha	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Gem	10000	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Gem	NonInoculated	0.20	0.18	0.19	0.02
AG-2-2 IV	0.43	2.7	60	Headline	0	0.65	0.38	0.52	5.19
AG-2-2 IV	0.43	2.7	60	Headline	0.1	0.57	0.35	0.46	3.55
AG-2-2 IV	0.43	2.7	60	Headline	1	0.55	0.37	0.46	2.47
AG-2-2 IV	0.43	2.7	60	Headline	10	0.56	0.32	0.43	4.17
AG-2-2 IV	0.43	2.7	60	Headline	100	0.43	0.22	0.31	3.37
AG-2-2 IV	0.43	2.7	60	Headline	1000	0.34	0.16	0.24	2.43
AG-2-2 IV	0.43	2.7	60	Headline	672.3ml/ha	0.20	0.18	0.19	0.02
AG-2-2 IV	0.43	2.7	60	Headline	10000	0.37	0.18	0.27	2.51
AG-2-2 IV	0.43	2.7	60	Headline	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Vertisan	0	0.65	0.38	0.52	5.19
AG-2-2 IV	0.43	2.7	60	Vertisan	0.1	0.68	0.44	0.56	4.53
AG-2-2 IV	0.43	2.7	60	Vertisan	1	0.50	0.22	0.35	5.46
AG-2-2 IV	0.43	2.7	60	Vertisan	10	0.37	0.18	0.27	2.51
AG-2-2 IV	0.43	2.7	60	Vertisan	100	0.53	0.22	0.36	7.41
AG-2-2 IV	0.43	2.7	60	Vertisan	1000	0.20	0.18	0.19	0.02
AG-2-2 IV	0.43	2.7	60	Vertisan	2192.3ml/ha	0.20	0.18	0.19	0.02
AG-2-2 IV	0.43	2.7	60	Vertisan	10000	0.20	0.18	0.19	0.02
AG-2-2 IV	0.43	2.7	60	Vertisan	NonInoculated	0.40	0.15	0.25	4.36
AG-2-2 IV	0.43	2.7	60	Proline	0	0.65	0.38	0.52	5.19

Table G.1. Non-parametric analysis for efficacy of fungicides at controlling of *Rhizoctonia solani* isolates (22-1, 393, 60, and 850) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IV	0.43	2.7	60	Proline	0.1	0.58	0.35	0.46	3.89
AG-2-2 IV	0.43	2.7	60	Proline	1	0.48	0.22	0.34	4.93
AG-2-2 IV	0.43	2.7	60	Proline	10	0.45	0.26	0.35	2.78
AG-2-2 IV	0.43	2.7	60	Proline	100	0.42	0.18	0.29	3.88
AG-2-2 IV	0.43	2.7	60	Proline	1000	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Proline	416.5ml/ha	0.20	0.18	0.19	0.02
AG-2-2 IV	0.43	2.7	60	Proline	10000	0.31	0.16	0.23	1.57
AG-2-2 IV	0.43	2.7	60	Proline	NonInoculated	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Quadris	0	0.86	0.64	0.77	3.49
AG-2-2 IIIB	3.52	5.85	850	Quadris	0.1	0.87	0.46	0.70	12.99
AG-2-2 IIIB	3.52	5.85	850	Quadris	1	0.85	0.42	0.67	15.19
AG-2-2 IIIB	3.52	5.85	850	Quadris	10	0.86	0.64	0.77	3.49
AG-2-2 IIIB	3.52	5.85	850	Quadris	100	0.53	0.22	0.36	7.10
AG-2-2 IIIB	3.52	5.85	850	Quadris	1000	0.31	0.16	0.23	1.57
AG-2-2 IIIB	3.52	5.85	850	Quadris	672.3 ml/ha	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Quadris	10000	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Quadris	NonInoculated	0.31	0.16	0.23	1.57
AG-2-2 IIIB	3.52	5.85	850	Gem	0	0.86	0.64	0.77	3.49
AG-2-2 IIIB	3.52	5.85	850	Gem	0.1	0.82	0.60	0.73	3.47
AG-2-2 IIIB	3.52	5.85	850	Gem	1	0.86	0.62	0.76	3.98
AG-2-2 IIIB	3.52	5.85	850	Gem	10	0.86	0.63	0.76	3.70
AG-2-2 IIIB	3.52	5.85	850	Gem	100	0.81	0.48	0.66	7.86
AG-2-2 IIIB	3.52	5.85	850	Gem	1000	0.54	0.32	0.43	3.61
AG-2-2 IIIB	3.52	5.85	850	Gem	255.9 ml/ha	0.50	0.18	0.32	7.34
AG-2-2 IIIB	3.52	5.85	850	Gem	10000	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Gem	NonInoculated	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Headline	0	0.86	0.64	0.77	3.49
AG-2-2 IIIB	3.52	5.85	850	Headline	0.1	0.88	0.67	0.80	3.21
AG-2-2 IIIB	3.52	5.85	850	Headline	1	0.86	0.65	0.77	3.03
AG-2-2 IIIB	3.52	5.85	850	Headline	10	0.85	0.35	0.63	20.88
AG-2-2 IIIB	3.52	5.85	850	Headline	100	0.78	0.26	0.52	23.48
AG-2-2 IIIB	3.52	5.85	850	Headline	1000	0.31	0.16	0.23	1.57

Table G.1. Non-parametric analysis for efficacy of fungicides at controlling of *Rhizoctonia solani* isolates (22-1, 393, 60, and 850) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration μg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IIIB	3.52	5.85	850	Headline	672.3ml/ha	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Headline	10000	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Headline	NonInoculated	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Vertisan	0	0.86	0.64	0.77	3.49
AG-2-2 IIIB	3.52	5.85	850	Vertisan	0.1	0.80	0.33	0.59	18.77
AG-2-2 IIIB	3.52	5.85	850	Vertisan	1	0.81	0.39	0.62	13.62
AG-2-2 IIIB	3.52	5.85	850	Vertisan	10	0.83	0.43	0.66	12.48
AG-2-2 IIIB	3.52	5.85	850	Vertisan	100	0.78	0.40	0.61	11.21
AG-2-2 IIIB	3.52	5.85	850	Vertisan	1000	0.64	0.26	0.44	10.81
AG-2-2 IIIB	3.52	5.85	850	Vertisan	2192.3ml/ha	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Vertisan	10000	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Vertisan	NonInoculated	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Proline	0	0.86	0.64	0.77	3.49
AG-2-2 IIIB	3.52	5.85	850	Proline	0.1	0.86	0.84	0.85	0.03
AG-2-2 IIIB	3.52	5.85	850	Proline	1	0.81	0.39	0.62	13.85
AG-2-2 IIIB	3.52	5.85	850	Proline	10	0.80	0.39	0.62	13.82
AG-2-2 IIIB	3.52	5.85	850	Proline	100	0.80	0.32	0.58	18.81
AG-2-2 IIIB	3.52	5.85	850	Proline	1000	0.74	0.25	0.49	20.12
AG-2-2 IIIB	3.52	5.85	850	Proline	416.5ml/ha	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Proline	10000	0.20	0.18	0.19	0.02
AG-2-2 IIIB	3.52	5.85	850	Proline	NonInoculated	0.20	0.18	0.19	0.02

Table G.2. Non-parametric analysis for efficacy of fungicides at controlling *Rhizoctonia solani* isolates (571, 946, 40-2, and 31-1) under greenhouse conditions.

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IIIB	876.58	876.63	571	Quadris	0	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Quadris	0.1	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Quadris	1	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Quadris	10	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Quadris	100	0.81	0.74	0.77	0.36
AG-2-2 IIIB	876.58	876.63	571	Quadris	1000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Quadris	672.3 ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Quadris	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Quadris	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Gem	0	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Gem	0.1	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Gem	1	0.94	0.77	0.88	1.75
AG-2-2 IIIB	876.58	876.63	571	Gem	10	0.90	0.42	0.71	18.04
AG-2-2 IIIB	876.58	876.63	571	Gem	100	0.92	0.54	0.78	10.30
AG-2-2 IIIB	876.58	876.63	571	Gem	1000	0.82	0.42	0.65	12.71
AG-2-2 IIIB	876.58	876.63	571	Gem	255.9 ml/ha	0.63	0.28	0.45	9.02
AG-2-2 IIIB	876.58	876.63	571	Gem	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Gem	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Headline	0	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Headline	0.1	0.93	0.87	0.91	0.21
AG-2-2 IIIB	876.58	876.63	571	Headline	1	0.94	0.54	0.82	11.28
AG-2-2 IIIB	876.58	876.63	571	Headline	10	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Headline	100	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Headline	1000	0.90	0.72	0.83	2.15
AG-2-2 IIIB	876.58	876.63	571	Headline	672.3ml/ha	0.57	0.24	0.39	8.02
AG-2-2 IIIB	876.58	876.63	571	Headline	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Headline	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Vertisan	0	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Vertisan	0.1	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Vertisan	1	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Vertisan	10	0.93	0.85	0.90	0.43
AG-2-2 IIIB	876.58	876.63	571	Vertisan	100	0.92	0.81	0.88	0.91

Table G.2. Non-parametric analysis for efficacy of fungicides at controlling *Rhizoctonia solani* isolates (571, 946, 40-2, and 31-1) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC_{50}	Trifloxystrobin EC_{50}	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IIIB	876.58	876.63	571	Vertisan	1000	0.88	0.42	0.69	16.70
AG-2-2 IIIB	876.58	876.63	571	Vertisan	2192.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Vertisan	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Vertisan	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	876.58	876.63	571	Proline	0	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Proline	0.1	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Proline	1	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Proline	10	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Proline	100	0.93	0.91	0.92	0.01
AG-2-2 IIIB	876.58	876.63	571	Proline	1000	0.85	0.42	0.67	15.21
AG-2-2 IIIB	876.58	876.63	571	Proline	416.5ml/ha	0.86	0.42	0.68	15.44
AG-2-2 IIIB	876.58	876.63	571	Proline	10000	0.56	0.29	0.42	5.49
AG-2-2 IIIB	876.58	876.63	571	Proline	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Quadris	0	0.85	0.73	0.79	0.89
AG-2-2 IIIB	4.21	3.36	946	Quadris	0.1	0.77	0.70	0.74	0.37
AG-2-2 IIIB	4.21	3.36	946	Quadris	1	0.71	0.65	0.68	0.23
AG-2-2 IIIB	4.21	3.36	946	Quadris	10	0.65	0.33	0.49	7.68
AG-2-2 IIIB	4.21	3.36	946	Quadris	100	0.46	0.26	0.36	2.89
AG-2-2 IIIB	4.21	3.36	946	Quadris	1000	0.46	0.26	0.36	2.89
AG-2-2 IIIB	4.21	3.36	946	Quadris	672.3 ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Quadris	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Quadris	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Gem	0	0.85	0.73	0.79	0.89
AG-2-2 IIIB	4.21	3.36	946	Gem	0.1	0.76	0.40	0.59	10.04
AG-2-2 IIIB	4.21	3.36	946	Gem	1	0.68	0.33	0.51	9.20
AG-2-2 IIIB	4.21	3.36	946	Gem	10	0.55	0.29	0.41	5.23
AG-2-2 IIIB	4.21	3.36	946	Gem	100	0.47	0.26	0.36	3.27
AG-2-2 IIIB	4.21	3.36	946	Gem	1000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Gem	255.9 ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Gem	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Gem	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Headline	0	0.85	0.73	0.79	0.89

Table G.2. Non-parametric analysis for efficacy of fungicides at controlling *Rhizoctonia solani* isolates (571, 946, 40-2, and 31-1) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IIIB	4.21	3.36	946	Headline	0.1	0.80	0.71	0.76	0.59
AG-2-2 IIIB	4.21	3.36	946	Headline	1	0.78	0.68	0.73	0.65
AG-2-2 IIIB	4.21	3.36	946	Headline	10	0.68	0.39	0.54	6.42
AG-2-2 IIIB	4.21	3.36	946	Headline	100	0.77	0.50	0.64	5.41
AG-2-2 IIIB	4.21	3.36	946	Headline	1000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Headline	672.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Headline	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Headline	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	0	0.85	0.73	0.79	0.89
AG-2-2 IIIB	4.21	3.36	946	Vertisan	0.1	0.72	0.48	0.61	4.18
AG-2-2 IIIB	4.21	3.36	946	Vertisan	1	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	10	0.57	0.24	0.39	8.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	100	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	1000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	2192.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	0	0.85	0.73	0.79	0.89
AG-2-2 IIIB	4.21	3.36	946	Vertisan	0.1	0.72	0.48	0.61	4.18
AG-2-2 IIIB	4.21	3.36	946	Vertisan	1	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	10	0.57	0.24	0.39	8.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	100	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	1000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	2192.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	10000	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Vertisan	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Proline	0	0.85	0.73	0.79	0.89
AG-2-2 IIIB	4.21	3.36	946	Proline	0.1	0.82	0.76	0.79	0.26
AG-2-2 IIIB	4.21	3.36	946	Proline	1	0.77	0.68	0.73	0.67
AG-2-2 IIIB	4.21	3.36	946	Proline	10	0.84	0.51	0.70	8.17
AG-2-2 IIIB	4.21	3.36	946	Proline	100	0.71	0.48	0.60	4.04
AG-2-2 IIIB	4.21	3.36	946	Proline	1000	0.31	0.30	0.30	0.02

Table G.2. Non-parametric analysis for efficacy of fungicides at controlling *Rhizoctonia solani* isolates (571, 946, 40-2, and 31-1) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC_{50}	Trifloxystrobin EC_{50}	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IIIB	4.21	3.36	946	Proline	416.5ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IIIB	4.21	3.36	946	Proline	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Quadris	0	0.87	0.42	0.69	16.40
AG-2-2 IV	830.42	888.41	40-2	Quadris	0.1	0.84	0.68	0.77	1.86
AG-2-2 IV	830.42	888.41	40-2	Quadris	1	0.86	0.69	0.79	1.93
AG-2-2 IV	830.42	888.41	40-2	Quadris	10	0.79	0.50	0.66	5.95
AG-2-2 IV	830.42	888.41	40-2	Quadris	100	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Quadris	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Quadris	672.3 ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Quadris	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Quadris	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Gem	0	0.87	0.42	0.69	16.40
AG-2-2 IV	830.42	888.41	40-2	Gem	0.1	0.77	0.40	0.60	10.95
AG-2-2 IV	830.42	888.41	40-2	Gem	1	0.69	0.33	0.51	9.87
AG-2-2 IV	830.42	888.41	40-2	Gem	10	0.68	0.33	0.50	9.05
AG-2-2 IV	830.42	888.41	40-2	Gem	100	0.54	0.24	0.38	6.52
AG-2-2 IV	830.42	888.41	40-2	Gem	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Gem	255.9 ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Gem	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Gem	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Headline	0	0.87	0.42	0.69	16.40
AG-2-2 IV	830.42	888.41	40-2	Headline	0.1	0.73	0.40	0.58	8.35
AG-2-2 IV	830.42	888.41	40-2	Headline	1	0.74	0.40	0.58	8.81
AG-2-2 IV	830.42	888.41	40-2	Headline	10	0.56	0.29	0.42	5.49
AG-2-2 IV	830.42	888.41	40-2	Headline	100	0.51	0.25	0.37	4.73
AG-2-2 IV	830.42	888.41	40-2	Headline	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Headline	672.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Headline	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Headline	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Vertisan	0	0.87	0.42	0.69	16.40
AG-2-2 IV	830.42	888.41	40-2	Vertisan	0.1	0.81	0.49	0.67	7.48
AG-2-2 IV	830.42	888.41	40-2	Vertisan	1	0.87	0.53	0.73	8.54

Table G.2. Non-parametric analysis for efficacy of fungicides at controlling *Rhizoctonia solani* isolates (571, 946, 40-2, and 31-1) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IV	830.42	888.41	40-2	Vertisan	10	0.81	0.41	0.63	12.22
AG-2-2 IV	830.42	888.41	40-2	Vertisan	100	0.65	0.33	0.49	7.40
AG-2-2 IV	830.42	888.41	40-2	Vertisan	1000	0.63	0.28	0.45	9.51
AG-2-2 IV	830.42	888.41	40-2	Vertisan	2192.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Vertisan	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Vertisan	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Proline	0	0.87	0.42	0.69	16.40
AG-2-2 IV	830.42	888.41	40-2	Proline	0.1	0.88	0.76	0.83	1.09
AG-2-2 IV	830.42	888.41	40-2	Proline	1	0.75	0.41	0.59	9.04
AG-2-2 IV	830.42	888.41	40-2	Proline	10	0.80	0.40	0.62	12.12
AG-2-2 IV	830.42	888.41	40-2	Proline	100	0.83	0.51	0.69	7.98
AG-2-2 IV	830.42	888.41	40-2	Proline	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Proline	416.5ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Proline	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	830.42	888.41	40-2	Proline	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Quadris	0	0.70	0.63	0.67	0.38
AG-2-2 IV	0.28	0.45	31-1	Quadris	0.1	0.47	0.26	0.36	3.27
AG-2-2 IV	0.28	0.45	31-1	Quadris	1	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Quadris	10	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Quadris	100	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Quadris	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Quadris	672.3 ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Quadris	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Quadris	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Gem	0	0.70	0.63	0.67	0.38
AG-2-2 IV	0.28	0.45	31-1	Gem	0.1	0.72	0.67	0.70	0.15
AG-2-2 IV	0.28	0.45	31-1	Gem	1	0.71	0.48	0.60	3.91
AG-2-2 IV	0.28	0.45	31-1	Gem	10	0.59	0.28	0.43	6.86
AG-2-2 IV	0.28	0.45	31-1	Gem	100	0.56	0.29	0.42	5.62
AG-2-2 IV	0.28	0.45	31-1	Gem	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Gem	255.9 ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Gem	10000	0.31	0.30	0.30	0.02

Table G.2. Non-parametric analysis for efficacy of fungicides at controlling *Rhizoctonia solani* isolates (571, 946, 40-2, and 31-1) under greenhouse conditions (Continued).

Subgroup	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Isolate	Fungicide	Concentration µg ml^{-1}	Upper limit	Lowerl imit	Disease severity	Variance
AG-2-2 IV	0.28	0.45	31-1	Gem	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	0	0.70	0.63	0.67	0.38
AG-2-2 IV	0.28	0.45	31-1	Headline	0.1	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	1	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	10	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	100	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	672.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Headline	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Vertisan	0	0.70	0.63	0.67	0.38
AG-2-2 IV	0.28	0.45	31-1	Vertisan	0.1	0.55	0.29	0.41	4.92
AG-2-2 IV	0.28	0.45	31-1	Vertisan	1	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Vertisan	10	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Vertisan	100	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Vertisan	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Vertisan	2192.3ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Vertisan	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Vertisan	NonInoculated	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	0	0.70	0.63	0.67	0.38
AG-2-2 IV	0.28	0.45	31-1	Proline	0.1	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	1	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	10	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	100	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	1000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	416.5ml/ha	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	10000	0.31	0.30	0.30	0.02
AG-2-2 IV	0.28	0.45	31-1	Proline	NonInoculated	0.31	0.30	0.30	0.02

APPENDIX H. STATISTICAL ANALYSIS SUMMARY FOR RATE OF MYCELIUM

RADIAL GROWTH OF *RHIZOCTONIA SOLANI*

Source	DF	Mean Square	F Value	Adj Pr > F Greenhouse-Geisser Epsilon
Rate	2	111.87	502.23	<0.0001
Rate x Trail	2	0.03	0.13	0.81
Rate x Rep(Trial)	12	0.04	0.18	0.99
Rate x EC50	2	6.73	30.23	<0.0001
Rate x AG	2	7.32	32.85	<0.0001
Rate x EC50 x AG	2	0.71	3.17	0.06
Error(rate)	170	0.22		

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APPENDIX I. RATE OF MYCELIUM RADIAL GROWTH OF *RHIZOCTONIA SOLANI*

ISOLATES WITH LOW AND HIGH EC$_{50}$ VALUES

Day	AG-2-2 subgroup	Isolate	EC$_{50}$ Group	Azoxystrobin EC$_{50}$	Trifloxystrobin EC$_{50}$	Growth Rate
1	IIIB	22-1	High	868.11	589.79	0.71
1	IIIB	571	High	876.58	876.63	0.88
1	IIIB	331	High	564.65	612.28	1.29
1	IIIB	946	Low	4.21	3.37	0.90
1	IIIB	850	Low	3.52	5.85	1.29
1	IIIB	68	Low	7.46	13.52	1.48
1	IV	40-2	High	830.42	888.41	0.58
1	IV	393	High	707.26	450.2	0.73
1	IV	300	High	666.84	303.72	1.02
1	IV	60	Low	0.43	2.70	1.19
1	IV	31	Low	5.20	3.81	1.20
1	IV	1103	Low	0.37	0.09	1.70
2	IIIB	331	High	868.11	589.79	3.55
2	IIIB	22	High	876.58	876.63	3.66
2	IIIB	571	High	564.65	612.28	3.70
2	IIIB	68	Low	4.21	3.37	2.59
2	IIIB	850	Low	3.52	5.85	2.77
2	IIIB	946	Low	7.46	13.52	3.39
2	IV	40-2	High	830.42	888.41	0.69
2	IV	300	High	707.26	450.2	2.49
2	IV	393	High	666.84	303.72	3.31
2	IV	60	Low	0.43	2.70	1.89
2	IV	1103	Low	5.20	3.81	2.13
2	IV	31	Low	0.37	0.09	2.54
3	IIIB	331	High	868.11	589.79	3.14
3	IIIB	571	High	876.58	876.63	3.21
3	IIIB	22	High	564.65	612.28	3.30
3	IIIB	68	Low	4.21	3.37	3.14
3	IIIB	850	Low	3.52	5.85	3.40
3	IIIB	946	Low	7.46	13.52	3.59
3	IV	40-2	High	830.42	888.41	0.51
3	IV	300	High	707.26	450.2	2.96
3	IV	393	High	666.84	303.72	3.56
3	IV	60	Low	0.43	2.70	2.84
3	IV	1103	Low	5.20	3.81	3.79
3	IV	31	Low	0.37	0.09	3.90

APPENDIX J. STATISTICAL ANALYSIS SUMMARY FOR SENSITIVITY OF

APHANOMYCES COCHLIOIDES TO FUNGICIDES

Table J.1. Combined analysis of variance for sensitivity of *Aphanomyces cochlioides* to hymexazol in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	0.12	0.0804
Isolate	55	0.54	<0.0001
Experiment x Isolate	55	0.01	1
Error	112	0.04	

Table J.2. Combined analysis of variance for sensitivity of *Aphanomyces cochlioides* to tetraconazole in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	0.24	0.6959
Isolate	55	12.84	<0.0001
Experiment x Isolate	55	0.62	0.9999
Error	112	1.54	

Table J.3. Combined analysis of variance for sensitivity of *Aphanomyces cochlioides* to prothioconazole in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	1.26	0.2187
Isolate	55	3.67	<0.0001
Experiment x Isolate	55	0.19	1
Error	112	0.82	

Table J.4. Combined analysis of variance for sensitivity of *Aphanomyces cochlioides* to pyraclostrobin in vitro.

Source of variation	DF	Mean square	*P*
Experiment	1	0.10	0.3304
Isolate	55	6.56	<0.0001
Experiment x Isolate	55	0.01	1
Error	112	0.11	

Isolate	State	Year	Tetraconazole	Prothioconazole	Hymexazol	Pyraclostrobin
105-5-5	-	1994	8.11	2.71	0.05	0.05
25-3-4	-	1994	2.20	1.85	0.44	0.50
55-8-23	MN	1994	3.65	1.97	0.69	0.97
K4-4W	-	1994	1.16	1.70	0.49	0.57
SOIL8R4#1	-	1994	7.53	2.57	0.74	0.80
SOIL9R3#1	MN	1994	6.06	2.65	0.29	0.42
24SS	TX	1997	7.52	5.05	0.24	0.31
24W	TX	1997	4.79	2.57	0.61	1.18
31ss	TX	1997	4.24	5.75	0.06	9.49
32SS	TX	1997	1.98	2.67	0.27	0.35
35ss	TX	1997	3.07	2.52	1.02	1.69
3SS	TX	1997	2.88	2.02	0.23	0.28
51SS	TX	1997	2.34	2.25	0.80	1.28
56SS	TX	1997	4.07	2.49	0.48	0.20
61SS	TX	1997	2.09	2.11	0.66	0.80
64SS	TX	1997	2.35	1.35	0.31	0.39
B18	MN	1997	3.40	2.26	0.22	0.29
B2	MN	1997	2.91	4.24	0.26	0.33
B22	MN	1997	3.05	2.11	0.92	1.14
B33	MN	1997	1.87	2.58	0.26	0.29
B35	MN	1997	2.02	1.88	0.32	0.47
B36	MN	1997	2.81	4.24	0.41	0.16
B39	MN	1997	2.36	1.70	0.32	0.62
B4	MN	1997	5.85	2.01	0.42	0.05
B44	MN	1997	2.87	1.86	0.67	0.80
B45	MN	1997	2.56	2.78	0.79	1.20
B48	MN	1997	6.56	4.70	0.33	0.44
B43	MN	1997	0.44	0.24	0.37	0.52
C10	ND	1997	2.86	1.65	0.97	1.24

Isolate	State	Year	Tetraconazole	Prothioconazole	Hymexazol	Pyraclostrobin
C12	ND	1997	2.94	1.74	0.49	0.38
C14	ND	1997	1.93	1.76	0.61	0.68
C16	ND	1997	3.49	4.54	0.06	0.06
C2	ND	1997	1.99	1.99	0.07	1.02
C32	ND	1997	2.43	1.85	0.46	0.55
C34	ND	1997	4.12	2.07	0.63	0.72
C54	ND	1997	3.54	2.61	0.27	0.36
C60	ND	1997	5.36	2.46	0.33	0.42
C64	ND	1997	5.26	2.27	0.24	0.29
C70	ND	1997	3.51	2.73	0.41	1.91
C84	ND	1997	1.39	1.51	0.22	0.26
C88	ND	1997	3.18	2.31	0.80	0.93
C95	ND	1997	2.04	1.58	0.43	0.48
10-15-2	-	2010	2.67	1.80	0.55	1.63
10-44-5	-	2010	2.08	1.02	0.22	0.27
10-54-7	-	2010	4.54	2.16	0.63	0.78
11-169-2	MN	2011	2.61	2.38	0.06	0.05
11-169-4	MN	2011	2.54	2.10	0.33	0.47
11-169-6	MN	2011	3.21	2.82	1.91	1.66
11-169-7	MN	2011	7.85	2.27	0.59	0.69
WL301	ND	2011	2.56	2.42	0.23	0.28
WL405	ND	2011	2.79	2.31	0.71	0.36
WL501	ND	2011	2.67	2.12	0.53	1.06
12-26-3	MN	2012	2.70	1.96	0.50	0.58
12-28-6	MN	2012	3.65	2.19	2.00	2.47
12-28-7	MN	2012	7.65	2.57	0.71	0.87
12-56-4	MN	2012	2.73	1.99	0.46	0.61

APPENDIX L. STATISTICAL ANALYSIS SUMMARY FOR DETERMINING THE

SUSCEPTIBLE STAGES OF SUGARBEET TO *APHANOMYCES COCHLIOIDES* AND

EFFICACY OF FUNGICIDES IN VIVO

Table L.1. Test statistic for determining the susceptibile stages of sugarbeet plants to *Aphanomyces cochlioides*, using seed and 1to 7 week old stagesplants. Two types of seeds were used treated and nontreated with hymexazol.

Effect	df_N [a]	df_D [b]	*F*	*P*
Hymexazol	3	160	7563.03	<0.0001
Stage	7	160	3314.56	<0.0001
Hymexazol x Stage	21	160	1488.27	<0.0001

[a] Degree freedom of numerator
[b] Degree freedom of denominator

Table L.2. Non-parametric analysis for the effect of hymexazol (With and without) and sugarbeet stage (seed and 1 to 7 weeks old) on susceptibility to *Aphanomyces cochlioides*.

Treatment	Stage	Mean rank severity	Relative effect	Upper limit	Lower limit
Without Hymexazol	Non-inoculated	75.50	0.39	0.39	75.50
Without Hymexazol	Seed	187.50	0.97	0.98	187.50
Without Hymexazol	1 Week	183.90	0.96	0.97	183.90
Without Hymexazol	2 Weeks	172.30	0.89	0.91	172.30
Without Hymexazol	3 Weeks	156.50	0.81	0.81	156.50
Without Hymexazol	4 Weeks	75.50	0.39	0.39	75.50
Without Hymexazol	5 Weeks	75.50	0.39	0.39	75.50
Without Hymexazol	6 Weeks	75.50	0.39	0.39	75.50
Without Hymexazol	7 Weeks	75.50	0.39	0.39	75.50
Hymexazol	Non-inoculated	75.50	0.39	0.39	75.50
Hymexazol	Seed	75.50	0.39	0.39	75.50
Hymexazol	1 Week	174.60	0.91	0.93	174.60
Hymexazol	2 Weeks	169.20	0.88	0.89	169.20
Hymexazol	3 Weeks	156.50	0.81	0.81	156.50
Hymexazol	4 Weeks	75.50	0.39	0.39	75.50
Hymexazol	5 Weeks	75.50	0.39	0.39	75.50
Hymexazol	6 Weeks	75.50	0.39	0.39	75.50
Hymexazol	7 Weeks	75.50	0.39	0.39	75.50

Table L.3. Test statistic for the efficacy of hymexazol, tetraconazole, prothioconazole, and pyraclostrobin at controlling *Aphanomyces cochlioides* when sugarbeet plants were inoculated at seed stage.

Effect	df_N [a]	df_D [b]	*F*	*P*
Fungicides	5	30	29.73	<0.0001

[a] Degree freedom of numerator
[b] Degree freedom of denominator

Table L.4. Non-parametric analysis for efficacy of hymexazol, tetraconazole, prothioconazole, and pyraclostrobin at controlling *Aphanomyces cochlioides* when sugarbeet plants were inoculated at seed stage.

Treatment	Mean rank severity	Relative effect	Variance	Upper limit	Lower limit
Non-inoculated	3.50	0.08	0		
Inoculated	29.33	0.80	0.076	0.866	0.679
Hymexazol	9.67	0.25	0.001	0.266	0.244
Prothioconazole	26.50	0.72	0.114	0.811	0.592
Tetraconazole	23.67	0.64	0.144	0.751	0.509
Pyraclostrobin	18.33	0.50	0.051	0.568	0.423

Table L.5. Test statistic for the efficacy of hymexazol, tetraconazole, prothioconazole, and pyraclostrobin at controlling *Aphanomyces cochlioides* when sugarbeet plants were inoculated at two weeks old.

Effect	df_N [a]	df_D [b]	*F*	*P*
Fungicides	5	30	19.2	<0.0001

[a] Degree freedom of numerator
[b] Degree freedom of denominator

Table L.6. Non-parametric analysis for efficacy of hymexazol, tetraconazole, prothioconazole, and pyraclostrobin at controlling *Aphanomyces cochlioides* when sugarbeet plants were inoculated at two weeks old.

Treatment	Mean rank severity	Relative effect	Variance	Upper limit	Lower limit
Non-inoculated	3.50	0.08	0		
Inoculated	30.58	0.84	0.05	0.89	0.72
Hymexazol	19.17	0.52	0.09	0.62	0.42
Prothioconazole	15.42	0.41	0.11	0.52	0.32
Tetraconazole	27.50	0.75	0.07	0.82	0.65
Pyraclostrobin	14.83	0.40	0.28	0.58	0.25

APPENDIX M. STATISTICAL ANALYSIS FOR SPORE PRODUCTION FOR

CERCOSPORA BETCOLA ISOLATES FROM DIFFERENT TEMPERATURE

REGIMES

Table M.1.Combined analysis of variance for spore production of tetraconazole-sensitive
Cercospora beticola isolate (07-230) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	2333333	0.41
Replicate temperature	2	2607143	0.47
Trial x Replicate temperature	2	5511905	0.21
Temperature regimes	6	2579365	0.61
Trial x Temperature regimes	6	4888889	0.22
Replicate temperature x Temperature regimes	12	4871032	0.19
Trial x Replicate temperature x Temperature regimes	12	5025794	0.17
Residual	42	3404762	

Table M.2. Combined analysis of variance for spore production of tetraconazole-sensitive
Cercospora beticola isolate (08-640) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	761905	0.69
Replicate temperature	2	5190476	0.35
Trial x Replicate temperature	2	1333333	0.76
Temperature regimes	6	4956349	0.43
Trial x Temperature regimes	6	7845238	0.17
Replicate temperature x Temperature regimes	12	3384921	0.75
Trial x Replicate temperature x Temperature regimes	12	4833333	0.47
Residual	42	4857143	

Table M.3. Combined analysis of variance for spore production of tetraconazole-resistant
Cercospora beticola isolate (09-347) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	107143	0.86
Replicate temperature	2	1107143	0.72
Trial x Replicate temperature	2	7750000	0.11
Temperature regimes	6	2107143	0.71
Trial x Temperature regimes	6	2162698	0.70
Replicate temperature x Temperature regimes	12	4940476	0.18
Trial x Replicate temperature x Temperature regimes	12	3805556	0.37
Residual	42	3392857	

Table M.4.Combined analysis of variance for spore production of tetraconazole-resistant
Cercospora beticola isolate (07-981) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	761905	0.68
Replicate temperature	2	2654762	0.54
Trial x Replicate temperature	2	226190	0.95
Temperature regimes	6	4662698	0.38
Trial x Temperature regimes	6	2956349	0.66
Replicate temperature x Temperature regimes	12	2251984	0.89
Trial x Replicate temperature x Temperature regimes	12	6378968	0.17
Residual	42	4285714	

APPENDIX N. STATISTICAL ANALYSIS FOR SPORE GERMINATION FOR

CERCOSPORA BETCOLA ISOLATES FROM DIFFERENT TEMPERATURE

REGIMES

Table N.1. Combined analysis of variance for spore germination of tetraconazole-sensitive *Cercospora beticola* isolate (07-230) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	2.01	0.12
Replicate temperature	2	0.32	0.68
Trial x Replicate temperature	2	1.51	0.17
Temperature regimes	6	0.66	0.57
Trial x Temperature regimes	6	0.26	0.92
Replicate temperature x Temperature regimes	12	0.31	0.97
Trial x Replicate temperature x Temperature regimes	12	0.55	0.77
Residual	42	0.82	

Table N.2. Combined analysis of variance for spore germination of tetraconazole-sensitive *Cercospora beticola* isolate (08-640) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.05	0.81
Replicate temperature	2	0.88	0.58
Trial x Replicate temperature	2	4.74	0.06
Temperature regimes	6	2.48	0.80
Trial x Temperature regimes	6	3.62	0.62
Replicate temperature x Temperature regimes	12	1.95	1.00
Trial x Replicate temperature x Temperature regimes	12	8.10	0.62
Residual	42	34.00	

Table N.3. Combined analysis of variance for spore germination of tetraconazole-sensitive *Cercospora beticola* isolate (09-347) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	1.71	1.57
Replicate temperature	2	0.93	0.42
Trial x Replicate temperature	2	0.64	0.29
Temperature regimes	6	4.40	0.67
Trial x Temperature regimes	6	11.12	1.69
Replicate temperature x Temperature regimes	12	3.24	0.25
Trial x Replicate temperature x Temperature regimes	12	8.52	0.65
Residual	42	46.00	

Table N.4. Combined analysis of variance for spore germination of tetraconazole-sensitive *Cercospora beticola* isolate (07-981) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.05	0.11
Replicate temperature	2	0.02	0.03
Trial x Replicate temperature	2	0.17	0.18
Temperature regimes	6	1.24	0.46
Trial x Temperature regimes	6	5.62	2.07
Replicate temperature x Temperature regimes	12	3.48	0.64
Trial x Replicate temperature x Temperature regimes	12	3.67	0.68
Residual	42	19.00	

APPENDIX O. STATISTICAL ANALYSIS FOR RADIAL GROWTH OF *CERCOSPORA*

BETCOLA ISOLATES FROM DIFFERENT TEMPERATURE REGIMES

Table O.1. Combined analysis of variance for radial growth of tetraconazole-sensitive
Cercospora beticola isolate (07-230) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.006	0.5668
Replicate temperature	2	0.023	0.2816
Trial x Replicate temperature	2	0.003	0.8273
Temperature regimes	6	0.763	<0.0001
Trial x Temperature regimes	6	0.013	0.6401
Replicate temperature x Temperature regimes	12	0.014	0.6575
Trial x Replicate temperature x Temperature regimes	12	0.006	0.9713
Residual	42	0.018	

Table O.2. Combined analysis of variance for radial growth of tetraconazole-sensitive
Cercospora beticola isolate (08-640) after exposure to dfferent temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.0005	0.9098
Replicate temperature	2	0.0154	0.6605
Trial x Replicate temperature	2	0.0044	0.8871
Temperature regimes	6	0.6692	<0.0001
Trial x Temperature regimes	6	0.0571	0.1833
Replicate temperature x Temperature regimes	12	0.0045	0.9998
Trial x Replicate temperature x Temperature regimes	12	0.0269	0.7109
Residual	42	0.0366	

Table O.3. Combined analysis of variance for radial growth of tetraconazole-resistant
Cercospora beticola isolate (09-347) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.023	0.2973
Replicate temperature	2	0.010	0.6000
Trial x Replicate temperature	2	0.001	0.9289
Temperature regimes	6	3.964	<0.0001
Trial x Temperature regimes	6	0.019	0.4761
Replicate temperature x Temperature regimes	12	0.031	0.1658
Trial x Replicate temperature x Temperature regimes	12	0.008	0.9562
Residual	42	0.021	

Table O.4. Combined analysis of variance for radial growth of tetraconazole-resistant *Cercospora beticola* isolate (07-981) after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.0001	0.9568
Replicate temperature	2	0.0129	0.7276
Trial x Replicate temperature	2	0.0062	0.8575
Temperature regimes	6	0.2591	<0.0001
Trial x Temperature regimes	6	0.0243	0.7245
Replicate temperature x Temperature regimes	12	0.0572	0.193
Trial x Replicate temperature x Temperature regimes	12	0.0116	0.988
Residual	42	0.0401	

APPENDIX P. STATISTICAL ANALYSIS FOR SENSITIVITY OF *CERCOSPORA BETCOLA* ISOLATES FROM DIFFERENT TEMPERATURE REGIMES TO TETRACONAZOLE

Table P.1. Combined analysis of variance for sensitivity of tetraconazole-sensitive *Cercospora beticola* isolate (07-230) to tetraconazole after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.0000007	0.47
Replicate temperature	2	0.0000004	0.74
Trial x Replicate temperature	2	0.0000003	0.82
Temperature regimes	6	0.0000027	0.09
Trial x Temperature regimes	6	0.0000003	0.95
Replicate temperature x Temperature regimes	12	0.0000003	0.99
Trial x Replicate temperature x Temperature regimes	12	0.0000011	0.63
Residual	42	0.0000014	

Table P.2. Combined analysis of variance for sensitivity of tetraconazole-sensitive *Cercospora beticola* isolate (08-640) to tetraconazole after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.0000028	0.13
Replicate temperature	2	0.0000002	0.85
Trial x Replicate temperature	2	0.0000029	0.11
Temperature regimes	6	0.0000043	0.01
Trial x Temperature regimes	6	0.0000003	0.96
Replicate temperature x Temperature regimes	12	0.0000006	0.89
Trial x Replicate temperature x Temperature regimes	12	0.0000008	0.81
Residual	42	0.0000012	

Table P.3. Combined analysis of variance for sensitivity of tetraconazole-resistant *Cercospora beticola* isolate (09-347) to tetraconazole after exposure to different temperature regimes.

Source of variation	DF	Mean square	*P*
Trial	1	0.27	0.4727
Replicate temperature	2	0.89	0.1895
Trial x Replicate temperature	2	…0.16	0.7281
Temperature regimes	6	219.77	<0.0001
Trial x Temperature regimes	6	1.17	0.0556
Replicate temperature x Temperature regimes	12	0.51	0.4778
Trial x Replicate temperature x Temperature regimes	12	0.46	0.5583
Residual	42	0.52	

Table P.4. Combined analysis of variance for sensitivity of tetraconazole-resistant *Cercospora beticola* isolate (07-981) to tetraconazole after exposure to different temperature regimes.

Source of variation	DF	Mean square	P
Trial	1	0.64	0.6443
Replicate temperature	2	0.15	0.9507
Trial x Replicate temperature	2	2.94	0.3806
Temperature regimes	6	59.19	<0.0001
Trial x Temperature regimes	6	2.60	0.5203
Replicate temperature x Temperature regimes	12	4.69	0.1347
Trial x Replicate temperature x Temperature regimes	12	3.96	0.2371
Residual	42	2.97	

APPENDIX Q. STATISTICAL ANALYSIS SUMMARY FOR EFFECT OF DIFFERENT TEMPERATURE REGIMES ON TETRACONAZOLE-SENSITIVE AND -RESISTANT *CERCOSPORA BETICOLA* ISOLATES BASED ON DISEASE SEVERITY

Table Q.1. Test statistic for severity of Cercospora leaf spot caused by tetraconazole-sensitive *Cercospora beticola* isolate (07-230) after exposure to different temperature regimes.

Effect	$df_N{}^a$	$df_D{}^b$	F	P
Temperature regimes	6	105	3.25	0.006
Temperature replicate	2	105	1.30	0.277
Temperature replicate x Temperature regimes	12	105	0.97	0.482

Table Q.2. Test statistic for severity of Cercospora leaf spot caused by tetraconazole-sensitive *Cercospora beticola* isolate (08-640) after exposure to different temperature regimes.

Effect	$df_N{}^a$	$df_D{}^b$	F	P
Temperature regimes	6	105	6.06	<0.0001
Temperature replicate	2	105	0.99	0.3768
Temperature replicate x Temperature regimes	12	105	0.98	0.4735

Table Q.3. Test statistic for severity of Cercospora leaf spot caused by tetraconazole-resistant *Cercospora beticola* isolate (09-347) after exposure to different temperature regimes.

Effect	$df_N{}^a$	$df_D{}^b$	F	P
Temperature regimes	6	105	4.07	0.001
Temperature replicate	2	105	1.37	0.258
Temperature replicate x Temperature regimes	12	105	1.76	0.064

Table Q.4. Test statistic for severity of Cercospora leaf spot caused by tetraconazole-resistant *Cercospora beticola* isolate (07-981) after exposure to different temperature regimes.

Effect	$df_N{}^a$	$df_D{}^b$	F	P
Temperature regimes	6	105	0.90	0.49
Temperature replicate	2	105	0.42	0.65
Temperature replicate x Temperature regimes	12	105	0.47	0.93

APPENDIX R. NON-PARAMETRIC ANALYSIS SUMMARY FOR EFFECT OF DIFFERENT TEMPERATURE REGIMES ON TETRACONAZOLE-SENSITIVE AND -RESISTANT *CERCOSPORA BETICOLA* ISOLATES BASED ON DISEASE SEVERITY

Table R.1. Non-parametric analysis for severity of Cercospora leaf spot caused by tetraconazole-sensitive *Cercospora beticola* isolate (07-230) after exposure to different temperature regimes.

Temperature Regimes (˚C)	Temperature Replicate	Mean Rank Severity	Relative Effect	Variance	Upper Limit	Lower Limit
Original		45.33	0.36	2.00	0.62	0.16
-20ºC	1	54.00	0.43	1.31	0.63	0.25
	2	67.92	0.54	2.76	0.78	0.27
	3	74.83	0.59	2.43	0.81	0.32
4ºC	1	87.33	0.69	0.44	0.79	0.56
	2	66.58	0.52	1.74	0.73	0.31
	3	44.17	0.35	2.13	0.62	0.15
20ºC	1	50.92	0.40	1.07	0.59	0.24
	2	85.08	0.67	1.45	0.84	0.44
	3	55.25	0.44	0.69	0.58	0.30
-20ºC to 20ºC to -20ºC to 20ºC	1	43.33	0.34	2.81	0.66	0.13
	2	60.92	0.48	1.26	0.67	0.30
	3	54.00	0.43	1.31	0.63	0.25
-20ºC to 4ºC	1	74.75	0.59	0.77	0.73	0.43
	2	81.67	0.64	0.34	0.74	0.54
	3	55.25	0.44	0.69	0.58	0.30
-20ºC to 4ºC to 20ºC to 4ºC	1	95.67	0.76	0.63	0.86	0.59
	2	80.42	0.63	1.00	0.78	0.45
	3	80.42	0.63	1.00	0.78	0.45

Table R.2. Non-parametric analysis for severity of Cercospora leaf spot caused by tetraconazole-sensitive *Cercospora beticola* isolate (08-640) after exposure to different temperature regimes.

Temperature Regimes (˚C)	Temperature Replicate	Mean Rank Severity	Relative Effect	Variance	Upper Limit	Lower Limit
Original		56.58	0.45	1.56	0.66	0.25
-20ºC	1	53.00	0.42	0.08	0.47	0.37
	2	38.17	0.30	0.66	0.46	0.18
	3	38.17	0.30	0.66	0.46	0.18
4ºC	1	38.17	0.30	0.66	0.46	0.18
	2	53.00	0.42	0.08	0.47	0.37
	3	53.00	0.42	0.08	0.47	0.37
20ºC	1	88.50	0.70	1.00	0.84	0.50
	2	82.25	0.65	2.33	0.85	0.36
	3	65.00	0.51	1.10	0.69	0.34
-20ºC to 20ºC to -20ºC to 20ºC	1	85.67	0.68	0.82	0.81	0.50
	2	53.75	0.42	1.09	0.61	0.26
	3	46.33	0.36	1.50	0.59	0.19
-20ºC to 4ºC	1	67.58	0.53	2.33	0.77	0.28
	2	50.75	0.40	3.32	0.71	0.16
	3	61.92	0.49	1.56	0.69	0.29
-20ºC to 4ºC to 20ºC to 4ºC	1	93.83	0.74	0.55	0.85	0.59
	2	99.50	0.79	0.74	0.90	0.60
	3	83.17	0.66	1.39	0.82	0.43

Table R.3. Non-parametric analysis for severity of Cercospora leaf spot caused by tetraconazole-resistant *Cercospora beticola* isolate (09-347) after exposure to different temperature regimes.

Temperature Regimes (˚C)	Temperature Replicate	Mean Rank Severity	Relative Effect	Variance	Lower Limit	Upper Limit
Original		77.50	0.61	1.87	0.36	0.81
-20ºC	1	51.67	0.41	0.61	0.28	0.55
	2	69.00	0.54	1.03	0.37	0.71
	3	60.33	0.48	0.92	0.32	0.64
4ºC	1	113.50	0.90	0.23	0.76	0.95
	2	80.50	0.64	1.61	0.40	0.82
	3	56.25	0.44	2.48	0.21	0.71
20ºC	1	43.17	0.34	2.22	0.14	0.62
	2	25.50	0.20	0.45	0.11	0.35
	3	57.33	0.45	2.32	0.22	0.71
-20ºC to 20ºC to -20ºC to 20ºC	1	60.33	0.48	0.92	0.32	0.64
	2	69.00	0.54	1.03	0.37	0.71
	3	69.00	0.54	1.03	0.37	0.71
-20ºC to 4ºC	1	40.00	0.31	1.26	0.16	0.53
	2	60.33	0.48	0.92	0.32	0.64
	3	45.83	0.36	0.98	0.21	0.55
-20ºC to 4ºC to 20ºC to 4ºC	1	86.33	0.68	0.60	0.53	0.80
	2	77.67	0.61	0.92	0.44	0.76
	3	43.00	0.34	0.09	0.29	0.39

Table R.4. Non-parametric analysis for severity of Cercospora leaf spot caused by tetraconazole-resistant *Cercospora beticola* isolate (07-981) after exposure to different temperature regimes.

Temperature Regimes (˚C)	Temperature Replicate	Mean Rank Severity	Relative Effect	Variance	Lower Limit	Upper Limit
Original	1	60.83	0.48	1.07	0.31	0.65
-20ºC	1	70.17	0.55	0.71	0.41	0.69
	2	60.83	0.48	1.07	0.31	0.65
	3	76.67	0.61	1.17	0.41	0.77
4ºC	1	50.83	0.40	2.91	0.17	0.70
	2	47.92	0.38	1.56	0.20	0.61
	3	51.50	0.41	1.19	0.24	0.60
20ºC	1	75.08	0.59	2.44	0.32	0.81
	2	74.83	0.59	2.40	0.32	0.81
	3	49.67	0.39	2.32	0.18	0.66
-20ºC to 20ºC to -20ºC to 20ºC	1	60.83	0.48	1.07	0.31	0.65
	2	67.33	0.53	1.72	0.31	0.74
	3	51.50	0.41	1.19	0.24	0.60
-20ºC to 4ºC	1	60.83	0.48	1.07	0.31	0.65
	2	60.83	0.48	1.07	0.31	0.65
	3	58.67	0.46	2.16	0.24	0.71
-20ºC to 4ºC to 20ºC to 4ºC	1	86.67	0.68	0.43	0.56	0.78
	2	60.83	0.48	1.07	0.31	0.65
	3	70.17	0.55	0.71	0.41	0.69

APPENDIX S. EFFICACY OF DIFFERENT CONCENTRATIONS OF

AZOXYSTROBIN, TRIFLOXYSTROBIN, PYRACLOSTROBIN, PENTHIOPYRAD,

AND PROTHIOCONAZOLE AT CONTROLLING *RHIZOCTONIA SOLANI* AG-2-2 IIIB
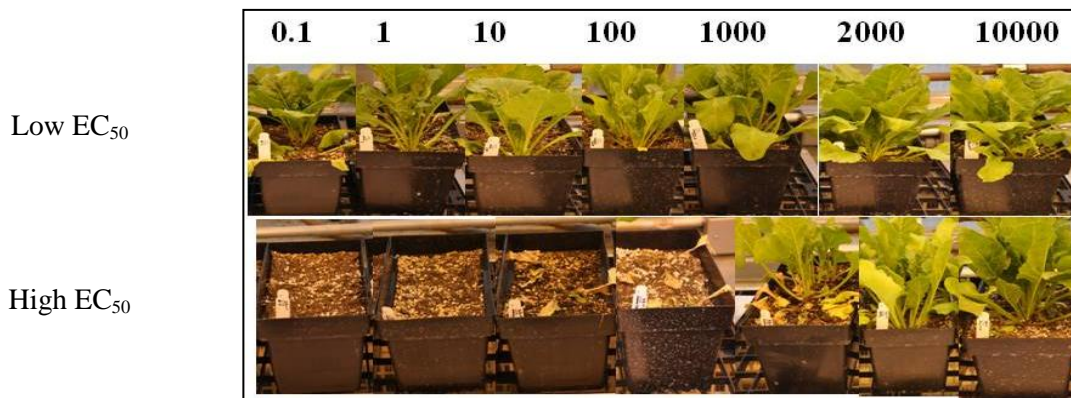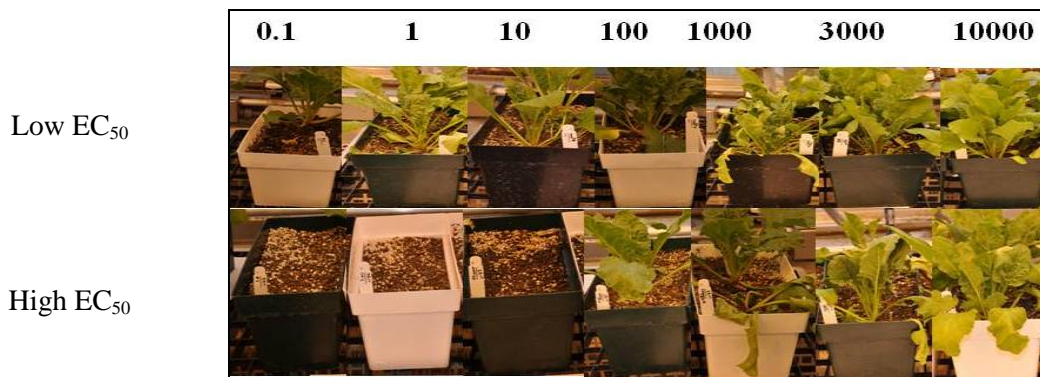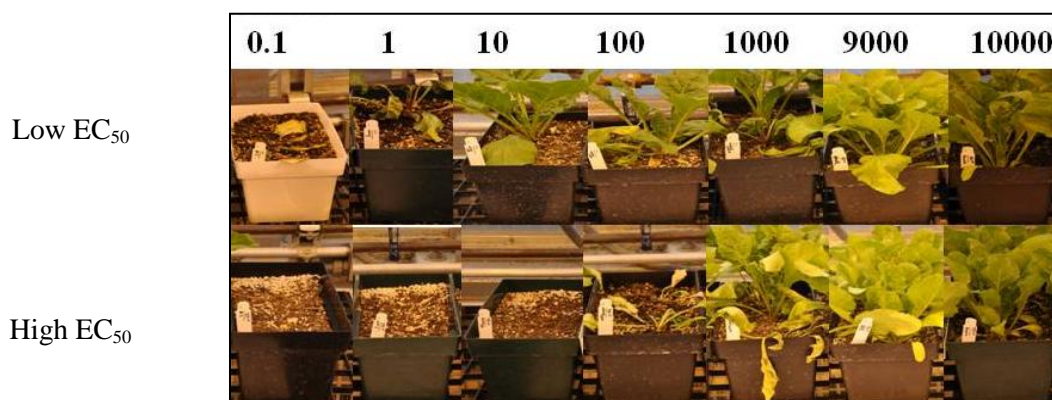
WITH HIGH AND LOW EC$_{50}$
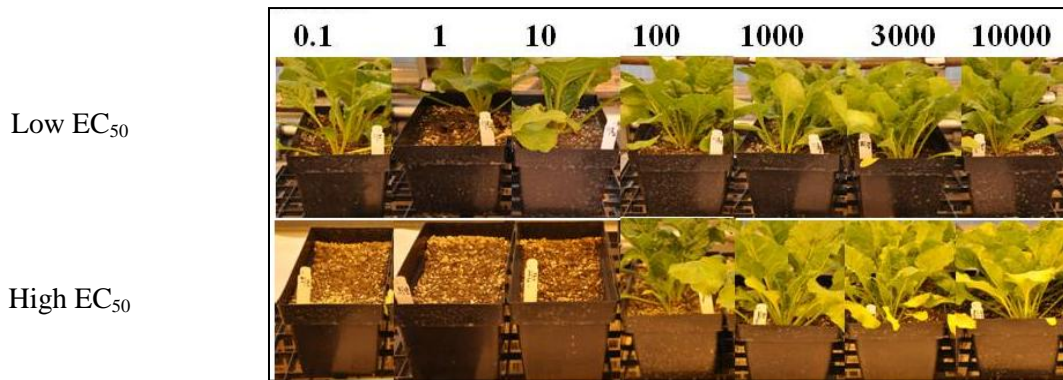
Figure S.1. Efficacy of different azoxystrobin concentrations at controlling *Rhizoctonia solani* isolates with high EC$_{50}$ value (22-1) and low EC$_{50}$ value (850).

Figure S.2. Efficacy of different trifloxystrobin concentrations at controlling *Rhizoctonia solani* isolates with high EC$_{50}$ value (22-1) and low EC$_{50}$ value (850).

Figure S.3. Efficacy of different pyraclostrobin concentrations at controlling *Rhizoctonia solani* isolates with high EC_{50} value (22-1) and low EC_{50} value (850).

Figure S.4. Efficacy of different penthiopyrad concentrations at controlling *Rhizoctonia solani* isolates with high EC_{50} value (22-1) and low EC_{50} value (850).
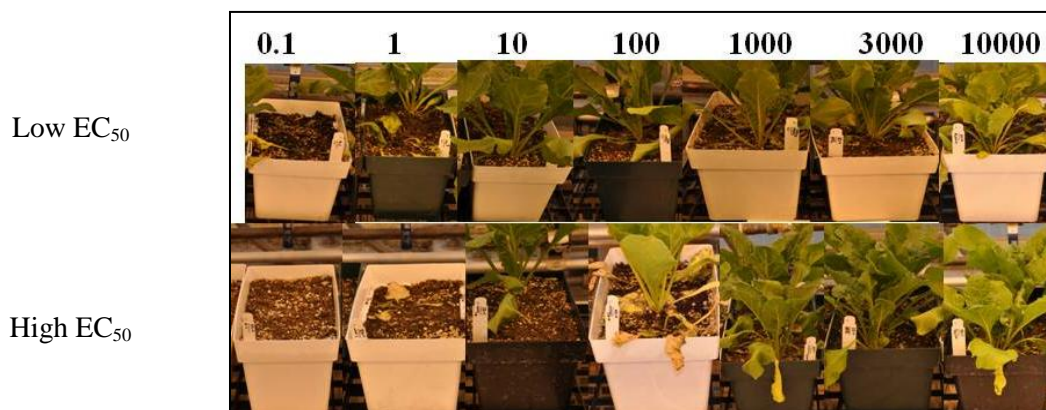

Figure S.5. Efficacy of different prothioconazole concentrations at controlling *Rhizoctonia solani* isolates with high EC_{50} value (22-1) and low EC_{50} value (850).
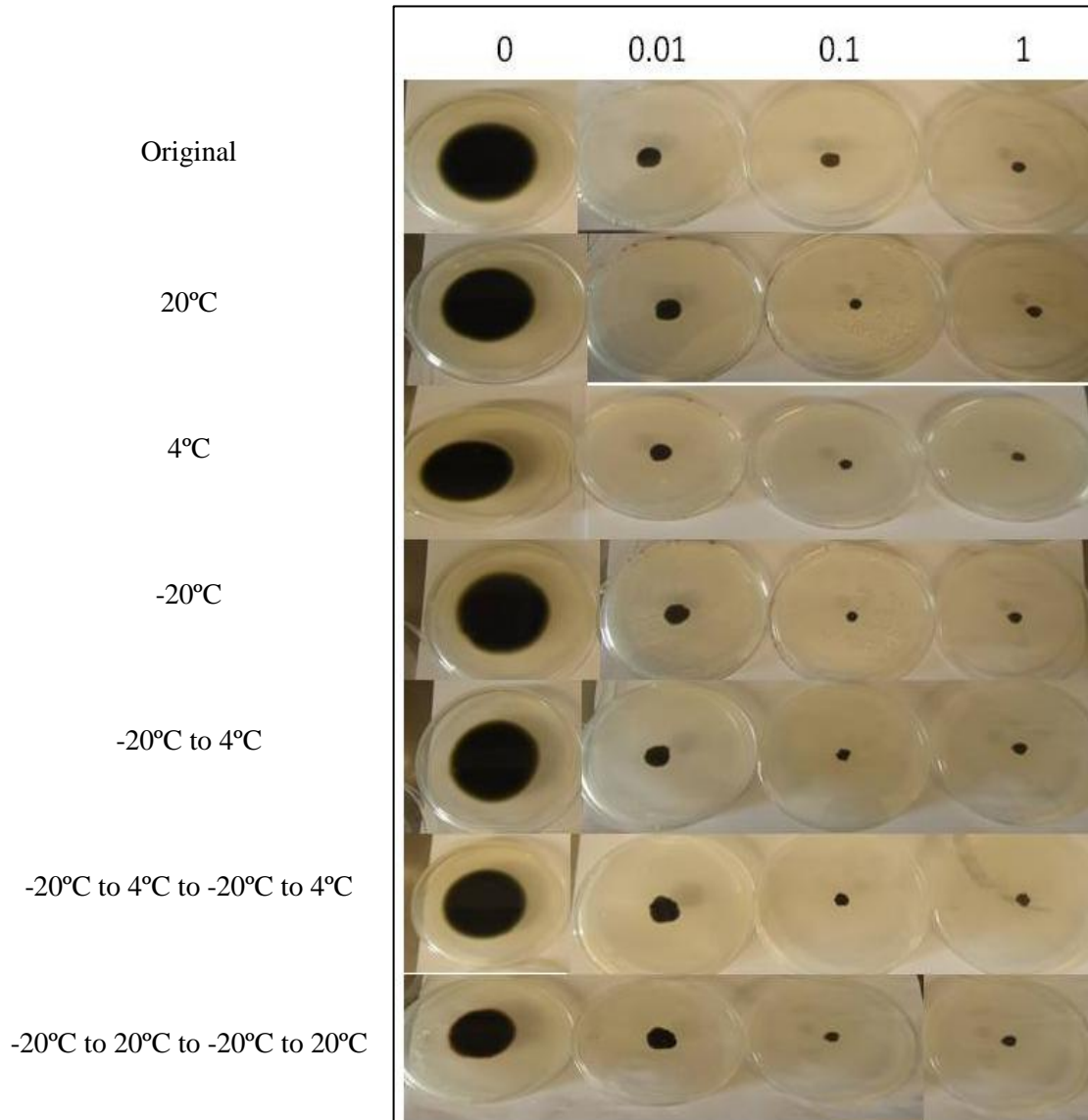
Figure T.1. Mycelium radial growth of tetraconazole-sensitive *Cercospora beticola* isolate (08-640) at different tetraconazole concentrations ($\mu g\ ml^{-1}$) after exposure to different temperature regimes.
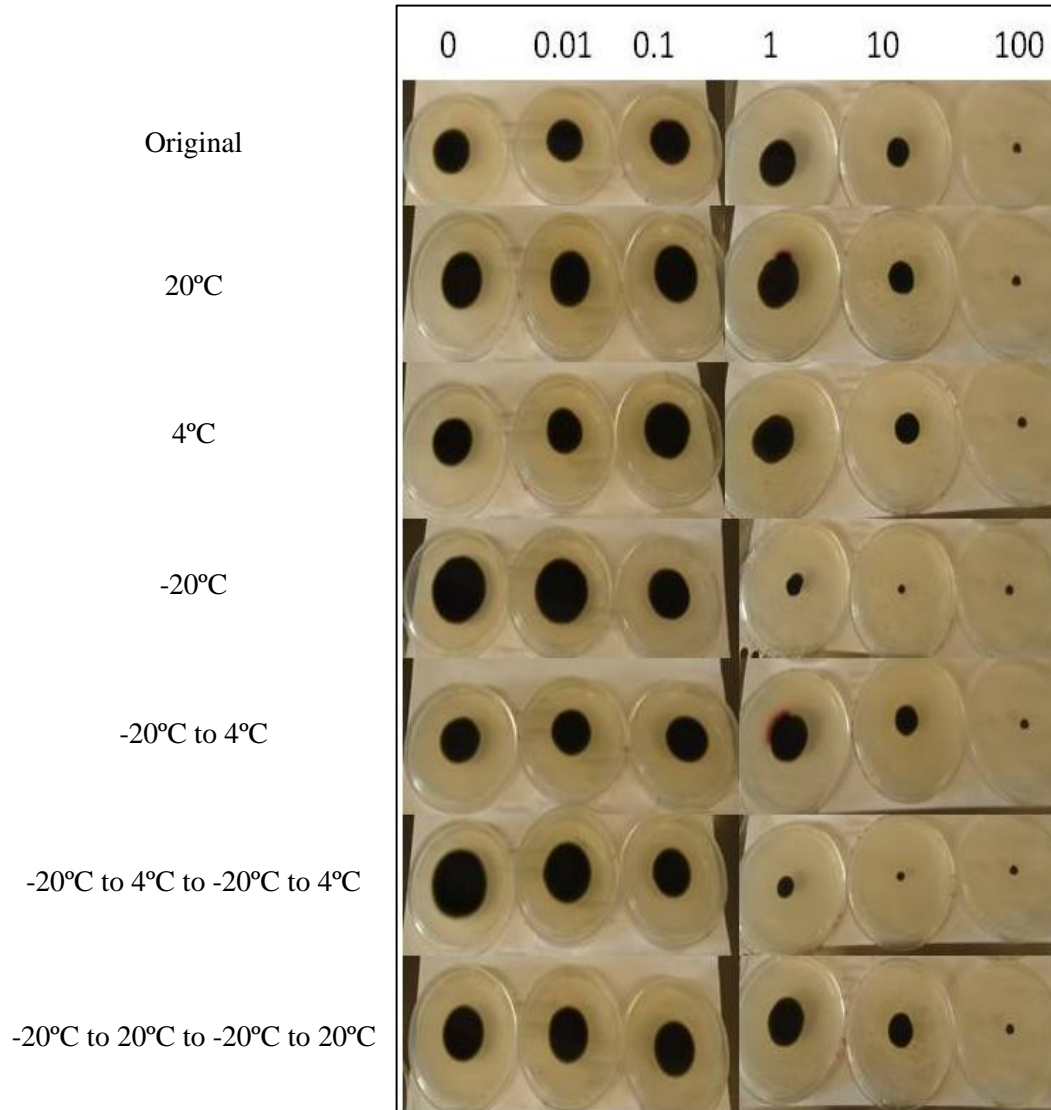
Figure T.2. Mycelium radial growth of tetraconazole-resistant *Cercospora beticola* isolate (09-347) at different tetraconazole concentrations (µg ml^{-1}) after exposure to different temperature regimes.
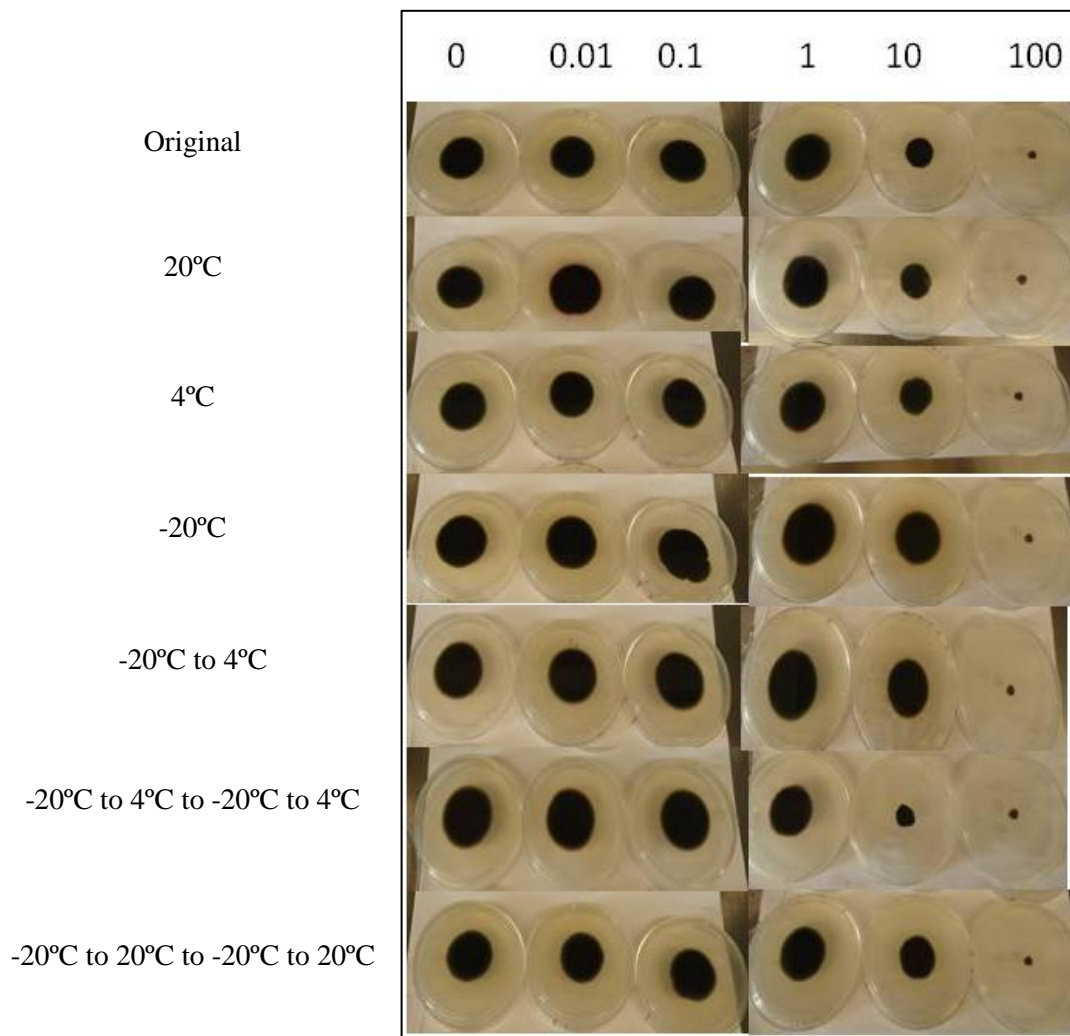
Figure T.3. Mycelium radial growth of tetraconazole-resistant *Cercospora beticola* isolate (07-981) at different tetraconazole concentrations (µg ml^{-1}) after exposure to different temperature regimes.